MANUAL OF METHODS

OF

ANALYSIS OF FOODS

BEVERAGES: TEA, COFFEE, CHICORY

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Note: The test methods given in the manual are standardized / validated and were 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.

	Determination of Moisture		
Method No.	FSSAI 04A.001:2021	**Revision No. & Date**	0.0
Scope	This method is applicable for Tea, Kangra Tea, Green Tea, Instant Tea, Coffee, Soluble Coffee Powder, Decaffeinated roasted and ground coffee, Decaffeinated soluble coffee powder, Chicory and coffee – chicory mixture Form and Decaffeinated coffee – chicory mixture		
Caution	Once sample is opened, seal it in airtight manner after taking test portion		
Principle	Moisture is the weight lost due to evaporation of water present in a sample. The sample is dried under controlled conditions to remove moisture during the analysis. To determine moisture content, the difference in sample weight before and after drying is calculated.		
Apparatus/Instrument	1. Aluminium dish (About 7.5 cm in dia and 2.5 cm deep) 2. Air Oven 3. Desiccator 4. Stop Clock 5. Weighing Balance		
Materials and Reagents	Desiccants (for Desiccators)		
Sample Preparation	Grind the sample in a grinder to pass through No. 30 mesh sieve. Mix well to get a homogenous sample. Store sample in a tightly stoppered bottle, withdraw portions for analytical determinations.		
Method of analysis	1. Weigh accurately about 5 g of sample in a pre-weighed aluminium dish. 2. Dry the sample in an air oven at 100 ±2 °C for 5 to 6 h. 3. Cool in a desiccator and weigh. 4. Dry again for 30 min, cool in a desiccator and weigh. 5. Repeat the process of heating and cooling in a Desiccator until the difference in two successive weighings is less than 1 mg. 6. Record the lowest weight. Carry out the analysis in duplicate.		
Calculation with units of expression	Moisture (%) (by weight) Moisture % (M) $= \frac{W_1 - W_2}{W1 - W} \times 100$ Where, W = Weight in g, of empty Aluminium dish W_1 = Weight in g, of empty Aluminium dish + sample before drying W_2 = Weight in g, of empty Aluminium dish + dried sample		
Reference	• IS: 3077 – 2009 (A Specification for Roasted and Ground Coffee)		

Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Moisture for roasted coffee and chicory mixture - Vacuum Oven method (Reference method)		
Method No.	FSSAI 04A.002:2021	**Revision No. & Date**	0.0
Scope	Roasted coffee, chicory and coffee – chicory mixture		
Caution	Once sample is opened seal it in air tight manner after taking test portion.		
Principle	Moisture is the weight lost due to evaporation of water present in a sample. The sample is dried in a vacuum oven under controlled conditions of pressure and temperature to remove moisture by passing dry air. To determine moisture content, the difference in sample weight before and after drying is calculated		
Apparatus/Instrument	1. Aluminium dish (7 cm diameter and about 3 cm height) with close fitting cover 2. Vacuum oven – connect with pump capable of maintaining partial vacuum in oven with pressure equivalent to 25 mm Hg and provided with thermometer passing into the oven in such a way that the bulb is near the test sample. Concentrated H_2SO_4 gas drying bottle with oven to admit dry air when releasing vacuum 3. Desiccator 4. Stop Clock 5. Weighing Balance		
Materials and Reagents	1. Conc. Sulphuric Acid 2. Desiccants (for Desiccator)		
Preparation of Reagents	NA		
Sample Preparation	Grind the sample in a grinder to pass through No. 30 mesh sieve. Mix well to get a homogenous sample. Store sample in a tightly stoppered bottle, withdraw portions for analytical determinations.		
Method of analysis	1. Accurately weigh about 5 g of sample, in a dish previously dried at 98 –100 °C, cooled in desiccator and weighed with cover soon after attaining room temperature. 2. Place in oven, lean cover against dish and heat to constant weight (about 5.5 hr at 98 – 100°C at pressure equal to 25 mm Hg. 3. During heating allow slow current of air (about two bubbles / second through H_2SO_4) into oven. 4. Carefully admit dry air into oven to bring to atmospheric pressure. 5. Cover dish, transfer to desiccator and weigh soon after room temperature is attained. 6. Repeat the operation until the difference between two successive weighing is less than 1 mg. Record the lowest mass. 7. Report percent loss in weight as moisture.		
Calculation with units of expression	Moisture (%) (by weight) $= \frac{W_1 - W_2}{W1 - W} \times 100$ Where, W = Weight in g, of empty Aluminium dish. W_1 = Weight in g, of empty Aluminium dish + sample before drying. W_2 = Weight in g, of empty Aluminium dish + dried sample.		

Reference	A.O.A.C 21st edn, Official Method of Analysis(2019) Method no. 968.11 Moisture (Loss on Drying in Roasted Coffee, Vacuum Oven method 1		
Approved by	Scientific Panel on Methods of Sampling and Analysis		
fssai FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA *Inspiring Trust, Assuring Safe & Nutritious Food*	**Determination Of Moisture For Soluble (Instant) Coffee Powder - Vacuum Oven Method (Reference Method)**		
Method No.	FSSAI 04A.003:2021	**Revision No. & Date**	0.0
Scope	Soluble (Instant) Coffee powder		
Caution	Once sample is opened seal it in air tight manner after taking test portion.		
Principle	Moisture is the weight lost due to evaporation of water present in a sample. The sample is dried in a vacuum oven under controlled conditions of pressure and temperature to remove moisture by passing dry air. To determine moisture content, the difference in sample weight before and after drying is calculated		
Apparatus/Instrument	General Apparatus and Glassware 1. Aluminium dish 7 cm diameter and about 3 cm height with close fitting cover. 2. Vacuum oven – connected with pump capable of maintaining partial vacuum in oven with pressure equivalent to 25 mm Hg and provided with thermometer passing into the oven in such a way that the bulb is near the test sample. Connect H_2SO_4 gas drying bottle with oven to admit dry air when releasing vacuum 3. Desiccator. 4. Stop Clock. 5. Weighing Balance		
Materials and Reagents	1. Conc. Sulphuric acid 2. Desiccants (for desiccator)		
Sample Preparation	Grind the sample in a grinder to pass through No. 30 mesh sieve. Mix well to get a homogenous sample. Store sample in a tightly stoppered bottle, withdraw portions for analytical determinations.		
Method of analysis	1. Accurately weigh about 5 g of sample in a dish, previously dried at 98 –100 °C, cooled in desiccator and weighed with cover soon after attaining room temperature. 2. Place in an oven, lean cover against dish and heat to constant weight (about 16 h) at 70 ± 1°C at pressure equal to 37.5 mm Hg. 3. During heating, admit slow current of air (about one bubble / second through H_2SO_4) into oven. 4. Carefully admit dry air into oven to bring to atmospheric pressure. 5. Cover dish, transfer to desiccator and weigh soon after room temperature is attained. 6. Repeat the operation until the difference between two successive weighing is less than 1 mg. Record the lowest mass. 7. Report % loss in weight as moisture.		
Calculation with units of expression	Moisture (%) = $\frac{(M_1 - M_2)}{(M_1 - M_0)} \times 100$ Where M_0 = Weight of empty dish M_1 = weight of dish + sample before drying M_2 = Weight of dish + sample after drying		
Reference	A.O.A.C 21st edn, Official Method of Analysis (2019) Method no. 979.12 Moisture (Loss on Drying) in Roasted Coffee – applicable to instant coffees.		

