Manual of Methods of Analysis-Trace Elements

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Note : *The test methods given in the manuals are validated/standardized test methods. However, it would be the responsibility of the respective testing laboratory to confirm that the above methods are verified in their laboratory and gives proper result in their laboratory.*

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 the analysis of trace elements Lead, Cadmium, Zinc, Copper, and Iron in food after microwave digestion by Atomic Absorption Spectrophotometer**		
Method No.	**FSSAI 09.001:2024**	**Revision No. & Date**	0.0
Scope	Applicable to determination of Zn, Cu, and Fe in a variety of foods by microwave digestion and flameatomic absorption spectrophotometry (FAAS), and Cd and Pb by microwave digestion and graphite furnaceatomic absorption spectroscopy (GFAAS). Method is capable of determining these elements at concentrations above approximately Pb (0.4), Cd (0.01), Zn (4), Cu (3), and Fe (7) mg/kg. **Note:** Method is not applicable to foods with a fat content ≥40%. Not applicable to milk powder.		
Caution	1) Use fume hood and wear full personal laboratory protective clothing, gloves, and appropriate eye protection (safety glasses) when using glassware and preparing standards or test portions with acid solutions. 2) Reagents should be regarded as potential health hazards and exposure to these materials should be minimized. Follow universal precautions. Wear gloves, a lab coat, and safety glasses while handling reagents. Material safety data sheets for these chemicals are to be available to the user. 3) Exercise caution when handling and dispensing concentrated acids. Always add acid to water. Acids are caustic chemicals that are capable of causing severe eye and skin damage. If acids or bases come in contact with any part of the body, quickly wash the affected area with copious quantities of water for at least 15 minutes. 4) Microwave digestion systems are dangerous. Vessels contain concentrated nitric acid at high temperatures and pressures. Analyst must be familiar with manufacturer's recommended safety precautions. Never remove hot vessels from microwave; wait until they are near room temperature. Keep microwave door closed while vessels are hot. 5) Digestion vessels must cool for an appropriate time before opening in order to avoid burns from hot and corrosive vapors. Maintain safe distance from furnaces equipped with Zeeman background correction when the magnet is on.		
Principle	Products are digested with HNO_3 and H_2O_2 under pressure in a closed vessel heated by microwaves. Solution is diluted with H_2O. Pb and Cd are determined by GFAAS. Zn, Cu, and Fe are determined by FAAS.		
Apparatus/Instruments	1) **Atomic absorption spectrophotometer**—With air-acetylene burner or nitrous oxide-acetylene burner for flame (FAAS; *see* Table-1) and a graphite furnace for electrothermal (GFAAS; *see* Table-2) determinations, with appropriate background (nonatomic) correction. 2) **Hollow cathode or electrodeless discharge lamp**—For Pb, Cd, Zn, Cu, and Fe. 3) **Microwave oven**—Designed for laboratory use. Check microwave oven regularly for delivered power. If the measured effect does not agree with the specification, adjust the program: Fill a plastic beaker (polypropylene or Teflon) with 1.000 kg water(room temperature) and measure temperature (Tb). Place beaker in microwave oven and heat water at full power for 2 min. Take beaker out of oven, stir water, and measure		

temperature (Ta). The delivered power in watts:

$$P = 35 \times (Ta - Tb)$$

4) **Teflon digestion vessels**—100 mL, withstanding a pressure of at least 1.4 MPa.
5) **Volumetric flasks**—25 and 1000 mL.
6) **Funnels**—Glass or plastic.
7) **Plastic bottles**—e.g., Polystyrene bottles with tightly □tting lids, 50–100 mL.
8) **Drying oven**—Or equipment for freeze-drying.

Table-1 (Instrumental parameters for FAAS)

Metal	Flame type	Wavelength, nm
Zinc (Zn)	Air-acetylene, oxidizing	213.9
Copper (Cu)	Air-acetylene, oxidizing	324.7
Iron (Fe)	Air-acetylene, oxidizing	248.3
Iron (Fe)	N2O-acetylene, oxidizing	248.3

Table-2 (Instrumental parameters for GFAAS)

		Temp. (°C)/ ramp-hold (s)		
Metal	**Wavelength, nm**	**Ashing step**	**Atomization step**	**Cleaning out step (°C)**
Lead(Pb)	Air-acetylene, oxidizing	650/15–10	1900/0–4	2500
Cadmium (Cd)	Air-acetylene, oxidizing	350/15–10	1200/0–4	2500

Note : Carefully clean all glassware and plasticware and rinse, e.g., with HNO3 or HCl, to avoid metal contamination.

Materials and Reagents

Reagents should be of at least analytical reagent grade (p.a.), preferably ultrapure (suprapur), or equivalent.

1) Water—Redistilled or deionized, ≥18 MΩ·cm.
2) Nitric acid—65% (w/w).
3) Nitric acid—0.1 M. Dilute 7 mL concentrated HNO3, with water to 1 L.
4) Nitric acid—3 M. Dilute 200 mL concentrated HNO3, with water to 1 L.
5) Hydrogen peroxide.—30% (w/w).

Preparation of Reagents

1) **Zinc standard solution**—1 mg/mL. Dissolve 1.000 g Zn in 14 mL water + 7 mL nitric acid, in 1 L volumetric flask. Dilute to volume with water.
   [Note: Commercially available standard solutions for AAS may be used for all metal standard solutions].
2) **Copper standard solution**—1 mg/mL. Dissolve 1.000 g Cu in 7 mL nitric acid, in 1 L volumetric flask. Dilute to volume with water.
3) **Iron standard solution**—1 mg/mL. Dissolve 1.000 g Fe in 14 mL water + 7 mL nitric acid, in 1 L volumetric flask. Dilute to volume with water.

	4) **Lead standard solution**—1 mg/mL. Dissolve 1.000 g Pb in 7 mL HNO3, in 1 L volumetric flask and dilute to volume with water. 5) **Cadmium standard solution**—1 mg/mL. Dissolve 1.000 mg Cd in 14 mL water + 7 mL HNO3, in 1 L volumetric flask and dilute to volume with water. 6) **Working standard solutions**— (a) **For flame analysis**—Dilute standard solutions, with 0.1 M HNO3, to a range of standards that covers the concentration of the element to be determined. (*b*) **For graphite furnace analysis**—Dilute standard solutions, with 0.1 M HNO3, to a range ofstandards that covers the linear range of the element to be determined. 7) **Cleaning procedure**— **(a)For glass and plasticwar**.—Acid solution: 500 mL concentrated HNO3, + 4500 mL deionized water, Wash first with water and detergent. Rinse with tap water, followed by deionized water, then with acid solution. Finally rinse 4–5 times with deionized water. **(b)For Tefon digestion vessels**—Rinse with acetone, wash with deionized water, keep vessels covered with 0.1 M HNO3, for at least 30 min, rinse with deionized water, and let vessels dry. Use separate vessels for different applications, depending on the concentration of metals. If, however, the same digestion vessels are used for heavily contaminated products, e.g., sludge, it may be necessary to use a more severe cleaning procedure, e.g., heating vessels together with concentrated HNO3.
Sample Preparation	1) **Pretreatment**—If product is to be analyzed fresh, proceed to, Homogenization. Otherwise, continue at, drying. 2) **Homogenization**—Homogenize products using noncontaminating equipment. Check for leached metals if the apparatus consists of metal parts. 3) **Drying**—Dry to constant weight in drying oven at 105°C, or freeze-dry. Freeze-drying is usually preferable because it renders the product less compact and easier to homogenize. If final result is based on fresh weight, weigh test portion before and after drying to obtain water content: $$H_2O = \frac{Wf - Wd \times 100}{Wf}$$ where H_2O, % = water content of the test portion (%); *W*f = weight of the test portion (g); *W*d = weight after drying (g). 4) **Digestion**—Weigh 0.2–0.5 g dry material into digestion vessel. If water-containing materials are used, maximum weight is restricted to 2 g, but dry matter content must never exceed 0.5 g. For example, if product has a water content of 50%, take a maximum of 1 g (= 0.5 g dry matter). If a product has a water content of 95%, take 2 g (<0.5 g dry matter). When unknown products aredigested, too much solids may cause the safety membrane in the digestion vessel to rupture. 5) Add 5 mL HNO3, and 2 mL 30% H2O2. Close vessels, place vessels in holder, place vessel holder in microwave oven, and close door. Set oven program according to the parameters given in Table-3 andstart program.

Table-3Parameters for microwave oven program

Step	Power, W	Duration, min
1	250	3
2	630	5
3	500	22
4	0	15

Note: The program is valid only when 12 vessels are being digested simultaneously. If fewer are being digested, the remaining vessels must be filled with reagent blank. When a microwave oven other than the one given as an example is used, it may be necessary to use a slightly different time/power program.

6) Remove digestion vessels from microwave oven and let cool thoroughly before opening them. Open vessel and rinse down lid and walls into container. Transfer solution to 25 mL volumetric flask and dilute to mark with deionized water. Then, transfer solution to plastic container. Treat blanks in the same way as tests. One blank should be included in every set.

7) **Dilution**—If test solution needs to be further diluted (due to high metal concentrations), dilute with 3 M HNO_3, in order to maintain same acid concentration prior to metal determination.

Note: High acid concentration is environmentally undesirable and may depress the analytical signal. Reduce acid strength by diluting the test solution 1/2 with 0.1 M nitric acid and standard solutions 1/2 with 3 M nitric acid. The tests and standards are thereby brought to the same acid concentration. Matching of acid concentrations is important when a calibration curve is used.

Method of analysis

1) **Atomic absorption spectrophotometry**—Use of flame or graphite furnace technique is determined by the concentration of the metal to be determined. Flame technique should be used as far as possible, since this technique is less sensitive to interference than the GFAAS. Always use background correction.

Measurements must be within the linear range when the method of standard addition is used. A standard addition curve consists of at least three points, of which at least two are standards. The concentration of the highest standard should be 3–5 times the concentration in the test solution. The lower standard should have a concentration approximately half of the highest standard. A simplified version of the method of standard addition is to use a matrix-matched standard curve, which is applicable to products with the same matrix:

The test and standard solutions are mixed and used to make a standard addition curve. This curve is thenparallel transferred to origin and is used as the standard curve for the tests that followed and that have beendiluted in the same proportions. The matrix-matched standard curve and the test solutions will thus havethe same matrix concentration.

(a) **Flame technique**—The concentration of Zn, Cu, and Fe are usually at levels suitable for determination by FAAS. When calibration curve is to be used, standards and test solutions must have the same acid concentration.

Note: Since Fe may be strongly affected by interferences from the matrix, use either the method of standard addition or matrix-matched standards. When experiencing severe interferences, an oxidizing nitrous oxide acetylene flame may be an alternative.

	(b) **Graphite furnace technique**—This technique is generally required for determination of Pb and Cd in foods. Use pyrolytically coated tubes with platforms. Since the method results in a fairly large dilution of the analyte, it may frequently be needed also for the determination of, e.g., Cu. The method of standard addition or matrix-matched standards should always be used unless shown to be unnecessary (i.e., no significant difference between the slopes of calibration curves of pure working standard and standard addition curves of the test product). Measurements must be made in the linear range when the method of addition is used. Program the autosampler to deliver a volume that gives as large an absorbance as possible within the linear range and producing a background absorbance not larger than approximately 0.5 absorbance units. Multiple injection may enhance the absorbance at very low concentrations. Evaluate each new matrix by means of ash- and atomization-curves in order to optimize the graphite furnace parameters.

Calculation with units of expression	Calculate the concentration (*C*) of metal in the test sample according to the formula:
	$$C = \frac{(a - b)df \times 25}{m}$$
	where *C* = concentration in the test sample (mg/kg);
	a = concentration in the test solutions (mg/L);
	df = dilution factor;
	b = mean concentration in the blank solutions (mg/L);
	m = weight of the test portion (g)
	If (*a* – *b*) is lower than the detection limit, DL, then (*a* – *b*) is replaced by DL for calculation of the limit of detection in the test sample.
	$$C_{FW=}C \times \frac{100 - H_20}{100}$$
	where C_{FW} = concentration in the test portion corrected to fresh weight (mg/kg)
	H_2O% = the water content of the test portion (%).
	Detection limit—The DL for each metal is calculated as DL = 3 × standard deviation of the mean of the blank determinations (*n* = ≥20). A large number of blanks must be analyzed before DL can be established. A DL is not static and will need to be reevaluated from time to time in accordance with changes in the blank levels.

Inference (Qualitative Analysis)	
Reference	**9.1.08 Official Methods of Analysis of AOAC INTERNATIONAL 999.10 (Final Action 2005)**
Approved by	Scientific Panel on Methods of Sampling and Analysis

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 the analysis of trace elements Lead, Cadmium, Zinc, Copper, and Iron in food after drying ashing in food by Atomic Absorption Spectrophotometer**		
Method No.	**FSSAI 09.002:2024**	**Revision No. & Date**	0.0
Scope	Applicable to determination of Zn, Cu, and Fe in a variety of foods by dry ashing and flame atomic absorption spectrophotometry (FAAS), and Cd and Pb by dry ashing and graphite furnace atomic absorption spectroscopy (GFAAS). Method is capable of determining these elements at concentrations above approximately Pb (0.3), Cd (0.1), Zn (1), Cu (5), and Fe (4) mg/kg. **Note:** Avoid environmental contamination by Pb. Store quartz crucibles in 20% HNO3 and rinse with deionized water before use. When necessary, crucibles may be boiled with 20% HNO3 before use. Heat platinum crucibles until red hot and boil with 50% (v/v) HCl prior to use.		
Caution	1) Use fume hood and wear full personal laboratory protective clothing, gloves, and appropriate eye protection (safety glasses) when using glassware and preparing standards or test portions with acid solutions. 2) Reagents should be regarded as potential health hazards and exposure to these materials should be minimized. Follow universal precautions. Wear gloves, a lab coat, and safety glasses while handling reagents. Material safety data sheets for these chemicals are to be available to the user. 3) Exercise caution when handling and dispensing concentrated acids. Always add acid to water. Acids are caustic chemicals that are capable of causing severe eye and skin damage. If acids or bases come in contact with any part of the body, quickly wash the affected area with copious quantities of water for at least 15 minutes.		
Principle	Test portions are dried and then ashed at 450°C under a gradual increase (≤50°C/h) in temperature. 6 M HCl (1 + 1) is added, and the solution is evaporated to dryness. The residue is dissolved in 0.1 M HNO3, and the analytes are determined by flame and graphite furnace procedures.		
Apparatus/Instruments	**1) Atomic absorption spectrophotometer**—With air-acetylene burner or nitrous oxide-acetylene burner for flame (FAAS; *see* Table-1) and a graphite furnace for electrothermal (GFAAS; *see* Table-2) determinations, with appropriate background (nonatomic) correction. **2) Hollow cathode or electrodeless discharge lamp**—For Pb, Cd, Zn, Cu, and Fe. **3) Furnace—**Programmable, or muffle furnace with thermostat maintaining 450 ± 25°C. If muffle furnace is used, a separate pre-ashing device is required. **4) Hot plate—**With heating control, to heat up to about 300°C. **5) Lamp—**IR 250 W, fixed to a retort stand in a way that allows adjustment of the distance to the hot plate. **6) Ceramic plate—**e.g., Desiccator plate on a low stand, with a diameter that suits the hot plate.		

7) **Glass cover—**e.g., Crystallizing dish, 185 mm diameter, 100 mm height, to fit on ceramic plate or equivalent.

8) **Wash bottle—**"Scrubber," containing H_2SO_4 for purification of air.

Table-1 (Instrumental parameters for FAAS)

Metal	Flame type	Wavelength, nm
Zinc (Zn)	Air-acetylene, oxidizing	213.9
Copper (Cu)	Air-acetylene, oxidizing	324.7
Iron (Fe)	N2O-acetylene, oxidizing	248.3

Table-2 (Instrumental parameters for GFAAS)

Temperature program						
Metal	**Wavelength (nm)**	**Step 1**	**Step 2**	**Step 3**	**Step 4**	**Test solution vol (µL)**
Lead (Pb)	283.3	Temp 130°C	650	1900	2500	20
		Ramp 10 S	5	0	2	
		Hold 30 S	10	2	2	
Cadmium (Cd)	228.8	Temp 130°C	350	1200	2500	10
		Ramp 1 S	5	0	2	
		Hold 19 S	10	2	2	

9) **Quartz or platinum crucibles**—50–75 mL.

