Manual of Methods of Analysis of Foods-Water

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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	**Determination of Colour – Platinum cobalt (visual comparison) method**		
Method No.	**FSSAI 14.001:2024**	**Revision No. & Date**	0.0
Scope	This method is applicable to: • Mineral water • Packaged drinking water • Drinking Water (Purified)		
Caution	Even a slight turbidity causes the apparent colour to be noticeably higher than the true colour; therefore, remove turbidity by the filtration procedure described in Sample Preparation. The colour value of water is extremely pH-dependent and invariably increases as the pH of the water is raised. When reporting a colour value, specify the pH at which colour is determined.		
Principle	Colour is determined by visual comparison of the sample with known concentrations of coloured solutions, The platinum-cobalt method of measuring colour is the standard method, one unit/Hazen of colour being that produced by 1 mg/L platinum in the form of the chloroplatinate ion.		
Apparatus/Instruments	1) Nessler cylinders 50 mL capacity. 2) pH meter 3) Filter and filter assembly with vacuum system (for true colour measurements): Use a 0.45 µm pore diam cellulose membrane filter of 22 or 47 mm diameter. Glass fiber filters also can be used. Rinse filters before use and monitor filter blanks. Smaller-pore filters of 0.2 or 0.22 µm or even ultrafiltration may be needed to remove colloidal particles for certain samples such as Mn or Fe oxides or other colloids. Use a glass, PTFE, or stainless-steel assembly to hold the selected filters.		
Materials and Reagents	1) Organic-free water: Type I reagent water or equivalent water 2) Potassium chloroplatinate (K_2PtCl_6), analytical grade 3) Potassium chloroplatinate (K_2PtCl_6), analytical grade 4) Cobaltous chloride (CoCl2 -6H2O), analytical grade 5) Hydrochloric acid (HCl), analytical grade		
Preparation of Reagents	Dissolve 1.246 gm potassium chloroplatinate and 1.0 gm crystalline cobaltous chloride in distilled water containing 100 mL concentrated hydrochloric acid. Dilute to 1000mL with distilled water. This standard solution is equivalent to 500 colour units (CU). Prepare standards having CU of 5, 10, 15, 20, 25, 30, 40, 50, and 100 by diluting 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0, 10.0, and 20.0 mL stock colour standard with reagent water in 100 mL volumetric flasks. Transfer to		

<table>
<tr><td></td><td>Nessler tubes for use as standards. Protect standards against evaporation and contamination when not in use. Keep in the dark when not in use and keep only for 1 month.</td></tr>
<tr><td>Sample Preparation</td><td>Representative samples shall be taken in clean glassware. Colour should be determined as early as possible after the collection of samples as biological activity or physical changes occurring during storage may affect the colour. Refrigeration of water samples at 4°C is recommended until analysis, and warm them up to room temperature before measurement.

Check sample pH. If outside the range of 4 to 10, preferably adjust sample to pH 7 and note the adjustment.

Filtration Method: If true colour is to be measured, wash membrane filter and filter assembly by passing at least 50 mL reagent water through filter. Filter about 25 mL sample and discard filtrate. Filter a further portion of about 50 mL through the same filter and retain for analysis.</td></tr>
<tr><td>Method of analysis</td><td>Observe sample colour by filling a matched Nessler tube to the 50 mL mark with sample and comparing it with standards. Look vertically downward through tubes toward a white or specular surface placed at such an angle that light is reflected upward through the columns of liquid. If turbidity is present and has not been removed, report as "apparent colour." If the colour exceeds 100 units, dilute the sample in known proportions until the colour is within the range of the standards.</td></tr>
<tr><td>Calculation with units of expression</td><td>Calculate colour units (CU) by the following equation:

Colour Units = $\frac{A \times 50}{B}$

A = estimated colour of a diluted sample, and
B = mL sample taken for dilution.

The correct units for true colour are CU. One CU is equivalent to one Hazen unit and to one Pt-Co unit. If samples are not filtered, report data as Apparent CU. Report colour results in whole numbers and record as follows:

<table>
<tr><th>SL.NO.</th><th>Colour units</th><th>Record to Nearest</th></tr>
<tr><td>1</td><td>1-50</td><td>1</td></tr>
<tr><td>2</td><td>51-100</td><td>5</td></tr>
<tr><td>3</td><td>101-250</td><td>10</td></tr>
<tr><td>4</td><td>251-500</td><td>20</td></tr>
</table>
</td></tr>
<tr><td>Reference</td><td>APHA (24th edition) 2120 B</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	**Determination of Colour – Spectrophotometric Method**		
Method No.	**FSSAI 14.002:2024**	**Revision No. & Date**	0.0
Scope	This method is applicable to: • Mineral water • Packaged drinking water • Drinking Water (Purified)		
Caution	The primary interference is from the presence of colloidal and suspended particles that absorb or scatter light. Remove turbidity, colloidal and suspended particles by filtration procedure described in Sample Preparation.		
Principle	Colour characteristics are measured at pH 7 and original pH of the sample by obtaining the visible absorption spectrum of the sample on a spectrophotometer. The percent transmission at certain wavelengths is used to calculate the results which are expressed in terms of dominant wavelength, hue, luminance, and purity.		
Apparatus/Instruments	1. Spectrophotometer, having absorption cells of a minimum of 10 mm, a narrow (10 nm or less) spectral band, and an effective operating range from 400 to 700 nm. 2. pH meter 3. Filter and filter assembly with vacuum system (for true color measurements): Use a 0.45 µm pore diam cellulose membrane filter of 22 or 47 mm diameter. Glass fiber filters also can be used. Rinse filters before use and monitor filter blanks. Smaller-pore filters of 0.2 or 0.22 µm or even ultrafiltration may be needed to remove colloidal particles for certain samples such as Mn or Fe oxides or other colloids. Use a glass, PTFE, or stainless-steel assembly to hold the selected filters.		
Materials and Reagents	Organic-free water: Type I reagent water or equivalent water		
Preparation of Reagents	NA		
Sample Preparation	Representative samples shall be taken in clean glassware. Color should be determined as early as possible after the collection of samples as biological activity or physical changes occurring during storage may affect the colour. Refrigeration of water samples at 4°C is recommended until analysis, and warm them up to room temperature before measurement. Bring two 50 mL samples to room temperature. Use one sample at the original pH; adjust pH of other to 7.0 by using sulfuric acid (H_2SO_4) and sodium hydroxide (NaOH) of such concentrations that the resulting volume		

	change does not exceed 3%. A standard pH is necessary because of the variation of color with pH. Remove particulate matter from samples before color determination by filtration method: Wash membrane filter and filter assembly by passing at least 50 mL reagent water through filter. Filter about 25 mL sample and discard filtrate. Filter a further portion of about 50 mL through the same filter and retain for analysis.
Method of analysis	Determination of light transmission characteristics: Thoroughly clean 10 mm absorption cells. Rinse twice with filtered sample, and fill cell with filtered sample. Determine transmittance values (in percent) at each visible wavelength value presented in Table 1, using the 10 ordinates delineated by the border-boxes for fairly accurate work and all 30 ordinates for increased accuracy. Set instrument to read 100% transmittance on the distilled water blank and make all determinations with a narrow spectral band. **Table l**: Selected Ordinates for Spectrophotometric Color Determinations*****

Ordinate No.	Wavelength (nm)		
	X	Y	Z
1	424.4	465.9	414.1
2	435.5*	489.5*	422.2
3	443.9	500.4	426.3
4	452.1	508.7	429.4
5	461.2*	515.2*	432.0
6	474.0	520.6	434.3
7	531.2	525.4	436.5
8	544.3*	529.8*	438.6
9	552.4	533.9	440.6
10	558.7	537.7	442.5
11	564.1*	541.4*	444.4
12	568.9	544.9	446.3
13	573.2	548.4	448.2
14	577.4*	551.8*	450.1
15	581.3	555.1	452.1
16	585.0	558.5	454.0
17	588.7*	561.9*	455.9
18	592.4	565.3	457.9
19	596.0	568.9	459.9
20	599.6*	572.5*	462.0

	21 | 603.3 | 576.4 | 464.1 22 | 607.0 | 580.4 | 466.3 23 | 610.9* | 584.8* | 468.7 24 | 615.0 | 589.6 | 471.4 25 | 619.4 | 594.8 | 474.3 26 | 624.2* | 600.8* | 477.7 27 | 629.8 | 607.7 | 481.8 28 | 636.6 | 616.1 | 487.2 29 | 645.9* | 627.3* | 495.2 30 | 663.0 | 647.4 | 511.2 Factors when 30 ordinates used 0.032 69 0.033 33 0.039 38 Factors when 10 ordinates used 0.098 06 0.100 00 0.118 14 *Insert in each column the transmittance value (%) corresponding to the wavelength shown. Where limited accuracy is sufficient, use only the 10 ordinates delineated by the border-boxes.
Calculation with units of expression	Tabulate transmittance values corresponding to wavelengths shown in Columns X, Y, and Z in Table: l. Sum each transmittance column and multiply the totals by the appropriate factors (for 10 or 30 ordinates) shown at the bottom of the table, to obtain tristimulus values X, Y, and Z. The tristimulus value Y is percent luminance. Calculate the trichromatic coefficients x and y from the tristimulus values X, Y, and Z by the following equations: $$x = \frac{X}{X+Y+Z}$$ $$y = \frac{Y}{X+Y+Z}$$ Locate point (*x, y*) on one of the chromaticity diagrams in Figure:1 and determine the dominant wavelength (in nanometers) and the purity (in percent) directly from the diagram. Determine the hue from the dominant-wavelength value, according to the ranges in Table:2. Expression of Results: Report color characteristics (at pH 7.0 and at the original pH) in terms of dominant wavelength (nanometers, to the nearest unit), hue (e.g., blue, blue-green, etc.), luminance (percent, to the nearest tenth), and purity (percent, to the nearest unit). Report type of instrument (i.e., spectrophotometer), number of selected ordinates (10 or 30), and the

spectral band width (nanometers) used.

Figure 1: Chromaticity diagrams. The larger diagram is used when purity values being determined will be at 20% or more, and the smaller inset diagram is used when purity values being determined will fall below 20%.

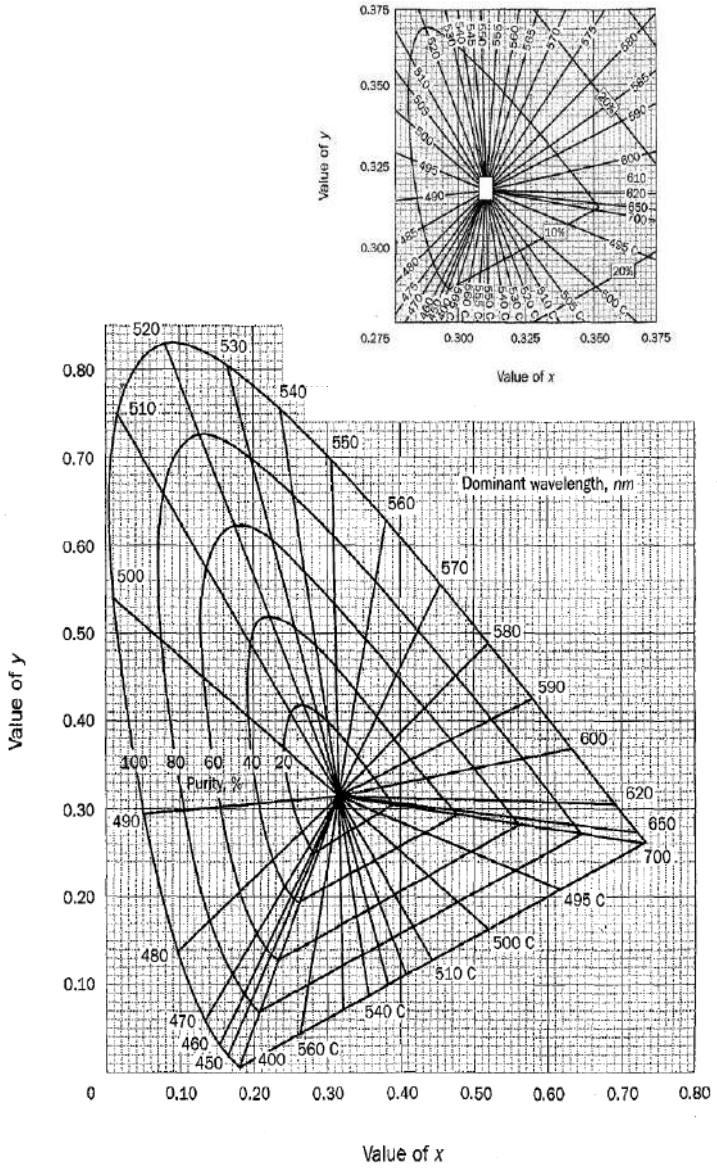

Table 2: Colour Hues for Dominant Wavelength ranges:

Dominant wavelength range(nm)	Colour hue
400-465	Violet
465-482	Blue
482-497	Blue green
497-530	Green

<table>
<tr><td></td><td>
<table>
<tr><td>530-575</td><td>Greenish yellow</td></tr>
<tr><td>575-580</td><td>Yellow</td></tr>
<tr><td>580-587</td><td>Yellowish orange</td></tr>
<tr><td>587-598</td><td>Orange</td></tr>
<tr><td>598-620</td><td>Orange red</td></tr>
<tr><td>620-700</td><td>Red</td></tr>
<tr><td>400-530c#</td><td>Blue purple</td></tr>
<tr><td>530c-700#</td><td>Red Purple</td></tr>
</table>
#The blue-purple and red-purple hues occur when the location of point (x, y) in Figure: 1 results in the dominant wavelength being obtained from the lower right scale having the "c" labeling after the wavelength values.
</td></tr>
<tr><td>Reference</td><td>APHA (24th edition) 2120 D</td></tr>
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</table>

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Method No.	**FSSAI 14.003:2024**	**Revision No. & Date**	0.0
Scope	This method can be used to evaluate samples from drinking water sources, various points in the water treatment train, and finished drinking water.		
Caution	Do not use this method for industrial or domestic wastes or other samples suspected to contain hazardous levels of chemical or biological contaminants.		
Principle	Odour is recognized as a quality factor affecting acceptability of drinking water and food prepared from it, tainting of fish and other aquatic organisms & aesthetes of recreational waters. Most organic and some inorganic chemicals contribute taste or odour. These chemicals may originate from municipal and industrial waste discharges, natural sources, such as decomposition of vegetable matter or from associated microbial activity. Odour of water, though very important, cannot be determined in absolute units. Olfactory sense, which is the most sensitive means of detecting small concentrations of odoriferous substances, lacks precision and mathematical expression nevertheless a qualitative test is prescribed. In case of doubt as to the intensity or character of odour, a majority opinion of several observers should be recorded		
Apparatus/Instruments	a. Sample and standard bottles b. Odor-free testing room c. Water bath		
Materials and Reagents	a. Sample and standard bottles: 1-L narrow-mouth amber glass, with PTFE-lined closure. (Note: 500-mL bottles can also be used.) b. Odor-free testing room: TIO sessions should take place in a clean, odor-free room with minimal distractions. c. Water bath: Capable of bringing samples to testing temperature within 30 min.		
Preparation of Reagents	Thoroughly clean the requisite number of wide mouth glass stoppered bottles of about one-liter capacity. Rinse them with hydrochloric acid and render them completely odour-free by repeated washing with odour-less distilled water, which can be prepared by passing distilled water through a column of granulated activated carbon.		
Sample Preparation	a. As soon as possible after collection of sample, fill a cleaned bottle half full of sample, insert the stopper, shake vigorously for 2 to 3 seconds and then quickly observe the odour. The sample taken for observation of odour shall be at a room temperature. b. When it is desired to record the odour at an elevated temperature, make the observation after warming the sample to about 60°C in a clean stoppered bottle.		
Method of analysis	• Working independently, each panelist gently shakes their sample		

	bottle, removes the cap, and sniffs the headspace. They then each assign the sample a TIO based on their familiarity with the intensity scale. • If evaluating additional samples, panelists then sniff odor-free water and rest for ≥ 1 min between samples. • **Recording and discussion:** One panel member compiles all results on one sheet. If the difference in individual TIO values is >2 for any sample (e.g., one panelist reports TIO of 2, and another reports TIO of 6), then the panel discusses the sample and may retest, again using the reference standards for calibration, so a closer consensus may be obtained.
Calculation with units of expression	Report the true odour of the sample at the mouth of the bottle as rotten egg, burnt, sugar, soapy, fishy, septic, aromatic, chlorinous, alcoholic odour or any other specific odour. In case it is not possible to specify the exact odour, report as agreeable or disagreeable.
Inference (Qualitative Analysis)	After individual results are discussed and recorded, the panel reaches a consensus via discussion and reports it.
Reference	IS:3025 (part 5) : 1983 (Reaffirmed 2002) - Methods of Sampling and Test (Physical and chemical) for water and Waste Water : Odour
Approved by	Scientific Panel on Methods of Sampling and Analysis

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Method No.	**FSSAI 14.004:2024**	**Revision No. & Date**	0.0
Scope	This method is applicable to: • Mineral water • Packaged drinking water • Drinking Water (Purified)		
Caution	1. At pH value above 10, high sodium concentrations interfere with the measurement. Correction for the sodium error may be made by consulting the chart supplied by the manufactures of electrodes being used. Sodium errors at pH value levels greater than 10 can be reduced or eliminated by using a low sodium error electrode. 2. Oil and grease may interfere by coating the pH electrode and causing a sluggish response. These coatings can usually be removed by gentle wiping or detergent washing, followed by distilled water rinsing. An additional treatment with hydrochloric acid (1%) may be necessary to remove any remaining film. 3. Temperature affects the pH values in two ways. The first is covered by the change in electrode output at various temperatures. This interference can be controlled with instruments having temperature compensation or by calibrating the electrode instrument system at the temperature of the samples. The second source is the change of pH inherent in the sample at various temperatures. This error is sample dependent and cannot be controlled. Therefore, the temperature at the time of analysis should be reported.		
Principle	The pH value is determined by measurement of the electromotive force of a cell consisting of an indicator electrode immersed into the test solution and a reference electrode. Contact between the test solution and the reference electrode is usually achieved by means of a liquid junction which forms part of the reference electrode. The electromotive force is measured with a pH meter i.e. a high impedance voltmeter calibrated in terms of pH. Several types of electrodes have been suggested for electrometric determination of pH value. Although the hydrogen gas electrode is recognized as primary standard, the glass electrode in combination with calomel electrode is generally used with reference potential provided by saturated calomel electrode. The glass electrode system is based on the fact that a change of 1 pH unit produces an electrical change of 59.1 mV at 25°C. The active element of glass electrode is membrane of a special glass. The membrane forms a partition between two liquids of differing hydrogen ion concentration and a potential is produced between the two sides of the membrane which is proportional to the difference in pH between the liquids.		
Apparatus/Instruments	a. pH Meter with glass and reference electrode (saturated calomel) preferably with temperature compensation.		

	b. Magnetic stirrer with polytetrafluoroethylene coated stirring bar. c. Thermometer with least count of 0.5°C.
Materials and Reagents	1. Borax buffer 2. Phosphate buffer 3. Tartrate buffer 4. Phthalate buffer 5. Tetraoxalate buffer 6. Calcium Hydroxide Buffer
Preparation of Reagents	Standard pH buffer solutions be prepared using commercially available tablets or powder with NIST traceability or known amount of chemicals. Procedures for the preparation of some standard pH buffer solutions are given below and Table 1 shows the pH value of these buffers at different temperatures. **1. Borax buffer** - 0.01 M solution, pH 9.18 at 25°C: Dissolve 3.814 gm borax ($Na_2B_4O_7.10H_2O$) in deionized or distilled water and dilute to 1 lt. Fresh borax may be used or it may be recrystallized, but, it should not be over dried. For preparation of dilution water, freshly boil and cool deionized or distilled water to expel carbon dioxide gas. Specific conductance of dilution water should be less than 2 µS at 25°C and pH value 5.6 to 6.0 for preparation of all standard solutions. **2. Phosphate buffer** - 1:1 solution, pH 6.865 at 25°C: For preparing 0.025M potassium dihydrogen phosphate and 0.025 M disodium hydrogen phosphate, dry potassium dihydrogen phosphate and sodium dihydrogen phosphate in an oven at 130°C for 2 hr and cool in a desiccator. Dissolve 3.388 gm potassium dihydrogen phosphate and 3.533gm sodium dihydrogen phosphate in deionized or distilled water and make up to 1 lt. **3. Tartrate buffer** – 0.034M solution, pH 3.56 at 25°C: Prepare a saturated solution of potassium hydrogen tartrate in deionized or distilled water. **4. Phthalate buffer** – 0.05M solution, pH 4.008 at 25°C: Dissolve 10.12gm potassium hydrogen phthalate in deionized water and dilute to 1 lt. **5. Tetraoxalate buffer**- 0.05M solution, pH 1.68 at 25oC: Dissolve 12.61 gm potassium tetraoxalate dihydrate in deionized water and dilute to 1 lt. **6. Calcium Hydroxide Buffer** – 0.0203M solution, pH 12.45 at 25°C: Ignite well washed calcium carbonate ($CaCO_3$) of low alkali grade in a platinum dish at 1000°C for 1hr. Hydrate the cooled calcium oxide

by adding deionized water slowly with stirring and heat to boiling. Filter the cooled suspension and collect the solid calcium hydroxide on fritted glass filter of medium porosity. Dry the collected calcium hydroxide in an oven at 110°C, cool and pulverize to uniformly fine granules. Vigorously shake an excess amount of this product in polyethylene bottle with distilled or demineralized water. Allow the gross excess to settle and filter by suction through a fritted glass funnel. Keep the securely stoppered bottle to prevent ingress of carbon dioxide.

Table 1: pH value of buffers at different temperatures:

S. No.	Calcium Hydroxide Saturated (0.0203 M)	Potassium Tetraoxlate (0.05 M)	Potassium Hydrogen Tartarate (Saturated) (0.034 M)	Potassium Hydrogen Phthalate (0.05 M)	Potassium Dihydrogen Phosphate & Disodium Hydrogen Phosphate (0.025 M)	Sodium Borate Decahydrate (Borax) (0.01 M)	Calcium Hydroxide Saturated (0.0203M)
1.	0	1.67	-	4.01	6.98	9.46	13.43
2.	5	1.67	-	4.01	6.95	9.39	13.21
3.	10	1.67	-	4.00	6.92	9.33	13.00
4.	15	1.67	-	4.00	6.90	9.27	12.31
5.	20	1.67	-	4.00	6.88	9.23	12.63
6.	25	1.68	3.56	4.01	6.86	9.18	12.45
7.	30	1.68	3.55	4.02	6.85	9.14	12.30
8.	35	1.69	3.55	4.03	6.84	9.10	12.04
9.	40	1.69	3.55	4.04	6.84	9.07	11.99
10.	50	1.71	3.55	4.06	6.83	9.01	11.70
11.	60	1.72	3.56	4.09	6.85	8.96	11.45

Sample Preparation

- Samples should be analyzed as soon as possible preferably in the field at the time of sampling.

	• High purity waters and waters not at equilibrium with the atmosphere (ground waters or lake waters collected at depth) are subject to changes when exposed to the atmosphere. Therefore, the sample containers should be filled completely and kept sealed prior to analysis.
Method of analysis	Follow the manufacturer's instructions for operation of pH meter. After required warm-up period, standardize the instrument with a buffer solution of pH near that of the sample and check electrode against at least one additional buffer of different pH value. Measure the temperature of the water and if temperature compensation is available in the instrument adjust it accordingly. Rinse and gently wipe the electrodes with solution. If field measurements are being made, the electrodes may be immersed directly in the sample stream to an adequate depth and moved in a manner to ensure sufficient sample movement across the electrode, the sensing element as indicated by drift free readings (<0.1 pH unit). If necessary, immerse them into the sample beaker or sample stream and stir at a constant rate to provide homogeneity and suspension of solids. Rate of stirring should minimize the air transfer rate at the air-water interface of the sample. Note and record sample pH and temperature. However, if there is a continuous drift, take a second reading with the fresh aliquot of sample without stirring and report it as the pH value.
Calculation with units of expression	Report pH to the nearest coefficient or 0.01 unit (if instrument reads up to 2 decimal places) and temperature to the nearest °C.
Inference (Qualitative Analysis)	NA
Reference	IS 3025 (part 11) - 1983 (Reaffirmed 2002)- Methods of Sampling and Test (Physical and chemical) for water and Waste Water : pH Value
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of pH by Colorimetric method		
Method No.	**FSSAI 14.005:2024**	**Revision No. & Date**	0.0
Scope	This method is applicable to: • Mineral water • Packaged drinking water • Drinking Water		
Caution	The temperature, some gases and organic materials interfere with the pH-measurement. - Suspended materials in the sample may cause significant errors (suspension effect).		
Principle	A series of indicators and buffer solutions are used for determination of pH value by visual comparison.		
Apparatus/Instruments	NA		
Materials and Reagents	**Indicators** Thymol blue (acid range) Bromophenol blue Bromocresol green Methyl red Bromocresol purple Bromothymol blue Phenol red Cresol red Thymol blue (alkaline range) Thymolphthalein Thymol violet		

Preparation of Reagents

Indicators –

1. Prepare universal Indicator by dissolving 0.05 gm of methyl orange, 0.15 gm of methyl red, 0.3 gm of bromothymol blue and 0.35 gm of phenolphthalein in one liter of alcohol (66 percent). The color changes are:

pH	**Color**
Upto 3	Red
4	Orange Red
5	Orange
6	Yellow
7	Yellowish green
8	Greenish Blue
9	Blue
10	Violet
11	Reddish Violet

2. Prepare indicator solution as given below:

Table 1: Preparation of indicator solution:

S. No.	**Name of Indicator**	**pH Range**	**Color Change**	**Method of Preparation**

1.	Thymol blue (acid range)	1.2 to 2.8	Red to yellow	Weigh 0.10 gm, add 10.75 mL of N/50 sodium hydroxide solution and dilute with water to 250 mL
2.	Bromoph enol blue	3.0 to 4.5	Yellow to blue violet	Weigh 0.10 gm, add 7.45 mL of N/50 sodium hydroxide solution and dilute with water to 250 mL
3.	Bromoc esol green	3.8 to 5.4	Yellow to blue	Weigh 0.10 gm, add 7.15 mL of N/50 sodium hydroxide solution and dilute with water to 250 mL
4.	Methyl red	4.2 to 6.3	Red to yellow	Weigh 0.10 gm, add 18.60 mL of N/50 sodium hydroxide solution and dilute with water to 250 mL
5.	Bromocr esol purple	5.2 to 6.8	Yellow to blue violet	Weigh 0.10 gm, add 9.25 mL of N/50 sodium hydroxide solution and dilute with water to 250 mL
6.	Bromoth ymolblue	6.0 to 7.8	Yellow to blue	Weigh 0.10 gm, add 8.00 mL of N/50 sodium hydroxide solution and dilute with waterto 250

				mL
7.	Phenol red	6.8 to 8.4	Yellow tored	Weigh 0.10 gm, add 14.20 mLof N/50 sodium hydroxide solution and dilute with waterto 250 Ml
8.	Cresol red	7.2 to 8.8	Yellow to red	Weigh 0.10 gm, add 13.10 mL of N/50 sodium hydroxide solution and dilute with waterto 250 mL
9.	Thymol blue (alkaline range)	8 to 9.5	Yellow to blue	Weigh 0.10 gm, add 10.75 mLof N/50 sodium hydroxide solution and dilute with waterto 250 mL
10.	Thymolphthal ein	9.3 to 10.5	Colorless to blue	Dissolve 0.10gm in 100mL of rectified spirit [see IS : 323-1959 Specifications forrectified spirit (revised)]
11.	Thymol violet	9.0 to 13.0	Yellow to green to violet	Dissolve 0.10gm of tropaeolin O in 100mL of water. Dissolve 0.04gm of thymolphthal ein in a mixture of 50mL of water.Mix one part of tropaeolin O solution with

	<table><tr><td></td><td></td><td></td><td></td><td>4 parts of thymophthalein solution.</td></tr></table>
Sample Preparation	NA
Method of analysis	Take 100 mL of the sample in a hard glass tube and determine the approximate pH by using the universal indicators. Repeat using a solution of the indicator (about 1/20 of the volume of the liquid being tested) which corresponds to the approximate pH found above. Compare the color produced with a series of buffer solutions of known pH each containing the same proportion of the indicators.
Calculation with units of expression	Report the pH of that buffer solution which matches with that of the sample to the nearest 0.1 unit.
Inference (Qualitative Analysis)	NA
Reference	IS 3025 (part 11) - 1983 (Reaffirmed 2002)- Methods of Sampling and Test (Physical and chemical) for water and Waste Water : pH Value
Approved by	Scientific Panel on Methods of Sampling and Analysis

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Method No.	**FSSAI 14.006:2024**	**Revision No. & Date**	0.0
Scope	This procedure has been used with samples from public sources in laboratory research and consumer surveys to recommend standards governing mineral content in drinking water.		
Caution	• Perform flavor tests only on samples known to be potable. Do not use samples that may be contaminated with bacteria, viruses, parasites, or hazardous chemicals, that contain dechlorinating agents such as sodium arsenate or that are derived from an unaesthetic source. • Do not perform flavor tests on wastewaters or similar untreated effluents or any other non-potable water. Observe all sanitary and aesthetic precautions with regard to apparatus and containers contacting the sample. • Glassware used for sensory testing are not to be used for other analysis. • Properly clean and sterilize containers before using them. Conduct analysis in a laboratory free from interfering background odors and, if possible, provide odor-free carbon-filtered air at constant temperature and humidity		
Principle	Each Panelist (tester) is presented with a list of 9 statements about the water ranging on a scale from very favorable to very unfavorable. The panelist's task is to select the statement that best expresses his or her opinion. The individual rating is the scale number of the statement selected. The panel rating for a particular sample is an appropriate measure of central tendency of the scale numbers for all testers for that sample.		
Apparatus/Instruments	• 50-mL beaker or ordinary drinking glass for each dilution and reference sample. (Between tests, clean containers in an automatic dishwasher supplied with water at not less than 60 ºC.)		
Materials and Reagents	Taste and odour-free water and 2000 mg/L solution of sodium chloride prepared with taste and odour -free water as reference sample		
Preparation of Reagents	NA		
Sample Preparation	• Present samples at a temperature that the testers will find pleasant for drinking water; maintain this temperature throughout testing. A temperature of 15 ºC is recommended, but in any case, do not let the test temperature exceed tap water temperatures customary at the time of the test.		
Method of analysis	**a. Panel selection and preparation:** • Give prospective testers thorough instructions and trial or orientation sessions followed by questions and discussion of procedures. In tasting samples, testers work alone. Select panel		

	members on the basis of performance in these trial sessions. • Do not let testers know the composition or source of specific samples. • Independently randomize the sample order for each tester. **b. Rating test:** • A single rating session may be used to evaluate up to 10 samples. Allow at least 30 min rest between repeated rating sessions. • Specify test temperature in reporting results. Independently randomize the sample order for each tester. Instruct each to complete the following steps: 1) Taste about half the sample by taking water into the mouth, holding it for several seconds, and discharging it without swallowing. 2) Form an initial judgment on the rating scale. 3) Make a second tasting in a similar manner. 4) Make a final rating and record result on an appropriate data form. 5) Rinse mouth with reference water. 6) Rest 1 min before repeating Steps 1 through 5 on next sample. **c. Characterization**: • If a characterization of flavor also is required, conduct a final rating session wherein each tester is asked to describe the flavor of each sample rated. • The value of characterization increases as observers become more experienced with a particular flavor category such as chlorophenolic, grassy, or musty.
Calculation with units of expression	Use the following scale for rating. Record ratings as integers ranging from 1 to 9, with 1 given the highest quality rating. Calculate the mean and standard deviation of all ratings if the distribution is reasonably symmetrical. Otherwise express the most typical rating of a group as the median or geometric mean of individual ratings. Action tendency scale: 1) I would be very happy to accept this water as my everyday drinking water. 2) I would be happy to accept this water as my everyday drinking water. 3) I am sure that I could accept this water as my everyday drinking water. 4) I could accept this water as my everyday drinking water. 5) Maybe I could accept this water as my everyday drinking water. 6) I don't think I could accept this water as my everyday drinking water. 7) I could not accept this water as my everyday drinking water. 8) I could never drink this water. 9) I can't stand this water in my mouth, and I could never drink it.
Inference (Qualitative Analysis)	NA
Reference	• APHA 2160 • IS 3025 part-8:1984 (Reaffirmed 2002)- Methods of Sampling and Test

	(Physical and chemical) for water and Waste Water : Taste Rating
Approved by	Scientific Panel on Methods of Sampling and Analysis

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Method No.	**FSSAI 14.007:2024**	**Revision No. & Date**	0.0
Scope	Turbidity can be determined for any water sample that is free of debris and rapidly settling coarse sediment.		
Caution	• Dirty glassware and the presence of air bubbles give false results. • True color (i.e., water color due to dissolved substances that absorb light) causes measured turbidities to be low. • Never handle the sample cells where the instrument's light beam strikes to avoid dirt and fingerprints in the light path. • Use either matched pairs of cells or the same cell for both standardization and sample measurement. • Sample cells may be coated on the outside with a thin layer of silicone oil to mask minor imperfections and scratches that may contribute to stray light. Use silicone oil with the same refractive index as glass. The cell should appear to be nearly dry with little or no visible oil. • Hydrazine sulfate is a carcinogen. Avoid inhalation, ingestion, and skin contact		
Principle	This method is based on a comparison of the intensity of light scattered by the sample under defined conditions with the intensity of light scattered by a standard reference suspension under the same conditions. The higher the intensity of scattered light, the higher the turbidity.		
Apparatus/Instruments	a. **Laboratory or process nephelometer** consisting of a light source for illuminating the sample and one or more photoelectric detectors with a readout device to indicate intensity of light scattered at 90º to the path of incident light. Use an instrument designed to minimize stray light reaching the detector in the absence of turbidity and to be free from significant drift after a short warmup period. The sensitivity of the instrument should permit detecting turbidity differences of 0.02 NTU or less in the lowest range in waters having a turbidity of less than 1 NTU. Several ranges may be necessary to obtain both adequate coverage and sufficient sensitivity for low turbidities. b. **Sample cells:** Use sample cells or tubes of clear, colorless glass or plastic. Keep cells scrupulously clean, both inside and out, by thoroughly washing with laboratory soap inside and out followed by multiple rinses with distilled or deionized water and discard if scratched or etched. Never handle them where the instrument's light beam strikes to avoid dirt and fingerprints in the light path.		
Materials and Reagents	a. Dilution water b. Stock primary standard formazin suspension: • Solution I		

