एफएसएसएआई fssai भारतीय खाद्य सुरक्षा और मानक प्राधिकरण Food Safety and Standards Authority of India स्वास्थ्य और परिवार कल्याण मंत्रालय Ministry of Health and Family Welfare	**Method for Determination of Iodine in Double Fortified Salt (Quantitative)**		
Method No.	FSSAI.FS.16.011.2023	**Revision No. & Date**	0.0
Scope	The iodine content can be measured by conventional iodometric titration using sulphuric acid, but H_2SO_4 interferes with the estimation of iodine leading to erroneous results. Hence a modified method with orthophosphoric acid has been validated for the estimation of iodine in DFS.		
Caution	Caution should be taken while preparing the solutions and also while analyzing the samples.		
Principle	Iodine estimation by Titration Method. The Iodine content in DFS is measured by a modified iodometric titration.		
Apparatus/Instruments	Weighing balance		
Materials and Reagents	***Materials*** 1. Burette 2. Erlenmeyer flask with stopper, 250 mL 3. Beakers, 250mL and 500 mL 4. Pipettes ***Reagents*** 1. Potassium Iodide 2. Orthophosphoric acid 3. Sodium thiosulphate 4. Starch 5. Sodium chloride 6. Potassium iodate 7. Double distilled water		
Preparation of Reagents	1. Potassium Iodide, KI (1% solution): Dissolve 1 g of KI (LR grade) in 100 mL water. Store in a cool, dark place. The solution is stable for at least 3 months if stored properly. 2. Orthophosphoric acid(H_3P0_4), 4 N: Slowly add 75.4 mL of AR grade orthophosphoric acid to 900 mL of ice-cold distilled water. Dilute and make to 1000 mL with water. The volume is sufficient for 200 samples. The solution is stable. ***Note:*** Always add acid to water dropwise, not water to acid. Stir the solution while adding acid. 3. Sodium thiosulphate ($Na_2S_2O_3$), 0.0005M: Dissolve 1.24 g $Na_2S_2O_3.5H_2O$ (AR grade) in 1000 mL water. Store in a cool place. This volume is sufficient for nearly 200 samples. The solution is stable at least for 1 month, if stored properly. 4. Starch indicator solution: 4a. Preparation of saturated NaCl solution Make 100 mL of a saturated NaCl solution, by adding NaCl in small quantities at a time, to approximately 80 mL water in a beaker, with stirring and heating, until no further Nacl dissolves. This solution is stable for one year.		

	5. Preparation of Starch: Weigh one gram soluble starch (potato, extra pure/LR grade) into a 100 mL beaker, add 10 mL water and make a paste, heat to dissolve. Add saturated NaCl solution to the hot starch solution to make to 100 mL. Store in a cool, dark place. This volume is sufficient for 200 samples. The solution is stable for up to one month, and should be heated (not boiled) each day before use to resuspend any solids. 6. Standard KIO_3 : Weigh accurately 0.167 g of KIO_3 (AR grade) and dissolve in water in a standard measuring flask (100 mL) and make up the volume to 100 mL. This will give a concentration of 1 mg of iodine/mL.
Sample Preparation	***A. DFS Sample Preparation:*** a. Weigh accurately 10 g DFS into a 250 mL Erlenmeyer flask with stopper b. Add 0.5 mL of 1% KI (CAUTION: Do not pipette by mouth) c. Add 46 mL of water. Swirl the flask to dissolve the salt. d. Add 5 mL of 4 N H_3PO_4. The solution will turn yellow if iodine is present. e. Stopper the flask and put it in the dark (cup board) for 10 min. (Caution: The reaction mixture should be kept in the dark before titration because a side reaction can occur when exposed to light that causes iodide ions to be oxidized to iodine) ***B. Standard KIO_3:*** Run standard KIO_3 (1 mg iodine/mL) with 10 g of non-iodized salt as part of quality control. Take 46 mL of water into a 250 mL Erlenmeyer flask with stopper. Add 1 mL of standard KIO_3 (1mg of iodine/mL) and 10 g of non-iodized salt. Add 0.5 mL 1% KI. Add 5 mL 4 N H_3PO_4. Stopper the flask and put in the dark for 10min. **Precautions:** Inaccurate results may occur if starch solution is used while still warm. If starch indicator is added too early, a strong iodine-starch complex is formed which reacts slowly and gives falsely elevated results. The reaction should be performed at room temperature (< 30 ° C), as iodine is volatile and the indicator solution will lose sensititvity when exposed to high temperature.
Method of analysis	***Sample Analysis*** a. Rinse and fill the burette with 0.005 M Sodium thiosulphate and adjust the level to zero. b. Remove the flask from the dark and titrate against $Na_2S_2O_3$ from the burette until the solution turns pale yellow (straw yellow) c. Add 0.5 mL of starch indicator solution and continue titration until the solution becomes colorless. d. Record the volume of thiosulfate in the burette and convert to ppm using the "conversion table". Refer to conversion table for iodine content. ***Standard KIO_3 Analysis*** i. Rinse and fill the burette with 0.005 M Sodium thiosulphate and adjust the level to zero. ii. Then titrate the standard KIO3 solution against 0.005 M $Na_2S_2O_3$ (repeat steps b to d as indicated above) to calculate the iodine content. This will give an iodine value of 100 ppm (100 µg/g).
Calculation with units of expression	The unit of expression is µg/g (ppm)
Inference	NA, Quantitative Analysis