10) **Polystyrene bottles**—With leak-proof closures, 100 mL.

11) **Cleaning procedure for glass and plasticware**—Acid solution: 500 mL concentrated HNO3, Nitric acid 65%, + 4500 mL deionized water. Wash first with water and detergent. Rinse with tap water, followed by deionized water, then with dilute acid. Finally rinse 4–5 times with deionized water.
Note : Carefully clean all glassware and plasticware and rinse, e.g., with HNO_3 or HCl, to avoid metal contamination.

Materials and Reagents

Reagents should be of at least analytical reagent grade (p.a.), preferably ultrapure or equivalent.

1) Water—Redistilled or deionized, ≥18 MΩ·cm.
2) Hydrochloric acid—6 M. Dilute 500 mL HCl (37%, w/w) with water to 1 L.
3) Nitric acid—65% (w/w).
4) Nitric acid—0.1 M. Dilute 7 mL concentrated HNO3, with water to 1 L.
5) Nitric acid—3 M. Dilute 200 mL concentrated HNO3, with water to 1 L.
6) Hydrogen peroxide.—30% (w/w).

Preparation of Reagents

1) Zinc standard solution—1 mg/mL. Dissolve 1.000 g Zn in 14 mL water + 7 mL nitric acid, in 1 L volumetric flask. Dilute to volume with water.
[Note: Commercially available standard solutions for AAS may be used for all metal standard solutions].

2) Copper standard solution—1 mg/mL. Dissolve 1.000 g Cu in 7 mL nitric

	acid, in 1 L volumetric flask. Dilute to volume with water. **3) Iron standard solution**—1 mg/mL. Dissolve 1.000 g Fe in 14 mL water + 7 mL nitric acid, in 1 L volumetric flask. Dilute to volume with water. **4) Lead standard solution**—1 mg/mL. Dissolve 1.000 g Pb in 7 mL HNO_3, in 1 L volumetric flask and dilute to volume with water. **5) Cadmium standard solution**—1 mg/mL. Dissolve 1.000 mg Cd in 14 mL water + 7 mL HNO_3, in 1 L volumetric flask and dilute to volume with water. **6) Working standard solutions**— (a) **For flame analysis**—Dilute standard solutions, with 0.1 M HNO3, to a range of standards that covers the concentration of the element to be determined. (*b*) **For graphite furnace analysis**—Dilute standard solutions, with 0.1 M HNO3, to a range of standards that covers the linear range of the element to be determined. ***7)*** **Cleaning procedure**— **(a) For glass and plasticware**—Acid solution: 500 mL concentrated HNO3, + 4500 mL deionized water, Wash first with water and detergent. Rinse with tap water, followed by deionized water, then with acid solution. Finally rinse 4–5 times with deionized water.
Method of analysis	1) **Pretreatment**—Homogenize product if necessary, using noncontaminating equipment. Check for leaching metals if the apparatus consists of metal parts. 2) **Drying**—In a crucible, weigh 10–20 g test portion to nearest 0.01 g. Dry in a drying oven, on a waterbath, or a hot plate at 100°C, if there is a risk of heavy boiling in the ashing step. Proceed according to type of furnace. 3) **Ashing**—(1) Ashing in a programmable furnace.—Place dish in furnace at initial temperature not higher than 100°C. Increase temperature at a maximum rate of 50°C/h to 450°C. Let dish stand for at least 8 h or overnight. 4) Ashing in a muffle furnace with thermostat following drying and pre-ashing in apparatus described in. Place crucible with the test portion covered with the glass cover on the ceramic plate, and let purified air coming through a glass tube sweep over the product. Put IR lamp down at the cover. Pre-ash product by increasing temperature slowly with IR lamp by gradually increasing temperature on hot plate to maximum. Final temperature on ceramic plate should then be about 300°C. Time required for pre-ashing varies with product. Put crucible in muffle furnace at 200–250°C and slowly raise temperature to 450°C at a rate of no more than 50°C/h. Let stand for at least 8 h or overnight. Take crucible out of furnace and let cool. 5) **Solution**—Wet ash with 1–3 mL water and evaporate on water-bath or hot plate. Put crucible back in furnace at no more than 200°C and raise temperature (50–100°C/h) to 450°C. Proceed with ashing at 450°C for 1–2 h or longer. Repeat procedure until product is completely ashed, i.e., ash should be white/grey or slightly colored. Number of repetitions necessary varies depending on type of product.

	Add 5 mL 6 M HCl, to crucible ensuring that all ash comes into contact with acid. Evaporate acid on water-bath or hot plate. Dissolve residue in 10.0–30.0 mL, to the nearest 0.1 mL, of 0.1 M HNO3. Swirl crucible with care so that all ash comes into contact with acid. Cover with watch glass and let stand for 1–2 h. Then stir solution in crucible thoroughly with stirring rod and transfer contents to plastic bottle. Treat blanks in the same way as products. Include two blanks with each analytical batch. 6) **Atomic absorption spectrophotometry**—Pb and Cd in foods generally require graphite furnace AAS for determination. Zn, Cu, and Fe can, in most foods, be determined by flame AAS. Wavelength, gas mixture/temperature program, and other instrumental parameters that are most appropriate for each metal are found in the manual provided with the instrument. Background correction must always be used in flameless AAS and for flame applications at low concentrations. When results are out tide of the linear range, dilute the test solutions with 0.1 M HNO3. (a) Flame technique.—Prepare calibration curves from a minimum of three standards. (b) Graphite furnace (flameless) technique—The method of addition should always be used. Measurements must be made in the linear range when method of addition is used. Measurements are preferably made with peak area rather than peak height.
Calculation with units of expression	Detection limit—Calculate the detection limit, DL, for each metal as: DL = 3 × standard deviation of the mean of the blank determinations (n = ≥ 20) Calculate the concentration (*C*) of metal in the test sample according to the formula: $$\frac{C=(a-b)x\ V}{m}$$ where *C* = concentration in the test sample (mg/kg); *a* = concentration in the test solutions (mg/L); *b* = mean concentration in the blank solutions (mg/L); *m* = weight of the test portion (g) V= Volume of the test solution (mL) If (*a* – *b*) is lower than the detection limit, DL, then (*a* – *b*) is substituted with DL for calculation of the limit of detection in the test portion. If the test solution has been diluted, the dilution factor (df) has to be taken into account. When running replicates, the average of the results should be given with two significant figures.
Inference	

(Qualitative Analysis)	
Reference	**9.1.08 Official Methods of Analysis of AOAC INTERNATIONAL 999.11 (Final Action 2005)**
Approved by	Scientific Panel on Methods of Sampling and Analysis

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Method No.	**FSSAI 09.003:2024**	**Revision No. & Date**	0.0

Scope

This method describes procedures for using inductively coupled plasma-optical emission spectrometry (ICP-OES) for determination of total element concentration (mass fraction) in food. The method was validated with the following foods: milk, cheese, bacon, tuna, eggs, peanut butter, corn, bread, pancakes, cereal, prune juice, lemonade, broccoli, sweet potato, spaghetti & meatballs, mayonnaise, beer, beef, baby food, haddock and pears. Other matrices may be analyzed by these procedures if performance is demonstrated for an applicable analyte in the matrix of interest, at the concentration levels of interest.

It should be noted that aluminum results could be biased low in some samples because of insoluble aluminum compounds especially if silica is present. Thallium is listed conditionally because although fortification recoveries were acceptable during method validation, no reference materials were available.

Table-1 Analytical limits

Element	Symbol	LOD (mg/kg)	LOQ (mg/kg)
Aluminum	Al	0.8	2
Arsenic	As	2	4
Barium	Ba	0.05	2
Boron	B	0.3	0.8
Cadmium	Cd	0.3	0.9
Calcium	Ca	8	30
Chromium	Cr	2	5
Cobalt	Co	0.3	0.8
Copper	Cu	0.1	0.3
Iron	Fe	0.2	0.3
Lead	Pb	3	6
Magnesium	Mg	2	6
Manganese	Mn	0.2	0.4
Molybdenum	Mo	0.4	1
Nikel	Ni	0.9	3
Phosphorus	P	2	6
Potassium	K	20	40
Sodium	Na	2	5
Strontium	Sr	0.03	0.07

	<table><tr><td>Thallium</td><td>Tl</td><td>2</td><td>6</td></tr><tr><td>Vanadium</td><td>V</td><td>0.2</td><td>0.5</td></tr><tr><td>Zinc</td><td>Zn</td><td>0.3</td><td>0.5</td></tr></table> Aluminum concentrations using the method do not account for aluminum bound to silicates. The method, especially using pneumatic nebulization, may not achieve quantitative measurement of typical concentrations in some foods for some elements. Using ultrasonic nebulization will improve analytical limits for most elements. The following elements appear prone to laboratory environmental contamination and therefore require extensive assessment of contamination control: aluminum, chromium, and lead.
Caution	6) Use fume hood and wear full personal laboratory protective clothing, gloves, and appropriate eye protection (safety glasses) when using glassware and preparing standards or test portions with acid solutions. 7) Inductively coupled plasmas should only be viewed with proper eye protection from ultraviolet emissions. 8) Reagents should be regarded as potential health hazards and exposure to these materials should be minimized. Follow universal precautions. Wear gloves, a lab coat, and safety glasses while handling reagents.Material safety data sheets for these chemicals are to be available to the user. 9) Exercise caution when handling and dispensing concentrated acids. Always add acid to water. Acids are caustic chemicals that are capable of causing severe eye and skin damage. If acids or bases come in contact with any part of the body, quickly wash the affected area with copious quantities of water for at least 15 minutes. 10) Microwave digestion systems are dangerous. Vessels contain concentrated nitric acid at high temperatures and pressures. Analyst must be familiar with manufacturer's recommended safety precautions. Never remove hot vessels from microwave; wait until they are near room temperature. Keep microwave door closed while vessels are hot.
Principle	An analytical portion (0.4 to 5 g dependent on food composition) is decomposed with nitric acid and hydrogen peroxide in a high-pressure Teflonlined digestion vessel using microwave heating and a feedback program to control temperature and pressure. A 50 mL analytical solution is prepared from the digest. Analytical solutions are nebulized and aerosol is transported to plasma where desolvation and excitation occur. Either pneumatic or ultrasonic nebulization sample introduction is used. Characteristic atomic emission spectra are produced by radio frequency inductively coupled plasma. Spectra are dispersed by a grating spectrometer, and line intensities are measured with a light sensitive detector such as a photomultiplier tube or charge transfer device. Photocurrents are processed by a computer system. A background

	correction technique is required to compensate for variable background emission contribution to analyte signal and should be applied except in cases of line broadening.
Apparatus/Instruments	1) **Inductively coupled plasma atomic emission spectrometer (ICP-AES)**—Simultaneous or sequential ICP-AES with associated glassware, which uses a mass flow controller to regulate argon nebulizer flow rate supplied by a Dewar of liquid argon or tank of gaseous argon. A variable speed peristaltic pump to deliver all solutions to nebulizer. Pneumatic nebulizer which can aspirate high dissolved solids (e.g., V-groove, cross flow, etc.) or an ultrasonic nebulizer. 2) **Microwave decomposition system**—requires temperature control to 200 °C, pressure control to at least 600 psi, power range of 0-100% in 1% increments, minimum 1000 watts for 12 position carousel, feedback control of temperature and pressure and multistep programming with ramp to temperature capability. Digestion vessels must be quartz or Teflon lined. System must be able to reach at least 200 °C and at least 600 psi. Vessels designed to vent and reseal can be used provided they vent at pressures >300 psi.
Materials and Reagents	Reagents may contain elemental impurities that can affect the quality of analytical results. Use of high purity or trace element (i.e., metals) grade reagents is usually required. 1) Reagent water—Water that meets specifications for ASTM Type I water 2) High purity nitric acid—Concentrated (sp gr 1.41), trace element grade or double distilled. 3) Nitric acid—Concentrated (sp gr 1.41), ACS reagent grade. 4) Nitric acid 1% (v/v)—Dilute 10 mL high purity nitric acid to 1000 mL with reagent water. 5) (5) Nitric acid 10% (v/v)—Dilute 100 mL high purity nitric acid to 1000 mL with reagent water. 6) (6) Hydrogen peroxide—30% H_2O_2 solution. High purity or trace metals grade.
Preparation of Reagents	**1) Stock standard solutions**—Commercially prepared single element solutions prepared specifically for spectrometric analysis (usually 1000 or 10,000 mg/L). Stock standard solutions may also be prepared in the laboratory from high purity (≥99.99%) metals or salts. Alternatively, commercial multi-element solutions prepared specifically for spectrometric analysis can be used. These multi-element solutions will be much lower in concentration (typically 10-500 mg/L) than single element solutions to avoid compatibility problems. **2) Intermediate standard solution(s)**—Prepared to contain appropriate concentration(s) of analytes for preparation of standard solutions. Pipet an appropriate volume of stock standard solution(s) into an acid rinsed

	volumetric flask and dilute to volume with 10% nitric acid. Alternatively, intermediate standard solutions may be prepared gravimetrically by measuring stock standard solution and 10% nitric acid masses multiplied by solution density in a 125 or 250 mL plastic bottle. The density of 10% (v/v) nitric acid is 1.04 g/mL and stock standard solution densities are provided by their commercial sources. Store prepared intermediate standard solutions in plastic bottles. Alternatively, commercial multi-element solutions prepared specifically for spectrometric analysis can be used. **3) Standard solutions**—prepare at least 3 standard solutions by combining appropriate volumes of stock standard solutions or intermediate standard solutions in volumetric flasks. Analyte concentration range should cover the LDR or a portion thereof. Lowest standard should be near the ASQL. Dilute to volume with 10% nitric acid. Many of the elements (cadmium, cobalt, molybdenum, etc.) have LDRs that far exceed the values expected in food analytical solutions. In addition, line-rich elements like iron may cause spectral interference on other emission lines if high concentrations are used to standardize the instrument. Therefore, the analyst may choose to work within part of the LDR. A recommended maximum concentration of an element in a standard solution is 10 mg/L. Exceptions would be elements usually present at high concentrations for example, calcium, sodium, potassium, magnesium and phosphorus. For convenience, each standard solution should contain all the analytes to be determined. Chemical compatibility (i.e., of analytes, acids, etc.) must be considered to avoid the formation of analyte precipitates when mixing single element stock solutions to prepare standard solutions. High quality custom-made multi-element solutions are commercially available and are recommended. Transfer prepared standard solutions to acid cleaned plastic bottles (Teflon FEP is preferred) for storage. Standard solutions may also be prepared by gravimetric dilution. Gravimetric dilution can be performed by measuring mass of stock or intermediate standard solution and 10% nitric acid masses in a 125 or 250 mL plastic bottle. Volumes are calculated from solution densities. At typical laboratory temperatures, the density of 10% (v/v) nitric acid is 1.04 g/mL and stock standard densities are provided by their commercial sources. Do not use standard solutions that are more than 30 days old since element concentrations can change with age. **4) Standard blank**—10% nitric acid. Prepare sufficient amount for use in standardization, determination of IDLs, and for nebulizer rinse between each measurement. **5) Independent check solution (ICS)**—Dilute appropriate volumes of analyte stock solutions or intermediate standard solutions obtained from a different source than used to prepare standard solutions with 10% nitric acid so analyte concentration will be several times the ASQL or in the range of 0.5 to 10 mg/L for most elements.

