File No. 11014/08/2020-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, the22nd June, 2021

ORDER

Subject: Revised FSSAI Manual of Methods of Analysis of Foods – reg.

Following Revised FSSAI Manual of Methods of Analysis of Foods have been approved by the Food Authority in its 33rd meeting held on 23.03.2021 and are enclosed herewith.

(i) Oils and Fats
(ii) Spices, Herbs and Condiments

2. The manuals shall be used by the laboratories with immediate effect. It supersedes the earlier manual on 'Oils and Fats' and 'Spices and condiments' issued vide Office Order No. 1-90/FSSAI/SP (MS&A)/2009 dated 25.05.2016.

3. 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

(Harinder Singh Oberoi)
Advisor (QA)

To:

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

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA
Inspiring Trust, Assuring Safe & Nutritious Food
Ministry of Health and Family Welfare, Government of India

MANUAL OF METHODS OF ANALYSIS OF FOODS

SPICES HERBS AND CONDIMENTS

PREFACE

Food safety requires an assurance that food will not cause any harm to the consumer, when it is prepared and/or consumed according to its intended use. There is a significant challenge in ensuring food safety to protect public health. Safeguarding food safety in today's complex world is a formidable task and is possible only with an intensive effort of all the stakeholders including regulatory authorities, industry and consumers.

The FSSAI Manual of Methods for Analysis of Spices, Herbs and Condiments is principally intended to provide unified, up-to-date testing methods for regulatory compliance. The manual brings together testing methodologies approved by FSSAI for use in surveillance and implementing the regulatory program. The objective here is to adopt "One Parameter - One Method" approach. These methods are dynamic and will be constantly updated,commensurate with the latest technological advancements in food analysis. The FSSAI notified laboratories shall use these testing methods only for analyzing samples under the Food Safety and Standards Act, 2006 and Food Safety and Standards Regulations, 2011.

Any suggestions/feedback from the stakeholders, which will contribute towards updating the manuals from time to time are welcome.

Shri ArunSinghal,
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ACKNOWLEDGEMENT

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Sincere thanks to the Panel, Chairman for their valuable guidance and encouragement and the Secretariat of this panel who have extended their support during this revision process.

Deepest appreciation to the Chairperson, FSSAI and CEO, FSSAI for their cooperation, support and constant encouragement without which the work would not have seen the light of day.

June 2021

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Note: The test methods given in the manual are standardized/ validated/ taken from national or international methods or recognized 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.

MANUAL FOR ANALYSIS OF SPICES, HERBS AND CONDIMENTS

Spices and condiments are added in food in small amounts but they make important contribution to the sensory qualities due to the presence of volatile and fixed oils. Standards for spices and condiments are laid under Food Safety and Standards (Food Products Standards and Food Additives) Regulations, 2011. The methods described in this manual are applicable for evaluating parameters such as proximate composition, volatiles, spice bioactives and adulteration. For analytical methods related to mycotoxins, pesticide residues, heavy metal and microbiological analysis of spices the analyst should refer the relevant FSSAI Manuals.

	Grinding of Sample for Chemical Analysis (Sample Preparation)		
Method No.	FSSAI 10.001:2021	**Revision No. & Date**	0.0
Scope	This method is applicable to the majority of spices and condiments. However, in view of their large number and their diversity, it may be necessary in certain special cases (such as considerable hardness, high content of water and essential oil or fat) to carry out some modifications or even to choose a more suitable method.		
Caution	None		
Principle	Spices should be ground so as to pass through 1-mm IS Sieve.		
Apparatus/ Instruments	1. Grinding Mill: It should be made of a material which does not absorb moisture, easy to clean and have as little dead space as possible. It should enable quick and uniform grinding without causing undue heating and avoid as far as possible contact with outside air. It should be adjustable to obtain particles of about 1 mm size. 2. Sieves: 1 mm IS sieve or equivalent 3. Sample Container - A clean dry air-tight glass container or any other suitable container on which the sample has no action and of such a size that it will be nearly but not completely filled with the ground sample.		
Procedure	1. Mix carefully the sample for analysis. 2. Using the grinding mill, grind a small quantity of this sample and reject. 3. Then, grind quickly an amount slightly larger than that required for the tests. 4. Avoid undue heating of the apparatus during grinding. 5. Mix carefully so as to avoid stratification. 6. Transfer the ground material to the previously dried sample container and immediately close the latter.		
Reference	I.S. Specification No. IS 1797 – 1985 (Reaffirmed in 2009) Methods of Test for Spices and Condiments (Second Revision		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

	Determination of Extraneous Matter and Other Refractions in Whole Spices		
Method No.	FSSAI 10.002:2021	**Revision No. & Date**	0.0
Scope	This method is applicable to most of the spices, condiments and dehydrated herbs. The method specifies a general procedure for visual examination of spices for the determination of visible filth. This method also covers the determination of other refractions such as: • Insect damaged matter, empty and malformed capsules, broken, damaged, immature and shriveled seeds, unripe, discolored and marked fruits in all spices • Tendrils, mother cloves, 'khoker' cloves and headless cloves • Light seeds in cardamom • Defective rhizomes in turmeric • Floral waste in saffron • Pinheads, broken berries, insect defiled berries, moldy berries in black pepper etc.		
Caution	None		
Principle	The method is based on the visual detection of extraneous matter mold infestation, animal excreta, whole insects and/or large insect fragments, sticks, stems, feathers, thread, paper, rubber, shells, wood, bones, dead insects, stones, glass, plastics, metal etc. in spices and condiments.		
Definitions	1. The extraneous matter wherever prescribed, shall be classified as follows: 2. **Organic extraneous matter** such as chaff, stems, straw, edible seeds from other plants (e.g. Mace in nutmeg), wood, bone, living and/or dead insects, insect fragments maggots, worms, larvae, droppings, excrements, hair, feathers, thread, paper, rubber etc. 3. **Inorganic extraneous matter** such as dust, dirt, glass, stones, hard plastic, soft plastic, paint and lumps of earth. 4. **Pinheads:** Pinhead means berry of very small size that has not developed. 5. **Broken berries:** Broken berry means berry that has been separated in two or more parts. 6. **Mother cloves**: A fruit in the form of an ovoid brown berry surmounted by four incurved sepals. 7. **Khoker cloves**: A clove which has undergone fermentation as a result of incomplete drying as evidenced by its pale brown color whitish mealy appearance and other wrinkled surface. 8. **Headless cloves**: A clove consisting of only the receptacle and sepals and which has lost the domed shaped head. 9. **Defective rhizomes** consist of shriveled fingers and or bulbs internally damaged, hollow or porous rhizomes scorched by boiling and other		

	types of damaged rhizomes. 10. **Floral waste**: Floral waste means yellow filaments that are unattached and separated pollens, stamens, parts of ovaries and other parts of flowers of *Crocus sativus* Linnaeus. 11. **Light seeds (cardamom)**: Seeds that are brown or red in color and broken immature and shriveled seeds.
Apparatus/ Instruments	1. Table and suitable lighting 2. Paper, large, clean white sheets (preferably glazed) 3. Neutral grey paper 4. Spatulas, selection, of small and large sizes 5. Balance, accurate to 0.001 g 6. Butcher's knife or any other suitable tool
Laboratory sample size	1. The laboratory sample should be a composite sample taken from different parts of the lot. 2. For high bulk density products, the laboratory sample size should be about 500 g (see Table 1). 3. For low bulk density products, the laboratory sample size should be about 250 g (see Table 1). 4. Saffron is an exception and the laboratory sample size should be 3 g.
Sample Preparation	1. The laboratory sample should be homogenized before taking the test portion. 2. The entire laboratory sample is to be analyzed unless a test portion is appropriate. The appropriateness is determined based on historical performance, the level of defect under investigation and homogeneity of the samples (see Table 1) **Table 1 — Laboratory sample and test portion size**

Bulk density of product	Product	Laboratory sample size g	Appropriate test portion size g	Minimum test portion size g
High	Allspice/pimento	500	100	100
	Anise seed		100	10
	Caraway seed		100	10
	Cardamom seed		100	100
	Cassia/cinnamon		100	50
	Celery seed		100	10
	Cloves		100	10
	Coriander seed		100	10
	Cumin seed		100	10
	Dill seed		100	10
	Fennel seed		100	10
	Garlic		100	10
	Ginger		100	100
	Juniper berries		100	100
	Nutmeg (whole and broken)	100 Nuts or 500 g if broken	100 Nuts or 500 g if broken	50 Nuts or 250 g if broken
	Onion	500	100	10
	Pepper (black and white)		100	100
	Poppy seed		100	10
	Sesame seed		100	10
	Turmeric		100	100
Low	Capsicums	250	100	100
	Mace		25	25
	Herb leaves		25	5
Other	Saffron	3	3	0,5

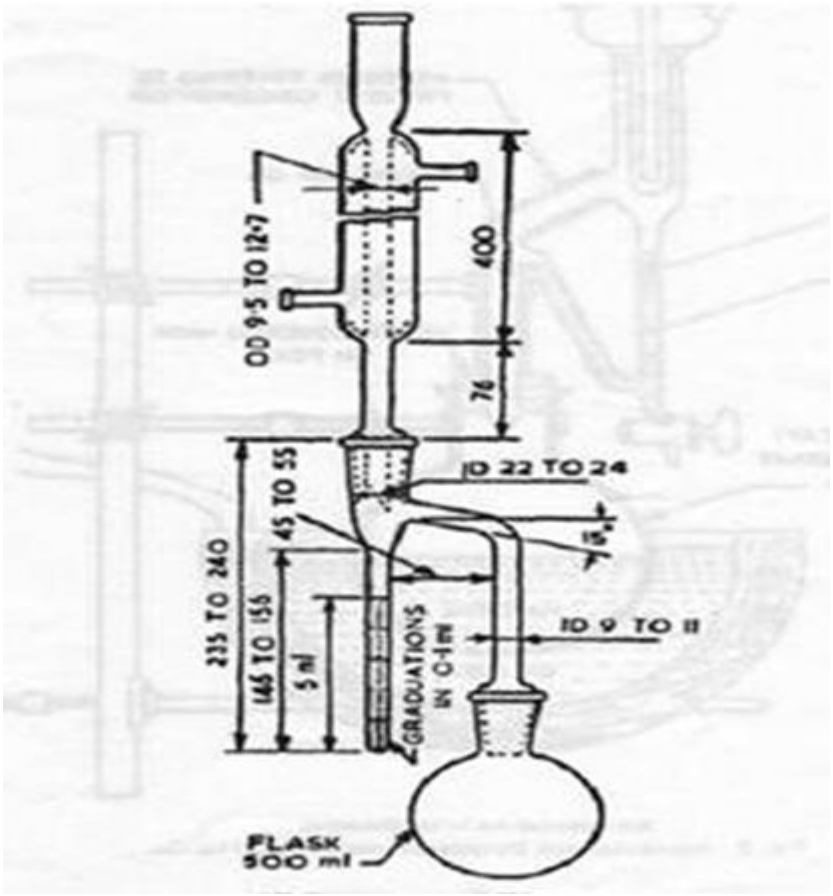
Examination procedure

1. **All spices and herbs including nutmeg (broken):**
   a) Weigh the sample prepared as in Table 1, to the nearest 0.01 g.
   b) Spread the product over a wide area of a well-lit, white sheet
   c) Using a spatula, move the product in such a manner that it is thoroughly examined.
   d) Separate all inorganic and organic extraneous matter.
   e) Weigh the extraneous matters obtained above separately.
   f) Calculate total extraneous matter as a sum of inorganic extraneous matter and organic extraneous matter.
   g) Report up to one decimal point as % mass fraction.
2. **Nutmeg (whole):**
   a) Break 100 nutmegs lengthwise using a butcher's knife.
   b) Examine the broken surfaces for insects, insect parts, insect channeling, excreta, or visible mold.
   c) Report extraneous matter as % by mass.
3. **Floral waste in saffron:**
   a) Reconstitute the test sample by reincorporating extraneous matter, homogenize and then weigh accurately 3 g sample.
   b) Spread on a sheet of neutral grey paper and separate all yellow

	filaments attached or unattached and other floral waste that might be present. c) Weigh in a watch glass and calculate as % by mass. **4. Determination of other refractions:** Proceed as ***above for Floral waste in saffron,*** for the determination of: a) Insect damaged matter, empty and malformed capsules, broken, damaged, immature and shriveled seeds, unripe, discolored and marked fruits in all spices. b) Tendrils, mother cloves, 'khoker' cloves and headless cloves. c) Light seeds in cardamom. d) Defective rhizomes in turmeric, e) Floral waste in saffron, f) Pinheads, broken berries, insect defiled berries, moldy berries in black pepper etc. Report each of them as % mass fraction up to one decimal point.
Calculation with units of expression	**1. Inorganic extraneous matter and organic extraneous matter:** The mass fraction of inorganic extraneous matter (W_{IM}), and mass fraction of organic extraneous matter (W_{OM}), expressed as %s, are given by: $$W_{IM} = 100 \times \frac{W_{IM}}{W_S}$$ $$W_{OM} = 100 \times \frac{W_{OM}}{W_S}$$ % extraneous matter (W_{EM}) = W_{IM} + W_{OM} Where, W_{IM} = mass, in g, of inorganic extraneous matter W_{EM} = mass, in g, of organic extraneous matter W_S = mass, in g, of the laboratory test sample or test portion, as appropriate.
Reference	1. ISO 927:2009, *Spices and condiments* — Determination of extraneous matter and foreign matter content. 2. Food Safety and Standards (Food Products Standards and Food Additives) Regulations, 2011. 3. IS Specification No IS 5453 (Part II) - 1966 / ISO 3632-2: 1993 Saffron – Methods of Test.
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of moisture content (Dean and Stark Distillation Method)		
Method No.	FSSAI 10.003:2021	**Revision No. & Date**	0.0
Scope	The quantity of water, expressed as a % by mass, distilled and collected in accordance with the method specified in this standard. The method is applicable to all spices as they contain volatile compounds.		
Caution	1. Toluene and hexane are highly flammable and hazardous in case of skin contact, eye contact, ingestion or inhalation. 2. Wear required personal protective equipment while working. 3. Avoid every possible source of ignition. 4. For disposal of solvent waste, follow good laboratory practices outlined by environment health and safety protocols in your institution.		
Principle	The amount of water is determined by distilling the material with an organic liquid not miscible with water and collecting the distillate in a graduated tube.		
Apparatus/ Instrument	1. Analytical balance, accurate upto 0.1 mg. 2. Moisture distillation apparatus: The apparatus consists of a glass flask heated by suitable means, provided with a reflux condenser discharging into a trap and connected to the flask. The connection between the trap and the condenser and the flask should be interchangeable ground glass joints. The trap serves to collect and measure the condensed water and to return the condensed solvent to the flask. a) **Flask,** of capacity 500 mL, made up of heat-resistance glass, well annealed and as free as possible from striae and similar effects. b) **Reflux condenser**, water cooled, made of glass, having a jacket approximately 400 mm long and an inner tube of diameter 9.5 to 12.5 mm. The tip of the condenser to be inserted in the receiver may be ground off at an angle of 30° from the vertical axis of the condenser. When inserted into the receiver, the tip of the condenser is 6 to 7 mm above the surface of the liquid in the receiver after distillation conditions have been established. c) **Receiver,** of capacity 5 mL, made of heat-resistant glass, well annealed and as free as possible from striae and similar defects, provided with ground glass joints, with the shape, dimensions and tolerances given in the figure, and consisting essentially of the upper chamber, together with the tube and ground joint leading to the flask, and the graduated tube. The graduated portion has a capacity of 5 mL when filled to the highest graduation mark. The scale covers the range of 0 to 5 mL and is graduated at intervals of 0.1 mL. The graduation marks corresponding to each milliliter are numbered and carried completely round the tube. The graduation marks midway between the numbered		

	marks are carried three-quarter-way, and the remaining marks are carried halfway, around the tube. The error at any indicated capacity does not exceed 0.05 mL. d) **Heat source**, either an oil bath or an electric heater provided with a sliding rheostat or other means of heat control. The temperature of the oil in the bath should not be very much higher than the boiling point of toluene. e) **Copper wire**, long enough to extend through the condenser and with one end twisted into a spiral. The diameter of the spiral is such that it fits snugly within the graduated portion of the receiver and yet can be moved up and down.
Materials and Reagents	***Note:*** Refer to Material Safety Data Sheets and ensure that safety guidelines are applied before using the solvents. 1. Toluene (For most spices) 2. Hexane (For capsicum, onion, garlic, and other spices containing large amount of sugar)
Preparation of Reagents	1. Toluene: Saturate the toluene by shaking with quantity of water and distil. Use the distillate for the determination of moisture.
Sample Preparation	Prepare test sample as described in Method No. FSSAI 10.001:2021.
Method of analysis	**Note**: Clean the entire apparatus with chromic acid cleaning solution to minimize adherence of water droplets to the sides of the condenser and the receiver. Rinse thoroughly with distilled water and dry completely before use. 1. Weigh to the nearest 0.01 g, about 20 - 40 g of prepared sample (or enough to yield 2 - 5 mL H_2O in the trap) and note down the exact weight. Transfer to the distilling flask with toluene. 2. Add enough toluene to cover test portion completely (about 75 mL). 3. Fill receiving tube with toluene pouring it through top of the condenser until it begins to overflow into the distillation flask. 4. Insert loose cotton plug in top of the condenser to prevent condensation of atmospheric moisture in the tube. 5. Add a few pumice stones to avoid bumping. Bring to boil and distill slowly about 2 drops per second (about 100 drops per min) until most of water distills over, then increase rate of distillation to 4 drops per second. 6. Continue distilling until 2 consecutive readings 15 min apart show no change. 7. Dislodge any water held up in the condensed with wire loop. Rinse condenser carefully with 5 mL toluene. 8. Continue distillation 3-5 min, cool receiver to 25 ± 2 °C allowing it to stand in air or cooling it in water.

	9. Solvent and water layers should now be clear, if not, let stand until clearing occurs. 10. Read volume of water estimating to nearest 0.1 mL and calculate %.  FIG:1: All Dimensions in millimeters MOISTURE DISTILLATION APPARATUS
Calculation with units of expression	The moisture content (% by mass) = $\frac{100*V}{M}$ Where, V = Volume of water collected in mL and M = Mass of the test portion in g ***Note:*** *It is assumed that density of water is 1 g/mL exactly.*
Reference	1. A.O.A.C 17th Edn, 2000 Official Method 986.21, Moisture in spices, Distillation method. 2. I.S. Specification No I.S 1797 – Methods of Test for Spices and Condiments. 3. Nielsen, S. S. (Ed.). (2003). *Food Analysis Laboratory Manual* (p. 557). Nueva York, USA: Kluwer Academic/Plenum Publishers.
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of moisture in spices and condiments (by Karl Fischer Method)		
Method No.	FSSAI 10.004:2021	**Revision No. & Date**	0.0
Scope	This method is applicable for the determination of the moisture content of spices and spice products such as seasonings.		
Caution	1. Karl-Fischer (KF) reagent is a powerful desiccant, the sample and reagent must be kept protected from atmospheric moisture in all conditions. 2. KF reagent must be added as precisely, accurately, reproducibly and with as high resolution as possible. 3. The water adhering to the walls of the titration cell (inner water film) must be removed by swirling about the conditioned contents of the titration vessel. 4. Do not use acetone to rinse glassware or equipment. The acetone residue reacts with methanol to form acetone dimethyl acetal and water, and thus bias water results high. 5. HYDRANAL® -Composite five contains five hazardous components—iodine, sulfur dioxide, imidazole, diethylene glycol monoethyl ether, and hydriodic acid, and should be handled with care.		
Principle	Karl Fischer (KF) titration is a widely used analytical method for quantifying water content in a variety of food products. The fundamental principle behind it is based on the Bunsen Reaction between iodine and sulfur dioxide in an aqueous medium. The alcohol reacts with sulfur dioxide (SO_2) and base to form an intermediate alkylsulfite salt, which is then oxidized by iodine to an alkylsulfate salt. This oxidation reaction consumes water. $ROH + SO2 + R'N \rightarrow [R'NH]SO_3R + H_2O + I_2 + 2R'N \rightarrow 2[R'NH]I + [R'NH]SO4R$ [alcohol] [base] [alkylsulfite salt] [water] [iodine] [hydroiodic acid salt] [alkylsulfate salt] Water and iodine are consumed in a 1:1 ratio in the above reaction. Once all of the water present is consumed, the presence of excess iodine is detected potentiometrically by the titrator's indicator electrode that signals the end-point of the titration. The amount of water present in the sample is calculated based on the concentration of iodine in the Karl Fisher titrating reagent (i.e., titer) and the amount of Karl Fisher Reagent consumed in the titration.		