Approved by	Scientific Panel on Methods of Sampling and Analysis		
	Determination Of Total Ash		
Method No.	FSSAI 04A.004:2021	**Revision No. & Date**	0.0
Scope	Tea, Kangra Tea, Green Tea, Instant Tea, Coffee, Soluble Coffee Powder, Decaffeinated roasted and ground coffee, Decaffeinated soluble coffee powder, Chicory and coffee – chicory mixture and Decaffeinated coffee – chicory mixture		
Caution	Once sample is opened, seal it in airtight manner after taking test portion Wear heat resistant gloves and face protection while doing analysis		
Principle	Ash is the inorganic residue remaining after destruction of organic matter at a temperature of 550 ± 10 °C. Sample is weighed before and after heat treatment to estimate total ash.		
Apparatus/Instrument	1. Silica / Platinum dish 2. Burner 3. Muffle furnace 4. Desiccator 5. Weighing balance		
Materials and Reagents	1. Desiccants (for Desiccator)		
Sample Preparation	Grind the sample in a grinder to pass through No. 30 mesh sieve. Mix well to get a homogenous sample. Store sample in a tightly stoppered bottle, withdraw portions for analytical determinations.		
Method of analysis	1. Weigh accurately about 5 g of sample in a tarred silica / platinum dish. 2. Char the material carefully on a burner. (Instead of Bunsen burner, hot plate can also be used for charring of samples). 3. Transfer the dish to a muffle furnace. 4. Ash at a temperature of 550 ± 10 °C until the ash is free of Carbon. 5. Heat the dish again at 550 ± 10 °C for 30 min. 6. Cool the dish in a desiccator and weigh. 7. Repeat this process of heating for 30 min, cooling in a desiccator and weighing until the difference between two successive weighing is less than 1 mg. 8. Record the lowest weight. **Note: –** Preserve the dish containing this ash for the determination of acid insoluble ash.		
Calculation with units of expression	Total ash (% on dry weight) = $\frac{(W_2 - W) \times 100 \times 100}{(W_1 - W) \times (100 - M)}$ Where, W_1 = Weight in g of empty Silica dish. + sample W_2 = Weight in g of empty Silica dish + ash W = Weight in g of empty Silica dish M = Moisture % of the sample		
Reference	• I S: 3077 – 2009(A Specification for Roasted and Ground Coffee Appendix F • I S 13854: 1994 (ISO 1575: 1987) Tea – Determination of Total Ash		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

	Determination of Total Ash (Alternate Method for Roasted and Ground Coffee)		
Method No.	FSSAI 04A.005:2021	**Revision No. & Date**	0.0
Scope	Roasted and ground coffee		
Caution	1. Once sample is opened, seal it in airtight manner after taking test portion 2. Wear heat resistant gloves and face protection while doing analysis		
Principle	Ash is the inorganic residue remaining after destruction of organic matter at a temperature of 550 ± 10 °C and sample is weighed before and after ash to estimate total ash.		
Apparatus/Instrument	1. Silica / Platinum dish 2. Muffle furnace (programmable) 3. Desiccator 4. Weighing balance		
Materials and Reagents	1. Desiccants (for desiccator)		
Sample Preparation	Grind the sample in a grinder to pass through No. 30 mesh sieve. Mix well to get a homogenous sample. Store sample in a tightly stoppered bottle, withdraw portions for analytical determinations.		
Method of analysis	1. Use a programmable muffle furnace that allows a gradual increase in temperature to 550 °C. [Charring with a Bunsen burner and inserting the sample into the furnace at 550 °C. The charring technique is often prone to losses and can have superheating of samples as they enter the furnace causing them to 'explode' (not in a dramatic way) and lose sample or contaminate surrounding samples. It's very quick but not as accurate as using a programmable muffle. It's also safer to use a programmable furnace for the analyst- handling crucibles at 550 °C, is prone to risk]. 2. Ash at a temperature of 550 ± 10 °C until the ash is free of Carbon. 3. Heat the dish again at 550 ± 10 °C for 30 min. 4. Cool the dish in a desiccator and weigh. 5. Repeat this process of heating for 30 min, cooling in a desiccator and weighing until the difference between two successive weighing is less than 1 mg. 6. Record the lowest weight.		
Calculation with units of expression	Total ash (% on dry weight) = $\frac{(W_2 - W) \times 100 \times 100}{(W_1 - W) \times (100 - M)}$ Where, W_1 = Weight in g of empty Silica dish + sample W_2 = Weight in g of Silica dish + ash W = Weight in g of empty Silica dish M = Moisture % of the sample		
Reference	• IS: 3077 – 2009 (A Specification for Roasted and Ground Coffee Appendix F		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

	Determination Of Total Ash (Instant Tea In Solid Form)		
Method No.	FSSAI 04A.006:2021	**Revision No. & Date**	0.0
Scope	Instant tea in solid form.		
Caution	Concentrated hydrochloric acid is corrosive, has an irritant vapour and causes burns. Wear mask and gloves during handling		
Principle	Ash is the inorganic residue remaining after destruction of organic matter at a temperature of 550 ± 10 °C and sample is weighed before and after ash to estimate total ash.		
Apparatus/Instrument	1. Dish: approximately 50 ml capacity made of platinum, porcelain or any other material unaffected by the conditions of the test. 2. Furnace: capable of being controlled at 550°C ± 25°C. 3. Hot-plate thermostatically controlled. 4. Desiccator, containing an efficient desiccant.		
Materials and Reagents	Hydrochloric acid, concentrated (Analytical grade).		
Sample Preparation	Thoroughly mix the instant tea sample as received, by shaking or inverting the sealed sample container.		
Method of analysis	1. **Preparation of the dish:** Ensure that the dish is completely clean, and then heat it in the furnace at 550 °C ± 25 °C for at least 30 min. Cool in the desiccator. Alter cooling to room temperature, weigh to the nearest 0,001 g. 2. Weigh about 2 g of the prepared test sample into the prepared dish. Spread the sample evenly over the base of the dish. 3. Add, drop by drop, to the test portion contained in the dish, sufficient (approximately 1 ml) of the concentrated hydrochloric acid solution to wet it completely. 4. Place the dish on the cool hot-plate, set the control to medium and heat for 30 min. Raise the hot-plate temperature to the highest setting in three successive steps, allowing the test portion to heat at each stage for 30 min. keep the test portion at the highest setting until no fuming has occurred for at least 5 min. 5. Place the dish containing the test portion in the furnace at 550°C ± 25°C for 16 h. Remove, leave to cool and add a few drops of water to moisten and disperse the ash. 6. Evaporate-to dryness on the hot-plate as before, and then return to the furnace for a further 30 min. 7. Remove, cool to room temperature in the desiccator and weigh to the nearest 0,001g. Determine the mass of the total ash. NOTE - instant tea ashed under these conditions should give a grey/white ash. 8. Carry out two determinations on the same test sample.		
Calculation with units of expression	The total ash, expressed as a percentage by mass of the sample on a dry basis, is given by the formula $$\frac{m_1}{m_o} \times 100 \times \frac{100}{RS}$$ Where, m_o is the mass, in grams, of the test portion; m_1 is the mass, in grams, of the total ash; RS is the dry matter content, expressed as a percentage by mass, of the test sample. It is equal to 100 minus the moisture content.		
Reference	IS 13860:1993 (ISO 7514:1990): Instant tea in solid form - Determination of total ash.		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

	Determination Of Water Soluble Ash		
Method No.	FSSAI 04A.007:2021	**Revision No. & Date**	0.0
Scope	Roasted coffee, Tea, Kangra Tea, Green Tea, Coffee Roasted /unroasted ground/green, Decaffeinated roasted and ground coffee		
Caution	1. Once sample is opened, seal it in airtight manner after taking test portion 2. Wear gloves and face protection while doing analysis.		
Principle	Water Soluble Ash is the part of the total ash dissolved by water. Difference between Total ash and water in-soluble ash is calculated as water soluble ash.		
Apparatus/Instrument	General Apparatus and Glassware 1. Beakers, 2. Silica dish, 3. Watch glass, 4. Filter Paper (Whatman No. 42 or its equivalent)and 5. Red litmus		
Materials and Reagents	1. Total ash after ashing of sample 2. Distilled water		
Preparation of Reagents	NA		
Sample Preparation	Continue after ashing of sample		
Method of analysis	1. Transfer the total ash with the aid of about 25 mL distilled water into a beaker. 2. Cover with a watch glass and boil for 5 min. 3. Filter through an ash less filter paper (Whatman No. 42 or its equivalent). 4. Collect the filtrate in a 150 mL beaker. 5. Wash the filter paper 4 -5 times with hot water until the filtrate no longer turns red litmus blue and collect the washings in the same beaker. (Note: Reserve the entire filtrate for the determination of alkalinity of soluble ash) 6. Dry the ash less paper with residue in an oven in a silica dish and transfer to muffle furnace and ignite at 550 °C for 2 h. 7. Cool in a desiccator and weigh (W_3). 8. Repeat the process till the difference in two consecutive weighing is less than 1 mg. Record the lowest weight.		
Calculation with units of expression	Water in-soluble ash on dry wt. basis (%) = $\frac{(W_3 - W) \times 100 \times 100}{(W_1 - W) \times (100 - M)}$ Where, W_3 = Weight in g of Silica dish + water insoluble ash. W = weight in g of empty dish. W_1 = weight in g of Silica dish with material. M = Percentage of Moisture Water soluble ash percent by wt = A – B Where, A = Total ash percent by wt B = Water insoluble ash percent by wt Water soluble ash of total ash (Percent by wt) = $\frac{\text{Water soluble ash}}{\text{Total ash}} \times 100$		
Reference	• IS: 3077 – 2009 (A Specification for Roasted and Ground Coffee) • IS 13855: 1993 (ISO 1576:1988) Tea – Determination of Water soluble ash and Water insoluble Ash		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