	6) Check solution—Use mid-concentration multi-analyte standard solution for the check solution. **7) Spike solution**—Prepared such that, when 1 mL is diluted to analytical solution volume (initial analytical solution volume usually 50 mL), analyte concentration is approximately at the middle of the LDR or appropriate for the expected sample analyte concentration. A fortification solution should not be prepared that would result in an analyte concentration in the analytical solution that is less than 10 times the ASQL. In addition, the fortification solution should not increase any analyte's concentration by more than 40 mg/L relative to the analytical solution because of potential problems caused by high analyte levels (nebulizer transport effects and spectral interference, etc.) and the challenge of minimizing the spike solution volume. Pipet an appropriate volume of stock standard solution(s) or intermediate standard solution(s) into an acid rinsed volumetric flask and dilute to volume with 10% nitric acid.
Sample Preparation	1) Weigh analytical portion into clean vessel liner and determine mass of analytical portion. Generally, for samples of unknown composition, weight the equivalent of about 0.5 dry material to an accuracy of 0.001 g. If maximum pressure attained for this unknown less than the vessel limit then a greater mass may be analyzed.Less than the maximum mass should be used for samples high in salt content. A maximum analytical portion of 5 g should not be exceeded even if calculations based on the food's energy indicate that a larger portion could be taken. Use 1 g reagent water for method blanks (MBKs). For dry samples and dry CRM materials adding 1 g of reagent water can help control exothermic reactions during the digestion. 2) Pipette 8.0 mL or weigh 11.3 g of high purity nitric acid (sp gr 1.41 g/mL) into vessel liner, washing down any material on walls. Weighing acid using a top loading balance and Teflon FEP wash bottle is suggested. Use double distilled grade for lowest method blank values. Acid should be added drop wise for the first few mL until it can be established that the sample will not react violently. Some foods, especially those high in sugar, will react with nitric acid within several minutes. If foaming or reaction with the acid is observed, let the vessels sit uncovered in a class 100 clean hood for 20 minutes or until reaction subsides. If a clean hood is unavailable, place caps on vessels without pressing down fully or, if so equipped, cap vessels but loosen the pressure relief nut (with the safety membrane) to allow pressure to escape. If, however, it appears that excessive foaming would result in the sample-acid mixture expanding out of the vessel then cap the vessel and tighten to appropriate torque to prevent loss of sample or acid. 3) Add 1 mL high purity 30% H2O2. Seal vessels, apply correct torque to cap (tighten pressure relief nuts if equipped) and run the digestion program as

given in table 2.

Table-2Microwave Digestion Programs

Digestion program with Ramp to Temperature feature and pressure control	
Maximum Power (Watts)	1200
Control Pressure (psi)	800
Ramp Time (min)	25
Hold Time (min)	15
Control Temperature (°C)	200
Power is applied for the Ramp Time minutes or until Control Pressure or Control Temperature is met. If Control Pressure or Control Temperature are met before end of Ramp Time then program proceeds to Hold Time.	

4) After vessels have cooled to less than 50° C remove them to an exhausting clean hood and vent excess pressure slowly. Quantitatively transfer and dilute digestion solution with reagent water to 25 mL. This analytical solution should be transferred to a plastic bottle or a capped polypropylene centrifuge tube for storage.

Method of analysis

Instrument Setup

1) Setup inductively coupled plasma optical emission spectrometer according to the manufacturer's recommendations and with the following attributes:

- Set rinse time to at least 60 sec.
- Program instrument method for the analytes of interest. Include the following elements even if they are not analytes of interest to allow for interference correction: Al,Ca, Fe, Cr, Cu, Mn, Ti, and V.
- Suggested emission line wavelengths are listed in below table. Other wavelengths may be used but they may not achieve the same sensitivities.

Table-3Typical ICP-AES Instrument Conditions: Wavelengths

Element	Wavelength (nm)
Aluminum (Al)	308.22
Arsenic (As)	189.01
Barium (Ba)	493.41
Boron (B)	249.68
Cadmium (Cd)	226.50
Calcium(Ca)	317.93
Chromium (Cr)	267.72
Cobalt (Co)	228.62

Copper (Cu)	324.75
Iron (Fe)	259.94
Lead (Pb)	220.35
Magnesium (Mg)	383.83
Manganese (Mn)	257.61
Molybdenum (Mo)	202.03
Nickel	231.60
Phosphorus (P)	178.29
Potassium (K)	766.49
Sodium (Na)	589.59
Strontium (Sr)	407.77
Thallium (Tl)	190.86
Vanadium (V)	292.40
Zinc (Zn)	213.86

• Use background correction.

• Configure instrument for 3 integrations of emission. Use integration time appropriate for the particular instrument and emission line. Allow at least 10 sec after the solution reaches the plasma before starting integration. Report each emission reading and the mean and RSD.

• Program instrument to use a linear, least squares calculated intercept, curve fit algorithm for converting emission values to mg/L concentration units. Do not subtract standard blank response from standard solution response. Use the mean of the emission integrations to calculate concentration of analyte.

2) Optimize instrument

• Follow manufacturer's recommendations for optimizing the emission spectrometer.

• After instrument warm-up, perform optical profiling. Optical profiling is performed either with a built-in mercury lamp, a 2 mg/L Mn solution, or procedure recommended by instrument manufacturer.

3) Check instrument performance

• Verify emission counts are within 80-100% of expected value with a mid-range standard.

• Verify short term precision is less than 5% relative standard deviation with a mid-range standard (n=5).

• Verify IDL is within a factor of 3 of expected value.

Determination of Analyte Concentration Using Standard Curve

1) Standardize the instrument using the standard blank and at least 3 standard solution concentration levels. Allow at least 10 sec after the

	standard solution reaches the plasma before starting integration. Flush system with standard blank for at least 60 sec between each standard solution. 2) Check Standardization Performance • Correlation coefficient (r) of linear regression (emission intensity verses concentration) is ≥0.998. • ICS recovery within 100 ± 5% (initial calibration verification). • Standard blank <ASDL. 3) Analyze analytical solutions and quality control solutions. Interpolate analyte concentration from standard curve. Rinse sample introduction system by aspirating standard blank for a minimum of 60 sec between all analyses (or longer if necessary). Rinse time is appropriate if results of a standard blank are <ASDL when analyzed immediately after a high standard. 4) Check Instrument Measurement Performance • RSD of replicate integrations ≤7% for all solutions when instrument response ≥ASQL. • Check solution analyzed at a frequency of 10% and at the end of the analytical run has a recovery of 100 ± 10% (continuing calibration verification). • Standard blank analyzed at a frequency of 10% and at the end of the analytical run <ASDL (continuing calibration blank). • Measurements are below highest standard solution. Dilute analytical solution with standard blank if necessary to comply with criteria. • Wavelength scan indicates absence of spectral interference that is not corrected for by background correction or inter-element correction factors. 5) Inter-element Correction Factors • If analytical solution has or is expected to have Al, Ca, Fe, Cr, Cu, Mn, Ti or V at concentrations >20 mg/L then inter-element correction factors must be determined as outlined in manufacturer's Instructions. Program instrument to use these factors. • Analyze the solution(s) used to determine the inter-element correction factors as a sample to demonstrate proper correction for interference.
Calculation with units of expression	Calculate the concentration (mass fraction) of the analyte in the analytical portion according to the formula $$\text{Concentration}\left(\frac{\text{mg}}{\text{Kg}}\right)=\frac{[(\text{SxDF})-\text{MBKL}]\text{x V}}{\text{m x MCF}}$$ where S = concentration of analyte in analytical solution (or diluted analytical solution) (mg/L) MBKL = laboratory MBK (mg/L)

	V = volume (L) of analytical solution (usually 0.050 L) m = mass of analytical portion (kg) DF = dilution factor (1 if analytical solution not diluted) MCF = mass correction factor (1 if no water or other solvent was added to aid homogenization) Round calculated concentration to at most 3 significant figures. Concentration may be converted to other convenient units (*e.g.*, µg/kg, ng/kg).
Inference (Qualitative Analysis)	
Reference	U.S. Food and Drug Administration-(4.4)-Inductively Coupled Plasma-Atomic Emission Spectrometric Determination of Elements in Food Using Microwave Assisted Digestion (version 1.1) (August 1010)
Approved by	Scientific Panel on Methods of Sampling and Analysis

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 Calcium, Copper, Iron, Magnesium, Manganese, Potassium, Phosphorus, Sodium, and Zinc in Fortified Food Products by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES)**		
Method No.	**FSSAI 09.004:2024**	**Revision No. & Date**	0.0
Scope	Applicable to analysis of calcium, copper, iron, potassium, magnesium, manganese, phosphorus, sodium, and zinc in fortified food products. **Upper limits (mg/kg):** Ca (24 000); Cu (210); Fe (850); K (32 000); Mg (7500); Mn (20); Na (16 000); P (16 000); Zn (320). **Limit of quantitation (LOQ; mg/kg):**Ca (150); Cu (2); Fe (10); K (200); Mg (50); Mn (0.05); Na (100); P (100); Zn (5).		
Caution	1) Use fume hood and wear full personal laboratory protective clothing, gloves, and appropriate eye protection (safety glasses) when using glassware and preparing standards or test portions with acid solutions. 2) Inductively coupled plasmas should only be viewed with proper eye protection from ultraviolet emissions. 3) Reagents should be regarded as potential health hazards and exposure to these materials should be minimized. Follow universal precautions. Wear gloves, a lab coat, and safety glasses while handling reagent. 4) Exercise caution when handling and dispensing concentrated acids. Always add acid to water. Acids are caustic chemicals that are capable of causing severe eye and skin damage. If acids or bases come in contact with any part of the body, quickly wash the affected area with copious quantities of water for at least 15 minutes. 5) Application of microwave digestion systems involves hot pressurized acid solutions and concentrated acids. Follow manufacturer's directions for safety risk and safety environment of microwave systems. Never remove hot vessels from microwave; wait until they are near room temperature. Keep microwave door closed while vessels are hot.		
Principle	The principle involves the removal of organic matter of the sample through acid digestion to ensure the trace elements are in free form for their measurement by ICP-OES. Test portion is heated at 200°C either with nitric acid in a closed-vessel microwave digestion system (MDC) or with a combination of hydrogen peroxide, nitric acid, and hydrochloric acid in an open-vessel microwave digestion system (MDO).		
Apparatus/Instruments	**1) Microwave-** Commercial MDC or MDO designed for laboratory use at 200 ± 20°C, up to 600 psi, and controlled temperature or pressure ramping capability.It is recommended that vessel design be selected that will withstand the maximum possible pressure (600 psi) since organic residues of rich-fat or rich-carbohydrate samples, if not given sufficient time to predigest, will generate significant pressure during digestion. **2) ICP-OES Spectrometer**—Instrument with axial, radial, or dual view grating configurations and autosampler, capable of determining multiple wavelengths for each element of interest with the required sensitivity. A 3-		

	channel peristaltic pump with or without appropriate in-line addition system (e.g., T connector) are linked between the peristaltic pump and nebulizer to avoid having to manually add ionization buffer and internal standards to each sample solution. A thermostated cyclonic spray chamber equipped with a micro-concentric nebulizer or other components designed to optimize aerosol and maximize precision was used. Select peristaltic pump rotation speed, sample, and internal standard pump tubes of similar size to maximize mixing accuracy while maintaining the required detection levels. Ionization buffer (cesium chloride) is combined with the internal standard solution to compensate EIEs effects (e.g., K, Na, and Ca) in the plasma since certain food materials can contain substantial concentrations of these elements. This provides a significant source of electrons in the plasma. The presence of ionization buffer in all samples and standards will minimize the effects of varying concentrations of EIEs in the sample. The solution presented to the nebulizer contains a maximum of 5000 mg/kg cesium for high- salted food raw materials (e.g., culinary products or tastemakers) and a minimum of 500 mg/kg cesium (for main food samples); 20 mg/kg indium and 5 mg/kg strontium, yttrium, and chromium; less than half of each element concentration of the higher working standard Std6 and less than 0.5 g/kg total dissolved minerals. **3) ICP wavelengths**- A number of recommended and alternative wavelengths may be used for the nine elements to be determined and internal standards. As a minimum, select one recommended and one alternative wavelength for each element corrected by one recommended wavelength for appropriate internal standard. All responses for both recommended and alternative wavelengths for each element are corrected using only one internal standard line. The following is a list of wavelengths for each element (and its appropriate internal standard) in priority order that have been found acceptable for main foodstuffs: The following is a list of wavelengths for each element (and its appropriate internal standard) in priority order that have been found acceptable for main foodstuffs: Wavelength (nm): Ca: 317.933 (In: 303.936); Cu: 324.754 (In: 303.936); Fe: 259.94 (Sr: 338.071); K: 766.491 (Sr: 460.733); Mg: 285.213 (In: 303.936); Mn: 257.610 (Sr: 338.071); Na: 589.592 (Sr: 460.733); P: 213.618 (In: 303.936); Zn: 213.857 (Sr: 338.071). Other wavelengths that are acceptable for both elements and internal standards could be used as confirmatory analytical lines or alternative wavelengths as certain recommended lines may not be available on some ICP-OES systems: Wavelength (nm): Ca: 317.933 (Y: 371.028); Cu: 324.754 (Y: 371.028), 327.395 (In: 303.936 or Y: 371.028); Fe: 259.94 (Y: 371.028 or Cr: 283.563); Mg: 285.213 (Y: 371.028), 279.028 (In: 303.936); Mn: 257.610 (Sr: 460.733 or Y: 371.028); P: 178.222 (Sr: 460.733 or Y: 371.028); Zn: 213.857 (Sr: 460.733 or Y: 371.028).
Materials and Reagents	1) High-grade water, (18 MΩ).—For slurry preparation and/or dilution. 2) Nitric acid (HNO3), 65% (w/v).—Trace metal grade throughout.

3) Hydrochloric acid (HCl), 37% (w/v).—Trace metal grade throughout.
4) Hydrogen peroxide (H_2O_2), 97% (w/v).—Trace metal grade throughout.

Preparation of Reagents

1) Ionization buffer/internal standard solution—Weigh 1.27 g cesium chloride into a 1000 mL acid-washed volumetric flask [Note: This cesium 0.1% (w/v) solution was tested as the minimal recommended concentration required for element analysis in most food matrixes. Cs solution 1% (w/v) is recommended if an element is present at low concentration in high-salted food raw materials, e.g., culinary products or tastemakers, or if it is analyzed as an impurity in food-grade salts.] Add 40 mL indium 1000 mg/kg and 10 mL each of strontium, yttrium, and chromium 1000 mg/kg stock standard solutions, as internal standards. Add 10 mL HNO3. Dilute to volume with H_2O, mix, and transfer to an acid-washed polyethylene bottle.

(Note: Reagent concentrations assume the use of same pump tubing internal diameter for both internal standard/ionization buffer and sample pump tubes using automatic addition.)

2) Stock standard solution—Working standards can be prepared from ICP-grade individual element 1000 mg/kg (e.g., for copper, iron, manganese, and zinc) and 10 000 mg/kg (e.g., for calcium, magnesium, phosphorus, potassium, and sodium) commercial stock standard solutions. However, it is also acceptable to use commercially prepared, custom blended stock standard mixtures containing all of the nine elements at appropriate concentrations.

3) Intermediate stock solution—Suggested composition of the intermediate stock standard solution:

Table 1 (Preparation of intermediate solutions from stock solution)

S No	Metal	Stock solution conc. (mg/kg)	Intermediate stock solution conc. (mg/kg)	Volume of stock solution required for 500 ml
1	Calcium	10000	1500	75
2	Magnesium	10000	500	25
3	Phosphorus	10000	1000	50
4	Potassium	10000	2000	100
5	Sodium	10000	1000	50
6	Copper	1000	10	5
7	Iron	1000	50	25
8	Manganese	1000	0.25	0.125
9	Zinc	1000	20	10

4) Working standard solutions—Standards prepared from intermediate stock standard solution are designed to have the same acid concentration

as digested test solutions (i.e., 10%, v/v, HNO_3) for MDC or 15% (v/v) for MDO using combined acids (HNO_3, H_2O_2, and HCl).

(1) Std6—Pipet 15.0 mL intermediate stock standard solution into a 100 mL acid-washed volumetric flask. Add 10 mL HNO_3 (MDC) or 15 mL combined acids (MDO), dilute to volume with H_2O, mix, and transfer to acid-washed polyethylene bottle.

(2) Std5—Pipet 10 mL intermediate stock standard solution into a 100 mL acid-washed volumetric flask. Add 10 mL HNO_3 (MDC) or 15 mL combined acids (MDO), dilute to volume with H_2O, mix, and transfer to acid-washed polyethylene bottle.

(3) Std4—Pipet 5.0 mL intermediate stock standard solution into a 100 mL acid-washed volumetric flask. Add 10 mL HNO_3 (MDC) or 15 mL combined acids (MDO), dilute to volume with H_2O, mix, and transfer to acid-washed polyethylene bottle.