Apparatus/ Instruments	1. **Karl Fischer titration system**: Metrohm 720 KFS Titrino "snap-in" burette unit (10 mL), 703-titration stand, or equivalent. Incorporate a titration vessel with water jacket (50–150 mL) and condenser or a four-neck flask with a heating mantle. 2. **Circulating water bath**: maintaining 75 ± 1 °C. 3. **Glass weighing spoon**: With opening for dispensing test portion into the titration flask through the septum, stopper. 4. **Magnetic stirrer**. 5. **Analytical balance** with 0.001 g accuracy**.** 6. **Oven**: 103° ± 2 °C.
Materials and Reagents	1. **Karl Fischer reagent**: One component based on imidazole, with titer 5 mg H_2O/mL reagent, HYDRANAL® –Composite 5 or equivalent. 2. **Methanol**: Anhydrous, for moisture determinations, water content not to exceed 0.05% (HYDRANAL–Methanol Dry or equivalent). 3. **Sodium tartrate dihydrate**: Primary standard (water content, 15.66 ± 0.05%), HYDRANAL® –standard sodium tartrate–2-hydrate or equivalent. 4. **Water standard**: Water standard with traceable certificate (water content, 10 mg/g), HYDRANAL® –water standard 10 or equivalent.
Sample Preparation	Prepare test sample as described in Method No. FSSAI 10.001:2021
Method of analysis	**Follow the following steps as:** I. Drying or conditioning the cell II. Standardization III. System suitability IV. Determination
	I. Drying or Conditioning the Cell 1. Dispense 50 mL methanol into the titration vessel. 2. Close the cell to minimize the addition of atmospheric moisture. 3. Heat until the MeOH begins to boil. 4. Dry the cell (including solvent, cell walls, electrode walls, generator, and cell atmosphere) by titrating to dryness. 5. The end point is reached when no change in potential is observed for 10 s (titration system programmed for "stop criterion: time; delay: 10 s). A dried titration cell has a maximum drift consumption of 5–10 mL Karl Fischer reagent per min.
	$$\text{Titer} = \frac{\text{mg } H_2O}{\text{mL reagent}} \div \frac{\text{mg } Na_2C_4H_4O_6 \cdot 2H_2O \times 0.1566}{\text{mL reagent}}$$ where mg $Na_2C_4H_4O_6 \cdot 2H_2O$ is S – T, in mg. **II. Standardization** 1. Heat cell to 66° ± 1 °C (boiling point of methanol). 2. Dry the cell as in **I**. Depending on instrument, call up calibration

	mode. 3. Condition solvent by titrating background moisture (hit "start"). 4. Switch off the heating system and when the methanol stops boiling, quickly weigh 150–250 mg of sodium tartrate dihydrate standard into the glass weighing spoon and record weight of spoon and standard to the nearest 0.1 mg (S). 5. Quickly transfer the weighed test portion into the titration flask through the septum stopper. 6. Reweigh empty spoon to obtain tare weight (T) and obtain the weight of standard material added by subtracting tare weight (T) from weight of spoon plus standard (S). 7. Record weight of standard material (S – T) in mg to the nearest 0.1 mg. 8. Enter weight into instrument, start the stirrer and begin the titration. 9. Titrate to same end point as in **I,** recording volume of reagent required for the titration (mL reagent) in mL to the nearest 0.001 mL. 10. Repeat 4 times. Calculate titer and then average the 5 values. 11. The relative standard deviation should be <2%.
	III. System Suitability 1. Heat cell to 66° ± 1 °C (boiling point of methanol). 2. Dry the cell as in **I**. 3. Check drift in the titration cell. A dried titration cell should have a maximum drift consumption of 5–10 mL Karl Fischer reagent/min. 4. Analyze a water standard as follows: 5. Immediately after drying the cell, switch off the heating system and after the methanol stops an active boil, break open the standard ampoule at the white ring and take 1–2 mL of standard with syringe which has been pre-dried in a 103 °C oven. 6. Rinse the syringe and discard the standard solution. 7. Draw the remaining water standard (ca 6 mL) into the syringe and weigh accurately by placing the syringe into a beaker on the balance pan (SS). 8. Quickly add ca 2 mL water standard through the septum keeping the tip of the syringe below the surface of the solvent. 9. Carefully withdraw the syringe tip, reweigh the syringe and record the weight (S_1). 10. Obtain the weight of standard solution ($S_S - S_1$) by subtracting the weight (S_1) from the weight of the syringe plus test portion (S_S). 11. Record the water standard weight to the nearest 0.1 mg, and enter the weight into instrument. 12. Turn on the heating system, start the stirrer and begin the titration

as soon as the methanol returns to an active boil.

13. Record the volume of titrant (V_1).

$$\text{Water standard, g} = S_S - S_1,\ S_1 - S_2,\ S_2 - S_3$$

$$\text{Rec., \%} = \frac{\frac{V_s \times \text{titer}}{\text{g standard}}}{\text{certified value, mg / g}} \times 100 \dagger \frac{\text{mg / g } H_2O \text{ found}}{\text{certified value, mg / g}} \times 100$$

14. Carry out the titration procedure 2 additional times, recording weights of the syringe after each subsequent addition (S_2, S_3) and the respective volume of titrant (V_2, V_3).

15. Calculate the % recovery as follows:

Average % recovery should be 100 ± 1%. If system is not within specifications, correct before continuing with determinations. If the % recovery on the water standard is within specification, it is not necessary to perform a blank run (with no material), since the water standard indicates the condition of the system and running a blank will provide no additional information.

IV. Determination

1. Dry the cell as described in **I**. Depending on the instrument, call up the sample analysis mode.
2. After switching off the heating system and the methanol stops an active boil, quickly weigh ca 0.5 g test portion (to contain ca 25 to 50 mg water) into the glass weighing spoon and record weight of the spoon plus the test portion (W).
3. Quickly add weighed test portion into the titration flask through the septum stopper.
4. Reweigh empty spoon and record tare weight (T).
5. Obtain the test portion weight by subtracting tare weight (T) from weight of spoon plus test portion (W).
6. Record weight (W – T) in g to the nearest 0.1 mg.
7. Enter weight into instrument, start the stirrer, turn on the heating system, and begin the titration as soon as the methanol returns to an active boil.
8. The end point is reached when no change in potential is observed for 10 s (stop criterion: time; delay: 10 s).
9. Record the volume of titrant (V). Repeat determination in triplicate. The relative standard deviation of replicates should be <5%.
10. The cell need not be emptied between each titration. Usually about 3 titrations can be performed before the cell requires emptying and replenishing.

Calculations with units of expression	Moisture by Karl Fischer is the mass of total water content in a food. $$mg\ H_2O = V \times titer$$ $$\%\ H_2O = \frac{V \times titer}{10 \times test\ portion\ wt}$$ $$Dry\ matter, \% = 100 - \%\ H_2O$$ where V is the volume of titrant in mL and test portion weight is W – T, in g.
Reference	AOAC Official Method 2001.12 Water/Dry Matter (Moisture) in Animal Feed, Grain, and Forage (Plant Tissue).
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of moisture content in spices (Vacuum Oven Method)		
Method No.	FSSAI 10.005:2021	**Revision No. & Date**	0.0
Scope	This method is used to determine the moisture of capsicum spices (paprika, chili pepper, chilies, red pepper etc.), dehydrated onion and garlic and dehydrated herbs.		
Caution	1. Do not handle aluminum dishes with bare hands, use gloves or tongs. 2. Do not place samples of high moisture and low moisture in oven at the same time. 3. Do not stack dishes in oven as this may impair distribution of heat and flow of air around dishes. 4. Use safety gloves, tongs and protective eyewear while handling hot dishes.		
Principle	Samples are dried under vacuum at 70 °C to remove moisture. The weight loss relative to initial weight of the sample is interpreted as % (%) moisture.		
Apparatus/ Instruments	1. Aluminum dishes, 3'' diameter by ¾'' height with tight fitting aluminum lid covers 2. Analytical balance, accurate up to 0.1 mg 3. Airtight desiccator with orange silica gel beads 4. Vacuum oven with fittings for gas washing system, manometer and thermometer 5. Vacuum pump capable of maintain pressure to 50-100 mm Hg (2-4 inch) 6. Air drying system, connected in series to the vent of oven, consisting for example of a Gilmont No.10 flowmeter, two gas-drying jars with desiccant and a GelmanAcro 50, 0.2-micron filter 7. Gloves or tongs 8. Timer capable of measuring at least 6 h.		
Materials and Reagent	***Note:*** Refer to Material Safety Data Sheets and ensure that safety guidelines are applied before using chemical. 1. Orange indicating silica gel ***Note:*** Do not use blue indicating silica gel with cobalt chloride as it is a human toxin.		
Sample Preparation	Prepare sample as described in Method No. FSSAI 10.001:2021		

Method of analysis	1. Tare weigh an aluminum dish and cover. 2. Accurately weigh 2-5 g of sample into the dishes. Use only enough samples to sufficiently cover the bottom of the dish but not less than 2 g. 3. Replace cover and store in desiccator until all have been weighed. 4. Before placing dishes in oven, remove lid and place under dish. 5. Place dish and cover in vacuum oven previously warmed to 70 °C. 6. With vent closed, attach gas-washing system. 7. Open vacuum and adjust pressure to 50-100 mm Hg (2-4 inch). During drying, open vent and adjust airflow through flowmeter to 60-80 mL/min. 8. Dry for 6 h. 9. Close vacuum. Disconnect flowmeter from gas washing system. Slowly vent oven through desiccant until pressure in the oven returns to atmospheric pressure (760 mm Hg). 10. Remove sample dishes from oven, replace matching cover and immediately transfer to desiccator and cool to ambient temperature. 11. Weigh samples to nearest 0.0001 g and calculate to %.
Calculation with units of expression	Moisture content (% by mass) $= \frac{Initial\ \ Weight - Final\ \ Weight}{Initial\ \ Weight} \times 100$
Reference	Official Analytical Methods of American Space Trade Association, Fourth Edition 1997, Method 2.1, Moisture in spices (Vacuum oven method).
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of total ash of spices and condiments		
Method No.	FSSAI 10.006:2021	**Revision No. & Date**	0.0
Scope	Ash refers to the inorganic residues remaining after either ignition or complete oxidation of organic matter. This method is applicable to determination of total ash of most of spices and condiments, herbs and seasonings.		
Caution	1. Use safety gloves, tongs and protective eyewear while handling hot crucibles. 2. Warm crucibles will heat air within the desiccator and a vacuum may form on cooling. Remove desiccator's cover gradually by sliding to one side to prevent a sudden inrush of air at the end of cooling period. 3. Open and close desiccator slowly in order to avoid the danger of glass breakage. 4. Concentrated Nitric acid is corrosive open and use in fume hood.		
Principle	Destruction of organic matter by incinerating the sample to a constant mass at higher temperature of (550 ± 25) °C in Muffle furnace.		
Apparatus/ Instruments	1. Dish, flat-bottomed, with surface area of at least 15 cm^2, made of platinum, quartz, porcelain or any another material that remains unaffected by the conditions of the test. 2. Ashless filter paper (Whatman filter paper 42) 3. Muffle furnace, capable of being regulated at 550 ± 25 °C 4. Electrical hotplate or surface heater 5. Fume hood or equivalent venting system 6. Desiccator, containing desiccant such as orange indicating silica gel 7. Analytical balance, accurate up to 0.0001 g 8. Tongs for crucible, stainless steel 9. Thermal protection gloves, capable to resist temperature upto 550-600 °C 10. Bunsen burner 11. Tripod stand, iron 12. Wire gauge 13. Water bath		
Materials and Reagents	*Note:* Refer to Material Safety Data Sheets and ensure that safety guidelines are applied before using chemical. 1. Ethanol, ACS grade 2. Water, analytical laboratory use 3. Concentrated Nitric acid, analytical quality (for ground mustard)		
Sample Preparation	Prepare sample as described in Method No. FSSAI 10.001:2021		

Method of analysis	1. Accurately weigh ~2 g of the prepared sample into the tared dish. 2. Pour about 2 mL of ethanol on the material and ignite it. 3. When the ethanol is burnt off, heat the dish carefully over a small flame to char the material. 4. Then ignite in a Muffle furnace at 550 ± 25 °C for 3-4 h. Cool and wet the ash with a few drops of water, evaporate carefully to dryness and heat in the Muffle furnace for a further 1 h. 5. If the wetting shows the ash to be carbon free, remove the dish to a desiccator containing an efficient desiccant, allow to cool and weigh soon. If the wetting shows presence of carbon, repeat the wetting and heating until no specks of carbon are visible and ignite in the Muffle furnace for 1 h after the disappearance of carbon. 6. If carbon is still visible, leach the ash with hot water, filter through ashless filter paper, wash the filter paper thoroughly, transfer the filter paper and contents to ashing-dish, dry and ignite in Muffle furnace set at 550 ± 25 °C until the ash is white. 7. Cool the dish, add the filtrate and evaporate to dryness on a water bath. Heat in Muffle furnace again, cool in a desiccator and weigh as previously. 8. Heat again in the Muffle furnace for 1 h, cool and weigh. Repeat these operations until the difference in weight between two successive weighing is less than 0.001 g. Record the lowest weight. **NOTE: Saffron**: Take 2- 2.5 g sample for test and proceed as above. **Nutmeg, Mace, Ginger and Cloves**: Ignition should be carried out at 600 ± 25 °C. **Ground Mustard**: Proceed as above and ignite for 1 h after disappearance of carbon. Leach the ash with hot water, filter through ashless filter paper and wash filter paper thoroughly. Transfer the filter paper and contents to the dish, dry and ignite in Muffle furnace again for 1 h. Cool and add 5 - 10 drops of concentrated nitric acid of analytical quality, evaporate to dryness on a water bath and heat in Muffle furnace for 30 min. Repeat the addition of 5 – 10 drops of nitric acid, evaporating to dryness and heating in Muffle furnace for 1 h. Cool and weigh.
Calculation with units of expression	Calculate the total ash, expressed as a % by mass, using the following equation: Total ash,% by mass $= \frac{W_2 - W_0}{W_1 - W_0} \times 100$ Where, W_0 =mass of empty dish, in g W_1 =mass of dish and test portion, in g W_2 =mass of dish and total ash, in g For determination on a moisture-free basis, the value should be multiplied

	by $\frac{100\%}{100\%-M}$ Where, M = moisture content of sample as received, in % Calculate the mean of two determinations and express the result to one decimal place.
Reference	1. Nielsen, S. S. (Ed.). (1998). *Food Analysis* (Vol. 86). Gaithersburg, MD: Aspen Publishers. 2. Nielsen, S. S. (Ed.). (2003). *Food Analysis Laboratory Manual* (p. 557). New York, USA: Kluwer Academic/Plenum Publishers. 3. I.S. Specification No I.S 1797 – Methods of Test for Spices and Condiments 4. A.O.A.C. 17th edn , 2000 Official Method 941.12 Ash of Spices
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of acid insoluble ash of spices and condiments		
Method No.	FSSAI 10.007:2021	**Revision No. & Date**	0.0
Scope	Acid Insoluble Ash refers to the ash remaining after dissolution of the total ash in concentrated Hydrochloric acid. This method is applicable to determination of Acid Insoluble Ash of most of spices and condiments, herbs and seasonings.		
Caution	1. Wearing of required personal protective equipment is essential while working with hydrochloric acid as it is very corrosive in nature. 2. Use hydrochloric acid under adequate ventilation or fume hood as it emits significant amounts of fumes. 3. Concentrated Hydrochloric acid is corrosive and when spilt on skin can cause severe burns. Handle with care.		
Principle	Treatment of the total ash, with hydrochloric acid, filtration, incineration and weighing of the residue which is insoluble in acid.		
Apparatus/ Instruments	1. Dish, flat-bottomed, with surface area of at least 15 cm^2, made of platinum, quartz, porcelain or any another material that remains unaffected by the conditions of the test 2. Ashless filter paper (Whatman filter paper 42) 3. Muffle furnace, capable of being regulated at 550 ± 25 °C 4. Electrical hotplate or surface heater 5. Fume hood or equivalent venting system 6. Desiccator, containing desiccant such as orange indicating silica gel 7. Analytical balance, accurate up to 0.0001 g 8. Tongs for crucible, stainless steel 9. Thermal protection gloves, capable to resist temperature up to 550 – 600 °C 10. Bunsen burner 11. Tripod stand, iron 12. Wire gauge 13. Water bath 14. Watch glass 15. Funnel		
Materials and Reagents	***Note:*** Refer to Material Safety Data Sheets and ensure that safety guidelines are applied before using chemical. 1. Concentrated hydrochloric acid (relative density 1.19 at 20 °C), ACS grade 2. Silver nitrate, ACS grade 3. Distilled water		

Preparation of Reagents	1. **Hydrochloric acid solution (2:5 v/v):** Add one volume of conc. HCl to 2.5 volumes of distilled water. ***Caution:*** Do not add water to acid. Always add acid to water 2. **Silver nitrate solution (10% m/v)**: Dissolve 10 g of silver nitrate in distilled water to a total volume of 100 mL.
Sample Preparation	Prepare sample as described in Method No. FSSAI 10.001:2021
Method of analysis	1. Add 15 - 25 mL of HCl solution to total ash of sample and boil for 10 min in the boiling water bath, covering the dish with watch glass to prevent spattering. 2. Filter the contents of the dish through the ashless filter paper. 3. Wash the dish and the filter paper with hot water until the washings are free from hydrochloric acid (about 6 to 8 times). Test for the absence of hydrochloric acid with silver nitrate solution. **Note:** Lack of turbidity when a portion of silver nitrate solution is added to the filtrate indicates absence of hydrochloric acid. Return the filter paper with the residue to the dish. Evaporate it on water bath and ignite it in the Muffle furnace at 550 ± 25 °C for 1 h (or until the ash is carbon free). When carbon-free ash is obtained, transfer the dish to desiccator, cool to 25 ± 2 °C and weigh immediately. Repeat the operations of igniting, cooling and weighing until the difference between successive weighing does not exceed 0.001 g. **NOTE: Saffron**: Take 2 - 2.5 g sample for test and proceed as above.
Calculation with units of expression	Calculate the acid insoluble ash, expressed as a % by mass, using the following equation: Acid insoluble ash(% by mass) = $\frac{(W_2 - W_0)}{(W_1 - W_0)} \times 100$ Where, W_0 =mass of empty dish in g W_1 = mass of dish and test portion in g W_2 = mass of dish and acid insoluble ash in g For determination on a moisture-free basis, the value should be multiplied by $= \frac{100\%}{100\% - M}$ Where, M = moisture content of sample as received, in % Calculate the mean of two determinations and express the result to one decimal place.
Reference	1. I.S. Specification No I.S 1797 – Methods of Test for Spices and Condiments. 2. ISO 930:1997, Spices and Condiments — Determination of acid insoluble ash.
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of water soluble and insoluble ash of spices and condiments		
Method No.	FSSAI 10.008:2021	**Revision No. & Date**	0.0
Scope	Water Soluble and Insoluble Ash refers to the ash remaining after dissolution of the total ash in distilled. This method is applicable to determination of Water Soluble and Insoluble Ash of most of spices and condiments.		
Caution	1. Use safety gloves, tongs and protective eyewear while handling hot crucibles. 2. Warm crucibles will heat air within the desiccator and a vacuum may form on cooling. Remove desiccator's cover gradually by sliding to one side to prevent a sudden inrush of air at the end of cooling period. 3. Open and close desiccator slowly in order to avoid the danger of glass breakage.		
Principle	Treatment of the total ash, with deionized distilled water, filtration, incineration and weighing of the residue.		
Apparatus/ Instruments	1. Dish, flat-bottomed, with surface area of at least 15cm^2, made of platinum, quartz, porcelain or any another material that remains unaffected by the conditions of the test 2. Ashless filter paper (Whatman filter paper 42) 3. Muffle furnace, capable of being regulated at 550 ± 25 °C 4. Electrical hotplate or surface heater 5. Fume hood or equivalent venting system 6. Desiccator, containing desiccant such as orange indicating silica gel 7. Analytical balance, accurate up to 0.0001 g 8. Tongs for crucible, stainless steel 9. Thermal protection gloves, capable to resist temperature up to 550 – 600 °C 10. Bunsen burner 11. Tripod stand, iron 12. Wire gauge 13. Water bath 14. Watch glass 15. Funnel		
Reagent	1. Deionized, distilled water		
Method of analysis	To the total ash in the dish, add distilled water, heat nearly to boil. Place watch glass over the crucible in order to prevent loss due to splattering. Filter through an ashless filter paper.		

	Wash the filter paper with hot water until the combined filtrate and washings measure about 60 mL. Take the filter paper and content carefully from water bath and ignite at 550 ± 25 °C for 1 h. Cool in the desiccator and weigh. Ignite again, cool and weigh. Repeat the process of igniting, cooling and weighing until the difference in mass between two successive weightings is less than 0.001 g. Note the lowest mass.
Calculation with units of expression	Calculate the water insoluble ash, expressed as a % by mass, using the following equation: Water insoluble ash (% by mass), on dry basis = $\frac{W_2 - W_0}{W_1 - W_0} \times 100 \times \frac{100}{(100 - M)}$ Where, W_0 = mass of empty dish in g W_1 = mass of dish and test portion in g W_2 = mass of dish and water insoluble ash in g **Water soluble ash (% by mass), on dry basis =** 100 - % of water insoluble ash, on dry basis. Calculate the mean of two determinations and express the result to one decimal place.
Reference	1. I.S. Specification No I.S 1797 –Methods of Test for Spices and Condiments
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of cold water soluble extract of spices and condiments		
Method No.	FSSAI 10.009:2021	**Revision No. & Date**	0.0
Scope	The method is applicable to all spices and spice products.		
Caution	Use safety gloves, tongs and protective eyewear while handling hot dishes.		
Principle	Making a cold-water extract of the spice and evaporating the water gives the water-soluble extract.		
Apparatus/ Instruments	1. Volumetric flask with stopper (Class A),100 mL 2. Pipette (Class A), 50 mL 3. Dish, flat-bottomed 4. Filter paper, medium-fine 5. Funnel 6. Oven, capable of operating at 103 ±2 °C 7. Analytical balance, accurate up to 0.001 g 8. Steam bath		
Reagent	Distilled water or water of at least equivalent purity.		
Sample Preparation	Prepare sample as described in Method No. FSSAI 10.001:2021		
Method of analysis	1. Weigh, to the nearest 0.001 g, about 2 g of the test sample. 2. Transfer the test portion quantitatively with water to the volumetric flask of 100 mL capacity and fill to the mark with cold water. 3. Stopper the flask, shake at approximately 30 min intervals for 8 h, and allow to stand for a further 16 h without shaking. 4. Filter the extract through a dry filter paper. 5. Evaporate a 50 mL aliquot portion to dryness in the dish previously dried and weighed to the nearest 0.001 g, on the steam bath. 6. Heat in the oven at 103 ± 2 °C to constant mass, i.e. until two consecutive weighings separated by a period of 1 h in the oven do not differ by more than 0.002 g. 7. Record the final mass.		
Calculation with units of expression	The cold water-soluble extract, expressed mass on the dry basis, $$m_1 \times \left(\frac{100}{50}\right) \times \left(\frac{100}{m_0}\right) \times \left(\frac{100}{100 - H}\right)$$ Where: m_o= mass in gm, of the test portion; m_1 =mass in gm, of the extract obtained; H is the moisture content, expressed in mass of the sample as received. Report the results up to one decimal place.		
Reference	ISO 941-1980 (E), *Spices and Condiments* — Determination of cold water soluble extract		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

