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 Iron in Fortified Rice Kernel by AAS**		
Method No.	FSSAI.FRK.16.007.2023	**Revision No. & Date**	0.0
Scope	The Scope of this Method is applicable for Quantification of Iron at 500 mg/Kg LOQ Level (with respect to the Sample) by using AAS. Limit of Detection 2.5 mg/Kg with respective to the Standard. Limit of Quantification 5.0 mg/Kg in with respective to the Standard. Limit of Quantification 500 mg/Kg in with respective to the Sample.		
Safety & Precautions	**1. Concentrated Nitric Acid** It is a Chemical which is corrosive to Metals. It causes severe skin burns and eye damage. It is toxic if inhaled. It is corrosive to the respiratory tract Following safety measures need to be taken during Handling of Concentrated Nitric Acid: a) Do not breathe dust/fume/gas/mist/vapors/spray b) Wash face, hands and any exposed skin thoroughly after handling c) Wear protective gloves/protective clothing/eye protection/face protection d) Use only outdoors or in a well-ventilated area Keep away from heat/sparks/open flames/hot surfaces. e) No smoking f) Keep/Store away from clothing/ other combustible materials g) Take any precaution to avoid mixing with combustibles h) Keep only in original container i) Wear respiratory protection **2. Hydrogen Peroxide** It is Oxidizing, Corrosive and Irritant chemical. Following safety measures need to be taken during Handling of Hydrogen Peroxide: When handling moderate-to-high concentrations of Hydrogen Peroxide in the workplace, ensure eyewash stations and safety showers are accessible, and use splash goggles, gloves, and an approved Vapor Respirator.		
Principle	Weigh 0.50 g (± 0.05 g) of Grinded Sample Transfer to Microwave Digestion Cool Vessel. Add 2.0 mL Milli Q Water, 1.0 mL Hydrogen Peroxide, add 5 mL of Nitric Acid digest in microwave digestor, extract the analyte in Nitric acid make up to 50 mL, Filter and Inject in AAS.		
Apparatus/Instruments	1. Atomic Absorption Spectrometry (AAS) 2. Microwave Digester 3. Analytical Balance 4. Micro Pipettes (20 -200 µl) & (100 -1000 µl) **Note:** *The make & model of Instrument can be changed. However, the Instrument should be able to achieve the desired LOD value.*		
Materials and Reagents	1. Concentrated Nitric Acid (Purity- 69%) 2. Hydrogen Peroxide (Purity -30%)		

<table>
<tr><td></td><td>3. CRM Used: Iron</td></tr>
<tr><td>Preparation of solutions</td><td>
<u>A) PREPARATION OF INTERMEDIATE STOCK SOLUTION - 1 (100 mg/Kg)</u>
1. Transfer 10.0 ml from stock solution of iron (1000 mg/Kg) in 100 ml volumetric flask.
2. Add 5.0 ml nitric acid and made up the volume till 100 ml volumetric flask by Milli-Q water and mix by Vortex Shaker Mixer.
<u>B) PREPARATION OF BRACKETING STANDARD SOLUTION (10 mg/Kg)</u>
1. Transfer 1.00 ml from Intermediate Standard Solution-1 of Iron (100 mg/Kg) in 10 ml volumetric flask.
2. Add 0.5 ml Nitric Acid and made up the volume till 10ml volumetric flask by Milli-Q water and mix by Vortex Shaker Mixer.
C) <u>PREPARATION OF BLANK (5% NITRIC ACID)</u>
1. Transfer 7.25 mL of Nitric Acid (69%) in 100 mL Milli Q Water in Glass Bottle and Mix well.
D) <u>PREPARATION OF CALIBRATION STANDARD SOLUTIONS</u>
1. Use Intermediate Standard Solution-1 for preparing Calibration Standard Solutions as mentioned in below Table.
<table>
<tr><th>CAL. STANDARD SOLUTIONS</th><th>ISS - 1 (mg/Kg)</th><th>VOL. OF ISS - 1 (mL)</th><th>VOL. OF NITRIC ACID (mL)</th><th>FINAL VOL. (mL)</th><th>FINAL CONC. (mg/Kg)</th></tr>
<tr><td>LS 6</td><td>100</td><td>8.00</td><td>0.5</td><td>10</td><td>80.0</td></tr>
<tr><td>LS 5</td><td>100</td><td>6.00</td><td>0.5</td><td>10</td><td>60.0</td></tr>
<tr><td>LS 4</td><td>100</td><td>4.00</td><td>0.5</td><td>10</td><td>40.0</td></tr>
<tr><td>LS 3</td><td>100</td><td>2.00</td><td>0.5</td><td>10</td><td>20.0</td></tr>
<tr><td>LS 2</td><td>100</td><td>1.00</td><td>0.5</td><td>10</td><td>10.0</td></tr>
<tr><td>LS 1</td><td>100</td><td>0.50</td><td>0.5</td><td>10</td><td>5.0</td></tr>
</table>
CAL : Calibration
ISS : Intermediate Stock Solution
VOL : Volume
LS : Linearity Solution
NOTE: Use freshly prepared Standard solutions for the analysis.
</td></tr>
<tr><td>Sample Preparation</td><td>
<u>PREPARATION OF SAMPLE SOLUTION</u>
1. Grind 50g sample as fine as possible.
2. Weigh 0.50 g (± 0.05 g) Grinded Sample.
3. Transfer to Microwave Digestion Closed (MDC) Vessel.
4. Heat Milli Q Water at 60 ^{0}C.
5. Add 2.0 mL of Hot Milli-Q water.
6. Add 1.0 mL Hydrogen Peroxide.
7. Add 5.0 mL of Nitric Acid.
8. Close the Microwave Vessel tightly.
9. Keep at Room Temperature for 5 minutes.
10. Keep the Vessel rotor in Microwave Digester.
11. Cool the Vessel at Room Temperature after Digestion.
</td></tr>
</table>