(4) Std3—Pipet 2.0 mL intermediate stock standard solution into a 100 mL acid-washed volumetric flask. Add 10 mL HNO_3 (MDC) or 15 mL combined acids (MDO), dilute to volume with H_2O, mix, and transfer to acid-washed polyethylene bottle.

(5) Std2—Pipet 1.0 mL intermediate stock standard solution into a 100 mL acid-washed volumetric flask. Add 10 mL HNO_3 (MDC) or 15 mL combined acids (MDO), dilute to volume with H_2O, mix, and transfer to acid-washed polyethylene bottle.

(6) Std1—Pipet 0.5 mL intermediate stock standard solution into a 100 mL acid-washed volumetric flask. Add 10 mL HNO_3 (MDC) or 15 mL combined acids (MDO), dilute to volume with H_2O, mix, and transfer to acid-washed polyethylene bottle.

(7) Blank—Add 10 mL HNO_3 (MDC) or 15 mL combined acids (MDO) into a 100 mL acid-washed volumetric flask, dilute to volume with H_2O, mix, and transfer to acid-washed polyethylene bottle. All calibration solutions when made are stable for 1 week in glass volumetric flasks.

(a) Sampler wash solution- 10% HNO_3 (v/v).—Dilute 100 mL trace metal-grade HNO_3 to 1000 mL with H_2O.

Table-2 (Suggested concentration of the six standard solutions, mg/kg)

Element	Blank	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Calcium	0	7.5	15	30	75	150	225
Magnesium	0	2.5	0.5	10	25	50	75
Phosphorus	0	5	10	20	50	100	150
Potassium	0	10	20	40	100	200	300
Sodium	0	5	10	20	50	100	150
Copper	0	0.05	0.1	0.2	0.5	1.0	1.5
Iron	0	0.25	0.5	1.0	2.5	5	7.5
Manganese	0	0.00125	0.0025	0.005	0.0125	0.025	0.0375
Zinc	0	0.1	0.2	0.4	1.0	2.0	3.0

Sample Preparation

(a) Sample preparation—

(1) Test sample preparation—Homogenize a representative sample by

	grinding as finely as possible and/or by preparing a slurry with H_2O. For example: Infant cereals and fortified milk powders, preheat water at 50°C. Prepare the slurry by weighing 10.0 ± 0.1 g test sample and place into a 100 mL Erlenmeyer flask; add 90.0 ± 0.1 g H_2O. Mix well with stopper. **(2) Test portion preparation**—Accurately weigh 0.50 ± 0.01 g test portion or sample mass on a dry weight basis in the prepared slurry to MDC vessel (1.00 ± 0.01 g into a 100 mL volumetric flask for MDO). [Note: An optimal analytical test portion mass of 0.5 g (1.0 g for MDO) is based on an empirical maximum energy release by the food of 3 kcal and 90–110% recovery.] Line the MDC vessel walls or Pasteur pipet with weighing paper during sample transfer to keep sample from adhering to sides of vessel or use a Pasteur pipet to transfer liquid samples. (Weigh fluid samples or test portion from slurry test sample directly after mixing.) (Note: Remove weighing paper from sample prior to next step.) Carefully add 5.0 ± 0.1 mL HNO_3 into MDC/MDO vessel (and then 5 mL H_2O_2 only into MDO vessel).Loosely cap MDC vessel without sealing.Predigest for at least 10 min at room temperature or until vigorous foaming subsides. Close MDC vessels and distribute onto microwave carousel to ensure uniform microwave power application on all samples. **(3) Food-grade salt sample preparation**—Weigh 0.20 ± 0.01 g food-grade salt (a minimum dilution factor of 500 is recommended) into a 100 mL volumetric flask. Add deionized water and 10 mL HNO_3. Dissolve salt and dilute to volume with deionized water. **(b) Test portion digestion**— (1) **Sample digestion**—With power setting appropriate to MDC (maximum power of 1600 W) and MDO models (maximum power of 600 W), and number of vessels used, heat MDO vessels at 200 ± 20°C for 20 min or ramp MDC temperature from ambient to 200 ± 20°C in 15 min and hold at 200°C for 25 min. (Note: Yellow vapors will be emitted during the hydrolysis in MDO vessels.) Carefully remove the MDO vessels. Allow the vessels to cool down to room temperature. Add 5 ml HCl 35% (w/v) into MDO vessels and heat MDO vessels at 200 ± 20°C for 20 min. Cool vessels to room temperature before venting (MDC vessels). Transfer the MDC digests to 50 mL (100 mL for MDO) volumetric flasks. Dilute to volume with H_2O and mix. (Note: A digestion is judged complete when clear to yellow analytical solutions are produced.) Filter the digested solution using an ashless filter paper for turbid samples containing fat. Discard the first 20 mL filtrate and collect the remaining filtrate for analysis.

	(Note: Membrane disc filters (0.45 µm) are not recommended as they are generally not metal-free.) Transfer to polyethylene containers within 2 h. Dilute the samples that are found to be above the standard curve range or have total content of minerals higher than 1000 mg/L with H_2O.
Method of analysis	1) Make a calibration curve using either weighted linear or quadratic regression with correlation coefficients of at least 0.9999 from seven standards prepared from intermediate standard solution, including a blank (Std 0) and six suggested concentrations of the standard solution (Std1–Std6) shown in Table-2and expressed in mg/kg. 2) Analyze test solutions using an ICP-OES instrument calibrated with the working standard solutions. 3) Insert a working standard or other suitable quality control solution every 10 test portions to monitor for instrument drift. The inclusion of a digestion blank, a sample duplicate, and known reference materials is highly encouraged.
Calculation with units of expression	The concentration (C) of each element, in mg/kg, is calculated as follows: $$C = \frac{a \times V \times F}{m}$$ where C = concentration in the test portion sample (mg/kg); a = concentration (mg/L) of the element in the digest solution as obtained from instrument; V = volume (mL) of the test solution after being made up (i.e., 50 mL for MDC and 100 mL for MDO); F = dilution factor of the test solution; m = weight of the test portion (g).
Inference (Qualitative Analysis)	
Reference	AOAC Official Method 2011.14
Approved by	Scientific Panel on Methods of Sampling and Analysis

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Method No.	**FSSAI 09.005:2024**	**Revision No. & Date**	0.0
Scope	Applicable to the determination of As, Cd, Hg, and Pb in a variety of foods by pressure digestion and inductively coupled plasma-mass spectrometry (ICP-MS). Method is capable of determining As, Cd, Pb, and Hg at or above 0.06, 0.03, 0.04, and 0.09 mg/kg dry matter, respectively.		
Caution	1) Use fume hood and wear full personal laboratory protective clothing, gloves, and appropriate eye protection (safety glasses) when using glassware and preparing standards or test portions with acid solutions. 2) Inductively coupled plasmas should only be viewed with proper eye protection from ultraviolet emissions. 3) Reagents should be regarded as potential health hazards and exposure to these materials should be minimized. Follow universal precautions. Wear gloves, a lab coat, and safety glasses while handling reagent. 4) Exercise caution when handling and dispensing concentrated acids. Always add acid to water. Acids are caustic chemicals that are capable of causing severe eye and skin damage. If acids or bases come in contact with any part of the body, quickly wash the affected area with copious quantities of water for at least 15 minutes. 5) Digestion of carbon-rich materials (e.g., carbohydrates, fats, oils) can result in explosions.		
Principle	Foodstuffs are mineralized (digested) in closed vessels by nitric acid at elevated temperature and pressure by conventional or microwave-assisted heating. The mineralized sample is dilutedwith water to a defined volume to produce the test solution. Test samples may be either dry or wet. Test samples may be dried and results corrected for moisture. The test solution, obtained by pressure digestion, is transferred to the sample introduction system of the ICP-MS instrument and nebulized, and the aerosol is transferred to high frequency inductively coupled argon plasma. The high temperature of the plasma is used to dry the aerosol and to atomize and ionize the elements. The ions are extracted from the plasma by a set of sampler and skimmer cones and transferred to a mass spectrometer by vacuum, where the ions are separated by their mass/charge ratio (m/z) and determined by a pulse-count and/or analog detector.		
Apparatus/Instruments	1) **Pressure digestion**—Commercially available microwave digestion system or high-pressure asher for acid digestion in an acid-resistant sealed vessel with a low level of contamination. Capable of digestions by conventional or microwave-assisted heating in a sealed vessel in a pressure container. 2) **Inductively coupled plasma-Mass spectrometer (ICP-MS)**—Mass		

	spectrometer with inductively coupled argon plasma operating in a mass range from 5–240 amu. Mass spectrometers with additional reaction or collision cells may be used to reduce the influence of polyatomic ions. An ICP-MS instrument having a nebulizing system with a low pulsation peristaltic pump should be equipped with a mass flow controller for the nebulizer gas.
Materials and Reagents	1) Nitric acid—Not less than 65%, with a density of ca 1.4 g/mL. 2) Hydrogen peroxide (H_2O_2)—30%. 3) Water—Specific resistance >18 mega ohm-cm. 4) Element stock solutions- Commercially available single element or multi-element standards with a concentration of 1000 mg/L are recommended.
Preparation of Reagents	1) **Diluted Hg stock solution**—Hg = 10 mg/L, prepared by dilution of 1 mL Hg and 1 mL nitric acid with water to the mark of a 100 mL volumetric flask. 2) **Diluted multi-element stock solution**—The concentration levels of the elements in the diluted multi-element stock solution may be chosen with reference to the type of samples to be analyzed. The following descriptions are given as an example: As = 20 mg/L, and Cd and Pb = 10 mg/L. Pipet 2 mL As stock solution and 1 mL of the single element standards Cd and Pb each in a 100 mL volumetric flask, add 1 mL nitric acid, dilute with water to the mark, and transfer the solution into a suitable vessel. 3) **Multi-element calibration stock solution**—According to the example given, the multi-element calibration stock solution contains 100 µg As/L and 50 µg/L for Cd, Hg, and Pb. Pipet 0.5 mL of diluted Hg stock solution and 0.5 mL of the diluted multi-element stock solution into a 100 mL volumetric flask, add 1 mL nitric acid, dilute with water to the mark, and transfer the solution into a suitable vessel (PFA or quartz is recommended). 4) **Calibration solutions**—For calibration of the instrument a set of at least three different concentrations are used (in addition to the standard reagent blank). The concentration range should be chosen with respect to the concentrations expected and with respect to the linear dynamic range. It is important that the concentration of nitric acid in the sample solutions and the calibration solutions are approximately the same. The calibration solutions should be prepared freshly before use. **Example: Calibration solution 1** contains—1 µg As/L and 0.5 µg/L Cd, Hg, and Pb (each); pipet 0.5 mL of the multielement calibration stock solution, into a 50 mL volumetric flask, add 1 mL nitric acid, and dilute with water to the mark. **Calibration solution 2** contains—5 µg As/L and 2.5 µg/L Cd, Hg, and Pb (each); pipet 2.5 mL of the multielement calibration stock solution, into a 50 mL volumetric flask, add 1 mL nitric acid, dilute with water to the mark. **Calibration solution 3** contains.—20 µg As/L, 10 µg/L Cd, Hg, and Pb

	(each); pipet 10 mL of the multielement calibration stocksolution, into a 50 mL volumetric flask, add 1 mL nitric acid, and dilute with water to the mark. 5) **Standard reagent blank**—Standard reagent blank contains water and the same amount of acid used in the calibration stock solution. 6) **Internal standard stock solution**—Rh and Lu with a concentration of 1000 mg/L is recommended. Alternatively, other internal standards may also be used. Au is used to stabilize Hg in the solution and to reduce memory effects. The internal standards should cover the mass range used for determination of the elements. Their concentrations in the test solutions should be negligible. 7) **Diluted internal standard stock solution**—The concentration of the diluted internal standard solution should be high enough to give a sufficient signal intensity. For example, Au, Rh, and Lu at 5 mg/L can be used. Pipet 0.5 mL of Au, Rh, and Lu internal standard stock solution each into a 100 mL flask, add 1 mL nitric acid, dilute to volume with water, and transfer the solution into a suitable vessel. 8) **Optimization solution**—The optimization solution is used for check and optimizing procedures during set up of the ICP-MS instrument. It is used for mass calibration purposes and for adjustment of maximum sensitivity at low rates of oxides and doubly charged ions. The optimizing solution should contain elements that cover the whole mass range giving a high rate of oxides and double charged ions. The solutions recommended by the manufacturer of the ICP-MS instrument may be used. A solution containing, e.g., Y, Rh, Ce, and Pb is suitable for those purposes. The concentration of these elements should be chosen in order to achieve a count rate of 10 000–100 000 cps.
Sample Preparation	1) Equipment which does not impart any or least possible contamination particularly with respect to the analytes of the interest is used to homogenize the sample. 2) **Moisture content (optional)**—To avoid possible losses of volatile elements such as As and Hg, the determination of moisture content should be done on separate homogenized test portions rather than on test portions used for analysis. Determination of optimal drying temperatures and times are needed to avoid mass loss due to loss of volatile oils. Where previous drying studies have been conducted, recommended temperatures and drying times can be used. For oven

	drying, temperatures may range from 80°C to 110°C until constant mass is reached. Alternatively samples can be dried over $Mg(ClO_4)_2$in a sealed desiccators until constant mass is reached. The drying factor necessary to convert the mass of the stored material to a dry-mass basis should be determined at each use to account for changes in mass due to the exposure of the material to the laboratory atmosphere. 3) Food samples are digested in sealed pressure digestion vessels. The sample mass is chosen to match the capacity of the digestion vessel following the manufacturer's instructions which may also limit the carbon content to be digested. The test sample portion and the appropriate amounts of nitric acid and hydrogen peroxide are placed in the digestion vessel. The vessel is secured in the pressure digester and the temperature/pressure program implemented. **Microwave-assisted wet digestion:** **(a)** Weigh approximately 0.20 g dry weight sample material into the digestion container. **(b)** Each digestion series must contain two reagent blanks (nitric acid and hydrogen peroxide without sample materials). Include at least one certified reference material corresponding to the amount expected in the samples, in order to reveal systematic errors. **(c)** Digestion programs and amounts of acids will vary with different digestion systems. Add 2 mL concentrated nitric acid and 0.5 mL hydrogen peroxide to each container. **(d)** Seal the containers in the capping station. Place carousel with the digestion containers in the microwave oven and start the digestion program. **(e)** The digested solution is diluted by water to a known volume (test solution).
Method of analysis	1) **Preparation of calibration solutions and test solutions for ICP-MS measurement**—All solutions to be measured by ICP-MS during routine runs should contain one or a set of internal standards. The concentration of the internal standard(s) must be equal in all of the solutions. For the determination of Hg, Au must be added in order to stabilize the Hg and minimize memory effects in the tubing and during nebulization. The test solution obtained by pressure digestion should be analyzed after dilution to a known volume. **Example:** Pipet exactly 10 mL of standard reagent blank or calibration solution to a vessel; add 0.1 mL diluted internal standard stock solution and mix. Pipet exactly 2 mL of test solution to a vessel; add exactly 8 mL water and 0.1 mL diluted internal standard stock solution and mix. Every solution contains ca 10 µg/L of the internal standard Rh. The internal

	standard solution may also be added online by a different channel on the peristaltic pump used for the analytes. Adjust the concentration of the internal standard solution and the pump flow rate in order to achieve a concentration of the internal standard of ca 50 µg/L. 2) **Calibration of the ICP-MS instrument**—For calibration purposes, a minimum of three different concentrations must be used. Measure the standard reagent blank and then the calibration solutions. According to the instrument manual, calculate the calibration function. Different isotope ratios between calibration solutions and test solutions should be taken into account, if necessary. 3) **Analyses of test solutions**—After calibration of the instrument, the test solutions can be analyzed. The samples obtained by pressure digestion should be diluted before measurement in order to avoid interference by high concentrations of matrix elements. If the final volume of the digested solution is 20–30 mL, a dilution by a factor of 5–10 is recommended for the ICP-MS measurement. Within suitable short intervals (e.g., after 5 or 10 samples), the blank solution and one calibration solution should be checked regularly. The recovery of the calibration solution should range within 10%. For high concentrations of Hg, prolonged washout times have to be applied. The blank level for Hg should be checked regularly in order to detect any memory or washout effects. The system should be tested for washout times using the highest calibration standard. 4) **Control for matrix effects**—The amount of matrix present in the test solution to be analyzed may create more or less significant matrix effects compared to pure multi-element standards. To check for matrix effects, a known amount of the multi-element standard is added to the test solution. **Example:** Pipet exactly 2 mL test sample into a sample vessel, and add exactly 7 mL water and 1 mL Calibration Solution 3. Then add 0.1 mL internal standard stock solution and mix. The non-added sample is prepared in the same way by using 1 mL water instead of the calibration solution. The concentrations found by addition of the standard should not exceed 10% of the added concentration. In case of greater differences, the matrix effects must be compensated by a standard addition calibration. 5) **Standard addition calibration**—A standard addition calibration should consist of at least three points, of which two are standard additions. The concentration of the highest standard should be three to five times the concentration in the sample solution. The concentration of the lower standard should be half of the highest standard, i.e., 100, 200, and 400% of the initial concentration in the test sample. The non-spiked test