	• Solution II c. Dilute turbidity suspensions d. Secondary standards:
Preparation of Reagents	**a. Dilution water**: To obtain low-turbidity water for dilutions, nominal value 0.02 NTU, pass laboratory reagent-grade water through a filter with pore size sufficiently small to remove essentially all particles larger than 0.1 µm. Rinse collecting flask at least twice with filtered water and discard the next 200 mL. • Some commercial bottled demineralized waters have a low turbidity. These may be used when filtration is impractical or an adequate grade of water is not available to filter in the laboratory. **b. Stock primary standard formazin suspension:** • Solution I - Dissolve 1.0 g hydrazine sulfate in high grade reagent water and dilute to 100 mL in a volumetric. • Solution II - Dissolve 10.00 g hexamethylenetetramine in high grade reagent water and dilute to 100 mL in a volumetric flask. • In a flask, mix 5.0 mL Solution I and 5.0 mL Solution II. Let it stand for 24 h at 25 ± 3 ºC. This results in a 4000-NTU suspension. Transfer the stock suspension to an amber glass or other UV-light-blocking bottle for storage. Make dilutions from this stock suspension. The stock suspension is stable for up to 1 year when properly stored. **c. Dilute turbidity suspensions**: Dilute 4000 NTU primary standard suspension with high-quality dilution water. Prepare immediately before use and discard after use. **d. Secondary standards:** These are standards that the manufacturer has certified will give instrument calibration results equivalent to the results obtained when the instrument is calibrated with the primary standard. (i.e., user-prepared formazin). • Secondary standards provided by the instrument manufacturer (sometimes called permanent standards) may be necessary to standardize some instruments before each reading and in other instruments only as a calibration check to determine when calibration with the primary standard is necessary.
Sample Preparation	NA
Method of analysis	**a. General measurement techniques:** Measure turbidity immediately to prevent temperature changes and particle flocculation and sedimentation from changing sample characteristics. • Avoid dilution whenever possible. Particles suspended in the original sample may dissolve or otherwise change characteristics when the temperature changes or when the sample is diluted. • Remove air or other entrained gases in the sample before

<table>
<tr><td></td><td>measurement, by applying a partial vacuum, adding a non-foaming type surfactant, using an ultrasonic bath, applying heat or a combination of these techniques.
• Condensation may occur on the outside surface of a sample cell when a cold sample is being measured in a warm, humid environment. This interferes with turbidity measurement. Remove all moisture from the outside of the sample cell before placing the cell in the instrument. If fogging recurs, let the sample warm slightly by letting it stand at room temperature or by partially immersing it in a warm water bath for a short time. Make sure samples are again well mixed.
a. Nephelometer calibration: Follow the manufacturer's operating instructions. Run at least one standard in each instrument range to be used. Make certain the nephelometer gives stable readings in all sensitivity ranges used.
b. Measurement of turbidity: Gently agitate the sample. Wait until air bubbles disappear and pour the sample into a cell. When possible, pour the well-mixed sample into a cell and immerse it in an ultrasonic bath for 1 to 2 s or apply vacuum degassing, causing complete bubble release. Read turbidity directly from the instrument display.
c. Calibration of continuous turbidity monitors: Calibrate continuous turbidity monitors for low turbidities by determining the turbidity of the water flowing out of them, using a laboratory model nephelometer, or calibrate the instruments according to the manufacturer's instructions with a formazin primary standard or appropriate secondary standard.</td></tr>
<tr><td>Calculation with units of expression</td><td>Report turbidity readings as follows:
<table><tr><th>Turbidity Range (NTU)</th><th>Report to the Nearest (NTU)</th></tr><tr><td>0-1</td><td>0.05</td></tr><tr><td>1-10</td><td>0.1</td></tr><tr><td>10-40</td><td>1</td></tr><tr><td>40-100</td><td>5</td></tr><tr><td>100-400</td><td>10</td></tr><tr><td>400-1000</td><td>50</td></tr><tr><td>Greater than 1000</td><td>100</td></tr></table>NTU = nephelometric turbidity units.</td></tr>
<tr><td>Inference (Qualitative Analysis)</td><td>NA</td></tr>
<tr><td>Reference</td><td>APHA 2130 B</td></tr>
<tr><td>Approved by</td><td>Scientific Panel on Methods of Sampling and Analysis</td></tr>
</table>

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Method No.	**FSSAI 14.008:2024**	**Revision No. & Date**	0.0
Scope	Solids refer to matter suspended or dissolved in potable, surface, and saline waters, as well as domestic and industrial waste waters. Solids may adversely affect water or effluent quality in a number of ways. Waters with high dissolved solids generally are of inferior palatability and may induce an unfavorable physiological reaction in the transient consumer, so a limit of 500 mg/L dissolved solids is desirable for drinking waters. The following procedure is for checking analyses' correctness apply specifically to water samples with relatively complete analyses of total dissolved solids by gravimetric method.		
Caution	1. Highly mineralized waters containing significant concentration of calcium, magnesium, chloride and sulphate may be hygroscopic. These may require prolonged drying, desiccation and rapid weighing. However, prolonged drying may also cause loss of constituents, particularly nitrates and chlorides. 2. A large amount of residue in the evaporating basin may crust over and entrap water preventing its evaporation during drying. For this reason, the volume of the sample should be adjusted so that the residue left after drying should be about 100-200mg.		
Principle	The sample is filtered and the filtrate evaporated in a tarred dish on steam bath. The residue after evaporation is dried to constant mass at 103-105°C or 179-181°C.		
Apparatus/Instruments	1. Filter - Any one of the following filter may be used. • Glass fiber filter disc - (Whatman GF/C or equivalent) 2.1 to 5.5 cm in diameter, pore size 1.2 µm • Paper – Acid washed ashless hard filter finish; filter paper sufficiently retentive for the fine particles (Pore size 2-2.5 µm equivalent to Whatman filter no. 542). • Gooch crucible-30mL capacity with 2.1 or 2.4 cm diameter glass fibre filter disc (Whatman or equivalent). • Sintered disc-G-5 or its equivalent with pore size 1 to 2 µm. • Membrane filters 0.45 µm membrane. 2. Filtering assembly depending upon the type of filter selected. 3. Drying oven with thermostatic control for maintaining temperature up to 180 ±2°C. 4. Desiccators provided with a colour indicating desiccant. 5. Analytical Balance 200gm capacity and capable of weighing to nearest 0.1 mg. 6. Magnetic stirrer with Teflon coated stirring bars.		
Materials and Reagents	--		

Preparation of Reagents	NA
Sample Preparation	Preservation of the samples is not practical. Analysis should begin as soon as possible. Refrigeration or chilling to 4°C to minimize microbiological decomposition of solids is recommended.
Method of analysis	1. Heat the clean evaporating dish to 180°C for 1 hr. Cool in the desiccator, Weigh and store in the desiccators until ready for use. 2. Filter a portion of the sample through any of the filter mentioned. Select volume of the sample which has residue between 25 and 250mg preferably between 100 to 200mg. This volume may be estimated from values of specific conductance to obtain a measurable residue; successive aliquots of filtered sample may be added to the sample dish. 3. Stir volume of sample with a magnetic stirrer or shake it vigorously. Pipette this volume to a weighed evaporating dish placed on a steam bath. Evaporation may also be performed in a drying oven. The temperature of drying oven shall be lowered to approximately 98°C to prevent boiling and splattering of the sample. After complete evaporation of water from the residue, transfer this dish to an oven at 103-105°C or 179-181°C and dry to constant mass i.e. till the difference in the successive weighing is less than 0.5 mg. Drying for a long duration (usually 1-2 hr) is done to eliminate necessity of checking for constant mass. The time for drying to constant mass with a given type of sample when a number of samples of nearly same type are to be analyzed has to be determined by trial. 4. Weigh the dish as soon as it has cooled avoiding residue to stay for long time as some residues are hygroscopic and may absorb water form desiccant that is not absolutely dry.
Calculation with units of expression	Calculate filterable residue from the following equation. Filterable residue, mg/L = $\frac{1000M}{V}$ Where, M = Mass in mg of filterable residue V = volume in mL of the sample Report in whole numbers for less than 100 mg/L and to three significant figures for values above 100mg/L. Report the temperature of determination.
Inference (Qualitative Analysis)	NA
Reference	IS : 3025 part 16 – 1984 (Reaffirmed 2002)- Methods of Sampling and Test (Physical and chemical) for water and Waste Water : Filterable Residue (Total Dissolved Solids)
Approved by	Scientific Panel on Methods of Sampling and Analysis

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Method No.	**FSSAI 14.009:2024**	**Revision No. & Date**	0.0
Scope	Solids refer to matter suspended or dissolved in potable, surface, and saline waters, as well as domestic and industrial waste waters. Solids may adversely affect water or effluent quality in a number of ways. Waters with high dissolved solids generally are of inferior palatability and may induce an unfavorable physiological reaction in the transient consumer, so a limit of 500 mg/L dissolved solids is desirable for drinking waters. The following procedure is for checking analyses' correctness apply specifically to water samples with relatively complete analyses of total dissolved solids based on conductivity.		
Caution	1. Temperature affects conductivity, which varies by about 2% per degree Celsius. The temperature of 25°C is taken as standard. It is desirable to observe the conductivity at 25°C or as near to this temperature as possible, although compensation for variations from it can be made. In some instruments, this is made automatically. 2. Dissolved carbon dioxide increases conductivity without increasing the mineral salt content. However, the effect is not large and it is usual to ignore it. In low pH water, H+ ions and in high pH water OH- ions, may contribute substantially to conductivity owing to high equivalent conductivity of these ions. Water with high silica (SiO2) content give relatively low values of electrical conductivity to total dissolved solids ratio as SiO2 (H4SiO4) does not contribute significantly to electrical conductance values. 3. It is not convenient to use water containing large amount of suspended matter. It should be settled or filtered. High suspended matter also affects electrical conductance values. 4. Samples containing fat, grease, oil, tar, etc, may contaminate the electrodes causing erratic results.		
Principle	Specific conductance is determined by using a wheatstone bridge in which a variable resistance is adjusted so that it is equal to the resistance of the unknown solution between platinized electrodes of a standard conductivity cell. The cell constant is determined by the following relationship: Specific conductance = Conductance × Cell constant, or Specific conductance = $\frac{\text{Cell constant}}{\text{Resistance}}$ The cell constant is determined experimentally with a standard solution of known conductance.		
Apparatus/Instruments	1. **Conductivity Meter**- Wheatstone bridge type or equivalent direct reading meter. 2. **Conductivity Cells**- Cells of at least two different cell constants, for measurement of wide range of conductivities. Specific conductance ranges and corresponding values of cell constants are given below **SPECIFIC CONDUCTANCE CELL CONSTANT**		

	µS/cm at 25°C 20 – 1000 0.2 40 - 2000 0.5 100 - 4000 1.0 200 - 10000 2.0 400 – 20000 5.0 10000 - 40000 10.0 3. **Thermometer** - 0 to 50°C, graduated in 0.1°C. Note- some direct reading conductivity meters have automatic compensation built into the instrument
Materials and Reagents	Standard Potassium Chloride Solution
Preparation of Reagents	**Standard Potassium Chloride Solution** - Dissolve 0.5232gm potassium chloride dried at 180°C for 1 hr in demineralized water and dilute to 1000mL. The distilled water used for preparing standard solutions should have a very low conductivity. The specific conductance of this solution at 25°C is 1000µs/cm and the concentration of this solution is 0.00702 N. Alternatively, dissolve 0.7456 gm of anhydrous potassium chloride, dried at 180°C for 1 hour in distilled water and make up to 1000 mL at 25°C. The specific conductance of this solution at 25°C is 1408 µs/cm and the concentration of this solution is 0.01N.
Sample Preparation	--
Method of analysis	1. Platinizing of cell- Platinization of cell is required when readings become erratic. For platinizing, clean the cell in chromic acid solution once and rinse several times with distilled water. Place the cell in a commercial platinizing solution or dissolve 3 gm of chloroplatinic acid (H2PtCl6) in 10 mL water to which 20 mg lead acetate has been added. Connect it with two dry cells of 1.5 volts each in parallel and reverse the direction of the current once a minute for 6 minutes or till the shining platinum surface is covered. Repeat the electrolytic process using 10% sulphuric acid to remove chlorine. Wash with distilled water and keep the cell immersed in distilled water when not in use. 2. Set the instrument according to manufacturer's instruction. In some instruments correction for cell constant and temperature factor is provided. If this arrangement is not there, cell constant may be separately determined and values of specific conductance should be converted to 25: C by multiplying with the factor given in table1. Cell Constant, L = $\frac{K1 + K2}{K_x \times f}$ Where K1 = conductivity in µs /cm of the potassium chloride solution at 25°C; K2 = Conductivity in µs/cm of distilled water at 25:C used for preparing the reference solution;

Kx = measured conductance in µs/cm; and
f = temperature factor for converting specific conductance value to that at 25°C (see table 1)

Note - if K2 is very low, it may be ignored.

3. Determine conductivity of 0.00702 N potassium chloride or 0.01 N. Potassium chloride solution by use of instrument in accordance with manufacturer's instructions. Measure the temperature of the solution before and after the test and take the mean value (t°C).

4. Because the cell constants are subject to slow change even under ideal conditions and sometimes to more rapid change under adverse conditions, it is recommended that cell constant be periodically established.

5. Determine conductance of the unknown sample.

Calculation with units of expression

Calculate specific conductance as follows:

Specific conductance at 25°C, µs/cm = KL*f*

Where

K = conductivity, µs/cm;

L = Cell Constant; and

f = factor for converting specific conductance value to that at 25°C

Temperature :C	**Factor *f***	**Temperature :C**	**Factor *F***	**Temperature :C**	**Factor *F***
15.0	1.247	23.0	1.043	30.2	0.904
16.0	1.218	23.2	1.038	30.4	0.901
16.2	1.212	23.4	1.034	30.6	0.897
16.4	1.206	23.6	1.029	30.8	0.894
16.6	1.200	23.8	1.025	31.0	0.890
16.8	1.194	24.0	1.020	31.2	0.887
17.0	1.189	24.2	1.016	31.4	0.884
17.2	1.184	24.4	1.012	31.6	0.880
17.4	1.179	24.6	1.008	31.8	0.877
17.6	1.174	24.8	1.004	32.0	0.873
17.8	1.169	25.0	1.000	32.2	0.870
18.0	1.163	25.2	0.996	32.6	0.864
18.2	1.157	25.4	0.992	32.8	0.861
18.4	1.152	25.6	0.988	33.0	0.858
18.6	1.147	25.8	0.983	33.2	0.855
18.8	1.142	26.0	0.979	33.4	0.852
19.0	1.136	26.2	0.975	33.6	0.849
19.2	1.131	26.4	0.971	33.8	0.846
19.4	1.127	26.6	0.967	34.0	0.843
19.6	1.122	26.8	0.964	35.0	0.829
19.8	1.117	27.0	0.960	36.0	0.815
20.0	1.112	27.2	0.956	37.0	0.801
20.2	1.107	27.4	0.953	38.0	0.788
20.4	1.102	27.6	0.950	39.0	0.775

20.6	1.097	27.8	0.947	40.0	0.763	
20.8	1.092	28.0	0.943	41.0	0.750	
21.0	1.087	28.2	0.940	42.0	0.739	
21.2	1.082	28.4	0.936	43.0	0.727	
21.4	1.076	28.6	0.932	44.0	0.715	
21.6	1.073	28.8	0.929	45.0	0.705	
21.8	1.068	29.0	0.925	46.0	0.694	
22.0	1.064	29.2	0.921	47.0	0.683	
22.2	1.060	29.4	0.918			
22.4	1.055	29.6	0.914			
22.6	1.051	29.8	0.911			
22.8	1.047	30.0	0.907			

Calculation of TDS by conductivity

The ability of a solution to conduct an electric current is the functioning of the concentration and charge of ions in the solution and also depends on ionic mobility. Ionic mobility decreases with increase in number of ions per unit volume of solution due to interionic effect and other factors. Broadly, the relationship between conductivity and dissolved solids and conductivity and soluble cations is given by the following equations:

A K = S and, K = 100 C

Where

A = multiplication factor for converting conductivity values to total dissolved solids;

K = conductivity in µs/cm,

S = total dissolved solids in mg/L, and

C = total soluble cations in meq/L

Note 1- the value of A varies from 0.54 to 0.96 depending on the nature of ion present in water, and is usually taken as 0.65.

Note 2 -The relationship given above is approximate and is used for broad checking only and should not be used for accurate calculations. Types of ions present in solution effect these relationships. A pure solution of sodium bicarbonate with total dissolved solids 980 mg/L will have a conductivity of 1000µs/cm and a solution of sodium chloride with total dissolved solids 500 mg/L will have the same conductivity. Presence of relatively low conductivity particles or molecules like silicic acid and the presence of H^+ and OH^- ions effect the ratio between conductivity and total dissolved solids.

Inference (Qualitative Analysis)	NA
Reference	IS:3025 part 16 – 1984 (Reaffirmed 2002)- Methods of Sampling and Test (Physical and chemical) for water and Waste Water : Filterable Residue (Total Dissolved Solids)
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	**Determination of Anionic Surfactants as MBAS**		
Method No.	**FSSAI 14.010:2024**	**Revision No. & Date**	0.0
Scope	Determination of Anionic Surfactants as MBAS in Mineral water, Packaged Drinking Water (other than Mineral Water), Drinking Water (Purified)		
Caution	• Chloroform is toxic and a suspected carcinogen. Take appropriate precautions against inhalation and skin exposure. • Methanol vapors are flammable and toxic; take appropriate precautions.		
Principle	Methylene blue active substances (MBAS) bring about the transfer of methylene blue, a cationic dye, from an aqueous solution into an immiscible organic liquid upon equilibration. This occurs through ion pair formation by the MBAS anion and the methylene blue cation. The intensity of the resulting blue color in the organic phase is a measure of MBAS. Anionic surfactants are among the most prominent of many sub□stances, natural and synthetic, showing methylene blue activity. The MBAS method is useful for estimating the anionic surfactant content of waters and wastewaters, but the possible presence of other types of MBAS always must be kept in mind. This method is relatively simple and precise. It comprises 3 successive extractions from acid aqueous medium containing excess methylene blue into chloroform ($CHCl_3$), followed by an aqueous backwash and measurement of the blue color in the $CHCl_3$ by spectrophotometry at 652 nm. The method is applicable at MBAS concentrations down to about 0.025 mg/L.		
Apparatus/Instruments	a. Colorimetric equipment: One of the following is required: 1) Spectrophotometer, for use at 652 nm, providing a light path of 1 cm or longer. 2) Filter photometer, providing a light path of 1 cm or longer and equipped with a red color filter exhibiting maximum transmittance near 652 nm. b. Separatory funnels: 500-mL, preferably with inert PTFE stopcocks and stoppers.		
Materials and Reagents	a. Stock LAS solution b. 1000 mg/L LAS stock standard c. Phenolphthalein indicator solution d. Sodium hydroxide (NaOH), I M e. Sulfuric acid (H_2S0_4), 1 N and 6 N f. Chloroform ($CHCl_3$) g. Methylene blue reagent h. Wash solution i. Methanol (CH_3OH) j. Hydrogen peroxide (H_20_2), 30% k. Glass wool		

	l. Water, reagent-grade, MBAS-free
Preparation of Reagents	a. Stock LAS solution: Weigh an amount of the reference material equal to 1.00 g LAS on a 100% active basis. Dissolve in water and dilute to 1000 mL; 1.00 mL = 1.00 mg LAS. Store in a refrigerator to minimize biodegradation. If necessary, prepare weekly. Commercial stock standards are available; follow the manufacturer's recommendations for holding times. Preferably, obtain a 1000 mg/L LAS stock standard from a reputable commercial supplier. b. Standard LAS solution: Dilute 10.00 mL stock LAS solution to 1000 mL with water; 1.00 mL = 10.0 mg LAS. Prepare daily. c. Phenolphthalein indicator solution, alcoholic. d. Sodium hydroxide (NaOH), I M. e. Sulfuric acid (H_2S0_4), 1 N and 6 N. f. Chloroform ($CHCl_3$) g. Methylene blue reagent: Dissolve 100 mg methylene blue in 100 mL water. Transfer 30 mL to a 1000-mL flask. Add 500 mL water, 41 mL 6 N H_2SO_4, and 50 g sodium phosphate, mono-basic, monohydrate, NaH2PO4 · H_2O. Shake until dissolved. Dilute to 1000 mL. h. Wash solution: Add 41 mL 6 N H_2SO_4 to 500 mL water in a 1000 mL flask. Add 50 g NaH_2PO_4 · H2O and shake until dissolved. Dilute to 1000 mL. i. Methanol (CH_3OH). Caution: Methanol vapors are flammable and toxic; take appropriate precautions. j. Hydrogen peroxide (H_20_2), 30%. k. Glass wool: Pre-extract with $CHCl_3$ to remove interferences. l. Water, reagent-grade, MBAS-free. Use for making all reagents and dilutions.
Sample Preparation	--
Method of analysis	**a. Preparation of calibration curve:** Prepare an initial calibration curve consisting of at least 5 standards covering the referenced or desired concentration range. Provided that linearity is demonstrated over the range of interest (r = 0.995 or better), run daily check standards at the reporting limit and a concentration above the expected samples' concentration. Check standard results must be within 25% of original value at the reporting limit and 10% of original value for all others. Otherwise, prepare a new calibration curve. Prepare a series of separatory funnels for a reagent blank and selected standards. Pipet portions of the standard LAS solution into funnels. Add sufficient water to make the total volume 100 mL in each separatory funnel. Treat each standard as described in paragraphs d and e below, and plot a calibration curve of absorbance versus micrograms LAS taken, specifying the molecular weight of the LAS used. **b. Sample size:** For the direct analysis of waters and wastewaters, select the sample volume on the basis of expected MBAS concentration. The table below is for guidance; adjust volumes, if ne

	Expected MBAS Concentration (mg/L) — Sample Taken (mL) 0.025-0.080 — 400 0.08-0.40 — 250 0.4-2.0 — 100 If the expected MBAS concentration is more than 2 mg/L, dilute the sample containing 40 to 200 mg MBAS to 100 mL with water. For analysis of samples purified by sublation, dissolve sublate residue in 10 to 20 mL methanol; quantitatively transfer the entire amount (or a suitable portion if more than 200 g MBAS is expected) to 25 to 50 mL water; evaporate without boiling until methanol is gone, adding water as necessary to avoid going to dryness; and dilute to about 100 mL with water. **c. Peroxide treatment:** If necessary, to avoid decolorization of methylene blue by sulfides, add a few drops of 30% H_2O_2. **d. Ion pairing and extraction:** 1) Add the sample to a separatory funnel. Make alkaline by dropwise addition of 1 M NaOH, using phenolphthalein indicator. Discharge the pink color by dropwise addition of 2 M H2SO4. 2) Add 10 mL CHC13 and 25 mL methylene blue reagent. Rock the funnel vigorously for 30 s and let the phases separate. Alternatively, place a magnetic stirring bar in the separatory funnel; lay the funnel on its side on a magnetic mixer and adjust the speed of stirring to produce a rocking motion. Excessive agitation may cause emulsion formation. To break persistent emulsions add a small volume of isopropyl alcohol (<10 mL); add the same volume of isopropyl alcohol to all standards. Some samples require a longer period of phase separation than others. Before draining the $CHCl_3$ layer, swirl gently, then let settle. 3) Draw off the $CHCl_3$ layer into a second separatory funnel. Rinse the delivery tube of the first separatory funnel with a small amount of $CHCl_3$. Repeat the extraction 2 additional times, using 10 mL $CHCl_3$ each time. If the blue color in the water phase becomes faint or disappears, discard and repeat, using a smaller sample. 4) Combine all $CHCl_3$ extracts in the second separatory funnel. Add 50 mL wash solution and shake vigorously for 30 s. Emulsions do not form at this stage. Let settle, swirl, and draw off the $CHCl_3$ layer through a funnel containing a plug of glass wool into a 100-mL volumetric flask; the filtrate must be clear. Extract the wash solution twice with 10 mL $CHCl_3$ each and add to the flask through the glass wool. Rinse the glass wool and funnel with $CHCl_3$. Collect washings in volumetric flask, dilute to mark with $CHCl_3$, and mix well. **e. Measurement:** Determine absorbance at 652 nm against a blank of $CHCl_3$.
Calculation with units of expression	From the calibration curve, read micrograms of apparent LAS (mol wt) corresponding to the measured absorbance.

	mg/L= $\frac{\mu g \text{ apparent LAS}}{\text{mL original sample}}$ In laboratory records, record as LAS, mol wt.
Inference (Qualitative Analysis)	NA
Reference	APHA 5540 c
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Anionic Surface Active Agents		
Method No.	**FSSAI 14.011:2024**	**Revision No. & Date**	0.0
Scope	Surfactants are a large group of surface-active substances with a great number of (cleaning) applications. Most surfactants have degreasing or wash active abilities. They reduce the surface tension of the water so it can wet the fibers and surfaces, they loosen and encapsulate the dirt and, in that way, ensure that the soiling will not re-deposit on the surfaces. Surfactants have a hydrophobic (water repellent) part and a hydrophilic ('water loving') part. The hydrophobic part consists of an uncharged carbohydrate group that can be straight, branched, cyclic or aromatic. Most surfactants are more or less toxic to aquatic organisms due to their surface activity which will react with the biological membranes of the organisms. The biological degradability varies according to the nature of the carbohydrate chain. Generally, the linear chains are more readily degradable than branched chains. Also, the toxic effects vary with the chain structure. Generally, an increase of the chain length in the range of 10 to 16, leads to an increase in toxicity to aquatic organisms.		
Caution	• Chloroform is toxic and a suspected carcinogen. Take appropriate precautions against inhalation and skin exposure. • Methanol vapors are flammable and toxic; take appropriate precautions.		
Principle	Methylene blue a cationic dye forms the salts with anionic surfactants in an alkaline medium. These salts are extracted with chloroform. Any interference present is eliminated by extraction of anionic surfactant methylene blue complex from alkaline solutions and shaking with acidic methylene blue solution. The absorbance of the separated organic phase is measured at the maximum absorption wavelength of 650 nm. This method is applicable to limit of detection of about 0.05mg/L for solutions of standard surfactants in distilled water.		
Apparatus/Instruments	a. pH-Meter-With suitable electrodes made of glass. b. Spectrophotometer, capable of measurement at 650 nm, equipped with cells of optical path length of 10 mm &50 mm c. Gas stripping Apparatus- One liter capacity. d. Seperatory Funnels 500 mL capacity.		
Materials and Reagents	a. Sodium Chloride b. Ethyl Acetate c. Chloroform d. Ethanol, 95% e. Methanol (Freshly Distilled) f. Sulphuric Acid Solution- 0.5 ml g. Ethanolic Sodium Hydroxide - 0.1 mol/L h. Methylene Blue, Neutral Solution i. Methylene Blue, Acidic Solution		

	j. Buffer Solution, pH 10 k. Phenolphthalein Indicator Solution l. Dodecylbenzene Sulphonic Acid Methyl Ester (Tetra propylene Type), stock standard solution
Preparation of Reagents	• **Ethanolic Sodium Hydroxide** - 0.1 mol/L - Dissolve 4gm of sodium hydroxide pellets in ethanol and dilute to I000 mL with the same ethanol. • **Methylene Blue, Neutral Solution** - Dissolve 0.350 gm of methylene blue in water and dilute to 1000 mL. Prepare the solution at least 24 hr before use. • **Methylene Blue, Acidic Solution**-- Dissolve 0.350 gm of methylene blue in 500 mL of water and add 6.50 mL of sulphuric acid (Density - I.84 gm/mL). Dilute with water to 1000 mL after mixing. Prepare solution at least 24 hr before use. The absorbance of the chloroform phase of the blank test, measured against. 0.02/10 mm of optical path length at 650 nm. In the case of higher blank absorbance, either wash the methylene blue solution twice with chloroform or use other batches of Methylene Blue. • **Buffer Solution, pH 10**-- Dissolve 24 gm of sodium hydrogen carbonate (NaHCO3) and 27 gm of anhydrous sodium carbonate (Na2CO3) in water and dilute to 1000 mL. • **Phenolphthalein Indicator Solution**-- Dissolve 1.0 gm of phenolphthalein in 50 mL of ethanol and add 50 mL of water, with stirring continuously. Filter off any precipitates. • **Dodecylbenzene Sulphonic Acid Methyl Ester (Tetra propylene Type), stock standard solution** —Weigh 400 mg to 450 mg of Dodecylbenzene Sulphonic acid methyl ester to the nearest 0.1 mg, into a round-bottom flask, and add 50 mL of ethanol sodium hydroxide solution and some anti-bumping granules. Attach the reflux condenser and boil for 1 hr. After cooling, rinse the condenser and the ground-glass joint with about 30 ml of ethanol and add the rinsing to the contents of the flask. Neutralize the solution with sulphuric acid against phenolphthalein until it becomes colorless. Transfer the solution to a 1000 mL volumetric flask, dilute to the mark with water and mix. This standard solution is stable for 6 months.
Sample Preparation	--
Method of analysis	**1. Separation of the Surfactant** - Non-surfactant methylene blue active substances can cause errors in the test of methylene blue index. Stripping is recommended for concentrating small amount of surfactants from water samples. Separate suspended matter by centrifugation, but note that adsorbed surfactants on suspended matter will not be determined. Place a measured quantity of the test sample, up to 1000 mL in the gas-stripping apparatus. Install the stripping apparatus in well ventilated hood to carry off ethyl acetate vapour. Separation is improved by addition of

	sodium chloride. If sample volume exceeds 500 mL, add 100 gm of sodium chloride dissolve by passing nitrogen gas or air through it. If a smaller test sample volume is used, dissolve 100 gm of sodium chloride in 400 mL of water and add this solution to test sample. If necessary, add water to bring the sample surface up to the level of the upper stopcock. Add 100 mL ethyl acetate. Fill the wash bottle in the gas line (nitrogen or air) two-third full with ethyl acetate. Pass a gas stream of 20 L/h to 50 L/h through the gas stripping apparatus. Adjust the gas flow in such a way that the phases remain separate and no turbulence is produced at the interface. The significant mixing of the phases and consequent solution of ethyl acetate in the water is avoided. Stop the gas flow after 5 min. If a loss of more than 20 percent (v/v) of the organic phase has occurred due to solution in the water phase discard the test sample. Run off the organic phase completely into a separating funnel. Return any water in the separating funnel to the gas-stripping apparatus. Filter the ethyl acetate solution through a dry qualitative gas-filter paper into a 250 mL flask. Add a further 100 mL of ethyl acetate to the gas-stripping apparatus and again pass nitrogen or air through it for 5 min. separate the organic layer as described above, using the same separating funnel, filter, and add it to the first portion. Rinse the filter paper and funnel with 25 mL of ethyl acetate. Remove all the ethyl acetate solution on a water bath under a hood. To speed up the process direct a gentle air stream over the surface of the solution. Dissolve the residue in about 5 mL of methanol and 50 mL of water. Transfer the solution quantitatively to a 100 mL volumetric flask and dilute to the mark with water. **1. Blank Test** - Carry out a blank test at 650 nm and subtract the interpolated absorbance, A0 from the absorbance A1 of the test sample. Under the given conditions the absorbance A0 of the blank test shall not exceed 0.02 per 10 nm optical path length otherwise equipment and the reagents shall be checked carefully for any contamination. **2. Test with the sample** i. Transfer a measured volume of the test sample into a separating funnel. This test portion should contain 20 µg to 200µg of MBAS (methylene blue active substances). In the lower MBAS range, a test portion up to 100 mL may be used. If the volume of the test portion is less than 100 mL, dilute with water to 100 mL. ii. Add 5.0 mL of neutral methylene blue solution, 10 mL of buffer solution and 15 mL chloroform. iii. Shake evenly and gently about twice a second for 1 min, preferably in a horizontal plane. iv. Allow the layers to separate as completely as possible and swirl the funnel to dislodge droplets from the sides of the funnel. v. Allow to settle for 2 min, and then run as much as possible of the

	chloroform layer into a second separating funnel, containing 110 mL of water and 5.0 mL of acidic methylene blue solution. vi. Shake uniformly but not too vigorously for 1 min as previously described. vii. Filter the chloroform layer through a cotton or glass wool filter wetted with chloroform into a 50 mL volumetric flask. viii. Repeat the extraction of the alkaline and acidic solution using a 10 mL portion of chloroform for the extraction. ix. Separate the chloroform layer and filter it through the same filter, into the volumetric flask. x. Repeat the extraction using a further 10 mL of portion of chloroform and filter that into a 50 mL of volumetric flask. xi. Dilute to the mark with chloroform and mix. xii. For each test sample carry out the complete extraction for a blank determination with 100 mL water.
Calculation with units of expression	Measure the absorbance for the test sample as well as for the blank test at 650 nm in cells of optical path length 10 mm to 50mm against chloroform. The absorbance of the test sample should not be more than that of the blank.
Inference (Qualitative Analysis)	NA
Reference	IS: 13428 - 2005 (Reaffirmed- 2009) Packaged Natural Mineral water Specifications. Annex: K (Method of test for Anionic Surface Active A
Approved by	Scientific Panel on Methods of Sampling and Analysis

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Method No.	**FSSAI 14.012:2024**	**Revision No. & Date**	0.0
Scope	Determination of Boron in Mineral water, Packaged Drinking Water (other than Mineral Water), Drinking Water (Purified)		
Caution	Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information		
Principle	Reaction of azomethine-H, which is the condensation product of H-acid (8-amino-naphth-l-ol-3.6-disulfonic acid) and salicylaldehyde, with dissolved forms of borate at a pH of about 6, leads to the formation of a yellow complex that is measured spectrometrically at the absorption maximum in the range of 410 nm to 420 nm.		
Apparatus/Instruments	a. Ordinary laboratory apparatus made of polypropylene, polyethylene or polytetrafluoroethylene, where applicable. b. Spectrometer, for use in the wavelength range of 410 nm to 420 nm, with cells of an optical path length between 10 mm and 50 mm.		
Materials and Reagents	a. Azomethine-H, Solution b. Buffer Solution (pH 5.9) c. Reagent Solution d. Borate stock solution e. Boron standard solution-I f. Boron standard solution-II g. Calcium hydroxide [Ca $(OH)_2$]		
Preparation of Reagents	a. **Azomethine-H, Solution:** Dissolve 1.0 gm of azomethine-H sodium salt [8-N-2-hydroxybenzylidene)-amino naphth-l-o1-3.6 disulfonic acid] (CI7HI2NNaO3S2) and 3.0 gm of ± L ascorbic acid (C6H806) in water and dilute to 100 mL in a volumetric flask. The solution is stable for up to one week when stored in a polyethylene bottle at a temperature of between 4-6°C. b. **Buffer Solution (pH 5.9):** Mix 250 gm of ammonium acetate (CH3COONH4) 250 mL of water, 80 mL of sulphuric acid (H2SO4) (sp.gr-1.21 g/mL), 5 mL of phosphoric acid (H2PO4) (sp.gr -1.71 g/mL), 1.0gm of citric acid (C6H8O7H2O) and add 1.0gm of disodiumethylenediamine-tetracetic acid dehydrate (C10H14N2Na2O8H2O) with stirring and gentle heating. c. **Reagent Solution:** Mix equal volumes of reagents prepared in a and b. Prepare this solution on the day of use and store in a Polyethylene bottle. d. **Borate, stock solution** corresponding to 1.0 of B per liter. Dissolve 5.719 gm of boric acid (H3BO3) in 1000 mL of water. Store it in a polyethylene bottle. 1 mL of this stock solution contains 1.0 mg of borate, expressed as B.		