	Determination of alcohol soluble extract		
Method No.	FSSAI 10.010:2021	**Revision No. & Date**	0.0
Scope	This method is applicable for all spices and spice products.		
Caution	1. Follow all safety precautions for the safe handling of organic solvents and special chemical hazards- ethanol. See Material Safety Data Sheets, or equivalent, for each reagent. 2. Ethanol is a flammable liquid, hazardous in case of skin or eye contact (irritant), ingestion, and inhalation. 3. Use safety gloves, tongs and protective eyewear while handling hot dishes.		
Principle	The test sample is extracted in alcohol and filtered. The obtained extract is dried and determined gravimetrically.		
Apparatus/ Instruments	1. Volumetric flask 100 mL / Conical flask 250 mL 2. Filter paper Whatman No. 2 or equivalent 3. Water bath 4. Oven, capable of operating at 103 ± 3 °C 5. Analytical balance, accurate up to 0.001 g		
Reagent	Ethyl alcohol, Reagent grade – 90% v/v.		
Sample Preparation	Prepare sample as described in Method No. FSSAI 10.001:2021		
Method of analysis	**Extraction** For spices expected to contain more than 5% volatile oil content, complete extraction in Soxhlet apparatus with 90% alcohol. Moisture should be determined by Dean and Stark Distillation method and the real alcohol extract should be determined by difference. For spices expected to contain less than 5% volatile oil content, follow the procedure as described below: 1. Weigh accurately about 2 g of test sample, transfer to a 100 mL volumetric flask/ 250 mL conical flask, and fill to mark with 90% alcohol. 2. Stopper the flask and shake it approximately 30 min interval for 4 h and allow to stand for 16 h longer without shaking. 3. Filter the extract through a dry filter paper. 4. Evaporate a 50 mL aliquot to dryness on a water bath. 5. Heat in an oven at 103 ± 2 °C to constant weight, that is until two consecutive weights separated by a period of 1 h in the oven do not differ by more than 0.001 g. 6. Record the final weight. NOTE: Determine alcoholic extract in Asafoetida by the method described		

	below: • Place accurately weighed about 2 g of asafetida (10 g of compounded asafetida) in a tared extraction thimble • Extract with 90% alcohol in either a Soxhlet or other suitable extraction apparatus for about 3 h. • Dry the insoluble residue at 100 °C for 30 min or until constant mass is obtained. • Alcohol extract, % by mass = 100 - (A + B) where A = % of residue B = % of moisture
Calculation with units of expression	Alcohol soluble extract (dry basis), % by mass is = $$W_1 \times \frac{100}{50} \times \frac{100}{W_0} \times \frac{100}{100 - M}$$ Where, W_1= Weight of the dried extract obtained W_0= Weight of the sample taken for the test M = % moisture in the sample Report the results up to one decimal place.
Reference	1. IS 1797 : 2017, *Spices and Condiments- Methods of Test (Third revision)* 2. IS Specification No IS 7807 – 1975 (Reaffirmed in 2003) Methods of Test for Asafoetida)
Approved by	Scientific Panel on Methods of Sampling and Analysis

FSSAI FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India	**Determination of calcium oxide**		
Method No.	FSSAI 10.011:2021	**Revision No. & Date**	0.0
Scope	This method determines calcium oxide content in spices.		
Caution	Use safety gloves, tongs and protective eyewear while handling hot dishes. Concentrated Hydrochloric acid and Sulphuric acid are corrosive and can cause severe burns.		
Principle	The total ash is treated with hydrochloric acid to precipitate the Calcium as Calcium oxalate, which is then titrated against Potassium Permanganate by using Bromocresol Green as indicator.		
Apparatus/ Instruments	1. Dish, flat-bottomed, with surface area of at least 15cm^2, made of platinum, quartz, porcelain or any another material that remains unaffected by the conditions of the test 2. Muffle furnace, capable of being regulated at 550 ± 25 °C 3. Analytical balance, accurate up to 0.0001 g 4. Water bath 5. Ashless filter paper 6. Funnel 7. Tongs for crucible, stainless steel 8. Thermal protection gloves, capable to resist temperature up to 550 – 600 °C		
Materials and Reagents	***Note:*** Refer to Material Safety Data Sheets and ensure that safety guidelines to be applied while using chemicals. • Conc. Hydrochloric acid • Ammonium hydroxide • Ammonium oxalate • Potassium permanganate • Sodium oxalate • Conc. sulphuric acid • Bromocresol Green indicator • Glacial acetic acid		
Preparation of Reagents	1. **Dilute hydrochloric acid** –Add two volumes of conc. HCl (Specific gravity 1.19) to five volumes with distilled water 2. **Standard potassium permanganate solution**- 0.1 N standardized against sodium oxalate 3. **Dilute sulphuric acid** – Add one volume of conc. H_2SO_4 (Sp. gr. 1.84) to four volumes of distilled water 4. **Bromocresol Green indicator solution** – 0.04% - Weigh accurately 0.1 g of Bromocresol green powder and grind it with 14.3 mL 0.01N NaOH in an agate mortar. Transfer the contents of the mortar		

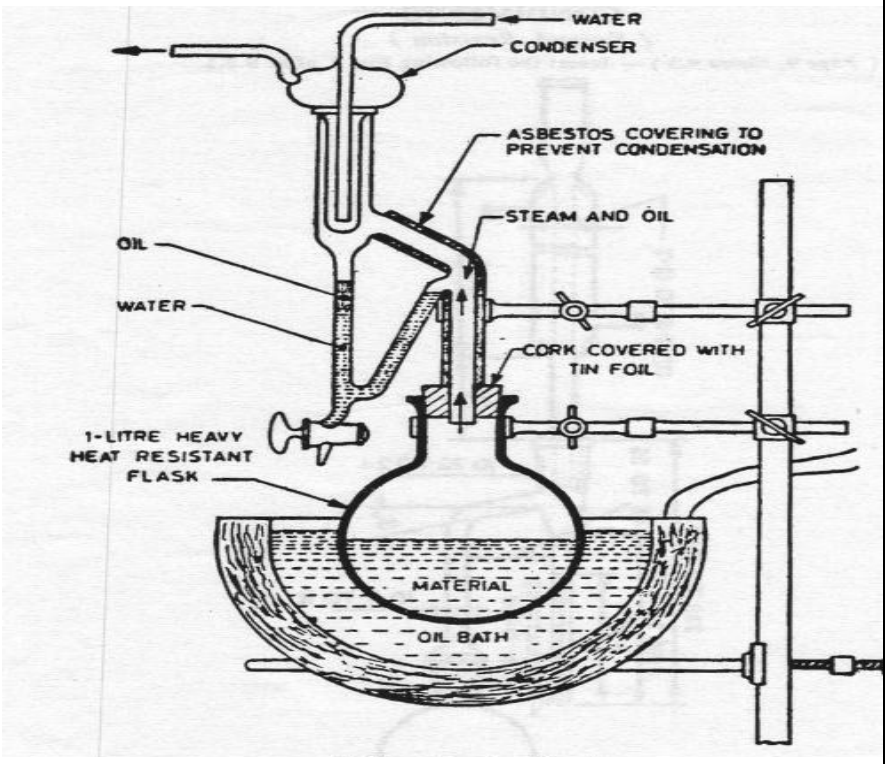
	quantitatively to a 250 mL graduated flask and make up the volume with distilled water. 5. **Dilute Acetic acid** – Add one volume of glacial acetic acid to two volumes of distilled water.
Sample Preparation	Prepare sample as described in Method No. FSSAI 10.001:2021
Method of analysis	1. Weigh accurately about 2-4 g of test sample and ash it in a Muffle furnace. 2. Dissolve the ash with dilute HCl and evaporate to dryness. 3. Digest the dry ash again with dilute HCl and again evaporate to dryness on a water bath. 4. Treat the residue with 5 -10 mL of conc. HCl, add about 50 mL water, allow to stand on water bath for few min and filter in a 250 mL beaker. 5. Wash the insoluble residue with hot water and collect the washings in the same beaker. 6. Add 0.5 mL of Bromocresol green indicator and then ammonium hydroxide until the color of the solution is distinctly blue. 7. Adjust the pH of the solution to 4.4 – 4.6 by adding acetic acid drop by drop until the color changes to distinctly green. 8. Filter and wash the filter paper with hot water. Collect the washings in the same beaker and bring the solution to boil. 9. While still hot, add saturated ammonium oxalate solution drop wise as long as any precipitate forms and then add an excess. 10. Heat to boiling. 11. Allow to stand for 3 h or longer. 12. Decant the clear solution through an ashless filter paper. 13. Pour 15-20 mL of hot water on the precipitate and again decant the clear solution through filter paper. 14. Dissolve any precipitate remaining on the filter paper by washing with hot dilute HCl into the original beaker. 15. Wash the filter paper thoroughly with hot Water. 16. Then precipitate while boiling hot by addition of ammonium hydroxide and a little of saturated ammonium oxalate solution. 17. Allow to stand for 3 h or longer, filter through the same filter and wash with hot water until it is chloride free. 18. Perforate the apex of the filter cone and wash the precipitate into the beaker used for precipitation. 19. Wash filter paper with dilute sulphuric acid and titrate with standard potassium permanganate solution at temperature not less than 70 °C.
Calculation with units of expression	Calcium (as CaO) % by mass = $\frac{2.8 \times V \times N}{W}$

	Where, V = Volume of standard potassium permanganate used for titration N = Normality of standard potassium permanganate solution W = Mass of the sample taken for test Report the results up to one decimal place.
Reference	I.S. specification No. IS 1797 – 2017: Methods of Test for Spices and Condiments.
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of nonvolatile ether extract		
Method No.	FSSAI 10.012:2021	**Revision No. & Date**	0.0
Scope	This method determines nonvolatile ether extract content and is applicable to most spices and condiments.		
Caution	1. Follow all safety precautions for the safe handling of organic solvents and special chemical hazards– diethyl ether**.** See Material Safety Data Sheets, or equivalent, for each reagent. 2. Diethyl ether is a flammable liquid, hazardous in case of skin or eye contact (irritant), ingestion, and inhalation.		
Principle	Non-volatile ether extract content is the extract of the material with diethyl ether. The volatile fractions are removed and insoluble non-volatile residue obtained.		
Apparatus/ Instruments	1. Extractor (Goldfisch, Bailey- Walker or equivalent) 2. Rotary evaporator 3. Oven, capable of operating at 110 ± 2 °C (Forced draft oven recommended) 4. Analytical balance, accurate to 0.001 g 5. Paper extraction thimble, Alundum crucible (porosity RA 360), or cup made of Whatman No. 1 filter paper.		
Materials and Reagents	*Note:* Refer to Material Safety Data Sheets and apply safety guidelines before using chemicals. 1. Diethyl ether, anhydrous, ACS grade or equivalent 2. Anhydrous calcium sulfate (Drierite) or silica gel		
Sample Preparation	Grind laboratory sample as quickly as possible in a grinding mill to pass sieve with 1 mm diameter aperture. Avoid undue heating of apparatus during grinding. Mix carefully to avoid stratification (layering).Store in a dry stoppered container.		
Method of analysis	1. Weigh 2.0 g of the ground sample into an extraction thimble. 2. Place sample and the container in the extractor. Assemble apparatus and extract with diethyl ether for 20 h. If residue is to be weighed in the extraction flask, tare the flask before assembling. 3. Quantitatively transfer extract to a tared beaker or other suitable container such as an aluminum dish of suitable dimensions with minimum quantity of diethyl ether. Evaporate the diethyl ether on a steam bath under suitable fume hood. Avoid bringing to boil. 4. When last traces of diethyl ether have disappeared, place container in hot air oven at 110 ± 2 °C until two consecutive weighing taken at 1.5 h intervals differ by no more than 1 mg. Sample should be cooled to 25 ± 2 °C in desiccator containing drying agent before weighing.		

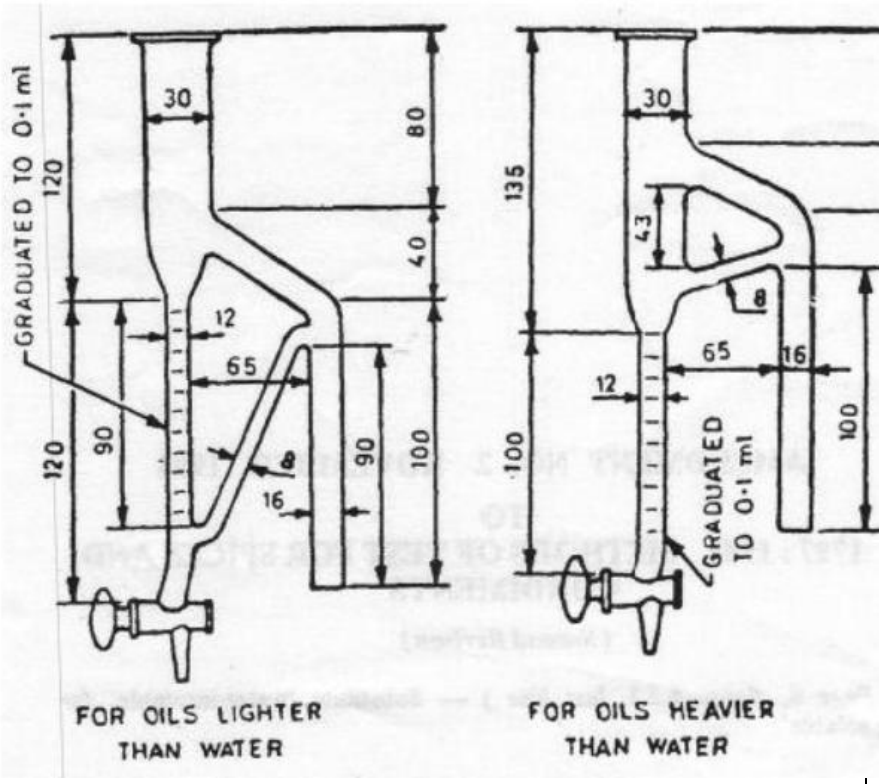
	5. The dried residue is the non- volatile ether extract. ***Note***: 1. In case of spices with low bulk density such as sage, it may be necessary to reduce sample size to accommodate certain extractors. 2. Observe extreme caution due to flammability of diethyl ether and avoid splattering of extract due to the low boiling point of diethyl ether during evaporation. Methylene chloride is the more efficient and the solvent of choice for black and white pepper for piperine extraction.
Calculation with units of expression	Non-volatile ether extract, expressed as % by mass (dry basis), is equal to: $$\left(\frac{W_0}{W}\right) \times \left(\frac{100}{100 - H}\right) \times 100$$ Where W = Mass of the sample in g W_0 = Mass of the residue in g H = % moisture content determined by Dean and Stark (Toluene distillation method).
Reference	1. I.S. Specification No. IS 1797-1985: Methods of Test for Spices and Condiments. 2. Official Analytical Methods of American Spice Trade Association, Fourth Edition, 1997, Method 11.0, Determination of Non- Volatile Methylene Chloride extract.
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Method for determination of volatile oil		
Method No.	FSSAI 10.013:2021	**Revision No. & Date**	0.0
Scope	This method specifies method of determination of volatile oil content of most of the spices and condiments.		
Caution	See Material Safety Data Sheets, or equivalent, for each reagent. Xylene is a flammable liquid, hazardous in case of skin or eye contact (irritant), ingestion, and inhalation.		
Principle	The determination of volatile oil in a spice is made by distilling the spice with water, collecting the distillate in a graduated tube in which the aqueous portion of the distillate is automatically separated and returned to the distilling flask, and measuring the volume of the oil. The content of volatile oil is expressed as % v/w.		
Apparatus/ Instruments	1. Volatile oil trap, Clevenger type lighter than water (Figure 2a) heavier than water (Figure 2b) 2. Flask, distilling, 1000 mL, preferably with magnetic stirrer 3. Heating source: Oil bath/ heating mantle/ stirring hotplate 4. Glass beads, if stirring hotplate is not used **NOTE:** Once a year, calibrate the volatile oil trap for xylene retention. Run 3 blanks and take their average. It is essential to wash the apparatus with acetone and water and leave it to stand in chromic- sulphuric acid mixture with complete rinsing prior to use.		



All dimensions in millimeters

FIG. 1 APPARATUS FOR DETERMINATION OF VOLATILE OIL

	 **All dimensions in millimeters** **FIGURE. 2 OIL SEPARATORY TUBE OF THE APPARATUS USED FOR DETERMINATION OF VOLATILE OIL**
Materials and Reagents	1. Xylene (Reagent grade) 2. Sodium chloride (NaCl) solution: 10% (w/v). 3. Cleaning solutions: (i) Acetone (for fatty residues) (ii) Chromic-sulphuric acid mixture (iii) Liquid glassware cleaner: Such as Fisher brand Versa-Clean Concentrate. 4. Antifoam (preferably Antifoam B)
Method of analysis	1. Grind the sample to pass through No. 20 (850 micron) sieve. Regrind oversize until the test portion passes the sieve. 2. Weigh, but accurately 20 – 50 g of the spice enough to yield 2 - 4 mL of oil if possible. 3. Place in the flask with glass beads or porous earthenware pieces, if a magnetic stirrer is not used. 4. Add about 300 mL water and a drop of antifoam if necessary. 5. Fill the trap with water. 6. Place an efficient water-cooled condenser on top of the trap and heat the flask with good stirring or agitation until boiling starts and continue boiling moderately briskly, but so that the lower part of the

	condenser remains cold. 7. Set the apparatus so that the condensate will not drop directly on the surface of the liquid in the trap but run down the sidewalls. 8. Rotate the flask occasionally to wash down any material adhering to the upper part of the walls. 9. Distill until two consecutive readings taken at 1 h intervals show no change in oil content (more than 6 h). 10. Remove the source of heat and read the volume of oil ten min or so later. Calculate as v / w and express the results in %. 11. If the oil separates in the graduated portion of the trap or clings to the walls, add several drops of a saturated aqueous detergent solution through the top of the condenser. Repeat if necessary. Distill for at least 10 min after adding detergent in order to wash out of the trap. **NOTE**: Some oils (e. g Cassia) have a density close to 1 or separate into two fractions in the trap (allspice, nutmeg). For these, prior to adding sample to the flask, add 1.0 mL xylene to the trap and distill without sample for at least half an h. Cool and read the volume of xylene after 2 min. Add the sample and distill for up to six h as before. Subtract the volume of xylene from the total volume of the organic layer in the trap. Calculate as before. 12. The oil obtained (without the use of xylene) may be recovered, dried with a small amount of sodium sulphate and its characteristics such as density and refractive index can be determined.
Calculation with units of expression	Calculate volatile content % (v/ w), in the test portion as: Volatile oil% (v/w) = $\frac{V}{W} \times 100$ Where, V= volume of oil collected in the trap (mL) W= mass of the test portion (g) NOTE: The results should be reported on dry basis, by multiplying the value obtained with:$\frac{100\%}{100\%-M}$ Where, M=moisture content of sample as received, in %
Reference	1. FAO Manual of Food Quality Control (1986)14 / 8 page 239) 2. AOAC 17th edn., 2000 Official Method 962.17 3. IS 1797 : 2017, Spices and Condiments- Methods of Test (Third revision)
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of crude fiber of spices and condiments		
Method No.	FSSAI 10.014:2021	**Revision No. & Date**	0.0
Scope	This method is for determination of crude fiber in spices and condiments.		
Caution	1. It is recommended to use fume-hoods. 2. Ethyl alcohol is flammable, handle with care. 3. Ensure neutralization of the acid/base used prior to disposal. 4. Use protective wear while handling sulphuric acid and Sodium hydroxide. 5. During digestion, heating shall be performed with care in order to avoid over-heating and too rapid boiling. 6. The foam formed in the vessel should never be allowed to exceed a height of 10 mm.		
Principle	Successive digestions of samples with sulphuric acid and sodium hydroxide of specified concentrations to degrade macromolecules. Separation of the residue by filtration followed by drying and ashing of the residue. The loss in weight resulting from ashing corresponds to the crude fiber content of the sample.		
Apparatus/ Instruments	1. Soxhlet apparatus (optional) 2. **Digestion apparatus**: With condenser to fit one-litre, digestion flask and hot plate adjustable to temperature that will bring 200 mL H_2O at 25 °C to rolling boil in 15 ± 2 min 3. **Digestion flask** of such a size and shape that the solution will not be less than 1 inch (25 mm), nor more than 1.5 inch (38 mm) in depth. A one-litre Erlenmeyer flask with 45/50 ground joint is recommended. 4. **Ashing dishes**: Silica, vitreosil 70 x 16 mm; or porcelain, or equivalent 5. **Filtering device:** California modified Buchner Funnel. Alternatively, a filter cloth, of such character that no appreciable solid matter can pass through it during rapid filtration, may be used. Retention may be tested by running filtrate through a Gooch crucible. Butcher's linen, dress linen with ca. 45 threads to an inch, or No. 40 filter cloth made by the National Filter Media Corporation, Hamden, connection 06514, or equivalent may be used. 6. **Desiccator** with fresh and efficient desiccant (preferably, orange silica gel beads with moisture indicator). ***Note:*** Do not use silica with blue cobalt indicator, as it is not suitable for food applications**.** 7. **Antifoam:** Antifoam A compound diluted 1 + 4 with mineral spirits or petroleum ether, or H_2O-diluted antifoam B emulsion (1 + 4). Do		

	not use antifoam spray. 8. **Bumping chips or granules:** Broken Alundum crucibles or equivalent granules are satisfactory 9. Blue litmus paper 10. Analytical balance, accurate upto 0.0001 g 11. Drying oven, capable of being controlled at 105 ± 1 °C 12. Muffle furnace, capable of being regulated at 500 ± 25 °C
Materials and Reagents	***Note:*** Before using chemicals, refer to chemical safety and/or safety data sheets approved by authorities. 1. Sulfuric acid, specific gravity 1.84 at 60 °F 2. Sodium hydroxide pellets 3. Ethyl alcohol, 95%, ACS grade 4. Methylene chloride, anhydrous (dichloromethane), ACS grade 5. Demineralized water 6. Petroleum ether, initial boiling temperature, 35°–38 °C; dry-flask end point, 52°–60 °C; 95% distilling <54°C, specific gravity at 60 °F, 0.630–0.660
Preparation of Reagents	1. **Sulphuric acid (H_2SO_4) solution, 0.255N**: Into a 1000 mL volumetric flask add about 200 mL of demineralized water then slowly introduce 12.5 g of conc. sulphuric acid and make up to the mark with demineralized water. 2. **Sodium hydroxide (NaOH) solution, 0.312N:** Into a 1000 mL volumetric flask introduce 12.5 g of carbonate free sodium hydroxide pellets and make up to the mark with demineralized water. 3. **Prepared ceramic fiber**: Place 60 g ceramic fiber in blender, add 800 mL H_2O, and blend 1 min at low speed. Determine blank by treating 2 g (dry weight) of prepared ceramic fiber with acid and alkali as in determination. Correct crude fiber results for any blank, which should be negligible (2 mg). **Note:** Concentration of 1 and 2 must be checked by titration. If the concentration differs by more than ± 0.01 N from the nominal values adjust it within the range.
Sample Preparation	Prepare sample as described in Method No. FSSAI 10.001:2021
Method of analysis	1. Extract 2 g of sample with methylene chloride or use the fat free residue from method 1. Transfer the residue together with ca. 0.5 g of ceramic fiber to the digestion flask. 2. Add 200 mL of the H_2SO_4solution connect the digestion flask to the condenser and place on a preheated hot plate or digestion rack adjusted so that the acid will boil in ca. 5 min. Continue boiling briskly for 28 ± 1 min with frequent rotation of the flask to ensure thorough wetting and mixing of the sample. Material should not be allowed to remain on the sides of the flask out of contact with the

	solution. Add one drop diluted antifoam (Excess antifoam may give high results; use only if necessary, to control foaming.). Bumping chips or granules may also be added. Successive sample digestions should be started at ca. 3 min intervals to facilitate accurate timing. 3. After boiling 28 min, remove the flask and filter immediately through the California Modified Buchner funnel or through a filter cloth in a fluted funnel using a suction flask to speed filtration. Wash with boiling water until washings are no longer acid. Check alkalinity with litmus paper. 4. Transfer the sample and ceramic fiber quantitatively in digestion flask, washing the filter cloth or Buchner filter with 200 mL of NaOH solution. A wash bottle to deliver 200 mL is convenient. 5. Connect the flask to the reflux condenser, place on the preheated hot plate or heating mantle or digestion rack, bring to a boil in ca. 5 min, and boil exactly 28 min. Successive sample digestions should be started at ca. 3 min intervals to facilitate accurate timing. 6. After 28 min, remove the flask and immediately filter through a Gooch crucible. 7. Wash the residue thoroughly with water and then with ca. 15 mL of ethyl alcohol 8. Dry the crucible and contents at 110 ± 2 °C to a constant weight (ca. one h). Cool in a desiccator and weigh. 9. Ignite the crucible and contents in an electric Muffle furnace at ca. 600 °C. Cool in a desiccator and weigh. Determine the loss in weight on ignition.
Calculation with units of expression	Carry out a blank test under the same conditions but without the test portion. Calculate the loss in weight resulting from ashing, according to the following formula: $B = R_{1Blank} - R_{2Blank}$ where: B = blank test, in g R_{1Blank} = mass of the dried blank residue, in g R_{2Blank} = mass of the dried and ashed blank residue, in g $\text{Crude fiber (\% m/m on dry basis)} = \frac{(R_1-R_2)-B \times 100}{m} \times \frac{100}{100-H}$ where: R_1 = mass of the dried residue, in g R_2 = mass of the dried and ashed residue, in g B = blank test, in g m = mass of the test portion, in g.