(Qualitative Analysis)	
Reference	S. Ranganathan & M. G. Karmarkar, Indian Journal of Medical Research 123, April 2006, p,531-540; Estimation of Iodine in salt fortified with Iodine & Iron
Approved by	Scientific Panel on Methods of Sampling and Analysis

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Method No.	FSSAI.FS.16.012.2023	**Revision No. & Date**	0.0
Scope	This method is used for the estimation of Iron calorimetrically in Double Fortified Salt (DFS).		
Caution	Caution should be taken while preparing the solutions and also while analyzing the samples.		
Principle	Iron is determined calorimetrically by the principle that ferric ion (Fe^{3+}) gives a blood red color with potassium thiocyanate (KCNS).		
Apparatus/Instruments	1. Weighing Balance 2. Colorimeter		
Materials and Reagents	1. Sulphuric Acid 2. Potassium Persulphate 3. Potassium thiocyanate 4. Standard Iron solution 5. Working standard solution		
Preparation of Reagents	1. Sulphuric Acid, H_2SO_4 (30%): Take 60 mL distilled water in a beaker. Keep in an ice bath and add slowly drop-wise 30 mL of concentrated H_2SO_4 with constant stirring. Make the volume to 100 mL with distilled water. 2. Potassium persulphate, $K_2S_2O_8$ (7%): Dissolve seven grams of $K_2S_2O_8$ in distilled water and make up the volume to 100 mL with distilled water. 3. Potassium thiocyanate, KCNS (40%): Dissolve 40 g of KCNS in 90 mL distilled water. Add four mL acetone and make up the volume to 100 mL. 4. Standard Iron Solution: Dissolve 702.2 mg ferrous ammonium sulphate in 100 mL distilled water. Add five mL of 1:1 hydrochloric acid (HCL) and make up the volume to 100 mL (0.1 mg/mL). The standard solution is prepared fresh and can be kept for 6 months. From this prepare the working standard. 4a. Working Standard (10 µg iron/mL): Dilute 10 mL of the standard iron solution (0.1 mg/mL) to 100 mL with distilled water. This will give 1010 µg iron/mL concentration.		
Sample Preparation	Take one gram of DFS in a 100 mL standard measuring flask using a glass funnel. Add 2.5 mL of concentrated HCL and make up the volume to 100 mL with distilled water. Mix and use 1 mL – 2 mL aliquots for the estimation of iron as given below.		

Reagent	**Test Tube 1**	**Test Tube 2**	**Test Tube 3**	**Test Tube 4**	**Test Tube 5**	**Test Tube 6**	**Test Tube 7**	**Test Tube 8**
Distilled water (mL)	6.5	5.5	4.5	6.0	5.5	4.5	3.5	2.5
Iron working standard(mL)	0	0	0	0.5	1.0	2.0	3.0	4.0
DFS Solution(mL)	0	1.0	2.0	0	0	0	0	0
30% H_2SO_4(mL)	1	1	1	1	1	1	1	1

	7% $K_2S_2O_8$(mL)	1	1	1	1	1	1	1	1
	40% KCNS(mL)	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Method of analysis	Prepare the test tubes as above adding all other solutions except 40% KCNS solution. Add 40% KCNS solution just before taking the readings. Measure the red color developed within 20 min of addition of 40% KCNS at 540 nm.								
Calculation with units of expression	Draw a standard graph of the iron standards by taking iron concentration (µg) on the X-axis and the OD on the Y-axis and calculate the iron content from the standard graph.								
Inference (Qualitative Analysis)	NA, Quantitative Analysis								
Reference	Wong, SY, Hawk' s. Physiological Chemistry,14th Edition, New York: McGraw Hill, 1965, page 1094								
Approved by	Scientific Panel on Methods of Sampling and Analysis								