	Determination Of Ash Insoluble in Dilute Hydrochloric Acid		
Method No.	FSSAI 04A.008:2021	**Revision No. & Date**	0.0
Scope	Tea, Kangra Tea, Green Tea, Instant Tea, Coffee Roasted /unroasted ground/green, Chicory, coffee – chicory mixture, Instant Coffee - Chicory Mixture, Decaffeinated Roasted and Ground coffee-chicory, Decaffeinated Instant coffee-chicory mixture		
Caution	1. Once sample is opened, seal it in airtight manner after taking test portion 2. Concentrated hydrochloric acid is corrosive, has an irritant vapour and causes burns. Wear mask and gloves during analysis		
Principle	The proportion of ash that is not hydrolyzed by acid is known as the acid insoluble ash(silica and oxalates). Acid insoluble ash is evaluated by dissolving total ash in dilute hydrochloric acid (5N)and ignited in muffle furnace @ 550 °C .		
Apparatus/Instrument	General Apparatus and Glassware: 1. Beakers, 2. Silica dish, 3. Watch glass, 4. Filter Paper (Whatman No. 42 or its equivalent) and 5. Red litmus		
Materials and Reagents	1. Total ash after ashing of sample 2. Conc. Hydrochloric acid 3. Distilled water		
Preparation of Reagents	1. Hydrochloric acid (5N) - Hydrochloric acid (10 mL) is dissolved in 25 mL distilled water.		
Sample Preparation	Continue after ashing of the sample.		
Method of analysis	1. Boil the total ash with 25 mL of 5N Hydrochloric acid for 5 min, covering the Silica dish with a watch glass to prevent spattering. 2. Filter through ash less filter paper (Whatman No. 42 or equivalent). 3. Wash the entire residue with hot water (> 85 °C) until the filtrate does not turn blue litmus paper to red. 4. Dry the ash less paper with the residue in silica dish and transfer to muffle furnace and ignite at 550 °C for 2 h. 5. Repeat the process of igniting in the muffle furnace, cooling and weighing at 30 min intervals until the difference in two successive weighing is less than 1 mg. 6. Cool in a desiccator and weigh (W_4).		
Calculation with units of expression	Ash insoluble in dilute HCl (%) (on dry wt.) = $\frac{(W_4 - W) \times 100 \times 100}{(W_1 - W) \times (100 - M)}$ Where, W_4 = weight of empty dish + acid insoluble ash W_1 = weight of dish + sample W = weight of dish M = Percent moisture		
Reference	• IS: 3077 – 2009 A Specification for Roasted and Ground Coffee • IS 13857: 1993 (ISO 1577: 1987) Tea – Determination of Acid insoluble Ash		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

	Determination Of Alkalinity Of Soluble Ash: Coffee		
Method No.	FSSAI 04A.009:2021	**Revision No. & Date**	0.0
Scope	Coffee Roasted /unroasted ground/green, Decaffeinated roasted and ground coffee		
Caution	1. Once sample is opened, seal it in airtight manner after taking test portion 2. Concentrated hydrochloric acid is corrosive, has an irritant vapour and causes burns. Wear mask and gloves during analysis		
Principle	Alkalinity of soluble ash, indicate the amount of acid required to neutralize the aqueous extract of the total ash. The ash obtained mixed with water and heated to boiling and filtered through ash less filter paper. The filtrate of water soluble ash is titrated against 0.l N HCl using methyl orange as an indicator to calculate alkalinity of soluble ash.		
Apparatus/Instrument	General Apparatus and Glassware 1. Calibrated Burette 2. Dropper		
Materials and Reagents	1. Methyl orange indicator 2. Conc. Hydrochloric Acid		
Preparation of Reagents	1. Methyl orange indicator (0.1% w/v) - 0.1 g of methyl orange dissolved in 100 mL of distilled water. 2. Hydrochloric acid (0.1 N) – Concentrated (1 mL) diluted to 116.5 mL with distilled water.		
Sample Preparation	1. Filtrate reserved during the determination of water soluble ash		
Method of analysis	1. To the filtrate reserved during the determination of water soluble ash, add 3-4 drops of methyl orange indicator (0.1% w/v in water). 2. Titrate with 0.1 N hydrochloric acid to an orange end point. Note down the titre value.		
Calculation with units of expression	Alkalinity of soluble ash % per = g of sample (on dry wt.) $\frac{\text{Titre value x Normality of HCl}}{\text{Wt. of sample } (W_1 - W) \times (100 - M)}$ Where, W = weight of empty dish W_1 = weight of dish + sample M = % Moisture of the sample		
Reference	• IS: 3077 – 2009 (A Specification for Roasted and Ground Coffee) •		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

	Determination Of Alkalinity Of Soluble Ash : Tea		
Method No.	FSSAI 04A.010:2021	**Revision No. & Date**	0.0
Scope	Tea/ Instant Tea, Kangra Tea, Green Tea		
Caution	Concentrated hydrochloric acid is corrosive, has an irritant vapour and causes burns. Wear mask and gloves during analysis		
Principle	Alkalinity of soluble ash, indicate the amount of acid required to neutralize the aqueous extract of the total ash. The ash obtained mixed with water and heated to boiling and filtered through ash less filter paper. The filtrate of water soluble ash is titrated against 0.l N HCl using methyl orange as an indicator to calculate alkalinity of soluble ash.		
Apparatus/Instrument	General Apparatus and Glassware 1. Calibrated Burette. 2. Dropper.		
Materials and Reagents	1. Methyl orange indicator 2. Concentrated Hydrochloric acid (36%)		
Preparation of Reagents	1. Methyl orange indicator - 0.1 g of methyl orange dissolved in 100 mL of distilled water. 2. Hydrochloric acid (0.1 N) – Concentrated hydrochloric acid (1 mL) diluted to 116.5 mL with distilled water.		
Sample Preparation	Filtrate reserved during the determination of water soluble ash		
Method of analysis	1. To the filtrate reserved during the determination of water soluble ash, add 3-4 drops of methyl orange indicator (0.1% in water). 2. Titrate with 0.1 N hydrochloric acid to an orange end point. Note down the titre value.		
Calculation with units of expression	Express the result as KOH (m/m) on dry basis: $\text{Alkalinity of soluble ash \%} = \frac{0.0056 \times \text{titer value} \times \text{Normality HCl} \times 100 \times 100}{\text{Weight of sample} \times 0.1 \times (100 - \text{moisture \%})}$		
Reference	I.S 13856: 1993 (ISO 1578: 1975) - Tea Determination of Alkalinity of Water soluble ash		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

	Determination of Aqueous Extract		
Method No.	FSSAI 04A.011:2021	**Revision No. & Date**	0.0
Scope	Tea/ instant tea, Kangra tea, green tea, coffee roasted /unroasted ground/green, decaffeinated roasted and ground coffee, chicory, coffee – chicory mixture, decaffeinated roasted and ground coffee -chicory mixture		
Caution	1. Once sample is opened, seal it in airtight manner after taking test portion 2. Wear gloves and face protection during analysis		
Principle	Sample is refluxed in water for one h and filtered the water soluble portion/ extract and calculated as % Aqueous Extract.		
Apparatus/Instrument	General Apparatus and Glassware: 1. Flask -500 mL, 2. Water jacketed condenser – 50 cm length, 3. Burner / hot plate, 4. Whatman No 1filter paper, 5. Pipette – 50 mL, 6. Aluminum dish, 7. Steam bath and 8. Hot air oven		
Materials and Reagents	1. Distilled water		
Sample Preparation	Grind the sample in a grinder to pass through No. 30 mesh sieve. Mix well to get a homogenous sample. Store sample in a tightly stoppered bottle, withdraw portions for analytical determinations.		
Method of analysis	1. Accurately weigh around 2 g of sample and transfer to a 500 mL flask. 2. Add 200 mL distilled water and connect the flask with a 50 cm long water jacketed condenser. Reflux for one h over low flame with occasional mixing. 3. Cool, and filter through Whatman No. 1 filter paper or equivalent, wash three times with 10 – 15 mL distilled water and finally make upto 250 mL in a volumetric flask. 4. Shake well and pipette 50 mL of aliquot to a tarred aluminium dish. 5. Evaporate on a steam bath. 6. Transfer to 100 °C air oven and dry for two h. 7. Dry again for 30 min, cool in a desiccator and weigh. 8. Repeat this process of heating for 30 min, cooling in desiccator and weighing until the loss in weight between two successive weighing is less than 1 mg. 9. Record the lowest weight.		
Calculation with units of expression	$\text{Aqueous extract (\%) (on dry wt.)} = \frac{(W_2 - W_1) \times 250 \times 100 \times 100}{W \times 50 \times (100 - M)}$ Where, W= Weight of sample. W_1 = Weight of empty aluminium dish. W_2 = Weight of empty aluminium dish + dried extract. M = Moisture %		
Reference	• IS: 3077 – 2009 (A Specification for Roasted and Ground Coffee)		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