Reference	1. American Spice Trade Association (ASTA) (1997), Official Analytical Methods (Method 7.0 for Crude Fiber) American Spice Trade Association, New York. 2. Official Methods of Analysis of the AOAC, Method 962.09.
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Detection of argemone seeds in mustard		
Method No.	FSSAI 10.015:2021	**Revision No. & Date**	0.0
Scope	This is qualitative method to check the presence of argemone seeds in mustard.		
Caution	Concentrated Hydrochloric acid is highly corrosive and may cause severe burns.		
Principle	The hydrochloric acid extract of the oil sample containing argemone oil when subjected to TLC for separation of alkaloid gives fluorescent spot under UV light.		
Apparatus/ Instruments	1. Erlenmeyer flask 2. Separating funnel – 50 mL capacity 3. Whatman filter paper 1 4. Drying oven regulated at 100 ± 1 °C 5. Grind mill 6. Sieve with circular opening of 1mm diameter 7. Air tight container for storage		
Materials and Reagents	***Note:*** Refer to Material Safety Data Sheets and ensure that safety guidelines are applied before using chemical. • Ethyl ether		
Sample Preparation	Prepare sample as described in Method No. FSSAI 10.001:2021		
Method of analysis	1. Weigh 50 g of powdered sample sufficient to yield 5-10 g nonvolatile ether extract 2. Extract with 125 mL ethyl ether in a closed Erlenmeyer flask for 24 h with occasional shaking. 3. Filter the contents through Whatman filter paper 1 and wash the residue with two 50 mL portions of ethyl ether. 4. Evaporate the combined ether extract and dry the residual oil at 100 °C. 5. Test the extracted oil for argemone oil as per the procedure in the manual on oils and fats.		
Reference	Manual Methods of Analysis for Adulterants and Contaminants in Foods I.C.M.R. 1990, page24.		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

	Determination of allylisothiocyanate / volatile oil in mustard		
Method No.	FSSAI 10.016:2021	**Revision No. & Date**	0.0
Scope	This method determines allylisothiocyanate content in mustard.		
Caution	See Material Safety Data Sheets, or equivalent, for each reagent.		
Principle	Titrimetric method After two successive soakings of the sample, the first in water at a temperature of 70 °C and the second in alcoholic medium, distillation of the allyl isothiocyanate liberated into an alcoholic ammonium hydroxide solution, addition to the distillate of a standard volumetric silver nitrate solution, and titration of the excess silver nitrate with standard volumetric potassium, or ammonium, thiocyanate solution in the presence of ammonium iron (III) sulphate.		
Apparatus/ Instruments	1. Gas Chromatograph- with flame ionization detector 2. Approximate operating conditions: Column 145 °C, detector 200 °C, injector 160 °C, N_2 flow rate 100 mL/min. Optimum conditions are obtained when not less than 10 cm peak is obtained for 8 µL standard injection solution. 3. Column and packing- 3.7 m ×4 mm i.d., Carbowax 4000 on Fluoropak 80, 20 - 40 mesh or capillary column (30 m x 0.53 mm x 3.0 µ DB-WAX)		
Preparation of Reagents	1. Allylisothiocynate standard solution- 30.5 mg/100 mL. 2. Measure 30 µL of allylisothiocynate in 50 µL syringe with 0.5% accuracy. 3. Add to 50 mL 10% alcohol in 100 mL volumetric flask and shake intermittently until dissolved. 4. Dilute to volume with water.		
Extraction of Allyisothiocyanate	1. Grind about 10 g of sample to pass through No. 20 sieve. 2. Immediately weigh 6 g into 300 mL Erlenmeyer flask, add 150 mL 5% alcohol. 3. Stopper tightly and stir magnetically 90 ± 5 min in water bath maintained at 37 °C.		
Gas Chromatography Method	1. Distill about 70 mL of extract into 100 mL volumetric flask containing 20 mL 5% alcohol (v/v) taking care that the end of the condenser dips below surface of solution. 2. Dilute to volume with water. Inject 4–10 µL into gas chromatograph. Compare peak height of sample with that from same volume of standard solution.		
Titration Method	1. Distill about 60 mL of extract into 100 mL volumetric flask containing 10 mL NH_4OH (1+2) taking care that the end of the condenser dips below surface of solution. 2. Add 20 mL 0.1 M $AgNO_3$ to distillate and let stand overnight, heat to boiling point on water bath (boil behind safety barrier) to		

	agglomerate Ag_2S, cool dilute to 100 mL with water and filter. 3. Acidify 50 mL filtrate with about 5 mL HNO_3 and titrate with 0.1 M NH_4SCN using 5 mL ferrous ammonium sulphate as indicator.
Calculation with units of expression	1 mL 0.1M $AgNO_3$ = 0.004958 g allylisothiocyanate **Note:** 1. Before discarding Ag_2S and filter paper, treat with 25 mL 0.5 M sodium thiosulphate in 1 M sodium hydroxide. 2. During storage mustard becomes moist – conditions which encourage production of allylisothiocyanate, which tends to be lost by volatilization. 3. Include moisture content of the sample in test report.
Reference	1. AOAC 17th edn.,2000 Official Method 970.55, Volatile oil in Mustard Seed 2. Pearson's Composition and Analysis of Foods 9th edn. 1991, Page 417.
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of p-hydroxybenzyl isothiocyanate in *Sinapsis alba* (white mustard)		
Method No.	FSSAI 10.017:2021	**Revision No. & Date**	0.0
Scope	This method determines the content of p-hydroxybenzyliso- thiocyanate in *Sinapsis alba* (white mustard).		
Caution	Concentrated Sulphuric acid and Nitric acid are highly corrosive and can cause severe burns. Always add acid to water when making dilute solutions		
Principle	Decomposition, by enzymatic hydrolysis, of the sinalbin (glucoside of *Sinapis alba*) into glucose, the hydrogen sulphate of sinapin and ρ-hydroxybenzyl isothiocyanate, the last mentioned giving ρ-hydroxybenzyl and thiocyanate. Colorimetric determination of the thiocyanate so formed.		
Apparatus/ Instruments	1. 250 mL volumetric flask 2. 1000 mL volumetric flask 3. UV- visible spectrophotometer 4. Analytical balance 5. Grinding Mill		
Materials and Reagents	1. Calcium carbonate, pulverized 2. NaOH solution (1M): Dissolve 40 g of NaOH in 1000 mL of distilled water 3. Potassium ferrocyanide (106 g/L): Dissolve 106 g of Potassium ferrocyanide in 1000 mL of distilled water 4. Zinc acetate (219 g/L containing 3 g acetic acid: — Dissolve 21.9 g of zinc acetate in water, add 3 mL of glacial acetic acid and dilute to 100 mL with water 5. Ammonium Iron (lll) Sulphate, 200 g/L solution in approximately 0.5 mol/L sulphuric acid solution 6. Sulphuric acid (0.5 M) 7. Mercuric chloride solution (50 g/L) 8. Nitric Acid 1M 9. Potassium Thiocyanate or Ammonium Thiocyanate, standard volumetric solution, c(KCNS) or c(NH4CNS) = 0.l mol/L, that is containing 5.808 g of CNS — per litre		
Method of analysis	**Hydrolysis**: Weigh 5 g of ground sample into a 250 beaker; add 100 mL water at 70 °C and at least 100 mg calcium carbonate. Cover the beaker, keep at 70 °C for 15 min, add 20 mL NaOH (about 1M), mix and stand for 15 min. **Clarification**: Adjust the pH to 6- 6.5 with 1 M HNO_3 and transfer to a 250 mL volumetric flask. Add 2 mL potassium ferrocyanide solution and 2 mL zinc acetate solution with shaking. Make up to mark and pipette in a further 2 mL water to take into account the insoluble matter. Shake, and filter through a rapid filter shaded from bright light. The filtrate (F) should		

<table>
<tr><td></td><td>be clear and colorless.
Determination:
Add to a 50 mL volumetric flask,
a) 5 mL of the filtrate (F); and
b) 5 mL of the ammonium iron (III) sulphate solution.
Dilute to 50 mL with water, shake, and measure the absorbance at a wavelength of 450 nm
The test should be repeated with the addition of 2 drops of mercuric chloride solution (50 g/L) to correct for any absorbance due to phenols present.

Calibration curve: Dilute 5 mL of 0.1M potassium or ammonium thiocyanate to 1 L. Prepare calibration graph, giving absorbance as a function of number of micrograms of thiocyanate. Into a series of five 50 mL volumetric flasks, transfer the volumes of this diluted potassium, or ammonium, thiocyanate solution indicated in the following table:

<table>
<tr><th>Volume of Diluted Potassium, or Ammonium, Thiocyanate Solution
ml</th><th>Corresponding Mass of Thiocyante Ion
µg</th></tr>
<tr><td>5</td><td>145.2</td></tr>
<tr><td>10</td><td>290.4</td></tr>
<tr><td>15</td><td>435.6</td></tr>
<tr><td>20</td><td>580.8</td></tr>
<tr><td>25</td><td>726</td></tr>
</table>

Add to each flask, 5 mL of the ammonium iron (III) sulphate solution, dilute to the mark with water, shake, and measure the absorbance at 450 nm.

Plot a calibration curve, giving the absorbance as a function of the number of micrograms of thiocyanate.</td></tr>
<tr><td>Calculation with units of expression</td><td>p- hydroxybenzyl isothiocyanate content, expressed as % by mass on dry basis $= 2.84 \times \frac{m}{10^6} \times \frac{250}{5} \times \frac{100}{M} \times \frac{100}{100-H}$

Where,
m = µg thiocyanate from calibration graph
M =Mass of sample in g
H = moisture content of the sample</td></tr>
<tr><td>Reference</td><td>1. Pearson’s Composition and Analysis of Foods 9th edn. 1991 page 418
2. IS 2323:2011, Spices and Condiments- Mustard Whole and ground – Specification, Second Revision</td></tr>
<tr><td>Approved by</td><td>Scientific Panel on Methods of Sampling and Analysis</td></tr>
</table>

	Determination of bulk density (mass/ litre) of black pepper		
Method No.	FSSAI 10.018:2021	**Revision No. & Date**	0.0
Scope	This method is applicable for determining the bulk density of black pepper.		
Caution	None		
Principle	The method is based on the basic principle of density which is defined as mass per unit volume.		
Apparatus/ Instruments	1. One litre cylindrical measure with lid made of aluminum alloy, brass or stainless (internal diameter 95 mm, internal height 142 mm) 2. A thin strip of straight metal sheet of about 10 mm width and 150 mm length.		
Method of analysis	1. Fill the 1-liter cylindrical measure with the test sample. 2. Lightly shake the measure horizontally three times and fill again as much as possible to the brim. 3. Tap the measure on a level hard surface three times by changing the position each time and fill again as much as possible to a little over the brim. 4. By moving the metal strip in level with the top of the measure, remove the excess material. 5. Weigh the contents in a balance to the nearest g and record the mass. 6. The mass of one litre of sample provides the Bulk density.		
Calculation with units of expression	Bulk density (g/L) = Mass of sample		
Reference	I.S. Specification No. IS 1797 – 1985 (Reaffirmed in 2009) Methods of Test for Spices and Condiments		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India	**Determination of the percentage of light berries in whole black/ White pepper**		
Method No.	FSSAI 10.019:2021	**Revision No. & Date**	0.0
Scope	This method is applicable for determining the presence of light berries in whole black and white pepper (*Piper nigrum L.)/ (*Kalimirch), sample.		
Definition	**Light Berry —** Berry that has reached an apparently normal stage of development but floats in alcohol with a specific gravity of 0.80 to 0.82 at 25 ºC**.**		
Caution	Follow all safety precautions for the safe handling of organic solvents and special chemical hazards- ethanol. See Material Safety Data Sheets, or equivalent, for each reagent. Ethanol is a flammable liquid, hazardous in case of skin or eye contact (irritant), ingestion, and inhalation		
Apparatus/ Instruments	1. Electronic balance 2. 600 mL beaker 3. Blotting paper or other absorbent material		
Reagents	Alcohol – water solution (specific gravity of 0.80 – 0.82). The alcohol may be ethyl alcohol, denatured spirit or isopropanol.		
Sample Preparation	For the purpose of the test, remove extraneous matter and pinheads from a suitable mass of black pepper, whole black or white pepper, whole.		
Method of analysis	1. Weigh accurately 50 g of prepared sample and transfer to a 600 mL beaker. 2. Add 300 mL of alcohol – water solution and stir the material with a spoon stirrer. 3. Allow the material to settle for 2 min and then spoon off the berries that float on the surface. 4. Repeat the process of stirring, settling and removal of floating berries until no more berries float on the surface in two successive stirrings. **Note: -** Only berries which float on the surface should be removed and not those, which may stay suspended some distance below the surface of the alcohol- water solution. 5. Blot the removed berries to remove excess liquid and then spread them to air dry on a piece of paper, towel or any other absorbent material. Air dry these berries for one hr and then weigh them accurately. 6. If the range of two replications is not over 0.8% then the average of these two determinations can be reported as light berries. If the difference is more than 0.8% then third replication should be performed and average of all three values can be reported as light berries.		
Calculation with units of expression	Light berries (% by mass) =$W_1 \times \frac{100}{W_2}$ Where, W_1= Mass of the light berries removed (Step 5)		

	W_2=Mass of the sample taken for the test
Reference	1. I.S. Specification No. IS 1798 – 1982 Specification for Black Pepper, Whole and Ground revised in IS 1798 2010 2. ASTA method 14.2
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of piperine content of black pepper by UV-VIS spectrophotometry		
Method No.	FSSAI 10.020:2021	**Revision No. & Date**	0.0
Scope	This method determines the piperine content in black and white pepper.		
Caution	Follow all safety precautions for the safe handling of organic solvents and special chemical hazards for ethylene dichloride. See Material Safety Data Sheets, or equivalent, for each reagent.		
Principle	Piperine is extracted into denatured alcohol and absorbance is measured at the maximum absorbance between 342 nm and 345 nm. Other isomers of piperine and related compounds such as piperedine and piperyline, which also absorb at 340-345 nm will also be included.		
Apparatus/ Instruments	1. UV spectrophotometer – any suitable model 2. Analytical balance 3. 1 cm path length silica cuvettes 4. 125 mL amber Erlenmeyer flask/round bottom flask 5. Reflux condenser 6. Volumetric flasks – 100 mL, glass stoppered, amber colored to reduce photo degradation of piperine in solution 7. Anti-bumping granules 8. Funnel 9. Filter paper – Whatman No.2 or equivalent 10. Pipettes		
Reagents	1. Standard denatured alcohol (SDA) – this can be prepared by mixing 1000 mL of 95% ethyl alcohol and 50 mL of methyl alcohol. 2. Piperine – pure		
Preparation of Standard solution	1. Weigh 100 mg of pure piperine into 100 mL volumetric flask, add about 70 mL SDA, shake to dissolve and make up to volume. 2. Pipette 10 mL into 100 mL volumetric flask and dilute to volume. 3. Pipette 1, 2, 3, 4, 5, 6 mL aliquots into six 100 mL volumetric flasks and dilute to volume with SDA. 4. These solutions represent concentrations of 1, 2, 3, 4, 5, and 6 µg/ mL in the standard solutions. 5. Set the wavelength of the spectrophotometer between 342-345 nm 6. Adjust to zero absorbance s with SDA in both reference and sample cell 7. Determine the absorbance readings A1, A2, A3, A4, A5, A6 of corresponding solutions of Step 3 at absorbance maxima between 342-345 nm with SDA in reference cell. 8. Plot a graph of concentration against observed absorbance.		

	9. Determine the average absorbance obtained from the readings and express as µg/mL $$A_{avg}\,(\mu g/mL) = \left[\frac{A_1}{1} + \frac{A_2}{2} + \frac{A_3}{3} + \frac{A_4}{4} + \frac{A_5}{5} + \frac{A_6}{6}\right] \div 6$$
Method of analysis	**For Black and white pepper** 1. Grind sample to pass 60-mesh sieve and blend uniformly. Accurately weigh 0.5 g test sample and transfer to 125 Erlenmeyer flask. Protect from light. 2. Add about 70 mL SDA. Reflux and stir for 1 h, cool to 25±2 °C and filter quantitatively through paper into 100 mL volumetric flask. 3. Transfer rest of the extracted residue to filter, wash thoroughly and dilute to volume. 4. Pipette 2 mL of this solution into 100 mL volumetric flask and dilute to mark with SDA. 5. Shake well, using SDA as reference solution, measure the absorbance reading of the solution at 342-345 nm within 15 min. **For oleoresins** 1. Weigh 1.000 g of well mixed sample and transfer into 100 mL volumetric flask. 2. Make up to volume with SDA. Shake well until dissolved. 3. Pipette 10 mL of solution in a. into 100 mL volumetric flask and fill to mark with SDA. Shake well. 4. Pipette 1 mL of solution in Step 2 into 100 mL volumetric flask and fill to mark with SDA Shake well. 5. Using SDA as the reference solution, record absorbance of solution at maxima 342-345 nm within 15 min.
Calculation with units of expression	Piperine (%) is calculated using the following formula: Where: $$\%\ \text{Piperine} = \frac{A_s}{A_{avg}} \times \frac{V}{W_s \times 10^6} \times 100$$ A_s = absorbance of sample A_{avg} = Average of standard absorbances, each normalized to 1µg/mL V = dilution volume, milliliters W_s = sample weight, grams
Reference	1. AOAC 17th edn, 2000 Official Method 987.07, Piperine in Pepper Preparations, Spectrophotometric Method 2. Piperine Content of Black and White Pepper, Their Oleoresins and Soluble Pepper Seasonings, ASTA Analytical Methods Method 12.1
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Piperine Content in Black Pepper by GC Method		
Method No.	FSSAI 10.021:2021	**Revision No. & Date**	0.0
Scope	This is an alternate method to determine the Piperine content in black pepper sample by using GC equipped with a Flame Ionisation Detector (FID).		
Caution	Follow all safety precautions for the safe handling of organic solvents and special chemical hazards for Dichloromethane. See Material Safety Data Sheets, or equivalent, for each reagent.		
Apparatus/ Instruments	1. A GC equipped with a FID detector. 2. Capillary polar column BP1 (50 m × 0.22 mm I.D., 0.25 µm film thickness)		
Material and Reagents	1. Dichloromethane 2. Piperine of purum grade (98%) 3. Hexacosane (C26) 99% pure		
Gas Chromatography	**Operating parameters** • Carrier gas: hydrogen at 1 mL/min, • Split at 25 mL/min, • Injector temperature at 300 °C, • Detector temperature at 300 °C,		
	Program conditions • 250°C to 280 °C at 0.5 °C/min. • Run time up to 35 min, • Piperine retention time c.a. 20 min • Hexacosane retention time c.a. 13 min.		
Method of analysis	1. Quantify the alkaloids by internal standard method. The internal standard used is hexacosane. 2. Prepare the standard solution adding 2 mL of pure piperine solution (4 g/L in dichloromethane) to 1 mL of hexacosane solution (3 g/L in pentane).		
Reference	Noyer et al. Analysis, 1999, 27, 69-74		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

	Determination of piperine content in pepper and pepper oleoresins using high performance liquid chromatography		
Method No.	FSSAI 10.022:2021	**Revision No. & Date**	0.0
Scope	This high-performance liquid chromatography (HPLC) method for the determination of the piperine content is applicable to pepper (*Piper nigrum* Linnaeus), whole or powdered, as well as their extracts (oleoresins). This method enables the separation and, if necessary, the determination of the other alkaloids of pepper (isochavicine, isopiperine and piperittin).		
Caution	Handle Piperine standard with care as it is a strong irritant.		
Principle	Extraction of piperine from ground pepper with ethanol under reflux, followed by Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) and detection at 343 nm. **Oleoresins of pepper:** Dilution of the oleoresin in ethanol, then determination of piperine by RPHPLC as above.		
Apparatus/ Instruments	1. Volumetric flasks (25 mL, 50 mL and 100 mL capacity) 2. Filtration diaphragms, non-adsorbing. 3. Test sieve of aperture size 500 pm 4. HPLC equipped with i. *Pumping System*: which enables a flow rate, which is constant or programmed at high pressure to be obtained and maintained. ii. Degassing system, for solvents iii. *Detector*: adjustable to a wavelength of 343 nm. iv. Recorder or integrator, the performance of which is compatible with the apparatus as a whole. v. *Column*: Octadecyl column of Length 10-25 cm, i.d. 4-5 mm and particle size 5 µm vi. *Isocratic elution* vii. *Elution solvent:* Mix 52 volumes of 1% (v/v) acetic acid solution and 48 volumes of acetonitrile viii. *Injection system***:** Injection valve with 10µL loop or any other system giving the same injection accuracy.		
Materials and Reagents	• *Reference substance***:** Piperine of at least 98% purity, determined by the spectrometric method described in ISO 5564. *Caution: This product should be handled with care as it is strongly irritating.* • Ethanol, 96% *(V/V).* • Acetonitrile, HPLC grade. *Caution: This product should be handled with care as it is lachrymatory,* • Distilled water (Milli Q or equivalent)		