	solution is used as the lowest level in the calibration curve. The linear regression through these points crosses the negative concentration axis. The absolute value of this point is the concentration of the element in the sample solution. **Example:** For a test solution containing ca 0.5 µg Cd/L, pipet into 4 different sample vessels exactly 2 mL of each test sample. To the first sample vessel, add exactly 8 mL water (= non-spiked test solution). To the second sample vessel, add exactly 7.5 mL water and 0.5 mL Calibration Solution 3 (= Sample Spike 1, with an added concentration of 0.5 µg Cd/L). To the third sample vessel, add exactly 7 mL water and 1 mL Calibration Solution 3 (= Sample Spike 2, with an added concentration of 1 µg Cd/L). To the fourth sample vessel, add exactly 6 mL water and 2 mL Calibration Solution 3 (= Sample Spike 3, with an added concentration of 2 µg Cd/L).
Calculation with units of expression	Calculation of the concentration is generally done automatically by the software of the ICP-MS instrument. The following steps are performed for each element: The count rates are corrected according to the correction functions chosen, the count rates are measured in the standard reagent blank, and calibration and test solutions are normalized on the count rates of the internal standard. The calibration function is then calculated. By the use of the count rates, the calibration function and the dilution factor of the concentrations of the elements are calculated. The content, W, as mass fraction, of the element to be determined in mg/kg of sample is calculated using the following equation: $$W = \frac{a \times V \times F}{m \times 1000}$$ Where, a is the content (µg/L) of the element in the test solution, V is the volume (mL) of the digestion solution after being made up to volume, F is the dilution factor of the test solution, and m is the mass of the test portion (**g**). Report moisture content if test samples were dried and indicate mass fraction (W) as dry matter. Alternatively, correct dry matter result for moisture content.
Inference (Qualitative Analysis)	
Reference	AOAC Official Method 2013.06
Approved by	Scientific Panel on Methods of Sampling and Analysis

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Method No.	**FSSAI 09.006:2024**	**Revision No. & Date**	0.0
Scope	Applicable to the determination of Cr, Se, and Mo in Infant Formula and Adult Nutritional Productsby pressure digestion and inductively coupled plasma-mass spectrometry (ICP-MS). Method is capable of determining Cr, Se, and Mo at or above 0.06, 0.03, and 0.09 mg/kg dry matter, respectively.		
Caution	1) Use fume hood and wear full personal laboratory protective clothing, gloves, and appropriate eye protection (safety glasses) when using glassware and preparing standards or test portions with acid solutions. 2) Microwave operation involves hot pressurized acid solution. Use appropriate face protection and laboratory clothing. 3) Inductively coupled plasmas should only be viewed with proper eye protection from ultraviolet emissions. 4) Reagents should be regarded as potential health hazards and exposure to these materials should be minimized. Use normal laboratory safety precautions (laboratory coats and safety glasses with side shields) when handling concentrated acids, bases, and organic solvents. Additional protections such as face shields, neoprene gloves, and aprons should be used where splashing may occur. 5) Exercise caution when handling and dispensing concentrated acids. Always add acid to water. Acids are caustic chemicals that are capable of causing severe eye and skin damage. If acids or bases come in contact with any part of the body, quickly wash the affected area with copious quantities of water for at least 15 minutes.		
Principle	Test portion is heated with nitric acid in a closed vessel microwave digestion system at 200°C. Digested test solution, or an appropriate dilution, is presented to the inductively coupled plasma-mass spectrometer (ICP-MS) instrument standardized with acid matched standard calibrant solutions. An ionization buffer (potassium) is used to minimize easily ionizable element (EIE) effects, methanol is added to normalize the carbon content, and nickel and tellurium are used as internal standards.		
Apparatus/Instruments	1) **Microwave**—Commercial microwave designed for laboratory use at 0–300°C, with closed vessel system and controlled temperature ramping capability. It is recommended that the vessel design be selected that will withstand the maximum possible pressure, since organic material, and also carbonates if not given sufficient time to predigest, will generate significant pressure during digestion. (Vessels can reach 700 psi or more on occasion.) Vessels must be designed to operate with only 6 mL solution volume, or the volume must be adjusted accordingly. 2) **ICP-Mass Spectrometer**—With collision reaction cells (CRCs).		

	3) Various plastic ware and pipets.
Materials and Reagents	1) **Laboratory water**—Use 18 MΩ water throughout for dilution. 2) **Concentrated nitric acid (HNO3)—**65–70% trace metal-grade HNO_3 throughout. 3) **Hydrogen peroxide**—30% ACS reagent grade. 4) **Methanol**—99.99% analytical reagent grade for matrix matching. 5) **Potassium**—10 000 mg/L in nitric acid for matrix matching.
Preparation of Reagents	1) 2 mg/L Cr and Mo and 1 mg/L Se multi-element stock standard solution in nitric acid—High-Purity Standards, or equivalent. 2) 5 mg/L Ni and Te multi-element stock standard solution in nitric acid.—High-Purity Standards, or equivalent. 3) **Standard preparation**— **(a)** Prepare intermediate standards from commercial stock standards at 40 ng/mL Cr and Mo, and 20 ng/mL Se. Custom-blended multi-element stock standard in HNO_3 is acceptable. **(b)** Prepare a minimum of three multi-element working standards containing 0.8, 4.0, and 20 ng/mL Cr and Mo and 0.4, 2.0, and 10 ng/mL Se, plus blank, with both Ni and Te internal standard, in HNO_3. Ni is used as the internal standard for both Cr and Mo, and Te must be used for Se.
Sample Preparation	1) Prepare powder samples by reconstituting approximately 25 g sample in 200 ml warm laboratory water (60°C). 2) Accurately weigh approximately 1.8 g reconstituted test portion into the digestion vessel. This represents approximately 0.2 g original powder sample. 3) Fluid samples may be prepared by accurately weighing approximately 1 g test portion weighed directly into the digestion vessel after mixing. **For 1-step digestion**, add 4) Microwave digestion- (a) Add 0.5 mL 5000 ng/mL Ni and Te internal standard solution and 5 mL trace metal-grade HNO_3 followed by 2 mL H_2O_2 to the microwave digestion vessels. (b) Seal vessels according to manufacturer's directions and place in microwave. (c) Ramp temperature from ambient to 180°C in 20 min, and hold for 20 min in stage 1. In stage 2, the microwave will automatically ramp to 200°C in 20 min, and hold for 20 min. For microwave ovens without the 2-stage program and where it is more convenient, use the 2-step digestion. Add 0.5 mL 5000 ng/mL Ni and Te internal standard solution and 5 mL trace metal-grade HNO3. With power settings appropriate to microwave model and number of vessels, ramp temperature from ambient to 200°C in 20 min. Hold at 200°C for 20 min. Cool vessels according to

manufacturer's directions, approximately 20 min.

(d) Cool vessels according to manufacturer's directions, approximately 20 min.

(e) Slowly open the microwave vessels, venting the brownish nitrogen dioxide gases.

(f) Add 1 mL H_2O_2 and redigest samples by ramping the temperature from ambient to 180°C in 15 min. Hold at 180°C for 15 min and cool for 20 min.

5) **Preparation of test solution**—Add approximately 20 mL laboratory water to the contents of the vessel with the digested samples and transfer to a 50 mL sample vial. Rinse the vessel and transfer the rinsate into the sample vial. Add 0.5 mL methanol to the sample vial and dilute to 50 mL with laboratory water.

Stage 1 sample digestion		
1	Power	100% (1600W)
2	Ramp to temperature	20 min
3	Hold Time	20 min
4	Temperature	180°C
5	Cool down	20 min
Stage 2 sample digestion		
1	Power	100% (1600W)
2	Ramp to temperature	20 min
3	Hold Time	20 min
4	Temperature	200°C
5	Cool down	20 min

Method of analysis

1) Analyze test solutions using an ICP-MS instrument standardized with standard solutions. Ni is used as the internal standard for both Cr and Mo (helium mode), and Te must be used for Se (hydrogen mode).

2) Analyze a 4 ng/mL Cr and Mo, and 2 ng/mL Se working standard or other suitable quality control solution every 10 test portions to monitor for instrument drift and linearity (result 100 ± within 5% of nominal).

3) The inclusion of a method blank (run as a sample), a duplicate sample [relative percent difference (RPD) ≤ within 10%], and known reference materials serving as control samples (recovery check within control limits) are considered mandatory for good method performance. If any of these QC checks fails, results should be considered invalid.

Calculation with units of expression

Sample concentrations were automatically calculated by the ChemStation software using a nonweighted least-squares linear regression calibration analysis to produce a best-fit line:

$$Y = ax + \text{blank}$$

	$$x = \frac{y - \text{blank}}{a} \text{ x DF}$$ where *x* = analyte concentration (ng/g); *y* = sample response ratio (ng/mL), which is the measured count of each analyte's standard solution data point in the calibration curve divided by the ratio of the counts/concentration of the internal standard at the same level; blank = blank standard solution (ng/mL), which is the measured count of the blank standard solution data point in the calibration curve divided by the ratio of the counts/concentration of the internal standard at the same level as the blank standard solution; a = slope of the calibration curve; DF = dilution factor of the sample solution divided by sample weight (mL/g).
Inference (Qualitative Analysis)	
Reference	**AOAC Official Method 2011.19**
Approved by	Scientific Panel on Methods of Sampling and Analysis

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 the analysis of Total Iodine in Infant Formula and Adult/Pediatric Nutritional formula by Inductively Coupled Plasma–Mass Spectrometry**		
Method No.	**FSSAI 09.007:2024**	**Revision No. & Date**	0.0
Scope	Applicable to the measurement of total iodine in infant formula and adult/pediatric nutritional formula from 0.5 to 1500 µg/100 g reconstituted final product and for ready-to-feed (RTF) products from 2.5 to 1000 µg/100 g using ICP-MS. This method is not applicable to products containing FD&C Red Dye No. 3 (erythrosine). The iodine from erythrosine is also quantitatively determined by this method; thus, accurate quantification of fortified levels of iodine is not possible.		
Caution	1) Use fume hood and wear full personal laboratory protective clothing, gloves, and appropriate eye protection (safety glasses) when using glassware and preparing standards or test portions with acid solutions. 2) Oven and microwave digestion procedures involve moderately elevated temperatures. Carefully remove samples and allow cooling before removing the lids from the digestion vessels. 3) Inductively coupled plasmas should only be viewed with proper eye protection from ultraviolet emissions. 4) The method involves the use of strong bases and concentrated acids. Avoid spills, inhalation, and exposure to human tissues. Use normal laboratory safety precautions (laboratory coats and safety glasses with side shields) when handling concentrated acids, bases, and organic solvents. Additional protections such as face shields, neoprene gloves, and aprons should be used where splashing may occur. 5) Exercise caution when handling and dispensing concentrated acids. Always add acid to water. Acids are caustic chemicals that are capable of causing severe eye and skin damage. If acids or bases come in contact with any part of the body, quickly wash the affected area with copious quantities of water for at least 15 minutes.		
Principle	Digestion occurs using a potassium hydroxide (KOH) solution in an oven or open-vessel microwave system. Iodine is stabilized with ammonium hydroxide and sodium thiosulfate after digestion. The solution is brought to volume followed by filtration. The filtrate is analyzed directly or after dilution by ICP-MS.		
Apparatus/Instruments	1) Polypropylene (PP) tubes—Assorted sizes, use as received. 2) Oven (i.e., warming/drying oven). 3) Open-vessel microwave digestion unit (optional). 4) Analytical and top-loader balances.—Sensitive to 0.0001 and 0.01 g, respectively. 5) ICP-MS system. 6) Auto sampler for ICP-MS. 7) Adjustable (electronic or manual) volumetric pipets and pipet tips. 8) Re-pipet volumetric dispenser—Adjustable volume.		

	9) Polypropylene or Teflon bottles for storage of reagents. 10) Disposable plastic syringes. 11) Syringe filters with 1 µm membrane (e.g., 0.25 or 0.45 µm) may be used. **Note:** All laboratory plasticware should be single-use whenever possible. If reuse is necessary, wash using 10% nitric acid, then rinse thoroughly with purified water prior to use. When needed, general laboratory acid-washed glassware may also be used.
Materials and Reagents	1) KOH (KOH) pellets, certified ACS—KOH may contribute background levels of iodine. 2) KOH solution—50% (w/v). 3) Ammonium hydroxide (NH_4OH)—Certified ACS. 4) Sodium thiosulfate ($Na_2S_2O_3$)—99.99+% metal basis. 5) Surfactant (i.e., Triton® X-100). 6) Nitric acid (HNO_3)—High purity. 7) Perchloric acid ($HClO_4$)—High purity. 8) Purified water—18 MΩ/cm. 9) Iodide 1000 ppm standard solution in H_2O. 10) Iodide 1000 ppm standard solution in 1% triethanolamine (TEA). **Note:** Either stock iodide reference solutions may be used for intermediate and working standard solutions preparation. The remaining source may be used as a continuing calibration verification (CCV) standard.
Preparation of Reagents	**Iodine stock standard solutions**—Certified ICP-MS or ICP grade single- or multi-element standard solutions (or other certified reference materials; CRM) are used to prepare calibration, calibration verification standards, internal standards, and spiking solutions. Internal standards are prepared using certified ICP-MS or ICP-grade single- or multielement standard solutions whenever possible. When applicable, choose elements for internal standards within ±40 atomic mass units (amu) from the mass to be quantified. Likely choices for use as internal standards for iodine analysis are praseodymium (Pr), samarium (Sm), tellurium (Te), and rhodium (Rh). Concentrations used for analysis are 30.0 ppb Pr, Sm, Rh, and 500 ppb Te. The internal standard solution reagent's concentration is 2% HNO_3, 0.1% $HClO_4$, 0.01% Triton X-100, 0.25% KOH, 0.1% NH_4OH, and 0.01% $Na_2S_2O_3$ in purified water. 1) **5% KOH solution**—Dissolve 25 g KOH pellets in an appropriate amount of purified water, then dilute to 500 mL with purified water. Store at room temperature. Reagent expires 6 months after preparation date. Alternatively, dilute 50 mL 50% (w/v) KOH solution to a final volume of 500 mL with purified water. Store at room temperature. 2) **50% KOH solution**—Dissolve 250 g KOH pellets in an appropriate amount of purified water, then dilute to 500 mL with purified water. Store at room temperature. **Note:** Use caution when preparing this solution as a significant amount of heat is generated. Alternatively, use purchased 50% (w/v) KOH solution. Store at room temperature.