	Determination of Caffeine Content (Bailey Andrew Method)		
Method No.	FSSAI 04A.012:2021	**Revision No. & Date**	0.0
Scope	Coffee roasted /unroasted ground/green, decaffeinated roasted and ground coffee, Soluble coffee Powder, decaffeinated Soluble coffee Powder, coffee – chicory mixture, decaffeinated coffee – chicory mixture, Instant coffee – chicory mixture and decaffeinated Instant coffee – chicory mixture		
Caution	1. Once sample is opened, seal it in airtight manner after taking test portion 2. Wear gloves and face protection during Analysis		
Principle	Caffeine is a naturally occurring stimulant found in coffee. Caffeine from coffee sample is extracted followed by digestion using Micro Kjeldhal flask. The conversion factor is used to convert the estimated nitrogen to caffeine content.		
Apparatus/Instrument	1. Erlenmeyer flask – 250 mL 2. Reflux condenser 3. Filter papers. 4. Volumetric flask – 50 mL. 5. Filtration set. 6. Separating funnels – 125 mL. 7. Kjeldahl flask (100 mL) and distillation assembly. 8. Beaker - 125 mL. 9. Burette. Space-1.0		
Materials and Reagents	1. Magnesium oxide. 2. Distilled water. 3. Concentrated Sulphuric acid (98%). 4. Chloroform. 5. Potassium hydroxide. 6. Potassium sulphate. 7. Mercuric oxide. 8. Vaseline. 9. Sodium hydroxide. 10. Methyl red indicator.		
Preparation of Reagents	1. Diluted sulphuric acid– Concentrated sulphuric acid (1 mL) diluted by mixing with 9 mL of distilled water. 2. Potassium hydroxide solution (1%) - Potassium hydroxide (1 g) dissolved in distilled water (100 mL). 3. Sulphuric acid (0.05 N) – conc. Sulphuric acid (1 mL) is added to 735 mL distilled water. 4. Sodium hydroxide (concentrate) (1:2) - Sodium hydroxide (5 g) dissolved in 10 mL distilled water.		

	5. Sodium hydroxide (0.1 M / 0.1 N) - Sodium hydroxide (0.4 g) dissolved in distilled water (100 mL). 6. Methyl Red Indicator Solution: Dissolve 50 mg of methyl red in a mixture of 1.86 mL of 0.1 M sodium hydroxide and 50 mL of ethanol (95 %, v/v). After the solution is effected, add sufficient water to produce 100 mL 7. Methyl Red Indicator Solution: Dissolve 50 mg of methyl red in 100 mL of 95% ethanol.
Sample Preparation	Grind the sample in a grinder to pass through No. 30 mesh sieve. Mix well to get a homogenous sample. Store sample in a tightly stoppered bottle, withdraw portions for analytical determinations.
Method of analysis	1. Weigh accurately about 5 g of sample, transfer to a 250 mL Erlenmeyer flask and add 3 g of magnesium oxide and 100 mL of distilled water. 2. Weigh the flask with contents and boil under a reflux condenser for 45 min, shaking occasionally. 3. Cool and weigh the flask again and add water till the original weight is obtained. 4. Mix well and filter through a dry filter paper directly into a 50 mL graduated flask until exactly 50 mL of the solution (equivalent to half the quantity of the sample taken for test) is obtained. 5. Transfer the solution to a 125 mL separator. Wash the graduated flask with 2 mL of water and add the washings to the separator. 6. Add 4 mL of dilute Sulphuric acid (1: 9). 7. Extract with five 10 ml portions of chloroform shaking vigorously for 1 minute for each extraction. Let the emulsion break, then drain the chloroform into a 125 mL separator. 8. Add 5 mL of Potassium hydroxide solution (1%). 9. Shake vigorously for 1 min, let the emulsion break and drain the chloroform through a cotton plug into a 100 mL Kjeldahl flask. 10. Extract the Pot hydroxide solution with 5 mL of chloroform and add to the Kjeldahl flask. 11. To the digestion flask add 1.3 ± 0.5 g of potassium sulphate and 40 ± 5 mg mercuric oxide. Rinse down the neck of the flask with 3 mL chloroform. 12. Place the flask on the digestion rack and concentrate chloroform to about 20 mL 13. Distil off chloroform. Add 2 ± 0.1 mL conc. sulphuric acid of Sp. gravity 1.84, digest for one h after the acid begins to boil. 14. Cool and add minimum quantity of water to dissolve the solids. 15. Cool and place a thin film of Vaseline at the rim of the flask. 16. Transfer the digest with a few boiling chips to the distillation apparatus and rinse the flask five-six times with 1 – 2 mL distilled water. 17. Place a 125 mL beaker containing a known quantity of standard sulphuric acid (0.05 N).

	18. Add 6 mL of conc. sodium hydroxide solution (1:2) carefully through the side of the still so that it does not mix, and assemble the distillation apparatus taking care that the dip tube extends well within the standard sulphuric acid solution contained in the beaker. 19. Mix the contents of the distillation flask and distill until all ammonia has passed over into the standard sulphuric acid. 20. Shut off the heater and immediately detach the flask from the condenser. 21. Rinse the condenser thoroughly with water into the beaker. Wash the dip tube carefully so that all traces of the condensate are transferred to the beaker. 22. When all the washings have drained into the beaker, add 2-3 drops of methyl red indicator and titrate with standard sodium hydroxide solution (0.1 N). 23. Carry out a blank determination using reagents in the same proportion without the sample.
Calculation with units of expression	Caffeine on dry basis (%) by weight $= \frac{486.96\ (B- A)\ N}{W\ (100 – M)}$ Where, B = Volume of standard sodium hydroxide used to neutralize acid in the blank determination A = Volume of standard sodium hydroxide used to neutralize the excess acid in the test with the sample N = Normality of standard sodium hydroxide solution W = Weight in g of the sample in the aliquot M = Percentage of moisture in the sample **Note**: - For soluble coffee (instant coffee) the quantity of sample for test should be 1 g only.
Reference	• IS: 3077 – 2009 (A Specification for Roasted and Ground Coffee) • A.O.A.C 21st edn, Official Method of Analysis (2019) Method no.960.25 Caffeine in Roasted Coffee.
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Caffeine (Alternate Chromatographic – Spectrophotometric Method)		
Method No.	FSSAI 04A.013:2021	**Revision No. & Date**	0.0
Scope	Coffee roasted /unroasted ground/green, decaffeinated roasted and ground coffee, Soluble coffee Powder, decaffeinated Soluble coffee Powder, coffee – chicory mixture, decaffeinated coffee – chicory mixture, Instant coffee – chicory mixture and decaffeinated Instant coffee – chicory mixture		
Caution	1. Once sample is opened, seal it in airtight manner after taking test portion 2. Wear gloves and face protection during Analysis		
Principle	Caffeine is a natural stimulant most commonly found in tea, coffee, and cacao plants Caffeine is separated using column chromatography using chloroform solvent and optical density (OD) is measured using spectrophotometer at 276nm using caffeine standard.		
Apparatus/Instrument	General Apparatus and Glassware 1. Glass columns – 25 x 250 mm size 2. UV – VIS Spectrophotometer – To record 250 – 350 nm range with matched 1 cm cells.		
Materials and Reagents	1. Ammonia solution 2. Concentrated Sulphuric acid (98%) 3. Diethyl ether 4. Chloroform 5. Celite 545 6. Caffeine 7. Sodium hydroxide		
Preparation of Reagents	1. Ammonium hydroxide solution (1:2)– Ammonia (100 mL) is added to distilled water (200 mL) 2. Sulphuric acid (4 N) – Concentrated sulphuric acid (10 mL) is diluted to 92 mL with distilled water. 3. Diethyl ether (Water Saturated) – Diethyl ether (100 mL) is mixed with distilled water and shaken well. Top layer is diethyl ether saturated with water and taken is extracted. 4. Chloroform – Chloroform (100 mL) is mixed with distilled water and shaken well. Bottom layer is chloroform saturated with water and taken. 5. Caffeine standard solution (10, 20, 30 µg /mL in Chloroform) - Accurately weigh 100 mg of caffeine (USP, anhydrous) into 100 mL volumetric flask, dissolve in chloroform and make upto volume. Dilute 10 mL aliquot to 100 mL with chloroform. Further dilute 10, 20, and 15 mL aliquots to 100, 100 and 50 mL respectively with chloroform to obtain standard solutions of 10, 20, and 30 µg /mL 6. Sodium hydroxide (2 N) – Sodium hydroxide (8 g) dissolved in distilled water (100 mL).		