	e. **Boron, standard solution-I** corresponding to 10.0 mg of B per liter. Dilute 10 mL of borate stock solution (see 2.4) to 1000 mL with water. 1 mL of this standard solution contains 10.0 µg of borate, expressed as B. f. **Boron, standard solution-II** corresponding to 1.0 mg of B per liter. Dilute 10 mL of borate solution (see 2.5) to 100 mL with water. 1mL of this standard solution contains 1.0 µg of borate, expressed as B. **g. Calcium hydroxide [$Ca(OH)_2$]**
Sample Preparation	--
Method of analysis	**1. Determination** • Transfer 25.0 mL of the sample, or a smaller amount of the sample diluted to 25mL with distilled water, into a 100 mL polyethylene flask. Add 10mL of Azomethine-H. • Mix and allow to stand in the dark for 2 hr at 20 ± 1°C, then measure the absorbance at the absorption maximum in the range of 410 nm to 420 nm against distilled water in a cell of optical path length 10mm, using the spectrometer set up according to the manufacturer's instructions and after setting the zero with distilled water in the cell. • Alternatively use a cell of 50 mm optical path length for low boron concentrations of up to about 0.2 mg of boron per litre. Check the wavelength of the absorption maximum whenever a new batch of this reagent is used. **2. Blank Test** • Carry out a blank test by treating 25 mL of water as described in 4.1. Ensure that the blank value is in the range of 0.1 absorption units to 0.17 absorption units per 10 mm. If the absorption is higher, then check the reagents and the distilled water for their borate content. • Measure it into three separate borate-free beakers (preferably poly tetrafluorethylene), 25 mL, 100 mL and 250mL aliquots of the distilled water. Make slightly alkaline by the addition of the same small (for example 200 mg) amount of calcium hydroxide to each. Evaporate the 100 mL and 250 mL aliquots to a volume of just less than 25 mL and adjust their volumes to precisely 25 mL by the addition of a little extra distilled water, as necessary. Carry out the procedure given in 4.1 on these aliquots. • Carry out a blank determination with each of the aliquots. If borate is present in the distilled water, the borate found increases in proportion to the volume of the aliquot taken. Erratic results indicate external borate contamination. Relatively high but constant results indicate impure reagents. **3. Prevention of Contamination:** • As borate is widespread in the environment, significant contamination may occur during trace determinations. The

	following sources of contamination, and remedies, should be considered. • Borosilicate glassware should be avoided to the extent possible as it may leads to positive contamination. Borosilicate glass, well rinsed in hydrochloric acid, may be used for acidic solutions, but should never be used for neutral or alkaline solutions, or for prolonged storage at any pH value. (Borosilicate glassware previously used with alkaline solutions shall not be used without very thorough acid rinsing.) Polyethylene flasks and plastics pipettes are preferable. • Detergents and soaps used for glassware and lab coats should be borate free, and the use of towels and tissues, for drying shall be avoided. • Toiletries, talcum powder and cosmetics used by technicians often contain borate and should be avoided or removed, especially prior to undertaking accurate low-level determinations. • Water and reagents may contain borate and blanks should be carried out at least in duplicate and should agree. **4. Calibration** **a. Zero mg/L to 0.20 mg/L of Boron Calibration Graph** • To a series of six 25 mL one mark plastics flasks add respectively 0 mL, 1 mL, 2 mL, 3 mL, 4 mL and 5 mL of boron standard solution-II, dilute to the mark with distilled water and mix. This gives concentrations of 0 mg; 0.04 mg; 0.08 mg; 0.12 mg; 0.16 mg and 0.20 mg of boron per liter respectively. Analyze each standard solution as described in 1, measuring the absorbance values in a 50 mm optical path length cell compared against distilled water. Prepare a calibration graph by plotting the absorbance values against the known concentrations in milligrams of boron per liter for each standard. **b. Zero mg/L to 1.00 mg/L of Boron Calibration Graph** • Repeat the above calibration, using 0 mL, 5 mL, 10mL, 15 mL, 20 mL and 25 mL of boron standard solution-II (see 2.6) respectively to give concentrations of 0mg; 0.2mg; 0.4mg; 0.6mg; 0.8mg and 1.0mg of boron per liter respectively. Analyze each standard Solution as described in 4.1, but this time measuring the absorbance values using a 10mm optical path length cell compared against distilled water. Prepare a separate Calibration graph. **c. Calculation of Factor f** • It is essential that a linear calibration graph be achieved in both cases; if not then check the solutions and repeat the calibration. Calculate the reciprocal value for the slope & factor f, for each graph.
Calculation with units of expression	Calculate the borate content, in milligrams of boron per liter, from the formula $$= \frac{(A1 - A0)\, f\, V\, 1\ \text{max}}{V1}$$ Where

	A1 = absorbance of the sample A0= absorbance of the blank V1 = volume, in milliliters, of the sample V1Max = maximum volume, in milliliters, of the sample f = calibration factor, determined from the appropriate calibration curve (reciprocal value of the slope, in milligrams of boron per liter)
Inference (Qualitative Analysis)	NA
Reference	IS: 13428 - 2005 (Reaffirmed- 2009) Packaged Natural Mineral water Specifications. Annex: H (Determination of Borate).
Approved by	Scientific Panel on Methods of Sampling and Analysis

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Method No.	**FSSAI 14.013:2024**	**Revision No. & Date**	0.0
Scope	Mineral water, Packaged Drinking Water (other than Mineral Water), Drinking Water (Purified)		
Caution	Follow all safety procedures while handling and disposing solutions. Wear laboratory apron, shoes, safety goggles and mask while working with chemicals. Perform work in fume hood while working with solvents. Refer to MSDS (Material Safety Data Sheets) for specific information		
Principle	In the presence of boron, a solution of carmine or carminic acid in concentrated sulfuric acid changes from a bright red or bluish red, depending on the concentration of boron present.		
Apparatus/Instruments	Colorimetric equipment: One of the following is required: 1. Spectrophotometer, for use at 585 nm, with a minimum light path of 1 cm. 2. Filter photometer, equipped with an orange filter having a maximum transmittance near 585 nm, with a minimum light path of 1 cm.		
Materials and Reagents	• Standard boron solution • Hydrochloric acid, HCI, conc. and 1 + 11 • Sulfuric acid, H2S04, conc. • Carmine reagent		
Preparation of Reagents	Store all reagents in polyethylene or boron-free containers • Standard boron solution: Dilute 10.00 ml stock boron solution to 1000 mL with distilled water; 1.00 mL = 1.00 µg Boron. • Hydrochloric acid, HCI, conc. and 1 + 11 • Sulfuric acid, H2S04, conc. • Carmine reagent: Dissolve 920 mg carmine N.F. 40, or carmine acid, in 1 L conc. H2S04, (If unable to zero spectrophotometer, dilute carmine 1+1 ·with conc. H2S04 to replace above reagent)		
Sample Preparation	--		
Method of analysis	**1. Low-level sample concentration:** • If sample contains less than 1mg Boron/L, pipet a portion containing 2 to 20 µg B into platinum dish, make alkaline with 1N NaOH plus a slight excess, and evaporate to dryness on a steam or hot water bath. • If necessary, destroy any organic material by ignition at 500 to 550°C. Acidify cooled residue (ignited or not) with 2.5 mL 1 + 11 HCI and triturate with a rubber policeman to dissolve. • Centrifuge if necessary to obtain a clear solution. Pipet 2.00mL clear concentrate into a small flask or 30mL test tube. Treat reagent blank identically. **2. Color development:** • Prepare a series of boron standard solutions (100, 250, 500, 750,		

	and 1000 µg) in 100 mL with distilled water. • Pipet 2.00 mL of each standard solution into a small flask or 30 mL test tube. Treat blank and calibration standards exactly as the sample. • Add 2 drops (0.1 mL) conc. HCI, carefully introduce 10.0 mL conc. H2S04, mix, and let cool to room temperature. • Add 10.0 mL carmine reagent, mix well, and after 45 to 60 min measure absorbance at 585 nm in a cell of 1cm or longer light path, using the blank as reference. • To avoid error, make sure that no bubbles are present in the optical cell while photometric readings are being made. Bubbles may appear as a result of incomplete mixing of reagents. • Because carmine reagent deteriorates, check calibration curve daily.
Calculation with units of expression	mg Boron/L= µg B X D mL sample Where: D = dilution correction.
Inference (Qualitative Analysis)	NA
Reference	1. IS: 13428 - 2005 (Reaffirmed- 2009) Packaged Natural Mineral water Specifications. Annex: H (Determination of Borate). 2. APHA 4500-B
Approved by	Scientific Panel on Methods of Sampling and Analysis

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Method No.	**FSSAI 14.014:2024**	**Revision No. & Date**	0.0
Scope	Mineral water, Packaged Drinking Water (other than Mineral Water), Drinking Water (Purified)		
Caution	Closely control such variables as volumes and concentrations of reagents, as well as time and temperature of drying. Use evaporating dishes identical in shape, size, and composition to insure equal evaporation time because increasing the time increases intensity of the resulting color.		
Principle	When a sample of water containing boron is acidified and evaporated in the presence of Curcumin, a red-colored product called rosocyanine is formed. The rosocyanine is taken a suitable solvent and the red color is compared with standards visually or photometrically.		
Apparatus/Instruments	a. Colorimetric equipment: One of the following is required: b. Spectrophotometer, for use at 540 nm, with a minimum light path of 1 cm. c. Filter photometer, equipped with a green filter having a maximum transmittance near 540 nm, with a minimum light path of 1 cm. d. Evaporating dishes, 100 to 150mL capacity, of high-silica glass, platinum, or other suitable material. e. Water bath, set at 55 ± 2°C. f. Glass-stoppered volumetric flasks, 25 and 50 mL capacity. g. Ion-exchange column, 50 cm long by 1.3 cm in diameter.		
Materials and Reagents	a. Stock boron solution b. Standard boron solution c. Curcumin reagent d. Ethyl or isopropyl alcohol 95% e. Reagent for removal of high hardness and cation interference: f. Strongly acidic cation- exchange resin g. Hydrochloric acid, HCl 1+5		
Preparation of Reagents	Store all reagents in polyethylene or boron-free containers. a. **Stock boron solution**: Dissolve 571.6 mg anhydrous boric acid, H3B03, in distilled water and dilute to 1000 mL; 1.00 mL = 100µg Boron. Because H3B03 loses weight on drying at 105°C, use a reagent meeting ACS specifications and keep the bottle tightly stoppered to prevent entrance of atmospheric moisture. b. **Standard boron solution:** Dilute 10.00 ml stock boron solution to 1000 mL with distilled water; 1.00 mL = 1.00 µg Boron. c. **Curcumin reagent**: Dissolve 40 mg finely ground Curcumin and 5.0 gm oxalic acid in 80 mL 95% ethyl alcohol. Add 4.2 mL conc. HCl, make up to 100 mL with ethyl alcohol in a 100 mL volumetric flask, and filter if reagent is turbid (isopropyl alcohol, 95% may be used in place of ethyl alcohol). This reagent is stable for several days if stored in a refrigerator.		

Sample Preparation	Same as above
Method of analysis	**1. Preparation of calibration curve:** • Pipet 0 (blank), 0.25, 0.50, 0.75, and 1.00 µg boron into evaporating dishes of the same type, shape, and size. Add distilled water to each standard to bring total volume to 1.0 mL. • Add 4.0 mL curcumin reagent to each and swirl gently to mix contents thoroughly. • Float dishes on a water bath set at 55 ± 2°C and let them remain for 80 min, which is usually sufficient for complete drying and removal of HCI. Keep drying time constant for standards and samples. • After dishes cool to room temperature, add 10 mL 95% ethyl alcohol to each dish and stir gently with a polyethylene rod to insure complete dissolution of the red-colored product. • Wash contents of dish into a 25mL volumetric flask, using 95% ethyl alcohol. Make up to mark with 95% ethyl alcohol and mix thoroughly by inverting. • Read absorbance of standards and samples at a wavelength of 540 nm after setting reagent blank at zero absorbance. • The calibration curve is linear from 0 to 1.00 µg boron. Make photometric readings within 1 h of drying samples. **2. Sample treatment:** • For waters containing 0.10 to 1.00 mg B/L, use 1.00mL sample. For waters containing more than 1.00 mg B/L, make an appropriate dilution with boron-free distilled water, so that a 1.00 mL portion contains approximately 0.50 µg boron. • Pipet 1.00 mL sample or dilution into an evaporating dish. Unless the calibration curve is being determined at the same time, prepare a blank and a standard containing 0.50 µg boron and run in conjunction with the sample. Proceed as in 1 above, beginning with Add 4.0 mL Curcumin reagent. • If the final solution is turbid, filter through filter paper before reading absorbance. Calculate boron content from calibration curve. **3. Visual comparison:** • The photometric method may be adapted to visual estimation of low boron concentrations, from 50 to 200 µg/L, as follows: • Dilute the standard boron solution 1 + 3 with distilled water; 1 mL = 0.20µg Boron. Pipette 0, 0.05, 0.10, 0.15, and 0.20 µg B into evaporating dish indicated in 2 above. • At the same time add an appropriate volume of sample (1.00 mL or portion diluted to 1.00 mL) to an identical evaporating dish. The total boron should be between 0.05 and 0.20µg. Proceed as in 1 above, beginning with "Add 4.0 mL curcumin reagent. ..." • Compare color of samples with standards within 1 hr of drying samples.

	4. Removal of high hardness and cation interference: • Prepare an ion-exchange column of approximately 20 cm X 1.3 cm diameter. • Charge column with a strongly acidic cation-exchange resin. Backwash column with distilled water to remove entrained air bubbles. • Keep the resin covered with liquid at all times. Pass 50 mL I + 5 HCI through column at a rate of 0.2 mL acid/mL resin in column/min and wash column free of acid with distilled water. • Pipet 25 mL sample, or a smaller sample of known high boron content diluted to 25mL, onto the resin column. • Adjust rate flow to about 2 drops/s and collect effluent in a 50mL volumetric flask. Wash column with small portions of distilled water until flask is filled to mark. • Mix and transfer 2.00 mL in evaporating dish. Add 4.0 mL Curcumin reagent and complete the analysis as described above.
Calculation with units of expression	Use the following equation to calculate boron concentration from absorbance readings: $$\text{Mg B/L} = \frac{A2 \times C}{A1 \times S}$$ Where: A1 = absorbance of standard, A2 = absorbance of sample, C = µg B in standard taken, and S = mL sample.
Inference (Qualitative Analysis)	NA
Reference	• IS: 13428 - 2005 (Reaffirmed- 2009) Packaged Natural Mineral water1Specifications. Annex: H (Determination of Borate). • APHA 4500-B
Approved by	Scientific Panel on Methods of Sampling and Analysis

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Method No.	**FSSAI 14.015:2024**	**Revision No. & Date**	0.0
Scope	Cadmium Reduction Method for determination of Ammoniacal Nitrogen in water prescribes cadmium reduction method for determination of Nitrate. This method is suitable for concentration below 0.1 mg per liter of nitrate nitrogen.		
Caution	Higher concentrations of copper, iron etc lower the reduction efficiency. Add EDTA to remove this interference. Oil and grease & residual chlorine can interfere. Remove oil and grease by extraction with organic solvents and residual chlorine by adding sodium thiosulphate.		
Principle	Nitrate is reduced to nitrite in presence of cadmium. The nitrite produced is determined by diazotizing with sulphanilamide and coupling with N-(1naphthyl) ethylenediamine to from a highly colored azo dye which is measured colorimetrically.		
Apparatus/Instruments	1. Reduction column – commercially available one or construct the column from a 100 mL volumetric pipette by removing the top portion. The column can also be constructed by two pieces of tubing joined end to end (join a 10 cm length of 3 cm internal diameter tubing to a 25 cm length of 3.5 cm Internal diameter tubing). A liquid leveling device is useful. 2. Colorimeter- One of the following 3. Spectrophotometer- for use near 543 nm with a light path of 1 cm or longer. 4. Filter photometer – provided with a yellow green filter having maximum transmittance near 540 nm and a light path of 1 cm or longer.		
Materials and Reagents	• Nitrate free water • Copper cadmium granules • Sulphanilamide reagent • N-(1-naphthyl)-ethylenediamine dihydrochloride (NED dihydrochloride) solution • Ammonium chloride – EDTA solution • Hydrochloric acid 6 N • Copper suphate solution • Stock nitrate solution • Stock nitrite solution		
Preparation of Reagents	• **Nitrate free water-** the absorbance of a reagent blank prepared with this water should not exceed 0.01. Use for all solutions and dilution.		

	• **Copper cadmium granules –** Wash 25 gm of 40-60 mesh cadmium granules with 6N hydrochloric acid and rinse with water. Swirl cadmium with 100 mL of 2 percent copper sulphate solution for 5 minutes or until blue color partially fades. Decant, repeat with fresh copper sulphate until a brown colloidal precipitate develops. Wash copper cadmium copiously with water (at least 10 times) to remove all precipitated copper. • **Sulphanilamide reagent –** Dissolve 5 gm of sulphanilamide in a mixture of 50 mL concentrated hydrochloric acid and 300 mL of water. Dilute to 500 mL with water. The reagent is stable for months. • **N-(1-naphthyl)-ethylenediamine dihydrochloride (NED dihydrochloride) solution-** Dissolve 500 mg of NED dihydrochloride in 500mL of water. Store in dark colored bottle. Replace as soon as brown color develops. • **Ammonium chloride – EDTA solution-** Dissolve 13 gm ammonium chloride 1.7 gm of disodium ethylenediamine tetracetate in 900 mL of water. Adjust pH to 8.5 with liquid ammonia and dilute to 1 liter. • Dilute 300 mL of the above solution to 500 mL with water to get a dilute solution. • **Hydrochloric acid 6 N** • **Copper suphate solution-** 2 percent (m/v). • **Stock nitrate solution** – Dissolve 0.7218 gm of dry potassium nitrate in water and dilute to 1000 mL. Preserve with 2 mL of chloroform per liter (1 mL = 100µg of nitrate nitrogen). • Dilute 50 mL of stock nitrate solution to 500 mL with water to get standard solution. 1.0 mL equal to 10.0 µg nitrate nitrogen. • **Stock nitrite solution**- Dissolve 0.6072 of dried potassium nitrite in nitrate free water and make up to 1000 mL. (1 mL = 100µg of nitrite nitrogen). Preserve with 2 mL of chloroform and keep in a refrigerator. The solution is stable for 3months. • Dilute 50.0 mL of above stock nitrite solution to 500 mL with nitrite free water (1 mL = 10µg of nitrite nitrogen).
Sample Preparation	If turbidity or suspended solids are present, remove by filtering through a 0.45 µm pore diameter membrane or glass fiber filter. Adjust pH to between 7 & 9 as necessary. To 25.0 mL sample or a portion diluted to 25.0 mL add 75 mL of ammonium chloride- EDTA solution and mix. Pour mixed sample into column and collect at the rate of 7 to 10 mL/minute. Discard first 25 mL. Collect the rest in original sample flask. There is no need to wash the column between samples but if columns are not to be reused for several hours or longer, pour 50 mL dilute ammonium chloride - EDTA solution on to the top and let it pass through the system. Store Cu-Cd column in this

	solution and never allow it to dry. As soon as possible and not more than 15 min after reduction add 2.0 mL sulphanilamide reagent to 50 mL of sample. Let the reagent react for 2 to 8 min. add 2 mL of NED dihydrochloric acid solution and mix immediately. Measure absorbance between 10 min to 2 hr at 540 nm against a distilled water reagent blank. Using the standard nitrate nitrogen solution prepare standards in the range of 0.05 to 1.0 mg of nitrate nitrogen per liter by diluting the following volumes of standards to 100 mL in volumetric flasks: 0.5, 1.0, 2.0, 5.0 and 10.0 mL. Carry out reduction of standards exactly as described for samples. Compare at least one nitrite standard to a reduced nitrate standard at the same concentration to verify reduction column efficiency. Reactivate copper cadmium granules when reduction efficiency falls below 75 percent.
Method of analysis	**Preparation of reduction column** – insert a glass wool plug into the bottom of the reduction column and fill with water. Add sufficient copper cadmium granules to produce a column 18.5 cm long. Maintain water level above Cu-Cd granules to prevent entrapment of air. Wash column with 200 mL dilute ammonium chloride EDTA solution. Activate column by passing, 100 mL of a solution comprising of 25 mL of 1.0 mg nitrogen (nitrate) per liter standard and 75 mL of ammonium chloride EDTA solution, through it, at 7 to 10 mL/ minute.
Calculation with units of expression	Obtain a standard curve by plotting absorbance at standards against nitrate nitrogen concentration, compute sample concentration directly from standard curve report as milligrams of oxidized nitrogen per liter(sum of nitrate nitrogen plus nitrite nitrogen) unless the concentration of nitrite nitrogen is separately determined and corrected for .
Inference (Qualitative Analysis)	Higher concentrations of copper, iron etc. lower the reduction efficiency. Add EDTA to remove this interference. Oil and grease & residual chlorine can interfere. Remove oil and grease by extraction with organic solvents and residual chlorine by adding sodium thiosulphate.
Reference	APHA 4500-B
Approved by	Scientific Panel on Methods of Sampling and Analysis

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Method No.	**FSSAI 14.016:2024**	**Revision No. & Date**	0.0
Scope	This method is suitable for concentration below 0.1 mg per liter of nitrate nitrogen.		
Caution	Residual chlorine, certain oxidants and nitrites yield yellow colour with chromotropic acid. Addition of sulphite removes interference from residual chlorine and oxidants. Urea coverts nitrites to nitrogen gas. The minimum detectable quantity is 50 µg of nitrate nitrogen per litre.		
Principle	Two moles of nitrate nitrogen react with one mole of chromotropic acid to form a yellow reaction product having maximum absorbance at 410 nm.		
Apparatus/Instruments	Spectrophotometer- for use of 410 nm and with a light path of 1 cm or longer. Photometer- having maximum transmittance at 410 nm and having a light path of 1 cm or longer and equipped with violet filter.		
Materials and Reagents	Nitrate free water Stock nitrate solution Standard nitrate solution Antimony reagent Chromotropic acid reagent Sulphuric acid		
Preparation of Reagents	1. Nitrate free water- The absorbance of a reagent blank prepared with this water should not exceed 0.01. Use for all solution and dilution. 2. Stock nitrate solution – Dissolve 0.7218 gm of dry potassium nitrate in water and dilute to 1000 mL preserve with 2 mL of chloroform per liter (1 mL = 100µg of nitrate nitrogen). 3. Standard nitrate solution- Dilute 50 mL of stock nitrate solution to 500 mL with water to get standard solution 100 mL equal to 10.0 µg nitrate nitrogen. Sulphite urea reagent- Dissolve 5 gm of urea and 4 gm of anhydrous sodium sulphite in water and dilute to 1000 mL. 4. Antimony reagent- Dissolve 500 mg antimony metal by heating in 80 mL concentrated sulphuric acid. Cool and cautiously add to 20 mL of iced water. If crystals from upon standing overnight redissolve by heating 5. Chromotropic acid reagent- Dissolve 100 mg of purified chromotropic acid crystals in 100 mL of concentrated sulphuric acid and store in a brown bottle. Prepare every 2 weeks. A colorless reagent solution signifies the absence of nitrate contamination from sulphuric acid. 6. Sulphuric acid- concentrate nitrate free.		
Sample Preparation	If appreciable amount of suspended matter is present, filter suitably. Pipette 2.0 mL portions of the standard nitrate solutions samples and a water blank into dry 10 mL volumetric flasks.		
Method of analysis	Prepare nitrate standards in the range of 0.10 to 5.0 mg/L by diluting 0, 1.0,		

	5.0 10, 25, 25, 40 and 50 mL of standard nitrate solution to 100 mL with water. If appreciable amount of suspended matter is present, filter suitably. Pipette 2.0 mL portions of the standard nitrate solutions samples and a water blank into dry 10 mL volumetric flasks. To each flask, add 1 drop of sulphite urea reagent. Place flask in tray of cold water (10 to 20°C) and add 2 mL of antimony reagent. Swirl flasks during addition of each reagent. After about 4 minutes in the bath, add 1 mL of chromotropic acid reagent, swirl and let stand in cooling bath for 3 minutes. Add concentrated sulphuric acid to bring volume near the 10 mL mark. Stopper the flasks and mix by inverting each flask four times. Let it stand for 45 minutes at room temperature and adjust volume to 10 mL with concentrated sulphuric acid. Perform final mixing very carefully and gently to avoid introducing gas bubbles. Read absorbance at 410 nm between 15 minutes and 24 hours after last volume adjustment. Use nitrate free water in the reference cell of the spectrophotometer.
Calculation with units of expression	Nitrate nitrogen (as NO3), mg/L= µg of nitrate nitrogen in 10 mL final volume Volume in ml of sample taken for test
Inference (Qualitative Analysis)	Residual chlorine, certain oxidants and nitrites yield yellow colour with chromotropic acid. Addition of sulphite removes interference from residual chlorine and oxidants. Urea coverts nitrites to nitrogen gas. The minimum detectable quantity is 50 µg of nitrate nitrogen per litre.
Reference	IS 3025 (part 34) 1998: (Reaffirmed 2003) - Methods of Sampling and Test (Physical and chemical) for water and Waste Water: Nitrogen.
Approved by	Scientific Panel on Methods of Sampling and Analysis

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Method No.	**FSSAI 14.017:2024**	**Revision No. & Date**	0.0
Scope	This method comprises of • Nesslerization Method • Titrimetric Method The above two represent the ammonia produced from reduction of nitrate and nitrite. To get nitrate nitrogen determine nitrite separately and subtract.		
Caution	Ammonia is to be removed from sample by preliminary distillation. Nitrite also gets reduced to ammonia by this method. Therefore, a separate determination is made for nitrite and subtracts the result. This method is not recommended for levels of nitrate nitrogen below 2 mg/L.		
Principle	The nitrate and nitrite is reduced to ammonia under hot alkaline conditions in the presence of the reducing agent (Devarda's Alloy). The ammonia formed distils and is trapped in a receiving flask containing boric acid. The ammonia can be determined either by direct nesslerization or acidimetrically. This method is recommended for nitrate nitrogen and nitrite nitrogen		
Apparatus/Instruments	1. Distillation assembly- Kjeldahl assembly is suitable 2. Measuring scoop- to contain 1 gm of Devarda's alloy 3. Spectrophotometer or photometer- suitable for use at 400-425 nm. The photometer should be equipped with a blue filter.		
Materials and Reagents	• Ammonia free water • Borate buffer solution • Sodium hydroxide • Devarda's alloy		
Preparation of Reagents	• Ammonia free water • Borate buffer solution – add 88 mL of 0.1 N sodium hydroxide to 500 mL of 0.025 M sodium tetra borate (50 gm Na2B1O7 or 9.5 gm Na2B1O7.H2O) and make up to 1litre • Sodium hydroxide- 6 N • Devarda's alloy (An alloy of 50 percent Cu, 45 percent Al and 5 percent Zn)- 20 mesh or smaller containing less than 0.005 percent nitrogen • Mixed indicator solution – Dissolve 200 mg of methyl red indicator in 100 mL 95%ethyl or isopropyl alcohol. Dissolve 100 mg of methylene blue in 50 mL of 95 % ethyl or isopropyl alcohol. Combine these two. Prepare monthly • Indicating boric acid solution - dissolve 20 gm hydroboric acid in ammonia free water, add 10 mL of mixed indicator solution and dilute to 1 liter • Standard sulphuric acid titrant – 0.02 N (1mL=280 µg of nitrogen)		

	• Nessler's reagent- Dissolve 100 gm of mercuric iodide and 70 gm of potassium iodide in a small quantity of water and add this mixture slowly with stirring to a cool solution of 160 gm of sodium hydroxide dissolved in 500 mL of water. Dilute to 1 liter. Store in brown rubber stopper glass bottle. Reagent is stable up to one year. It is toxic and so avoid ingestion. • Stock ammonia solution- Dissolve 3.819 gm of anhydrous ammonium chloride in water and dilute to 1 liter (1.00 mL = 1.00 mg of nitrogen = 1.22 mg of ammonia). 4.6.3 Standard ammonia solution- Dilute 10.00 mL of stock solution to 1000 mL with water (1.00 mL = 12.2 µg of ammonia = 10.0 µg of N).
Sample Preparation	If appreciable amount of suspended matter is present, filter suitably .
Method of analysis	-- If ammonia has not been determined by a method involving preliminary distillation dilute a portion of the sample to 500 mL with ammonia free water. Add 25 mL of borate buffer and adjust to pH 9. 5 with 6 N sodium hydroxide using a pH meter or short-range pH paper. Distil 250 to 300 mL into a dry receiving flask and discard. Make sure that the last part of the distillation is conducted with condenser tip out of the liquid in receiving flask. To the residue after removing ammonia, add 1 gm of Devarda's alloy and sufficient ammonia- free distilled water to bring total volume to 350 mL. Place in a receiving flask of 50 mL boric acid absorbent for each milligram of nitrate nitrogen in sample. Immerse the end of condenser in the absorbent. Heat distillation flask until boiling or vigorous bubbling occurs. Reduce heat and distil at the rate of 5 to 10 mL/min until at least 150 mL distillate have been collected. Lower receiver so that liquid is below the end of the condenser and continue distillation for 1 to 2 minutes to cleanse condenser. Determine ammoniacal nitrogen either by nesslerization or titration with standard strong acid as given in Nesslerization or Titrimetric Method.
Calculation with units of expression	Nesslerization Method Ammoniacal Nitrogen (NH3 - N), mg/L = (A X B)/ (V X C) Where A = µg of ammoniacal nitrogen (51 mL of final volume); B = total volume of distillate collected, in mL, including acid absorbent; C = volume distillate taken for nesslerization in mL, and V = volume in mL of sample taken for test. Titrimetric Method Ammoniacal nitrogen (NH3 - N), mg/L = (A- B) X 280/V

	Where A = volume in mL of sulphuric acid titrated for sample B = volume in mL of sulphuric acid titrated for blank, and V = volume in mL of sample taken for test. The above two represent the ammonia produced from reduction of nitrate and nitrite. To get nitrate nitrogen determine nitrite separately and subtract.
Inference (Qualitative Analysis)	Ammonia is to be removed from sample by preliminary distillation. Nitrite also gets reduced to ammonia by this method. Therefore, a separate determination is made for nitrite and subtracts the result. This method is not recommended for levels of nitrate nitrogen below 2 mg/L.
Reference	• IS 3025 (part 34) 1998: (Reaffirmed 2003) - Methods of Sampling and Test (Physical and chemical) for water and Waste Water: Nitrogen. • APHA 4500 NO3
Approved by	Scientific Panel on Methods of Sampling and Analysis

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Method No.	**FSSAI 14.018:2024**	**Revision No. & Date**	0.0
Scope	The presence of chloride in natural waters can be attributed to dissolution of salt deposits, discharges of effluents from chemical industries, oil well operations and seawater intrusion in coastal areas. Each of these sources may result in local contamination of both surface water and groundwater. The salty taste produces by chloride depends on the chemical compositions of the water. A concentration of 250 mg/L may be detected in some waters containing sodium ions. On the other hand, the typical salty taste may be absent in water containing 1000mg/L chloride when calcium and magnesium ions are predominant. High chloride content may harm pipes and structures as well as agricultural plants. This method prescribes the determination of chloride. This method is suitable for use in relatively clear waters when 0.15 to 10mg of chloride is present in the portion titrated.		
Caution	Bromide, iodide and cyanide register equivalent chloride concentrations. Sulphite, thiosulphate and sulphide ions interfere but can be removed by treatment with hydrogen peroxide. Orthophosphates in excess of 25mg/L interfere. Iron in excess of 10mg/l interferes by masking the end point.		
Principle	In a neutral or slightly alkaline solution, potassium chromate can indicate the end point of the silver nitrate titration of chloride. Silver chloride is precipitated before red silver chromate is formed.		
Apparatus/Instruments	1. Erlenmeyer flask — 250 mL. 2. Burette — 50 mL.		
Materials and Reagents	1. Potassium chromate 2. silver nitrate 3. sodium chloride 4. Aluminium hydroxide 5. Phenolphthalein indicator 6. Sodium hydroxide 7. Sulphuric acid 8. Hydrogen peroxide		
Preparation of Reagents	**1. Potassium chromate indicator solution —** Dissolve 50 gm of potassium chromate in a little distilled water. Add silver nitrate solution until a definite red precipitate is formed. Let it stand for 12 hr, filter and dilute to 1 liter with distilled water. **2. Standard silver nitrate titrant —**0.0141 N. Dissolve 2.395 gm of silver nitrate in distilled water and dilute to 1 liter. Standardize against		

	0.0141N sodium chloride solution. 1.00 mL = 500 µg of chloride. Store in a brown bottle. **3. Standard sodium chloride solution —** 0.0141 N. Dissolve 824.0 mg of sodium chloride (dried at 140°C) in distilled water and dilute to 1 liter. 1 mL = 500 µg ofchloride. **4. Aluminium hydroxide suspension —** Dissolve 1.25 gm of aluminum potassium sulphate or aluminium ammonium sulphate [AlK $(SO4)2$ $.12H2O$ or Al $NH4$ $(SO4)2.12H2O$] in 1 liter of distilled water. Warm to 60°C and add 55 mL of concentrated ammonium hydroxide slowly with stirring. Let it stand for 1 hr, transfer to a large bottleand wash precipitate by successive additions, with thorough mixing and decanting with distilled water, until free from chloride. When freshly prepared, the suspension occupies a volume of about 1 liter. **5.** Phenolphthalein indicator solution **6.** Sodium hydroxide- 1 N **7.** Sulphuric acid -1N **8.** Hydrogen peroxide - 30 percent
Sample Preparation	Use 100 mL sample or a suitable portion diluted to 100 mL. If the sample is highly colored, add 3 mL of aluminium hydroxide suspension, mix, let settle and filter.
Method of analysis	1. If sulphide, sulphite or thiosulphate is present, add 1 mL of hydrogen peroxide and stir for 1 minute. Directly titrate the samples in the pH range 7 to 1 0. 2. Adjust sample pH to 7-10 with sulphuric acid or sodium hydroxide if it is not in the range. 3. Add 1.0 mL of potassium chromate indicator solution. 4. Titrate with standard silver nitrate solution to a pinkish yellow end point. Standardize silver nitrate solution and establish reagent blank value by titration method.
Calculation with units of expression	Chloride, mg/L = $\frac{(\upsilon 1 - \upsilon 1) \times N \times 35450}{V3}$ Where V1 = Volume in mL of silver nitrate used by the sample V2 = Volume in mL of Silver nitrate used in the blank titration V3= volume in mL of sample taken for titration N= Normality of silver nitrate solution
Interference	Bromide, iodide and cyanide register equivalent chloride concentrations. Sulphite, thiosulphate and sulphide ions interfere but can be removed by treatment with hydrogen peroxide. Orthophosphates in excess of 25mg/L interfere. Iron in excess of 10mg/l interferes by masking the end point.

