Manual of Methods of Analysis of Foods-Metals and Minerals

Table of Contents

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 in food by Inductively Coupled Plasma-Optical Emission Spectroscopy Using Microwave Assisted Digestion**		
Method No.	FSSAI 09.001:2024	**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.

Element	**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	0.9	3
Phosphorus (P)	2	6
Potassium (K)	20	40
Sodium (Na)	2	5

<table>
<tr><td></td><td>
<table>
<tr><td>Strontium (Sr)</td><td>0.03</td><td>0.07</td></tr>
<tr><td>Thallium (Tl)</td><td>2</td><td>6</td></tr>
<tr><td>Vanadium (V)</td><td>0.2</td><td>0.5</td></tr>
<tr><td>Zinc (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.
</td></tr>
<tr><td>Caution</td><td>
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) 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. The door is the primary safety device if a vessel vents.
</td></tr>
<tr><td>Principle</td><td>An analytical portion (0.4 to 5 g dependent on food composition) is decomposed with nitric acid and hydrogen peroxide in a high-pressure Teflon® lined 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.</td></tr>
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	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% H2O2 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. 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. 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. **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. 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

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<tr><td></td><td>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.

<table><tr><th colspan="2">Digestion program with Ramp to Temperature feature and pressure control</th></tr><tr><td>Maximum Power (Watts)</td><td>1200</td></tr><tr><td>Control Pressure (psi)</td><td>800</td></tr><tr><td>Ramp Time (min)</td><td>25</td></tr><tr><td>Hold Time (min)</td><td>15</td></tr><tr><td>Control Temperature (°C)</td><td>200</td></tr><tr><td colspan="2">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.</td></tr></table>
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.</td></tr>
<tr><td>Method of analysis</td><td>1) Instrument Setup -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.</td></tr>
</table>

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) Determination of Analyte Concentration Using Standard Curve

i. 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. ii. 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. iii. 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. iv. 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. v. 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

	$$\text{Concentration (mg/kg)} = \frac{[(S \times DF) - MBKL] \times V}{m \times MCF}$$ 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-(4.8) High Pressure Liquid Chromatographic-Inductively Coupled Plasma-Mass Spectrometric Determination of Methylmercury and Total Mercury in Seafood (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 09.002:2024	**Revision No. & Date**	0.0
Scope	Applicable to analysis of calcium, copper, iron, potassium, magnesium, manganese, phosphorus, sodium, and zinc in fortified food products. **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. The door is the primary safety device if a vessel vents.		
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 auto sampler, 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. 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:

<table>
<tr><td></td><td>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).</td></tr>
<tr><td>Materials and Reagents</td><td>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 (H2O2), 97% (w/v).—Trace metal grade throughout.</td></tr>
<tr><td>Preparation of Reagents</td><td>(a) 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 H2O, 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.)
(b) 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.
(c) Intermediate stock solution—Suggested composition of the intermediate stock standard solution:
Table 1 (Preparation of intermediate solutions from stock solution)
<table>
<tr><th>S.No.</th><th>Metal</th><th>Stock solution conc. (mg/kg)</th><th>Intermediate stock solution conc. (mg/kg)</th><th>Volume of stock solution required for 500 ml</th></tr>
<tr><td>1</td><td>Calcium</td><td>10000</td><td>1500</td><td>75</td></tr>
<tr><td>2</td><td>Magnesium</td><td>10000</td><td>500</td><td>25</td></tr>
</table>
</td></tr>
</table>

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

(d) ***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, HNO3) for MDC or 15% (v/v) for MDO using combined acids (HNO3, H2O2, and HCl).

(*1*) *Std6*—Pipet 15.0 mL intermediate stock standard solution into a 100 mL acid-washed volumetric flask. Add 10 mL HNO3 (MDC) or 15 mL combined acids (MDO), dilute to volume with H2O, 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 *HNO3* (MDC) or 15 mL combined acids (MDO), dilute to volume with H2O, 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 *HNO3* (MDC) or 15 mL combined acids (MDO), dilute to volume with H2O, 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 $H2_O$, 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.

(e) ***Sampler wash solution-*** *10% HNO_3 (v/v).*—Dilute 100 mL trace metal-grade HNO3 to 1000 mL with H2O.

