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 Vitamin Mineral Premix for Preparation of Fortified Rice Kernel (FRK)**		
Method No.	FSSAI.VMP-FRK.16.008.2023	**Revision No. & Date**	0.0
Scope	The Scope of this Method is Applicable for Quantification of Iron in Premix at 5000 mg/Kg LOQ Level (with respect to the Sample) by Using Atomic Absorption Spectroscopy (AAS). Limit of Detection 12.5 mg/Kg with respective to the Standard. Limit of Quantification 25.0 mg/Kg with respective to the Standard. Limit of Quantification 5000 mg/Kg with respective to the Sample.		
Caution (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) Keep/Store away from clothing/ other combustible materials f) Take any precaution to avoid mixing with combustibles g) Keep only in original container h) Wear respiratory protection **2. Hydrogen Peroxide** It is Oxidizing, Corrosive and Irritant chemical. 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	Nitric acid, and hydrogen peroxide are added to homogenized Vitamin Premix sample in microwave vessels, and digested using a preprogramed temperature control. Analysis is performed by AAS.		
Apparatus/Instruments	1. Atomic Absorption Spectroscopy - AAS 2. Microwave Digester 3. Analytical Balance 4. Micro Pipettes (20 -200 µL) & (100 -1000 µL)		
Materials and Reagents	1. Concentrated Nitric Acid (Purity- 69%) - Suprapure 2. Hydrogen Peroxide (Purity -30%) – LR Grade 3. CRM / Standard Stock Solution - Iron (Purity - 1000 mg/Kg)		

<table>
<tr><td></td><td>4. Purity of Argon and other gas, if used must fulfill the standard of instrument requirement</td></tr>
<tr><td>Sample Preparation</td><td><u>PREPARATION OF SAMPLE SOLUTION</u>

1. Weigh 0.50 g (± 0.05 g) of Homogenized Sample.
2. Transfer to Microwave Digestion Closed (MDC) Vessel.
3. Heated Milli Q Water at 60 °C.
4. Add 2.0 mL of Hot Milli-Q water.
5. Add 1.0 mL Hydrogen Peroxide.
6. Add 5.0 mL of Nitric Acid.
7. Close the Microwave Vessel tightly.
8. Kept at Room Temperature for 5 minutes.
9. Kept the Vessel rotor in Microwave Digester.
10. Cool the Vessel at Room Temperature after Digestion.
11. Add 10 mL of Milli Q water.
12. Mixed well.
13. Transfer to 100 mL Volumetric Flask.
14. Volume make-up to 100 mL with Milli-Q water.
15. Filter and use this for injecting on AAS.

<u>Note</u>: If required, dilute the sample for the desired concentration.</td></tr>
<tr><td>Method of Analysis
(a) Preparation of Standard solutions</td><td>A) <u>PREPARATION OF BLANK (5% NITRIC ACID)</u>

Transfer 7.25 mL of Nitric Acid (69%) in 100 mL Milli Q Water in Glass Bottle Mix well. Shake Vigorously.
B) <u>PREPARATION OF CALIBRATION STANDARD SOLUTIONS</u>
Use Intermediate Standard Solution-1 for Preparing Calibration Standard Solutions as mentioned in below Table.

<table>
<tr><th>CAL. STANDARD SOLUTIONS</th><th>SSS (mg/Kg)</th><th>VOL. OF SSS (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>1000</td><td>1.50</td><td>0.5</td><td>10</td><td>150</td></tr>
<tr><td>LS 5</td><td>1000</td><td>1.25</td><td>0.5</td><td>10</td><td>125</td></tr>
<tr><td>LS 4</td><td>1000</td><td>1.00</td><td>0.5</td><td>10</td><td>100</td></tr>
<tr><td>LS 3</td><td>1000</td><td>0.75</td><td>0.5</td><td>10</td><td>75</td></tr>
<tr><td>LS 2</td><td>1000</td><td>0.50</td><td>0.5</td><td>10</td><td>50</td></tr>
<tr><td>LS 1</td><td>1000</td><td>0.25</td><td>0.5</td><td>10</td><td>25</td></tr>
</table>
CAL : Calibration
SSS : Standard Stock Solution
VOL : Volume
LS : Linearity Solution

NOTE: Use Freshly Prepared Standard solutions for the Analysis.

C) <u>PREPARATION OF BRACKETING STANDARD SOLUTION (50 mg/Kg)</u>
1. Transfer 0.5 ml from Standard Stock Solution of Iron (1000 mg/L) in 10 ml volumetric flask.
2. Add 0.5 ml nitric acid and made up the Volume till 10 ml volumetric flask by Milli-Q water and mix by Vortex Shaker Mixer.</td></tr>
</table>

(b) Instrument Details

a) **Instrument** : Atomic Absorption Spectrometer

b) **Equipment Conditions** : As detailed in below Table

Hollow cathode Lamp	Iron (Fe)
Lamp Current (mA)	5.0
Absorption Wavelength (nm)	372.0
Slit Width (nm)	0.2
Signal-Type	Atomic Absorption
Signal -Measurement	Integration
Oxidant	Air
Oxidant Flow (L/min)	13.5
Acetylene Flow (L/min)	2
Equation	Linear
Read Parameter	
Time (sec)	10
Delay time (sec)	10

c) Microwave Digestion Program

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

Note: *The make & model of Instrument can be changed. However, the Instrument should be able to achieve the desired LOD &LOQ value.*

<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>8</td><td>Blank</td><td>2</td></tr>
<tr><td>9</td><td>Sample Solution</td><td>1</td></tr>
<tr><td>10</td><td>Blank</td><td>2</td></tr>
<tr><td>11</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>Carry out analysis and calculate Regression coefficient (R^2) by analyzing the calibration standards by fitting the data into a linear regression curve, including zero.

Calculate the Iron Content in Vitamin Premix using the following equation:

Iron (mg/Kg) = <u>Instrument Conc. (mg/Kg) X Make-up Volume (mL)</u>
Sample Weight (gm)</td></tr>
<tr><td>LOD & LOQ</td><td>Limit of Detection 12.5 mg/Kg with respective to the Standard.
Limit of Quantification 25.0 mg/Kg with respective to the Standard. Limit of Quantification 5000 mg/Kg with respective to the Sample.</td></tr>
<tr><td>Inference (Qualitative Analysis)</td><td>PRT/MT/PRM/2023/001, Method Validation Protocol for Estimation of Iron in Premix Using Atomic Absorption Spectroscopy.

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>