Reference	• IS : 3025 (Part 32) – 1988 (Reaffirmed 2003)- Methods of Sampling and Test (Physical and chemical) for water and Waste Water : Chloride • APHA 4500-Cl
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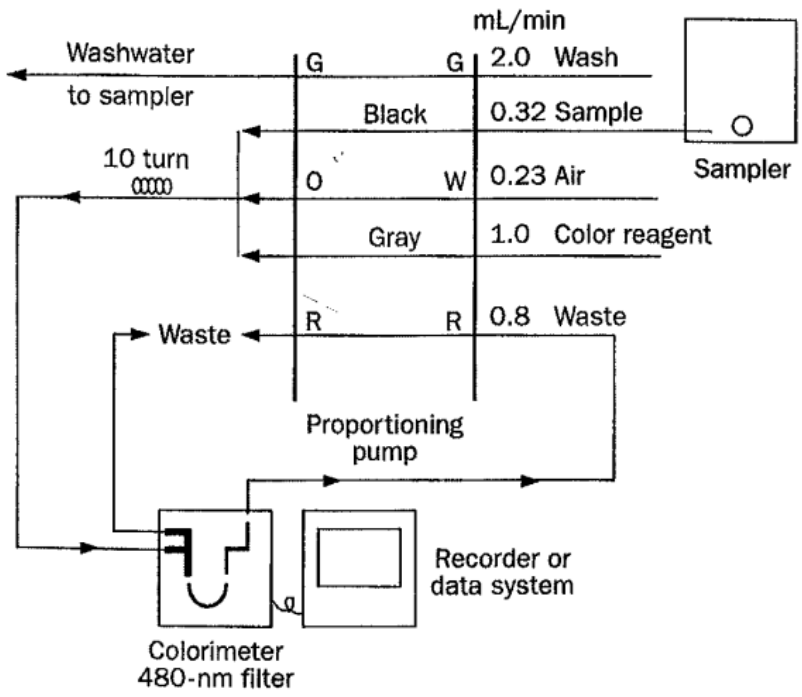
Method No.	**FSSAI 14.019:2024**	**Revision No. & Date**	0.0
Scope	This method prescribes the determination of chloride. This method is suitable for use in relatively clear waters.		
Caution	Iodide and bromide also are titrated as chloride. Ferricyanide causes high results and must be removed. Chromate and dichromate interfere and should be reduced to the chromic state or removed. Ferric iron interferes if present in an amount substantially higher than the amount of chloride. Chromic ion, ferrous ion, and phosphate do not interfere. Grossly contaminated samples usually require pretreatment where contamination is minor, some contaminants can be destroyed simply by adding nitric acid.		
Principle	Chloride is determined by potentiometric titration silver nitrate solution with a glass and silver-silver .chloride electrode system. During titration an electronic voltmeter used to detect the change in potential between the two electrodes. The end point of the titration is that instrument reading at which the greatest change in voltage has occurred for a small and constant increment of silver nitrate added.		
Apparatus/Instruments	1. Glass and silver-silver chloride electrodes: Prepare in the laboratory or purchase a silver electrode coated with AgCl for use with specified instruments. Instructions on use and care of electrodes are supplied by the manufacturer. 2. Electronic voltmeter, to measure potential difference between electrodes: A pH meter may be converted to this use by substituting the appropriate electrode. 3. Mechanical stirrer, with plastic-coated or glass impeller.		
Materials and Reagents	Sodium chloride Nitric acid, HNO_3 Sulfuric acid H_2SO_4 Hydrogen peroxide, H_2O_2, 30 % Sodium hydroxide		
Preparation of Reagents	**1.1** Standard Sodium chloride solution, 0.0141M (0.0141N): Dissolve 824.0 mg NaCl (dried at 140°C) in distilled water and dilute to 100 mL: 1.00 mL= 500 µg Cl^- **1.2** Nitric acid, HNO_3, conc. **1.3** Standard silver nitrate titrant, 0.0141 (0.0141 N): Dissolve 2.395 gm $AgNO_3$ in distilled water and dilute to 1000 mL. Standardize against NaCl by the procedure; 1.00 mL= 500 µg Cl^-		

	1.4 Pretreatment reagents 1.4.1 Sulfuric acid H_2SO_4, 1+1 1.4.2 Hydrogen peroxide, H_2O_2, 30 % 1.4.3 Sodium hydroxide NaOH, 1N.
Sample Preparation	Pipet 100.0 mL sample or a portion containing not more than 10 mg Cl, into a 250mL beaker. In the absence of interfering substances, proceed with above. In the presence of organic compounds, sulfite, or other interferences (such as large amounts of ferric iron, cyanide, or sulfide) acidify sample with H_2SO_4, using litmus paper. Boil for 5 min to remove volatile compounds. Add more H_2SO_4, if necessary, to keep solution acidic. Add 3 mL H_2O_2 and boil for 15 min, adding chloride-free distilled water to keep the volume above 50 mL. Dilute to 100 mL, add NaOH solution dropwise until alkaline to litmus, then 10 drops in excess. Boil for 5 min, filter into a 250mL beaker, and wash precipitate and paper several times with hot distilled water. Add conc. HNO_3 drop wise until acidic to litmus paper, then 2.0 mL in excess. Cool and dilute to 100 mL if necessary. Immerse stirrer and electrodes and start stirrer. Make any necessary adjustments according to the manufacturer's instructions and set selector switch to appropriate setting for measuring the difference of potential between electrodes. Complete determination by titrating according to 4.1above. If an end-point reading has been established from previous determination for similar samples and conditions, use this predetermined end point. For the most accurate work, make a blank titration by carrying chloride-free distilled water through the procedure.
Method of analysis	**1.1** Standardization: The various instruments that can be used in this determination differ in operating details; follow the manufacturer's instructions. Make necessary mechanical adjustments. Then, after allowing sufficient time for warm-up (10 min), balance internal electrical components to give an instrument setting of 0 mV or, if a pH meter is used, a pH reading

	of 7.0. 1.1.1 Place 10.0 mL standard NaCl solution in a 250mL beaker, dilutes to about 100 mL, and adds 2.0 mL conc HNO3. Immerse stirrer and electrodes. 1.1.2 Set instrument to desired range of mill volts or pH units Start stirrer 1.1.3 Add standard AgN03 titrant, recording scale reading after each addition. At the start, large increments of AgNO3 may be added; then, as the end point is approached, add smaller and equal increments (0.1 or 0.2 mL) at longer intervals, so the exact end point can be determined. Determine volume of AgNO3 used at the point at which there is the greatest change in instrument reading per unit addition of AgNO3· 1.1.4 Plot a differential titration curve if the exact endpoint cannot be determined by inspecting the data. Plot change in instrument reading for equal increments of AgNO3 against volume of AgNO3 added, using average of burette readings before and after each addition.
Calculation with units of expression	$$\text{mg Cl/L} = \frac{(A - B) \times N \times 35450}{\text{mL sample}}$$ Where A = mL AgNO3, B = mL blank, and N = normality of titrant
Interference (Qualitative Analysis)	Iodide and bromide also are titrated as chloride. Ferricyanide causes high results and must be removed. Chromate and dichromate interfere and should be reduced to the chromic state or removed. Ferric iron interferes if present inan amount substantially higher than the amount of chloride. Chromic ion, ferrous ion, and phosphate do not interfere. Grossly contaminated samples usually require pretreatment where contamination is minor, some contaminants can be destroyed simply by adding nitric acid.
Reference	• IS : 3025 (Part 32) – 1988 (Reaffirmed 2003)- Methods of Sampling and Test (Physical and chemical) for water and Waste Water : Chloride • APHA 4500-Cl
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	**Determination of Chloride by Automated Ferricyanide Method**		
Method No.	**FSSAI 14.020:2024**	**Revision No. & Date**	0.0
Scope	The method is applicable to potable, surface, and saline waters, and domestic and industrial wastewaters. The concentration range is 1 to 200 mg Cl/L; it can be extended by dilution.		
Caution	Remove particulate matter by filtration-or centrifugation before analysis. Guard against contamination from reagents, water, glassware, and sample preservation process. No chemical interferences are significant.		
Principle	Thiocyanate ion is liberated from mercuric thiocyanate by the formation of soluble mercuric chloride. In the presence of ferric ion, free thiocyanate ion forms a highly colored ferric thiocyanate, of which the intensity is proportional to the chloride concentration.		
Apparatus/Instruments	Automated analytical equipment: An example of the continuous-flow analytical instrument consists of the interchangeable components shown in the figure .		
Materials and Reagents	1. mercuric thiocyanate 2. ferric nitrate 3. Color reagent 4. Stock chloride solution 5. Standard chloride solutions		
Preparation of Reagents	1.1 Stock mercuric thiocyanate solution: Dissolve 4.17 gm $Hg(SCN)_2$ in about 500 mL methanol, dilute to 1000 mL with methanol, mix, and filter through filter paper. 1.2 Stock ferric nitrate solution: Dissolve 202 gm $Fe(NO_3)_3 \cdot 9H_2O$ in about 500mL distilled water, then carefully add 21 mL conc HNO_3. Dilute to 1000mL with distilled water and mix. Filter through paper and store in an amber bottle. 1.3 Color reagent: Add 150 mL stock $Hg(SCN)_2$ solution to 150 mL stock $Fe(NO_3)_3$ solution. Mix and dilute to 1000 mL with distilled water. Add 0.5mL polyoxyethylene 23 lauryl ether. 1.4 Stock chloride solution: Dissolve 1.6482 gm NaCl, dried at 140°C, in distilled water and dilute to 1000 mL; 1.00 mL = 1.00 mg Cl. 1.5 Standard chloride solutions: Prepare chloride standards in the desired concentration range, such as 1 to 200 mg/L, using stock chloride solution.		
Sample Preparation	Set up manifold and follow general procedure described by the manufacturer.		
Method of analysis	Set up manifold and follow general procedure described by the manufacturer.		

	 **Fig**: Flow scheme for automated chloride analysis.
Calculation with units of expression	Prepare standard curves by plotting response of standards processed through the manifold against chloride concentrations in standards. Compute sample chloride concentration by comparing sample response with standard curve.
Interference	Remove particulate matter by filtration-or centrifugation before analysis. Guard against contamination from reagents, water, glassware, and sample preservation process. No chemical interferences are significant.
Reference	• IS : 3025 (Part 32) – 1988 (Reaffirmed 2003)- Methods of Sampling and Test (Physical and chemical) for water and Waste Water : Chloride • APHA 4500-Cl
Approved by	Scientific Panel on Methods of Sampling and Analysis

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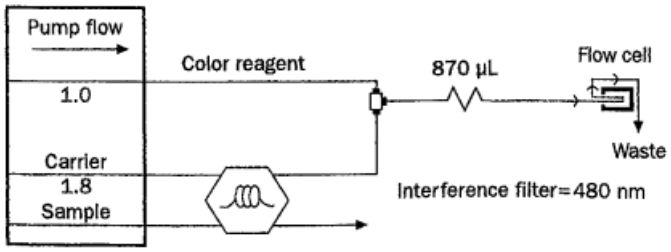
Method No.	**FSSAI 14.021:2024**	**Revision No. & Date**	0.0
Scope	Determination of Chloride in water by Mercuric Thiocyanate Flow Injection Analysis method		
Caution	Mercuric thiocyanate is toxic. Wear gloves!		
Principle	A water sample containing chloride is injected into a carrier stream to which mercuric thiocyanate and ferric nitrate are added. The chloride complexes with the Hg(ll), displacing the thiocyanate anion, which forms the highly colored ferric thiocyanate complex anion. The resulting peaks absorbance is measured at 480 nm. The peak area is proportional to the concentration of chloride in the original sample		
Apparatus/Instruments	a. Flow injection analysis equipment consisting of b. FIA injection valve with sample loop. c. Multichannel proportioning pump. d. FIA manifold with flow cell. e. Absorbance detector, 480 nm, 10nm band pass. f. Valve control and data acquisition system.		
Materials and Reagents	a. Water b. Mercuric thiocyanate c. Ferric Nitrate d. chloride standard		
Preparation of Reagents	**a.** Use reagent water (> 10 mega ohm) to prepare carrier and all solutions. **b.** Stock mercuric thiocyanate solution: In a 1L volumetric flask, dissolve 4.17gm mercuric thiocyanate, Hg(SCN)2, in about 500 mL methanol. Dilute to mark with methanol and mix. **c.** Stock ferric nitrate reagent, 0.5M: In a 1L volumetric flask, dissolve 202 gm ferric nitrate, Fe(NO3)3 .9H2O, in approximately 800 mL water. Add 25 mL conc. HNO3 and dilute to mark. Invert to mix. **d.** Color reagent: In a 500mL volumetric flask, mix 75 mL stock mercuric thiocyanate solution with 75 mL stock ferric nitrate reagent and dilute to mark with water. Invert to mix. Vacuum filter through a 0.45 µm membrane filter. The color reagent also is available as a commercially prepared solution that is stable for several months. **e.** Stock chloride standard, 1000 mg Cl/L: In a 105°C oven, dry 3 gm primary standard grade sodium chloride, NaCI, overnight. In a 1L volumetric flask, dissolve1.648 gm primary standard grade sodium chloride in about 500 mL water. Dilute to mark and invert to mix. **f.** Standard chloride solutions: Prepare chloride standards for the calibration		

	curve in the desired concentration range, using the stock standard (as above), and diluting with water
Sample Preparation	Set up a manifold and follow method supplied by manufacturer, or laboratory standard operating procedure for this method.
Method of analysis	Set up a manifold equivalent to that in Figure and follow the method supplied by the manufacturer or laboratory standard operating procedure for this method. 
Calculation with units of expression	Prepare standard curves by plotting absorbance of standards processed through the manifold versus chloride concentration. The calibration curve gives a good fit to a second-order polynomial.
Interference (Qualitative Analysis)	Remove large or fibrous particulates by filtering sample through glass wool. Guard against contamination from reagents water, glassware, and the sample preservation process. Substances such as sulfite and thiosulfate, which reduce iron (lll) to iron (ll) and mercury (II) to mercury (I), can interfere. Halides, which also form strong complexes with mercuric ion (e.g., Br^-, I^-), give a positive interference.
Reference	• IS : 3025 (Part 32) – 1988 (Reaffirmed 2003)- Methods of Sampling and Test (Physical and chemical) for water and Waste Water : Chloride • APHA 4500-Cl
Approved by	Scientific Panel on Methods of Sampling and Analysis

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Method No.	**FSSAI 14.022:2024**	**Revision No. & Date**	0.0
Scope	Magnesium occurs commonly in the minerals magnetite and dolomite. Magnesium is used in alloys, pyrotechnics, flash photography, drying agents, refractories, fertilizers, pharmaceuticals, and foods. The common aqueous species is Mg2+ The carbonate equilibrium reactions from magnesium are more complicated than for calcium and conditions for direct precipitation of dolomite in natural waters are not common. Important contributors to the hardness of a water, magnesium salts break down when heated, forming scale in boilers. Chemical softening, reverse osmosis, ion exchange reduces magnesium and associated hardness to acceptable levels. Magnesium is an essential element in chlorophyll and in red blood cells. Some salts of magnesium are toxic by ingestion or inhalation. Concentrations greater than 125 mg/L also can have a cathartic and diuretic effect. The following methods are suitable for determination of Magnesium in water.		
Caution	The solution should be reasonably free from aluminum, calcium, iron, manganese, silica, strontium and suspended matter. It should not contain more than about 3.5 gm ammonium chloride		
Principle	**Calculation Method:** Water sample containing Calcium as $CaCO_3$ is estimated and Total Hardness is the sum of $CaCO_3$ and $MgCO_3$. Magnesium hardness is determined by subtracting Calcium hardness from total hardness. **Gravimetric Method**: Diammonium hydrogen phosphate quantitatively precipitates magnesium in ammoniacal solution as magnesium ammonium phosphate. The precipitate isignited and weighed as magnesium pyrophosphate. Below 1 mg/L atomic absorption Spectrophotometric method is desirable.		
Apparatus/Instruments	**Gravimetric Method** Vacuum Pump or Other Source of vacuum Filter Flasks Filter Crucibles – medium porosity; 30 Ml		
Materials and Reagents	**Gravimetric Method** 1. Methyl Red Indicator 2. Hydrochloric acid 3. Ammonium Oxalate 4. Ammonium Hydroxide 5. Nitric Acid- Concentrated 6. Diammonium hydrogen phosphate 7. Urea		

Preparation of Reagents	**Gravimetric Method** 1. Methyl Red Indicator Solution: Dissolve 100 mg of methyl red sodium salt in distilled water and dilute to 100 Ml 2. Hydrochloric acid: 1:1, 1:9 and 1:99 3. Ammonium Oxalate Solution: Dissolve 10gm (NH4)2 C2O4 + H2O in 250mL distilled water. Filter if necessary 4. Ammonium Hydroxide- Concentrated- 1:19 5. Nitric Acid- Concentrated 6. Diammonium hydrogen phosphate solution Dissolve 30gm of Diammonium pyrophosphate (NH4)2 HPO4 in distilled water and make up to 100 mL. 7. Urea.
Sample Preparation	**Gravimetric Method** • **Pretreatment of Polluted Water and Wastewater Samples:** Mix the sample pretreated, if so required, and transfer a suitable volume (50 to 100 mL) to 250 mL conical flask or a beaker. Add 5 mL concentrated nitric acid and a few boiling chips or glass beads. Bring to a slow boil and evaporate on a hot plate to the lowest volume possible (about 10 to 20 mL) before precipitation or salting occurs. Add 5 mL concentrated nitric acid cover with a watch glass and heat to obtain a gentle refluxing action. Continue heating and adding concentrated nitric acid as necessary until digestion is complete as shown by a light coloured clear solution. Do not let sample dry during digestion. Add 1 to 2 mL concentrated nitric acid and warm slightly to dissolve any remaining residue. Wash down beaker walls and watch glass with water and then filter if necessary . Transfer filtrate to 100 mL volumetric flask with two 5 mL portions of water adding these risings to the volumetric flask. Cool dilute to mark and mix thoroughly. Take portions of this solution for the determination. • **Removal of calcium and other Metals as Oxalates:** To 200 mL of the sample pretreated if so required containing about 50 mg of calcium, add a few drops of methyl red indicator and 1:1 hydrochloric acid. Sufficient acid must be present in the solution WATER ANALYSIS 2016 89 to prevent the precipitation of calcium oxalate when ammonium oxalate solution is added. Introduce 50 mL of ammonium oxalate solution and 15 gm of urea. Boil the solution gently until the methyl red changes its colour to yellow. Filter the precipitate and wash with small volume of cold water until free from chloride.

Method of analysis	To the combined filtrate and washings from 5.2 containing not more than 60 mg magnesium add 50 mL of concentrated nitric acid and evaporate carefully to dryness on a hot plate. Do not let reaction become too violent during the later part of the evaporation stay in constant attendance to avoid losses through spattering. Moisten residues with 2 to 3 mL of concentrated hydrochloric acid, add, 20 mL of distilled water, warm, filter and wash. To the filtrate add 3 mL of concentrated hydrochloric acid 2 to 3 drops of methyl red solution, and 10 mL of (NH4)2HPO4 solution. Cool and add concentrated ammonium hydroxide drop by drop stirring constantly until color changes to yellow, stir for 5minutes and add again 5mL of concentrated ammonium hydroxide and stir vigorously for 10 min more. Let it stand overnight and filter through filter paper. Wash with 1:19 ammonium hydroxide. Transfer to an ignited, cooled and weighed crucible. Dry precipitate thoroughly and burn paper off slowly, allowing circulation of air. Heat at about 500°C until residue is white. Ignite for 30 minutes at 1100°C to constant mass.
Calculation with units of expression	**Calculation Method:** 1. **Calculate the Total hardness as follows**: Analysis for Total Hardness is carried out at pH 10 using Erichrome Black T indicator. Total hardness (CaCO3) mg/L = [1000(V1 –V2)/V3] x CF Where V1 = volume in mL of the EDTA standard solution used in the titration for the sample V2 = volume in mL of the EDTA solution used in the titration for blank. V3 = volume in mL of the sample taken for the test CF = X1/X2 correction factor for standardize ion of EDTA. X1 = volume in mL of standard calcium solution taken for standardization X2 = volume of mL of EDTA solution used in the titration. 2. **Calculate the Calcium hardness as follows**: Analysis for Calcium is carried out at pH 12-14 using Patton & Reader Indicator. Calcium hardness (CaCO3) mg/L = [1000(V1 – V2)/V3] x CF Where V1 = volume in mL of the EDTA standard solution used in the titration for the sample V2 = volume in mL of the EDTA solution used in the titration for blank. V3 = volume in mL of the sample taken for the test CF = X1/X2 correction factor for standardize ion of EDTA. X1 = volume in mL of standard calcium solution taken for standardization and X2 = volume of mL of EDTA solution used in the titration. **Calculate the Magnesium hardness as follows**

	Magnesium hardness = Total hardness - Calcium hardness (mg/L) Magnesium (as Mg+2) = Magnesium hardness * 0.2428 mg/L **Gravimetric Method:** Calculation Magnesium, mg/L= $\frac{M \times 218.4 \times 103}{V}$ Where M= mass in mg of magnesium pyrophosphate, and V= volume in mL of sample.
Inference (Qualitative Analysis)	NA
Reference	IS : 3025 (Part 46) – 1994 Methods of Sampling and Test (Physical and chemical) for water and Waste Water
Approved by	Scientific Panel on Methods of Sampling and Analysis

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Method No.	**FSSAI 14.023:2024**	**Revision No. & Date**	0.0
Scope	Mineral water, Packaged Drinking Water (other than Mineral Water), Drinking Water (Purified)		
Caution	Volumetric measurement of sample and reagent is extremely important to analytical accuracy. Use samples and standards at the same temperature or at least within 2 ºC. Maintain constant temperature throughout the color development period. Prepare different calibration curves for different temperature ranges.		
Principle	The SPADNS colorimetric method is based on the reaction between fluoride and a zirconium-dye lake. Fluoride reacts with the dye lake, dissociating a portion of it into a colorless complex anion (ZrF_6^{2-}) and the dye. As the amount of fluoride increases, the color produced becomes progressively lighter. The reaction rate between fluoride and zirconium ions is influenced greatly by the acidity of the reaction mixture. lf the proportion of acid in the reagent is increased, the reaction can be made almost instantaneous. Under such conditions, however, the effect of various ions differs from that in the conventional alizarin methods. The selection of dye for this rapid fluoride method is governed largely by the resulting tolerance to these ions.		
Apparatus/Instruments	Colorimetric equipment: One of the following is required: a. Spectrophotometer, for use at 570 nm, providing a light path of at least 1 cm. b. Filter photometer, providing a light path of at least 1 cm and equipped with a greenish yellow filter having maximum transmittance at 550 to 580 nm.		
Materials and Reagents	a. Standard fluoride solution b. SPADNS solution c. Zirconyl-acid reagent d. Acid zirconyl-SPADNS reagent e. Reference solution f. Sodium arsenite solution		
Preparation of Reagents	**a. Standard fluoride solution:** Dilute 100 mL stock fluoride solution to 1000 mL with reagent water; 1.00 mL = 10.0 µg F- (Stock fluoride solution: Dissolve 221.0 mg anhydrous sodium fluoride, NaF, in reagent water and dilute to 1000 mL; 1.00 mL = 100 mg F-.) **b. SPADNS solution:** Dissolve 958 mg SPADNS, sodium 2 (parasulfophenylazo)-1,8-dihydroxy-3,6-naphthalene disulfonate, also called 4,5-dihydroxy-3-(parasulfophenylazo)-2,7naphthalenedisulfonic acid trisodium salt, in reagent water and dilute to 500 mL. This solution is stable for at least 1 year if protected from direct sunlight.		

	c. Zirconyl-acid reagent: Dissolve 133 mg zirconyl chloride octahydrate, ZrOC12 • 8H2O, in about 25 mL reagent water. Add 350 mL conc HCl and dilute to 500 mL with reagent water. **d. Acid zirconyl-SPADNS reagent**: Mix equal volumes of SPADNS solution and zirconyl-acid reagent. The combined reagent is stable for at least 2 years. **e. Reference solution:** Add 10 mL SPADNS solution to 100 mL reagent water. Dilute 7 mL conc HCl to 10 mL and add to the diluted SPADNS solution. The resulting solution, used for setting the instrument reference point (zero), is stable for at least 1 year. Alternatively, use a prepared standard of 0 mg/L F^- as a reference. **f. Sodium arsenite solution:** Dissolve 5.0 g $NaAsO_2$ and dilute to 1 L with reagent water. (Caution: Toxic-avoid ingestion.)
Sample Preparation	If the sample contains residual chlorine, remove it by adding 1 drop (0.05 mL) $NaAsO_2$ solution per 0.1 mg residual chlorine and mix. (Sodium arsenite concentrations of 1300 mg/L produce an error of 0.l mg/L at 1.0 mg/L F^-.)
Method of analysis	a. **Preparation of standard curve:** Prepare fluoride standards in the range of O to 1.40 mg/L F- by diluting appropriate quantities of standard fluoride solution to 50 mL with reagent water. Pipet 5.00 mL each of SPADNS solution and zirconyl-acid reagent, or 10.00 mL mixed acid-zirconyl-SPADNS reagent, to each standard and mix well. Avoid contamination. Set the photometer to zero absorbance with the reference solution and obtain absorbance readings of the standards. Plot a curve of the milligrams fluoride-absorbance relationship. Prepare a new standard curve whenever a fresh reagent is made or a different standard temperature is desired. As an alternative to using a reference, set the photometer at sorne convenient point (0.300 or 0.500 absorbance) with the prepared O mg/L p-standard b. **Color development:** Use a 50.0-mL sample or a portion diluted to 50 mL with reagent water. Adjust the sample temperature to that used for the standard curve. Add 5.00 mL each of SPADNS solution and zirconyl-acid reagent, or 10.00 mL acid-zirconyl-SPADNS reagent. Mix well and read the absorbance, first setting the reference point of the photometer as above. If the absorbance falls beyond the range of the standard curve, repeat using a diluted sample.
Calculation with units of expression	$$mg/L\ F^- = \frac{A}{mL\ sample} \times \frac{B}{C}$$ where: A= µg F- determined from plotted curve, B = final volume of diluted sample (mL), and C = volume of diluted sample used for color development (mL). When the prepared O mg/L p- standard is used to set the photometer,

	alternatively calculate fluoride concentration as follows; $\text{mg/L F} = \frac{A_0 - A_X}{A_0 - A_1}$ where: Ao = absorbance of the prepared O mg/L F- standard, Ax= absorbance of the prepared sample, and A_i = absorbance of a prepared 1.0 mg/L F- standard.
Inference (Qualitative Analysis)	NA
Reference	APHA 4500 – F^-
Approved by	Scientific Panel on Methods of Sampling and Analysis

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Method No.	**FSSAI 14.024:2024**	**Revision No. & Date**	0.0
Scope	Fluoride ions have dual significant in water supplies. High concentration of F- causes dental fluorosis (disfigurement of the teeth). At the same time, a concentration less than 0.8 mg/L results in 'dental caries'. Hence it is essential to maintain the F- concentration between 0.8 to 1.0 mg/L in drinking water. Among the many methods suggested for the determination fluoride ion in water, the Colorimetric method (SPANDS) and the ion selective electrode method are the most satisfactory and applicable to variety of samples. Because all of the Colorimetric methods are subject to errors due to presence of interfering ions, it may be necessary to distill the sample before making the fluoride estimation. This standard method prescribes three methods of test for determination of fluoride content in water. • Zirconium alizarin method without distillation • Zirconium alizarin method with distillation • Electrochemical probe method		
Caution	Iron, alkalinity, phosphates interfere, if present above the values given in Table - 1. Interference of free residual chlorine can be removed by adding sodium arsenite. Aluminium gives negative error because of formation of Al-F complex which withdraws fluoride from the reaction of zirconium.		
Principle	The color (red to yellow with increasing concentration of fluoride) obtained with zirconium alizarin reagent is matched against that produced with a series of standard fluoride solutions.		
Apparatus/Instruments	• Nessler Tubes, 100 mL capacity. • Distillation Apparatus - The distillation apparatus shall consist of a Claisen flask of 100 mL capacity, a large flask for generating steam and an efficient condenser. The main neck of the Claisen flask shall be fitted with a two-holed rubber stopper through which shall pass a thermometer and a glass tube (for connecting with the steam supply), both the thermometer and the tube extending almost to the bottom of the flask. The side neck of the flask shall be closed with a rubber stopper and the side arm connected with the condenser. Steam shall be generated from water made alkaline with sodium hydroxide. Local overheating of the Claisen flask shall be avoided by use of an asbestos board with a hold which shall fit closely to the lower surface of the flask.		
Materials and Reagents	• Sodium Thiosulphate • Standard Sodium Fluoride • Zirconium Alizarin Reagent • Silver Sulphate • Perchloric Acid — 60 percent.		

<table>
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Phenolphthalein Indicator
Sodium Hydroxide Solution — 10 percent w/v.
Concentrated Sulphuric Acid

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<tr><td>Preparation of Reagents</td><td>
1. Sodium Thiosulphate Solution — approximately 0.1 N. 1.5.2

2. Standard Sodium Fluoride Solution — Dissolve 0.221 gm of dry sodium fluoride in distilled water and make up to 1000 mL. Dilute 1000 mL of the solution to 1000 mL distilled water. One millilitre of this diluted solution contains 0.01 mg of fluoride (as F). The solution shall be kept in polyethylene or wax-lined glass bottles.

3. Zirconium Alizarin Reagent
Dissolve 0.3 gm of zirconium oxychloride (ZrOCl2.8H2O), or 0.25 gm of zirconium oxynitrate [ZrO(NO3)2.2H2O] in 50 mL of distilled water. Dissolve 0.07 gm of alizarin sodium monosulphonate (alizarin S) in another 50 mL quantity of distilled water and add the latter solution slowly to the zirconium solution with continuous stirring. The resulting solution clears on standing for a few minutes.

4. Dilute 112 mL of concentrated hydrochloric acid to 500 mL with distilled water. Also add 37 mL of concentrated sulphuric acid to 400mL of distilled water and then dilute to 500 mL. Mix the two diluted acids when cool.

5. Dilute the clear zirconium solution prepared in 3. to 1000 mL with the mixed acid solution prepared in 4. The reagent is at first red, but within an hour it changes to orange-yellow and is ready for use. The solution shall be stored in the dark, if kept in a refrigerator it is stable for 2 to 3 months. When 5 mL of this reagent is added to 100 mL of distilled water containing no fluorides, it soon turns pink. Fluorides discharge the pink colour of the lake so that the solution acquires a more yellow tint.

6. Silver Sulphate
7. Perchloric Acid — 60 percent.
8. Phenolphthalein Indicator
9. Sodium Hydroxide Solution — 10 percent w/v.
10. Concentrated Sulphuric Acid
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<tr><td>Sample Preparation</td><td>
The method without distillation and electrochemical probe method is reliable for samples of potable and lightly polluted water in which the interfering substances are not in excess of the limits given below:

<table>
<tr><td>Chlorides (as CI)</td><td>2000 mg/L</td></tr>
<tr><td>Sulphates (as SO4)</td><td>300 mg/L</td></tr>
<tr><td>Alkalinity (as CaCO3)</td><td>400 mg/L</td></tr>
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</table>

	<table><tr><td>Iron (as Fe)</td><td>2 mg/L</td></tr><tr><td>Aluminium (as Al)</td><td>0.5 mg/L</td></tr><tr><td>Phosphates (as PO4)</td><td>5 mg/L</td></tr></table> Where the sample is highly coloured or turbid or has interfering substances in excess of the limits given above, the method with distillation shall be used or the sample shall be appropriately diluted before this test. With samples of unknown composition or where greater accuracy is needed, the method with distillation shall be employed. The sample shall not contain free chlorine; if necessary, it shall be dechlorinated with a slight excess of sodium thiosulphate solution before use.
Method of analysis	**Method without Distillation** Take 100 mL of the clear sample and a series of dilutions of standard sodium fluoride solution in 100 mL of distilled water in Nessler tubes and add 5.0 mL of the zirconium alizarin reagent to each. The sample and standards shall be at the same temperature to within 1°C to 2°C. Mix and compare the colours after standing for 1 hr exactly. Note the volume of standard sodium fluoride solution contained in the tube, with which a match with the sample under test is obtained. **Method with Distillation** Introduce into the Claisen flask a number of fragments of Pyrex glass or glass beads, 0.2 gm of silver sulphate, 7 mL of distilled water and 15 mL of Perchloric acid. Heat the flask until the temperature reaches 120°C to 125°C, connect to the steam supply and regulate the gas and steam so that the distillation proceeds at a temperature of 137°C to 140°C. Distil 150 mL in 25 to 35 min and steam out the condenser towards the end of the distillation. Discard the first distillate. Distil a further 150mL and determine the fluorides in it by the method given above. The figure for this blank shall not exceed 0.0015 mg and shall be approximately constant for any further 150 mL fraction. Make 150 mL of the sample alkaline to phenolphthalein indicator with sodium hydroxide solution, add a few drops in excess and concentrate to 20 mL. When cool, transfer quantitatively to the distillation flask and carefully add 15 mL of concentrated sulphuric acid. If the amount of chloride in the aliquot exceeds 5 mg, add about 5 mg of silver sulphate for each milligram of chlorine. Connect up the apparatus and distil 150 mL as above. Determine the fluoride content of the total 150 mL of distillate.

Calculation with units of expression	**Method without Distillation** Fluoride (as F), mg/L = $\frac{1000W}{V}$ W - Weight of fluorides (as F) in the standard solution matched by the sample in mg; V - Volume of the sample taken in mL. **Method with Distillation** Fluoride (as F), mg/L = $\frac{1000W}{V}$ W = weight of fluorides (as F) in the standard solution matched by 150 mL of the Distillate, in mg V= volume of the sample taken in mL
Inference (Qualitative Analysis)	NA
Reference	• IS : 3025 (Part 60) – 2008 Methods of Sampling and Test (Physical and chemical) for water and Waste Water : Fluoride • APHA 4500 F
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	**Determination of Fluoride by electrochemical probe method**		
Method No.	**FSSAI 14.025:2024**	**Revision No. & Date**	0.0
Scope	The electrochemical technique method is directly suitable for measuring fluoride concentrations from 0.2 mg/L to 2.0 g/L. After the addition of a known amount of fluoride, concentrations as low as 0.02 mg/L can be detected.		
Caution	The electrode will respond directly to hydroxide ions. The formation of HF under acidic conditions will reduce the measured fluoride concentration. Therefore, buffer all test aliquots to a pH between 5 and 7 to prevent such interference. Cations such as calcium, magnesium, iron and aluminium form complexes with fluoride or precipitates to which the electrode does not respond. Therefore the buffer solution also contains trans- 1, 2-diaminocyclohexane-N,N,N',N' - tetraacetic acid (CDTA) as a decomplexing agent to free bound fluoride. The boron tetrafluoride anion, is not decomplexed by the addition of buffer.		
Principle	When a fluoride ion-selective electrode comes into contact with an aqueous solution containing fluoride ions, a potential difference develops between the measuring electrode and the reference electrode. The value of this potential difference is proportional to the logarithm of the value of the fluoride ion activity in accordance with the Nernst equation.		
Apparatus/Instruments	1. Millivolt Meter — a Millivolt meter with an impedance of not less than 1012Ω, capable of resolving potential differences of 0.1 mV or better. 2. Fluoride Ion-selective Electrode — the e.m.f. response, using standard solutions, shall not be less than 55 mV per decade change in fluoride concentration at 25°C. 3. Reference Electrode -- Either a calomel electrode, filled with saturated potassium chloride (KCl) solution, or a silver/silver chloride electrode shall be used. 4. Measuring Cells — capacity 100 mL, made of polypropylene and fitted with a thermo stated jacket. 5. Water Bath — capable of supplying water to the jacket of the measuring cell at a temperature of 25°C±0.2°C. 6. Magnetic Stirrer, with a polytetrafluoroethylene (PTFE) -coated stirring bar. 7. Polyethylene Beaker, of capacity 100 mL.		
Materials and Reagents	1. Purity of the Reagents 2. Sodium Hydroxide (5M) 3. Total Ionic Strength Adjustment Buffer (TISAB)		

	4. Fluoride, Stock Solution, 1000 mg/L 5. Fluoride, working standard
Preparation of Reagents	1. Purity of the Reagents —Unless specified otherwise, only pure chemicals and fluoride free distilled water shall be used in tests. 2. Sodium Hydroxide (5M) — Dissolve cautiously 100 ± 0.5 gm of sodium hydroxide in water, cool and dilute to 500 mL. 3. Total Ionic Strength Adjustment Buffer (TISAB) — Add 58 gm of sodium chloride (NaCI) and 57 mL of glacial acetic acid [p(CH3C00H) = 1.05 g/mL] to 500 mL of water in a 1 liter beaker. Stir until dissolved. Add 150 mL of the sodium hydroxide solution and 4 gm of CDTA (trans-1, 2-diaminocyclohexane-N,N,N,N'tetraacetic acid). Continue stirring until all the solids have dissolved and adjust the solution to pH 5.2 with sodium hydroxide solution using a pH meter. Transfer to a 1000 mL one mark volumetric flask, make up to the mark with water and mix. The solution is stable for about 6 months, but do not use if precipitation occurs in solution. 4. Fluoride, Stock Solution, 1000 mg/L - Dry a portion of sodium fluoride (NaF) at 150°C for 4 hr and cool in a desiccator. Dissolve 2.210 ± 0.001 gm of the dried material in water contained in a 1000mL one-mark volumetric flask. Make up to the mark with water and mix. Store the solution in a screw-capped polyethylene container. 5. Fluoride, working standard solution-I, 10 mg/L - Pipette 10 mL of the fluoride stock solution into a 1000 mL one-mark volumetric flask. Make up to the mark with water and mix. Standard solutions should be stored in plastic bottles and are usable for one month. 6. Fluoride, working standard solution-II, 5 mg/L - Pipette 5 mL of the fluoride stock solution into a 1000 mL one-mark volumetric flask and make up to the mark with water. 7. Fluoride, working standard solution-III, 1 mg/L - Pipette 100 mL of the working standard solution I into a 1000 mL one-mark volumetric flask and make up to the mark with water. 8. Fluoride, working standard solution-IV, 0.5 mg/L - Pipette 100mL of the working standard solution-II into a 1000 mL one-mark volumetric flask and make up to the mark with water. 9. Fluoride, working standard solution-V, 0.2 mg/L - Pipette 20 mL of the working standard solution-I into a 1000 mL one-mark volumetric flask and make up to the mark with water.

