FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India	**Determination of Iron as Fe in Fortified Rice Kernel (FRK)**		
Method No.	FSSAI.FRK.16.004.2023	**Revision No. & Date**	0.0
Scope	The method is applicable for estimating the iron content in FRK using Inductively Coupled Plasma (ICP)-Optical Emission Spectrometer (ICP-OES).		
Caution	**Concentrated Nitric Acid** is highly corrosive and can cause irritation to the eyes, skin, and mucous membrane. Always add acid to water to prevent splattering from overheating and boiling. Clean-up spills promptly with appropriate materials. Handle only inside a fume hood **Hydrogen Peroxide:** Hydrogen Peroxide is a strong oxidising agent that also has corrosive properties. Keep hydrogen peroxide away from sources of ignition, heat, and moisture, storing in a tightly closed container. Keep away from incompatible materials such as organic materials, metals, acids, alkalis, combustible materials, and oxidizing agents. **Operation of Microwave Digester** involves a hot pressurized acid solution. Use appropriate personal protective equipment, face protection such as a laboratory coat, safety glasses, rubber gloves, and a fume hood.		
Principle	Nitric acid, and hydrogen peroxide are added to the sample in microwave vessels, and the samples are digested using preprogrammed temperature control. The addition of hydrogen peroxide helps reduce carbon and nitrous oxide levels in the digestate. Analysis is performed by an ICP-OES. Quantitation of Fe is achieved essentially simultaneously by comparing the analyte–ISTD response ratios in the unknown samples with a standard curve constructed from the response ratios of calibration standards		
Apparatus/Instruments	1. Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) 2. Microwave digester. —A commercial microwave designed for laboratory use at 0–300°C, with a closed-vessel system and controlled temperature ramping capability. Use manufacturer recommended vessels. 3. Analytical Balance (capable of weighing 0.0001 g) 4. Fume hood. 5. Bottle-top dispenser. —PTFE; Adjustable volume 0.5–5 mL. 6. Volumetric pipets. —Class A, assorted sizes. 7. Digital pipets- 1 mL adjustable, to deliver 500 µL with accuracy tolerance of better than 0.8% and precision of better than 0.2% RSD.		
Materials and Reagents	1. Concentrated Nitric acid (Purity- 69%) 2. Hydrogen peroxide (Purity -30%) 3. CRM / Stock Solution - Iron (Purity - 1000 mg/Kg) 4. Purity of Argon and other gas, if used must fulfill the standard of instrument requirement		

Sample Preparation

1. Grind 50 g of FRK sample.
2. Weigh 0.25 g (± 0.05 g) of ground kernels.
3. Transfer to microwave digestion closed vessel.
4. Add 2.0 mL of Hot (60 °C) Milli-Q Water
5. Add 1.0 mL H_2O_2.
6. Add 0.5 mL of Nitric acid.
7. Loosely cap the vessel and keep at 25 °C for 5 min to predigest the sample.
8. Close the microwave vessel tightly.
9. Keep at 25 °C for 5 min.
10. Place the vessel rotor in microwave digester.
11. Keep the vessel rotor in microwave digester* and execute a heating program equivalent to that shown in the Table below for total digestion of the sample

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	-	-

12. Cool the vessel to 25 °C after digestion.
13. Add 10 mL of Milli Q water and mix well using a vortex.
14. Transfer to a 100 mL volumetric Flask.
15. Make-up the volume to 100 mL with Milli-Q water.
16. Filter and use for ICP-OES analysis.

Preparation of Standard solutions

A) Preparation of intermediate stock solution - 1 (ISS-1) (100 mg/kg)

1. Transfer 1.0 mL from stock solution of iron (1000 mg/kg) in 10 mL volumetric flask.
2. Add 0.5 mL Nitric acid and make up the volume to 10 mL using Milli-Q water and mix using a vortex.

B) Preparation of blank (5% Nitric acid)

1. Transfer 7.25 mL of Nitric Acid (69%) into 92.75 mL of Milli Q water in a glass bottle. Mix well**.**

C) Preparation of calibration standard solutions

Prepare the calibration standard solutions using the ISS-1 as indicated in the Table below.

Cal. Standard Solution	ISS - 1 (100 mg/mL	VOL. OF ISS – 1 (mL)	VOL. OF Nitric acid (mL)	Final vol. (mL)	Final Conc. (mg/mL)
LS 7	100	2.00	0.5	10	20.0

LS 6	100	1.50	0.5	10	15.0
LS 5	100	1.00	0.5	10	10.0
LS 4	100	0.75	0.5	10	7.5
LS 3	100	0.50	0.5	10	5.0
LS 2	100	0.20	0.5	10	2.0
LS 1	100	0.10	0.5	10	1.0

NOTE: Use freshly prepared calibration standard solutions for the analysis.

Method of analysis

Instrument: ICP-OES

Equipment conditions:

Plasma condition	Plasma flow (Argon 12 L/min) Nebulizer flow (0.7 L/min) RF power 1.2 kW
Uptake Delay	25 sec
Pump Speed	12 rpm
Stabilization	15 sec
Numbers of Replicates	3.0
Resolution	Normal
Wavelength	238.204 nm For Iron
Read Time	5 sec
Aux flow	1.0 L/min
Viewing Mode	Radial

Note: ***The make & model of instrument may be changed. Instrument tuning varies with make and model. Set parameter as per manufacturer's instructions and optimize for best resolution to obtain the desired LOD e.***

The injection sequence for standards and sample is given below

SL.NO.	Sample	Number of injections
1	Blank	2
2	Linearity Solution (LS) - 1	1
3	Linearity Solution (LS) - 2	1
4	Linearity Solution (LS) - 3	1
5	Linearity Solution (LS) - 4	1
6	Linearity Solution (LS) - 5	1
7	Linearity Solution (LS) - 6	1
8	Linearity Solution (LS) - 7	1
9	Blank	2
10	Sample Solution	1
11	Blank	2

<table>
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<tr><td>12</td><td>Spike sample</td><td>1</td></tr>
<tr><td colspan="2">TOTAL INJECTIONS</td><td>15</td></tr>
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<tr><td>Calculation with units of expression</td><td colspan="3">
a) Carry out a regression analysis and calculate Regression coefficient (R^2) by analyzing the calibration standards including zero as the response for the reagent blank. Should be >0.99
Calculate the Fe content in FRK using the following equation:

$Iron\frac{(Fe)\ mg}{kg} = \frac{C\ \times Makeup\ volume}{Sample\ weight\ (g)}$
Where
C= concentration from instrument software

The LOD and LOQ are determined by considering the S/N of 3 and 10, respectively, for the Iron in the matrix.
i. Limit of Detection 0.5 mg/Kg with respective to the Standard.
ii. Limit of Quantification 1.0 mg/Kg in with respective to the Standard.
iii. Limit of Quantification 400 mg/Kg in with respective to the sample
b) Determine the recovery of Iron by the external spiking method at a spike level of 2000 mg/Kg in six replicates.
Calculate the recovery value using the following equation:
$$Recovery(\%) = \frac{(\mathrm{A} - \mathrm{B})}{\mathrm{C}} \times 100$$
where
A = the concentration of Iron in the spiked sample (mg/kg)
B = the natural content of Iron in the control sample (mg/kg)
C = the spiked concentration of Iron (mg/kg)
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<tr><td>Reference</td><td colspan="3">PRT/MT/FRK/2022/006, Method Validation Protocol for Estimation of Iron in Fortified Rice Kernel by Using ICP OES.
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 colspan="3">Scientific Panel on Methods of Sampling and Analysis</td></tr>
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