	3) **Stabilizer concentrates—**Dissolve 5 g $Na_2S_2O_3$ in an appropriate amount of purified water, add 50 mL NH_4OH, then dilute to 500 mL with purified water. The resulting concentrationis 10% NH_4OH and 1% $Na_2S_2O_3$ in purified water. Store at room temperature. 4) **Wash solution (rinse*)***—Dissolve 2 g Triton X-100 in an appropriate amount of purified water, add 20 mL NH_4OH, then dilute to 2 L with purified water. The resulting concentration is 1% NH_4OH and 0.1% Triton X-100 in purified water. Store at room temperature. 5) **Diluent**—Dissolve 10 g KOH pellets and 0.4 g of $Na_2S_2O_3$ in an appropriate amount of purified water, add 4 mL NH4OH, then dilute to 2000 mL with purified water. Store at room temperature. Alternatively for a smaller volume, dilute 50 mL 5% KOH and 10 mL stabilizer concentrate to 500 mL with purified water. Store at room temperature. **Note:** The resulting concentration for both preparations is 0.5%KOH, 0.2% NH4OH, and 0.02% $Na_2S_2O_3$ in purified water. 6) **Conditioning solution**—Prepare by aliquoting 25 mL 5% KOH (2.5 mL 50% KOH) solution, then diluting to 250 mL with purified water. This solution is used to prepare the instrument for analysis. The resulting concentration is 0.5% KOH. Store at room temperature. 7) **Carrier solution**—Equivalent to the wash solution. The carrier solution is used to deliver the sample solution to the nebulizer through the ICP-MS auto sampler introduction system. The carrier solution is introduced via a peristaltic pump using black/black two-stoppolyvinyl chloride pump tubing (0.76 mm id). Store at room temperature. **Note:** All above reagent expires in 6 months after preparation date.
Sample Preparation	**1) Oven digestion (preferred)—Note:** The following oven digestion procedure is for a final volume of 100 mL. It is critical that the final reagent concentration in all vessels be equivalent to that of the calibration standards. Samples expected to contain levels of iodine below 10 000 µg/kg may be digested using the 5% KOH solution. However, if samples are expected to contain >10 000 µg/kg iodine and are anticipated to be detectable after an appropriate dilution, the 50% KOH solution may be used. Vitamin/mineral dietary supplements or premixes or other certain matrixes should be digested using only the 50% KOH solution. For the testing of vitamin/mineral tablets or premixes, it is recommended (due to potential homogeneity issues) that a reconstitution be performed. Unless a specific reconstitution procedure is required, use the following reconstitution procedure as a guide. **2) Suggested reconstitution procedure—** **(a)** Accurately weigh approximately 5.00 g of sample into an appropriate vessel (150 mL or 250 mL beaker) and record the sample weight. Without zeroing the balance, add water to make approximately 100 g. Record the sample + water weight. Place a stir bar in the mixture and stir on a stir plate to form a homogenous slurry/suspension. While stirring, weigh 5–10 g of the slurry/suspension into an appropriate digestion vessel, add approximately 10 mL water, then proceed with the addition of the KOH as stated below. For the testing of other matrixes, visually evaluate the samplefor

homogeneity before weighing an appropriate amount. If asample does not appear to be homogenous, perform additionalhomogenizing (i.e., blending, grinding, etc.). If applicable, thesample may be reconstituted.

(b) Accurately weigh or aliquot an appropriate amount (0.2500 to 2.50 g or 0.50 to 10 mL) of sample into a labeled 100 mL digestion vessel. Add 20 mL purified water to the vessel.

Accurately weigh an appropriate amount (0.2500 to 1.00 g) of an appropriate CRM, i.e., National Institute of Standards and Technology Standard Reference Material (NIST SRM) 1549 or 3280, if applicable, in the same manner as the samples. SRM 1549 may be digested using either 5 or 50% KOH solution. SRM 3280 should be digested using only the 50% KOH solution.

(c) Designate at least one digestion vessel as the digest blank. The digestion blank(s) should be treated in the same manner as the samples. If both the 5 and 50% KOH solutions will be used,prepare at least one blank with each concentration.Place an aliquot of spiking solution (if applicable) into an appropriately labeled digestion vessel.

(d) Add either 10 mL 5% KOH solution or 10 mL 50% KOH solution to each digestion vessel.

Note: If values well below 10 000 µg/kg are anticipated, add 5 mL 5% KOH solution.

(e) Dilute to 50 mL. Seal the vessels and swirl or use a vortex apparatus to mix. Avoid inverting as this may allow sample to adhere to the inner walls of the vessel above the level of the digestion solution. Digest samples in an oven set to maintain 105 ± 5°C until the dissolution of iodine is complete, approximately 1 h.

(f) After removal from the oven, add 2 mL stabilizer concentrate, then allow the samples to cool before bringing to volume with purified water.

Alternatively, allow samples to cool first, then add2 mL stabilizer concentrate and bring to volume with purifiedwater.

Note: If the final volume will be 50 mL, add 1 mL stabilizer concentrate.

Cap the vessels, then invert to mix thoroughly.

(g) Filter the sample solution by filling a disposable syringe with the digested sample solution, attach a 1 µm membrane filter, then filter an adequate amount (i.e., several milliliters) into appropriate vessel (i.e.,15 mL PP centrifuge tube) to be used for analysis. Store samples at ambient temperature.

3) Open vessel microwave digestion (optional)-

Note: Use only 50 mL disposable PP centrifuge tubes. The following procedure is written for a final volume of 50 mL. It is critical that the final reagent concentration in all vessels be equivalent to that ofthe calibration standards. Follow the first several steps as outlinedabove in Oven Digestion (preferred), then proceed as directedbelow.

Note: For reconstitution of samples, prepare as outlined inOven Digestion (preferred), but weigh 5 g (instead of 5–10 g) of theslurry/suspension, and do not add additional water. Proceed

	withthe addition of KOH as described below. **(a)** Accurately weigh or aliquot an appropriate amount (0.2500 to 1.00 g or 0.50 to 2 mL) of sample into a labeled microwave digestion vessel already contains 5 mL purified water. **(b)** Accurately weigh an appropriate amount (0.2500 to 1.00 g) of an appropriate CRM (i.e., NIST SRM 1549 or 3280), if applicable, inthe same manner as the samples. SRM 1549 may be digested usingeither 5 or 50% KOH solution. SRM 3280 should be digested usingonly the 50% KOH solution. **(c)** Designate at least one digestion vessel as the digest blank. The digestion blank(s) should be treated in the same manner as the samples. If both the 5 and 50% KOH solutions will be used, prepare at least one blank with each concentration. Place an aliquot of spiking solution (if applicable) into an appropriately labeled microwave digestion vessel. **(d)** Add either 5 mL 5% KOH solution or 5 mL 50% KOH solution to each digestion vessel. **Note:** If values well below 500 µg/kg are anticipated, add 5 mL of 5% KOH solution. **(e)** Seal the vessels and swirl or use a vortex apparatus to mix. Avoidinverting as this may allowsample to adhere to the inner walls of thevessel above the level of the digestion solution. Place the digestionvessels into the carousel of the open-vessel microwave digestionunit. If less than the maximum capacity is to be digested, distributethe vessels evenly throughout the carousel. Digest the samples inthe microwave until the dissolution of iodine is complete. Thevessel caps should be loosened slightly (from fully tightened) duringthe digestion procedure. **(f)** After removal from the oven, allow sample to cool first, then add 1 mL stabilizer concentrate and bring to volume with purified water. Cap the vessels, and then invert to mix thoroughly. Alternatively, allow sample to cool first, then add 1 mL stabilizer concentrate and bring to volume with purified water. Cap the vessels, then invert to mix thoroughly. **(g)** Filter the sample solution by filling a disposable syringe with the digested sample solution, attach a 1 µm membrane filter, then filter an adequate amount (i.e., several milliliters) into appropriate vessel (i.e., 15 mL PP centrifuge tube) to be used for analysis. Store samples at ambient temperature.
Method of analysis	The digested samples are analyzed directly or diluted so that the iodine concentration will fall within the calibration range. 1) Samples digested with 50% KOH solution must be diluted 1 to 10 mL to achieve the desired final concentration of 0.5% KOH. 2) Aliquot 1 mL of the filtrate into an appropriate vessel (i.e. 15 mL PP centrifuge tube), add 0.18 mL stabilizer concentrate, then dilute to 10 mL with purified water. 3) If samples digested with 50% KOH solutionneed more than a 1 to 10 mL dilution to obtain a reading on thecalibration curve, an additional dilution must be prepared from theoriginal 1 to 10 mL dilution.

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4) Aliquot the desired amount into anappropriate vessel (i.e., 15 or 50 mL PP centrifuge tube), then diluteto volume with diluent.
5) Condition the ICP-MS sample introduction system.Analyze conditioning solution while concomitantly introducing internal standard solution on-line through a mixing block until conditioned (approximately 1 h). The internal standard solution is introduced via a peristaltic pump using orange/green two-stop PVC pump tubing (0.38 mm id). After conditioning, begin to aspirate carrier solution while continuing to add internal standard. Analyze samples using ICP-MS.

Isotope selection and interferences- The internal standards listed below can use for method development:
<table>
<tr><th>Analyte</th><th>Mass, amu</th></tr>
<tr><td>Iodine</td><td>126.900</td></tr>
<tr><td>Praseodymium</td><td>140.907</td></tr>
<tr><td>Samarium</td><td>146.915</td></tr>
<tr><td>Rhodium</td><td>102.906</td></tr>
<tr><td>Tellurium</td><td>146.915</td></tr>
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<td>Calculation with units of expression</td>
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If a reconstitution was performed, use the following equation:
$$S = [\frac{\frac{\{(C \times V) \times D\}}{WRA}}{10}]$$
Where,
C = sample concentration (ng/mL, sample solution reading on the curve);
V = volume (mL, final volume after digestion);
D = dilution factor (if not applicable, enter 1);
WRA =weight (g) of reconstitution aliquoted during sample preparation,; and S = sample concentration of iodine (µg/100 g reconstituted

If a reconstitution was not performed, use the following equation:
$$S = [\frac{\frac{\{(C \times V) \times D\}}{W}}{10}]$$
Where,
C = sample concentration (ng/mL, sample solution reading on the curve);
V = volume (mL, final volume after digestion);
D = dilution factor (if not applicable, enter 1);
W= sample size (g); and
S = sample concentration of iodine (µg/100 g)
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<tr>
<td>Inference
(Qualitative Analysis)</td>
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Reference	**50.8.02 AOAC Official Method 2012.15 (Final Action 2015)**
Approved by	Scientific Panel on Methods of Sampling and Analysis

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 the analysis of Iodine in Foods using Tetramethyl Ammonium Hydroxide Extraction followed by Inductively Coupled Plasma–Mass Spectrometry**		
Method No.	**FSSAI 09.008:2024**	**Revision No. & Date**	0.0
Scope	This method describes a procedure for the determination of iodine in foods and dietary supplements by alkaline extraction followed by inductively coupled plasma-mass spectrometric detection (ICP-MS). LOD- 4.1 µg/kg LOQ- 36.5 µg/kg		
Caution	1) Use fume hood and wear full personal laboratory protective clothing, gloves, and appropriate eye protection (safety glasses) when using glassware and preparing standards or test portions with acid solutions. 2) Oven and microwave digestion procedures involve moderately elevated temperatures. Carefully remove samples and allow cooling before removing the lids from the digestion vessels. 3) Inductively coupled plasmas should only be viewed with proper eye protection from ultraviolet emissions. 4) The method involves the use of strong bases and concentrated acids. Avoid spills, inhalation, and exposure to human tissues. Use normal laboratory safety precautions (laboratory coats and safety glasses with side shields) when handling concentrated acids, bases, and organic solvents. Additional protections such as face shields, neoprene gloves, and aprons should be used where splashing may occur. 5) Exercise caution when handling and dispensing concentrated acids. Always add acid to water. Acids are caustic chemicals that are capable of causing severe eye and skin damage. If acids or bases come in contact with any part of the body, quickly wash the affected area with copious quantities of water for at least 15 minutes.		
Principle	An analytical portion (0.5 to 5.0 g dependent on food composition) is mixed with tetramethyl-ammonium hydroxide (TMAH) and a hot block extraction system at 85°C is used to extract the available iodine. The supernatant contains extractable iodine in 1% TMAH at pH>9. The analytical solution is analyzed using an ICP-MS. Iodine mass fraction is quantified using an external calibration and quality controls are incorporated to ensure data quality.		
Apparatus/Instruments	1) **Inductively coupled plasma-mass spectrometer (ICP-MS)**—Capable of scanning the mass-to-charge (m/z) range 5 – 240 amu with a minimum resolution of 0.9 amu at 10% peak height, mass flow controller for nebulizer gas. 2) Nebulizer and spray chamber 3) **Hot block extraction system**—Requires uniform temperature control to at least 85 ± 2 °C.		

	4) **Laboratory centrifuge**– Capable of 3000 rpm (capable at least of max RCF of 1300 g) and 50 mL centrifuge tubes. 5) **Labware—**All reusable laboratory ware must be sufficiently clean for trace metals analysis. The recommended cleaning procedure for all laboratory ware includes washing in special clean-rinsing laboratory detergent such as Micro-90, reagent water rinse, soaking in 10% nitric acid and final reagent water rinse immediately before use. Glass should not be used for dilution or storage of sample or standard solutions because of possible contamination. 6) **Plastic labware**—This includes disposable plastic laboratory ware such as autosampler tubes and capped centrifuge tubes. Plastic bottles for solution storage should be tested for contamination before using a particular lot with 1% TMAH rinse immediately before use. Items can also be cleaned, dried and stored in a dust free environment for later use. FEP, PFA, PP, LDPE or HDPE are recommended materials for bottles and tubes. FEP, FEP coated or polypropylene spatulas should be used for sampling food portions. 7) **Gloves**—Use powder free vinyl or nitrile. Do not use powdered gloves or latex because of possible contamination. Gloves manufactured for clean room use that are free from trace metals contamination are suggested. 8) **Analytical balance**—Capable of measuring to 0.1 mg. 9) **Top Loading balance**—Capable of measuring from 0.01 g to 2500 g. 10) Peristaltic pump tubing 11) Drain tubing 12) Optional plastic syringes 13) Optional PTFE syringe filter
Materials and Reagents	1) Reagent water—Water processed to meet specifications for ASTM Type-I water. 2) Argon supply for instrument—High purity (99.99%) liquid argon. Argon compressed gas tanks can also be used but is more expensive than liquid argon. 3) High purity tetramethyl-ammonium hydroxide —25% (m/m), electronics grade (99.9999% purity). 4) High purity isopropanol—Electronic grade or equivalent. 5) Triton X-100 (ACS grade)
Preparation of Reagents	1) **TMAH 5% (m/m)**—Dilute 100 g electronic grade 25% TMAH to 500 g with reagent water. **Recommendation:** Use an empty Teflon bottle originally used for concentrated nitric acid. To minimize contamination, dilute gravimetrically on a top loading balance with a capacity of a least 1000 g. Tare bottle. Fill with approximately 300 mL reagent water. Note mass. Add 100 g TMAH while pouring slowly from the stock bottle. Add reagent water until a total solution mass of 500 g is reached (400 g water + 100 g TMAH). Place bottle cap on and mix. 2) **TMAH 1% (m/m)**—Dilute 80 g high purity TMAH to 2,000 g with reagent water. **Recommendation:** Use an empty Teflon bottle originally used for concentrated nitric acid. To minimize contamination dilute gravimetrically

	on a top loading balance with a capacity of a least 2500 g. Tare bottle. Fill with approximately 1000 mL reagent water. Note mass. Add 80 g TMAH while pouring slowly from the stock bottle. Add reagent water until a total solution mass of 2000 g is reached (1920 g water + 80 g TMAH). Place bottle cap on and mix. 3) **Internal standard solution (ISTD)**—Multi-element solution prepared by diluting an appropriate mass of stock standard. ISTD matrix is 1% TMAH, 6% isopropanol, 0.01% triton X-100. The presence of isopropanol will help equalize iodine sensitivity due to residual carbon remaining in solution after the extraction. The dilution factor of the internal standard solution is 1:1 if the autosampler and internal standard peristaltic pump tubes are equal inner diameter. The analytical solution pumped into the nebulizer will be approximately 3% isopropanol. ISTD elements and suggested mass fractions: 2±0.5 µg/kg Rh, 20±2 µg/kg Te. ISTD solution must be prepared daily because of instability of Rh in alkaline solutions longer than 48 h. 4) **Suggested Tuning Solution** — 1 µg/kg iodine solution in 1% TMAH used to tune and optimize instrument. Typical sensitivity for 1 µg/kg I should be better than 50,000 cps/ppb. 5) **Analyte stock standard solutions** — commercially prepared single element NIST traceable standard solutions prepared specifically for plasma mass spectrometric or ion chromatography analysis should be used. Due to the low mass fractions of solutions required for ICP-MS standardization, starting from 10 mg/kg stock solutions is recommended to minimize the number of dilutions and intermediate solutions. 6) **Standard solutions**—Dilute stock standard with 1 % TMAH to prepare iodine standards. Depending on the mass fraction of the stock standard the use of serial dilutions is recommended in preparing the calibration standards. Store in Teflon® FEP, PP or HDPE bottles. A minimum of 4 calibration standard levels should be used for calibration. 7) Standard blank— 1 % TMAH 8) **Initial (independent) calibration verification (ICV)**— Dilute an appropriate volume of stock iodine solution obtained from a different (second) source gravimetrically with 1 % TMAH so that the analyte mass fraction will be at the approximate midpoint of the calibration curve.
Sample Preparation	Typically, only the edible portions of foods are analyzed. However, if an assignment requires mass fractions as a function of dry mass, dry a minimum of 10 g of the homogenized, ground samples in a laboratory oven at 85 °C until a constant mass is obtained. Standard reference materials (SRM) should be dried according to the manufacturer's recommendations. Calculate the moisture content of the original sample. Store dried samples in a desiccator. **Extraction Procedure using DigiPrep hot block** 1) Weigh each 50 mL centrifuge tube and record mass with cap. 2) In each extraction batch, a minimum of two method blanks must be included to check contamination from the vessels. The method blanks

