fssai FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA *Inspiring Trust, Assuring Safe & Nutritious Food*	**Method for Determination of Iron in Fortified Rice**		
Method No.		**Revision No. & Date**	
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. **3. Zinc** Zinc metal used to coat steel . Following safety measures need to be taken during Handling of Zinc:		

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(to be written depending upon concerned manual)

	Zinc oxide fumes may cause milk local irritation to eyes, nose, throat and upper airways. Acute over exposure of zinc oxide fume may cause meal fume fever, flu like symptoms such as chills, fever, nausea and vomiting which may be delayed 3- 10 hrs in onset. Store in dry covered area, Sepreate from in compatible material. Zinc ingots suspected of containing moisture should be thoroughly dried before being added to a molten bath. Entrained moisture will expand explosively when immersed in a molten bath.
Scope	The Scope of this Method is applicable for Quantification of Iron at 10 PPM LOQ Level (with respect to the Sample) by using ICP-MS.
Principle	Weigh 0.25 g (± 0.02 g) Grinded Sample. Transfer to Microwave Digestion Closed (MDC) Vessel. Transfer to Microwave Digestion Cool Vessel. Heat Milli Q Water at 60 ^{0}C. Add 2.0 mL of Hot Milli-Q water, 1.0 mL Hydrogen Peroxide, Add 5 mL of Nitric Acid. Close the Microwave Vessel tightly. Keep at Room Temperature for 5 minutes. Keep the Vessel rotor in Microwave Digester , Cool it, Add 10 mL of Milli Q water & Mix well. Make upto 50 mL with Milli- Q Water.
Apparatus/Instruments	1. Inductively Coupled Plasma Mass Spectrometry (ICP-MS) 2. Microwave Digester 3. Analytical Balance
Materials and Reagents	1. Concentrated Nitric Acid (Purity- 69%) (Make: Finar) 2. Hydrogen Peroxide (Purity -30%) (Make: Rankem) 3. CRM Used : Iron (1.19781.0500, Merck)
Preparation of solutions	**PREPARATION OF INTERMEDIATE STOCK SOLUTION - 1 (100 PPM)** 1. The Standard of 974 PPM is used directly as Stock Solution. 2. Pipette out 1.027 mL of Stock Solution. 3. Transfer to a 10 mL Amber Colored Volumetric Flask containing 2 mL of Milli Q Water. 4. Add 0.5 mL Nitric Acid. 5. Add Milli Q Water for Volume make-up to 10 mL. 6. Mixed by using Vortex Shaker Mixer. **PREPARATION OF INTERMEDIATE STOCK SOLUTION-2 (10 PPM)** 1. Pipette out 1.0 mL of Intermediate Stock Solution -1. 2. Transfer to a 10 mL Amber Colored Volumetric Flask containing 2 mL of Milli Q Water. 3. Add 0.5 mL Nitric Acid.

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4. Add Milli Q Water for Volume make-up to 10 mL.
5. Mix by using Vortex Shaker Mixer.

PREPARATION OF BLANK (5% NITRIC ACID)

1. Transfer 72.50 mL of Nitric Acid (69%) in 1000 mL Milli Q Water in Glass Bottle of Mobile Phase.
2. Mix well.

PREPARATION OF CALIBRATION STANDARD SOLUTIONS

1. Use Intermediate Stock Solution - 1 for preparing Calibration Standard Solutions as mentioned in below Table.

Cal. Standard Solution	ISS - 1 (100 PPM)	VOL. OF ISS – 1 (mL)	VOL. OF NITRIC ACID (mL)	FINAL VOL. (mL)	FINAL CONC. (PPM)
LS 7	100	2.00	5	100	2.00
LS 6	100	1.50	5	100	1.50
LS 5	100	1.00	5	100	1.00
LS 4	100	0.50	5	100	0.50
LS 3	100	0.25	5	100	0.25
LS 2	100	0.10	5	100	0.10
LS 1	100	0.05	5	100	0.05

CAL : Calibration
ISS : Intermediate Stock Solution
VOL : Volume
LS : Linearity Solution

NOTE: Use freshly prepared Standard solutions for the analysis.