<table>
<tr><td>Sample Preparation</td><td>Please refer to the sample preparation section of Determination of Fluoride in water by Zirconium alizarin method without distillation.
METHOD NO : FSSAI 14.024:2024(Sample Preparation)

The method without distillation and electrochemical probe method is reliable for samples of potable and lightly polluted water in which the interfering substances are not in excess of the limits given below:

<table>
<tr><td>Chlorides (as CI)</td><td>2000 mg/L</td></tr>
<tr><td>Sulphates (as SO4)</td><td>300 mg/L</td></tr>
<tr><td>Alkalinity (as CaCO3)</td><td>400 mg/L</td></tr>
<tr><td>Iron (as Fe)</td><td>2 mg/L</td></tr>
<tr><td>Aluminium (as Al)</td><td>0.5 mg/L</td></tr>
<tr><td>Phosphates (as PO4)</td><td>5 mg/L</td></tr>
</table>

Where the sample is highly colored or turbid or has interfering substances in excess of the limits given above, the method with distillation shall be used or the sample shall be appropriately diluted before this test. With samples of unknown composition or where greater accuracy is needed, the method with distillation shall be employed.
The sample shall not contain free chlorine; if necessary, it shall be dechlorinated with a slight excess of sodium thiosulphate solution before use.</td></tr>
<tr><td>Method of analysis</td><td>Preparation for Measurement: Since the electrode characteristics of a fluoride ion selective electrode generally vary with time, check the calibration curve on the day of use. To accelerate the establishment of the equilibrium potential, condition the electrode prior to measurement in the following way. Prior to measurement, immerse the electrode for 1 h in the cell which contains the reference solution-V After rinsing with the first solution to be measured, the electrode is ready for use.

Measurement: Pipette 25 mL of the buffer solution, followed by 25mL of the water sample, into a measuring cell. Ensure that the pH is 5.2 ± 0.2; if necessary, adjust the pH with hydrochloric acid or sodium hydroxide solution, using as little as possible. For a series of determinations, start the measurement with the lowest concentration and finish with the highest following the anticipated concentration of the samples. After measuring the high concentrations, recondition the electrode before measuring the low concentrations. Measure all the solutions according to the following procedure. Wait until constant temperature (for example 25 ± 0.5°C) is reached and carry out all the measurements at this temperature. Put a stirring bar into the measuring cell and place it on the magnetic stirrer.</td></tr>
</table>

Insert the electrodes into the solution and fix them in place. Adjust the stirring rate to about 180 min/L to 200 min/L. When the potential does not change by more than 0.5 mV in 5 min, switch off the stirrer. After at least 15 sec, record the value obtained. Rinse the stirring bar and the electrodes with the next solution to be measured, before starting the next measurement.

Measurement after Concentration Enhancement: If a water sample contains less than 0.2 mg/L F, proceed as follows: Add 500mL of the fluoride standard solution-I to 25 mL of the sample using a piston pipette, and 25 mL of the buffer solution with a volumetric pipette; continue as described in 2.6.2. When calculating the result, subtract the amount of fluoride ions added from the total result.

Calibration: Establish a calibration function using the five reference solutions in the corresponding concentration range. For the range 0.2 mg/L to 10 mg/L, proceed as follows:
Pipette 25 mL of the buffer solution into each of five measuring cells. Pipette the respective volumes of the working standard fluoride solutions specified into the measuring flasks. For the establishment of the calibration function proceed step by step from the most dilute solution to the most concentrated solution, rinsing after each measurement with the solution of the next highest concentration. After the above measurements have been completed, recondition the electrode for 5 to 10 min, using the reference solution-V (see Table) in order to eliminate memory effects.

Preparation of Reference Solutions

Sl.no.	Reference Solution	Buffer Solution	Working Standard Solution	
	ml		No	ml
(1)	(2)	(3)	(4)	(5)
i)	1	25	I	25
ii)	2	25	11	25
iii)	3	25	III	25
iv)	4	25	IV	25
v)	5	25	V	25

Use the following order of measurement (the numbers refer to the reference solutions in this Tablet.. 5 - rinse - 4 - rinse - 3 - rinse - 2 - rinse - I - rinse with 5 - recondition - repeat measuring run.

If the individual values of the parallel series vary from the first series by more than I ± 0.5 mV. repeat the measuring run Regular checking of the calibration graph is essential. Ensure that the slope is not less than 55 mV, otherwise cheek the equipment and establish a new calibration graph.

Calculation with units of expression	Calculation and Expression of Result: Plot the calibration values on semi logarithmic paper, with the fluoride concentrations, in milligrams per liter, on the abscissa and the cell potential, in millivolts, on the ordinate and establish the regression line. Read the value for the samples by using the regression line and express the mass concentration of fluoride in milligrams per liter.
Inference (Qualitative Analysis)	NA
Reference	• IS : 3025 (Part 60) – 2008 Methods of Sampling and Test (Physical and chemical) for water and Waste Water : Fluoride • APHA 4500 F
Approved by	Scientific Panel on Methods of Sampling and Analysis

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Method No.	**FSSAI 14.026:2024**	**Revision No. & Date**	0.0
Scope	Water hardness is a traditional measure of the capacity of water to precipitate soap. Hardness of water is not a specific constituent but is a variable and complex mixture of cations and anions. It is caused by dissolved polyvalent metallic ions. In fresh water, the principal hardness causing ions are calcium and magnesium which precipitate soap. Other polyvalent cations also may precipitate soap, but often are in complex form, frequently with organic constituents, and their role in water hardness may be minimal and difficult to define. Total hardness is defined as the sum of the calcium and magnesium concentration both expressed as CaCO3 in mg/L. The degree of hardness of drinking water has been classified in terms of the equivalent CaCO3 concentration as follows: • Soft : 0-60 mg/L • Medium: 60-120 mg/L • Hard: 120-180 mg/L • Very Hard: >180 mg/L Although hardness is caused by cation, it may also be discussed in terms of carbonate (temporary) and non-carbonate (permanent) hardness. Carbonate hardness refers to the amount of carbonates and bicarbonates in solution that can be removed or precipitated by boiling. This type of hardness is responsible for the deposition of scale in hot water pipes and kettles. When total hardness is numerically greater then that of total alkalinity expressed as CaCO3 the amount of hardness is numerically equal to less than total alkalinity is called carbonate hardness. When the hardness is numerically equal to less than total alkalinity, all hardness is carbonate hardness. The amount of hardness in excess of total alkalinity expressed as CaCO3 is non-carbonate hardness. Non-carbonate hardness is caused by the association of the hardness-causing cation with sulphate, chloride or nitrate and is referred to the “permanent hardness”. This type of hardness cannot be removed by boiling. Public acceptability of the degree may vary considerably from community depending on local conditions, and the association. The taste threshold for magnesium is less than that for cation. Methods for determination of total hardness in water are prescribed:- • Titrimetric Method • Method based on Analytical Data		
Caution	The EDTA forms stable complexes with iron, manganese, copper, lead, cobalt, zinc and nickel. Heavy metal interferences can be eliminated by complexing the metals with cyanide. In the presence of cyanide the procedure may be used even when iron, copper, zinc or lead concentrations		

	are as high as 10mg/L. The higher oxidation states of manganese above Mn++ react rapidly with the indicator to form discolored oxidation products hydroxylamine hydrochloride reagent may be used to reduce manganese to divalent state. The divalent manganese interference can be removed by adding of one or two crystal of potassium ferrocyanide. In presence of high aluminum concentrations, the blue color near end point starts disappearing and reverts to red. Phosphate and carbonate ion may precipitate calcium at the pH of titration.
Principle	**Titrimetric Method:** Principle: (EDTA method for determination of total hardness) depends on ability of ethylenediamine tetra acetic acid ($C_{10}H_{16}O_8N_2$) or its disodium salt to form stable complexes with calcium and magnesium ions. When the dye eriochrome black T (EBT) ($C_2OH_{13}.N_3O_7S$) is added to solution containing calcium and magnesium ions at pH 10.0, a Wine red complex is formed, this solution is titrated with standard solution of disodium salt of EDTA, which extracts calcium and magnesium from the dye complex and the dye is changed back to its original blue colour. Eriochrome black T is used to indicate the end point for the titration of calcium and magnesium together. **Method based on Analytical Data** Total hardness computed from the concentration of the different metallic cation (other than alkali metals) in the sample but most often the cations taken into account are calcium, magnesium iron, aluminum zinc, strontium, barium and manganese.
Apparatus/Instruments	1. Burette 2. Polyethylene bottle with Stopper 3. Conical Flask
Materials and Reagents	Purity of the reagents. Unless specified otherwise only pure chemicals and tannin free distilled water shall be used in tests.
Preparation of Reagents	**Buffer solution**: Dissolve 16.9 gm ammonium chloride in 143 mL concentrated ammonium hydroxide, add 1.25gm of magnesium salt of EDTA and dilute to 250 mL with distilled water. Store the solution in a polyethylene bottle tightly stopper to prevent loss of ammonia or pick up of carbon dioxide for no longer than 1 month. Dilute 10 mL of the solution to 100mL with distilled water and check that the pH value is 10.0 ±0.1. In the absence of magnesium salt of EDTA dissolve 1.179 gm disodium salt of EDTA and 780 mg magnesium sulphate or 644 mg magnesium chloride in 50mL of distilled water. Add this solution to 16.9 gm ammonium chloride and 143 mL concentrated ammonium hydroxide with mixing and dilute to 250mL with distilled water. To attain the highest accuracy adjust to exact

	equivalence through appropriate addition of a small amount of EDTA or magnesium sulphate or chloride the exact amount can be determined by taking an appropriate aliquot of buffer and titrate it with disodium salt of EDTA as above. Keep the solutions tightly Stoppard to prevent loss of ammonia or absorbance of carbon dioxide and do not store for more than a month. Dilute 10 mL of the solution to 100 mL with distilled water and check that the pH value is 10.0 ±0.1 **Standard calcium solution:** 1.0mL = 1.00mg calcium carbonate. Dry analytical grade calcium carbonate in a oven at 180°C for 1 hr. weigh 1.0gm, suspend it in distilled water and add 1:1 hydrochloric acid AR quality drop, wise slowly to dissolve the solid. Use minimum amount of acid. Boil for a few minutes, cool add a few drop of methyl red indicator and adjust to orange color with 3N ammonium hydroxide or 1:1 hydrochloric acid. Dilute to 1000mL with distilled water. **Eriochrome black T indicator solution:** Dissolve 0.40 gm Eriochrome black T and 4.5 gm hydroxylamine hydrochloride in 100mL 95% ethanol. This indicator is stable for more than 2 months. Alternatively dissolve 0.5 gm Eriochrome black T in 100mL trietthanolamine or 2 methoxyethanol or mixed 0.5 gm EBT dye and 100gm sodium chloride in pestle and mortar. Store in tightly Stoppard bottle. All indicator formulation tends to deteriorate especially when exposed to moisture. If the end point color change is not sharp enough it is either due to the presence of some interfering ions or due to deterioration of the indicator. In the latter case, addition of inhibitor sodium cyanide or sodium sulphide does not sharpen the end point color change. Standard EDTA solution: Dissolve 3.723gm EDTA which has been dried overnight in sulphuric acid desiccators, in demineralized water and dilute to 1000mL. The reagent is stable for several weeks and large volume is usually prepared. Check the reagent by titrating 25 mL of standard calcium solution as described above. Store in polyethylene bottles.
Sample Preparation	--
Method of analysis	1. Standardization: Pipette 25 mL of standard calcium solution in a porcelain basin and adjust the volume to 50 mL with distilled water. Add 1 mL buffer solution, add 1 to 2 drops of indicator, titrate slowly with continuous stirring until the reddish tinge disappears. adding last few drops at 3 to 5 second interval. At the end point the color is sky blue. 2. Pipette an aliquot of water sample maximum 50 mL in a porcelain dish or 150 mL beaker and adjust the volume to approximately 50 mL. Add 1 mL hydroxylamine hydrochloride solution.

	3. Add 1 to 2 mL buffer solution so as to achieve pH of 10.0 to 10.1. 4. Add 2 mL Eriochrome black T indicator solution 5. Titrate with standard EDTA solution stirring rapidly in the beginning and slowly towards the end till end point is reached when all the traces of red and purple color disappear and solution is clear sky blue in color. Blank titration carried out in a same way as that for sample may be used for comparison. Note- Selection of sample volume may be made such that the result lies between 200 to 300 mg/L of hardness (as $CaCO_3$)
Calculation with units of expression	**Titrimetric Method** Total hardness as ($CaCO_3$), mg/L = [1000(V1 –V2)/V3] x CF Where V1 = volume in mL of the EDTA standard solution used in the titration for the sample V2 = volume in mL of the EDTA solution used in the titration for blank. V3 = volume in mL of the sample taken for the test CF = X1/X2 correction factor for standardization of EDTA. X1 = volume in mL of standard calcium solution taken for standardization X2 = volume of mL of EDTA solution used in the titration Report hardness in mg/L as $CaCO_3$ rounded to the first decimal place when the value is less than 10 mg/L and to the nearest unit if the value is more than 10mg/L. **Method based on Analytical Data:** Total hardness (as $CaCO_3$), mg/L= (2.497 x mg/L Ca) + (4.116 x mg/L Mg) + (2.69 x mg/L Fe) + (5.567 x mg/L Al) + (1.531 x mg/L Zn) + (1.822 x mg/L Mn) + (0.894 x mg/L Ba) + (1.319 x mg/L Sr).
Inference (Qualitative Analysis)	NA
Reference	IS: 3025 (Part 21) – 1983 (Reaffirmed 2002) - Methods of Sampling and Test (Physical and chemical) for water and Waste Water: Total Hardness
Approved by	Scientific Panel on Methods of Sampling and Analysis

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Method No.	**FSSAI 14.027:2024**	**Revision No. & Date**	0.0
Scope	Alkalinity of sample can be estimated by titrating with standard sulphuric acid (0.02N) at room temperature using phenolphthalein and methyl orange indicator. Titration to decolourisation of phenolphthalein indicator will indicate complete neutralization of OH- and ½ of CO3 - while sharp change from yellow to orange of methyl orange indicator total alkalinity (complete neutralization of OH-, CO3 - , HCO_3) This method is applicable to determine alkalinity in water in the range of 0.5 to 500mg/L alkalinity as CaCO3. The upper range may be extended by dilution of the original sample.		
Caution	Sulphuric Acid can be corrosive to metals, causes severe skin burns and eye damage.		
Principle	Alkalinity of water is the capacity of the water to accept protons. It may be defined as the quantitative capacity of an aqueous medium to react with hydrogen ions to pH 8.3 (phenolphthalein alkalinity) and then to pH 3.7 (total alkalinity or methyl orange alkalinity). The equation in its simplest form is as follows: $CO_3^{2-} + H+ = HCO_3$ (pH 8.3) From pH 8.3 to 3.7 the following reaction may occur: $HCO_3 - + H+ = H2CO_3$ 3		
Apparatus/Instruments	1. pH meter 2. Burette- 50 mL capacity 3. Magnetic stirrer assembly		
Materials and Reagents	1. Distilled water 2. Sulphuric acid 3. Phenolphthalein indicator 4. Mixed indicator solution		
Preparation of Reagents	1. **Distilled Water** : Distilled water used should have pH not less than 6.0. If the water has pH less than 6.0, it shall be freshly boiled for 15 minutes and cooled to room temperature. Deionized water may be used provided that it has a conductance of less than 2 µs/cm and a pH more than 6.0. 2. **Sulphuric Acid** : Dilute 5.6 mL of concentrated sulphuric acid (relative density 1.84) to 1 liter with distilled water.		

	3. **Standard solution of sulphuric acid**: 0.02N 4. **Phenolphthalein indicator**: Dissolve 0.5 gm of phenolphthalein in 100mL, 1:1 (v/v) alcohol water mixture 5. **Mixed indicator solution**: Dissolve 0.02gm methyl red and 0.01gm bromocresol green in 100mL, 95 percent, ethyl or isopropyl alcohol.
Sample Preparation	The sample aliquot used for analysis should be either free from turbidity or should be allowed to settle prior to analysis.
Method of analysis	1. Pipette 20 mL or a suitable aliquot of sample into 100 mL beaker. 2. If the pH of the sample is over 8.3 then add 2 to 3 phenolphthalein indicator and titrate with standard sulphuric acid solution till the pink color observed by indicator just disappears (equivalence of pH 8.3). 3. Record the volume of standard sulphuric acid solution used. Add 2 to 3 drops of mixed indicator to the solution in which the phenolphthalein alkalinity has been determined. 4. Titrate with the standard acid to light pink color (equivalence of pH 3.7). Record the volume of standard acid used after phenolphthalein alkalinity
Calculation with units of expression	Calculate alkalinity in the sample as follows Phenolphthalein alkalinity (as mg/L of $CaCO_3$) = $\frac{A \times N \times 50000}{V}$ Total alkalinity (as mg/L $CaCO_3$) = $\frac{(A+B) \times N \times 50000}{V}$ Where, A= mL of standard sulphuric acid used to titrate to pH 8.3 B=mL of standard sulphuric acid used to titrate form pH 8.3 to pH 3.7 N= normality of acid used V= Volume in mL of sample taken for test
Inference (Qualitative Analysis)	NA
Reference	1. IS : 3025 (Part 23) – 1986 (Reaffirmed 2003)- Methods of Sampling and Test (Physical and chemical) for water and Waste Water : Alkalinity 2. APHA 24TH EDITION 2023
Approved by	Scientific Panel on Methods of Sampling and Analysis

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Method No.	**FSSAI 14.028:2024**	**Revision No. & Date**	0.0
Scope	Turbidity method is applicable to surface and ground water in the range of 1 to 40 mg/L $SO4^{-}$. Samples having higher concentrations than this can be measured by appropriate dilution of sample.		
Caution	• Color or suspended mater in large amounts will interfere. • In waters containing large quantities of organic material, it may not be possible to precipitate barium sulphate satisfactorily.		
Principle	Sulphate ion is precipitated in hydrochloric acid medium with barium chloride in such a manner as to form barium sulphate crystals of uniform size. The absorbance of barium sulphate suspension is measured by a nepholometer or transmission photometer (turbidity meter) and the sulphate ion concentration is determined by comparison of the reading with a standard curve.		
Apparatus/Instruments	1. Turbidity meter or spectrophotometer- for use at 420 nm 2. Usual laboratory glass apparatus		
Materials and Reagents	1. Barium chloride 2. Gelatin powder 3. Conditioning reagent (1) 4. Conditioning reagent (2) 5. Stock sulphate solution 6. Standard sulphate solution 7. Hydrochloric acid (1+9)		
Preparation of Reagents	1. Conditioning reagent (1) - Add 0.3 gm gelatin in 100mL distilled water and warm it on hot plate till it is dissolved. The gelatin solution is kept for about 12 hours, or overnight preferably, at 4°C after bringing the solution to room temperature, 3.0 gm barium chloride is added to gelatin solution and dissolved by mixing. The turbid solution is kept standing for 2 hours and mixed before use. 2. Conditioning reagent (2) - Mix 50 mL glycerol with a solution containing 30mLconcentration hydrochloric acid, 300mL distilled water, 100 mL 95% ethyl or isopropyl alcohol and 75 gm sodium chloride. 3. Stock sulphate solution - Dissolve 0.1479 gm of anhydrous sodium sulphate ($Na2SO4$) in distilled water and dilute to one liter. 4. Standard sulphate solution - prepare a series of standards by diluting stock solution of sulphate to cover the desired range in between 1 to 40 mg/L. 5. Hydrochloric acid (1+9) - dissolve one volume of concentrated hydrochloric acid with 9 volumes of distilled water		
Sample Preparation	Filter the sample through 0.45 µm, filter, if there is any turbidity.		
Method of analysis	1. Take 20 mL of clear aliquot of the water sample of suitable amount		

	diluted to 20 mL in 100mL conical flask. 2. Add 1.0 mL hydrochloric acid solution and 1.0 mL conditioning reagent and mix well for 30 sec. 3. Read the absorbance on spectrophotometer after 10 min if glycerol conditioning reagent is used or 30 min, if gelatin is used, at 420 nm or read the turbidity occurred on turbidity meter following the manufacturer instruction to operate. If water sample is turbid take 20 mL sample or suitable amount dilute to 20 mL with distilled water. Do not add conditioning reagent. Read the absorbance of this sample and subtract this value form the above measured absorbance 4. Calibration curve: prepare a series of standards taking at least 4 standards and run a blank and follow the steps 2 and 3 and prepare a calibration curve of standards mg/L vs. absorbance
Calculation with units of expression	Read the sulphate concentration of sample directly from the calibration curve
Inference (Qualitative Analysis)	NA
Reference	IS: 3025 (Part 24) – 1986 (Reaffirmed 1992) - Methods of Sampling and Test (Physical and chemical) for water and Waste Water : Sulphate
Approved by	Scientific Panel on Methods of Sampling and Analysis

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Method No.	**FSSAI 14.029:2024**	**Revision No. & Date**	0.0
Scope	This method is applicable for all the waters having sulphate concentrations above 10 mg/L; however, it is a time consuming method.		
Caution	Suspended matter, silica, barium chloride precipitant, nitrate and sulphate are the principal factors in positive error. Alkali metal sulphates and heavy metals, such as chromium and iron cause low results. To minimize solubility of barium sulphite, the acid concentration while precipitating barium sulphate, should be minimized.		
Principle	Sulphate is precipitated in hydrochloric acid medium as barium sulphate the addition of barium chloride solution. The precipitation is carried out near boiling temperature and after a period of digestion, the precipitate is filtered, washed with water until free of chlorides, ignited or dried and weighed as barium sulphate (BaSO4). The reaction in its simplest form is: Hcl medium SO4+ BaCl2 ⟶ Ba S04 + 2Cl		
Apparatus/Instruments	1. Steam bath 2. Drying oven - equipped with thermostatic control. 3. Muffle furnace - with heat indicator. 4. Desiccator 5. Analytical balance - capable of weighing to 0·1 mg. 6. Filter paper - acid washed. Ashless hard finish filter paper sufficiently retentive for fine precipitates (preferably Whatman No. 42). 7. Crucible - Porous bottom silica or porcelain crucible with a maximum pore size of 5 microns. 8. Ion-exchange column -. The exchange column should be regenerated by passing hydrochloric acid (6.2) solution after five or six samples have passed through the column followed by washing with distilled water		
Materials and Reagents	1. Methyl red indicator 2. Hydrochloric acid (1: 4) 3. Barium chloride solution 4. Silver nitrate-nitric acid reagent 5. Ion exchange resin		
Preparation of Reagents	1. Methyl red indicator - Dissolve 100 mg methyl red sodium salt in distilled water and dilute to 100 mL. 2. Hydrochloric acid (1: 4) - Dilute one volume of concentrated hydrochloric acid with four volumes of distilled water. 3. Barium chloride solution - Dissolve 100 gm of barium chloride (BaCl2.2H2O) in 1 litre distilled water. Filter through a membrane		

	filter or hard finish filter paper (1 mL of this reagent is capable of precipitating approximately 40 mg S04). 4. Silver nitrate-nitric acid reagent- Dissolve 8·5 gm of silver nitrate and 0·5 mL of nitric acid in 500 mL distilled water. 5. Ion exchange resin - Strong cation exchange resin, Amberlite IR-120 or equivalent
Sample Preparation	• The sample used for analysis should either be free from turbidity or filtered through 0.45 µm filter. • If, the total cation concentration in the sample is more than 250mg/L or if the total heavy metal ion concentration is more than 10 mg/L, pass the sample through a cation removing ion exchange column. • If the silica concentration exceeds 25 mg/L, evaporate the sample nearly to dryness in a platinum dish on a steam bath. Add 2 mL hydrochloric acid tilt the dish and rotate it until the acid comes in contact with the residue; continue the evaporation to dryness. Complete the drying in an oven at 180°C and if organic matter is present, char over the flame of a burner. Moisten the residue with 2 mL distilled water and 2 mL hydrochloric acid and evaporate to dryness on steam bath. Add 5 mL hydrochloric acid, take up the soluble residue in hot water and filter. Wash the insoluble silica with several small portions of hot distilled water. Combine the filtrate and washings.
Method of analysis	1. Adjust the clarified sample, treated if, necessary to remove interfering agents, to contain approximately 100 mg of sulphate ion in 500 mL volume. 2. Add 2 to 3 drops of methyl red indicator solution. Add hydrochloric acid drop till an orange red colour appears. Lower concentrations of sulphate ion may be tolerated if it is impracticable to concentrate the sample to the optimum level, but in such cases it is better to fix the total volume at 150 mL after concentration on hot plate. 3. Heat the solution to boiling, while stirring gently, add warm barium chloride solution slowly until precipitation appears to be complete, then add about 2 mL in excess. Digest the precipitate at 80-90°C for at least 2 hours. 4. Filtration - Filter the precipitate through filter paper and wash the precipitate with small portion of warm distilled water until me washings are free of chloride ions as indicated by testing with silver nitrate-nitric acid reagent. 5. Dry the precipitate in crucible and ignite at 800°C for 1 hour. NOTE: Do not allow the filter paper to flame. 6. Cool in a desiccator and weigh
Calculation with units of expression	Calculate the sulphate concentration in the sample from the equation: Sulphate concentration as mg/L BaS04 = $\frac{\text{mg BaS04 X 411.5}}{\text{mL of sample}}$

Inference (Qualitative Analysis)	NA
Reference	IS: 3025 (Part 24) – 1986 (Reaffirmed 1992) - Methods of Sampling andTest (Physical and chemical) for water and Waste Water : Sulphate
Approved by	Scientific Panel on Methods of Sampling and Analysis

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Method No.	**FSSAI 14.030:2024**	**Revision No. & Date**	0.0
Scope	This method is applicable to surface and groundwater's with sulphate concentrations in the range 5 to 150 mg/L. Samples having higher concentrations can be measured by appropriate dilution of sample.		
Caution	chloride ions in concentrations greater than 1000 mg/L cause an indistinct end point when the sulphate present is Iow (less than 10 mg/L SO_4^{2-}). To overcome this interference, a known amount of sulphate present is added to sample to increase the sulphate concentration		
Principle	Sulphate ion is titrated in an alcoholic solution under controlled acid conditions with a standard barium chloride solution, using thorin as the indicator.		
Apparatus/Instruments	1. White porcelain basin-100 to 125mL capacity. 2. Burette - along with titration assembly. 3. Ion exchange column		
Materials and Reagents	1. Ethyl alcohol- 95%. 2. Ammonium hydroxide solution (1 + 99) 3. Hydrochloric acid solution (1 + 99). 4. Hydrochloric acid solution (1 + 4). 5. Thorin solution 6. Ion exchange resin 7. Stock sulphate solution (100 mg/L SO_4^2). 8. Standard sulphate solution 9. Standard barium chloride solution		
Preparation of Reagents	1. Ammonium hydroxide solution (1 + 99) - Mix one volume of concentrated ammonia with 99 volumes of distilled water. 2. Hydrochloric acid solution (1 + 99) - Mix one volume of concentrated hydrochloric acid with 99 volumes of distilled water. 3. Hydrochloric acid solution (1 + 4) - Dilute one volume of concentrated hydrochloric acid with 4 volumes of distilled water. 4. Thorin solution - Dissolve 0·2 gm thorin (2, 2·Hydroxy-3, 6-disulpho-1-naphthylazo benzene arsenic acid) in 100 mL of distilled water. 5. Ion exchange resin - Strong cation-exchange resin. Aberlite IR-120 or equivalent. 6. Stock sulphate solution (100 mg/L SO42-- Dissolve 1.479 gmanhydrous sodium sulphate (Na2SO4) (dried at 110°C for 1 hour) in distilled water and make up to 1 litre in volumetric flask. 7. Standard sulphate solution - Prepare a series of standard solutions by diluting stock solution of sulphate with distilled		

	water. The concentrations of standard solutions are 0 (blank). 10, 20, 30, 40, 50, 80, 100 and 150 mg/L SO_4^{2-}. 8. Standard barium chloride solution - Dissolve 0·4 gm barium chloride (BaCl2. 2H20) in 800 mL of distilled water and adjust the pH to 3·5 to 4·0 with dilute hydrochloric acid (6.3) or ammonia solution (6.2) and finally make up to one litre.
Sample Preparation	The sample should be free from turbidity or filtered through a 0·45 µm filter.
Method of analysis	1. Pass the sample through ion exchange column (50 mL at a time). Discard the first 10 mL effluent and then collect in a 100mL beaker. Pipette 10 mL of this sample into a porcelain basin 2. Add 40 mL alcohol and 2 drops of thorin indicator. Adjust the pH to 3·8 to 4-0 by carefully adding drop by drop ammonia solution until the solution just turns pink. Then add hydrochloric acid solution drop by drop until the pink colour disappears; a drop is usually sufficient. Note -If the ammonia is added too fast. it is possible to overrun the colour change from yellow to pink and the sample continues to be yellow. It is then impossible to develop the pink colour by addition of ammonia solution. 3. Titrate with standard barium chloride solution (6.9) until sample just turns pink.
Calculation with units of expression	Prepare a calibration curve, mL of standard barium chloride solution needed to titrate standard sulphate solution vs mg/L SO4 and read the sulphate concentration of sample directly from the graph.
Inference (Qualitative Analysis)	NA
Reference	IS: 3025 (Part 24) – 1986 (Reaffirmed 1992) - Methods of Sampling and Test (Physical and chemical) for water and Waste Water : Sulphate
Approved by	Scientific Panel on Methods of Sampling and Analysis

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Method No.	**FSSAI 14.031:2024**	**Revision No. & Date**	0.0
Scope	Sulfide is often present in groundwater and sediment. It is produced by decomposition of organic matter and bacterial reduction of sulfate. It is sometimes found in industrial or municipal wastewater. Hydrogen sulfide escaping into the air from sulfide-containing wastewater causes odor nuisances. The threshold odor concentration of H_2S in clean water is between 0.025 and 0.25 µg/L. Gaseous H_2S is very toxic and has claimed the lives of numerous workers. At levels toxic to humans it interferes with the olfactory system, giving a false sense of the safe absence of H_2S. It attacks metals directly and indirectly has caused serious corrosion of concrete sewers because it is oxidized biologically in the presence of oxygen to H_2SO_4 on the pipe wall. Dissolved H_2S is toxic to fish and other aquatic organisms.		
Caution	Reduced sulphur compounds such as sulphite thiosulphate and hydrosulphite which decompose in acid may yield erratic results. Volatile iodine consuming substances will give high results. Eliminate interferences due to sulphite, thiosulphate, iodide and many other soluble substances but not ferro-cyanide, by first precipitating zinc sulphide removing the supernatant, and replacing it with distilled water. Use the same procedure even when not needed for removal of interferences, to concentrate sulphide.		
Principle	Sulphides are stripped from the acidified sample with an inert gas and collected in zinc acetate solution. Excess iodine solution added to the zinc sulphide suspension reacts with the sulphide under acidic condition. Thiosulphate is used to measure unreacted iodine to indicate the quantity of iodine consumed by sulphide. The reaction may be given as follows: 1.1. $S + I_2 = S_2 + 2I$ 1.2. I_2 (excess) $+ 2S_2O_3 = S_4O_6 + 2I$		
Apparatus/Instruments	**1. Reaction Flask:** Wide mouth bottle of 1 litre capacity with a 2 hole stopper, fitted with a fritted gas-diffusion tube (plastic, ceramic or glass and a gas outlet tube). **2. Absorption flasks**: Two 250 mL capacity long necked flask with 2 hole stoppers fitted with glass tubes and suitable connections to pass gas through in series.		
Materials and Reagents	**1. Zinc acetate solution (2 N) –** Dissolve 110 gm Zn $(C_2H_3O_2)_2.2H_2O$ on 400 mL distilled water and		

	finally make up to 1 litre **2. Inert gas**- A cylinder of nitrogen [pure grade, see IS: 1747- 1972 Specification for nitrogen (first revision)] or CO2 or a CO2 gas generator [Grade 1 see IS: 307- 1966 specification for carbon dioxide (second revision)] **3. Sulphuric Acid** concentrated **4. Standard iodine solution (0.025 N**) – Dissolve 20-25 gm potassium iodide (Kl) in a little water and add 3.175 gm iodine. After iodine has dissolved, dilute to 1 litre with distilled water, standardize this solution against 0.025 N sodium thiosulphate using starch indicator. **5.** Hydrochloric acid concentrated **6. Standard thiosulphate solution (0.025 N):** Dissolve 6.205gm Na2S2O25H2O in 800 mL boiled and cooled distilled water. Add 0.4 gm NaOH or 5 mL chloroform as a preservative and finally make up to 1 litre **7. Starch indicator solution**: Add 5.0 gm starch to 800 mL boiling distilled water & stir. Dilute to one litre and boil for few minutes and let settle over night. Use the clear supernatant. (This solution may be preserve by adding 1.25 gm of salicylic acid/litre or by adding a few drops of toluene) **8. Aluminum Chloride solution (6 N**): Take the 100 gm AlCl3.6H2O from a previously unopened reagent bottle and dissolve in 144mL distilled water. Note- because of the hygroscopic and caking tendencies of this chemical it will be convenient to purchase in small packing **9. Sodium hydroxide (6 N):**Dissolve 240 gm NaOH in distilled water and dilute to 1 litre
Preparation of Reagents	Same as above.
Sample Preparation	Put required quantity of 2 N zinc acetate solution into 500 mL glass bottle, fill with sample and add required quantity of 6 N sodium hydroxide solution. Stopper with no air bubbles under stopper and mix by rotating back and forth vigorously about a transverse axis. Addition of reagents may be varied in volume so that the resulting precipitate is not excessively bulky and settles rapidly. Add enough sodium hydroxide to produce a pH above 9. Let the precipitate settle for 30 minutes. Filter the precipitate through glass fiber filter paper and carry out titration immediately.
Method of analysis	**1 Total sulphide:** 1.1 Take 5 mL zinc acetate solution and 95 mL distilled water into each of the two absorption flasks 1.2 Connect the reaction flask and two absorption flasks in series and purge

	the system with CO2 or N2 for 2 minutes. Measure 500 mL well mixed with sample into the reaction flask 1.3 Acidify the sample with 10 mL concentrated H2SO4 and replace the prepared 2 holes stopper tightly pass N2 or CO2 (Not air or oxygen) through the sample for 1 hour or until the experiments show no more sulphide coming over 1.4 To each of the absorption flasks, then add iodine solution well in excess of the amount necessary to react with collected sulphide 1.5 Add 2.5 mL concentrated HCl acid to each flask, stopper and shake to mix thoroughly 1.6 Transfer contents of bath flasks and back titrate with 0.025 N sodium thiosulphate solution using starch solution as indicator. Run a blank parallel for accurate results. **2.2 Dissolved Sulphide** 2.1 Remove suspended solids in the sample by flocculation and settling. 2.2 Fill 1 litre bottle with flowing sample in such a way that the sample, which has had the least possible contact with air. Add 2 mL aluminium chloride solution and 2 mL NaOH solution and stopper with no air bubbles under the stopper. Rotate back and forth about a transverse axis as vigorously as possible for at least 1 minute in order to flocculate the contents thoroughly. Note- The volume of these chemicals may be varied according to experience, the idea being to get good clarification without using excessively large amounts. 2.3 Allow to settle for 15minutes, or until supernant liquid is reasonably clear. Alternatively remove suspended matter by centrifugation. 2.4 Proceed as for total sulphide after taking 500 mL sample into the reaction flask.
Calculation with units of expression	$$\text{Mg/L Sulphide} = \frac{(V1 - V2) \times 400}{V}$$ Where, V1= volume in mL of standard iodine solution added V2 = Volume in mL of standard thiosulphate solution used, and V = Volume in mL of sample taken
Interferences	1. Reduced sulphur compounds such as sulphite thiosulphate and hydrosulphite which decompose in acid may yield erratic results. 2. Volatile iodine consuming substances will give high results.