	• Acetic acid, 1% (*V/V)* aqueous solution.
Preparation of standards and Calibration curve	**Reference solution**: Prepare for immediate use a 1 g/L stock solution of the piperine in ethanol. ***Note*:** Throughout the operation, it is imperative that the solutions are not exposed to light (for instance, wrap the flasks in aluminium foil) and are used as quickly as possible because of the instability of the piperine solutions**.** Working Standard • From the reference solution, prepare at least three working standard solutions of piperine with concentrations ranging from 0.05 g/L to 0.2 g/L. • Equilibrate the column by washing with the elution solvent for 20 min or till the baseline is stable. • Inject each solution into the HPLC and elute isocratically with elution solvent. Repeat the determination at least once. Measure the peak areas and repeat the tests if the results deviate by more than 5%**.** • Plot the calibration curve, i.e., the mass of piperine injected versus the peak area. • Conduct a regression analysis and obtain mean slope of the curve. **Calculation of Response Factor, K** Calculate the response factor, *K* ,using the following formulae: K=m'/A $m' = m \times P_r$ where: m : mass of piperine, in milligrams; A :area of the piperine peak, in integrator units; P_r : purity of the reference piperine; m' is the corrected mass of piperine, in milligrams.
Sample preparation	**Extraction** Caution: It is imperative to operate away from light because of the instability of the piperine solutions**.** **Peppers in powder form** 1. Check that the entire test sample passes through the 500 µm sieve 2. From the test sample prepared, weigh, to the nearest 0.01 g, 0.5 g to 1 g, and transfer to a 100 mL round-bottom flask fitted with a reflux condenser. Add 50 mL of ethanol and bring it to the boil. Maintain for 3 h under reflux. 3. Allow the solution to cool. Filter the solution and collect it in a 100 mL volumetric flask. 4. Rinse the extraction flask and filter with successive portions of ethanol, add the wash liquids to the 100 mL volumetric flask and make up to the mark with more ethanol. **Whole peppers**

	1. Grind the test sample 2. Using the test sample prepared, proceed as described above for peppers in powder form. **Note: 1.** Carefully mix the laboratory sample. Using a grind mill, grind a small quantity of sample and reject it. Then quickly grind an amount slightly larger than the amount required for the test, avoiding undue heating during the operation. Pass the sample through sieve with circular openings of 500 µm diameter. Carefully mix as to avoid stratification. Transfer the ground material in previously dried, clean, airtight container made of glass or any suitable material, which has no action on sample.
	Sample preparation for RP-HPLC 1. Take with the pipette, 10 mL of the extract and transfer it to a 25 mL one-mark volumetric flask. 2. Make up to the mark with the elution solvent. ***NOTE:*** This dilution may be modified, if necessary, by the analyst depending on the assumed piperine content of the sample and the sensitivity of the detector. **Oleoresins of pepper** 1. Thoroughly homogenize the test sample of oleoresin. 2. Weigh into a 100 mL one-mark volumetric flask, to the nearest 0.1 mg, 0.2 g of oleoresin and make up to the mark with ethanol. 3. Pipette 10 mL of this mixture into a 50 mL one-mark volumetric flask and make up to the mark with the elution solvent. *NOTE:* This dilution may be modified, if necessary, by the analyst depending on the assumed piperine content of the sample and the sensitivity of the detector.
	Sample preparation for HPLC 1. The final dilution should be clear. If this is not the case, filter it on the filtration diaphragm, Inject (20 µL) the test solution into the HPLC. 2. On the chromatogram obtained, measure the area of the piperine peak. 3. Carry out two tests and repeat the determination if the results deviate by more than 5%.
Calculation with units of expression	**Peppers, whole or ground** Calculate the piperine content, as a % by mass, using the following formula: $$A \times K \times (\frac{25}{10}) \times (\frac{100}{m_x}) \times 100$$ where *A:* is the area of the piperine peak, in integrator units; m_x *:* is the mass of the sample, in milligrams;

	K: is the response factor determined for the reference substance. NOTE: The ratio 25/10 is related to the dilution and should be modified accordingly when another dilution is used. **Oleoresins of pepper** Calculate the piperine content, as a % by mass, using the following formula: $$A \times K \times (\frac{50}{10}) \times (\frac{100}{m_x}) \times 100$$ where *A:* is the area of the piperine peak, in integratorunits; m_x *:*is the mass of the sample, in milligrams; *K*: is the response factor determined for the reference substance. NOTE: The ratio 50/10 is related to the dilution and should be modified accordingly when another dilution is used.
Reference	1. IS 15695:2006, *Indian standard:* Pepper and pepper oleoresins —Determination of piperine content —Method using high-performance liquid chromatography. 2. ISO 11027:1993, International Standard: Pepper and pepper oleoresins —Determination of piperine content —Method using high-performance liquid chromatography. 3. ISO 2825:1981 Spices and condiments - Preparation of a ground sample for analysis.
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Detection of mineral oil in black pepper		
Method No.	FSSAI 10.023:2021	**Revision No. & Date**	0.0
Scope	This is qualitative method applicable for the detection of Black pepper coated with mineral oil.		
Caution	Petroleum ether is a highly flammable solvent. Keep away from heat/ sparks/open flames/hot surfaces. Wear protective gloves/protective clothing/eye protection/face protection Inhaling chloroform vapors may cause drowsiness or dizziness. Use in a well ventilated hood		
Principle	Mineral oil gives yellow fluorescent spots when visualized under UV light.		
Apparatus/ Instruments	1. Glass slides (7.6 x 2.5 cm) or glass plates of 20 x 5 cm or 20 x 10 cm may be used. 2. TLC Developing tank 3. Ultra-violet lamp (365 nm). This should be placed in a darkened enclosure. 4. Hot air oven operating at 110 °C 5. Glass stoppered conical flask 6. Funnel 7. Glass wool 8. Water bath		
Materials and Reagents	***Note:*** Before using chemicals, refer to chemical safety and/ or safety data sheets approved by authorities. a. Silica-gel 'G' b. Petroleum ether with boiling point of 40-60 °C c. Chloroform d. Ethyl alcohol, 95% ACS grade c. Spray reagent: 0.1% solution of 2,7-dichloro-fluoresein dissolved in 95% ethanol.		
Preparation of TLC plates	Hold two slides together face to face and dip them in slurry of silica gel G (45 g) in a mixture of chloroform and methanol (80 + 20 mL). Withdraw the slides, separate them and allow drying in air and activating at 110 °C for 15 min and cooling in a desiccator. Commercially available Silica G TLC plates may be used		
Method of analysis	1. Weigh 10 g sample and transfer to 100 mL glass stoppered conical flask 2. Add 25 mL petroleum ether. 3. Shake well for 30 seconds and immediately filter in a 50 mL beaker.		

	Evaporate the solvent in the beaker using a water bath. 4. Dissolve the residue in 1 mL chloroform and spot about 10 µL on activated Silica gel G plate using capillary tube, leaving 1 cm from the base line of the plate. 5. Allow to dry and place the slide in a developing tank containing petroleum ether. 6. Cover the tank and allow the solvent to travel for 10 cm from the origin. Remove the plate from the tank, dry in air, spray with the fluorescein solution and view under UV light. 7. Appearance of a yellow fluorescent spot on the solvent front indicates the presence of mineral oil. **Note**: If desired, run a standard mineral oil alongside and report the result. The vegetable oil forms a yellow streak about 2-3 cm long from the point of spotting.
Interpretation of results	Appearance of a yellow fluorescent spot on the solvent front indicates the presence of mineral oil. Presence of a vegetable oil shows a yellow streak about 2-3 cm from the origin.
Reference	J. S. Pruthi, "Quality Assurance in Spices and Spice Products, Modern Methods of Analysis," Allied Publishers Ltd, New Delhi, 1999. Page 219
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Qualitative detection of papaya seeds in black pepper		
Method No.	FSSAI 10.024:2021	**Revision No. & Date**	0.0
Scope	The method is applicable for detecting the adulteration of black pepper with papaya seeds		
Caution	None		
Principle	Papaya seeds float in ethyl alcohol of 0.8 sp. gr. along with immature seeds and light berries whereas mature seeds of black pepper sink.		
Reagents	1. Ethyl alcohol (Specific gravity 0.8) 2. 2% Iodine solution n Potassium iodide		
Method of analysis	1. Weigh about 25 g of black pepper seed 2. Float the sample in ethyl alcohol of sp. gr. 0.8, separate all the floaters and examine them as under: • The morphological characteristics of papaya seeds are quite different from black pepper. The papaya seed is a dicotyledon and pepper is a monocotyledon. 3. Cut the seed into two halves and put a drop of iodine solution. The pepper seed gives blue color due to presence of starch while papaya seed gives pale color due to presence of dextrins.		
Interpretation of results	Seeds that float and do not show a blue color with iodine indicate adulteration with papaya seeds.		
Reference	Manual Methods of Analysis for Adulterants and Contaminants in Foods I.C.M.R. 1990, page23.		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

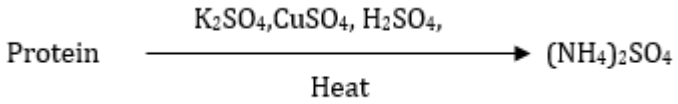
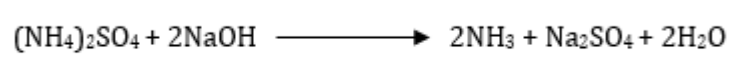
	Identification of Saffron (Filaments and Powder)		
Method No.	FSSAI 10.025:2021	**Revision No. & Date**	0.0
Scope	This method is applicable for identification of saffron. It applies to saffron in both of the following forms a) filaments and cut filaments and b) powder.		
Caution	Concentrated Sulphuric acid is highly corrosive and can cause severe burn. Handle with care.		
Principle	Saffron filaments are visually examined with a magnifying glass. For saffron powder a colorimetric reaction is used.		
Apparatus/ Instruments	1. Magnifying glass with a magnification of 10 times Max 2. Porcelain dish with flat bottom		
Reagents	1. Diphenylamine 2. Sulphuric acid		
Preparation of reagents	1. Diphenylamine solution: Add 0.1 g diphenylamine to 20 mL sulphuric acid (sp. gr. 1.19) and 4 mL water. ***Note***: the diphenylamine should not produce any color with Sulphuric acid.		
Method of analysis	**Filaments**–Spread out the saffron filaments and cut filaments and examine with a magnifying glass. **Powder** 1. Add the diphenylamine solution to porcelain dish with flat bottom and gradually add 0.2 g of the powder. 2. The development of a blue color, which rapidly turns reddish brown, shows presence of pure saffron.		
Interpretation of results	Pure saffron immediately produces a blue color, which rapidly turns reddish brown. In the presence of nitrates the blue color persists.		
Reference	IS 5453 (Part 2): 2016, Indian Standard, Spices-Saffron (*Crocus sativus* L.), Part 2 Test methods, First revision.		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

	Determination of extraneous matter in saffron		
Method No.	FSSAI 10.026:2021	**Revision No. & Date**	0.0
Scope	This method is to check the presence of extraneous matter in saffron.		
Caution	None		
Principle	Saffron filaments are visually examined for extraneous matter.		
Apparatus/ Instruments	1. Watch glass 2. Small laboratory tongs 3. Analytical balance		
Sample Preparation	Homogenize the sample well before weighing.		
Method of analysis	1. Weigh to the nearest 0.01 g, about 3 g of homogenized sample and spread it on a sheet of neutral grey paper. 2. With the help of a small tong, separate the extraneous matter. 3. Weigh the separated matter in a previously tared watch glass in an analytical balance and calculate the extraneous matter as % mass fraction.		
Calculation with units of expression	The extraneous matter content of the sample, expressed as a % by mass, is equal to: $$\textbf{Extraneous Matter}\ (\%) = \frac{\mathbf{W_2 - W_1}}{\mathbf{W_0}} \times \mathbf{100}$$ Where: W_2 = Weight of the watch glass and extraneous matter W_1= Weight of the empty *w*atch glass W_0= Weight of the test portion		
Reference	I.S. 5453 (Part 2): 2016, Indian Standard, Spices-Saffron (*Crocus sativus* L.), Part 2 Test methods, First revision.		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India	**Determination of moisture and volatile matter in saffron**		
Method No.	FSSAI 10.027:2021	**Revision No. & Date**	0.0
Scope	This method is used to determine the moisture and volatile matter in Saffron and applicable to filaments and powder.		
Caution	Use gloves and tong when handling hot moisture dishes.		
Principle	Oven drying at 103 ±2 °C to constant mass.		
Apparatus/ Instruments	1. Convection Oven: capable of operating at 103 ± 2 °C 2. Analytical balance, accurate to 0.001 g 3. Moisture dish 4. Desiccator		
Method of analysis	1. Weigh to the nearest 0.001 g, about 2.5 g homogenized sample or sample reconstituted after determination and reincorporation of extraneous matter and floral waste and transfer to a moisture dish with a slip-on cover. 2. Place the dish uncovered in an oven maintained at 103 ± 2 °C for 2 h and cool in a desiccator. 3. Note down the weight of the dish. Leave for 16 h. 4. Cover the dish, cool it in a desiccator, and weigh to the nearest 0.001 g. 5. Carry out two determinations and take the arithmetic mean of both determinations.		
Calculation with units of expression	Moisture and volatile matter (%) = $\frac{(\mathbf{m_0}-\mathbf{m_1})\times\mathbf{100}}{(\mathbf{m_0})}$ Where, m_0 = Mass in g of test portion m_1= Mass in g of the dry residue		
Reference	I.S. 5453 (Part 2): 2016, Indian Standard, Spices-Saffron (*Crocus sativus* L.), Part 2 Test methods, First revision.		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India	**Determination of picrocrocine, safranal and crocin in saffron**		
Method No.	FSSAI 10.028:2021	**Revision No. & Date**	0.0
Scope	This method enables determination of the bitterness as picrocrocine, flavor content as safranal and coloring strength as crocine of saffron. The method is applicable to 1) whole filaments as a loose, sample, 2) elastic and hygroscopic mass of filaments, and 3) powder form.		
Definitions	**Coloring strength:** Mainly due to its crocin content, it is defined by measurement of the absorbance at the maximum, about 440 nm. **Bitterness:** Mainly due to its picrocrocine content, it is defined by measurement of the optical density at the maximum, about 257 nm. **Flavour:** Mainly due to its safranal content, it is defined by measurement of the optical density at the maximum, about 330 nm.		
Caution	None		
Principle	Picrocrocine, safranal and crocine have a characteristic absorption maximum (λmax) at 257, 330 and 440 nm respectively. The absorbance of an aqueous extract extract at their respective λmax is measured and the $E_{1cm}^{1\%}$ is calculated.		
Apparatus/ Instruments	1. UV-Visible Spectrophotometer (220-480 nm) 2. Quart cuvette with 1 cm path length 3. Volumetric flasks – 200 mL and 1000 mL 4. Pipettes – 20 mL 5. Filtration membrane – made of cellulose acetate of 50 mm diameter and porosity 0.45 µm 6. Magnetic stirrer		
Method of analysis	1. Weigh exactly 500 mg of homogenized sample crushed to pass through 500 µm sieve to the nearest 0.001 g 2. Transfer quantitatively to a 1000 mL volumetric flask and add about 900 mL of distilled water. 3. Stir with a magnetic stirrer away from light for 1 h. Remove the magnetic bar. Make upto mark with distilled water. 4. Close with a glass stopper and mix by inversion. 5. Take an aliquot part with the 20 mL pipette. 6. Transfer to a 200 mL volumetric flask. Adjust to mark with distilled water. Close with a stopper and mix by inversion. 7. Filter the solution rapidly and away from light through the membrane to obtain a clear solution. 8. Auto zero the spectrophotometer with distilled water n both cells. 9. Scan the sample against a distilled water blank between 220 nm and 480 nm. 10. Record the absorbance at 257, 330 and 440 nm		

	11. Calculate the respective $E_{1cm}^{1\%}$
Calculations and Expression of results	Convert the absorbance to Using the at 257 nm calculate $E_{1cm}^{1\%}$ of Using the at 330 nm calculate of Safranal Flavor Using the at 440 nm calculate of Crocine coloring matter Note is defined as the Absorbance of a 1% solution in a 1 cm light path Note: Include the % moisture content of saffron in the test report, obtained by the method specified in this manual. $E_{1cm}^{1\%} = \frac{D \times 10000}{m \times (100 - H)}$ D is the absorbance at 257 nm, 310 nm and 440 nm; m is the mass of the saffron sample, in grams; H is the moisture and volatile matter content of the sample; $E_{1cm}^{1\%}$ without unit.
Reference	I.S. 5453 (Part 2): 2016, Indian Standard, Spices-Saffron (*Crocus sativus* L.), Part 2 Test methods, First revision.
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Total Nitrogen in Saffron		
Method No.	FSSAI 10.029:2021	**Revision No. & Date**	0.0
Scope	The Kjeldahl method for nitrogen determination is used. The method is applicable to saffron filaments and powder form.		
Caution	Concentrated Sulphuric acid is highly corrosive and can cause severe burns. Handle with care.		
Principle	The method is based on the principle that sulphuric acid in the presence of a catalyst helps in the digestion of food. All of the nitrogen is converted ammonium sulphate. By distillation in the presence of a base such as NaOH it is converted into ammonia. The ammonia is trapped in an acid (e.g. Boric acid), which is titrated against 01N hydrochloric acid. The method involves the following reactions     		
Apparatus/ Instruments	1. **Kjeldhal flasks:** Kjeldahl, hard, moderately thick, well-annealed glass, 500 or 800 mL capacity 2. **Distillation apparatus** 3. **Digestion apparatus**. 4. **Conical or Erlenmeyer flask:** 500 mL capacity, graduated at every 200 mL 5. **Burette:** 50 mL capacity, graduated at least at every 0.1 mL or autotitrator 6. **Boiling aids**/Glass beads 7. **Measuring cylinders:**50, 100 and 500 mL capacities, graduated 8. **Catalyst**		
Materials and Reagents	1. Potassium sulfate (K_2SO_4): Nitrogen free or low in nitrogen content 2. Copper (II) sulfate solution: Dissolve 5.0 g of copper (II) sulfate pentahydrate ($CuSO_4.5H_2O$) in water and make up the final volume to 100 mL in a 100 mL volumetric flask. 3. Concentrated sulphuric acid: At least 95 - 98% (m/m), nitrogen free, ρ_{20} approximately = 1.84 g/mL 4. Sodium hydroxide solution, 50%, m/v (low in nitrogen): Dissolve 50		

	g NaOH pellets in water and finally make to 100 g 5. Indicator solution: Dissolve 0.1 g of methyl red in 95% (v/v) ethanol and dilute to 50 mL with ethanol. Dissolve 0.5 g of bromocresol green in 95% (v/v) ethanol and dilute to 250 mL with ethanol. Mix 1 part of methyl red solution with 5 parts of bromocresol green solution or combine all of both solutions. 6. Boric acid solution (H_3BO_3): Dissolve 40 g of boric acid in hot water, allow the solution to cool and dilute to 1 L. Add 3 mL of methyl red - bromocresol indicator solution, mix and store the solution in borosilicate glass bottle. The solution will be light orange in color. Protect the solution from light and sources of ammonia fume during storage. 7. Standard hydrochloric acid solution: 0.1 ± 0.0005 N. 8. Ammonium sulfate [$(NH_4)_2SO_4$]: Minimum assay 99.9% on dried material. Immediately before use dry the ammonium sulfate at 102 ± 2 °C for not less than 2 h. Cool to 25±2 °C in a desiccator. 9. Tryptophan ($C_{11}H_{12}N_2O_2$) or Lysine hydrochloride ($C_6H_{15}ClN_2O_2$): Minimum assay 99%, do not dry these reagents in an oven before use. 10. Sucrose with a nitrogen content of not more than 0.002% (m/m). Do not dry in an oven before use.
Method of analysis	**Test portion and pre-treatment:** Add to the clean and dry Kjeldahl flask, 5 – 10 boiling aids, 15 g K_2SO_4, 1.0 mL of the copper sulfate solution, approximately 5 ± 0.1 g of prepared sample weighed to the nearest 0.1 mg, and add 25 mL of concentrated sulfuric acid. Use the 25 mL acid also to wash down any copper sulfate solution, K_2SO_4 or sample left on the neck of the flask. Gently mix the contents of the Kjeldahl flask. **Digestion:** Turn on the fume extraction system of the digestion apparatus prior to beginning the digestion. Heat the Kjeldahl flask and its contents on the digestion apparatus using a heater setting low enough such that charred digest does not foam up the neck of the Kjeldahl flask. Digest at this heat-setting for at least 20 min or until white fumes appear in the flask. Increase the heater setting to half way to the maximum setting as determined previously (See digestion apparatus) and continue the heating period for 15 min. At the end of 15 min period, increase the heat to maximum setting. After the digest clears (clear with light blue-green color), continue boiling for 1 h to 1.5 h at maximum setting. The total digestion time will be between 1.8 – 2.25 h. **Note:** At the end of digestion, the digest shall be clear and free of undigested material. Allow the acid digest to cool to 25±2 °C over a period of approximately 25 min. If the flasks are left on hot burners to

	cool, it will take longer to reach 25±2 °C. The cooled digest should be liquid or liquid with a few small crystals at the bottom of the flask at the end of 25 min cooling period. Do not leave the undiluted digest in the flask overnight. The undiluted digest may crystallize during this period and it will be very difficult to get that back into the solution to avoid this situation. **Note:** Excessive crystallization after 25 min is the result of undue acid loss during digestion and can result in low test values. Undue acid loss is caused by excessive fume aspiration or an excessively long digestion time caused by an incorrect maximum burner setting. After the digest is cooled to 25±2 °C, add 300 mL of water to 500 mL Kjeldahl flask or 400 mL of water when using 800 mL Kjeldahl flask. Use the water to wash down the neck of the flask too. Mix the contents thoroughly ensuring that any crystals which separate out are dissolved. Add 5 - 10 boiling aids. Allow the mixture to cool again to 25±2 °C prior to the distillation. Diluted digests may be stoppered and held for distillation at a later time. **Distillation:** Turn on the condenser water for the distillation apparatus. Add 75 mL of 50% (m/m) sodium hydroxide solution to the diluted digest by carefully pouring the solution down the inclined neck of the Kjeldahl flask, so as to form a clear layer at the bottom of the bulb of the flask. There should be a clean interface between the two solutions. Immediately after the addition of sodium hydroxide solution to the Kjeldahl flask, connect it to the distillation apparatus, the tip of whose condenser outlet tube is immersed in 50 mL of boric acid solution with indicator contained in a 500 mL Erlenmeyer flask. Vigorously swirl the Kjeldahl flask to mix its contents thoroughly until no separate layers of solution are visible in the flask any more. Set the flask down on the burner. Turn on the burner to a setting high enough to boil the mixture. Continue distillation until irregular boiling (bumping) starts and then immediately disconnect the Kjeldahl flask and turn off the burner. Turn off the condenser water. The distillation rate shall be such that approximately 150 mL distillate is collected when irregular boiling (bumping) starts and the volume of the contents of the conical flask will be approximately 200 mL. If the volume of distillate collected is less than 150 mL, then it is likely that less than 300 mL of water is added to dilute the digest. The efficiency of the condenser shall be such that the temperature of the contents of conical flask does not exceed 35 °C during distillation. **Titration:** Titrate the boric acid receiving solution with standard