Sample Preparation

PREPARATION OF SAMPLE SOLUTION

1. Homogenize the Sample by Grinding as finely as possible.
2. Weigh 0.25 g (± 0.02 g) Grinded Sample.
3. Transfer to Microwave Digestion Closed (MDC) Vessel.
4. Transfer to Microwave Digestion Cool Vessel.
5. Heat Milli Q Water at 60 ^{0}C.
6. Add 2.0 mL of Hot Milli-Q water.
7. Add 1.0 mL Hydrogen Peroxide.
8. Add 5 mL of Nitric Acid.
9. Close the Microwave Vessel tightly.
10. Keep at Room Temperature for 5 minutes.
11. Keep the Vessel rotor in Microwave Digester
12. Cool the Vessel at Room Temperature after Digestion.

13. Add 10 mL of Milli Q water.
14. Mix well.
15. Transfer to 50 mL Volumetric Flask.
16. Volume make-up to 50 mL with Milli-Q water.

Method of analysis

a) Instrument : ICP-MS Spectrometer.

b) Make & Model : Agilent -7700.

c) Chromatographic Conditions : As detailed in below Table

Plasma condition	a) Plasma Flow-Argon (15L /min) b) Nebulizer pump uptake speed (0.5 rps) c) RF power 1550 watts	
S/C Temperature	2°C	
Uptake Time	40 Sec	
Delay Time	40 Sec	
Stabilize Time	40 Sec	
Nebulizer Flow	1.0 mL/Min	
Reaction Cell	ORS and KED with Helium Flow:3.8 mL/Min	
Numbers of Replicates	3.0	
Detector's parameters	5 mV	
Mode	He	
TMP Revolution	100 %	
Auto sampler conditions	Working Mode	Continuous
	Wash	Between runs

d) 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	-	-

Calculation with units of expression

$$\text{Iron (PPM)} = \frac{\text{Instrument Conc. X Make-up Volume}}{\text{Sample Weight X1000}}$$

a) Carry out a regression analysis and calculate Regression coefficient (R2) by analyzing the calibration standards by fitting the data into

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<table>
<tr><td></td><td>a linear regression curve, including zero as the response for the reagent blank.

b) The LOD and LOQ are determined by considering the S/N of 3 and 10, respectively, for the Zinc in the matrix.

c) Determine the recovery of folic acid by the external spiking method at three different spike levels (10,20 & 30 mg/kg) in six replicates.
d) Calculate the recovery value using the following equation:
e) Recovery (%) = $\frac{(A - B) \times 100}{C}$
where
A = the concentration of Zinc in the spiked sample (mg/Kg)
B = the natural content of Zinc in the control sample (mg/Kg)
C = the spiked concentration of Zinc (mg/Kg)</td></tr>
<tr><td>Results</td><td>
<table>
<tr><td>Sample Name</td><td>SAMPLE SOLUTION</td></tr>
<tr><td>Sample Type</td><td>Sample</td></tr>
<tr><td>Comment</td><td>---</td></tr>
<tr><td>Prep Dilution</td><td>1.0000</td></tr>
<tr><td>Auto Dilution</td><td>1.0000</td></tr>
<tr><td>Total Dilution</td><td>1.0000</td></tr>
<tr><td>Operator Name</td><td>saidula</td></tr>
<tr><td>Acq Mode</td><td>Spectrum</td></tr>
<tr><td>Cal Title</td><td>---</td></tr>
<tr><td>Cal Type</td><td>External Calibration</td></tr>
<tr><td>Last Calib</td><td>02/11/2022 18:41:21</td></tr>
<tr><td>Bkg File</td><td>---</td></tr>
<tr><td>Bkg Mode</td><td>Count Subtraction except for ISTD</td></tr>
<tr><td>FQ BlankFile</td><td>---</td></tr>
<tr><td>VIS Fit</td><td>Point to Point</td></tr>
</table>
FullQuant Table
<table>
<tr><th>Element</th><th>Mass</th><th>Tune Mode</th><th>Conc.</th><th>Units</th><th>RSD(%)</th><th>CPS</th><th>Det.</th><th>Rep</th></tr>
<tr><td>Fe</td><td>56</td><td>He</td><td>41.609</td><td>ppb</td><td>2.3</td><td>708422.23</td><td>Pulse</td><td>3</td></tr>
</table>
</td></tr>
<tr><td>LOD & LOQ</td><td>a) Limit of Detection 4 mg/Kg in with respective to the Sample.
b) Limit of Quantification 10 mg/Kg in with respective to the Sample.</td></tr>
<tr><td>Reference</td><td>Method Protocol: PRT/MT/FTR/2022/002, Report for Determination of Iron content.

