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8. Chemical Analysis Methods for Fortified Foods

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8.1 Fortified Foods:

Substances called fortificants are added to food in order to increase the quantity of essential nutrients in foods so as to improve the quality of nutrition thereby providing a better public health. Foods that have undergone the process of fortification are called Fortified foods.

The essential dietary nutrients include Vitamins, Minerals and trace elements which are required for the health benefits of the public. Fortified foods are produced by using the micro-nutrients and raw materials specified under the Food Safety and Standards (Food product standards and Food Additives) Regulations, 2011. The fortified foods have become a necessity to address the nutrient deficient malnutrition.

8.2 Articles and Fortificants:

In India the following articles are fortified which are being consumed by a large number of populations.

Table 8.1: Articles and Fortificants

Articles	Fortificants
Salt	Iron, Iodine
Rice	Iron, Folic acid, Vitamin B12
Milk	Vitamin A, D
Oil	Vitamin A, D
Wheat Flour, Maida	Iron, Folic acid, Vitamin B12
Fruit Juice	Vitamin C

8.2.1 Process of Fortification Double Fortified Salt:

The double fortified Salt can be prepared by mixing of the Raw salt with Ferrous Sulphide Premix, to this mixture the Sodium hex metaphosphate is added and blended to prepare the double fortified Salt, another method is raw salt is added to premix of ferrous fumarate and is blended smoothly to get fortified salt as fine powder.

8.2.2 Fortified Rice Kernel:

The Vitamin/mineral premix is added to the pulverized milled rice and by Extrusion Processing; the fortified rice kernels are produced.

8.2.3 Fortified Wheat Flour:

Micronutrients such as Iron, Folic acid and Vitamin B12 are added in the form of a premix through a feeder at the end of milling process.

8.2.4 Fortified Milk:

Prior to pasteurization, the liquid ilk is fortified and is essential to ensure an equal distribution of the nutrients in milk before any heat treatment. The best way to fortify dried milk is to blend Vitamins and Minerals in dry form with dried milk powder, even oily forms can also be added.

8.2.5 Fortified Oil:

The preblend (premix of vitamins and a small amount of oil) is added to the refined oil and by the process of mixing either by batch process or continuous process, the fortified oil is produced.

8.3 Determination of Fortified Foods:

By using appropriate methods of Chromatography, Spectroscopy and Spectrometry various types of fortified foods can be identified. Some of these methods are available with the schedule of Food Safety and Standards Authority of India (FSSAI),

Fortification of foods that having phenolic content has increased because of their beneficial physiological effects. However, the analysis of polyphenols is a difficult task due to the complexity of the matrix. The main challenge is that polyphenols can interact with other food components, such as carbohydrates and proteins.

The chemical reactions that occur during the baking technologies in the bakery and biscuit industry may also affect the results of measurements. The analysis of polyphenols found in fortified foods can be done by several techniques, such as liquid chromatography (HPLC and UPLC), gas chromatography (GC), or spectrophotometry (TPC, DPPH etc.).

8.3.1 Method for Determination of Iron in Fortified Rice:

Weigh 0.25 g (± 0.02 g) of the ground sample and transfer to Microwave Digestion Closed (MDC) Vessel. Heat Milli Q Water at 60°C. Add 2.0 ml of Hot Milli-Q water, 1.0 ml Hydrogen Peroxide, 5 ml of Nitric Acid to it. 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 up to 50 ml with Milli- Q Water. 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. Limit of Detection 4 mg/kg in with respective to the Sample. Limit of Quantification 10 mg/kg in with respective to the Sample.

8.3.2 Method for Determination of Folic Acid (Vitamin B9) in Fortified Rice:

Powder samples were reconstituted by dissolving 5 g powder and add 0.1 gm. of Ascorbic acid and 15 ml of 0.1 M Potassium Hydrogen Phosphate Buffer. Maintain the pH of the Sample Solution between 8.0-9.0 using 1M Potassium Hydroxide Solution (KOH).

Make pH of the sample solution to 7.0 with 2 N. Add 0.125 g of α-amylase into the sample solution. Place 25 ml amber colored volumetric flask containing Sample Solution on the Water Bath at 55°C. Do Volume make-up to 25 ml with 0.1 M Potassium Hydrogen Phosphate Buffer. Shake vigorously and centrifuge at 6000 rpm. Filter through 0.45 µm membrane into an amber LC Vial for UHPLC MS/MS Analysis.