	3. Eliminate interferences due to sulphite, thiosulphate, iodide and many other soluble substances but not ferro-cyanide, by first precipitating zinc sulphide removing the supernatant, and replacing it with distilled water. Use the same procedure even when not needed for removal of interferences, to concentrate sulphide.
Reference	1. APHA 24TH EDITION 2023 2. IS 3025 (PART 29) 1986
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Method No.	**FSSAI 14.032:2024**	**Revision No. & Date**	0.0
Scope	Sulfide is often present in groundwater and sediment. It is produced by decomposition of organic matter and bacterial reduction of sulfate. It is sometimes found in industrial or municipal wastewater. Hydrogen sulfide escaping into the air from sulfide-containing wastewater causes odor nuisances. The threshold odor concentration of H2S in clean water is between 0.025 and 0.25 µg/L. Gaseous H2S is very toxic and has claimed the lives of numerous workers. At levels toxic to humans it interferes with the olfactory system, giving a false sense of the safe absence of H2S. It attacks metals directly and indirectly has caused serious corrosion of concrete sewers because it is oxidized biologically in the presence of oxygen to H2SO4 on the pipe wall. Dissolved H2S is toxic to fish and other aquatic organisms.		
Caution	Humic substances may interfere with Ag/S-ISE measurements. For highly colored water (high concentration of humic substances), use the method of standard additions to check results. Sulfide is oxidized by dissolved oxygen. Sulfide oxidation may cause potential readings to drift in the direction of decreasing concentration, i.e., to more positive values. Flush surface of samples and standards with nitrogen to minimize contact with atmospheric oxygen for low-level measurements. Temperature changes may cause potentials to drift either upward or downward. Therefore, let standards and samples come to the same temperature. If samples cannot be analyzed immediately, preserve dissolved sulfide by precipitating with zinc acetate (4500-S 2- .C).		
Principle	The potential of a silver/sulfide ion-selective electrode (ISE) is related to the sulfide ion activity. An alkaline antioxidant reagent (AAR) is added to samples and standards to inhibit oxidation of sulfide by oxygen and to provide a constant ionic strength and pH. Use of the AAR allows calibration in terms of total dissolved sulfide concentration. All samples and standards must be at the same temperature. Sulfide concentrations between 0.032 mg/L (1 x 10-6M) and 100 mg/L can be measured without preconcentration. For lower concentrations, preconcentration is necessary.		
Apparatus/Instruments	1. Silver/sulfide electrode. * 2. Double-junction reference electrode. 3. Electrode polishing strips. 4. pH meter with mill volt scale, capable of 0.1-m V resolution. Meters that can be calibrated in concentration and that perform standard-additions calculations are available. 5. Electrochemical cell: Make suitable cell from a 150mL beaker and a sheet of rigid plastic (PVC or acrylic) with holes drilled to allow insertion of the electrodes and a tube for flushing the headspace		

	with nitrogen. Alternatively, purchase a polarographic cell with gas transfer tube. 6. Gas dispersion tube: Use to deaerate water for preparing reagents and standards. 7. Magnetic stirrer and stirring bar: Use a piece of Styrofoam or cardboard to insulate the cell from the magnetic stirrer
Materials and Reagents	1. Alkaline antioxidant reagent (AAR). 2. Lead per chlorate, 0.1 M 3. Sulfide stock solution 4. Sulfide standards
Preparation of Reagents	Alkaline antioxidant reagent (AAR): To approximately 600 mL deaerated reagent water (DRW) in a 1L volumetric flask, add 80 gm NaOH, 35 gm ascorbic acid, and 67 gm Na2H2EDTA. Swirl to dissolve and dilute to 1L. The color of freshly prepared AAR will range from colorless to yellow. Store in a tightly capped brown glass bottle. Discard when solution becomes brown. Lead per chlorate, 0.1 M: Dissolve 4.60 gm Pb(CIO4)2 . 3H2O in 100 mL reagent water. Standardize by titrating with Na2H2EDT A. Alternatively, use commercially available 0.1 M Pb(CIO4)2 solutions. Sulfide stock solution: Dissolve 3.75 gm of NA2S.9H2Oand diluted to 500 mL will give a stock solution of which 1.00 mL= 1 mg S-2. Dilute 13.0 mL of 1.00 mg S2- /mL stock to 100.0 mL with AAR. Alternatively, add 500 mL AAR and 1 g Na2S.9H2O to a 1L volumetric flask; dissolve. Dilute to 1L with DRW. Use deaerated artificial seawater (DASW) or 0.7M NaCl if sulfide concentrations are to be determined in seawater. Standardize stock solution by titrating with 0.1MPb(CIO4)2 . Pipet 50 mL sulfide stock solution into the electrochemical cell. (Use 10 mL with a small-volume polarographic cell.) Insert Ag/S electrode and reference electrode and read initial potential. Titrate with 0.1 MPb(CIO4)2 .Let electrode potential stabilize and record potential after each addition. Locate equivalence point as in Section 45OO-Cl-.D 4a. Alternatively, linearize the titration curve1 Calculate the function F1for points before the equivalence point $F1 = (V0 + V)\ 10^{E/m}$ where: V 0= volume of stock solution, mL, V = titrant volume, mL, E = potential, mV, and m = slope of calibration curve, mV/log unit. Plot F1 as a function of titrant volume. Extrapolate to find the intersection with the x-axis; that is, the equivalence point. Calculate sulfide concentration in the stock solution from:

	C= Veq [Pb] V0 where: C = sulfide concentration, mg/L, Veq = equivalence volume, mL, [Pb] = concentration of Pb in titrant, mg/L, and Vo = volume of stock solution, mL. Store stock solution in a tightly capped bottle for 1 week or less. The stock solution also can be standardized iodometrically. CAUTION: Store in a fume hood. Sulfide standards: Prepare sulfide standards daily by serial dilution of stock. Add AAR and Zn (C2H302)2 solutions to100mL volumetric flasks. Add sulfide solutions and dilute to volume with DRW (or DASW). Prepare at least one standard with a concentration less than the lowest sample concentration.
Sample Preparation	NA
Method of analysis	Check electrode performance and calibrate daily. Check electrode potential in a sulfide standard every 2 hr. The procedure depends on the sulfide concentration and the time between sample collection and sulfide determination. If the total sulfide concentration is greater than 0.03 mg/L (1 X 10-6M) and the time delay is only a few minutes, sulfide can be determined directly. Otherwise, precipitate ZnS and filter as described in 4500-S 2- .C. Check electrode performance: Pipet 50 mL AAR, 50 mL DWR, and 1 mL sulfide stock solution into the measurement cell. Place Ag/S and reference electrodes in the solution and read potential. Add 10 mL stock solution and read potential. The change in potential should be -28 ±2 mV. If it is not, follow the troubleshooting procedure in the electrode manual. Calibration: Place electrodes in the most dilute standard but use calibration standards that bracket the sulfide concentrations in the samples. Record potential when the rate of change is less than 0.3 m V/min (This may take up to 30 min for very low sulfide concentrations, i.e., less than 0.03 mg/L.) Rinse electrodes, blot dry with a tissue, and read potential of the next highest standard. For a meter that can be calibrated directly in concentration, follow manufacturer's directions. For other meters plot potential as a function of the logarithm (base 10) of the sulfide concentration. For potentials in the linear range, calculate the slope and intercept of the linear portion of the calibration plot. Sulfide determination by comparison with calibration curve, no ZnS precipitation: Add 40 mL AAR, 0.15 mL (3 drops) zinc acetate, and 50mL sample to a 100mL volumetric flask. Dilute to 100 mL with AAR. Pour into

	the electrochemical cell and insert the electrodes. Record potential when the rate of change is less than 0.3 mV/min. Read sulfide concentration from the calibration curve. Alternatively, for potentials in the linear range, calculate the sulfide concentration from: $STot = 10^{(E-b)/m}$ Where: E = electrode potential and B and m are the intercept and slope of the calibration curve. For a meter that can be calibrated directly in concentration, follow the manufacturer's directions. Sulfide determination by comparison with calibration curve, with ZnS precipitation: Place filter with ZnS precipitate in a 150mL beaker containing a stir bar. Wash sample bottle with 50 mL AAR and 20 mL DRW and pour the washings into the beaker. Stir to dissolve precipitate. Remove filter with forceps while rinsing it into the beaker with a minimum amount of DRW. Quantitatively transfer to a 100mL volumetric flask and dilute to mark with DRW. Pour into the electrochemical cell and place the electrodes in the solution. Measure potential as in 4.3 above. Calculate sulfide concentration (above) Sulfide determination by standard addition with or without ZnS precipitation: Measure the Ag/S-ISE electrode potential as in 4.3 or 4.4 above. Add sulfide stock solution and measure potential again. Calculate sulfide concentration as follows: $Co = \frac{fCS}{(1+f)10^{(E_s - E_o)/m} - 1}$ where: Co and Cs= sulfide concentrations in sample and known addition, Eo and Es= potentials measured for sample and known addition, m = slope of calibration curve (approximately 28 mV/log S^{2-} and f = ratio of known-addition volume to sample volume
Calculation with units of expression	As mentioned above
Interferences	NA
Reference	IS 3025 (PART 29) 1986. APHA 4500S-2
Approved by	Scientific Panel on Methods of Sampling and Analysis

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Method No.	**FSSAI 14.033:2024**	**Revision No. & Date**	0.0
Scope	Cyanide refers to all of the CN groups in cyanide compounds that can be determines as the cyanide ion, CN; by the methods used. The cyanide compounds in which cyanide can be obtained as CN- are classed as simple and complex cyanide. Two methods for determination of total cyanides in water have been given.		
Caution	Potassium cyanide is highly toxic, take care to avoid ingestion; use gloves while preparing solution.		
Principle	Distillation of sample in the presence of sulphuric acid converts simple and complex cyanides into hydrocyanic acid. The hydrogen cyanide gas is absorbed in a solution of sodium hydroxide and the cyanide is determined colorimetrically. $Fe(CN)6^{-4} + 6^{H+} \Longrightarrow 6HCN + Fe^{+2}$ $HCN + NaOH \Longrightarrow NaCN + H_2O$ In the colorimetric measurement the cyanide in the sodium hydroxide solution after distillation is converted to cyanogen chloride by reaction with chloramine-T. The cyanogen chloride then forms a blue dye on the addition of pyridine-pyrazolone reagent and the absorbance is measured at 620 nm or pyridine-barbituric acid reagent and the absorbance is measured at 575 and 582 nm.		
Apparatus/Instruments	1. Boiling flask- 1 litre with inlet tube and provision for water cooled condensers 2. Heating mantle 3. Gas absorber- with gas dispersion tube equipped with medium- porosity fritted outlet 4. Ground glass ST joints- TFE sleeved or with an appropriate lubricant for the boiling flask and condenser. Neoprene stopper and plastic threaded joints may also be used. 5. Spectrophotometer- for use at 620 nm, providing a light path of 1 cm used in pyridine-Pyrazolone reagent Method 6. One of the following is required in case of Pyridine-barbituric acid reagent: i) Spectrophotometer, for use at 578 nm, providing a light path of 10 mm or longer ii) Filter photometer, providing a light path of at least 10 mm and equipped with a red filter having maximum transmittance at 570 to 580 mm.		
Materials and Reagents	1. Sodium hydroxide solution- Dissolve 50 gm sodium hydroxide in 1 litre distilled water. 2. Lead carbonate- Powdered. 3. Sulphamic acid (NH2SO3H).		

	4. Magnesium Chloride solution- Dissolve 51 gm magnesium chloride ($MgCl_2.6H_2O$) in 100 mL distilled water. 5. Sulphuric acid concentrated. 6. Sodium Hydroxide solution (0.2 N) - Dissolve 8.0 gm sodium hydroxide in 1 litre distilled water. 7. Acetic Acid- Make by diluting 1 part of glacial acid with 4 parts of water 8. Pyridine-Pyrazolone reagent Method: • Stock cyanide solution • Standard cyanide solution • Chloramine- T • Pyridine • 1-phenyl-3-methyl-5 pyrazolone solution • Bis-pyrazolone (3,3'-dimethyl-1-diphenyl) (4,4'-bis 2pyrazolone)-(5,5' dione) • Mixed pyridine-Pyrazolone reagent • Standard silver nitrate solution 9. Pyridine-barbituric acid reagent Method: • CholoramineT- Solution • Stock cyanide solution • Standard cyanide solution • Pyridine-barbituric acid reagent • Acetate buffer • Sodium Hydroxide dilution solution
Preparation of Reagents	1. Sodium hydroxide solution- Dissolve 50 gm sodium hydroxide in 1 litre distilled water. 2. Lead carbonate- Powdered. 3. Sulphamic acid (NH_2SO_3H). 4. Magnesium Chloride solution- Dissolve 51 gm magnesium chloride ($MgCl_2.6H_2O$) in 100 mL distilled water. 5. Sulphuric acid concentrated. 6. Sodium Hydroxide solution (0.2 N) - Dissolve 8.0 gm sodium hydroxide in 1 litre distilled water. 7. Acetic Acid- Make by diluting 1 part of glacial acid with 4 parts of water 8. **Pyridine-Pyrazolone reagent Method**: • Stock cyanide solution- Dissolve 2.51 gm potassium cyanide, in 1 litre water, standardise this solution with 0.019 2 N silver nitrate solution. This solution loses strength gradually and must be rechecked every week (1 mL of this solution = 1 mg CN) • Standard cyanide solution - Dilute 10 mL stock solution to 1 litre with distilled water, mix and make a second dilution of 10mL to 100 mL. One mL = 1 µg CN Note = this solution must be prepared daily

(Caution: Toxic, take care to avoid ingestion)

- Chloramine- T-Dissolve 1 gm of chloramines- T in 100 mL distilled water. Prepare daily.
- Pyridine
- 1-phenyl-3-methyl-5 pyrazolone solution - Prepare a saturated aqueous solution (approximately 0.5 g/100 mL) by adding the pyrazolone to water at 75°C. Agitate occasionally as the solution cools to room temperature. If necessary, the pyrazolone (melting point 127° to 128°C) can be purified by recrystallisation from ethyl alcohol. Usually this is not required.
- Bis-pyrazolone (3,3'-dimethyl-1-diphenyl) (4,4'-bis-2pyrazolone)-(5,5' dione)
- Mixed pyridine-Pyrazolone reagent- Mix 125 mL of the filtered saturated aqueous solution of pyrazolone with a filtered solution containing 0.025 gm bis□pyrazolone dissolved in 25 mL pyridine. Several minutes of mixing is usually necessary to dissolve the bis-pyrazolone in pyridine.

Note- Prepare the reagent daily. This reagent develops a pink colour on standing

- Standard silver nitrate solution- Dissolve 3.27 gm of silver nitrate in 1 litre of distilled water. Store in dark bottle.

1 mL of this solution= 1 mg CN

9. Pyridine-barbituric acid reagent Method:

- CholoramineT- Solution: Dissolved 1.0 gm white, water soluble powder in 100 mL water. Prepare a weekly and store in refrigerator.
- Stock cyanide solution: Dissolve approximately 1.6 gm NaOH and 2.51 gm KCN in 1 L distilled water. standardized against standard silver nitrate ($AgNO_3$) titrant using 25 mL KCN solution. Check titer weekly because the solution gradually loses strength; 1mL= 1 mg CN^-
- Standard cyanide solution: Based on the concentration determined for the KCN stock solution (as above) calculate volume required (approximately 10 mL) to prepare 1 liter of 10 µg CN- /mL. Dilute with NaOH dilution solution .Dilute 10 mL of the 10 µg CN^- /mL solution to 100 mL with the NaOH dilution solution; 1.0 mL=1.0 µg CN^-. Prepare fresh daily and keep in a glass-Stoppered bottle. (CAUTION- toxic; take care to avoid ingestion)
- Pyridine-barbituric acid reagent: Place 15 gm barbituric acid in a 250mL volumetric flask and add just enough water to wash sides of flask and wet barbituric acid. Add 75 mL pyridine and mix. Add 15 mL conc. hydrochloric acid (HCI), mix, and cool to room temperature. Dilute to volume and mix until barbituric acid is dissolved. The solution is stable for approximately 6 months if stored in an amber bottle under refrigeration; discard if precipitate develops.
- Acetate buffer: Dissolve 410 gm sodium acetate trihydrate.

	$NaC_2H_3O_2.3H_2O$ in 500 mL of water. Add glacial acetic acid to adjust to pH 4.5, approximately 500 mL. • Sodium Hydroxide dilution solution: Dissolve 1.6 gm NaOH in 1 L distilled water.
Sample Preparation	1. The sample should be collected in 2- litre polyethylene bottle and analyzed as soon as possible after collection 2. Samples should be preserved by addition of sufficient hydroxide to raise the pH to 11.0 or above and be stored in a cool place.
Method of analysis	**1. Distillation** • Add 500 mL sample to the boiling flask. Add 10 mL of sodium hydroxide solution to gas scrubber and dilute, if necessary, with distilled water to obtain an adequate liquid depth in the absorber. Do not use more than 225 mL total volume of absorber solution. When sulphide generation from the distilling flask is anticipated, add 50 or more mg powdered lead carbonate to the absorber solution to precipitate sulphide. Connect the train, consisting of boiling flask air inlet, flask condenser, gas washer, suction flask trap and aspirator. Adjust suction so that approximately 1 air bubble per second enters the boiling flask. The air rate will carrying hydrogen cyanide gas from flask to absorber and usually will prevent a reverse flow of hydrogen cyanide gas through the air inlet. If this air rate does not prevent sample backup in the delivery tube, increase air flow rate to 2 air bubbles per second. Observe air purge rate in the absorber where the liquid level should be raised not more than 6.5 to 10 mm. Maintain airflow through the reaction • Add 2 gm of Sulphamic acid through the air inlet tube and wash down with distilled water • Add 50 mL of concentrated sulphuric acid through the air inlet tube with distilled water and let air mix flask contents for 3 minutes. Add 20 mL of magnesium chloride reagent through air inlet and wash down with stream of water. A precipitate that may form re-dissolves on heating. • Heat with rapid boiling, but do not flood condenser inlet or permit vapors to rise more than halfway into condenser. Adequate refluxing is indicated by reflux rate of 40 to 50 drops/min from the condenser lip. Reflux for at least 1 hour. Discontinue heating but continue air flow. Cool for 15 minutes and drain gas washer contents into separate container. Rinse connecting tube between condenser and gas washer with distilled water, and rinse water to drained liquid, and make upto 250 mL in a volumetric flask. **2. For colorimetric measurement (By pyridine-Pyrazolone reagent)** • Transfer 15 mL of distillate to a 50 mL beaker. • To prepare standard solutions for the calibration curve, use cyanide standard 1 mL = 1 mg CN. Pipette 0 (blank), 0.2, 0.5, 0.8 & 1.0 mL

	into 50 mL beaker and make up to 15 mL with 0.2 N sodium hydroxide solution proceed with 4.8.3 to 4.8.7, treating samples and standards in the same manner. • Adjust pH at 6-7 with acetic acid (4.7); transfer to 25 mL volumetric flask • Add 0.2 mL chloramines- T solution and mix. Allow 2 minutes for the reaction. • Add 5.0 mL mixed pyridine- pyrazolone reagent (4.8.7) and make up to the mark, mix allow 20 minutes for colour development • Read absorbance at 620 nm in a 1 cm cell 5.2.7 As a check on the distillation step, periodically process cyanide standard solutions through the complete procedure **3. For colorimetric measurement (By Pyridine-barbituric acid reagent)** • **Preparation of standard curve:** Pipette a series of standards containing 1 to 10 µg CN^- into 50mL volumetric flasks (0.02 to 0.2 µg CN-/mL). Dilute to 40 mL with NaOH dilution solution. Use 40 mL of NaOH dilution solution as blank. Develop and measure absorbance in 10mm cells as described below for both standards and blank. For concentrations lower than 0.02 µg CN^-/ ml use 100 mm cells. Recheck calibration curve periodically and each time a new reagent is prepared • **Color Development**: Pipette a portion of absorption solution into a 50mLvolumetric flask and dilute to 40 mL with NaOH dilution solution. Add 1 mL acetate buffer and 2 mL chloramines-T solution, stopper, and mix by inversion twice. Let stand exactly 2 min. • Add 5 mL pyridine-barbituric acid reagent, dilute to volume with distilled water, mix thoroughly and let stand exactly 8min. Measure absorbance against distilled water at 578 nm. Measure absorbance of blank (0.0 mg CN^-/L) using 40 mL NaOH dilution solution and procedures for color development.
Calculation with units of expression	**For colorimetric measurement (By pyridine-Pyrazolone reagent)** • Prepare a calibration curve derived by plotting concentrations versus Absorbances • Determine the micrograms of cyanide in the samples by comparing on calibration curve. • Calculate the cyanide concentration as follows: $$\text{mg/L, CN} = \frac{A \times B}{C \times D}$$ Where A= cyanide determined in mg by calibration graph B= diluted absorbing solution in mL C= original sample in mL, and D= sample taken for colorimetric measurement in mL **For colorimetric measurement (By Pyridine-barbituric acid reagent)**

	• Use the linear regression feature available on most scientific calculators, or compute slope and intercept of standard curve as follows: $M = \frac{n \sum ca - \sum c \sum a}{n \sum a2 - (\sum a)2}$ $b = \frac{\sum a2 \sum c - \sum a \sum a\, c}{2n \sum a2 - (\sum a)2\, a}$ Where: a= absorbance of standard solution, c= concentration of CN⁻ in standard, mg/L n= number of standard solutions, m= slope of standard curve, and b= intercept on c axis Include the blank concentration, 0.0 mg CN-/L and blank absorbance in the calculations above. $\mathbf{CN^-, mg/L = (ma_1 + b) \times \frac{50}{X} \times \frac{250}{Y}}$ Where: X= absorption solution, mL, Y= original sample mL and a1= absorbance of sample solution.
Inference (Qualitative Analysis)	NA
Reference	• IS 3025 (PART 27)-1986 • APHA 4500 CN⁻
Approved by	Scientific Panel on Methods of Sampling and Analysis

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Method No.	**FSSAI14.034:2024**	**Revision No. & Date**	0.0
Scope	Cyanide refers to all of the CN groups in cyanide compounds that can be determines as the cyanide ion, CN; by the methods used. The cyanide compounds in which cyanide can be obtained as CN- are classed as simple and complex cyanide. Two methods for determination of total cyanides in water have been given.		
Caution	Cyanide is highly toxic, take care to avoid ingestion; use gloves while preparing solution		
Principle	Cyanide in the alkaline distillate from the preliminary treatment, as given in distillation step of cyanide estimation by colorimetric method can be determined potentiometrically by using a cyanide in selective electrode in combination with a double junction reference electrode and a pH meter having an expanded millivolt scale, or specific ion meter.		
Apparatus/Instruments	1. Expanded scale pH meter or specific ion meter 2. Cyanided-ion-selective electrode 3. Reference electrode, double junction 4. Magnetic mixer with TFE coated stirring bar		
Materials and Reagents	1. Stock standard cyanide solution 2. Sodium hydroxide dilutent 3. Intermediate standard cyanide solution 4. Dilute standard cyanide solution 5. Potassium nitrate solution		
Preparation of Reagents	1. Stock standard cyanide solution 2. Sodium hydroxide dilutent- Dissolve 1.6 gm sodium hydroxide in water and dilute to 1 litre 3. Intermediate standard cyanide solution – Dilute a calculated volume (approx. 100 mL) of stock potassium cyanide solution, based on the determined concentration, to 1000 ml with sodium hydroxide diluent. Mix thoroughly; 1 mL = 100 µg CN^- 4. Dilute standard cyanide solution- Dilute 100 mL intermediate cyanide standard solution to 1000 mL with sodium hydroxide diluents; 1.00 mL = 10.0 µg CN^- . Prepare daily and keep in a dark, glass Stopperd bottle 5. Potassium nitrate solution- Dissolve 100 gm potassium nitrate in water and dilute to 1 liter. Adjust to pH 12 with potassium hydroxide. This is the outer filling solution for the double-junction reference electrode.		
Sample Preparation	--		
Method of analysis	**1. Calibration** Use the dilute and intermediate standard cyanide solutions and sodium hydroxide diluent to prepare a series of three standards, 0.1, 1.0 and 10.0 mg CN-/1. Transfer approximately 100 mL of each of these standard solutions into a 250 mL beaker pre-rinsed with a small portion of standard		

	being tested. Immerse CN- and double-junction reference electrodes. Mix well on a magnetic stirrer at 27°C maintaining as closely as possible the same stirring rate for all solutions. Always progress from the lowest to the highest concentration of standard otherwise equilibrium is reached only slowly. The electrode membrane dissolves in solutions of high cyanide concentration; do not use with a concentration above 10 mg/L. After making measurements remove electrode and soak in water. After equilibrium is reached (at least 5 min and not more than 10 min) record potential (millivolt) readings and plot CN- concentrations versus readings on semi-logarithmic graph paper. A straight line with a slope approximately 59 m V per decade indicates that the instrument and electrodes are operating properly. Record slope of line obtained (millivolts/decade of concentration). The slope may vary somewhat from the theoretical value of 59.2 mV per decade because of manufacturing variation and reference electrode (liquid junction) potentials. The slope should be a straight line and is the basis for calculating sample concentration **2. Measurement of sample** Place 100 mL of absorption liquid obtained into a 250 mL beaker. When measuring low cyanide concentrations, first rinse beaker and electrodes with a small volume of sample. Immerse cyanide and double- junction reference electrodes and mix on a magnetic stirrer at the same stirring rate used for calibration. After equilibrium is reached (at least 5 min and not more than 10 min) record values indicated on ion meter or found from graph prepared above. Calculate concentration as given below.
Calculation with units of expression	Cyanide, mg/L = $\frac{A \times B}{C}$ Where A= mg cyanide per liter found from meter reading or graph B= total volume of absorption solution after dilution, mL; and C= volume of original sample used in the distillation, mL
Inference (Qualitative Analysis)	NA
Reference	• IS 3025 (PART 27)-1986 • APHA 4500 CN
Approved by	Scientific Panel on Methods of Sampling and Analysis

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Method No.	**FSSAI14.035:2024**	**Revision No. & Date**	0.0
Scope	The average abundance of Ca in the earth's crust is 4.9%: in soils it is 0.07 to 1.7 % in streams it is about 15 mg/L; and in groundwater it is from 1 to >500 mg/L. The most common forms of calcium are calcium carbonate (calcite) and calcium-magnesium carbonate (dolomite). Calcium compounds are widely used in pharmaceuticals photography, lime, de-icing salts, pigments, fertilizers, and plasters. Calcium carbonate solubility is controlled by pH and dissolved CO2. The CO2, HCO3 – and CO3 2- equilibrium is the major buffering mechanism in fresh waters. Hardness is based on the concentration of calcium and magnesium salts, and often is used as a measure of potable water quality. This method explains the titrimetric process of calcium.		
Caution	Under conditions of this test, the following concentrations of ions cause no interference with the calcium determination: Copper, 2 mg/L; ferrous iron 20 mg/L; ferric iron, 20 mg/L; manganese 20 mg/L; zinc 5 mg/L. Orthophosphate precipitates calcium at the pH of the test. Strontium and barium give a positive interference and alkalinity in excess of 300 mg/L may cause an indistinct end point in hard waters.		
Principle	In a solution containing both calcium and magnesium, calcium can be determined directly with EDTA (ethylenediamine tetra-acetic acid or its salts) when the pH is made sufficiently high (12 to 13) so that the magnesium is largely precipitated as the hydroxide and an indicator is used which combines, only with calcium.		
Apparatus/Instruments	Hot plate- One 30 x 50 cm heating surface is adequate		
Materials and Reagents	a. Quality of Reagents. Unless specified otherwise, pure chemicals and distilled water shall be used in tests. b. Sodium Hydroxide Solution- 1N c. Hydrochloric Acid- 0.1 N d. Indicator solution: Any of the following indicates shall be used. e. Murexide (ammonium purpurate) indicator f. Patton and Reeder's indicator g. Standard EDTA		
Preparation of Reagents	a. Quality of Reagents. Unless specified otherwise, pure chemicals and distilled water shall be used in tests. b. Sodium Hydroxide Solution- 1N c. Hydrochloric Acid- 0.1 N d. Indicator solution: Any of the following indicates shall be used. e. Murexide (ammonium purpurate) indicator solution: This indicator changes from pink to purple at the end point. An indicator solution can be prepared by dissolving 150 mg of the dye in 100 gm of absolute ethylene glycol. Water solutions of the dye are not stable for longer than		

	a day. A ground mixture of the dye powder and sodium chloride provides a stable form of the indicator. It is prepared by mixing 200 mg of murexide with 100 gm of solid sodium chloride and grinding the mixture to 300 to 425 microns. The titration should be performed immediately after the addition of the indicator because it is unstable under alkaline conditions. End point recognition is facilitated by the preparation of color comparison blank containing 2.0 ml of sodium hydroxide solution, 0.2 gm of solid indicator mixture (or 1 to 2 drops if a solution is used), and sufficient standard EDTA titrant (0.05 to 0.10 mL) to produce an unchanging color. f. Patton and Reeder's indicator solution: This indicator solution permits the direct titration of calcium in the presence of magnesium. It produces a sharp color change from wine red to pure blue at the end point. It is prepared by mixing 1 gm of Patton and Reeder's (Eriochrome blue Black R) reagent with 100 gm of sodium sulphate or potassium sulphate. g. Standard EDTA Solution- 0.01 M: Dissolve 3.75 gm of disodium ethylenediamine tetra-acetate, dihydrate in water and make up to 1000 mL in a volumetric flask. Standardize this with standard zinc solution. Pipette out 25 mL of standard zinc solution in a 250 mL conical flask. Adjust the pH to approximately 10 with buffer solution. Dilute to about 100 mL and add 3 to 4 drops of Eriochrome Black T indicator solution. This will give red color. Titrate with 0.01 M EDTA solution to a clear blue end point free from violet tinge. This solution will be slightly stronger than 0.01 M, dilute the solution to exactly 0.01 M by adding calculated amount of water and recheck the strength by titrating 25 mL of standard zinc solution by exactly the same manner as mentioned above. This should consume exactly 25.0 mL of standard EDTA solution. Alternatively, calcium solution may be used for standardization of EDTA subject to the availability of certified CaCO3 according to the method given below: WATER ANALYSIS 2016 147 Weigh 3.723 gm of dry analytical reagent grade disodium ethylene diamine tetra acetate, dihydrate, dissolve in distilled water and dilute to 1000 mL. Check the strength by standardizing against standard calcium solution. An exactly 0.01 M solution is equivalent to 0.4008 mg of calcium per milliliter. h. Stock Calcium Solution: Dry calcium carbonate (Ca CO3) at 180°C for one hour and allow it to cool in a desiccator. Suspend 2.50±0.01 gm of the dried material in 100 mL of water. Add slowly the minimum amount of 0.1N hydrochloric acid to dissolve the calcium carbonate (approximately 500 mL). Boil briefly to expel dissolved carbon dioxide, cool and transfer the solution quantitatively to a 1000 mL volumetric flask and dilute to mark with 0.1N hydrochloric Acid. i. Standard Calcium Solution: Dilute 100 mL of the stock solution (5.5) to 250 mL using 0.1N hydrochloric acid. This solution is equivalent to 1.00mg of calcium carbonate or 0.400 8 gm of calcium per milliliter.