	should be placed in random vessels. 3) Place 0.5 g analytical portion into clean centrifuge tube and record mass of tube and sample. a) Less than the maximum mass should be used for samples high in salt content. b) For most beverage and liquid samples, use an analytical portion mass of 5 g. c) Use 0.5 g reagent water for method blanks (MBK) and optional fortified method blanks (FMB). d) For dry samples and dry SRM materials adding 1 g of reagent water can help control reactions during the extraction. 4) Pipette 10 mL of 5% TMAH into centrifuge tube, washing down any material on walls. Using a bottle top dispenser is suggested. 5) Vortex each centrifuge tube containing sample and TMAH for 1 min. 6) Place capped samples on hot block. The hot block extraction of iodine temperature program contains a 30 min ramp to 85 °C and 150 min hold at 85 °C. 7) After vessels have cooled to less than 30 °C (approximately 2 h if the samples are left to cool on the hot block) remove tubes and vortex for 1 minute. 8) Add reagent water to 50 mL mark, vortex for 1 minute, weight samples and record final mass. 9) Centrifuge samples at 3000 rpm for 3 min. Collect supernatant and analyze by ICP-MS using the procedure listed below. 10) (optional step) If large amount of solids are present, filtering of the supernatant can prevent clogging of the nebulizer or sample probe. Uptake 5 mL of supernatant and filter into a clean sample tube for analysis. Discard 1 mL of the supernatant to prevent contamination from the filter.
Method of analysis	**Instrument Setup** 1) The sample introduction system need to be rinsed with 1% TMAH or 1% optima grade NH4OH for a minimum of 4 hours before the first use for iodine measurements in order to wash out iodine from all glass and plastic components. Once the ICP-MS is switched to alkaline sample introduction it is recommended that several batches are run before switching to acid solution mode. Frequent acid to base switching may require a prolonged iodine washout in order to obtain the above mentioned iodine count rates. 2) Perform manufacturer recommended instrument start up procedure or laboratory specific procedures. 3) Ignite plasma and perform initiation procedures as instructed in the owner's manual. a) Fill a rinse bottle with 1% TMAH. Send autosampler probe to the rinse bottle while instrument is warming up. Place internal standard line in tube containing reagent water during warm up. Rinse and warmup the

instrument for a minimum of 1 hour.

b) Program autosampler sequence table to run standards and samples of the batch.

4) Set up method to include analytes and internal standard elements
   (a) Use 3 points per peak and at least 3 replicates for integration. Use the mean of the integrations for reporting.
   (b) Be sure there is adequate rinse time programmed in between samples. Program the autosampler probe to go to the rinse station for at least 10 seconds after analyzing an analytical solution and then to the rinse bottle filled with 1% TMAH. The rinse time must be great enough so that a standard blank solution produces stable iodine baseline signal. A minimum of a three-minute rinse is recommended.
5) Optimize instrument
   (a) Configure the tune to monitor ^{103}Rh, ^{125}Te and ^{127}I. Introduce calibration blank solution. Pump speed during tuning and analyses should be set at 0.1 rev/s.
   Note: During tuning, the internal standard tubing is placed in the ISTD solution containing 1 % TMAH, 0.01% triton-x100 and 6% IPA.
   (b) Tune for highest stability while maintaining optimal sensitivity for the m/z 125 and 103, and lowest cps at m/z 127. Save updated and optimized tune file.
   (c) Check instrument performance using a 1 mg/kg I standard.
   (d) Precision Check: Demonstrate instrument stability by analyzing a midrange iodine standard solution (*e.g.* CCV). The resulting relative standard deviation (RSD) of ion signals must be ≤10%.

Determination of Analyte Mass Fraction Using External Standard Calibration Curve

Calibrate using the standard blank and at least four iodine standards. A calibration blank is used as a point on the calibration curve (0 µg/kg calibrant). Additionally a high standard check at or around 50 µg/kg iodine should be analyzed as a sample to ensure linearity to 50 µg/kg iodine. The high standard linearity check should be within 10 % of the calculated mass fraction.

1) Use linear regression with blank offset and no weighing factor.

2) Check standardization performance
   (a) Linear regression correlation coefficient (r) (intensity - (analyte counts/sec):(internal standard counts/sec)) versus mass fraction) is ≥ 0.9975.
   (b)Analyze initial calibration verification (ICV) to verify standardization. Recovery must be 100 ± 10%.
3) Check instrument measurement performance and analyze analytical solutions
   (a) Interpolate analyte mass fraction from standard curve. Start samples analysis sequence and analyze the highest standard, standard blank and ICV in that order. This will verify proper autosampler rinse time and

	valid calibration curve. (b) Continuing calibration verification (CCV) must be analyzed at every 10 samples and at the end of the analytical run. Recovery must be 100 ± 10%. (c) RSD of the measurements of replicate integrations must be ≤ 10% for all solutions when instrument response > ASQL. (d) Continuing calibration blank (CCB) analyzed at a frequency of 10% and at the end of the analytical run. CCB solutions should be ≤ ASQL. (e) Analytical solutions producing mass fractions which are greater than the high linearity check solution should be diluted with 1% TMAH and re-analyzed at a level falling within the lowest non-zero standard and the high standard. 4) Suppression or enhancement of internal standard isotope response may indicate a matrix effect is present. Monitor internal standard signals and dilute any analytical solution 5) Where the internal standard signal differs by more than 40% from the standard blank. Use 1 % TMAH for diluent. Rh is suggested to be used as a primary internal standard element. 6) Elevated internal standard isotope response may indicate the presence of the internal standard element in the sample or an interference on the internal standard isotope. If the internal standard signal is greater than 140% of the standard blank, choose a different internal standard and reprocess the data. 7) Analyze duplicate analytical portions every 10 samples. The duplicate analytical portions must have relative percent difference < 20% when analyte mass fractions are > LOQ. If it fails, repeat analysis of the duplicate portion. If it fails again, re-digest and re-analyze. 8) At least one fortified analytical portion (FAP) should be included in each analytical run and if more than 10 samples are extracted, an FAP should be included for every 10 samples. The marginal method of calculating percent recovery is used for fortification recovery calculations FAP preparation: Spike 50-300% of the native elemental mass fraction, FAP % marginal recovery: 80 - 120%. If it fails, re-analyze one time. If the FAP fails again, re-digest and re-analyze.
Calculation with units of expression	Calculate the mass fraction of the analyte in the analytical portion according to the formula $$Mass\ fraction\ \left(\frac{\mu g}{kg}\right) = [(S \times DF) - MBK_L] \times \frac{M}{m \times MCF}$$ where *S* = mass fraction of analyte in analytical solution (or diluted analytical solution) (µg/kg) *MBKL* = laboratory MBK (mg/kg) (subtract if MBK is greater than ASQL) *DF* = dilution factor (1 if analytical solution not diluted)

	MCF = mass correction factor (1 if no water or other solvent was added to aid homogenization) M = Mass (g) of analytical solution (usually 50 – 100 g) *m* = mass of analytical portion (g) Round calculated mass fraction to at most 3 significant figures. Mass fractions may be converted to other convenient units (*e.g.*, µg/kg, ng/g for solids or ng/L for liquids). Calculate the marginal recovery (%) in the fortified analytical portion according to the formula $$\%\ Recovery = \left[\frac{C_{x+s} - C_x}{\frac{C_s M_s}{M_x}}\right] \times 100$$ where *Cx+s*= concentration determined in spiked sample (µg/kg) *Cx*= concentration determined in unspiked sample (µg/kg) *Cs* = concentration of spiking solution (µg/kg) *Ms* = mass of spiking solution added to analytical portion (g) *Mx*= mass of analytical portion (g)
Inference (Qualitative Analysis)	
Reference	U.S. Food and Drug Administration-(4.13) Inductively Coupled Plasma-Mass Spectrometric Determination of Iodine in food Using Tetramethyl Ammonium Hydroxide Extraction (version 1.0)
Approved by	Scientific Panel on Methods of Sampling and Analysis

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 the Determination of Methyl Mercury and Total Mercury in Seafood by High Performance Liquid Chromatographic-InductivelyCoupled Plasma–Mass Spectrometry**		
Method No.	**FSSAI 09.009:2024**	**Revision No. & Date**	0.0
Scope	This method describes procedures for using high performance liquid chromatography (HPLC) and inductively couple plasma-mass spectrometry (ICP-MS) to determine methyl mercury and total mercury in seafood. Total mercury in this method is calculated as the sum of inorganic and methyl mercury determined in analytical solution. **Table 1 Analytical Limits** Analytical parameters | LOD (µg/kg) | LOQ (µg/kg) Methymercury | 3.8 | 28 Total Mercury | 6.5 | 47		
Caution	1) Use fume hood and wear full personal laboratory protective clothing, gloves, and appropriate eye protection (safety glasses) when using glassware and preparing standards or test portions with acid solutions. 2) Inductively coupled plasmas should only be viewed with proper eye protection from ultraviolet emissions. 3) Reagents should be regarded as potential health hazards and exposure to these materials should be minimized as much as possible. Use normal laboratory safety precautions (laboratory coats and safety glasses with side shields) when handling organic solvents. Additional protections such as face shields, neoprene gloves, and aprons should be used where splashing may occur. 4) Exercise caution when handling and dispensing concentrated acids. Always add acid to water. Acids are caustic chemicals that are capable of causing severe eye and skin damage. If acids or bases come in contact with any part of the body, quickly wash the affected area with copious quantities of water for at least 15 minutes.		
Principle	Hg species are isolated from 0.5 g non-dried, finely comminuted seafood or 0.2 g dried reference material by extracting with 50 mL aqueous solution of 1% (w/v) L-cysteine.HCl.H_2O for 120 minutes at 60 °C. The seafood-cysteine mixture is cooled to room temperature and filtered to remove particles > 0.45 µm diameter. 50µL portions of filtered extract are injected onto a C-18 column where Hg species are separated by HPLC using a mobile phase of aqueous 0.1% (w/v) L-cysteine.HCl.H_2O + 0.1% (w/v) L-cysteine. Hg at mass-to-charge ratio 202 vs. time is recorded and analyte peak areas are measured. Hg species are identified by retention times of peaks. Methylmercury and inorganic Hg concentrations are calculated using response factors determined for standard solution prepared in aqueous 1% (w/v) L-cysteine.HCl.H_2O and peak areas measured in extracts. Total Hg is calculated as the sum of methyl and inorganic Hg concentrations determined in extracts. Quality control procedures include (a) determination of methylmercury and inorganic Hg in check solutions		

	with known analyte concentrations, in independent check solutions, and in method blanks, (b) determination of percent recovery of analyte added to portions of samples, (c) determination of analyte concentrations in certified reference materials, and (d) determination of inorganic Hg contamination in methylmercury stock standard solution.
Apparatus/Instruments	1) **Inductively coupled plasma-mass spectrometer**—Capable of measuring mass-to-charge ratio 202 in time resolved (chromatographic) mode. Instrument should electronically interface with or can be configured to remote start by standard HPLC instruments for integrated operation. 2) **High performance liquid chromatography**—An integrated or modular system consisting of an analytical pump and autosampler capable of delivering aqueous mobile phase through analytical column isocratically and programmed injection of acidic aqueous solutions. 3) HPLC analytical column—150 x 4.6 mm, 4 µm particle size. 4) Glass vials for extracting analytical samples—Amber, borosilicate glass vials, 60 mL capacity, with screw caps. 5) **Heated water bath**—Capable of temperature control with sufficient water and thermal capacity to allow immersion of extraction vials to cap level and maintain water temperature at 60 ± 4 °C for 120 minutes. 6) **Syringe for filtering extracts**—Disposable, general use and non-sterile. 7) **Syringe filters for filtering extracts**—Disposable, 0.45 µm polypropylene membrane with polypropylene housing.
Materials and Reagents	1) Reagent water-Water that meets specifications for ASTM Type I water 2) Methylmercury(II) chloride—CH_3HgCl crystals, purity ≥ 95%, formula wt. 251.08. 3) Mercury(II) chloride—$HgCl_2$ crystals, ACS grade, formula wt. 271.50. 4) L-cysteine hydrochloride monohydrate (L-cysteine.HCl.H2O)—Purity > 98.5%, formula wt. 175.64. 5) L-cysteine (free base)—Purity ≥ 99.8%, formula wt. 121.16.
Preparation of Reagents	1) **Extraction solution, aqueous 1% (w/v) L-cysteine.HCl.H2O**—Dissolve 10 ± 0.1 g L-cysteine.HCl.H_2O crystals in 1000 ± 10 mL reagent water. 2) **Cysteine solution for preparation of standard solutions, aqueous 10% (w/v) L-cysteine.HCl.H_2O**—Dissolve 5 ± 0.05 g L-cysteine.HCl.H_2O crystals in 50 ± 0.5 mL reagent water. 3) **Mobil phase, aqueous 0.1% (w/v) L-cysteine + 0.1% (w/v) L-cysteine.HCl.H_2O**—Dissolve 0.5 ± 0.01 g L-cysteine and 0.5 ± 0.01 g L-cysteine.HCl.H_2O in 500 ± 5 mL reagent water. 4) **Methylmercury stock solution, CH_3HgCl in H_2O that may contain up to 20% (v/v) methanol, Hg=1000 mg/L**—Tare 100-mL volumetric flask on analytical balance in chemical fume hood. Weigh 0.1252 g CH_3HgCl (FW=251.08) in flask with stopper in place. Add ≤ 20 mL methanol and swirl stoppered flask to dissolve CH_3HgCl. Dilute to 100.0 mL with reagent water. Discard solution in which inorganic Hg is >3% of the theoretical methylmercury concentration. 5) **Inorganic Hg stock solution, $HgCl_2$ in 0.1% (v/v) HCl, Hg = 2000 mg/L**—Tare 50-mL polypropylene centrifuge tube. Weigh 0.1354 g $HgCl_2$ (FW = 271.50) in tube. Add 5.0 ± 0.1 mL 1% (v/v) HCl and swirl to dissolve. Dilute to 50.0 ± 0.5 mL with reagent water.