	hydrochloric acid solution (0.1 N) to the first trace of pink color. Take the burette reading to at least the nearest 0.05 mL. A lighted stir plate may aid visualization of the end point. **Blank test:** Simultaneously carry out a blank test by following the procedure as described above taking all the reagents and replacing the sample with 5 mL water and about 0.85 g of sucrose. **Note:** • The purpose of sucrose in a blank or a recovery standard is to act as organic material to consume an amount of sulfuric acid during digestion that is roughly equivalent to a test portion. If the amount of residual free sulfuric acid at the end of digestion is too low, the recovery of nitrogen by both recovery tests (See below i.e. Nitrogen recovery test) will be low. If the amount of residual acid present at the end of the digestion is sufficient to retain all the nitrogen, but the temperature and time conditions during digestion were not sufficient to release all the nitrogen from a sample, then the nitrogen recovery will be acceptable. • The amount of titrant used in the blank should always be greater than 0.00 mL. Blanks within the same laboratory should be consistent across time. If the blank is already pink before the beginning of titration, something is wrong. Usually, in such cases, the conical flasks are not clean or water from the air that may condense on the outside of the condenser apparatus has dripped down into the collection flask to cause the contamination. **Nitrogen recovery test** • The accuracy of the procedure should be checked regularly by means of following recovery tests, carried out in accordance with procedure as in the preceding steps. • Check that no loss of nitrogen occurs by using a test portion of 0.12 g of ammonium sulfate along with 0.85 g of sucrose. Add all other reagents (except sample). Digest and distill under same conditions as for a sample. • The % of nitrogen recovered shall be between 99.0 and 100.0% for the given apparatus. In the case recoveries of nitrogen exceed 100%, ammonium sulfate is only useful to determine whether nitrogen loss has occurred or the normality of titrant is lower than the stated value. For recoveries less than 99%, the loss could be in the digestion or distillation step. It is possible to use a mixture of ammonium sulfate and small amount of sulfuric acid (the amount of residual remaining

	at the end of digestion) in a Kjeldahl flask. Dilute it with the normal value of water, add the normal amount of NaOH solution and distill. If the nitrogen recovery is still low by the same amount, the loss of nitrogen is in the distillation apparatus and not in that of the digestion. The probable cause might be leaky tubing in a traditional system or the tips of the condensers not submerged under the surface of boric acid solution early in the distillation. The apparatus should pass this test before going on to check recoveries by the procedure described below. • Check the efficiency of digestion procedure by using 0.16 g of lysine hydrochloride or 0.18 g of tryptophan along with 0.67 g of sucrose. Add all other reagents. Digest and distill under same conditions as for a sample. At least 98% of the nitrogen shall be recovered. If the recovery is lower than 98% after having a 99 - 100% recovery on ammonium sulfate, then the temperature or time of digestion is insufficient or there is undigested sample material (i.e., char) on the inside of the Kjeldahl flask. • The final evaluation of performance is best done by participation in a proficiency testing system, where within and between laboratories statistical parameters are computed based on analysis of samples. • Lower results in either of the recovery tests (or higher than 100% in case of ammonium sulfate) will indicate failures in the procedure and/or inaccurate concentration of the standard hydrochloric acid solution. ***Note***: Fully automated Kjeldahl Analyzer (digestion unit, distillation unit with integrated colorimetric titrator), can be used in place of the conventional system described.
Calculation with units of expression	Calculate the nitrogen content, expressed as a % by mass, by following formula $$W_n = \frac{1.4007 \text{ x } (V_S - V_B) \text{x N}}{W}$$ W_n=nitrogen content of sample, expressed as a % by mass; V_S=volume in mL of the standard hydrochloric acid used for sample; V_B=volume in mL of the standard hydrochloric acid used for blank test; N=Normality of the standard hydrochloric acid expressed to four decimal places;

	W= mass of test portion in g, expressed to nearest 0.1 mg. Express the nitrogen content to four decimal places. The crude protein content, expressed as a % by mass, is obtained by multiplying the nitrogen content by 6.25. Express the crude protein results to three decimal places.
Reference	ISO 8968-1/IDF 020-1:2001 - Milk - Determination of nitrogen content - Part 1: Kjeldahl method.
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Curcumin Content in Turmeric		
Method No.	FSSAI 10.030:2021	**Revision No. & Date**	0.0
Scope	This method specifies a spectrophotometric method for the determination of curcumin in turmeric and is applicable to powders and tubers.		
Caution	Follow all safety precautions for the safe handling of organic solvents and special chemical hazards- ethyl alcohol. See Material Safety Data Sheets, or equivalent, for each reagent.		
Principle	Curcumin is extracted into ethyl alcohol and absorbance at 425 nm measured.		
Apparatus/ Instruments	1. Extraction flask – Flat bottom, 100 mL with TS 24/40 ground glass joint 2. Condenser – water cooled, drip tip 300-400 mm length TS24 /40 ground glass joint 3. Volumetric flasks – 100 and 250 mL 4. Analytical balance 5. UV-Visible Spectrophotometer – any suitable type capable of measuring absorbance at 425 nm 6. Cuvette/ cell: 1 cm light path, silica		
Reagents	1. Ethyl alcohol – 95% 2. Standard curcumin solution – Weigh 25 mg of standard curcumin into a 100 mL volumetric flask. Dissolve and dilute to mark with alcohol. • Transfer 1 mL of the solution to a 100 mL volumetric flask and dilute to mark with alcohol. This standard solution contains 2.5 mg (0.0025 g)/L.		
Method of analysis	1. Grind sample as quickly as possible in a grinding mill to pass sieve with 1 mm diameter aperture. 2. Weigh accurately about 0.1 g, add 30 mL alcohol and reflux for 2.5 h. 3. Cool the extract and filter quantitatively into a 100 mL volumetric flask. 4. Transfer the extracted residue to the filter. Wash thoroughly and dilute to mark with alcohol. 5. Pipette 20 mL of the filtered extract into a 250 mL volumetric flask and dilute to volume with alcohol. 6. Measure the absorbance of the extract and the standard solution at 425 nm in 1 cm cell against an alcohol blank.		
Calculation with units of expression	Calculate the Molar Absorptivity of curcumin using the equation $A = \frac{a_1}{L \times c}$ Where, a_1 =absorbance of standard solution at 425 nm		

	L = cell length in cm (=1 cm) c = concentration in g/l Using this value calculate the concentration of curcumin in the sample Curcumin (%) $=\frac{a_2 \times 125 \times 100}{L \times A \times m}$ a_2 =absorbance of extract at 425 nm m = mass of sample in g **NOTE**: The results should be reported on dry basis, by multiplying the value obtained with: $\frac{100\%}{100\%-M}$, where M is the % moisture content of the sample.
Reference	1. IS: 3576- 2010, Indian Standard, Specification for Turmeric Whole and Ground 2. IS: 10925- 1984, Reaffirmed- 2012, Indian Standard, Specification for Turmeric Oleoresin
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Total Curcuminoid content of Turmeric and Oleoresins by UV-VIS Spectrophotometry		
Method No.	FSSAI 10.031:2021	**Revision No. & Date**	0.0
Scope	This method determines percent curcumin content in turmeric preparations/ oleoresins.		
Caution	Follow all safety precautions for the safe handling of organic solvents and special chemical hazards for acetone. See material safety data sheets, or equivalent, for each reagent.		
Principle	Curcumin is extracted into acetone and absorbance at 425 nm measured. Analogues of curcumin, namely, demethoxy curcumin and bis-demethoxy curcumin also absorb at 425 nm and will be included.		
Apparatus/ Instruments	1. UV spectrophotometer 2. Analytical balance 3. 1 cm path length silica cuvettes 4. 125 mL Erlenmeyer flask/round bottom flask 5. West condenser 6. Volumetric flasks – 200 mL, 100 mL, glass stoppered 7. Anti-bumping granules 8. Funnel 9. Filter paper – Whatman No.1 or equivalent 10. Pipettes 11. Sieve		
Reagents	1. Acetone (AR grade) 2. Curcumin pure (Purity=99%)		
Sample Preparation	**Rhizomes**: Grind dry turmeric rhizomes to pass through 40-mesh sieve and blend uniformly. Accurately weigh 1.000 g test sample. **Oleoresin**- stir well, use as it is		
Method of analysis	**A. Raw spice** 1. Grind dry turmeric sample to pass through 40-mesh sieve and blend uniformly. Accurately weigh 1.000 g test sample and transfer to 125 mL Erlenmeyer flask. 2. Add about 75 mL of acetone. Reflux for 1 h with West Condenser on a water bath, cool to 25 ± 2 °C and filter quantitatively into 200 mL volumetric flask. 3. Transfer rest of the extracted residue to filter, wash thoroughly with acetone and dilute to volume. 4. Pipette 1 mL of (step 3) solution into 100 mL volumetric flask and dilute to volume with acetone and mix well. 5. Using acetone as reference solution, measure the absorbance of the solution at 425 nm within 15 min. **B. Oleoresins** 1. Weigh to the nearest 0.001 g appropriate weight of well mixed sample		

	and transfer to 100 mL volumetric flask. Dissolve in acetone and dilute to volume with acetone and mix. 2. Pipette 1 mL of the solution (step 1) into 100 mL volumetric flask and dilute to volume with acetone and mix well. 3. Using acetone as reference solution, measure the absorbance of the solution at 425 nm within 15 minutes.
Calculation with units of expression	Percent curcumin content = $\frac{\text{As} \times \text{V}}{\text{Ws} \times 1650 \times 100} \times 100$ Where As = absorbance of test solution; V = dilution volume in mL, where if one uses the dilution schedule as presented in this method ,V=20,000 for raw spice and V=10,000 for oleoresin; Ws= Test portion weight in gram. **Note:** Appropriate weight determined as: (0.03 g x 100%) / expected %
Reference	ASTA analytical Methods, Curcumin content in Turmeric spice and oleoresins, ASTA method 18.0 Revised Oct. 2004
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Starch content in Turmeric		
Method No.	FSSAI 10.032:2021	**Revision No. & Date**	0.0
Scope	This method determines the starch content of Turmeric (Not applicable to products with added sugars).		
Caution	Ether is a highly flammable liquid. Exercise extreme caution when handling.		
Principle	The starch is hydrolysed to reducing sugar using hydrochloric acid and reducing sugars estimated.		
Apparatus / Instruments	1. Erlenmeyer flask 500 mL, with T.S 24/40 ground joint 2. West type condenser, 400 mm in length, with water- cooled drip tip 24/40 ground joint 3. Volumetric flask 250 mL, with ground glass stopper 4. Gooch crucible 5. Pipettes, transfer type, 5, 25 and 50 mL 6. Burette, 50 mL, graduated in 0.1 mL		
Reagents / Preparation of Reagents	1. Hydrochloric acid solution, Sp.gr. 1.125 - Dilute 680 mL of 37% HCl by weight (Sp. gr. = 1.19 at 20 °C) to 1 liter 2. Sodium hydroxide solution, ca. 2.5 N, (10% w/v) 3. Ethyl ether, anhydrous, ACS grade or equivalent 4. Ethyl alcohol solution, 10% by volume 5. Ethyl alcohol absolute, ACS grade or equivalent 6. Ceramic fiber- Place 60 g ceramic fiber (Cerafiber or equivalent) in blender, add 800 mL H_2O and blend 1 min at low speed. 7. Indicator paper (universal) 8. Modified Fehling's solution: (a) Copper sulfate solution- Dissolve 34.639 g of copper sulfate ($CuSO_4.5H_2O$) in distilled water, dilute to 500 mL and filter through prepared ceramic fiber. (b) Alkaline tartrate solution- Dissolve 173 g of sodium tartrate, $NaKC_4H_4O_6.4H_2O$ (Rochelle salt) and 50 g of NaOH in distilled water. Dilute to 500 mL, allow standing for 2 days and filtering through prepared ceramic fiber.		
Sample Preparation	1. Grind laboratory sample as quickly as possible in a grinding mill to pass sieve with 1 mm diameter aperture. Avoid undue heating of apparatus during grinding. 2. Mix carefully to avoid stratification (layering). 3. Store in a dry stoppered container.		
Method of analysis	1. Weigh 4 g to the nearest 0.01 g of sample and transfer to a funnel containing a Whatman No. 2 filter paper or equivalent. 2. Extract the sample with 5 successive 10 mL portions of ethyl ether. Allow the ether to evaporate from the residue, wash with 150 mL of the		

<table>
<tr><td></td><td>10% alcohol solution, and then with 15 to 20 mL of absolute ethyl alcohol.
3. Carefully transfer the insoluble residue from the filter paper to the 500 mL Erlenmeyer flask with water, using a wash bottle and gently rubbing the paper with a rubber policeman. Add distilled water to make the total volume 200 mL and then add 20 mL of HCl solution (Sp. Gr. 1.125). Connect the flask to a reflux condenser and boil for 2.5 h.
4. Cool and add NaOH solution slowly with stirring until the solution is almost neutral to indicator paper (pH 6-7). Solution must not be alkaline at any time. Transfer to the 500 mL volumetric flask, make to volume at 25±2 °C and mix well.
5. To determine the amount of reducing sugars, filter the hydrosylate through a dry filter paper, discarding the first 10 mL portion of the filtrate. Pipette 25 mL each of the copper sulfate and alkaline tartrate solutions into a 400 mL beaker and then add an aliquot of the filtered sample solution. If the aliquot is less than 50 mL, add distilled water to make the final volume 100 mL.
6. Cover the beaker with a watch glass and heat on iron-wire gauze over a Bunsen burner or on a hot plate. The burner or hot plate must be pre-set to bring the solution to a boil in exactly 4 min. Continue boiling exactly 2 min.
7. Filter the hot solution immediately through the prepared, tared Gooch crucible with the aid of suction. Wash the precipitated Cu_2O thoroughly with water at ca. 60 °C. Then wash the precipitate with 10 mL of absolute alcohol and finally with 10 mL of ether. Dry the precipitate 30 min in an oven at 110 ± 2 °C, cool to 25±2 °C in a desiccator and weigh thoroughly.</td></tr>
<tr><td>Calculation with units of expression</td><td>Refer to the standard Munson and Walker table to find the mg of dextrose corresponding the weight of Cu_2O found.
$$\text{Dextrose}, \% = \frac{\text{Weight of dextrose(mg)} \times 500 \times 0.1}{\text{Weight of sample (g)} \times \text{aliquot(ml)}}$$
$$Starch, \% = dextrose \times 0.90$$</td></tr>
<tr><td></td><td>Notes:
1. The optimum aliquot depends on the starch content of the sample being analyzed. The aliquot should contain between 100 to 200 mg of dextrose.
<table>
<tr><td>Expected starch content</td><td>Aliquot in mL</td></tr>
<tr><td>60</td><td>25</td></tr>
<tr><td>50</td><td>35</td></tr>
<tr><td>40</td><td>50</td></tr>
<tr><td>30</td><td>50</td></tr>
<tr><td>20</td><td>50</td></tr>
</table>
2. Conduct a blank using 50 mL of the reagent and 50 mL of water. If the weight of the Cu_2O obtained exceeds 0.5 mg, correct the result of the</td></tr>
</table>

	determination accordingly. The alkaline tartrate solution deteriorates on standing and the quantity of Cu_2O obtained in the blank determination increases. 3. The procedure is very empirical and needs to be followed exactly.
Reference	Official Analytical Methods of the American Spice Trade Association, 4th edition, 1997, Method 8.0: Starch (Direct Acid Hydrolysis)
Approved by	Scientific Panel on Methods of Sampling and Analysis

Munson and Walker Table for Calculating Dextrose

(Applicable when Cu_2O is weighed directly)

(Expressed in mg)

Cuprous Oxide (Cu_2O)	Dextrose (d-Glucose)	Cuprous Oxide (Cu_2O)	Dextrose (d-Glucose)	Cuprous Oxide (Cu_2O)	Dextrose (d-Glucose)	Cuprous Oxide (Cu_2O)	Dextrose (d-Glucose)	Cuprous Oxide (Cu_2O)	Dextrose (d-Glucose)	Cuprous Oxide (Cu_2O)	Dextrose (d-Glucose)
10	4.0	90	38.9	170	75.1	250	112.8	330	152.2	410	193.7
12	4.9	92	39.8	172	76.0	252	113.7	332	153.2	412	194.7
14	5.7	94	40.6	174	76.9	254	114.7	334	154.2	414	195.8
16	6.6	96	41.5	176	77.8	256	115.7	336	155.2	416	196.8
18	7.5	98	42.4	178	78.8	258	116.6	338	156.3	418	197.9
20	8.3	100	43.3	180	79.7	260	117.6	340	157.3	420	199.0
22	9.2	102	44.2	182	80.6	262	118.6	342	158.3	422	200.1
24	10.0	104	45.1	184	81.5	264	119.5	344	159.3	424	201.1
26	10.9	106	46.0	186	82.5	266	120.5	346	160.3	426	202.2
28	11.8	108	46.9	188	83.4	268	121.5	348	161.4	428	203.3
30	12.6	110	47.8	190	84.3	270	122.5	350	162.4	430	204.4
32	13.5	112	48.7	192	85.3	272	123.4	352	163.4	432	205.5
34	14.3	114	49.6	194	86.2	274	124.4	354	164.4	434	206.5
36	15.2	116	50.5	196	87.1	276	125.4	356	165.4	436	207.6
38	16.1	118	51.4	198	88.1	278	126.4	358	166.5	438	208.7
40	16.9	120	52.3	200	89.0	280	127.3	360	167.5	440	209.8
42	17.8	122	53.2	202	89.9	282	128.3	362	168.5	442	210.9
44	18.7	124	54.1	204	90.9	284	129.3	364	169.6	444	212.0
46	19.6	126	55.0	206	91.8	286	130.3	366	170.6	446	213.1
48	20.4	128	55.9	208	92.8	288	131.3	368	171.6	448	214.1
50	21.3	130	56.8	210	93.7	290	132.3	370	172.7	450	215.2
52	22.2	132	57.7	212	94.6	292	133.2	372	173.7	452	216.3
54	23.0	134	58.6	214	95.6	294	134.2	374	174.7	454	217.4
56	23.9	136	59.5	216	96.5	296	135.2	376	175.8	456	218.5
58	24.8	138	60.4	218	97.5	298	136.2	378	176.8	458	219.6
60	25.6	140	61.3	220	98.4	300	137.2	380	177.9	460	220.7
62	26.5	142	62.2	222	99.4	302	138.2	382	178.9	462	221.8
64	27.4	144	63.1	224	100.3	304	139.2	384	180.0	464	222.9
66	28.3	146	64.0	226	101.3	306	140.2	386	181.0	466	224.0
68	29.2	148	65.0	228	102.2	308	141.2	388	182.0	468	225.1
70	30.0	150	65.9	230	103.2	310	142.2	390	183.1	470	226.2
72	30.9	152	66.8	232	104.1	312	143.2	392	184.1	472	227.4
74	31.8	154	67.7	234	105.1	314	144.2	394	185.2	474	228.3
76	32.7	156	68.6	236	106.0	316	145.2	396	186.2	476	229.6
78	33.6	158	69.5	238	107.0	318	146.2	398	187.3	478	230.7
80	34.4	160	70.4	240	108.0	320	147.2	400	188.4	480	231.8
82	35.3	162	71.4	242	108.9	322	148.2	402	189.4	482	232.9
84	36.2	164	72.3	244	109.9	324	149.2	404	190.5	484	234.1
86	37.1	166	73.2	246	110.8	326	150.2	406	191.5	486	235.2
88	38.0	168	74.1	248	111.8	328	151.2	408	192.6	488	236.3

U.S. Bureau of Standards Circular 44.

	Qualitative method to test for presence of Chromate in Turmeric		
Method No.	FSSAI 10.033:2021	**Revision No. & Date**	0.0
Scope	This method is to check the presence of Lead chromate in turmeric powder.		
Caution	a) Concentrated Sulphuric acid is a highly corrosive liquid, which can cause severe burns. Excise caution when handling it b) Always add acid to water while making dilute acid solutions c) Diphenyl carbazide can cause both skin and eye irritation when exposed		
Principle	Chromate reacts with 1,5-diphenylcarbazide (DPC) dye, which, in acidic conditions, forms a purple-colored species. As a result of a redox reaction, Cr VI is reduced to Cr III, and DPC is oxidised to 1, 5-diphenylcarbazone (DPCA). Cr III and DPCA form a purple-colored species with lambda max of 540 nm.		
Apparatus	Test tube		
Reagents	1. Dilute sulphuric acid – 1:7 (v/v) Dilute one volume of concentrated sulphuric acid with seven volumes of distilled water 2. Diphenylcarbazide solution – 0.2% (m/v) in 95% (v/v) ethyl alcohol		
Procedure	1. Ash about 2 g of the ground sample. 2. Dissolve the ash in 4-5 mL of dilute Sulphuric acid in a test tube 3. Add 1 mL of diphenyl carbazide solution.		
Interpretation	The development of a violet color indicates the presence of chromate in the turmeric powder.		
Reference	IS: 3576- 2010, Indian Standard, Specification for Turmeric Whole and Ground.		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India	**Detection of Galbnum, Ammoniacum and other Foreign resins in Asafoetida**		
Method No.	FSSAI 10.034:2021	**Revision No. & Date**	0.0
Scope	This method is to check the presence of galbnum, ammoniacum and other foreign resins in asafoetida.		
Caution	See Material Safety Data Sheets, or equivalent, for each reagent. Bromine, upon contact, causes skin burns and eye damage, is fatal if inhaled. Wear protective gloves/ protective clothing/ eye protection/ face protection Hydrochloric acid is corrosive and can cause severe burns.		
Principle	The detection is based on color reactions.		
Apparatus	100 mL volumetric flask		
Reagents	1. Dilute Hydrochloric acid 2. Sodium hypobromite solution – Prepare fresh by dissolving 20 g sodium hydroxide in 75 mL water, adding 5 mL bromine and making upto 100 mL with water 3. Ferric chloride: 9% aqueous solution		
Method of analysis	**Detection of Galbnum** 1. Place about 2 g of asafoetida (10 g of compounded asafoetida) in a tared extraction thimble and extract with 90% alcohol (v/v) in either a Soxhlet or other suitable extraction apparatus for about 3 h. 2. Add dilute HCl drop wise to 10 mL of alcoholic extract of the sample until a faint turbidity appears. 3. Appearance of a bluish green color in the mixture which fades on standing indicates absence of Galbanum. **Detection of Ammoniacum**: 1. Mix well about 4 g asafoetida (20 g of compounded asafoetida) with 90 mL of distilled water in a mortar. 2. Filter and make up the filtrate to 100 mL. 3. Mix 2 mL of the extract with 5 mL water in a test tube and add 5 mL of hypobromite reagent cautiously down the side of the test tube so as to form a separate layer. 4. Nonappearance of a red color in the mixture shows absence of ammoniacum. **Detection of Foreign resins:** 1. Add a few drops of 9% aqueous ferric chloride solution to 5 mL of alcoholic extract (step 1 of Galbanum detection). 2. Appearance of olive green color in the mixture shows absence of foreign resins. 3. Appearance of blackish precipitate or coloration in the mixture shows absence of foreign resins in compounded asafoetida.		