AOAC 2011.14: Determination of Minerals and Trace elements in Milk & Milk Products, Infant Formula, and Adult Nutrition.</td></tr>
</table>

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Approved by	Scientific Panel on Methods of Sampling and Analysis

The following 'note' need to be added in all manuals:

Note: The test methods given in the manual are standardised/ validated/ taken from national or international methods or recognised 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".

Editorials (For Reference purpose while writing methods)

Abbreviations to be used

Microgram	µg
Milligram	mg
Gram	g
Kilogram	kg
Milliliter	mL
Litre	L
Second	sec
Minute	min
Hour	h
Celsius	°C
Kelvin	°K
Centimeter	cm
Millimeter	mm
Molar	M
Millimolar	mM
Micromolar	µM
Mole	mol
Normal	N
Wavelength	nm

Some Editorials for the manuals

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Space between numbers and units

- Mass and volume need spaces 12 g not 12g, 100 mL not 100mL
- Time also needs space 10 h not 10h, 15 min not 15min
- Temperatures need spaces
  - between value and degree sign: **37 °C**, not 37° C or 37°C
  - but the degree sign for angles goes with the number: 90º angle
- Centrifugal forces need spaces
  - on both sides of the "×" (remember not x)
  - 10,000 × g, not 10,000g or 10,000xg
- Other "places for spaces"
  - around equals sign: **n = 3**, not n=3
    - also around >, <, ~, etc
  - around plus/minus: 29 ± 7, not 29±7
- Percentages is the only exception
  - **5%** serum, **0.01%** bromophenol blue
  - This is because % is not really a unit, just an indication that the value is presented as the "ratio to 100"
  - **a <u>space</u> is required**: 10 mM or 6 M, never 10mM or 6M
- Use numerals to express numbers 10 and above.
- Use words to express numbers below 10.
- Use numerals when you have 3 or more numbers in a series, even if each of the numbers is below 10.
- When numbers begin a sentence, you must write them out in words.
- Situations in which Numbers Should be Given as Numerals

General Guideline	Examples
All numbers 10 and above	Trial 14; 35 animals; 16 genera of legumes
All numbers that immediately precede a unit of measurement	A wing 10 cm long; 5 mg of drug; 21days
Numbers with decimals; fractions that include whole numbers	7.38 mm; $4^1/_2$ hours
Numbers that represent statistical or mathematical functions or results, percentages, ratios	Multiply by 5; fewer than 6%; 3.75 times as many; the 2nd quartile
Numbers that represent exact times or dates; ages; size of samples, subsamples or populations; specific numbers of subjects in an experiment; scores and points on a scale; exact sums of money; and numerals as numerals	About 3 weeks ago, at 1:00 a.m. on January 25, 2000, the 25-year-old patients with IQ scores above 125 all awoke simultaneously in the nursing home at 125 Oak Street. They were paid $25 apiece to go back to sleep
Numbers below 10 that are grouped for comparison with numbers 10 and above in the same paragraph	4 of 16 analyses, the 1st and 15th of the 25 responses; lines 2 and 21
Numbers that denote a specific place in a numbered series, parts of books and tables, and each number in a list of four or more numbers	Trial 6; Grade 9 (but the ninth grade); the groups consisted of 5, 9, 1, and 4 animals respectively

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17. Homogenize the Sample by Grinding as finely as possible.