The Scope of this Method is applicable for Quantification of Folic Acid (Vitamin B9) at 10 ppb LOQ Level (with respect to the Sample) by using LC-MS/MS in Fortified Rice Limit of Detection (5 ppb) with respective to the Sample. Limit of Quantification (10 ppb) with respective to the Sample.

8.3.3 Method for Determination of Cyanocobalamin (Vitamin B12) in Fortified Rice:

Weigh 10 g (± 0.5 g) of homogenized sample. Add 50 mg α-amylase and 20 ml of 0.25 M Sodium Acetate buffer. Vertex & sonicate for 20 minutes, add 50 ml of 0.25 M Sodium Acetate Buffer. Sonicate & Centrifuge @ 6000rpm at 4ºC, pass through 900 mg of C18 SPE cartridge, Pass 20 ml of filtrate.

Elute the solution and transfer the collected Sample Solution in to the Vial and use this for injecting into LC-MS/MS. The scope of this method includes for Quantification of Cyanocobalamin (Vitamin B12) at 0.5 ppb LOQ Level (with respect to the Sample) by using LC-MS/MS. Limit of Detection is 0.25 µg/kg with respect to the sample. Limit of Quantification is 0.5 µg/kg with respect to the sample.

8.3.4 Determination of Folic Acid (Vitamin B9) in Wheat Flour:

Folic acid is extracted from the sample using pancreatic and L-ascorbic acid. After incubation the extract is filtered, diluted with water, and applied to an immuno-affinity column (IAC) containing antibodies specific to folic acid. Folic acid is quantified by reversed-phase liquid chromatography (RP-HPLC) with UV detection, the method is applicable for the determination of Folic acid, in fortified rice and wheat flour.

8.3.5 Method for Determination of Zinc in Fortified Wheat & Rice Flour Using ICP-MS:

Nitric acid, and hydrogen peroxide are added to homogenized wheat/rice flour sample in microwave vessels, and digested using a preprogrammed temperature control. Analysis is performed by ICP-MS. Polyatomic interferences with the low mass elements are reduced or eliminated by analysis in the collision mode using kinetic energy discrimination (KED).

Quantitation of Zinc is achieved essentially simultaneously by comparing the analyte–ISTD response ratios in the unknown samples with a standard curve constructed from response ratios of calibration standards.

Table 8.2: Method of analysis for Formulated Supplements for Children mentioned in Regulation No. 2.4.11.4 of FSS (Food Product Standards and Food Additives) Regulation, 2011

Sr. No	Parameter	Specifications	Tests
1	Vitamin A as retinol	120-400 µg/100gm	Foodstuffs - Determination of vitamin A by high performance liquid chromatography - Part 1: Measurement of all-E-retinol and 13-Z-retinol
2	Vitamin D (expressed as Cholecalciferol or Ergocalciferol)	3-10 µg/100gm	Infant formula and adult nutritionals - Determination ofvitamin D by liquid chromatography-mass spectrometry
3	Vitamin C	12-40 mg/100gm	Infant formula and adult nutritionals - Determination of vitamin C by (ultra) high performance liquid chromatography with ultraviolet detection ((U) HPLC-UV)
4	Thiamine (Vitamin B_1)	250-500 µg/100gm	Foodstuffs - Determination of vitamin B1 by high performance liquid chromatography
5	Riboflavin (Vitamin B_2)	180-600 µg/100gm	Foodstuffs - Determination of vitamin B2 by high performance liquid chromatography
6	Niacin (Vitamin B_3)	2.5-8 mg/100gm	Foodstuffs - Determination of niacin by HPLC

Sr. No	Parameter	Specifications	Tests
7	Pyridoxine (Vitamin B_6)	270-900 µg/100gm	Foodstuffs - Determination of vitamin B6 by high performancechromatography
8	Folic acid (Vitamin B_9)	14.5-48 µg/100gm	Total Folates in Infant Formula and Adult Nutritionals
9	Pantothenic acid (Vitamin B_5)	0.6-2 mg/100gm	Infant formula and adult nutritionals – Determination of pantothenic acid by ultra-high performance liquid chromatography and Tandem masss pectrometry method (UHPLC-MS/MS)
10	Vitamin B_{12} (Cyanocobalamin)	0.15-0.5 µg/100gm	Vitamin B12 in Infant Formula and Adult/ Pediatric Formulas
11	Choline	>32 mg/100gm	Free and Total Choline and Carnitine in Infant Formula andAdult/ Pediatric Nutritional Formula
12	Vitamin K	4.5-15 µg/100gm	Infant formula and adult nutritionals - Determination of trans and total (cis + trans) vitamin K1 content - Normal phase HPLC
13	Biotin	2.5-8 µg/100gm	Biotin in Infant Formula And Adult/Pediatric Nutritional Formulas
14	Vitamin E as L-Tocopherols	1.5-5 mg/100gm	Foodstuffs - Determination of vitamin E by high performance liquid chromatography - Measurement of α-, ß-, y- and δ- tocopherol
15	Sodium	90-300 mg/100gm	Calcium, Copper, Iron, Magnesium, Manganese, Potassium, Phosphorus, Sodium, and Zinc in Fortified Food Products. Microwave Digestion and Inductively