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 H2O. 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 H2O. 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 HNO3 into MDC/MDO vessel (and then 5 mL H2O2 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 HNO3. 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 H2O 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 H2O.
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

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 in Water by Inductively Coupled Plasma Mass Spectrometry (ICP-MS)**		
Method No.	FSSAI 09.003:2024	**Revision No. & Date**	0.0
Scope	This method specifies the determination of the elements aluminium, antimony, arsenic, barium, beryllium, bismuth, boron, cadmium, cesium, calcium, cerium, chromium, cobalt, copper, dysprosium, erbium, europium, gadolinium, gallium, germanium, gold, hafnium, holmium, indium, iridium, lanthanum, lead, lithium, lutetium, magnesium, manganese, molybdenum, neodymium, nickel, palladium, phosphorus, platinum, potassium, praseodymium, rubidium, rhenium, rhodium, ruthenium, samarium, scandium, selenium, silver, sodium, strontium, terbium, tellurium, thorium, thallium, thulium, tin, tungsten, uranium, vanadium, yttrium, ytterbium, zinc, and zirconium in water [for example drinking water, surface water, groundwater, wastewater and elutes. The working range depends on the matrix and the interferences encountered. In drinking water and relatively unpolluted waters, the limit of application is between 0.1µg/l and 1.0 µg/l for most elements. The detection limits of most elements are affected by blank contamination and depend predominantly on the laboratory air-handling facilities available.		
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.		
Principle	Multi-element determination of 62 elements by inductively coupled plasma mass spectrometry (ICP-MS) consists of the following steps: -introduction of a measuring solution into a radiofrequency plasma (for example by pneumatic nebulization) where energy transfer processes from the plasma cause dissolution, atomization and ionization of		

	elements; -extraction of the ions from plasma through a differentially pumped vacuum interface with integrated ion optics and separation on the basis of their mass-to-charge ratio by a mass spectrometer (for instance a quadrupole MS); -transmission of the ions through the mass separation unit (for instance a quadrupole) and detection, usually by a continuous dynode electron multiplier assembly, and ion information processing by a data handling system; -quantitative determination after calibration with suitable calibration solutions. The relationship between signal intensity and mass concentration is usually a linear one over at least five orders of magnitude.
Apparatus/Instruments	The stability of samples, and measuring and calibration solutions depends to a high degree on the container material. The material shall be checked according to the specific purpose. For the determination of elements in a very low concentration range, glass or polyvinyl chloride (PVC) should not be used. Instead, it is recommended to use perfluoroalkoxy (PFA), hexafluoroethene propene (FEP) or quartz containers, cleaned with hot, concentrated nitric acid in a closed system. For the determination of elements in a higher concentration range, high density polyethene (HDPE) or polytetrafluoroethene (PTFE) containers are also allowed for the collection of samples. Immediately before use, all glassware should be washed thoroughly with warm diluted nitric acid [for example *w*(HNO3) = 10 %], and then rinsed several times with water. The use of piston pipettes is permitted and also enables the preparation of lower volumes of calibration solutions. The application of dilutors is also allowed. Every batch of pipette tips and disposable plastics vessels shall be tested for impurities. **(1) Mass spectrometer**. A mass spectrometer with inductively coupled plasma (ICP) suitable for multi-element and isotope analysis isrequired. The spectrometer should be capable of scanning a mass range from 5 *m*/*z* (AMU) to 240 *m*/*z* (AMU) with a resolution of at least 1 *m*r /*z* peak width at 5 % of peak height (*m*r = relative mass of an atom species; *z* = charge number).