Sample Preparation	Grind the sample in a grinder to pass through No. 30 mesh sieve. Mix well to get a homogenous sample. Store sample in a tightly stoppered bottle, withdraw portions for analytical determinations.
Method of analysis	**For Green/roasted Coffee** 1. Accurately weigh about 1 g ground sample and transfer to 100 mL beaker. 2. Add 5 mL NH_4OH (1:2) and warm on boiling water-bath for 2 min. 3. Cool, transfer to 100 mL volumetric flask and make up to volume with water. To 5 mL aliquot of the turbid solution add 6 g celite 545 and mix carefully. **For decaffeinated green/roasted coffee** 1. Accurately weigh 1 g of ground sample. **2.** Transfer to 100 mL beaker, add 5 mL NH_4OH (1:2) and warm on boiling water bath for 2 min. Add 6 g celite 545 and mix carefully. **For soluble Coffee** 1. Proceed as in green/roasted coffee except 0.5 g sample and an aliquot of 3 mL **For decaffeinated soluble coffee** 1. Proceed as in decaffeinated green/roasted coffee except 0. 5 g sample.
	Column Chromatography **Acid column:** 1. Place fine glass wool and plug into the base of 25 x 250 mm column. 2. Add 3 mL 4 N H_2SO_4 to 3 g celite 545 and mix well by kneading with spatula. Transfer into the tube and tamp using gentle pressure and place small glass wool above the surface. **Basic Column:** **Layer I:** 1. Mix 3 g celite 545 and 2 mL 2 N NaOH and place in 25 x 250 mm tube. Transfer over glass wool plug as in Acid column. **Layer II:** 1. Transfer sample plus celite 545 mixtures in about 2 g portions to tube directly over layer I, taping before adding mixture portion of sample until homogenous and compact layer is obtained. 2. Dry wash beaker with about 1 g celite 545, transfer to tube and tap to uniform mass. 3. Dry wash beaker with wad of glass wool and transfer to top of basic column. 4. Mount basic column above acid column. 5. Pass 150 mL water saturated ethers sequentially through basic column to acid column and discard ether. Then pass 50 mL water saturated ether through acid column and discard ether. 6. Place 50 mL volumetric flask under acid column. 7. Pass 48 mL water saturated $CHCl_3$ through acid column washing tip of basic column with first portions.

Calculation with units of expression	1. Dilute contents of volumetric flask (100 mL) to volume with water saturated chloroform, mix, and read O.D at 276 nm against water saturated chloroform $CHCl_3$ blank, by scanning from 350 to 250 nm. 2. Determine O.D of standards and use this value to calculate the caffeine percentage.
Reference	A.O.A.C 21st edn, Official Method of Analysis(2019) Method no. 979.11 Caffeine in Roasted Coffee, Chromatographic – Spectrophotometer method.
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Caffeine (Alternate method By HPLC)		
Method No.	FSSAI 04A.014:2021	**Revision No. & Date**	0.0
Scope	Coffee roasted /unroasted ground/green, decaffeinated roasted and ground coffee, Soluble coffee Powder, decaffeinated Soluble coffee Powder, coffee – chicory mixture, decaffeinated coffee – chicory mixture, Instant coffee – chicory mixture and decaffeinated Instant coffee – chicory mixture		
Caution	1. Once sample is opened, seal it in airtight manner after taking test portion 2. The cartridge should not be dry during elution.		
Principle	Caffeine is a natural stimulant most commonly found in tea, coffee, and cacao plants is usually extracted by C-18 cartridges and quantified by HPLC (absorbance measured at 280 nm)		
Apparatus/Instrument	1. General Apparatus and Glassware (Page 3 and Analytical Balance (0.0001g) 2. Millipore filters (0.45 µm). 3. Bond C 18 cartridges technical details??? 4. Volumetric flasks -10 mL. 5. HPLC system with UV-VIS 6. Column: Spherisorb ODS, C 18, 5 um packed column 25 cm long x 4 mm internal Dia.		
Materials and Reagents	1. Distilled water. 2. Sodium acetate. 3. Tetrahydrofuran. 4. Standard Caffeine		
Preparation of Reagents	1. Sodium acetate (0.005 M) 2. Standard Caffeine solutions: Caffeine (0.2, 0.4, 0.6, 0.8 and 1.0 mg) in 10 mL mobile phase (0.005 M Sodium acetate: tetrahydrofuran – 95: 5 at pH 5).		
Sample Preparation	Grind the sample in a grinder to pass through No. 30 mesh sieve. Mix well to get a homogenous sample. Store sample in a tightly stoppered bottle, withdraw portions for analytical determinations.		
Method of analysis	1. Dissolve 1 g of sample in 100 mL hot water 2. Filter 20 mL through a Millipore filter (0.45 µm) under vacuum. 3. To a Bond Elute C 18 cartridge or equivalent under vacuum. 4. Elute the caffeine with 5 mL of mobile phase (0.005 M Sodium acetate: tetrahydrofuran – 95: 5 at pH 5). 5. Collect in a 10 mL flask and make upto volume.		

	6. Inject 20 µL into a Spherisorb ODS, C 18, 5 um packed column 25 cm long x 4 mm internal dia. 7. Elute with the mobile phase at 1 mL/min, read the absorbance at 280 nm. 8. Calibrate with standard Caffeine solution, 0 - 1 mg Caffeine in 10 mL mobile phase. Note: For routine purposes the HPLC step can be eliminated and the absorbance of eluent from the cartridge measured at 280 nm in a spectrophotometer.
Calculation with units of expression	1. Calibration curve of Caffeine is prepared using absorbance standard solutions of caffeine (280 nm) solutions versus concentration. 2. Caffeine in sample solution is determined using the calibration curve.
Reference	Pearson's Composition and Analysis of Foods 9th edn, 1991, page 373
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of adulterants (Microscopic Examination)		
Method No.	FSSAI 04A.015:2021	**Revision No. & Date**	0.0
Scope	Coffee roasted /unroasted ground/green, soluble coffee powder and coffee – chicory mixture, instant coffee - chicory mixture		
Caution	1. Roasted cereals such as barley, oats and wheat and soya may be mixed with coffee and coffee and chicory as coffee substitutes. 2. Once sample is opened, seal it in airtight manner after taking test portion		
Principle	Sample is first heat treated to extract color present in the sample and microscopically examined to check the presence of any adultrant.		
Apparatus/Instrument	General Apparatus and Glassware 1. Filtration set. 2. Microscope. 3. Microscopic slide.		
Materials and Reagents	1. Sodium hydroxide. 2. Distilled water. 3. Glycerine. 4. Chloral hydrate. 5. Phloroglucinol. 6. Hydrochloric acid.		
Preparation of Reagents	1. Sodium hydroxide (2%) - Sodium hydroxide (2 g) is dissolved in distilled water (100 mL)		
Sample Preparation	Grind the sample in a grinder to pass through No. 30 mesh sieve. Mix well to get a homogenous sample. Store sample in a tightly stoppered bottle, withdraw portions for analytical determinations.		
Method of analysis	1. Boil about 1 g of sample with 50 mL of 2% sodium hydroxide for about 2 - 3 min. 2. Dilute and filter then wash the residue with water till the filtrate is free of alkali. 3. Repeat till the residue gives no colour with water (treatment with calcium chloride solution and then washing with water may be done in case, decant still shows some colouring matter). 4. Place a drop of residue material in glycerine on a clear microscopic slide. 5. Place a cover slip on the drop of the suspension and see under microscope. **Alternatively** 1. Boil sample with water so that most of the colour is extracted. 2. Drain and replace with chloral hydrate. Heat until sufficiently cleared. 3. Wash out chloral hydrate and stain with *phloroglucinol/ hydrochloric acid*. The microscopic structure as shown in the photomicrograph given below can be seen:		

Fig. 4 Roasted Coffee Powder x 100 (Photomicrograph) Coffee Showing Sclerenchymatous Fibres of Endocarp (Seed Coat)

Fig. 5 Roasted Coffee Powder x 100 (Photomicrograph) Coffee Showing Sclerenchymatous Fibres of Endocarp (Seed Coat)