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Method No.	FSSAI.FS.16.013.2023	**Revision No. & Date**	0.0
Scope	**Method for Determination of Phosphorous as (P_2O_5) in Fortified Salt**		
Caution	1. Sodium molybdate: It May cause eye, skin, and respiratory tract irritation. May be harmful if swallowed, inhaled, or absorbed through the skin. 2. Hydrazine sulfate: Hydrazine sulfate is a hazardous chemical. It May irritate and burn the eyes and skin. Breathing Hydrazine Sulfate can irritate the nose, throat and lungs causing coughing and shortness of breath. Exposure can cause dizzy and lightheaded. Higher levels can cause trembling, a feeling of excitement, and even convulsions.		
Principle	The method determines Phosphorous as **(P2O5)** in Fortified Salt in the presence of Hydrazine sulfate and Sodium molybdate followed by spectrophotometer measurement of phosphorous as blue phosphomolybdic acid.		
Apparatus/Instruments	1. General glassware and apparatus 2. Volumetric flasks – 50 mL, 100 mL, 250 mL and 500 mL with glass stoppers 3. Pipette – Mohr 's type 10 mL with 0.1 mL subdivision. 4. Spectrophotometer with 1.0 cm cuvettes. For use in the visible region		
Materials and Reagents	1. Sodium molybdate, reagent grade 2. Hydrazine sulphate, reagent grade 3. Potassium dihydrogen phosphate, reagent grade dried for 2 h at 101°C 4. Distilled Water		
Preparation of Reagents	1. Sodium molybdate - Carefully add 140 mL of concentrated sulphuric acid to 300 mL distilled water. Cool to room temperature and add 12.5 g of Sodium molybdate. Dilute to 500 mL with distilled water. Mix thoroughly and allow to stand for 24 h before use. 2. Hydrazine sulphate – 0.015% Dissolve 0.150 g hydrazine sulphate in 1 L water. 4. Standard Phosphate solution: **Stock solution(A)** – Dissolve 1.0967 g of dry Potassium dihydrogen phosphate in distilled water and make up to 250 mL in a volumetric flask The solution contains 1 mg phosphorous per Ml 5. **Working Solution (B)** – Dilute 5 mL of standard stock solution A with distilled water to 500 mL in a volumetric flask. This solution contains 0.01 mg phosphorous per mL. **Preparation of the standard curve: (0, 1, 2, 4, 8 & 10 µg/mL):** Pipette 0.0, 1.0, 2.0, 4.0, 8.0 and 10.0 mL of standard working solution into 50 mL volumetric Flasks & dilute to 10mL with water.		
Sample Preparation	1.Weigh accurately 3 – 4 gm of sample in 100mL Volumetric Flask. 2.Dilute to volume with water and mix thoroughly.		
Method of analysis	1.Take 10 mL sample solution in clean 50 mL volumetric flask.		

	2.Add 8 mL of hydrazine sulphate solution and 2 mL of sodium molybdate solution in this order in standard solution and sample solution. 3.Stopper and invert 3 – 4 times. Loosen the stopper and heat for 10 ± 0.5 minutes in a vigorously boiling water bath. 4.Remove from water bath and cool at room temperature. 5.Make the volume upto 50 ml with distilled water and mix thoroughly. 6.Transfer the solution to a clean dry cuvette and measure the absorbance at 650 nm in a spectrophotometer adjusted to read 0 % absorbance (100 % transmittance) for distilled water. 7.Plot curve the absorbance of each standard against its phosphorous content on a linear graph paper. 8.Measure the phosphorus content of the sample and the blank by comparison with the standard curve.
Calculation with units of expression	Concentration from calibration curve (µg/mL) X Volume made X 141.94 Phosphorous as P2O5 = ---------- (mg/Kg) Sample Weight (g) X 30.97
Inference (Qualitative Analysis)	*****
Reference	FSSAI 02.038:2021 of FSSAI Manual of methods of analysis of Food (oil and Fat)2021
Approved by	Scientific Panel on Methods of Sampling and Analysis