	The instrument may be fitted with a conventional or extended dynamic range detection system. **(2) Mass-flow controller**. A mass-flow controller on the nebulizer gas supply is required. Mass-flow controllers for the plasma gas and the auxiliary gas are also useful. A water cooled spray chamber may be of benefit in reducing some types of interferences (for example from polyatomic oxide species). NOTE The plasma is very sensitive to variations in the gas flow rate. **(3) Nebulizer with variable speed peristaltic pump,** for which information on different types of nebulizers is given in ISO 17294-1:—, 5.1.2. **(4) Argon gas supply**, of high purity grade, for instance 99,99 %. **(5) Glassware,** consisting of the following: -volumetric flasks, for example 50 ml, 100 ml, 500 ml and 1 000 ml; -conical (Erlenmeyer) flasks, for example 100 ml; -pipettes, for example 1 ml, 2,5 ml, 10 ml, 20 ml and 25 ml. **(6) Storage bottles**, for the stock, standard, calibration and sample solutions. For the determination of elements in a normal concentration range, high density polyethene (HDPE) or polytetrafluoroethene (PTFE) bottles are sufficient for the storage of samples. For the determination of elements in an ultratrace level bottles made from perfluoroalkoxy (PFA) or hexafluoroethene propene (FEP) should be preferred. In any case the user has to check the suitability of the chosen containers.
Materials and Reagents	For the determination of elements at trace and ultratrace level, the reagents shall be of adequate purity. The concentration of the analyte or interfering substances in the reagents and the water should be negligible compared to the lowest concentration to be determined. For preservation and digestion, nitric acid should be used to minimize interferences by polyatoms. 1) **Water,** Grade 1,for all sample preparation and dilutions. 2) **Nitric acid,** sp.g.(HNO_3) = 1,4 g/ml. 3) **Hydrochloric acid**, sp.g. (HCl) = 1,16 g/ml. 4) **Hydrochloric acid**, *dilute*(HCl) = 0,2 mol/l. 5) **Sulfuric acid**, sp.g.(H_2SO_4) = 1,84 g/ml. 6) **Hydrogen peroxide**, *w*(H_2O_2) = 30 %.

	7) **Element stock solutions,**sp.g.= 1 000 mg/l each of Ag, Al, As, Au, B, Ba, Be, Bi, Ca, Cd, Ce, Co, Cr, Cs,Cu, Dy, Er, Eu, Ga, Gd, Ge, Hf, Ho, In, Ir, K, La, Li, Lu, Mg, Mn, Mo, Na, Nd, Ni, P, Pb, Pd, Pr, Pt, Rb, Re, Rh, Ru, Sb, Sc, Se, Sm, Sn, Sr, Tb, Te, Th, Tl, Tm, U, V, W, Y, Yb, Zn, Zr. Both single-element stock solutions and multi-element stock solutions with adequate specification stating the acid used and the preparation technique are commercially available. Element stock solutions with different concentrations of the analytes (for example 1000 mg/l) are also allowed. **Anion stock solutions**, = 1 000 mg/l each of Cl, $PO4^{-3}$, $SO4^{-2}$. Prepare these solutions from the respective acids. The solutions are also commercially available. Anion stock solutions with different concentrations of the analytes (for example 100 mg/l) are also allowed.
Preparation of Reagents	**1) Multi-element calibration solutions** Depending on the scope, different multi-element standard solutions may be necessary. In general, when combining multi-element standard solutions, their chemical compatibility and the possible hydrolysis of the components shall be regarded. Care shall be taken to prevent chemical reactions (for example precipitation). The multi-element standard solutions are considered to be stable for several months, if stored in the dark. This does not apply to multi-element standard solutions that are prone to hydrolysis, in particular solutions of Bi, Mb, Mo, Sn, Sb, Te, W, Hf and Zr. **(a) Multi-element standard solution A**, consisting of the following: ρ(As, Se) = 20 mg/l ρ (Ag, Al, B, Ba, Be, Bi, Ca, Cd, Ce, Co, Cr, Cs, Cu, La, Li, Mg, Mn, Ni, Pb, Rb, Sr, Th, Tl, U, V, Zn)= 10 mg/l Pipette 20 ml of each element stock solution (As, Se) and 10 ml of each element stock solution (Ag, Al, B,Ba, Be, Bi, Cd, Ce, Co, Cr, Cs, Cu, La, Li, Mn, Ni, Pb, Rb, Sr, Th, Tl, U, V, Zn) into a 1 000 ml volumetricflask. Add 10 ml of nitric acid. Bring to volume with water and transfer to a suitable storage bottle. Multi-element standard solutions with more elements may be used provided it is verified that these solutions are stable and no chemical reactions occur. This shall be checked again a few days after the first