	 
Calculation with units of expression	Coffee is characterized by longitudinal and transverse schlerenchymatous fibres (from pericarp) Chicory has large vessels upto 115 microns across which have short pits.
Reference	• IS: 3077 – 2009(A Specification for Roasted and Ground Coffee) • FAO Manuals of Food Quality Control 14 /8 pages 318 and 319
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Presence of Chicory in Coffee		
Method No.	FSSAI 04A.016:2021	**Revision No. & Date**	0.0
Scope	Coffee roasted /unroasted ground/green, soluble coffee powder		
Caution	1. Once sample is opened, seal it in airtight manner after taking test portion 2. Concentrated hydrochloric acid is corrosive, has an irritant vapour and causes burns. Wear mask and gloves during analysis		
Principle	Chicory contains inulin, which hydrolyses to laevulose. Coffee contains no inulin. The presence of chicory is shown by a positive reaction with Seliwanoff's reagent.		
Apparatus/Instrument	General Apparatus and Glassware 1. Filtration set.		
Materials and Reagents	1. Neutral lead acetate 2. Conc. HCl. 3. Resorcinol 4. Hydrochloric acid. 5. Distilled water.		
Preparation of Reagents	1. Neutral lead acetate (10%) – Neutral lead acetate (10 g) dissolved in water (100 mL). 2. Seliwanoff reagent – Dissolve 0.05 g of resorcinol in 100 mL of mixture of hydrochloric acid: distilled water (1:2).		
Sample Preparation	Grind the sample in a grinder to pass through No. 30 mesh sieve. Mix well to get a homogenous sample. Store sample in a tightly stoppered bottle, withdraw portions for analytical determinations.		
Method of analysis	1. Clarify 25 mL of 2% aqueous extract of the sample with neutral lead acetate and filter. 2. To 5 mL of filtrate add 5 mL of Seliwanoff reagent and 1 mL of conc. HCl. 3. Boil for 2 min. 4. Appearance of distinct red color on standing shows the presence of Chicory in coffee.		
Calculation with units of expression	Absent/Present of chicory in coffee		
Reference	FAO Manuals of Food Quality Control 14 / 8 pages317 and 318		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

	Determination of Solubility in boiling water		
Method No.	FSSAI 04A.017:2021	**Revision No. & Date**	0.0
Scope	Soluble (Instant) Coffee powder, Decaffeinated soluble coffee powder, Instant Coffee - Chicory Mixture, Decaffeinated Instant coffee- chicory mixture		
Caution	1. Once sample is opened, seal it in airtight manner after taking test portion 2. Wear gloves and face protection during analysis		
Principle	Instant coffee / coffee-chicory powder are dissolved in hot water and solubility time is recorded.		
Apparatus/Instrument	General Apparatus and Glassware 1. Beaker -500 mL 2. Heating equipment. 3. Weighing balance. 4. Stop clock. 5. Stirring equipment.		
Materials and Reagents	Instant coffee powder. 1. Instant coffee- chicory powder. 2. Freshly boiled water.		
Sample Preparation	Grind the sample in a grinder to pass through No. 30 mesh sieve. Mix well to get a homogenous sample. Store sample in a tightly stoppered bottle, withdraw portions for analytical determinations.		
Method of analysis	1. Weigh 2.5 g of instant coffee powder/coffee- chicory powder in a 500 mL beaker. 2. Then pour 150 mL of freshly boiled water, stir. Check the solubility time of sample. The product should dissolve in 30 sec.		
Calculation with units of expression	Record the time taken by the sample to get dissolved in boiled water.		
Reference	IS 3309:2016 Soluble Coffee -Chicory Powder— Specification		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

	Determination of Solubility in Cold water		
Method No.	FSSAI 04A.018:2021	**Revision No. & Date**	0.0
Scope	Soluble (Instant) Coffee powder, Decaffeinated soluble coffee powder, Instant Coffee - Chicory Mixture, Decaffeinated Instant coffee- chicory mixture		
Caution	Once sample is opened, seal it in airtight manner after taking test portion		
Principle	Instant coffee / coffee-chicory powder are dissolved in cold water and solubility time is recorded.		
Apparatus/Instrument	General Apparatus and Glassware 1. Beaker-500 mL 2. Weighing balance 3. Stop Clock 4. Stirring equipment		
Materials and Reagents	Instant coffee powder/coffee- chicory powder Distilled water.		
Sample Preparation	Grind the sample in a grinder to pass through No. 30 mesh sieve. Mix well to get a homogenous sample. Store sample in a tightly stoppered bottle, withdraw portions for analytical determinations.		
Method of analysis	1. Weigh 2.5 g of instant coffee powder/coffee- chicory powder in a 500 mL beaker. 2. Pour 50 mL of water (16 ± 2 °C) and stir. The product should dissolve in 3 min with moderate stirring, leaving no appreciable sediments. Numbering		
Calculation with units of expression	Record the time taken by the sample to get dissolved in cold water		
Reference	IS 2791:2016 Soluble Coffee Powder— SPECIFICATION		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

	Determination of Crude Fibre in Tea		
Method No.	FSSAI 04A.019:2021	**Revision No. & Date**	0.0
Scope	Tea, Kangra Tea, Green Tea		
Caution	1. Once sample is opened, seal it in airtight manner after taking test portion 2. Wear gloves and face protection during analysis		
Principle	Crude fiber is determined gravimetrically after chemical digestion and solubilization of other materials present. The fiber residue weight is then corrected for ash content after ignition. The loss in mass resulting from ashing is called the crude fibre content		
Apparatus/Instrument	General Apparatus and Glassware 1. Condenser – Use condenser that will maintain constant volume of refluxing solutions. 2. Digestion Flask-700-750 mL, Erlenmeyer flask is recommended. 3. Filtering cloth–Use filtering cloth such character that no solid matter passes through when filtering is rapid. Fine linen or dress linen with about 18 threads/cm or 45 threads per inch (i.e. the aperture size 0.14 mm and thread thickness 0.42 mm) or its equivalent may be used (Whatman filter Paper No. 54 or equivalent may also be used). 4. Muffle Furnace maintained at 525 ± 20 °C.		
Materials and Reagents	1. Sulphuric acid. 2. Caustic soda (free from sodium carbonate).		
Preparation of Reagents	3. Sulphuric acid (1.25%, v/v) - Sulphuric acid (1.25 g) dissolved in distilled water (100 mL) (w / v). 4. Caustic soda (1.25%, w/v) - Caustic soda (1.25 g) dissolved in distilled water (100 mL) (w / v).		
Sample Preparation	Grind the sample in a grinder to pass through No. 30 mesh sieve. Mix well to get a homogenous sample. Store sample in a tightly stoppered bottle, withdraw portions for analytical determinations.		
Method of analysis	**1.** Weigh accurately 2 g fat free of prepared sample. **2.** Dry in an air oven maintained at 100 ± 2 °C for 4 h. **3.** Transfer to the digestion flask. Add 200 mL of boiling 1.25% sulphuric acid. Immediately connect to the condenser and heat (it is essential that the solution boils within one minute and boiling continues briskly for exactly 30 min). Rotate flask frequently until sample at sides is thoroughly wetted, taking care to keep material from remaining on the sides of the flask. **4.** Immediately filter through linen in fluted funnel, and wash with boiling water until washings are acid free.		

	5. Wash the residue back into the flask with 200 mL of boiling 1.25% Caustic soda solution using wash bottle marked to deliver 200 mL. **6.** Connect flask to reflux condenser and boil briskly, exactly for 30 min. **7.** After 30 min remove flask immediately, filter via prepared asbestos mat and carefully transfer, all the residue into the Gooch crucible with hot water. Wash the residue thoroughly with hot water until the filtrate is alkali free. Then, wash with about 10 mL alcohol. **8.** Dry the Gooch crucible at 110 °C to constant weight. Cool and weigh (W_1). **9.** Transfer the Gooch crucible to a muffle furnace controlled at 525 – 550 °C and ash the material. **10.** Cool, weigh (W_2). Loss in weight represents crude fibre.
Calculation with units of expression	Crude fibre % = (on dry weight) $\frac{(W_1 - W_2) \times 100 \times 100}{\text{Wt. of sample} \times (100\text{-Moisture content})}$
Reference	• IS 16041:2012- Tea — Determination of Crude Fibre Content, IS 10226
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Catechins in Tea — HPLC Method		
Method No.	FSSAI 04A.020:2021	**Revision No. & Date**	0.0
Scope	Green Tea, Instant Tea and Black Tea		
Caution	1. Once sample is opened, seal it in airtight manner after taking test portion 2. The cartridge should not be dry during elution.		
Principle	Catechin is a plant secondary metabolite of Flavonoids family is extracted from the tea by Methanol- Acetonitrile mixture and the extract is quantified on HPLC at 278nm.		
Apparatus/Instrument	1. Analytical Balance (± 0.0001 g). 2. Water Bath (70±1°C.) 3. Dispenser — set at 5 ml for methanol/water extraction mixture Centrifuge — capable of 3500 rev/min. 4. Vortex Mixer — 5. Extraction Tubes —Centrofuged tubes 15ml capacity, 6. Graduated Tubes — glass, 10ml capacity with 0.1 ml graduations. 7. Automatic Pipettes — (10-100ul, 1ml, 10ml) 8. Filters — membrane filter 0.45 pm pore size. 9. HPLC with ultraviolet detector (wavelength of 278 nm) NOTES 1. Phenyl bonded phases give additional selectivity over reversed phase packings, and result in improved resolution of the catechins. 2. In this standard the chromatographic conditions and composition of the mobile phase specified are suitable for a Phenomenex Lures 5 µm Phenyl-Hexyl column of dimensions 250 mm x 4.6 mm fitted with a Phenomenex Security Guard 4 mm x 3.0 mm Phenyl-Hexyl cartridge. If other types of column are used, an alternative mobile phase and alternative chromatographic conditions may be necessary.		