12. Add 10 mL of Milli Q water.
13. Mix well.
14. Transfer to 50 mL Volumetric Flask.
15. Volume make-up to 50 mL with Milli-Q water.
16. Filter and use for the injection on AAS.

Method of analysis

a) Instrument : AAS

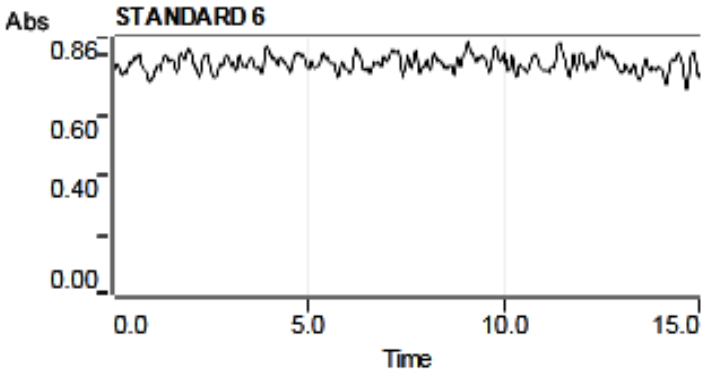
b) Equipment Conditions : As detailed in below Table

Hallow Cathode Lamp	Iron (as Fe)
Lamp Current	5 (mA)
Absorption Wavelength	372.0
Slit Width(nm)	0.2
Signal – Type	Atomic Absorption
Signal Measurement	Integration
Oxidant	Air
Oxidant Flow(L/Min)	13.5
Acetylene Flow	2
Equation	Linear
Read Parameters	
Time(Sec)	10
Delay time(Sec)	10

c) Microwave Digestion Program

SL. NO	RAMPING STAGE	HOLD TIME (Minutes)	TEMP (^{0}C)	POWER (Watt)
1	01	20	180	800
2	02	10	160	800
3	03	10	140	800
4	COOL DOWN	10	-	-

<table>
<tr><td>Batch Organization</td><td><u>Injection Sequence</u>
<table>
<tr><th>SL.NO.</th><th>NAME OF INJECTIONS</th><th>NUMBER OF INJECTIONS</th></tr>
<tr><td>1</td><td>Blank</td><td>2</td></tr>
<tr><td>2</td><td>Linearity Solution (LS) - 1</td><td>1</td></tr>
<tr><td>3</td><td>Linearity Solution (LS) - 2</td><td>1</td></tr>
<tr><td>4</td><td>Linearity Solution (LS) - 3</td><td>1</td></tr>
<tr><td>5</td><td>Linearity Solution (LS) - 4</td><td>1</td></tr>
<tr><td>6</td><td>Linearity Solution (LS) - 5</td><td>1</td></tr>
<tr><td>7</td><td>Linearity Solution (LS) - 6</td><td>1</td></tr>
<tr><td>9</td><td>Blank</td><td>2</td></tr>
<tr><td>10</td><td>Sample Solution</td><td>1</td></tr>
<tr><td>11</td><td>Blank</td><td>2</td></tr>
<tr><td>12</td><td>Bracketing Standard Solution</td><td>1</td></tr>
<tr><td colspan="2">TOTAL INJECTIONS</td><td>14</td></tr>
</table></td></tr>
<tr><td>Calculation with units of expression</td><td>a) Carry out a regression analysis and calculate Regression coefficient (R^2) by analyzing the calibration standards by fitting the data into a linear regression curve, including zero as the response for the reagent blank.

Iron (mg/Kg) = <u>Instrument Conc.(mg/Kg) X Make-up Volume (mL)</u>
Sample Weight (g)</td></tr>
<tr><td>Results</td><td> 
</td></tr>
<tr><td>LOD & LOQ</td><td>Limit of Detection 2.5 mg/Kg with respective to the Standard.
Limit of Quantification 5.0 mg/Kg in with respective to the Standard.
Limit of Quantification 500 mg/Kg in with respective to the Sample.</td></tr>
<tr><td>Reference</td><td>RPT/MT/FRK/2023/001, Method Validation Report for Estimation of Iron in Fortified Rice Kernel by Using AAS.

AOAC 2011.14: Determination of Minerals and Trace elements in Milk & Milk Products, Infant Formula, and Adult Nutrition.</td></tr>
<tr><td>Approved by</td><td>Scientific Panel on Methods of Sampling and Analysis</td></tr>
</table>