	use (sometimes precipitation can occur after preparation) **(b) Multi-element standard solution B**, consisting of the following: ρ (Au, Mo, Sb, Sn, W, Zr) = 5 mg/l. Pipette 2,5 ml of each element stock solution (Au, Mo, Sb, Sn, W, Zr) into a 500 ml volumetric flask. Add 40 ml of conc. hydrochloric acid. Bring to volume with water and transfer to a suitable storage bottle. **(c) Reference-element solution** (internal standard solution). The choice of elements for the reference-element solution depends on the analytical problem. Solutions of these elements should cover the mass range of interest. The concentrations of these elements in the sample should be negligibly low. The elements In, Lu, Re, Rh and Y have been found suitable for this purpose. For example, the following reference-element solutions may be used: ρ(Y, Re) = 5 mg/l Pipette 5 ml of each element stock solution (Y, Re) into a 1000 ml volumetric flask. Add 10 ml of nitric acid. Bring to volume with water and transfer to a suitable storage bottle. **2) Multi-element calibration solutions**. Choose the mass concentrations of the calibration solutions to allow for a sufficient precision and reproducibility and ensure that the working range is covered. The stability of the calibration solutions should be checked regularly. Due to their rather low respective mass concentrations, they should be replaced by freshly prepared solutions at least every month or more frequently for elements which are prone to hydrolysis. In special cases, daily preparation is necessary. The user has todetermine the maximum stability period of the calibration solutions. Transfer the calibration solution(s) A and B to suitable storage bottles. If the determination is carried out after previous digestion the matrix of the multi-element calibrationsolution(s) A and B shall be adjusted to that of the digests. The working range in general may cover the range of 0.1 µg/l to 50

	µg/l or a part of this. **(a) Multi-element calibration solution(s) A**. Prepare the calibration solution(s) A that cover the required working range by diluting the multi-element standard solution A. Add 10 ml of nitric acid per litre and bring up to volume with water. If necessary, add reference-element solutionto a concentration of for example 50 µg/l of the reference elements before bringing up to volume. **(b) Multi-element calibration solution(s) B**. Prepare the calibration solution(s) B that cover the required working range by diluting the multi-elementstandard solution B Add 5 ml of hydrochloric acid per litre and bring up to volume with water. If necessary add reference-element solution to a concentration of, for example, 50 µg/l of the reference-elements before bringing up to volume. **3) Blank calibration solutions**. High demands shall be set concerning the purity. The user should ensure that the background levels of the analytes are not significant to the results of the analysis. **(a) Blank calibration solution A**. Pipette 0,5 ml of nitric acid to a 100 ml volumetric flask made for example from perfluoroalkoxy (PFA) or hexafluoroethene propene (FEP) and bring to volume with water. If necessary, add reference-elementsolution to a concentration of, for example, 50 µg/l of the reference-elements before bringing up to volume. If the determination is carried out after previous digestion the matrix of the blank calibration solution A shall be adjusted to that of the digests. **(b) Blank calibration solution B**. Pipette 1,0 ml of conc. hydrochloric acid to a 100 ml volumetric flask made for example from perfluoroalkoxy (PFA) or hexafluoroethene propene (FEP) and bring to volume with water. If necessary add reference elementsolution to a concentration of for example 50 µg/l of the reference-elements before bringing up to volume. If the determination is carried out after previous digestion the matrix of the blank calibration solution Bshall be adjusted to that of the digests. **4) Optimization solution**.

	The optimization solution serves for mass calibration and for optimization of the apparatus conditions, for example adjustment of maximal sensitivity with respect to minimal oxide formation rate and minimal formation of doubly charged ions. It should contain elements covering the entire mass range, as well as elements prone to a high oxide formation rate or to the formation of doubly charged ions. For example, an optimization solution containing Mg, Cu, Rh, In, Ba, La, Ce, U and Pb is suitable. Li, Be and Bi are less suitable because they tend to cause memory effects. The mass concentrations of the elements used for optimization should be chosen to allow count rates of more than 10 000 counts/s. **5) Matrix solution**. The matrix solutions serve to determine the correction factors for the corresponding equations. High demandsare made concerning the purity of the basic reagents due to the high mass concentrations. The user shouldensure that the background levels of the analytes in the matrix solution are not significant to the results of theanalysis. The composition may be as follows: $\rho(Ca)$ = 200 mg/l; $\rho(Cl-)$ = 300 mg/l; $\rho(PO_4)^{-3}$= 25 mg/l; $\rho(SO_4)^{-2}$ = 100 mg/l. Pipette 200 ml of element stock solution (Ca), 300 ml of anion stock solution (Cl^-), 25 ml of anion stock solution $(PO_4)^{-3}$ and 100 ml of anion stock solution $(SO_4)^{-2}$ to a 1 000 ml volumetric flask. Add 10 ml of nitric acid. Bring to volume with water and transfer to a suitable storage bottle.
Sample Preparation	**Sampling** Due to the extremely high requirements concerning purity in trace and ultra trace analysis any impurity shall be avoided. The mass concentrations of the elements may change rather rapidly after sampling due to adsorption or desorption effects. This is of special importance, for example in the case of Ag, As, B, Se and Sn. The choice of the container material depends on the mass concentration of the elements to be determined. For the determination of the dissolved fraction of the elements, filter the sample through a membrane filter, nominal pore size 0.45 µm. Every batch of membrane filters shall be tested for impurities. Use several portions of the sample to rinse the filter assembly, discard and then collect the required volume of filtrate.