Materials and Reagents	1. Water —HPLC grade 2. Acetonitrile — HPLC Grade. 3. Methanol - HPLC Grade 4. Glacial Acetic Acid — HPLC Grade. 5. EDTA (Ethylenediaminetetraacetic Acid Disodium Salt, Dihydrate) 6. L-ascorbic Acid — Free acid. 7. Methanol/Water Extraction Mixture, 70 percent v/v Methanol — Add 700 ml of the methanol to a 1 litre mark volumetric flask. Dilute to the mark with water and mix. 8. HPLC Mobile Phase. 8.1 Mobile Phase A — Add 180 ml of acetonitrile and 40 ml acetic acid to a 2 litre mark volumetric flask. Dilute to the mark with water, mix, and filter through a filter of 0.45 µm pore size. 8.2 Mobile Phase B — Add 800 ml acetonitrile to a 1 Iitre mark volumetric flask. Dilute to the mark with water, mix and filter through a filter of 0.45 µm pore size.
Preparation of Reagents	1. Stabilizing Solution- Weigh, to the nearest 0.01 g, 0.25 g of EDTA and 0.25g of ascorbic acid into a 1 litre mark volumetric flask and dissolve in approximately 500 ml water. Add 100 ml acetonitrile dilute to the mark with water and mix. Prepare fresh stabilizing solution on the day of use. 2. Stock Standard Solutions 2.1 Weigh standards (>20mg) on an analytical balance in a volumetric flask and dissolved in stabilizing solution, gently warming (if required, 40°C maximum).The cool solution is diluted to the mark with stabilizing solution. Same procedure shall be followed for the preparation of the following stock standard solution. 2.2 Gallic Acid Stock Standard Solution — corresponding to 2.00 mg/ml. 2.3 Caffeine Stock Standard Solution — corresponding to 2.00 mg/ml. 2.4 (+) –Catechin, (C), Stock Standard Solution — corresponding to 1.00 mg/ml. 2.5 (–)-Epicatechin, (EC), Stock Standard Solution — corresponding to 1.00 mg/ml, 2.6 (–) –Epigallocatechin, (EGC), Stock Standard Solution — corresponding to 2.00 mg/ml. 2.7 (–) –Epigallocatechingallate, EGCG, Stock Standard Solution — corresponding to 2.00 mg/ml. 2.8 (–) –EpicatechingaIlate, ECG, Stock Standard Solution — corresponding to 2.00 mg/ml.

3. Dilute Gallic Acid Standard Solution — corresponding to 200 µg/ml. Using a pipette transfer 10 ml of the gallic acid stock standard solution to a 100 ml one-mark volumetric flask. Dilute to the mark with stabilizing solution and mix.
4. Mixed Working Standard Solutions
   - 4.1 Prepare three mixed working standard solutions, with concentrations selected to cover the range of compositions typically found in tea.
   - 4.2 Following Table 1, carefully pipette the given aliquots of dilute gallic acid standard solution and stock standard solutions into three separate 20 ml one-mark volumetric flasks, dilute to volume with stabilizing solution and mix. These mixed working standard solutions correspond to the nominal concentrations shown in Table 1. Use the actual standard weights taken to obtain the actual concentrations at each standard level.
   - 4.3 Pipette 1.0ml aliquots of each mixed standard solution into labeled small amber glass vials, gently flush with nitrogen prior to sealing and store frozen at –20°C. NOTES
     i. Mixed working standard solutions are stable for at least 2 months when stored frozen at –20”C.
     ii. Only thaw sufficient mixed working standard solution vials for each chromatographic run. Discard any remaining solution, do not re-freeze

Table 1: Composition of Mixed Working Standard Solutions Standard 1 to Standard 3

Sr. No.	Component	Solution	Aliquot, ml		
			Standard 1	Standard 2	Standard 3
i.	Gallic acid	200 µg/ml dilute stock standard solution	0.5	1.0	2.5
ii.	Caffeine	2.00 mg/ml stock standard solution	0.5	1.0	1.5
iii.	+C	1.00 mg/ml stock standard solution	1.0	2.0	3.0
iv.	EC	1.00 mg/ml stock standard solution	1.0	2.0	3.0
v.	EGC	2.00 mg/ml stock standard solution	1.0	2.0	3.0
vi.	EGCG	2.00 mg/ml stock standard solution	1.0	2.0	4.0

vii.	ECG	2.00 mg/ml stock standard solution	0.5	1.0	2.0

Table 2: Nominal Concentrations in Mixed Working Standard Solutions Standard 1 to Standard 3

Sr. No.	Component	Nominal concentration		
		Standard 1	Standard 2	Standard 3
i.	Gallic acid	5	10	25
ii.	Caffeine	50	100	150
iii.	+C	50	100	150
iv.	EC	50	100	150
v.	EGC	100	200	300
vi.	EGCG	100	200	400
vii.	ECG	50	100	200

Sample Preparation

Sample is prepared by grinding a small quantity of the sample and reject it, then quickly grind an amount slightly greater than that required for the specified tests and for the determination of dry matter content. Store all samples in well sealed containers, protected from light, and cool.

NOTE — Grinding of instant tea is only required for samples with a coarse granular structure.

Method of analysis

1. Determination of Dry Matter Content: Calculate the dry matter content from the moisture content (loss in mass at 103°C/1hr)determined on a portion of the test sample
2. Test Portion
   - 2.1 **Instant tea:** Weigh, to the nearest 0.0001 g, 0.5 g of the test sample into a 50 ml one-mark volumetric flask.
   - 2.2 **Green and Black Tea**: Weigh, to the nearest 0.0001 g, 0.2 g of the test sample into an extraction tube.
3. Extraction
   - **3.1 Instant tea:**
     - 3.1.1 a. Add, to the instant tea in the flask from 2.1, approximately 25 ml hot water (maximum temperature of 60°C), mix to dissolve the sample and allow to cool to room temperature.
     - 3.1.2 Add, 5 ml acetonitrile, dilute to the mark with water and mix.
   - **3.2 Green and Black Tea:**
     - 3.2.1 Place the methanol/water extraction mixture contained in the dispenser into the waterbath set at 70°C, and allows 30 min for the extraction mixture to reach temperature.
     - 3.2.2 Place the extraction tube containing the tea sample into the

water bath set at 70°C. Add 5 ml hot methanol/water extraction mixture from the dispenser, stopper the tube and carefully mix on the vortex mixer. NOTE — it is important to mix samples thoroughly to ensure complete extraction.

3.2.3 Continue heating the extraction tube in the water bath for 10 min, mixing on the vortex mixer at 5 min and 10 min.

3.2.4 Remove the extraction tube from the water bath, and allow cooling to room temperature. Remove stopper and place in the centrifuge at 3500 rev/min

3.2.5 Carefully decant the supernatant into a graduated tube.

3.2.6 Repeat extraction steps 3.2.2 to 3.2.5. Combine extracts, make up to 10ml with cold methanol/ water extraction mixture and mix contents.

NOTE — The extract from 3.2.6 is stable for at least 24 h if stored at 4°C. Allow extract to reach room temperature before proceeding with the assay. Resuspension of the small amount of fine particulate material settled during storage is not necessary.

4. Dilution: Using a pipette, transfer 1.0 ml of the sample extract into a graduated tube and dilute to 5 ml with stabilizing solution. Mix solution then filter through 0.45 µm filter.
5. Determination

5.1 Adjustment of the Apparatus: Set up the chromatography in accordance with the manufacturer's instructions and adjust it as follows:

5.1.1 Flow rate of the mobile phase: 1.0 ml/min

5.1.2 Binary gradient conditions: 100 percent mobile phase A for 10 min, then over 15min a linear gradient to 68 percent mobile phase A, 32 percent mobile phase B and hold at this composition for 10 min. Then reset to 100 percent mobile phase A and allow to equilibrate for 10min before next injection.

5.1.3 Temperature of the column: 35 ± 0.5°C.

Notes: 1 Column temperature control is recommended (chromatography column oven or recirculating water jacket) if major drifts in retention times are to be avoided. UV detector setting: wavelength 278 nm.

2 Ensure that the detector sensitivity range selected is able to keep all peaks from the highest mixed working standard (Standard 3) within the scale of the data collection system used.