	Store the solution in a polyethylene bottle.
Sample Preparation	Mix the sample pretreated, if so required and transfer a suitable volume (50 to 100 mL) to 250 mL conical flask or a beaker. Add 5 mL of concentrated nitric acid and evaporate on a hotplate at a slow boil to the lowest volume possible (about 15 to 20 mL) before precipitation or salting occurs. Add 5 mL of concentrated nitric acid, cover with a watch glass and heat to obtain a gentle refluxing action. Continue heating and adding concentrated nitric acid as necessary until digestion is complete as shown by a light colored clear solution. Do not let sample dry during digestion. Add 1 to 2 mL of concentrated nitric acid and warm slightly to dissolve any remaining residue. Wash down beaker walls and watch glass with water and then filter, if necessary. Transfer the filtrate to a 100 mL volumetric flask. Cool, dilute to mark and mix thoroughly. Take a portion of this solution for the determination of calcium.
Method of analysis	Because of the high pH used in this procedure, the titration should be performed immediately after the addition of the alkali and indicator. Use 50mL of sample or a smaller portion diluted to 50 mL so that the calcium content is about 5 to 10 mg. Analyze hard waters with alkalinity higher than 300 mg/LCaCO3 by taking a smaller aliquot and diluting to 50 mL or by neutralization of the alkalinity with acid, boiling for one minute and cooling before beginning the titration. Add 2.0 mL of sodium hydroxide solution or a volume sufficient to produce pH of 12 to 13. Stir. Add 0.1 to 0.2 gm of the indicator murexide-sodium chloride mixture selected (or 1 to 2 drops if a solution is used). Alternatively, approximately 1 gm of the mixture of Patton and Reeder's reagent and sodium sulphate or potassium sulphate may be used. Add EDTA titrant slowly with continuous stirring to the proper end point. Check the end point by adding 1 to 2 drop of titrant in excess to make certain that no further color change occurs.
Calculation with units of expression	Calcium (CaCO3) mg/L= $\frac{A \times CF \times 1000}{V}$ Calcium (Ca2+) mg/L = $\frac{A \times CF \times 1000 \times 0.4004}{V}$ Where A= volume in mL of EDTA solution used for titration. CF= mass in mg of calcium equivalent to 1 mL of EDTA solution, (X1/X2 correction factor for standardize ion of EDTA) X1 = volume in mL of standard calcium solution taken for standardization X2 = volume of mL of EDTA solution used in the titration V= volume in mL of the sample taken for the test.
Inference (Qualitative Analysis)	NA

Reference	IS 3025 (PART 40)
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Calcium by Permanganate Titration Method		
Method No.	**FSSAI 14.036:2024**	**Revision No. & Date**	0.0
Scope	The average abundance of Ca in the earth's crust is 4.9%: in soils it is 0.07 to 1.7 % in streams it is about 15 mg/L; and in groundwater it is from 1 to >500 mg/L. The most common forms of calcium are calcium carbonate (calcite) and calcium-magnesium carbonate (dolomite). Calcium compounds are widely used in pharmaceuticals photography, lime, de-icing salts, pigments, fertilizers, and plasters. Calcium carbonate solubility is controlled by pH and dissolved CO2. The CO2, HCO3 – and CO3 2- equilibrium is the major buffering mechanism in fresh waters. Hardness is based on the concentration of calcium and magnesium salts, and often is used as a measure of potable water quality. This method explains the titrimetric process by Permanganate of calcium in water.		
Caution	The sample should be free of interfering elements of strontium, silica, aluminium, iron, manganese, phosphate and suspended matter. Strontium may precipitate as oxalate and cause high results. In such cases, determine strontium by flame photometry. Interference of silica may be eliminated by classical dehydration procedure. Precipitate aluminum, iron, and manganese by ammonium hydroxide after treatment with persulphate. Precipitate phosphate as the ferric salt. Remove suspended matter by centrifuging or by filtration through sintered glass crucible or a cellulose acetate membrane.		
Principle	The calcium present in the solution is precipitated as oxalate filtered off and washed. The washed precipitate is dissolved in dilute sulphuric acid and the oxalic acid liberated is titrated against standard potassium permanganate solution. The homogeneous precipitation approach using the urea hydrolysis method is best suited for the precipitation of calcium oxalate. Initially the pH of the solution is adjusted to approximately 1.0 by adding sufficient amount of acid. This is followed by ammonium oxalate and urea. Upon boiling the solution, the urea gradually undergoes hydrolysis and the pH rises to the point of calcium oxalate precipitation. The precipitate is filtered off immediately after formation. This eliminates the digestion period which is otherwise required. The solution must remain clear until boiling is commenced to hydrolyse the urea.		
Apparatus/Instruments	Beakers with Glass Rod - 400mL capacity and cover glass. Filtration Set Up - A coarse filter paper or a small filter paper supported in a Gooch crucible with suction.		
Materials and Reagents	• Hydrochloric Acid • Methyl Red Indicator • Ammonium Oxalate Solution		

	• Urea 4.6 Dilute Sulphuric Acid • Sodium Oxalate • Potassium Permanganate Solution
Preparation of Reagents	a. Quality of Reagents Unless specified otherwise pure chemicals and distilled water shall be used in the tests. NOTE - Pure Chemicals shall mean chemicals that do not contain impurities which affect the results of analysis. b. Hydrochloric Acid - 1 N. c. Methyl Red Indicator Solution Dissolve 100 mg of methyl red sodium salt in 100 mL of hot water or dissolve in 60 mL of ethanol dilute with 40 mL of water. d. Ammonium Oxalate Solution- Saturated solution in water. e. Urea 4.6 Dilute Sulphuric Acid - 1 N f. Sodium Oxalate g. Standardization of Potassium Permanganate Solution. Weigh about 1.6 gm of AR grade potassium permanganate on a watch glass, transfer it to a 1500mL beaker, add 1 litre of water, cover the beaker with a watch glass, heat the solution to boiling; boil gently for 15-30 minutes and allow the solution to cool to the laboratory temperature. Filter the solution through a funnel, containing a plug of purified glass wool, or through a Gooch crucible provided with a pad of purified asbestos, or most simply, through a sintered glass or porcelain filtering crucible. Collect the filtrate in a vessel which has previously been cleaned with chromic acid mixture and then thoroughly washed with distilled water. Store the filtered solution in a clean, glass stoppered bottle. Keep it in the dark or in an amber coloured bottle or in diffused light except while in use. Weigh out accurately about 1.7 gm of dry sodium oxalate into a 250mL volumetric flask, dissolve it in water and make up to the mark. Pipette out 25mL of this solution into a 400mL beaker and add 150mL of 1 N sulphuric acid. Titrate this solution rapidly at room temperature with potassium permanganate solution tobe standardized while stirring, to a slight pink end point that persists 'for at least 1 minute. Do not let the temperature fall below 85°C. If' necessary, warm beaker contents during titration. Repeat the titration with two more aliquots of the oxalate solution. Calculate the normality of the permanganate solution using the following relationship: Normality of potassium = $\frac{100 \times m1}{67 \times V1}$ Permanganate solution Where m1= mass in gm of sodium oxalate taken, and

	V1= volume in mL of the potassium permanganate solution consumed by 25mL of the oxalate solution.
Sample Preparation	Mix the sample pretreated, if so required and transfer a suitable volume (50 to 100 mL) to 250 mL conical flask or a beaker. Add 5 mL of concentrated nitric acid and evaporate on a hotplate at a slow boil to the lowest volume possible (about 15 to 20 mL) before precipitation or salting occurs. Add 5 mL of concentrated nitric acid, cover with a watch glass and heat to obtain a gentle refluxing action. Continue heating and adding concentrated nitric acid as necessary until digestion is complete as shown by a light colored clear solution. Do not let sample dry during digestion. Add 1 to 2 mL of concentrated nitric acid and warm slightly to dissolve any remaining residue. Wash down beaker walls and watch glass with water and then filter, if necessary. Transfer the filtrate to a 100 mL volumetric flask. Cool, dilute to mark and mix thoroughly. Take a portion of this solution for the determination of calcium.
Method of analysis	Pipette out 50 mL of the sample (containing about 10 mg of calcium) into a 250mL beaker. Add dilute hydrochloric acid drop by drop to a pH of approximately 1.0. Add a few drops of methyl red indicator solution (sufficient acid must be present in the solution to prevent the precipitation of calcium oxalate when ammonium oxalate solution is added). Add about 10 mL of saturated ammonium oxalate solution gently until the methyl red changes colour to yellow (pH 5). Filter through a coarse filter paper or with suction on a small filter paper supported in a Gooch crucible. Wash the precipitate with cold water till the filtrate is free from chloride. Transfer the filter paper and the precipitate (or the Gooch crucible and precipitate) to the original beaker, dissolve the precipitate in hot dilute sulphuric acid and titrate immediately with standard 0.05N potassium permanganate solutions.
Calculation with units of expression	Calcium (as Ca) mg/L = $\frac{A \times B \times 100}{V}$ where A = volume in mL of permanganate solution used for the titration, B = mass in mg of calcium equivalent to 1 mL of potassium permanganate solution, and V = volume of the sample taken for the test.
Inference (Qualitative Analysis)	NA
Reference	IS 3025 (PART 40)
Approved by	Scientific Panel on Methods of Sampling and Analysis

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Method No.	**FSSAI 14.037:2024**	**Revision No. & Date**	0.0
Scope	Mineral water, Packaged Drinking Water (other than Mineral Water), Drinking Water (Purified)		
Caution	Phenol Stock Solution CAUTION - 'TOXIC, HANDLE WITH CARE',		
Principle	Steam-distillable phenols react with 4-aminoantipyrine at pH7.9 ± 0.1 in the presence of potassium ferricyanide to form a colored antipyrine dye. This dye is extracted from water with chloroform and the absorbance is measured at 460nm. This method covers the phenol concentration range from 1.0 mg/L to over 250 mg/L with a sensitivity of 1 mg/L.		
Apparatus/Instruments	a. Photometric equipment: A spectrophotometer for use at 460 nm equipped with absorption cells providing light paths of 1 to 10 cm, depending on the absorbances of the colored solutions and the individual characteristics of the photometer. b. Filter Funnels: Buchner type with fritted disk. c. Filter Paper: Alternatively use an appropriate 11-cm filter paper for filtering CHC13 extracts instead of the Buchner-type funnels and anhydrous Na2SO4. d. pH Meter. e. Separating Funnel: 1000-mL, Squibb form, with ground glass stoppers and PTFE stopcocks. At least 8 are required.		
Materials and Reagents	a. Stock Phenol Solution b. Intermediate Phenol Solution c. Standard Phenol Solution d. Bromate-bromide solution e. Hydrochloric acid (HCl), conc. f. Standard sodium thiosulfate titrant, 0.025 M g. Starch solution h. Ammonium hydroxide (NH4OH), 0.5 N i. Phosphate buffer solution j. Potassium ferricyanide solution k. Chloroform (CHCl3). l. Sodium sulfate, anhydrous NazSO4, granular. m. Potassium iodide (Kl), crystals.		
Preparation of Reagents	All reagents should be prepared with distilled water free from phenols and chlorine. a. **Stock phenol solution**: Dissolve 100 mg phenol in freshly boiled and cooled reagent water and dilute to 100 mL.. Ordinarily this direct weighing yields a standard solution. If extreme accuracy is required, standardize as follows: 1) To 100 mL water in a 500-mL glass-stoppered conical		

	flask, add 50.0 mL stock phenol solution and 1 O.O mL bromate□bromide solution. Immediately add 5 mL conc HCl and swirl gently. If the brown color of free bromine does not persist, add 10.0-mL portions of bromate-bromide solution until it does. Keep the flask stoppered and let stand for 10 min; then add approximately 1 g KI. Usually four 10-mL portions of the bromate□bromide solution are required if the stock phenol solution contains 1000 mg/L phenol. 2) Prepare a blank in exactly the same manner, using reagent water and 10.0 mL bromate-bromide solution. Titrate the blank and sample with 0.025 M sodium thiosulfate, using a starch solu□tion indicator. 3) Calculate the concentration of the phenol solution as follows: mg/L phenol = 7.842[(A x B)-C] where: A = mL thiosulfate for blank, B = mL bromate-bromide solution used for sample divided by 10, and C = mL thiosulfate used for sample. b. **Intermediate phenol solution:** Dilute 1.00 mL stock phenol solution in freshly boiled and cooled reagent water to 100 mL; 1 mL = 10.0 mg phenol. Prepare daily. c. **Standard phenol solution:** Dilute 50.0 mL intermediate phenol solution to 500 mL with freshly boiled and cooled reagent water; 1 mL = 1.0 mg phenol. Prepare within 2 h of use. d. **Bromate-bromide solution:** Dissolve 2.784 g anhydrous KBrO3 in water, add 10 g KBr crystals, dissolve, and dilute to 1000 mL. e. **Standard sodium thiosulfate titrant, 0.025 M:** Dissolve 6.205 g Na2S2O3 · 5H2O in reagent water. Add 1.5 mL 6 M NaOH or 0.4 g solid NaOH and dilute to 1000 mL. Standardize with potassium bi-iodate [KH(I03)z] solution. f. **Starch solution:** Use either an aqueous solution or soluble starch powder mixtures. To prepare an aqueous solution, dissolve 2 g laboratory-grade soluble starch and 0.2 g salicylic acid (as a preservative) in 100 mL hot reagent water. g. **Ammonium hydroxide (NH4OH), 0.5 N:** Dilute 35 mL fresh, conc NH4OH to 1 L with water. h. **Phosphate buffer solution:** Dissolve 104.5 g K2HPO4 and 72.3 g KH2PO4 in water and dilute to 1 L. The pH should be 6.8. i. **4-Aminoantipyrine solution:** Dissolve 2.0 g 4-aminoantipyrine in water and dilute to 100 mL. Prepare daily. j. **Potassium ferricyanide solution:** Dissolve 8.0 g K3Fe(CN)6 in water and dilute to 100 mL. Filter if necessary. Store in a brown glass bottle. Prepare fresh weekly.
Sample Preparation	Preliminary Step of Steam Distillation 1. Measure 500 mL of sample into a beaker. Lower the pH to approximately 4.0 with 8.5 percent phosphoric acid. If the sample was already preserved using phosphoric acid, omit the addition of

	phosphoric acid again. 2. Transfer to the distillation apparatus made up of glass, consisting of a 1 litre borosilicate glass distilling apparatus with Graham condenser. Distil 450 mL of sample and stop the distillation. When boiling ceases, add 50 mL of warm distilled water to the distilling flask and resume distillation until 500 mL have been collected. 3. If the distillate is turbid, filter through a pre washed membrane filter.
Method of analysis	1. Place 500 mL of distillate or a suitable portion containing not more than 50 mg phenol, diluted to 500 mL in 1 litre beaker. 2. Prepare a 500 mL distilled water blank and a series of 500 mL phenol standards containing 5, 10, 20, 30, 40 and 50 mg phenol. 3. Adjust samples, blank, and standards to pH 1 O± 0.1 with 1O mL of the following buffer solution (16.9 g NH4Cl in 143 mL conc NH4OH diluted to 250 mL with reagent water.) 4. Alternatively, for greater sensitivity to chlorinated phenols, adjust samples, blank, and standards to pH 7.9: Add 12.0 mL 0.5 N NH4OH and immediately adjust pH to 7.9 ± 0.1 with phosphate buffer. About 10 mL phosphate buffer are required. 5. Transfer to a 1 litre separating funnel, add 3.0 mL aminoantipyrine solution, mix well and add 3.0 mL of potassium ferricyanide and let color develop for 15 minutes. The solution should be clear and light yellow. 6. Extract immediately with chloroform using 25 mL for 1 to 5 cm cells and 50 mL for 10 cm cell. Shake separatory funnel at least 1O times, let chloroform settle, shake again for 10 minutes and let the chloroform settle again. 7. Filter each chloroform extract through filter paper or fritted glass funnels containing a 5 gm layer of anhydrous sodium sulphate. 8. Collect dried extracts in clean cells for absorbance measurements. Do not add more CHC13 or wash the filter papers or funnels with CHC13 9. Read absorbance of sample and standards against the blank at 460 nm. 10. Calibration Curve: Prepare a standard curve by plotting the absorbance values of standards versus corresponding phenol concentrations. Construct a separate calibration curve for each photometer and check each curve periodically to ensure reproducibility. **For infrequent non regulatory analysis:** 11. For infrequent analysis, prepare only one standard phenol solution. Prepare 500 mL standard phenol solution of strength approximately equal to the phenolic content of that portion of original sample used for final analysis. Also prepare a 500mL distilled water blank. 12. Measure absorbance of sample and standard phenol solution against the blank at 460 nm

Calculation with units of expression	After obtaining the absorbance values, depending upon the volume of sample chosen for test, calculate the amount of phenol present in 1000 mL as given below: Using calibration curve: $\mu g/L, \text{phenol} = \frac{A \times 1000}{B}$ Where, A = concentration of phenol in µg in sample from the calibration curve. B = volume in mL of original sample **For infrequent non regulatory analysis:** $\mu g/L, \text{phenol} = \frac{C \times D \times 1000}{E \times B}$ Where, C = µg standard phenol solution, D = absorbance reading of sample, E = absorbance of standard phenol solution, and B = mL original sample
Inference (Qualitative Analysis)	NA
Reference	APHA 5530
Approved by	Scientific Panel on Methods of Sampling and Analysis

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Method No.	**FSSAI 14.038:2024**	**Revision No. & Date**	0.0
Scope	This standard prescribes method for determination of sodium by flame emission photometric method using flame photometry method. Sodium ranks sixth among the elements in order of abundance and is present in most natural water. The levels may vary from less than 1 mg Na/L to more than 500 mg Na/L. Relatively high concentrations may be found in brines and hard water softened by the sodium exchange process. The ratio of sodium of total cations is important in agriculture and human pathology. Soil permeability can be harmed by a high sodium ratio. Persons afflicted with certain diseases require water with low sodium concentration. A limiting concentration of 2 to 3 mg/L is recommended in feed water destined for high pressure boilers. Ehen necessary, sodium can be removed by the hydrogen-exchange process or by distillation. Sodium compounds are used in many applications, including caustic soda, sat fertilizers and water treatment chemicals.		
Caution	Radiation interference caused by elements other than those being determined is the chief contributing factor for error in flame photometry. Of the elements encountered in these analyses, the major effect is due to interference of one alkali-metal on another. Some effects are positive and some are negative. Among the other common ions capable of causing interference are CI- , SO4 2- and HCO3- in relatively higher concentration. The foreign element effects cannot be entirely compensated without employing calibration standards closely duplicating the composition of the samples or by applying an experimentally determined correction in those instances where the sample contains a single important interference. However, the effects may be minimized by operating at the lowest practical sodium concentration range or by removal of the interfering elements. For example, aluminum has a depressing effect on alkali-metal emission, which may be of serious consequence. Suspended matter which may interfere mechanically by clogging the burner shall be removed by filtration prior to the analysis. Organic colouring matter does not cause interference and need not be removed. Flame photometers operating on the internal standard principle may require adding a standard lithium solution to each working standard and sample. Follow the manufacturer's instructions for the optimum lithium concentration. Incorporate a non-ionic detergent in the standard lithium solution to assure proper aspirator function when using the internal standard type flame photometer.		
Principle	A flame photometer measures photo electrically the intensity of color imparted to the flame of a Meker- type burner where the sample is introduced into the flame under carefully standardized conditions. The		

	intensity of color is proportional to the sodium content in the sample. Sodium is determined at a wavelength of 589 nm.
Apparatus/Instruments	a. Flame photometer: Either direct-reading or internal standard type or an atomic absorption spectrophotometer in the flame emission mode. b. Glassware: Rinse all glassware's with dilute nitric acid (1:15) followed by several portions of deionized distilled water.
Materials and Reagents	a. Reagents: Use deionized distilled water to prepare all reagents, calibrations, standards and dilution water.
Preparation of Reagents	b. Stock sodium solution: Dissolved in deionized distilled water, 2.542 gm of sodium chloride dried to constant mass at 140°C and make up to 1000 mL with water, 1 mL = 1mg of sodium. c. Standard lithium Solution: Weigh rapidly 6.109 gm of lithium chloride (LiCl) or 9.93 gm of lithium nitrate (LiNO3) dried overnight in an oven at 105°C. Dissolve in water and make up to 1000 mL with water, 1 mL = 1 mg of lithium. NOTE- prepare a new calibration curve whenever the standard lithium solution is changed.
Sample Preparation	**Direct Intensity Measurement**: Prepare a blank and sodium standards in stepped amounts by diluting the stock solutions described 4.4.1and for any of the following applicable ranges: 0 to 1.0 mg/L, 0 to 10 mg/L or 0 to 100 mg/L so that within each range there are equally spaced standards in tenths of the maximum. Starting with the highest calibration standard and working towards the most dilute standard, measure emission at 589 nm for sodium. Repeat the operation with both calibration standards and samples enough number of times to secure a reliable average reading for each solution. Construct a calibration curve, by plotting emission intensity (scale reading) versus concentration of each calibration standard on a linear graph paper. Determine the Sodium concentration of the sample solution from the respective calibration curve. Internal Standard Measurement: Add an appropriate volume of standard lithium solution to carefully measured volume of sample (or diluted portion), each sodium calibration standard and the blank and then follow all the steps described above.
Method of analysis	--
Calculation with units of expression	For direct intensity measurement and internal standard measurements Sodium = Sodium in mg/L in portion x D. Where D = dilution Ratio
Inference (Qualitative Analysis)	NA
Reference	IS 3025(Part 45)
Approved by	Scientific Panel on Methods of Sampling and Analysis

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Method No.	**FSSAI 14.039:2024**	**Revision No. & Date**	0.0
Scope	This standard prescribes Diphenylcarbbazide method for the determination of hexavalent chromium. The average abundance of Cr in the earth's crust is 122 pm; in soils Cr ranges from 11 to 22 ppm; in streams it averages about 1 µg/L. Chromium is found chiefly in chrome-iron ore (FeO.Cr2O3). Chromium is used in alloys, in electroplating, and in pigments. Chromate compounds frequently are added to cooling water for corrosion control. In natural waters trivalent chromium exists as Cr 3+ Cr(OH)2+ Cr(OH)4 - ; in the hexavalent from chromium exists as CrO4 2- and Cr2O7 2- Cr3+ would be expected to form strong complexes with amines and would be absorbed by clay minerals. Chromium is considered nonessential for plants, but an essential trace element for animals. Hexavalent compounds have been shown to be carcinogenic by inhalation and are corrosive to tissue. The chromium guidelines for natural water are linked to the hardness or alkalinity of the water (i.e the softer the water, the lower the permitted level for chromium). The United Nations Food and Agriculture organization recommended maximum level for irrigation water is 100 µg/L. The U.S. EPA primary drinking water standard MCL is 100 µg/L for total chromium. This standard prescribes Diphenylcarbbazide method for the determination of hexavalent chromium.		
Caution	The reaction with Diphenylcarbazide is nearly specific for chromium. Hexavalent molybdenum and mercury salts will react to form color with the reagent, but the intensities are much lower than that for chromium at the specified pH. Concentrations as high as 200 mg/L of Mo or Hg can be tolerated. Pentavalent vanadium interferes, strongly but concentrations up to 10 times that of chromium will not cause trouble. Potential interference from permanganate is eliminated by prior reduction with sodium azide. Iron in concentrations greater than 1 mg/L may produce a yellow color, but the color is not strong and no difficulty is encountered normally if the absorbance is measured spectrophotometrically at 540 nm.		
Principle	This procedure measures only hexavalent chromium (Cr6+). The hexavalent chromium is determined spectrophotometrically by reaction with Diphenylcarbazide in acid solution. A red violet color of unknown composition is produced. The colored complex obeys Beer's law and is suitable for spectrophotometric measurements at 540 nm. This method is applicable in range of 30 to 20000µg/l of chromium.		
Apparatus/Instruments	a. Spectrophotometer, for use at 540 nm, with a light path of 1 cm b. pH meter c. Standard volumetric glassware NOTE: Thoroughly cleaned glassware with nitric acid or hydrochloric acid to remove chromium traces. Do not use glassware previously		

	treated with chromic acid. New and unscratched glassware will minimize chromium absorption on glassware during oxidation procedure.
Materials and Reagents	a. Stock Chromium Solution b. Standard Chromium Solution: c. Nitric acid- Concentrated (16N). d. Sulphuric acid e. Methyl orange indicator f. Ammonium hydroxide g. Potassium permanganate h. Sodium Azide solution i. Diphenylcarbazide Solution. j. Acetone
Preparation of Reagents	a. Stock Chromium Solution: Dissolve 141.4 mg of K2Cr2O7 in water and dilute to 100 mL (1.0 mL = 500 µg of Cr). b. Standard Chromium Solution: dilute 1 mL of stock Chromium Solution to 100mL; (1 mL = 5 µg of Cr). c. Nitric acid- Concentrated (16N). d. Sulphuric acid - Concentrated 36 N; 1:1;6 N and 0.2 N. e. Phosphoric acid- concentrated (41N). f. Methyl orange indicator solution – Dissolve 50 mg of methyl orange in 100mL of Distilled water. g. Ammonium hydroxide- concentrated (14N) h. Potassium permanganate solution- Dissolve 4 gm of KMnO4 in 100mL Distilled water. i. Sodium Azide solution-Dissolve 0.5gm of sodium Azide (NaN3) in 100mL distilled water. j. Diphenylcarbazide Solution- Dissolve 250 mg of 1, 5-diphenylcarbazide in 50 mL acetone. Store in an amber colored bottle. Discard when the solution becomes discolored.
Sample Preparation	Preparation of calibration Curve: Pipette out measured volumes of standard chromium solution ranging from 2 to 20 mL (to give standards for 10-100 µg of Cr), into 100 mL beakers. Make up the volume to about 50 mL with water. Use 0.2 N H2SO4 and a pH meter to adjust the pH of each solution to 1.0 ± 0.3. Transfer quantitatively each of these solutions into 100 mL volumetric flasks and add 2.0 mL of diphenylcarbazide solution. Dilute to 100 mL with water, mix and let these stand for 5 to 10 min for full color development. Meanwhile, prepare a reagent blank in an identical manner using 10mL of water. Measure the absorbance of the standard solutions at 540 nm, using reagent blank as reference solution. Construct a calibration curve by plotting absorbance values against micrograms (µg) Cr in 100 mL of the final volume. Determination of Hexavalent Chromium (Cr6+): Pipette out a portion of filtered sample (filtered through 0.45 µm membrane filter), containing 10 to

	100 µg of Cr into a 100 mL beaker. Make up the volume to about 50 mL with water. Adjust pH of this solution to 1.0 ± 0.3 using 0.2 N H_2SO_4, and a pH meter. Transfer quantitatively into a 100 mL volumetric flak, add 2.0 mL of diphenylcarbazide solution. Dilute to 100 mL water, mix well and allow to stand for 5 to 10 min. Measure absorbance at 540 nm, using reagent blank as reference solution. From the absorbance data, determine the micrograms of chromium present in 100 mL of the final solution using the calibration curve.
Method of analysis	--
Calculation with units of expression	Soluble Hexavalent Chromium $\frac{(Cr^{6+})\ mg/L = \mu g\ of\ Cr\ (in\ 100\ mL\ of\ the\ final\ solution)}{V}$ Where V = volume in mL, of the sample used.
Inference (Qualitative Analysis)	NA
Reference	IS: 3025 part 52 –2003- Methods of Sampling and Test (Physical and chemical) for water and Waste Water: Chromium & Hexavalent chromium.
Approved by	Scientific Panel on Methods of Sampling and Analysis

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Method No.	**FSSAI 14.040:2024**	**Revision No. & Date**	0.0
Scope	The term 'Solid' refers to the matter either filterable or non- filterable that remains as residue upon evaporation and subsequent drying at a defined temperature. Further categorization depends upon the temperature employed for drying and ignition. Different forms of solids are defined on the basis of method applied for their determination. Solids may affect water or effluent quality adversely in number of ways. Water with high dissolved solids may include an unfavorable physiological reaction in the transient consumer and generally are of inferior palatability. Highly mineralized waters are unsuitable for many industrial applications. High suspended solids in waters may be aesthetically unsatisfactory for such purposes as bathing. Analysis of total solids are important to decide upon the various unit operations and processes in physical and biological wastewater treatment and to assess its performance evaluation. For assessing compliance with regulatory agency, wastewater effluent limitations for various form of solids act as indicating parameters. This following method describes the Total solid content in water by evaporation through heat		
Caution	Highly mineralized waters containing significant concentration of calcium, magnesium, chloride and/or sulphate may be hygroscopic. These may require prolonged drying, desiccation and rapid weighing. However, prolonged drying may also cause loss of constituents, particularly nitrates and chlorides. A large amount of residue in the evaporating basin may crust over and entrap water preventing its evaporation during drying. For this reason, the volume of the sample should be adjusted so that the residue left after drying should be about 100-200 mg. Preservation of the samples is not practical. Analysis should begin as soon as possible. Refrigeration or chilling to 4°C, to minimize microbiological decomposition of solids is recommended.		
Principle	The sample is evaporated in a weighed dish on a stream-bath and is dried to a constant mass in an oven either at 103-105oC or 179-181°C. Total residue is calculated from increase in mass. NOTE- In general by evaporating and drying water samples at 103-105°C or 179-181°C values are obtained which conform more closely to those obtained by summation of individually determined mineral salts.		
Apparatus/Instruments	a. Evaporating Dish- of 90 mm diameter, 100 mL capacity made of platinum, nickel, porcelain, silica or borosilicate glass. Platinum is suitable for all tests. Nickel is satisfactory if residue is not to be ignited. Porcelain, silica and glass may be used for samples with a pH value less		

	than 9.0. b. Steam-Bath. c. Drying Oven- Drying oven with thermostatic control for maintaining temperature up to 180 ±2°C. d. Desiccator- Provided with a color indicating desiccants. e. Analytical Balance- 200gm capacity and capable of weighing to nearest 0.1 mg
Materials and Reagents	--
Preparation of Reagents	--
Sample Preparation	Heat the clean evaporating dish to 180°C for 1 hour. Cool desiccate, weigh and store in desiccator until ready for use. Select volume of the sample which has residue between 25 and 250 mg, preferably between 100 and 200 mg. This volume may be estimated from values of specific conductance. To obtain a measurable residue; successive aliquots of sample may be added to the sample dish. Pipette this volume to a weighed evaporating dish placed on a steam-bath. Evaporation may also be performed in a drying oven. The temperature should be lowered to approximately 98°C to prevent boiling and splattering of the sample. After complete evaporation of water from the residue, transfer the dish to an oven at 103- 105°C, or 179-181°C and dry to constant mass, that is, till the difference in the successive weighing is less than 0.5 mg. Drying for a long duration (usually 1 to 2 hours) is done to eliminate necessity of checking for constant mass. The time for drying to constant mass with a given type of sample when a number of samples of nearly same type are to be analyzed should be determined by trial. Weigh the dish as soon as it has cooled avoiding residue to stay for long time as some residues are hygroscopic and may absorb water from desiccant which may not be absolutely dry.
Method of analysis	--
Calculation with units of expression	Calculate the total residue using following equation: Total residue, mg/L = $\frac{1000\ M}{V}$ Where, M= Mass in mg of total residue, and V= volume in mL of the sample. Report in whole numbers for less than 100 mg/L and above 100 mg/L to three significant figures. Report the temperature of determination also.
Inference (Qualitative Analysis)	NA
Reference	IS:3025 part 15 – 1984 (Reaffirmed 2003)- Methods of Sampling and Test (Physical and chemical) for water and Waste Water : (Total Solids)
Approved by	Scientific Panel on Methods of Sampling and Analysis

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Method No.	**FSSAI 14.041:2024**	**Revision No. & Date**	0.0
Scope	Nitrite in water is either due to oxidation of ammonium compounds or due to reduction of nitrate. As an intermediate stage in the nitrogen cycle it is unstable. A usual concentration in natural water is in the range of some tenths of mg/L. Higher concentrations are present in industrial wastes. Sewage and in biologically purified effluents and in polluted streams. In chlorinated supplies, levels of nitrite are often less than the limit of detection, i.e. 0.005mg/L NO2 - N but high levels may occur in unchlorinated water. Very high nitrite levels are usually associated with water of unsatisfactory microbiological activity.		
Caution	Nitrogen trichloride (NCl3) imparts a false red color when normal order of reagents addition is followed. It can be minimized by adding NED dihydrochloride first and then sulphanalic acid. Ions like Sb3+, Au3+,Fe3+,Bi3+,Pb2+,Hg2+ ,Ag+, PtCl6 2- interfere. Cupric ions cause low results.		
Principle	Nitrite is determined through formation of a reddish purple azo dye produced at pH 2.0 to 2.5 by coupling diazotized sulphanalic acid with N- (1 napthyl)- ethylene diamine dihydrochloride (NED dihydrochloride). The color obeys Beer's law up to 180 µg/L with 1 cm path length at 543 nm.		
Apparatus/Instruments	Spectrophotometer or photometer- for use at 543 nm in case of spectrophotometer or photometer having a green filter and having maximum absorbance near 540 nm. Nessler tubes-matched, 50 mL capacity.		
Materials and Reagents	a. Nitrite free water- b. Sulphanilamide reagent c. NED dihydrochloride d. Hydrochloric acid- 1:3. e. Sodium oxalate f. Ferrous ammonium sulphate g. nitrite solution		
Preparation of Reagents	1. Nitrite free water- If the distilled water is not nitrite free, prepare as follows: a. Add to 1 liter of distilled water, a small crystal each of potassium permanganate and barium hydroxide or calcium hydroxide. Redistill in a borosilicate glass bottle. b. Add 1 mL of concentrated sulphuric acid and 0.2 mL of manganese sulphate (36.48 gm MnSO4.H2O/100 mL) solution to each 1 liter of distilled water and make pink with 1 to 3 mL of potassium permanganate solution (400 mg/L). Redistill as in 4.1.1 above. Use this water in making all		

<table>
<tr><td></td><td>reagents and dilutions.

2. Sulphanilamide reagent- Dissolve 5 gm of the material in a mixture of 50 mL of concentrated hydrochloric acid and 300 mL of water. Dilute to 500 mL with water. The reagent is stable for several months.

3. NED dihydrochloride- Dissolve 500 mg of the material in 500 mL of water. Store in colored bottle in dark. Replace monthly or when it turns dark brown in color.

4. Hydrochloric acid- 1:3.

5. Sodium oxalate- 0.05 N. Dissolve 3.350 gm of sodium oxalate (primary standard grade) in 1000 mL of water.

6. Ferrous ammonium sulphate- 0.05 N. Dissolve 19.607 gm of ferrous ammonium sulphate in 20 mL of concentrated sulphuric acid and water and dilute to1 litre. Standardize with standard dichromate.

7. Stock nitrite solution- Dissolve 1.232 gm of sodium nitrite in water and dilute to 1000 mL (1 mL = 250 µg of N). Preserve with 1 mL of chloroform. Standardize using sodium oxalate (4.5) and standard potassium permanganate solution.

8. Intermediate nitrite solution – Calculate the volume, G, of stock nitrite solution required for intermediate nitrite solution from G = 12.5/A, where A is the stock solution in mg/L. Dilute the volume G to 250 mL with water (1.00 mL= 50.0 µg N).