	Add 0.5 ml of nitric acid per 100 ml of sample. Ensure that the pH is less than 2; otherwise, add nitric acid as required. In the case of determination of elements forming compounds that tend to be hydrolysed, for example Sb, Sn,W or Zr, add to an additional sample 1.0 ml of hydrochloric acid per 100 ml of water. Ensure that the pHis less than 1; otherwise, add more hydrochloric acid as required. **Sample pre-treatment** **1) Determination of the mass concentration of dissolved elements without digestion** Continue according to sampling procedure,using the acidified filtrate specified in sampling. If experience has shown that no significant amounts of particles occur, the filtration may be omitted. Those samples shall be colourless and shall have a turbidity <1.5 FNU (formazinenephelometric unit). **2) Determination of the total mass concentration after digestion** The mass concentration determined according to this clause does not in all cases represent the total mass concentration. Instead, only the portion that is determinable according to the distinct digestion for a given element composition will be analyzed. Some elements and their respective compounds (for example, silicates and aluminum oxide) will be dissolved incompletely using this procedure. For the determination of tin, the following digestion may be used: a) Add 0.5 ml of sulfuric acid and 0.5 ml of hydrogen peroxide to 50 ml of the homogenized water sample. b) Evaporate the mixture until SO3vapors is formed. c) In case of incomplete digestion, add a small portion of water after cooling, add hydrogen peroxide once more and repeat the treatment. d) Dissolve the residue in diluted hydrochloric acid and adjust the volume to 50 ml with water. e) Treat a blank in the same way. Special digestion methods may be necessary if Sb, W or Zr is to be determined. If experience has shown that the elements will be recovered quantitatively without decomposition, the digestion may be omitted.
Method of analysis	1) **General-** In ICP-MS methods, the relationship between measured count rates and mass concentrations of an element is known to be linear over several orders of magnitude. Therefore, linear calibration curves may

	be used for quantification. About 30 min prior to measurement, adjust the instrument to working condition. Before each series of measurement the sensitivity and the stability of the system should be checked using the optimization solution. Adjust the instrument with the aid of the optimization solution to minimize interfering effects (for example oxide formation, formation of doubly charged ions) allowing sufficient sensitivity. Define the rinsing times depending on the length of the flow; in the case of large variations in mass concentrations in the measuring solutions, allow for longer rinsing periods. The use of a reference-element solution is recommended. Add the reference-element solution to the matrix solution, to all multi-element calibration solutions, to the blank calibration solutions, and to all measuring solutions. The mass concentration of the reference-elements shall be the same in all solutions. A mass concentration of ρ□(Y, Re) = 50 μg/l is often suitable. ICP-MS has excellent multi-element capability. The sensitivity of determination depends on a number of parameters (nebulizer flow, radiofrequency power, lens voltage, lens voltage mode, etc.). The optimal instrument settings cannot be achieved for all elements simultaneously. **2) Calibration of the ICP-MS system** When the analytical system is first evaluated, and at intervals afterwards, establish a calibration curve for each element to be determined using at least five measuring points (for example, the blank calibration solution and four calibration solutions). For work on a daily basis, one blank solution and one to two calibration solutions are enough but check the validity of the calibration curve with a certified reference sample, a standard sample, or a suitable internal control sample. Typically proceed as follows: Prepare and measure the blank calibration solutions and the multi-element calibration solutions. According to the manufacturer's instruction, set up a calibration graph. Each reference point should be themean of at least two replicates. Take into account possible discrepancies in the isotope composition between the calibration solutions and themeasuring solutions (for example relevant for Li, Pb, U).

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3) **Measurement of the matrix solution for evaluation of the correction factors**

In order to evaluate and to update the correction factors, measure the matrix solutions at regular intervals within a measuring cycle.