Sr. No	Parameter	Specifications	Tests
			Coupled Plasma-Optical Emission Spectrometry
16	Potassium	270-900 mg/100gm	Calcium, Copper, Iron, Magnesium, Manganese, Potassium, Phosphorus, Sodium, and Zinc in Fortified Food Products. Microwave Digestion and Inductively Coupled Plasma-Optical Emission Spectrometry
17	Chloride	240-800 mg/100gm	Chloride in Milk, Milk Powder, Whey Powder, Infant Formula,and Adult Nutritionals
18	Calcium	180-600 mg/100gm	Calcium, Copper, Iron, Magnesium, Manganese, Potassium, Phosphorus, Sodium, and Zinc in Fortified Food Products. Microwave Digestion and Inductively Coupled Plasma-Optical Emission Spectrometry
19	Phosphorous	135-450 mg/100gm	Calcium, Copper, Iron, Magnesium, Manganese, Potassium, Phosphorus, Sodium, and Zinc in Fortified Food Products. Microwave Digestion and Inductively Coupled Plasma-Optical Emission Spectrometry
20	Magnesium	15-50 mg/100gm	Calcium, Copper, Iron, Magnesium, Manganese, Potassium, Phosphorus, Sodium, and Zinc in Fortified Food Products. Microwave Digestion and Inductively Coupled Plasma-Optical Emission Spectrometry

Sr. No	Parameter	Specifications	Tests
21	Iron	2.5-9 mg/100gm	Calcium, Copper, Iron, Magnesium, Manganese, Potassium, Phosphorus, Sodium, and Zinc in Fortified Food Products. Microwave Digestion and Inductively Coupled Plasma-Optical Emission Spectrometry
22	Iodine	27-90 µg/100gm	Infant formula and adult nutritionals - Determination of total iodine - Inductively coupled plasmamass spectrometry (ICP-MS)
23	Copper	102-340 µg/100gm	Calcium, Copper, Iron, Magnesium, Manganese, Potassium, Phosphorus, Sodium, and Zinc in Fortified Food Products. Microwave Digestion and Inductively Coupled Plasma-Optical Emission Spectrometry
24	Zinc	1.5-5.0 mg/100gm	Calcium, Copper, Iron, Magnesium, Manganese, Potassium, Phosphorus, Sodium, and Zinc in Fortified Food Products. Microwave Digestion and Inductively Coupled Plasma-Optical Emission Spectrometry
25	Selenium	5-17 µg/100gm	Infant formula and adult nutritionals - Determination of chromium, selenium and molybdenum-Inductively coupled plasma mass spectrometry (ICP-MS)

Antioxidants			
Sr No.	**Parameter**	**Specifications**	**Tests**
1	Mixed Tocopherols Concentrate	300 mg/kg fat oroil basis, singly or in combination	Foodstuffs - Determination of vitamin E by high performance liquidchromatography - Measurement of α-, ß-, y- and δ-tocopherol
2	Alpha Tocopherol	300 mg/kg fat oroil basis, singly or in combination	Foodstuffs-Determination of vitamin E by high performance liquid chromatography-Measurement of α-, ß-, y- and δ-tocopherol
3	L-Ascorbic acid	50 mg	Infant formula and adult nutritionals - Determination of vitamin C by (ultra) high performance liquid chromatography with ultraviolet detection ((U)HPLC-UV)
4	Sodium Ascorbate	50 mg	Infant formula and adult nutritionals - Determination of vitamin C by (ultra) high performance liquid chromatography with ultraviolet detection ((U)HPLC-UV)
5	Potassium ascorbate	50 mg	Infant formula and adult nutritionals - Determination of vitamin C by (ultra) high performance liquid chromatography with ultraviolet detection ((U)HPLC-UV)
6	Calcium ascorbate	20 mg	Infant formula and adult nutritionals - Determination of vitamin C by (ultra) high performance liquid chromatography with ultraviolet detection ((U)HPLC-UV)

8.4 References:

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