	6) **Multi-analyte intermediate solution, Hg due to CH3HgCl= 1000 µg/L and Hg due to $HgCl_2$ = 1000µg/L in 0.02% (w/v) L cysteine.HCl.H_2O**—Mix approximately 40mL reagent water and 0.1 mL 10% (w/v) L-cysteine.HCl.H_2O in 50-mL polypropylene tube. Add 50.0 µLmethylmercury stock solution and 25.0 µL inorganic Hg stock solution. Dilute to 50.0 ± 0.5 mL with reagent water. 7) **Multi-analyte working standard solution, Hg due to CH_3HgCl =1 µg/L and Hg due to $HgCl_2$=1µg/L in 1% (w/v) L-cysteine.HCl.H_2O**—Mix approximately 40 mL reagent water and 5.0 ± 0.05 mL 10% (w/v) L-cysteine.HCl.H_2O in 50-mL polypropylene tube. Add 50.0µL multi-analyte intermediate solution. Dilute to 50.0 ± 0.5 mL with reagent water. Mix and immediately transfer a portion to glass HPLC autosampler vial for storage before use. 8) **Check solution**—Use multi-analyte working standard solution for the check solution. 9) **Independent check solution (ICS)**—Prepare independent inorganic and methylmercury stock solutions, and independent multi-analyte intermediate and working standard solutions according to steps (4) – (7) from a different starting material than that used to prepare the primary stock solutions.
Sample Preparation	1) Weigh analytical portion into 60-mL amber glass extraction vial and determine mass of analytical portion. Generally, weigh 0.5 ± 0.1 g edible portion of seafood. Use 0.2 ± 0.01 g for reference materials. 2) Add 50.0 ± 0.5 mL extraction solution (aqueous 1% (w/v) L-cysteine.HCl.H_2O) to extraction vials, cap tightly, and shake vigorously by hand. 3) Heat extraction vials 120 ± 5 min in water bath at 60 ± 4 °C. Shake each vial vigorously by hand after 60 minutes of heating and again after 120 minutes of heating. 4) Remove extraction vials from water bath and allow cooling to room temperature. 5) Filter a portion of extract through 0.45 µm filter directly into HPLC autosampler vial. **Note:** Methylmercury in extraction solution decomposes over time. To ensure accurate quantification of methylmercury, extracts must be analyzed within 8 hours of preparation. **Note:** To assist homogenization of the analytical sample, reagent water ≤20% of the mass of seafood may be added if its addition provides a more visually homogenous and easier-to-manipulate material. If reagent water is added to assist homogenization, record to 4 significant figures the weights of edible portion and reagent water that are combined to prepare the analytical sample and apply mass correction factor (MCF) in calculation of concentration of analyte in analytical portion. Reserve a portion of reagent water used for homogenization to prepare method blanks.
Method of analysis	The optimum operating settings and conditions must be determined for the equipment used. **Instrument Setup**

	1) Setup and configure HPLC and ICP-MS separately before connecting equipment together. Follow instrument standard operating procedures for startup and initialization. • Tune ICP-MS normally. Ensure instrument performance meets default specifications for sensitivity, precision, stability, and/or other established system suitability requirements. • Set ICP-MS data acquisition for mass-to-charge ratio 202 in time resolved mode with 1 replicate (read) per point and use an initial dwell (integration) time of 1 second per point. • Purge and condition HPLC and analytical column with mobile phase. 2) Connect HPLC to ICP-MS. • Enable communication between instruments to synchronize ICP-MS data acquisition with HPLC injection start. • Stop HPLC flow and connect column output directly to ICP nebulizer using tubing and fittings. 3) Optimize operating conditions. • Start HPLC flow and ensure proper liquid flow through ICP nebulizer and drainage of spray chamber. • Analyze a multi-analyte standard solution and adjust acquisition parameters to obtain 10-20 data points across narrowest analyte peak. • Monitor instrument conditions to ensure operation is stable and within normal functioning range. 4) Check instrument performance. • Verify baseline resolution between inorganic and methylmercury peaks and that peaks are not tailing excessively. • Analyze a multi-analyte standard solution 3 or more times and verify short term precision is less than 5% relative standard deviation (peak area) for all analyte(s) of interest. • Verify absence of instrument carry-over. **Determination of Analyte Concentration Using Response Factor** **1)** Analyze a multi-analyte standard solution (or single analyte standard solutions separately) and extraction solution 2 or more times each. **2)** Calculate response factors and check accuracy of working standard(s). • Analyze independent check solution(s). Acceptance criteria: recovery within 100 ± 5%. **3)** Analyze analytical solutions and quality control solutions. **4)** Check instrument measurement performance • Check solution analyzed at a frequency of 10% and at end of the analytical run has a recovery of 100 ± 10% (continuing calibration verification). • Extraction solution analyzed following each check solution analysis is < ASDL (verify absence of carry-over). • Measurements do not surpass the LDR. Dilute analytical solution with extraction solution if necessary to comply with criteria. Retention time of analyte peaks of analytical solution is comparable to standard solution.
Calculation with units of	

expression	Calculate response factor of analyte, RF (cps-s/µg/L) $$\mathrm{RF} = \frac{(\mathrm{Astd} - \mathrm{ave}) - (\mathrm{Aes} - \mathrm{ave})}{\mathrm{Cstd}}$$ where Astd-ave= average peak area of n > 2 injections of standard solution(s) (cps-s) Aes-ave= average peak area of n > 2 injections of extraction solution (cps-s) (0 if no peak is detected) Cstd= analyte concentration (µg/L) in standard solution(s) Calculate concentration of analyte (inorganic mercury or methylmercury) in analytical solution, S (µg/L) $$\frac{\mathrm{S} = \mathrm{Aas} - \mathrm{Aes\_ave}}{\mathrm{RF}}$$ where Aas= peak area of analyte in analytical solution (cps-s) Aes-ave= average peak area of analyte in extraction solution (cps-s) (0 if no peak is detected) RF = response factor of analyte (cps-s / µg/L) Calculate concentration of total Hg in analytical solution, ST (µg/L) $$\mathrm{ST} = \mathrm{Sinorg} + \mathrm{Smethyl}$$ ST = Sinorg+Smethyl where Sinorg= concentration of inorganic Hg in analytical solution (µg/L) Smethyl= concentration of methyl Hg in analytical solution (µg/L) Calculate the concentration (mass fraction) of analyte in the analytical portion according to the formula $$\mathrm{Concentration}\left(\frac{\mu g}{kg}\right) = \frac{[(\mathrm{ST\ x\ DF}) - \mathrm{MBKL}]\mathrm{x\ V}}{\mathrm{m\ X\ MCF}}$$ where ST = concentration of analyte (S or total Hg, ST) in analytical solution (or diluted analytical solution) (µg/L) MBKL = laboratory MBK (µg/L) V = volume (L) of analytical solution (0.050 L) m = mass of analytical portion (kg) DF = dilution factor (1 if analytical solution not diluted) MCF = mass correction factor (1 if water or other solvent not added to aid homogenization)
Inference (Qualitative Analysis)	
Reference	U.S. Food and Drug Administration-(4.8) High Pressure Liquid Chromatographic-

	Inductively Coupled Plasma-Mass Spectrometric Determination of Methylmercury and Total Mercury in Seafood (version 1.0)
Approved by	Scientific Panel on Methods of Sampling and Analysis

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 the analysis of Minerals and Trace Elements in Milk, Milk Products, Infant Formula, and Adult/Pediatric Nutritional Formula by Inductively Coupled Plasma–Mass Spectrometry**		
Method No.	**FSSAI 0.010:2023**	**Revision No. & Date**	0.0
Scope	This method Applicable for determination of Na, Mg, P, K, Ca, Cr, Mn, Fe, Cu, Zn, Se, and Mo in infant formula and adult/pediatric nutritional formula. Also applicable to the determination of Na, Mg, P, K, Ca, Mn, Fe, Cu, Zn, Se and Mo in dairy products (milk, milk powder, whey powder, whey protein concentrate, butter and cheese). **Note:** Cr is excluded because none of the dairy products contained Cr above the LOQ, and so reproducibility could not be determined). **LOQ- mg/100 g-**Na (0.11), Mg(0.0049), P(0.13),K (0.56), Ca(0.53), Mn (0.00050), Fe(0.0073),Cu (0.00048),Zn (0.056), Cr(0.00073),Se (0.00031), Mo(0.0004).		
Caution	1) Use fume hood and wear full personal laboratory protective clothing, gloves, and appropriate eye protection (safety glasses) when using glassware and preparing standards or test portions with acid solutions. 2) Oven and microwave digestion procedures involve moderately elevated temperatures. Carefully remove samples and allow cooling before removing the lids from the digestion vessels. 3) Inductively coupled plasmas should only be viewed with proper eye protection from ultraviolet emissions. 4) The method involves the use of strong bases and concentrated acids. Avoid spills, inhalation, and exposure to human tissues. Use normal laboratory safety precautions (laboratory coats and safety glasses with side shields) when handling concentrated acids, bases, and organic solvents. Additional protections such as face shields, neoprene gloves, and aprons should be used where splashing may occur. 5) Exercise caution when handling and dispensing concentrated acids. Always add acid to water. Acids are caustic chemicals that are capable of causing severe eye and skin damage. If acids or bases come in contact with any part of the body, quickly wash the affected area with copious quantities of water for at least 15 minutes.		
Principle	Nitric acid, internal standard (ISTD), 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. The presence of carbon in the samples causes signal enhancement ofSe. Therefore, to matrix-match the samples, carbon in the form of methanol is added to both the standard solutions and the digestate before analysis. Analysis is performed by inductively coupled plasma-mass spectrometry (ICP-MS). Polyatomic interferences with the low mass elements are reduced or eliminated by analysis in He collision mode using kinetic energy discrimination (KED). For Se measurements, the H2 gas mode is preferred for increasedsensitivity. Quantitation of 12 elements 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.
Apparatus/Instruments	1) Polypropylene (PP) tubes.—Assorted sizes, use as received. 2) ICP-MS system- With quartz spray chamber, quartz torch, Ni/Pt sample cone, Ni/Pt skimmer cone. 3) Auto sampler for ICP-MS. 4) Microwave oven—Commercial microwave designed for laboratory use at 0–300°C, with closed vessel system and controlled temperature ramping capability. Use manufacturers-recommended vessels. 5) Hydrogen generator (hydrogen is recommended for better Se sensitivity)—On-demand supply of >99.999% pure hydrogen at >150 mL/min. Alternatively, a high-pressure cylinder (99.999% purity) may be used. 6) Magnetic stir plate and polytetrafuoroethylene (PTFE)-coated magnetic stir bars. **Note:** All laboratory plastic ware should be single-use whenever possible. If reuse is necessary, wash using 10% nitric acid, then rinse thoroughly with purified water prior to use.
Materials and Reagents	1) **Multielement standard stock solution**—National Institute of Standards and Technology (NIST) or NIST-traceable, containing Se at 20 µg/L; Cr and Mo at 40 µg/L; Mn and Cu at 0.25 mg/L; Zn at 1 mg/L; Fe at 2.5 mg/L; Mg at 10 mg/L; P at 25 mg/L; Ca and K at 50 mg/L; and Na at 25 mg/L in 2% HNO3 + trace hydrofluoric (HF) acid. 2) **Multielement ISTD stock solution**—NIST or NIST-traceable, containing Ge and Te at 5 mg/L in 2% HNO3 + trace HF acid. 3) **Quality control sample (QCS)**—Standard Reference Material (SRM) 1849a (NIST) milk-based hybrid infant/adult nutritional powder with certi□ed values for Ca, Cu, Cr, Fe, Mg, Mn, Mo, P, K, Se, Na, and Zn. 4) Methanol—99.99%, analytical reagent grade. 5) Nitric acid—Concentrated, ultrapure reagent grade. 6) Hydrogen peroxide, 30%—ACS reagent grade. 7) Laboratory water—Metal-free, organic-free, pyrogen-free, fltered 18 MΩ cm quality. 8) Tergitol—Type 15-S-9, Sigma, or equivalent surfactant (optional).
Preparation of Reagents	**1) Tergitol solution (optional, approximately 5%, v/v)**—Add about 700 mL laboratory water to a 1 L plastic bottle containing a PTFE-coated stirring bar. Place the bottle on a magnetic stirrer and begin stirring at a moderate speed. Slowly add 50 mL Tergitol from a graduated cylinder. When the Tergitol is dissolved, fill the bottle to approximately 1000 mL with laboratory water. **2)** Nitric acid rinse solution (2%, v/v) for autosampler rinse port with Tergitoladded.—Mix 20 mL concentrated nitric acid (ultrapure reagent grade) with 20 mL Tergitol solution, (1), and laboratory water to prepare a total volume of 1000 mL. **3) Calibration blank (Cal Blk) and preparation blank (PB) solution**—Add approximately 15 mL laboratory water to a 50 mL volumetric flask. Dispense (using bottle dispenser or pipet) 5 mL nitric acid (ultrapure

	reagent grade) into the same volumetric flask. Pipet (using digital pipet) 0.500 mL ISTD stock and 0.500 mL methanol into the flask. Dilute to volume with laboratory water. This solution serves as both the Cal Blk and PB. The Cal Blk is used as the initial calibration point, whereas the PB is used as the QCS. **4) Calibration standard solution set**—Prepare Cal Blk, Cal Std 1, Cal Std 2, Cal Std 3, and Cal Std 4 standard solutions by pipetting 0.00, 1.00, 5.00, 20.00, and 40.00 mL, respectively, multielement standard stock solution into separate 50 mL volumetric flasks or sample tubes. Add 0.500 mL ISTD stock, 5 mL nitric acid, and 0.5 mL methanol to each flask. Dilute the flasks to volume with laboratory water.
Sample Preparation	1) Prepare samples in duplicate. In sample vessels, weigh test portions to the nearest 0.0001 g. For liquid products, the test portion size is 1.0 g. Thoroughly shake liquid samples (5 min in a mechanical shaker is appropriate), open the container, and dump the contents into a plastic container into which a magnetic stir bar is placed. While stirring, remove the 1 g sample with a disposable pipet for weighing directly into the tared microwave vessel. For ingredients such as whole milk powder, whey powder, or whey protein concentrate, use a direct weight of 0.3 g. For powdered products, the test portion size is net 0.20 g powder sample, which should be taken from a 25 g powder + 200 g warm(60°C) laboratory water reconstitution (i.e., 1.8 g of the 11.1% reconstitution). For butter or processed cheese (take a mold-free portion), use a direct weight of 0.3 g. After weighing the sample, add 0.500 mL ISTD stock using a calibrated digital pipet, 5 mL nitric acid, and 2 mL of 30% hydrogen peroxide. **Note:** The PB/ Cal Blk solution prepared with the standards is the correct sample blank for this method. Specifically, do not microwave-digest the sample blank, which can subject the blank to contamination. 2) Seal the vessels and place into microwave oven. Execute a heating program suitable for total digestion of the sample. 3) After digestion, place vessels in a fume hood, unscrew the cap/venting nut slowly to gradually release pressure, and then completely remove the cap. 4) Slowly add approximately 20 mL laboratory water to the contents of the vessel, swirl to mix, and transfer contents to a 50 mL sample vial. Add 0.5 mL methanol to the sample vial and dilute to approximately 50 mL with laboratory water. Shake briefly.
Method of analysis	1) Using the appropriate tuning solutions, tune the instrument for optimal sensitivity in KED mode and/or reaction mode according to the instrument design. Also, tune the instrument to find the P/A calibration factors needed for those calibration curves that will extend above roughly 100 µg/L (depending on instrument type). 2) Analyze test solutions using an ICP-MS instrument standardized with the standard solutions. Ge is used as the ISTD for the 11 elements not including Se. Those 11 elements are determined in the He collision mode, using KED. Te must be used as the ISTD for Se determinations, and it is

	recommend that low levels of Se be determined in H2 mode, i.e., reaction mode. 3) Typical calibration correlation coefficients are 0.9995 or better for all analytes, but suitability is determined by calibration residuals as follows: Analyze calibration standard (Cal Std) 3 or another suitable QC solution every 10 test portions to monitor for instrument drift and linearity (the result must be within 4% of the standard's nominal concentration). The inclusion of a method blank (run as a sample; its measured concentration must be less than half of the lowest calibration standard), and known reference materials serving as control samples (recovery check within control or certified limits) are mandatory for good method performance. 4) The order of analysis should be calibration standards, followed by rinse, blank check, check standard, control sample, sample, sample duplicate (if used), and finally a repeated check standard.
Calculation with units of expression	The analyte concentration in the sample is calculated: $$x = \frac{(y - \text{blank}) \times DF}{a}$$ where *x* = analyte concentration (ng/g); y=analyte-to-ISTD intensity ratio, which is the measured count of each analyte's standard solution data point in the calibration curve divided by the counts of the ISTD at thesame level; similarly, blank=analyte-to-ISTD intensity ratio, which is the measured count of the blank standard solution data point in the calibration curve divided by the counts of the ISTD at the same level as the blank standard solution; *a* = slope of the calibration curve (mL/ng); and DF=dilution factor, or the volume of the sample solution (mL) divided by sample weight (g).
Inference (Qualitative Analysis)	
Reference	**AOAC Official Method 2012.15**
Approved by	Scientific Panel on Methods of Sampling and Analysis