4) **Measurement of the samples**

After establishing the calibration curves, measure the blanks and the samples.

Within sufficient small intervals (for example, every ten samples) check the accuracy of at least one certified reference sample or one standard sample or one internal control sample. If necessary, re-calibrate.

Some elements (for example Ag, B, Be, Li, Th) are rinsed very slowly from the sample inlet system. After high count rates, these memory effects shall be checked by measuring a blank calibration solution.

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<tr><td>Calculation with units of expression</td><td>

Calculation

The mass concentrations for each element are determined with the aid of the instrument software. Carry out the following single steps for each element.

a) Correct the count rates according to the respective equations as below:**Examples for suitable isotopes with their relative atomicmasses and equations for correction**

Element	Recommended isotope and inter-element correction
As	75As -3.127 (77Se- 0.815 82Se) or 75As -3.127 (77Se + 0.322 0 78Se)
Ba	138Ba -0.0009008 139La - 0.002 825 140Ce
Cd	114Cd-0.02684 118Sn
Ge	74Ge -0.1385 82Se
In	115In -0.01486 118Sn
Mo	98Mo -0.1106 101Ru
Ni	58Ni -0.04825 54Fe
Pb	208Pb + 207Pb + 206Pb
Se	82Se- 1.009 83Kr
Sn	120Sn–- 0.013 44 125Te
V	51V - 3.127 (53Cr -0.113 4 52Cr)
W	184W -0,001 242 189Os

b) Make allowance for the count rates from the blank calibration, calibration and measuring solutions, and relate to the count rates of the reference-elements. Determine the slope and the intercept on the ordinate.

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	c) Determine the mass concentrations of samples with the aid of the count rates and the calibration graphs. d) Correct the results taking into account the mass concentrations from the blank calibration solutions and incorporate all dilution steps in the calculation. If the sample is digested a correction for the procedure blank shall be used if appropriate (digestion blank solution). Report the results to as many significant figures as are acceptable according to the precision of the measuring values. **Examples**- Copper (Cu) 0.142 mg/l, Cadmium (Cd) 0.50 µg/l
Inference (Qualitative Analysis)	
Reference	ISO 17294 Water quality — Application of inductively coupled plasma mass spectrometry (ICP-MS)
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 Arsenic, Cadmium, Mercury, and Lead in Foods by Pressure Digestion and Inductively Coupled Plasma-Mass Spectrometry**		
Method No.	FSSAI 09.004:2024	**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.		
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 diluted with 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 megohm-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. 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. **Caution:** Digestion of carbon-rich materials (e.g., carbohydrates, fats, oils) can result in explosions. 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(ClO4)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

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 Chromium, Selenium, and Molybdenum in Infant Foods and Adult Nutritional Food Products by Inductively Coupled Plasma-Mass Spectrometry**		
Method No.	FSSAI 09.005:2024	**Revision No. & Date**	0.0
Scope	Applicable to the determination of Cr, Se, and Mo in Infant Formula and Adult Nutritional Products by 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 HNO3 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 HNO3 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 HNO3. 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. 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. (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.
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: $Y = ax + \text{blank}$ The analyte concentration in the sample was then calculated: $x = \frac{y - \text{blank}}{a} \times DF$ 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 Foods and Adult/Pediatric Nutritional foods by Inductively Coupled Plasma–Mass Spectrometry**		
Method No.	FSSAI 09.006:2024	**Revision No. & Date**	0.0
Scope	Applicable for the determination of total iodine in infant formula and adult nutritional products by ICP/MS using microwave oven closed-vessel acid digestion.		
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	Test portion is digested with KOH solution in an oven at 105 ± 5°C in a closed vessel until the dissolution of iodine is complete. Mix the digested test solution with stabilizer concentrate. Filter the sample through a 1 µm membrane filter. This sample solution or an appropriate dilution, is presented to the inductively to inductively coupled plasma-mass spectrometer (ICP-MS) instrument standardized with matched standard calibrate solutions.		
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. **Note:** All laboratory plasticware should be single-use wheneverpossible. If reuse is necessary, wash using 10% nitric acid, thenrinse thoroughly with purified water prior to use.
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.
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. 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. Reagent expires 6 months after preparation date. *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. Reagent expires 6 months after preparation date. ***Note*:** Use caution when preparing this solution as a significant amount of heat is generated. *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 concentration is 10% NH_4OH and 1% $Na_2S_2O_3$ in purified water. Store at room temperature. Reagent expires 6 months after preparation date. *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 stop polyvinyl 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. 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. **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. **(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, allow samples to cool first, then add2 mL stabilizer concentrate and bring to volume with purified water. **Note:** If the final volume will be 50 mL, add 1 mL stabilizer concentrate. **(g)** Cap the vessels, then invert to mix thoroughly. 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. 2) **Open vessel microwave digestion (optional)-** **(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, weigh 5 g of the slurry/suspension, and do not add additional water. Proceed with the addition of KOH as described below. **(b)** 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. **(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. Avoid inverting as this may allow sample to adhere to the inner walls of the vessel above the level of the digestion solution. Place the digestion vessels into the carousel of the open-vessel microwave digestion unit. If less than the maximum capacity is to be digested, distribute the vessels evenly throughout the carousel. Digest the samples in the microwave until the dissolution of iodine is complete. The vessel caps should be loosened slightly (from fully tightened) during the 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. **(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 solution need more than a 1 to