5.2 HPLC Analysis

5.2.1 Once the flow rate of the mobile phase and temperature are stable, condition the column with a blank gradient run. Then inject onto the column 10 µl of each of the mixed working standard solutions Standard 1 Standard 2 and Standard 3 followed by an equal volume of the diluted test solution.

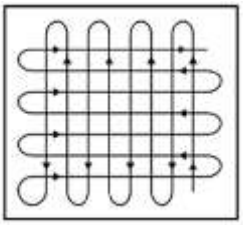
	Repeat injection of the mixed working standard solutions at regular intervals (typically after six test solutions). Collect and record the data for the peaks of all standards and test samples. 5.2.2 After each day's use and prior to storage, wash the column with approximately 50 percent acetonitrile, replacing the column sealing plugs after disconnection.
Calculation with units of expression	1. Identify and measure the peak areas or heights (area is preferable) for all standards and test samples. Construct linear calibration graphs for all components in the standards of concentration (~g/ml) against peak areas or heights and obtain the individual standard response factors (RF) automatically using a data collection/integration system or manually from a selected point on the calibration graph. $$RF = \frac{C_{std}}{A_{std}\ or\ h_{std}}$$ Where, RF = standard response factor C_{std} = concentration of the standard (µg/ml); A_{std} = peak area of the standard; and h_{std} = peak height of the standard. 2. Calculate response factors for all the individual components, that is Gallic acid, caffeine and the individual catechins EGC, +C, EC, EGCG and ECG. Calibration information obtained from a data collection/integration system will include an intercept value when the calibration is not forced through the origin and this should be included in the calculation. 3. The concentration of the individual components expressed as a percentage by mass on a sample as received basis is given by the formula: $$Percent\ individual\ component\ (m/m)(as\ received\ basis) = \left(A_{samp}\ \text{or}\ h_{samp}\right) \times RF\frac{Vd}{10{,}000m}$$ Where, A_{samp} = peak area for the test sample; h_{samp} = peak height for the test sample; RF = response factor for the individual component; V = sample extraction volume (50 for instant tea or 10 for leaf tea); d = dilution factor (see 4 in method of analysis), typically 5; and m = mass, in g, of the test sample. Percent total catechins (m/m) (as received basis) = (percent EGG) + (percent +G) + (percent EG) + (percent EGGG) + (percent EGG).

	Percent total catechins (m/m) (dry matter basis) $= \frac{\text{Percent total catechins m/m(as received basis)} \times 100}{\text{w}}$ Where, w = dry matter content of the test sample, determined in accordance with step 1 in method of analysis.
Reference	IS 15344:2003 (Green Tea - Specification)
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Added Color		
Method No.	FSSAI 04A.021:2021	**Revision No. & Date**	0.0
Scope	Tea, Coffee and Chicory products		
Caution	1. Once sample is opened, seal it in airtight manner after taking test portion 2. Wear gloves and face protection while doing analysis.		
Principle	Presence of added colors in foods, involve preliminary treatment of the food (Acidic/Alkali) and extraction of the color from the prepared solution of the food.		
Apparatus/Instrument	General Apparatus and Glassware 1. Pipette 2. Beaker 3. Flask. 4. Soxlet extractor. 5. Whatman No.1 filter paper. 6. Woolen thread.		
Materials and Reagents	1. White knitting wool. 2. Petroleum ether. 3. Distilled water. 4. Ammonia (0.88 sp. gr). 5. Acetic acid.		
Preparation of Reagents	1. White knitting wool: - Extract pure white wool in a soxhlet extractor with petroleum ether for 2-3 h to remove fat. Boil in very dilute solution of sodium hydroxide and then in water to free it from alkali. 2. Paper: Whatman No. 1 chromatographic paper or equivalent. 3. 1 mL (0.88 sp. gr) ammonia + 99 mL water. 4. Acetic acid solution in water (1:3).		
Sample Preparation	1. Grind the sample in a grinder to pass through No. 30 mesh sieve. Mix well to get a homogenous sample. Store sample in a tightly stoppered bottle, withdraw portions for analytical determinations. 2. ***Preliminary treatment of food*****:** Assuming that an acidic colour is present, the preliminary treatment involves removing interfering substances and obtaining the dye in acid solution prior to boiling with wool. To test the presence of basic color, treat the sample with ammonia to make alkaline solution prior to boiling with wool.		
Method of analysis	**Acidic Dyes**		

	1. Introduce about 20 cm length of woolen thread into a beaker containing about 35 mL of the prepared acidified solution of the sample and boil for a few min till the woolen thread is dyed. 2. Take out the woolen thread and wash it with tap water. 3. Transfer the washed woolen thread to a small beaker containing dilute ammonia and heat again. If the color is stripped by the alkali, the presence of an acid coal-tar dye is indicated. 4. Remove the woolen thread. Make the liquid slightly acidic and boil with a fresh piece of woolen thread. Continue boiling until the color is taken by the woolen thread. 5. Extract the dye from the woolen thread again with a small volume of dilute ammonia, filter through a small plug of cotton and concentrate the filtrate over a hot water bath. 6. This double stripping technique usually gives a pure color extract. Natural colors may also dye the wool during the first treatment, but the color is not usually removed by ammonia. **Basic dyes** 1. Basic dyes can be extracted by making the food alkaline with ammonia, boiling with wool and then stripping with dilute acetic-acid. 2. At present, all the permitted water soluble coal-tar dyes are acidic; hence an indication of the presence of a basic dye suggests that an unpermitted color is present.
Calculation with units of expression	Present/Absent
Reference	Manual Methods of Analysis for Adulterants and Contaminants in Food, I.C.M.R 1990 Page 56
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Iron Filings in Tea		
Method No.	FSSAI 04A.022:2021	**Revision No. & Date**	0.0
Scope	Tea, Kangra Tea.		
Caution	NA		
Principle	Iron filings or Iron particles may appear during the manufacturing/processing of tea and affects its quality. This method follows the gravimetric estimation of iron particles after separation using a magnet.		
Apparatus/ Instruments	Magnet (Strength: ~ 1000 gauss)		
Materials and Reagents	Analytical balance, Magnet, white sheet,		
Preparation of Reagents	Not Applicable		
Sample Preparation	❖ **Step-1:** Take whole unit pack (250 g) sample and homogenize/ mix properly and spread the mixed sample in thin layer (~ 5 mm) on white sheet. 		
Method of analysis	❖ **Step-2:** Collect the five sub-lot fractions of sample from five different regions (4 corners and centre) in total weighing 50 g. Remaining 200 g sample shall return to pack.  ❖ **Step-3:** Combine and mix all 5 sub-lot fractions into one.  ❖ **Step-4:** From the above, weigh and use 20 g of sample for next step. Spread it to very thin layer (close to uni-layer; around 2 - 3 mm) on white sheet.		

	 ❖ **Step-5:** Slowly move the magnet (~ 1000 gauss strength) over thinly spread (around 2 - 3 mm height) tea sample, as above in the flow manner indicated in below diagram. Repeat this manual magnet movement multiple times over 10 min duration. Collect the iron particles sticking to magnet each time of movement and pool in petri dish (Note: magnet should pass just above the surface of Tea powder).  ❖ **Step-6:** Spread the collected iron pieces (which may contain few tea particles also along, due electrostatic attraction) on white paper and use magnet movement (2nd time), above the distance of 0.5 - 1.0 cm from the spread layer on paper. This second action of magnet collects only iron particles, leaving tea sample on paper. ❖ **Step-7:** Take the weight of the collected iron particles, sticking on magnet, using analytical balance. ❖ **Step-8:** Repeat the entire process in triplicate for averaging.
Calculation with units of expression	Calculation (mg/Kg) : $\frac{\text{Weight of the iron filings (mg) X 1000}}{\text{Weight of the sample(g)}}$
Reference	IS 3633:2003 - Black Tea – Specification, 2nd Revision
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Extraneous matter		
Method No.	FSSAI 04A.023:2021	Revision No. & Date	0.0
Scope	Tea, Coffee and Chicory		
Caution	NA		
Principle	Sample is examined visually/using magnifying lens for extraneous matter like strings, stones, dirt, wood, glass and metallic pieces, twigs, bark and stems.		
Apparatus/Instrument	NA		
Materials and Reagents	Magnifying lens		
Sample Preparation	Mix whole sample Properly		
Method of analysis	Mix the whole sample and test visually for extraneous matter. The sample should be free from extraneous matter like strings, stones, dirt, wood, glass and metallic pieces		
Calculation with units of expression	Presence/Absence		
Reference	IS: 3077 – 2009 A Specification for Roasted and Ground Coffee		
Approved by	Scientific Panel on Methods of Sampling and Analysis		