Interpretation of results	1. Appearance of a bluish green color in the mixture which fades on standing indicates absence of Galbanum. 2. Nonappearance of a red color in the mixture shows absence of Ammoniacum. 3. Appearance of olive green color in the mixture shows absence of foreign resins. 4. Appearance of blackish precipitate or coloration in the mixture shows absence of foreign resins in compounded asafoetida.
Reference	IS :7807 – 1975 (Reaffirmed in 2003), Indian Standard: Methods of Test for Asafoetida
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Test for presence of Colophony resin in Asafoetida		
Method No.	FSSAI 10.035:2021	**Revision No. & Date**	0.0
Scope	The test is applicable for the detection of Colophony resin in Asafoetida and compounded Asafoetida.		
Caution	See Material Safety Data Sheets of benzene and Carbon tetrachloride. 1. Benzene is a carcinogen and highly flammable liquid. Avoid inhaling vapors and skin contact and carry out all reaction in a fume hood. 2. Carbon tetrachloride is a carcinogen. Avoid contact with skin and eyes. Avoid inhalation of vapour or mist. Carry out all reaction in a fume hood. 3. Petroleum ether is a highly flammable liquid. Do not use near open flames. 4. Bromine as a liquid or as a vapour is highly irritating to skin, mucous membranes, eyes and respiratory tract. Being a powerful oxidizing agent, it also constitutes a fire hazard. Exposure even at low concentrations may result in inflammatory reactions in the eyes and respiratory passages. Avoid contact with skin and eyes. Avoid formation of vapors, dusts, mists, and aerosols and use appropriate exhaust ventilation. 5. **Bromine handling areas should be clearly marked and restricted to qualified and trained personnel only**.		
Principle	The test is based on isolation and identification of abietic acid, which is a major constituent of colophony resin. Abietic acid spots are visualised by spraying with Helphen-Hicks reagent.		
Apparatus/ Instruments	1. Glass plates 20 x 10 cm or commercially available pre cast Silica Gel plates 2. TLC chamber		
Material and Reagents	1. Petroleum ether - B.P. 40 – 60 °C 2. Solvent: benzene – methanol (9:1) 3. Spray reagent (Halphen – Hicks Reagent): Carbon tetrachloride and phenol (2:1) 4. Standard abietic acid solution – Dissolve good quality colophony resin in 98% acetic acid and reflux for two h. Filter, cool and allow the crystals to separate out. Recrystallize in 95% alcohol. Dissolve 0.1 g of recrystallized abietic acid in 100 mL of petroleum ether. 1 µL of solution is equivalent to 1 µg of abietic acid. 5. Silica gel 6. Bromine		
Method of analysis	1. Extraction Take about 0.1 g asafoetida or 0.5 g compounded asafetida and mix with 10 mL of petroleum ether for 5 min. Filter and keep filtrate in a stoppered test tube. 2. Preparation of thin layer plates		

	Coat glass plates with a slurry of silica gel in water (1:2) to thickness of 250 µm. Allow to set and activate in an air oven at 100 °C for 1 hr. Store the plates in a desiccator. Alternately commercial precoated plates may be used. 3. Spotting and development: 1. Spot the plate with 10 µL of sample extract, 10 µL of standard abietic acid and 10 µL of sample + abietic acid (co - spotting). 2. Develop the plate in an ascending manner in a TLC chamber. 3. When the solvent front reaches 10 cm remove the plate, dry in air and spray with Halphens-Hicks reagent. 4. Expose the plates to bromine vapor in a saturated bromine chamber. The presence of blue – purple spots at an approximate R_f of 0.75 shows presence of abietic acid/colophony. 4. Sensitivity**:** 5 µg of abietic acid can be detected by TLC.
Interpretation of results	The appearance of blue-purple spots indicates the presence of Colophony resin.
Reference	IS: 7807 – 1975 (Reaffirmed in 2003), Indian Standard: Methods of Test for Asafoetida
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Qualitative detection of Turmeric in Chillies and Coriander		
Method No.	FSSAI 10.036:2021	**Revision No. & Date**	0.0
Scope	This method detects the presence of turmeric in chillies and coriander powder.		
Caution	Concentrated Hydrochloric acid is highly corrosive and can cause severe skin burns. Ammonium hydroxide: Handle with extreme care. Avoid contact with eyes and skin. Eye contact may result in eye burns and temporary loss of sight. If inhaled, mild exposure can cause nose irritation. Handle only inside a fume hood.		
Principle	Boric acid reacts with curcumin (the coloring matter found in turmeric) to form a 2:1 red colored complex, Rosocyanine in acidic solutions.		
Reagents	1. Hydrochloric acid 2. Ethyl alcohol 3. Boric acid 4. Ammonium hydroxide		
Method of analysis	1. Slightly acidify the aqueous or dilute ethanolic extract of the sample with hydrochloric acid and add a few boric acid crystals. 2. A brown red coloration appears in the presence of turmeric.		
Interpretation of results	The appearance of a red colored complex indicates the presence of Turmeric		
Reference	Manual Methods of Analysis for Adulterants and Contaminants in Foods I.C.M.R 1990 page 24		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

	Determination of Oil Soluble Dyes in Capsicum and Turmeric and their products by High Performance Liquid Chromatography		
Method No.	FSSAI 10.037:2021	**Revision No. & Date**	0.0
Scope/	This method is used to determine the presence of oil soluble dyes in capsicum and turmeric and their products using Reverse Phase High Performance Liquid Chromatography (RP-HPLC). It is applicable for the analysis of Sudan and related dyes.		
Caution	Avoid inhalation and contact with acetonitrile and methylene chloride. Methylene chloride: Higher levels of dichloromethane inhalation can lead to headaches, mental confusion, nausea, vomiting, dizziness and fatigue. Redness and irritation may occur if skin comes in contact with liquid dichloromethane. Handle only inside a fume hood. Hexane: Irritating to eyes, respiratory system and skin. Hexane is flammable and harmful. Avoid contact with skin and eyes. Store container in a designated flammable cabinet.		
Apparatus/ Instruments	(i) HPLC equipped with 1. Quartenary pumps for gradient elution 2. PDA/DAD detector, 3. Auto sampler/ Injector 4. Work station with appropriate software 5. Column, Octadecyl (C18), 250 mm x 4.6 mm, i.d, 5 µm Particle size (ii) Analytical Balance readable to 0.0001 g (iii) 50 mL culture tubes with Teflon lined cap (iv) Vortex mixer (v) Wrist action shaker (vi) 5 mL Luer- Lock disposable syringe (vii) Whatman Nylon 0.45 µ filter or equivalent (viii) Silica SPE columns(3 mL) or equivalent, 500 mg load, (ix) SPE Vaccum manifold (x) Evaporator / concentrator (xi) Volumetric pipettes, various sizes (xii) Volumetric flasks, various sizes (xiii) 25 mL graduated cylinder		
Reagents	1) Methylene chloride (HPLC grade) 2) Acetonitrile (HPLC grade) 3) Acetic acid glacial (HPLC grade) 4) Acetone (HPLC grade) 5) Methanol (HPLC grade) 6) Sodium chloride (A.R) 7) Solution A – Prepare a 200 g/L sodium chloride solution 8) Solution B – prepare a 12.5% methanol in sodium chloride solution using solution A		

	9) Ethyl ether (HPLC grade) 10) Hexane (HPLC grade) 11)Dyes standards i. Sudan -1 ii. Sudan –II iii. Sudan – III iv. Sudan –IV v. Sudan Orange G vi. Sudan Red B vii. Dimethyl Yellow viii. Para Red ix. Cis – bixin
Preparation of Calibration standards	**Stock Standard** 1. Prepare a stock standard containing Sudan dyes I-IV, Para Red and Dimethyl yellow dyes by accurately weighing 0.025 g of each dye in a 100 mL volumetric flask. Dissolve the dyes with methylene chloride. This is stock standard A. 2. Prepare a stock standard of Sudan Orange G and Sudan Red B dyes by accurately weighing 0.025 g of each dye into a 100 mL volumetric flask. Dissolve the dye with methylene chloride. This is stock standard B. 3. Prepare a stock standard of cis –bixin by accurately weighing 0.025 g of the dye into a 100 mL volumetric flask. Dissolve the dye with methylene chloride. This is stock standard C. **Note:** Cis- bixin will isomerise to its trans configuration in solution. A new stock cis- bixin standard and working standards must be prepared when this occurs. **Working standard** Prepare four calibration standards from stock standard A and four calibration standards from stock standard B and C in acetonitrile containing the following concentration of the dyes 0.1 µg/mL, 1 µg/mL, 5 µg/mL and 10 µg/mL of each dye. Transfer the calibration standards to autosampler vials or inject on the HPLC instrument. **Note:** (1) Correct each standard weight to pure dye content based on the declared purity of the dye (2) Store all standards in a freezer when not in use (3) Standard should be injected after each 4-6 sample injections (4) After the instrument linearity has been established by running the calibration standard series, then a single point standard calibration can be run with the 1.0 µg/ mL standard. However, the PDA detector must be capable of detecting a 0.10 µg/mL solvent standard.
Chromatographic	(1) Mobile Phase A – 1.0% acetic acid

<table>
<tr><td>conditions</td><td>(2) Mobile Phase B – 100% Acetonitrile
(3) Mobile Phase C - acetone
(4) Gradient time programme
<table><tr><th>Time (Min)</th><th>Mobile phase A
%</th><th>Mobile Phase B
%</th><th>Mobile Phase C
%</th></tr><tr><td>0</td><td>30</td><td>70</td><td>0</td></tr><tr><td>13.3</td><td>5</td><td>95</td><td>0</td></tr><tr><td>20.0</td><td>0</td><td>100</td><td>0</td></tr><tr><td>23.3</td><td>0</td><td>100</td><td>0</td></tr><tr><td>24.0</td><td>0</td><td>0</td><td>100</td></tr><tr><td>28.0</td><td>0</td><td>0</td><td>100</td></tr><tr><td>28.7</td><td>30</td><td>70</td><td>0</td></tr><tr><td>32.7</td><td>30</td><td>70</td><td>0</td></tr></table>a) Flow rate: 1.5 mL/min
b) Detection wavelength: 300 nm to 650 nm
c) Injection volume: 20 µL
d) Column temperature: 25 °C</td></tr>
<tr><td>Preparation of sample control and spikes</td><td>1. Accurately weigh 4.0 – 8.0 g of control sample into 5 separate 50 mL culture tubes. To one of the samples, pipette 10 µL of the stock standard A solution (the concentration of each dye in this spike sample will be between approx 0.3 -0.6 mg/kg).
2. To a second sample pipette 100 µL of stock standard A solution (the conc. of each dye in the sample will be between 3-6 mg / kg).
3. To a third sample pipette10 µL of stock standard B and 10 µL of stock standard C solutions (the conc. of each dye in this spiked sample will be approx 0.3- 0.6 mg/kg.
4. To a fourth sample pipette 100 µL of stock standard B and 100 µL of stock standard C solution (the conc. of each dye in this spiked sample will be between 3-6 mg /kg).
5. Pipette 20 mL of acetonitrile into each tube, cap and shake on a wrist action shaker for 1 h. Allow the solids to settle or centrifuge.
6. Filter through 0.45 µ nylon filter into autosampler vials and inject on the HPLC.
Note: - Spike recovery should be between 75-125% of the calculated amount of each dye spikes should be run with each different sample matrix to identify coeluting or interfering peaks from the sample matrix. For similar sample matrixes, a spiked sample should be run with every 10 sample extracts.</td></tr>
<tr><td>Sample Preparation</td><td>• Weigh 4.0 – 8.0 g of sample in a 50 mL culture tube.
• Pipette 20 mL of acetonitrile into each tube, cap and shake on a wrist action shaker for 1 hr. Allow the solids to settle or centrifuge.
• Filter through a 0.45 µ nylon filter into auto-sampler vials and inject</td></tr>
</table>

	on HPLC instrument. **Note:** - A sample clean up step may be necessary for concentrated or complex products in order to remove some of the compounds that interfere with the chromatographic peaks of interest. To perform this clean up proceed as below. When testing for Sudan Orange G, sample clean up must be performed to eliminate matrix interferences which coelute with the Sudan Orange G peaks in capsicum samples.
Sample and spike sample clean up	i. Pipette 5 mL of acetonitrile into a 50 mL centrifuge tube. ii. Pipette 5 mL of hexane into centrifuge tube. iii. Use a 25 mL graduated cylinder to add 25 mL of Solution B to the centrifuge tube. iv. Shake gently for 30 seconds, then centrifuge for 3 min v. Prepare a silica SPE column for each sample. vi. Initially prewash the silica SPE with one column volume of ethyl ether followed by two column volumes of hexane. vii. Discard the eluted solvent wash. **Note:** - Prewash each silica SPE column prior to use. Keep the silica bed wet with solvent and do not store prewashed SPE columns for more than 30 min Mild vacuum may be applied to SPE columns to pull the solvent through the column pipette 2 mL of hexane (top) layer into solvent washed SPE columns. Drain the sample extract into the column bed at 1-2 drips per second. Wash with one column volume of hexane and discard the hexane wash. Place clean collection tubes below the silica SPE columns and elute the dyes into the collection tubes with two column volumes of 10% acetone in hexane. Evaporate the solvent in the collection tubes to dryness under a stream of dried nitrogen or other inert gas. Redissolve the residue in each collection tube with 2 mL of acetonitrile. Filter through 0.45 µ nylon filter into auto sampler vials and inject on the HPLC instrument.
Processing/ Data Analysis Parameters	Set up a processing/data analysis method to process the calibration standards containing Sudan 1- 4, Para Red and Dimethyl Yellow. Use the following wavelengths: Dimethyl Yellow- 450 nm, Para Red – 450 nm, Sudan I -505nm, Sudan II – 505 nm, Sudan III – 530 nm, Sudan IV – 505 nm (550 nm for paprika oleo resin) Process the corresponding four calibration standards and samples for these six dyes. Set up a processing / data analysis method to process the calibration standards containing Sudan Orange G, cis – bixin and Sudan Red B. Use the following wavelengths Sudan Orange G -377 nm, cis – bixin – 460

	nm, Sudan red B – 505 nm (550 nm for paprika oleoresin) Process the corresponding four calibration standards and samples for these three dyes.
Calculation with units of expression	Using the data processing technique perform a linear regression analysis for each dye to determine the slope m of the dye's calibration curve. Force the line through the origin. Let the peak area be the y – variable and the concentration be the x – variable. Calculate the concentration of the dye in the samples with the following formula: $$Cx = \frac{Ax \times 20}{m \times W}$$ Where, C x = concentration of the dye (x) found µg/g (mg/kg) A x = peak area of dye (x) in the sample m = slope of the calibration curve for dye (x) 20 = sample extraction volume in mL W = sample mass in g
Reference	1. ASTA Analytical Method 28, 0/ European commission News notification 03 / 99 / Chinese National Quality Assurance and Inspection Bureau GB / t 19681 – 2005 2. FSSAI Manual of Methods- analysis of Foods: Food Additives
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Method of Measuring Color value in Chilliest and Paprika Oleoresin		
Method No.	FSSAI 10.038:2021	**Revision No. & Date**	0.0
Scope	This method is applicable for determination of color value in ground and crushed red pepper, chilli pepper, ground cayenne pepper, ground jalapeno pepper, and red pepper oleoresins. Not applicable to those chilli samples containing oregano or thyme.		
Caution	*N*-vanillyl-*n*-nonanamide is an extreme irritant, do not inhale. Dispose of waste solvents in an appropriate manner compatible with applicable environmental rules and regulations.		
Principle	Test sample is extracted in warm ethanol using reflux condenser. Extract is filtered and injected into liquid chromatography equipped with UV or fluorescence detector.		
Apparatus/ Instruments	1. High Performance Liquid Chromatography (HPLC) system equipped– • 20 µL sample injector, • UV detector set at 280 nm or fluorescence detector with excitation 280 nm and emission 325 nm. • Column- Octadecyl (C18), 150 x 4.6 mm i.d., packed with 5 µm particle size. And guard column, if desired. 2. Reflux condenser 3. Syringe filter- 0.45 µm 4. Solid-phase extraction cartridge (C18)		
Material and Reagents	1. Ethanol- 95% or denatured, suitable for chromatography 2. Acetone- ACS grade 3. Acetonitrile (HPLC Grade) 4. Mobile phase (40% Acetonitrile): Mix 400 mL acetonitrile with 600 mL H_2O containing 1% acetic acid (v/v). De-gas by suitable technique. 5. *N*-vanillyl-*n*-nonanamide standard, 99% pure, Keep solutions out of direct sunlight.		
Preparation of standard solutions	1. Standard solution A(0.15 mg/mL): Accurately weigh 75 mg *N*-vanillyl-*n*-nonanamide and transfer it into 500 mL volumetric flask. Dilute to volume with ethanol, and mix. Use standard solution A for analyzing all peppers except Chilli pepper. 2. Standard solution B (0.015 mg/mL): Transfer 10 mL standard solution A into 100 mL volumetric flask, dilute to volume with ethanol, and mix. Use standard solution B when analyzing chilli peppers		
Sample preparation	**(a) Ground or crushed peppers** 1. Accurately weigh ca 25 g of ground/crushed pepper into 500 mL boiling flask. 2. Place 200 mL ethanol into same flask, add several glass beads, and attach flask to reflux condenser. 3. Gently reflux test sample 5 h and then allow to cool.		

4. Filter 1–4 mL sample through 0.45 µm syringe filter into small glass vial.
5. Use for HPLC analysis.

(b) Red pepper oleoresin

1. Accurately weigh 1–2 g oleoresin into 50 mL volumetric flask.
2. Increase weight of sample, if total capsaicinoid concentration is <1%.

Note: Do not allow any oleoresin to coat sides of flask.

3. Add 5 mL acetone to flask and swirl contents of flask until test sample is completely dispersed (no oleoresin can coat bottom of flask when turning flask neck at 45° angle).
4. Add five 3–5 mL portions ethanol, swirling flask during each addition.
5. Dilute contents of flask to volume with ethanol and mix well.

(c) Solid phase clean-up

1. Hold C_{18} solid-phase extraction cartridge over 25 mL volumetric flask or place cartridge on 10 mL glass syringe and hold over 25 mL volumetric flask.
2. Transfer 5 mL solution from flask to cartridge or syringe. (Note: When using syringe, deliver solution to bottom of syringe so that sides of syringe are not coated with sample.)
3. Pass aliquot through cartridge and collect in 25 mL flask.
4. Wash cartridge 3 times with 5 mL ethanol, collecting washings in same flask. Dilute contents of flask to volume with ethanol and mix.
5. Filter 1–4 mL solution through 0.45 µm syringe filter into small glass vial.
6. Use for HPLC analysis.

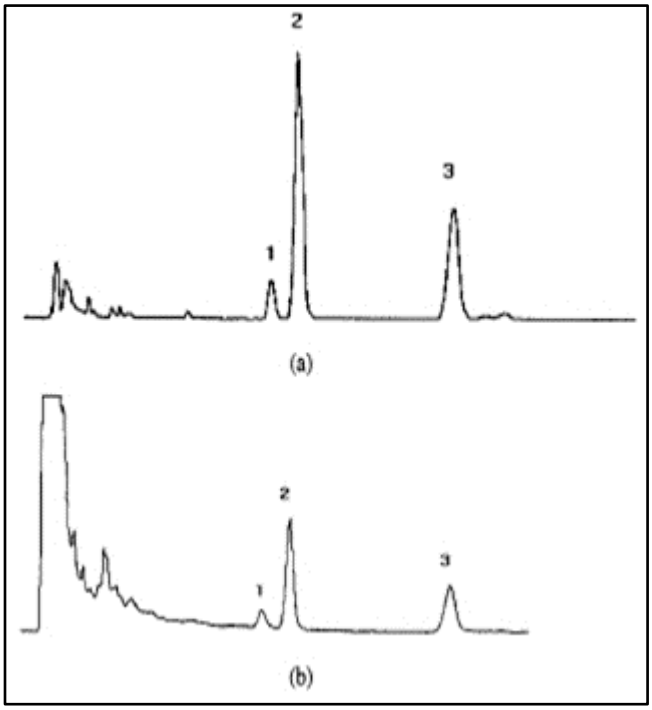
Table 995.03 Method Performance for Determination of Capsaicinoids in Capsicums and Their Extractives by Liquid Chromatography (SHU)

Sample	Mean	N[a]	s_r	s_R	RSD_r, %	RSD_R, %	r[b]	R[c]
Chili peper	900-2750	9	40-80	100-220	3.5-4.5	9.6-11.6	110-220	280-620
Red pepper	30140-41600	11	370-840	1600-1860	1.0-2.3	4.5-5.2	1040-2350	4480-5210
Red pepper oleoresins	305690-644060	13	6770-86870	23100-86870	2.2-14.8	7.6-14.8	18960-243200	64680-243240

[a] N = No. of laboratories.

[b] $r = 2.8 \times s_r$.