<table>
<tr><td></td><td>10 mL dilution to obtain a reading on the calibration curve, an additional dilution must be prepared from the original 1 to 10 mL dilution.
4) Aliquot the desired amount into an appropriate vessel (i.e., 15 or 50 mL PP centrifuge tube), then dilute to volume with diluent.
5) 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>
</table>
</td></tr>
<tr><td>Calculation with units of expression</td><td>Calculation of the concentration is done automatically by the software of the ICP-MS instrument.</td></tr>
<tr><td>Inference (Qualitative Analysis)</td><td></td></tr>
<tr><td>Reference</td><td>AOAC Official Method 2012.15</td></tr>
<tr><td>Approved by</td><td>Scientific Panel on Methods of Sampling and Analysis</td></tr>
</table>

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-Inductively Coupled Plasma–Mass Spectrometry**		
Method No.	FSSAI 09.007:2024	**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.		

Analytical parameters	LOD (µg/kg)	LOQ (µg/kg)
Methyl mercury	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.H2O for 120 minutes at 60 °C. The seafood-cysteine mixture is cooled to room temperature and filtered to remove particles > 0.45 µm diameter. Fifty µL 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.H2O + 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.H2O and peak areas measured in extracts. Total Hg is

	calculated as the sum of methyl and inorganic Hg concentrations determined in extracts.
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 2) Methylmercury(II) chloride—CH3HgCl crystals, purity ≥ 95%, formula wt. 251.08. 3) Mercury(II) chloride—HgCl2 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.H2O crystals in 1000 ± 10 mL reagent water. 2) Cysteine solution for preparation of standard solutions, aqueous 10% (w/v) L-cysteine.HCl.H2O—Dissolve 5 ± 0.05 g L-cysteine.HCl.H2O 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.H2O**—Dissolve 0.5 ± 0.01 g L-cysteine and 0.5 ± 0.01 g L-cysteine.HCl.H2O in 500 ± 5 mL reagent water. 4) **Methylmercury stock solution, CH3HgCl in H2O 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 CH3HgCl (FW=251.08) in flask with stopper in place. Add ≤ 20 mL methanol and swirl stoppered flask to dissolve CH3HgCl. 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, HgCl2 in 0.1% (v/v) HCl, Hg = 2000 mg/L**—Tare 50-mL polypropylene centrifuge tube. Weigh 0.1354 g HgCl2 (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 HgCl2 = 1000µg/L in 0.02% (w/v) L cysteine.HCl.H2O**—Mix approximately 40mL reagent water and 0.1 mL 10% (w/v) L-cysteine.HCl.H2O in 50-mL polypropylene tube. Add 50.0 µL methylmercury 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 CH3HgCl =1 µg/L and Hg due to HgCl2=1µg/L in 1% (w/v) L-cysteine.HCl.H2O**—Mix approximately 40 mL reagent water and 5.0 ± 0.05 mL 10% (w/v) L-cysteine.HCl.H2O 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 (9) – (12) 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.H2O) 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.
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) $RF = \frac{Astd\text{-}ave - Aes\text{-}ave}{Cstd}$ 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) $S = \frac{Aas - Aes\text{-}ave}{RF}$ 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) $ST = Sinorg + Smethyl$ 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 $Concentration\ (\mu g/kg) = \frac{[(ST \times DF) - MBKL] \times V}{m \times MCF}$ 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