Chromatography conditions	**Operating conditions**: 1. Injection volume: 20 µL 2. Temperature, ambient (20–25 °C); 3. Flow rate, 1.5 mL/min., 4. Isocratic elution; 5. Detector set at 280 nm

	Relative retention times: N-vanillyl-n-nonanamide, 1.00 min; Nordihydrocapsaicin, 0.90; Capsaicin, 1.00; Dihydrocapsaicin, 1.58. Typical baseline separation of major capsaicinoids as below:  **Typical chromatogram of Red pepper extract analyzed by (a)fluorescence detection, and (b) UV detection. Peak 1 = nordihydrocapaicin; peak 2 = capsaicin; Peak 3 = dihydrocapsaicin** Note Inject 20 µL standard solution Bonto LC column, when analyzing chili peppers. When analyzing other matrices inject 20 µL standard solution A, Re-inject standard solution at intervals of 6 sample injections, or less. Inject in duplicate 20 µL test sample After ≤ 30 sample injections, purge LC column 30 min with 100% acetonitrile at 1.5 mL/min flow rate and the re-equilibrate with 40% Acetonitrile.
Calculation with units of expression	Capsaicinoids contain 3 major compounds: nordihydrocapsaicin (N), capsaicin (C), and dihydrocapsaicin (D). Capsaicinoids =N + C + D **(a)UV detection:** *Ground peppers and chili pepper:* $N = (P_N/P_S) \times (C_S/W_T) \times (200/0.98) \times 9300$ $C = (P_C/P_S) \times (C_S/W_T) \times (200/0.89) \times 16100$ $D = (P_D/P_S) \times (C_S/W_T) \times (200/0.93) \times 16100$ *(2) Red pepper oleoresin*

	$N = (P_N/P_S) \times (C_S/W_T) \times (250/0.98) \times 9300$ $C = (P_C/P_S) \times (C_S/W_T) \times (250/0.89) \times 16100$ $D = (P_D/P_S) \times (C_S/W_T) \times (250/0.93) \times 16100$ **(b) Fluorescence detection:** (*1*) *Ground peppers and chili pepper* $N = (P_N/P_S) \times (C_S/W_T) \times (200/0.92) \times 9300$ $C = (P_C/P_S) \times (C_S/W_T) \times (200/0.88) \times 16100$ $D = (P_D/P_S) \times (C_S/W_T) \times (200/0.93) \times 16100$ (*2*) *Red pepper oleoresins:* $N = (P_N/P_S) \times (C_S/W_T) \times (250/0.92) \times 9300$ $C = (P_C/P_S) \times (C_S/W_T) \times (250/0.88) \times 16100$ $D = (P_D/P_S) \times (C_S/W_T) \times (250/0.93) \times 16100$ Where P_N, P_C, and P_D = average peak areas for nordihydrocapsaicin, capsaicin, and dihydrocapsaicin, respectively, from duplicate injections; P_S = average peak area of appropriate standard solution; C_S =Concentration of standard solution, mg/mL; W_T = mass of test sample, g
Reference	AOAC Official Method 995.03 Capsaicinoids in Capsicums and Their Extractives -Liquid Chromatographic Method AOAC Int.79**,** 738(1996)
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Microscopic examination of spices		
Method No.	FSSAI 10.039:2021	**Revision No. & Date**	0.0
Scope	Applicable for examination of all spices.		
Caution	Hydrochloric acid: Handle with extreme care. Concentrated HCl is corrosive. Avoid breathing vapors and avoid contact with skin and eyes. Handle only inside a fume hood.		
Principle	The spice is boiled with Chloral hydrate to make them transparent. Chloral hydrate has two-fold action: (1) it removes starch thereby concentrating other tissues and (2) it removes coloring matter from the tissues so that the outlines of the can be seen much more clearly. Phloroglucinol is used to stain Sclerenchymatous matter.		
Apparatus/ Instrument	Microscope		
Reagents	1. Ethanol 2. Iodine solution 3. Glycerol 4. Hydrochloric acid 5. Chloral hydrate 6. Phloroglucinol 7. Phloroglucinol solution (1% in 90% alcohol)		
Method of analysis	1. Preparation of water slide i. Prepare a water slide by dissolving finely powdered sample with a drop of alcohol and then adding one or two drops of glycerol solution (30% in water) before sliding on the cover slip. ii. The water slide is particularly suitable for detecting starch. iii. The presence of starch can be confirmed by adding a drop of very dilute solution of iodine which produces the usual dark blue color. iv. Some spices namely cumin, coriander, chillies and cloves do not contain true starch and the presence of extraneous starch can be easily detected in these powdered spices. 2. Preparation of cleared slide i. Gently boil the spice powder with chloral hydrate solution (prepared by warming 80 g of crystals in 50 mL water) in a tube until the particles look fairly transparent. ii. Sclerenchymatous matter can be stained red by warming the cleared material with excess of phloroglucinol solution (1% in 90 % alcohol) followed by a drop of conc. hydrochloric acid. iii. Examine under microscope		
Reference	Pearson's Composition and Analysis of Foods 9th edn 1991 page 394		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

	Peroxidase test in Dehydrated Garlic and Onion		
Method No.	FSSAI 10.040:2021	**Revision No. & Date**	0.0
Scope	This method tests the presence of peroxidase in dehydrated fruits and vegetables.		
Caution	Hydrogen peroxide is a strong oxidizer and a corrosive liquid. Safety glasses with side shields, face shield, or chemical splash goggles shall be worn. A chemical-resistant laboratory coat should be worn when working with hydrogen peroxide		
Principle	Peroxidase catalyzes the oxidation of guaiacol (colorless) in the presence of hydrogen peroxide to form tetraguaiacol (yellow brown) and water. Tetraguaiacol has an absorbance maximum around 450 nm and is reddish brown in color.		
Reagents	1. White porcelain dish 2. Ethyl alcohol 3. Hydrogen peroxide solution (3% solution) 4. Guaiacol 5. Guaiacol solution – 1% prepared by dissolving 1 g of 0.9 mL guaiacol in 50 mL ethyl alcohol and adding 50 mL water 6. Hydrogen peroxide – 1%. Dilute 1 part of 3% hydrogen peroxide with 2 parts of water		
Method of analysis	1. Take 25 g of the material and coarsely powder it. 2. Place 5 g on a white porcelain saucer or evaporating dish. 3. Add enough guaiacol solution to wet all the cut surfaces, then immediately add a similar amount of hydrogen peroxide solution. 4. At the end of three min note whether a reddish brown color has developed. 5. If none is observed the test is negative. 6. Neglect any color that may be developed after 3 min.		
Interpretation of results	Formation of a reddish-brown color indicates the presence of peroxidase activity.		
Reference	ISI Handbook of Food Analysis (Part VIII)1984, page 13		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

	Method for estimation of Coumarin content in Cinnamon		
Method No.	FSSAI 10.041:2021	**Revision No. & Date**	0.0
Scope	To estimate the coumarin content in cinnamon and cassia by HPLC.		
Caution	Coumarin is harmful. Handle with care.		
Principle	Coumarin is extracted and separated by HPLC. Using a calibration curve of pure coumarin the coumarin content of Cinnamon is calculated.		
Apparatus/ Instruments	1. Measuring cylinders, 50 mL, 100 mL capacity 2. Conical flask, 250 mL capacity 3. HPLC system equipped with (a) Injector/autosampler (b) Binary pumps (c) UV-Detector (d) Column: Octadecyl column (C18) 250 mm × 4.6 mm i.d. 5 µm particle size 4. Micro litre syringe capable of injecting 1-20 µL 5. Analytical Balance, readable to 0.001 g 6. Whatman No. 1 filter paper (90 mm)/ syringe filter 0.45 µm 7. Sample powdering mill or equivalent		
Material and Reagents	1. Methanol HPLC grade 2. Acetonitrile HPLC grade 3. Water HPLC grade 4. Acetic and HPLC grade 5. Ammonium acetate 6. Coumarin standard (>90%) 7. Mobile phase A: Water, 5mM ammonium acetate buffer with 0.2% (v/v) acetic acid. 8. Mobile phase B: Acetonitrile, methanol 1: 2 (v/v).		
Preparation of standard solution	1. Weigh accurately 0.1 g of the Coumarin standard and dissolve and make upto 100 mL with HPLC methanol. 2. Keep this solution as stock solution (1000 ppm) in standard flask wrapped in black cover. Shelf life is one year under refrigeration. 3. Working standard 10 ppm - From the stock solution pipette 1 mL to the 100 mL standard flask and make up to the mark with HPLC methanol. 4. Keep under refrigeration in standard flask wrapped in black cover. Shelf life is six months under refrigeration.		
Sample Preparation	Whole cassia & cinnamon: Finely grind 100 g of the sample and pass through the sieve ASTM No. 20. (850 µm) Ground cassia &cinnamon: Take a subsample of 100 g by mixing and quartering of the entire sample		
Extraction	1. Weight accurately 1.0 g of the above sample in duplicate into 250 mL conical flask. 2. Add 50 mL 90% (v/v) methanol using calibrated measuring cylinder.		

<table>
<tr><td></td><td>3. Shake for 30 min.
4. Filter 3 to 4 mL through Whatman no.1 filter paper or 0.45 µm syringe filter into stoppered test tubes.</td></tr>
<tr><td>HPLC conditions</td><td>Instrumentation conditions
(a) Set the UV detector at 275 nm
(b) Flow rate: 0.8 mL/min.
(c) Injection volume: 20 µL
(d) The gradient program is as follows.
<table><tr><td>Time (min)</td><td>%B</td></tr><tr><td>Start</td><td>0</td></tr><tr><td>14</td><td>22</td></tr><tr><td>16</td><td>70</td></tr><tr><td>22</td><td>70</td></tr><tr><td>25</td><td>30</td></tr><tr><td>30</td><td>Syop</td></tr></table></td></tr>
<tr><td>Calculation with units of expression</td><td>Construct a calibration curve with a range of standard (10, 20, 40 and 100 µg mL^{-1}. From the slope calculate the concentration of sample
Coumarin content (mg/Kg) $=\frac{X(\mu g)\times 50}{V\ (mL)\times W}$
Where
X= µg of Coumarin from calibration curve
V= volume of sample injected in mL
W= mass of sample in g
Report coumarin content to an accuracy of 0.0 mg/kg.</td></tr>
<tr><td>Reference</td><td>Evaluation of coumarin content and essential oil constituents in Cinnamomum cassia (Nees & T.Nees), (2019) J. Presl.A J Jose, N K Leela, T J Zachariah & J Rema, Journal of Spices and Aromatic Crops, 28 43–51.</td></tr>
<tr><td>Approved by</td><td>Scientific Panel on Methods of Sampling and Analysis</td></tr>
</table>

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India	**Method for estimation of Eugenol content in Clove extract by HPLC method**		
Method No.	FSSAI 10.042:2021	**Revision No. & Date**	0.0
Scope	To estimate the eugenol content in clove for evaluating quality and assurance of clove.		
Principle	Eugenol is extracted and separated by RP-HPLC. Using a calibration curve of pure eugenol, the eugenol content of cloves and other products is calculated.		
Apparatus/ Instruments	1. Measuring cylinders, 50 mL, 100 mL capacity 2. Conical flask, 250 mL capacity 3. HPLC system equipped with (a) Injector/autosampler (b) Binary pumps (c) UV/PDA/DAD-Detector (d) Column oven (e) Column: Octadecyl column (C18) 250 mm × 4.6 mm i.d. 5 µm particle size 4. Microlitre syringe capable of injecting 1-20 µL 5. Balance, readable to 0.001 g 6. Whatman No. 1 filter paper (90 mm)/ syringe filter 0.45 µm 7. Sample powdering mill or equivalent 8. Sieve ASTM No. 20. (850 µm)		
Material and Reagents	1. Methanol HPLC grade 2. Water HPLC grade 3. Ethanol HPLC grade 4. Eugenol standard (99%) 5. Mobile phase: Methanol: Water (60:40).		
Preparation of standard solutions	1. Weigh accurately 0.1 g of the above pure eugenol and dissolve and make upto 100 mL with HPLC grade methanol. 2. Keep this solution as stock solution 1mg/mL (1000 ppm) in standard flask wrapped in black cover. Shelf life is one year under refrigeration. 3. Working standard 10 µg/mL (10 ppm) - From the stock solution pipette 1 mL to the 100 mL standard flask and make up to the mark with HPLC methanol. 4. Keep under refrigeration in standard flask wrapped in black cover. Shelf life is six months under refrigeration.		
Sample preparation	1. Whole clove to be maintained in dark at 25 °C. 2. Grind to an optimum particle size using a commercial grinder and should pass through the sieve ASTM No. 20. (850 µm) 3. Reflux 0.3 g of ground clove 30 mL of 95% ethanol for 2 h using a reflux system. 4. Evaporated to dryness the extract and re-dissolve in 2 mL 95% ethanol. 5. Filter through a PTFE syringe filter (13mm, 1.45µm) 6. Inject the filtrate		

HPLC Conditions	(a) Set the detector at 280 nm (b) Column: Octadecyl (C18) column, 250 ×4.6 mm i.d. and Particle size. (c) Mobile phase methanol:water (60:40 v/v) (d) Flow rate: 0.8 mL/min, Isocratic elution (e) Column temperature: 30 °C (f) Volume for injection: 20 µL (g) Eugenol elutes within 10 min
Calculation	Quantification is carried out using a calibration curve and peak are measurements. External standard method is to be used to obtain the regression equation of the eugenol. Working range of eugenol to be used is 12.5-1000 ng/mL. The correlation coefficient of the equation should be > 0.99.
Reference	Yun et al., (2010) Journal of AOAC international, 93(6):1806-10
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Method for estimation of Eugenol content in Clove Extract by Gas Chromatography		
Method No.	FSSAI 10.043:2021	**Revision No. & Date**	0.0
Scope	To estimate the eugenol content in clove extract by GC for evaluating quality of clove.		
Caution	None		
Principle	Eugenol is extracted from the sample and estimated using gas chromatography with flame ionization detector		
Apparatus	1. Volumetric flasks with 50 mL, 100 mL capacity 2. GC system with accessories as mentioned under Instrument conditions 3. Micro litre syringe capable of injecting 1-20 µL 4. Balance, readable to 0.001 g 5. Sample powdering mill or equivalent 6. Centrifuge 7. Sonicator		
Chemicals	1. Methanol HPLC grade 2. Eugenol Standard (99%) 3. Magnesium sulfate		
Standard stock solution	1. Weigh accurately 0.100 g of eugenol standard, dissolve and make upto 100 mL with HPLC Methanol. 2. Keep this solution as stock solution (1000 ppm) in standard flask wrapped in black cover. Shelf life is one year under refrigeration. 3. Prepare Working standard by diluting the stock with HPLC Methanol. 4. Keep under refrigeration in standard flask wrapped in black cover. Shelf life is six months under refrigeration.		
Sample Preparation	Whole clove to be maintained in dark at 25 °C. An optimum particle size (850 µm) to be achieved by grinding in commercial grinder using the sieve ASTM No. 20.		
Extraction	1. One gram of homogenized sample is mixed with 20 mL of methanol. The mixture was sonicated for 10 min at room temperature. 2. After the addition of 1 g of $MgSO_4$, extraction by sonication was allowed to continue for 10 min. 3. Centrifuge at 3000 rpm for 5 min. 4. Filter the supernatant through a 0.45 µm PVDF syringe filter prior to GC analysis. 5. Perform all measurements in triplicate		

<table>
<tr><td>GC-FID conditions</td><td>GC coupled with a flame ionization detector.
Fused silica Polyethylene Glycol capillary GC-Column HP-INNOWAX (60 m × 0.32 mm × 0.5 µm) or equivalent.

<table>
<tr><td colspan="2">GC-FID Analysis parameter</td></tr>
<tr><td>Parameter</td><td>Condition</td></tr>
<tr><td>Injection volume</td><td>1 µL</td></tr>
<tr><td>Inlets condition</td><td>Heater 250 °C, split ratio 1/25</td></tr>
<tr><td>Column</td><td>HP-INNOWAX (60 m × 0.32 mm × 0.5 µm) or equivalent</td></tr>
<tr><td>Carrier gas</td><td>N_2</td></tr>
<tr><td>Flow</td><td>1.2 mL/min</td></tr>
</table>

<table>
<tr><td colspan="3">Oven Programme</td></tr>
<tr><td>°C/min</td><td>Next °C</td><td>Hold min</td></tr>
<tr><td></td><td>50</td><td>1</td></tr>
<tr><td>10</td><td>240</td><td>30</td></tr>
<tr><td>Run Time min</td><td></td><td>50</td></tr>
</table>

<table>
<tr><td colspan="2">Detector</td></tr>
<tr><td>Heater (°C)</td><td>250</td></tr>
<tr><td>H_2 Flow (mL/min)</td><td>35</td></tr>
<tr><td>Air Flow (mL/min)</td><td>350</td></tr>
<tr><td>Makeup Flow N_2</td><td>30</td></tr>
</table>
</td></tr>
<tr><td>Calculation</td><td>Using various concentrations obtain the regression equation of the eugenol. The correlation coefficient of the equation should be above 0.99. From the external standard equation calculate the content of eugenol in unknown sample and express as percentage by weight of ground clove.</td></tr>
<tr><td>Reference</td><td>Jin Lee et.al (2020) Development of a Gas Chromatography-Flame Ionization Method for the Detection and Quantification of 12 Flavoring Agents in Supplementary Feed, J. AOAC. International, 103, 710–714.</td></tr>
<tr><td>Approved by</td><td>Scientific Panel on Methods of Sampling and Analysis</td></tr>
</table>

	Determination of Gingerols in Ginger and Oleoresins by High Performance Liquid Chromatography		
Method No.	FSSAI 10.044:2021	**Revision No. & Date**	0.0
Scope	The method for the determination of gingerols and shogaols is applicable to ginger and oleoresins.		
Caution	None		
Principle	The gingerols are extracted using methanol and then separated using an octadecyl column with detection at 280 nm by using RP C18 column: length 10-25 cm 4-5 mm i.d. particle size 5 µm.		
Material and Reagents	**Reference substance:** N-vanillylnonanamide (>99% purity, Sigma). It has a retention time comparable to that of 6-gingerol. *Caution: This product should be handled with care as it is strongly irritating.* • HPLC grade water • Methanol (HPLC grade) • Acetonitrile (HPLC grade) • Acetic acid (HPLC grade) • Mobile phase: Acetonitrile: water containing 1% acetic acid (65:35)		
Apparatus/ Instruments	1. Volumetric flasks (10mL, 25 mL, 50 mL and 100 mL capacity) 2. Round bottom flasks-100 mL 3. Filtration diaphrams, non-adsorbing. 4. Suitable High performance liquid chromatography system with a. UV/PDA detector b. Degassing system, for solvents c. Injection system**:** Injection valve with 20 µL loop or any other system giving the same injection accuracy/ auto-sampler d. Suitable detector system, adjustable to a wavelength of 280 nm. e. Recorder or integrator, the performance of which is compatible with the apparatus as a whole. 5. RP C18 column: length 10-25 cm 4-5 mm i.d. particle size 5 µm		
Sample preparation	*i) Dried ginger* 1. Weigh 1.000 g of dry ginger powder 100 mL standard flask and made up the volume with HPLC grade methanol. 2. Shake for 2 h and leave standing overnight. 3. Without disturbing the solution, pipette 20 mL of the supernatant a 50 mL round-bottom flask 4. Concentrate at 40°C using a rotary evaporator. 5. Suspend the residue in HPLC methanol, transferred to a 5 mL standard flask and made up the volume with methanol and shaken well (40mg/mL). 6. Filter one mL of the extract is filtered using 0.2 µm syringe filter. 7. Inject 20 µL of this solution is injected in to HPLC		

	ii) Oleoresin Weigh 0.5 g of sample nearest to 0.001 g into 100 mL volumetric flask and note the exact weight. Dilute to volume with HPLC methanol and shake well and note the concentration in mg/mL.
HPLC conditions	All solutions are degassed and filtered through a 0.45 µm pore size filter. Flow rate: 1 mL/minute Run time: 20 minutes Detector wavelength: 280 nm Mobile phase: Acetonitrile: water containing 1% acetic acid (65:35) Elution: Isocratic Quantitation of gingerols is achieved after comparison with a calibration curve of authentic N-vanillylnonanamide (NVA).
Calculation of Response factor, K	*Determination of response factor for NVA* Standard stock solution (1mg /mL; 1000ppm) of NVA: Dissolve 100 mg of NVA in 100 mL of methanol. Pipette out 1 mL, 2 mL, 4 mL, 6 mL, and 8 mL of stock solutions in to 10 mL volumetric flasks and make up to the mark with methanol to yield 0.1mg/mL, 0.2mg/mL, 0.4mg/mL, 0.6mg/mL, 0.8mg/mL concentration. Inject 20 µL of each of the solution into HPLC The response factor of NVA is calculated as : $K_{NVA} = \frac{C_{NVA} \times 100}{A_{NVA}}$ mg/100mL/unit area where, C_{NVA} = Concentration of NVA in mg/mL and A_{NVA}= Mean peak area of NVA (For linear response, the value of K_{NVA} calculated for the two concentrations should not differ by more than 2%) Calculate the values of for gingerols and shogaols as indicated below: $K_{6G} = K_{NVA} \times \frac{\text{Mol.wt of 6G (294.38)}}{\text{Mol.wt. of NVA (293.41)}} = K_{NVA} \times 1.003$ mg/100mL/unit area Similarly, $K_{8G} = K_{NVA} \times 1.009$mg/100mL /unit area $K_{10G} = K_{NVA} \times 1.194$ mg/100mL /unit area $K_{6S} = K_{NVA} \times 0.942$ mg/100mL /unit area $K_{8}s = K_{NVA} \times 1.037$ mg/100mL /unit area $K_{10S} = K_{NVA} \times 1.133$ mg/100mL /unit area
Analysis of sample solutions	*i)Dried ginger :* Inject 20 µL of sample and record peak areas corresponding to each gingerols and shogaols *ii) Ginger oleoresin:* Inject 20 µL of each solution keeping a longer run time of 40 minutes and record peak areas corresponding to each gingerols and shogaols

	Calculation % 6-Gingerol $=\frac{A_{6G} x K_{6G}}{C}$ % Where A_{6G} = Area under the peak corresponding to 6-gingerol from chromatogram and C= Concentration of sample (in this case 40mg/mL). Similarly the percentage of other gingerols and shogaols are calculated.
Reference	ISO/DIS13685:1995 Ginger and its oleoresins - Determination of the main pungent components (gingerols and shogaols) – method using high performance Liquid Chromatography (HPLC)
Approved by	Scientific Panel on Methods of Sampling and Analysis

RAPID ANALYTICAL FOOD TESTING (RAFT) KIT/ EQUIPMENT

Alternate Rapid kits/equipments may be used to get quick results for screening and surveillance purposes, provided the kit/equipment is approved by FSSA(I). Details of the rapid food testing kit/ equipment approved by FSSA(I) are available at https://www.fssai.gov.in/cms/raft.php

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