9. Standard nitrite solution- Dilute 10.00 mL of intermediate nitrite solution to 1000 mL with water (1.00 mL = 0.500 µg N).</td></tr>
<tr><td>Sample Preparation</td><td>If the sample is turbid, filter through a 0.45 µm membrane filter. To 50.0 mL of clear sample neutralized to pH 7 or to a portion diluted to 50mL add 1 mL of sulphanilamide solution. Let the reagent react for 2 to 8 minutes. Add 1.0 mL of NED dihydrochloride solution and mix immediately. Let stand for at least 10 minutes but not more than 2 hours. Measure absorbance at 543 nm. As a guide, use the following light paths for the indicated nitrite nitrogen concentrations:

<table>
<tr><th>Light Path Length, cm</th><th>Nitrite Nitrogen, µg/L</th></tr>
<tr><td>1</td><td>2-25</td></tr>
<tr><td>5</td><td>2-6</td></tr>
<tr><td>10</td><td>2</td></tr>
</table>

Run parallel checks frequently against nitrite standards.</td></tr>
</table>

Method of analysis	**Color standards for visual comparison** – Prepare a suitable series of visual color standards in Nessler tubes by adding the following volumes of standard nitrite solutions and diluting to 50 mL with water: 0, 0.1, 0.2, 0.4, 0.7, 1.0, 1.4 1.7, 2.0 and 2.5 mL, corresponding, respectively to 0, 1.0, 2.0, 4.0, 7.0, 10, 14, 17, 20 and 25 µg of nitrite per liter. Develop color as described above. Compare samples to visual standards in matched Nessler tubes between 10 and 120 minutes after adding NED dihydrochloride reagent. Select the concentration where the sample tube color matches the standard tube color
Calculation with units of expression	Calculate nitrite nitrogen from the following: Nitrite Nitrogen (as NO2-N) per liter= $\frac{\mu g\ NO2{-}N\ (in\ 52\ mL\ final\ volume)}{mL\ of\ sample}$
Inference (Qualitative Analysis)	--
Reference	IS:3025 part 34 – 1988 (Reaffirmed 2003)- Methods of Sampling and Test (Physical and chemical) for water and Waste Water: Nitrogen
Approved by	Scientific Panel on Methods of Sampling and Analysis

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Method No.	**FSSAI 14.042:2024**	**Revision No. & Date**	0.0
Scope	Mineral oil in environmental matrices is one of the most often measured parameters. The present standard methods for the determination of mineral oil in environmental matrices give a total concentration. This concentration is not found to be a good estimate for ecotoxicological, human and agricultural risk.		
Caution	Thoroughly rinse cuvette with distilled TCA while reading blank(s) and sample(s).		
Principle	The sample of water is extracted with tetrachloroethylene (TCE) as extraction solvent followed by analysis by infra-red (IR) spectrometry using peak heights at 2930 ± 5cm^{-1}		
Apparatus/Instruments	Apparatus/Instruments: • Filter paper – Whatman No. 40 or equivalent • Cells – Infra- red, silica/quartz (1 or 5cm path length; for low range, 5cm path length will be appropriate). • FTIR- Fourier Transformer Infrared Spectrometer		
Materials and Reagents	1. TCE 2. Anhydrous Sodium Sulphate dried at 200 to 250°C. 3. HCl- 35% GR grade		
Preparation of Reagents	1. **Preparation of Stock solution** – Prepare a reference mixture by volume of 37.5 percent isooctane, 37.5 percent hexadecane and 25 percent benzene. Store in a stoppered 100mL volumetric flask to prevent loss of evaporation. 2. **Preparation of Calibration Solutions** - Take 20mL of TCE in 100mL volumetric flask, stopper it and weigh it. Add 1mL standard to it and obtain its exact weight by difference. Make up the volume with solvent and calculate the exact concentration in mg/L. Prepare the calibration standards in the range of 0-50 mg/L.		
Sample Preparation			
Method of analysis	1. Acidify sample using HCl to pH ~ 2. 2. Transfer 1L sample to 2L separating funnel. 3. Add 20mL TCE and shake vigorously for about 2 min. and leave the funnel undisturbed till layers separate. If emulsion will form, shake gently for 5 to 10 min or centrifuge. 4. Collect lower organic layer in glass vials after passing through anhydrous sodium sulphate and repeat the extraction step four times. Collect and combine the layers. 5. Prepare the method blank with reagent grade water adopting same extraction		

	process (without reference oil). 6. Scan the standards and samples from 3200 to 2700cm-1. Measure absorbance of standards at peak height at 2930 ±5 cm-1 on solvent TCE background. Similarly, measure the absorbance of samples at same peak height on blank background. 7. Prepare a calibration curve of absorbance against the concentration of standards. If the absorbance exceeds more than that of highest standard sample, dilute the sample as required. **NOTE:** Spiked Sample must be added with batch of 10 samples for quality check.
Calculation with units of expression	Mineral oil = Mass of oil in the extract as determined from calibration curve (mg) x 1000
Inference (Qualitative Analysis)	NA
Reference	Clause (6) of IS 3025 (part 39), Amendment No. 2
Approved by	Scientific Panel on Methods of Sampling and Analysis

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Method No.	**FSSAI 14.043:2024**	**Revision No. & Date**	0.0
Scope	Determination of Anions (F- , Cl- , NO2 - ,Br- , NO3 - , PO4 - , SO4 - -) in Drinking Water		
Caution	Sample with higher concentration may interfere which may lead to tailing and heading of the Peaks. Flush column before next analysis.		
Principle	A water sample is injected into a stream of effluent and passed through a series of ion exchangers. The anions of interest are separated on the basis of their relative affinities for a low capacity, strongly basic anion exchanger (guard & analytical columns). The separated anions are directed through a suppressor device that provides continuous suppression of effluent conductivity and enhance analyte response. In the suppressor the separated anions are converted to their highly conductive acid forms while the conductivity of the effluent is greatly decreased. The separated anions in their acid forms are measured by conductivity. They are identified on the basis of retention time as compared to standards. Quantitation is by measurement of peak area or peak height.		
Apparatus/Instruments	1. Ion Chromatograph, including an injection valve, a sample loop, Guard column, analytical column, suppressor device, a temperature – compensated small volume conductivity cell and detector and an electronic peak integrator or chromatography data acquisition system. Use an ion chromatography capable of delivering 2 to 5 mL eluent per minute at a pressure of 5600 to 28000 KPa (800 Psi). 2. Analytical Column: Any commercially available anion-exchange column capable of resolving Fluoride, Bromide, Chloride, Nitrate, Nitrite, Phosphate and Sulphate is acceptable. 3. Guard Column: Identical to separator column to protect analytical column from fouling by particulates or organics. 4. Suppressor device: Place this ion- exchange based device between column and detector to reduce background conductivity of the eluent and enhance conductivity of the target analytes. Several such devices with different operational principles are available commercially; any that provides the required sensitivity and baseline stability may be used.		
Materials and Reagents	1. Reagent water ASTM type 1 water 2. Sulphuric Acid 3. Eluent Solution 4. Regeneration solution 5. Standard Anion solutions		

Preparation of Reagents	1. Reagent water ASTM type 1 water 2. Sulphuric Acid 3. Eluent Solution: Appropriate to column used to resolve target anions. Prepare 1.7 mM sodium bicarbonate and 1.8 mM sodium carbonate as eluent. Dissolve 0.5712 gm sodium bicarbonate 0.7632 gm sodium carbonate in water and make up 4 L. Degas eluent before use either by vacuum filtration to simultaneously remove particle greater than 0.45 micron or by purging with helium for 10 mins. 4. Regeneration solution: Required with some types of suppressors. See manufacturer's recommendations. Standard Anion solutions:- Stock standards solutions traceable to NIST are available from a number of commercial suppliers (Merck / sigma) or alternatively prepare from salt.
Sample Preparation	--
Method of analysis	**System Equilibration**: Turn on ion chromatograph and adjust eluent flow rate to manufacturer's recommendations for the column/ eluent combination being used. Adjust detector to desired setting (10μs to 30 μs) and let system come to equilibrium (15-20 min). A stable base line indicates equilibrium conditions. Adjust detector offset to zero out eluent conductivity. If regenerant is used with the suppressor, adjust flow rate to manufacturer's specifications. **Calibration**: Inject standards containing a single anion or a mixture and determine approximate retention times. Observed times vary with conditions. Retention time always is in order F-, Cl- , NO2 - , Br- , NO3 - , HPO4 - and SO4 -. Inject at least three different concentrations for each anion to be measured. Construct a calibration by plotting peak height or area versus concentration using appropriate software. Verify calibration curve with a mid range check standard from a source independent of that of the calibration standards. Check validity of existing calibration curves daily with a mid range calibration standard. Result should be with in 10% of original curve at mid range. Recalibrate whenever the detector setting, eluent or regenerant is changed. To minimize the effect of the water dip on F- analysis. Eliminate water dip by diluting sample with eluent or by adding concentrated eluent to the sample to give the same concentration as in eluent. If sample adjustments are made, adjust standards and blanks identically. If linearity is established (r≥0.99) over the calibration range the average response factor is acceptable. Record peak height or area for calculations of the response factor, RF. HPO2 4 - is nonlinear below 1 mg/L. Sample analysis: If sample is collected with an auto sampler that does not automatically filter samples, remove particulates by filtering through a

	prewashed 0.45 µm pore membrane. With either manual or automated injection, flush loop with several volumes of sample. Take care to prevent carryover of analytes from samples of high concentration. After last peak has appeared and detector signal has returned to base line, another sample can be injected. **Special Precautions:** Do not inject any high concentration analyte samples or standards into the column. This may overload the column thereby leading to fronting or tailing of peaks. Only diluted samples and flow concentration standards are preferred. Even if high concentration sample were injected, they have to be completely flushed out of the column before next analysis. After the final analysis the column has to be flushed for 15-20 minutes with mobile phase to remove any ion present in the column. The column should not be stored with any ion.
Calculation with units of expression	Determine concentration of each anion, in milligrams per liter, by referring to the appropriate calibration curve. Alternatively, when the response is shown to be linear, use the following equation: C = H X RF X D , Where C = mg anion/L H = Peak height or area RF = response factor = concentration of standard/ height (or area) of standard D = dilution factor
Inference (Qualitative Analysis)	NA
Reference	APHA 4110
Approved by	Scientific Panel on Methods of Sampling and Analysis

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Method No.	**FSSAI 14.044:2024**	**Revision No. & Date**	0.0
Scope	Requirements for determining metals by atomic absorption spectrometry (AAS) vary according to metal and concentration. Metals by Flame Atomic Absorption Spectrometry encompasses the determination of- • antimony, bismuth, cadmium, calcium, cesium, chromium, cobalt, copper, gold, iridium, iron, lead, lithium, magnesium, manganese, nickel, palladium, platinum, potassium, rhodium, ruthenium, silver, sodium, strontium, thallium, tin, and zinc by direct aspiration into an air-acetylene flame • low concentrations of cadmium, chromium, cobalt, copper, iron, lead, manganese, nickel, silver, and zinc by chelation with 200 • Part 3000 ammonium pyrrolidjne dithiocarbamate (APDC), extraction into methyl isobutyl ketone (MIBK), and aspiration into an air-acetylene flame • aluminum, barium, beryllium, calcium, molybdenum, osmium, rhenium, silicon, thorium, titanium, and vanadium by direct aspiration into a nitrous oxide-acetylene flame • low concentrations of aluminum and beryllium by chelation with 8-hydroxyquinoline, extraction into MIBK, and aspiration into a nitrous oxide-acetylene flame		
Caution	Acetylene gas represents an explosive hazard in the laboratory. Follow instrument manufacturer's directions in plumbing and using this gas. Do not allow gas contact with copper, brass with >65% copper, silver, or liquid mercury. Do not use copper or brass tubing, regulators, or fittings with > 65 % copper content.		
Principle	In flame atomic absorption spectrometry, a sample is aspirated into a flame and the metals are atomized. A light beam is directed through the flame, into a monochromator, and onto a detector that measures the amount of light absorbed by the atomized metal in the flame. For some metals, atomic absorption exhibits superior sensitivity over flame emission. Because each metal has its own characteristic absorption wavelength, a source lamp composed of that element is used. This makes the method relatively free from spectral or radiation interferences. The amount of energy at the characteristic wavelength absorbed in the flame is proportional to the concentration of the element in the sample over a limited concentration range. Most atomic absorption instruments also are equipped for operation in an emission mode, which may provide better linearity for some elements.		
Apparatus/Instruments	**Atomic absorption spectrometer and associated equipment:** Atomic absorption spectrometer, consisting of a light source emitting the line spectrum of an element (hollow-cathode lamp or electrodeless discharge lamp), a device for vaporizing the sample (usually a flame), a means of		

isolating an absorption line (monochromator or filter and adjustable slit), and a photoelectric detector with its associated electronic amplifying and measuring equipment.

Burner: The most common type of burner is a premix, which introduces the spray into a condensing chamber for removal of large droplets. The burner may be fitted with a conventional head containing a single slot; a 3-slot Boling head, which may be preferred for direct aspiration with an air-acetylene flame; or a special head for use with nitrous oxide and acetylene. Recovery of the added metal should be between 85% and 115%.

Sl. No.	METHOD	METALS	BURNER
1.	Direct Air-Acetylene Flame Method	Calcium, Chromium, Copper ,Lead ,Magnesium , Manganese ,Nickel , Silver , Sodium ,Zinc	Use burner head recommended by the manufacturer.
2.	Extraction and Air-Acetylene Flame Method	Chromium, Copper ,Lead, Iron, Manganese , Nickel ,Silver , Zinc	Burner head, conventional. Consult manufacturer's operating manual far suggested burner .
3.	Direct Nitrous Oxide-Acetylene Flame Method	Calcium	Nitrous oxide burner head: Use special burner head as suggested in manufacturer's manual. At roughly 20-min intervals of operation it may be necessary to dislodge the carbon crust that forms along the slit surface with a carbon rod or appropriate alternative. **T-junction valve** or other switching valve for rapidly changing from nitrous oxide to air, so that flame can be turned on or off with air as oxidant to prevent flashbacks.

Analyze an additional standard solution after every 10 samples or with each batch of samples, whichever is less, to confirm that the test is in control.

Materials and Reagents	**a. Air:** Cleaned and dried through a suitable filter to remove oil, water, and other foreign substances. The source may be a compressor or commercially

	bottled gas. **b. Acetylene**: Standard commercial grade. Acetone, which always is present in acetylene cylinders, can be prevented from entering and damaging the burner head by replacing a cylinder when its pressure has fallen to 689 kPa (100 psi) acetylene. **c. Metal-free water** **d. Certified Reference Material For Standard metal solutions :** As per 17034:2017
Preparation of Reagents	1. Calcium solution: Dissolve 630 mg calcium carbonate (CaCO3) in 50 mL of 1 + 5 HCl. If necessary, boil gently to obtain complete solution. Cool and dilute to 1000 mL with water. 2. Hydrochloric acid (HCl), 1 %, 10%, 20% (all v/v), 1 + 5, 1 + 1, and concentrated. 3.Nitric acid (HNO3), 2% (v/v), 1 + 1, and conc. 4. Aqua Regia : Add 3 volumes concentrated HCl to 1 volume concentrated HNO^3 5. Sodium sulfate (Na2SO4) 6. lanthanum solution: Lanthanum solution: Dissolve 58.65 g lanthanum oxide (La2ü 3) in 250 mL concentrated HCl. Add acid slowly until the material is dissolved and dilute to 1000 mL with water. 7. Water-saturated MIBK: Mix 1 part purified MIBK with 1 part water in a separatory funnel. Shake 30 s and let separate. Discard aqueous layer. Save MIBK layer. 8. Sodium sulfate (Na2SO4), anhydrous. 9. Standard metal solutions: Prepare a series of standard metal solutions in the optimum concentration range by appropriate dilution of the fallowing stock metal solutions with water containing 1.5 mL concentrated HNO3 per liter.
Sample Preparation	**Direct Air-Acetylene Flame Method:** Required sample preparation depends on the metal form being measured. For all samples, make certain that the concentrations of acid and matrix modifiers are the same in both samples and standards. When determining Ca or Mg, dilute and mix 100 mL sample or standard with 10 mL lanthanum solution before aspirating. When determining Fe or Mn , mix 100 mL with 25 mL of Ca solution before aspirating. When determining Cr, mix 1 mL 30% H_2O_2 with each 100 mL before aspirating. Alternatively use proportionally smaller volumes. **Extraction and Air-Acetylene Flame Method:** Prepare samples in the same manner as the standards. Rinse atomizer by aspirating water-saturated MIBK. Aspirate organic extracts treated as above directly into the flame and record absorbance.

	Direct Nitrous Oxide-Acetylene Flame Method: Required sample preparation depends on the metal form being measured. For all samples, make certain that the concentrations of acid and matrix modifiers are the same in both samples and standards. When determining Ca or Mg, dilute and mix 100 mL sample or standard with 10 mL lanthanum solution before aspirating. When determining Fe or Mn , mix 100 mL with 25 mL of Ca solution before aspirating. When determining Cr, mix 1 mL 30% H_2O_2 with each 100 mL before aspirating. Alternatively use proportionally smaller volumes. When determining Al, Ba, or Ti, mix 2 mL KCl solution into 100 mL sample and standards before aspiration. When determining Mo and V, mix 2 mL Al(NO3)) • 9H2O into 100 mL sample and standards before aspiration.
Method of analysis	**Direct Air-Acetylene Flame Method :** Rinse nebulizer by aspirating water containing 1.5 mL of concentrated HNO_3 per liter. Aspirate blank and zero instrument. Aspirate sample and determine its absorbance. **Extraction and Air-Acetylene Flame Method :** After final adjusting of burner position, aspirate water-saturated MIBK into flame and gradually reduce fuel flow until flame is similar to that before aspiration of solvent. Prepare samples in the same manner as the standards. Rinse atomizer by aspirating water-saturated MIBK. Aspirate organic extracts treated as above directly into the flame and record absorbances. During extraction, if an emulsion forms at the water-MIBK interface, add anhydrous Na_2SO_4 to obtain a homogeneous organic phase. In that case, also add $N_{a2}SO_4$ to all standards and blanks. To avoid problems associated with instability of extracted metal complexes, determine metals immediately after extraction. **Direct Nitrous Oxide-Acetylene Flame Method:** Rinse atomizer by aspirating water containing 1.5 mL concentrated HNO_3 per liter and zero instrument. Aspirate a sample and determine its absorbance.
Calculation with units of expression	Determine concentration of each metal ion, in micrograms per liter for trace elements, and in milligrams per liter for more common metals, by using the appropriate calibration curve prepared. Alternatively, read concentration directly from the instrument readout if the instrument is so equipped. If the sample has been diluted, multiply by the appropriate dilution factor.
Inference (Qualitative Analysis)	NA
Reference	APHA 24TH Edition 2023
Approved by	Scientific Panel on Methods of Sampling and Analysis

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Method No.	**FSSAI 14.045:2024**	**Revision No. & Date**	0.0
Scope	This procedure is used for the determination of micro quantities of metals like aluminum, antimony ,arsenic ,barium, beryllium ,cadmium, chromium, cobalt, copper, iron, lead, manganese, molybdenum , nickel , selenium , silver and tin in water samples up to parts per billion (ppb) level.		
Caution	Do not mix hydrogen and other gases in the laboratory; hydrogen gas is very flammable-handle with caution. Use protective mask and/or dust collector. Prepare samples in area separate from analytical laboratory. Inhalation of solvent vapors can cause headaches, drowsiness, dizziness, and nausea. Disorientation, anesthetic effects, and loss of consciousness can occur at high concentrations. Wear laboratory coat, gloves, safety goggles and mask. Perform work in a fume hood when using solvents. Refer to MSDS for specific information.		
Principle	Electro-thermal atomic absorption spectroscopy is based on the same principle as direct flame atomization, but, an electrically heated atomizer or graphite furnace replaces the standard burner head. A discrete sample volume is dispensed into the graphite sample tube. Typically, determinations are made by heating the sample in three or more stages. First, a low current heats the tube to dry the sample. The second or charring stage destroys organic matter and volatizes other matrix components at an intermediate temperature. Finally, the current heats the tube to incandescence and in an inert atmosphere, atomizes the element being determined. Additional stages frequently are added to aid in drying and charring, and to clean and cool the tube between samples. The resultant ground-state atomic vapour absorbs monochromatic radiation from the source. A photoelectric detector measures the intensity of transmitted radiation. The inverse of transmittance is related logarithmically to the absorbance, which is directly proportional to the number density of vaporized ground state atom over a limited concentration range.		
Apparatus/Instruments	Electro-thermal atomic absorption spectroscopy or Atomic Absorption Spectrometer with Graphite furnace.		
Materials and Reagents	1. Reagent water (ASTM type-1) 2. Nitric acid (Suprapure 70%) 3. Standard of metals - stock standard solutions traceable to NIST are available from a number of commercial suppliers (Merck & Sigma) or alternatively prepare from reagent as mentioned in APHA 3111B 4. Air- Air is cleaned & dried through a suitable filter to remove oil, water and other foreign substances. The source may be a compressor or commercially bottled gas.		

	5. Argon Gas- Minimum purity 99.99% **6. Matrix modifier** 6.1 Magnesium nitrate- (10000 mg/L) 6.2 Palladium nitrate- (4000 mg/L) 6.3 Phosphoric acid- (10% v/v) 6.4 Nickel nitrate – (10000 mg/L) 6.5 Citric acid – (4%)
Preparation of Reagents	**1.Matrix modifier** Magnesium nitrate- (10000 mg/L): Dissolve 10.5 gm Mg $(NO_3)_2$. $6H_2O$ in water. Dilute to100 mL. Palladium nitrate- (4000 mg/L): Dissolve 8.89 gm Pd $(NO_3)_2.H_2O$ in water. Dilute to 1000 mL Phosphoric acid- (10% v/v): Add 10 mL conc. H_3PO_4 to water. Dilute to 100 mL. Nickel nitrate – (10000 mg/L): Dissolve 4.96 gm Ni $(NO_3)_2$. $6H_2O$ in water. Dilute to 100 mL Citric acid – (4%): Dissolve 40 gm citric acid in water. Dilute to 1L
Sample Preparation	Colorless & transparent water samples with turbidity of <1.0 can be directly analyzed by Electro-thermal atomic absorption spectroscopy for total metals after acidifying with conc. HNO_3 (1.5 mL HNO_3 /L of water). Sample digestion is not required.
Method of analysis	• **Standard Preparation:** Prepare a series of standard metal solution in the optimum concentration range by appropriate dilution from their stock solution with ASTM type 1 water containing 1.5 mL conc. HNO_3/L, using the following formula. $N_1V_1 = N_2V_2$ • **Sample Analysis**: Prepare standard solutions of at least three different concentrations, measure their absorbance and prepare a calibration curve. Then measure the absorbance of the test solution adjusted in concentration to a measurable range and determines the concentration of the element from the calibration curve. Before sample analysis, rinse nebulizer by aspirating ASTM type-1 containing 1.5 mL conc. HNO_3/L • **Determination by instrument:** Inject a measured portion of pretreated sample into the graphite furnace. Use same volume as was used to prepare the calibration curve. Add modifier immediately after adding the sample, preferably using an automatic sampler or a micropipette. Use the same volume and concentration of modifier for all standards and samples as given in the table. Dry, char, and atomize according to the preset program in the method. Repeat until reproducible results are obtained. Compare the average absorbance value or peak area to the calibration curve to determine concentration of the element of interest.

Table : Detection Levels and Concentration Ranges for Electrothermal Atomization Atomic Absorption Spectrometry

Element	Wavelength (nm)	Estimated Detection Level (1g/L)	Optimum Concentration Range (pg/L)
Al	309.3	3	20-200
Sb	217.6	0.8	20-300
As	193.7	0.5	5-100
Ba	553.6	2	10-200
Be	234.9	0.02	1-30
Cd	228.8	0.05	0.5-10
Cr	357.9	0.1	5-100
Co	240.7	0.7	5-100
Cu	324.7	0.7	5-100
Fe	248.3	1	5-100
Pb[a]	283.3	0.7	5-100
Mn	279.5	0.2	1-30
Mo	313.3	1	3-60
Ni	232.0	0.6	5-100
Se	196.0	0.6	5-100
Ag	328.1	0.2	1-25
Sn	224.6	1.7	20-300

a :The more sensitive 217.0-nm wavelength is recommended for instruments with background correction capabilities

Alternatively, read results directly if the instrument is equipped with this capability. If absorbance (or concentration) or peak area of the sample is greater than absorbance (concentration) or peak area of the most concentrated standard solution, dilute sample and reanalyze.

Table – Potential Matrix Modifiers for Graphite furnace AAS

Modifier	Analyses for which modifier May be Useful
1500 mg Pd/L + 100mg $Mg(NO_3)_2$	Ag, As, Cu, Mn, Hg, Sb, Se, Tl
00-2000 mg Pd/L + Reducing agent (Citric acid 1-2% preferred)	As, Cd, Cr, Cu, Fe, Mn, Hg, Ni, Pb, Sb
5000 mg $Mg(NO_3)_2$/L	Co, Cr, Fe, Mn,
100-500 mg Pd/L	As ,
50 mg Ni/L	As , Se , Sb
2% PO_4 + 1000mg$Mg(NO_3)_2$	Cd , Pb
Use 10µL modifier/ 10 µL sample	

Calculation with units of expression	• A continuing calibration verification (CCV) standard should be analyzed after every 10 injections and at the end of the run. The CCV standard should be a mid-range calibration standard. • An instrument blank should be analyzed after each CCV (called a continuing calibration blank, or CCB) to demonstrate that there is no carryover and that the analytical system is free from contamination. • Method of Standard Additions (MSA) calibration curves may be used any time matrix interferences are suspected. • Post-preparation spikes (PS) should be prepared and analyzed whenever there is an issue with the MS recoveries. • Export and process instrument data.
Inference (Qualitative Analysis)	• Electro thermal atomization determinations may be subjected to significant interferences from molecular absorption as well as chemical and matrix effect. Molecular absorption may occur when components of sample matrix volatize during atomization, resulting in broadband absorption. When such phenomena occurs use background correction to compensate for this interference. • Matrix modification can be useful in minimizing interference and increasing analytical sensitivity. Chemical modifier generally modifies relative volatilities of matrix and metal. Some modifiers inhibit metal volatization, allowing use of higher ashing/charring temperatures and increasing efficiency of matrix removal.
Reference	APHA 24th Edition 2023
Approved by	Scientific Panel on Methods of Sampling and Analysis

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Method No.	**FSSAI 14.046:2024**	**Revision No. & Date**	0.0
Scope	This procedure is used for the determination of mercury in Packaged Drinking water, drinking water and waste water. Lower detection limit of 0.2 ppb can be achieved using this technique.		
Caution	When possible, dedícate glassware for use in Hg analysis. Avoid using glassware previously exposed to high levels of Hg, such as those used in COD, TKN, or ci- analysis.		
Principle	The flameless atomic absorption procedure is a physical method based on the absorption of radiation at 253.7 nm by mercury vapour. The mercury is reduced to the elemental state and aerated from solution in closed system The mercury vapour passes through a cell positioned in the light path of Mercury lamp of an atomic absorption spectrophotometer. Absorbance (peak height) is measured as a function of mercury concentration and recorded.		
Apparatus/Instruments	• Atomic absorption spectrometer and associated equipment. Instruments and accessories specifically designed for measuring mercury via the cold vapor technique are available commercially and may be substituted. • Absorption cell, a glass or plastic tube approximately 2.5 cm in diameter. An 11.4-cm-long tube has been found satisfactory, but a 15-cm-long tube is preferred. Grind tube ends perpendicular to the longitudinal axis, and cement quartz windows in place. Attach gas inlet and outlet ports (6.4 mm diam) 1.3 cm from each end. • Cell support: Strap the cell to the flat nitrous-oxide burner head or other suitable support and align in the light beam to give maximum transmittance • Air pumps: Use any peristaltic pump with electronic speed control capable of delivering an air flow of 2 L/min. Any other regulated compressed air system or air cylinder also is satisfactory. • Flowmeter, capable of measuring an air flow of 2 L/min. • Aeration tubing, a straight glass frit having a coarse porosity for use in reaction flask. • Reaction flask, 250-mL Erlenmeyer flask or a BOD bottle, fitted with a rubber stopper to hold aeration tube. • Drying tube, 150-mm x 18-mm-diam, containing 20 g Mg (CIO4)i. A 60-W light bulb with a suitable shade may be substituted to prevent the condensation of moisture inside the absorption cell. Position the bulb to maintain the cell at 10 ºC above ambient temperature. • Connecting tubing, glass tubing to pass mercury vapor from the		

	reaction flask to absorption cell and to interconnect all other components. Clear vinyl plastic tubing (Tygon, or equivalent) may be substituted for glass.
Materials and Reagents	1. Metal-free water 2. Stock mercury solutio1J,: Dissolve 0.1354 g mercurié chloride (HgC12) in about 70 mL water, add 1 mL conc HNO3, and dilute to 100 mL with water; 1.00 mL = 1.00 mg Hg. 3. Standard mercury solutions: Prepare a series of standard mercury solutions containing O to 5 µg/L by the appropriate dilu□tion of stock mercury solution with water containing 10 mL/L conc HNO3. Prepare standards daily 4. Nitric acid (HNO3), conc 5. Potassium permanganate solution: Dissolve 50 g KMnO4 in water and dilute to 1 L. 6. Potassium persulfate solution: Dissolve 50 g K2S2O8 in water and dilute to 1 L. 7. Sodium chloride-hydroxylamine sulfate solution: Dissolve 120 g NaCl and 120 g (NH2OH)i · H2SO4 in water and dilute to 1 L. A 10% hydroxylamine hydrochloride solution may be substi□tuted for the hydroxylamine sulphate. 8. Stannous ion (Sn2 +) solution: Use either stannous chloride, paragraph hl below, or stannous sulfate, paragraph h2 below, to prepare this solution containing about 7.0 g Sn2 + per 100 mL. 1) Dissolve 10 g SnC12 in water containing 20 mL conc HCl and dilute to 100 mL. 2) Dissolve 11 g SnSO4 in water containing 7 mL conc H2SO4 and dilute to 100 mL. Both solutions decompose over time. If a suspension forms, stir the reagent continuously during use. Reagent volume is sufficient to process about 20 samples; adjust volumes prepared to accom□modate number of samples processed. 9. Sulfuric acid (H2SO4), conc.
Preparation of Reagents	As mentioned above.
Sample Preparation	Transfer 100mL of sample or portion diluted to 100 mL containing not than 5.0 µg/L and a blank of 100 mL water to a 300mL BOD bottles. Add 5 mL Sulphuric acid (98%) and 2.5 mL of Nitric acid (70%) to each bottle . Add 15 mL Potassium permanganate solution to each bottle and let it stand for at least 15 minutes. Add 8 mL Potassium persulphate solution to each bottle and heat for 2 h in a water bath at 95°C. Cool and add 6 mL of Sodium chloride-hydroxylamine sulphate to reduce the excess permanganate.
Method of analysis	**Standard Preparation**: Prepare a series of standard metal solution in the optimum concentration range (1µg/L to 5µg/L) by appropriate dilution from their stock solution with ASTM type 1 water using the following formula.

	Standardization: Transfer 100 mL each of the 1.0, 2.0, and 5.0 µg/L Hg standard solutions anda blank of 100 mL water to 250-mL Erlenmeyer reaction flasks. Add 5 mL conc H_2SO_4 and 2.5 mL conc HNO_3 to each flask. Add 15 mL $KMnO_4$ solution to each flask and let stand at least 15 min. Add 8 mL $K_2S_2O_8$ solution to each flask and heat for 2 h in a water bath at 90 to 95 ºC. Cool to ambient temperature. Treating each flask individually, add enough NaCl-hydroxyl□amine solution to reduce excess $KMnO_4$, then add 5 mL SnC_{12} or $SnSO_4$ solution and immediately attach flask to aeration apparatus. As Hg is volatilized and carried into the absorption cell, absorbance will increase to a maximum within a few seconds. As soon as the recorder returns approximately to the baseline, remove the stopper holding the frit from the reaction flask, and replace with a flask containing water. Flush the system for a few seconds, and run the next standard in the same manner. Construct a standard curve by plotting peak height versus micrograms of Hg. **Analysis of samples:** Transfer 100 mL sample or portion diluted to 100 mL containing not more than 5.0 µg Hg/L to a reaction flask.
Calculation with units of expression	Determine peak height of sample from recorder chart and read mercury value from standard curve prepared.
Inference (Qualitative Analysis)	NA
Reference	APHA 24TH Edition 2023
Approved by	Scientific Panel on Methods of Sampling and Analysis

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Method No.	**FSSAI 14.047:2024**	**Revision No. & Date**	0.0
Scope	This method is designed to determine trace metals and metalloids in surface, ground, and drinking waters via inductively coupled plasma-mass spectrometry (ICP-MS). The Inductively Coupled Plasma coupled with a mass spectrometer give very high sensitivity for the determination of multi elements and even isotopes ,metals like aluminum , antimony, arsenic, barium, cadmium, chromium, copper, iron, lead, manganese, molybdenum, nickel, selenium and silver in water can be determined up to ppb level.		
Caution	**1.** Concentrated nitric and hydrochloric acids present various hazards and are moderately toxic and extremely irritating to skin and mucus membranes. Use these reagents in a fume hood whenever possible and if eye or skin contact occurs, flush with large volumes of water. Always wear safety glasses or a shield for eye protection, protective clothing and observe proper mixing when working with these reagents. **2.** The acidification of samples containing reactive materials may result in the release of toxic gases, such as cyanides or sulfides. Acidification of samples should be done in a fume hood. **3.** Analytical plasma sources emit radiofrequency radiation in addition to intense UV radiation. Suitable precautions should be taken to protect personnel from such hazards. The inductively coupled plasma should only be viewed with proper eye protection from UV emissions.		
Principle	In this method, sample material is introduced to an argon-based, high-temperature radio-frequency plasma, usually via pneumatic nebulization. As energy transfers from the plasma to the sample stream, the target element dissolves, atomizes, and ionizes. The resulting ions are extracted from the plasma through a differential vacuum interface and separated based on their mass-to-charge (m/z) ratio by a mass spectrometer. Typically, either a quadrupole (with or without CCT or DRC) or magnetic sector (high-resolution) mass spectrometer is used. An electron multiplier detector counts the separated ions, and a computer-based data-management system processes the resulting information.		
Apparatus/Instruments	Inductively Coupled Plasma-Mass Spectrometry		
Materials and Reagents	Reagents may contain elemental impurities that might affect the integrity of analytical data. Owing to the high sensitivity of ICP-MS, high-purity reagents should be used whenever possible. All acids used for this method must be of ultra high-purity grade. Suitable acids are available from a number of manufacturers or may be prepared by sub-boiling distillation. Nitric acid is preferred for ICP-MS in order to minimize polyatomic ion interferences. Several polyatomic ion interferences result when hydrochloric acid is used, however, it should be noted that hydrochloric acid is required to maintain		

	stability in solutions containing antimony and silver. When hydrochloric acid is used, corrections for the chloride polyatomic ion interferences must be applied to all data . • Nitric acid (specific gravity 1.41) • Hydrochloric acid (specific gravity 1.19). • Ammonium hydroxide (specific gravity 0.902). • Reagent water - All references to reagent grade water in this method refer to ASTM Type I water • **Standard Stock Solutions** - Stock standards and tuning solution may be purchased from a reputable commercial source or prepared from a ultra-high-purity grade chemicals or metals (99.99 - 99.999% pure). All salts should be dried for one hour at 105°C, unless otherwise specified. Stock solutions should be stored in FEP bottles.
Preparation of Reagents	Same as above.
Sample Preparation	Colorless & transparent, water samples with turbidity of <1.0 can be directly analyzed by ICP-MS for total metals after acidifying with HNO3 (1.5 mL HNO3 /L of water). Sample digestion is not required.
Method of analysis	After preparation of sample • **Standard Preparation**: - Prepare a series of standard metal solution in the optimum concentration range (1µg/L to 5µg/L) by appropriate dilution from their stock solution with ASTM type 1 water using the following formula N1V1 = N2V2 • **Analysis of sample**: Follow manufacturer's standard operating procedure for initialization, mass calibration, gas flow optimization, and other instrument operating conditions. Maintain complete and detailed information on the operational status of the instrument whenever it is used. A suggested analytical run sequence, including instrument tuning/optimization, checking of reagent blanks, instrument calibration and calibration verification, analysis of samples, and analysis of quality control samples and blanks. Follow manufacture's instruction for optimizing instrument performance. The most important optimization criteria include nebulizer gas flows, detector and lens voltages, radio-frequency forward power, and mass calibration. Periodically check mass calibration and instrument resolution. Ideally, optimize the instrument to minimize oxide formation and doubly-charged species formation. Measure the CeO/Ce ratio to monitor oxide formation, and measure doubly-charged species by determination of the Ba2+/Ba+ ratio. Both these ratios should meet the manufacture's criteria. After optimization and tuning, calibrate instrument using an appropriate range of calibration standards. Use appropriate regression techniques to determine calibration lines or curves for each analyte. For acceptable calibrations. Correlation coefficients for regression curves are ideally 0.995 or greater. Immediately after calibration,

run initial calibration verification standard; acceptance criteria are +10% of known analyte concentration. Next run initial calibration verification blank; acceptance criteria are ideally ± the absolute value of the instrument detection limit for each analyte , but in practise ± the absolute value of laboratory reporting limit or the laboratory method detection limit for each analyte is acceptable. Verify low-level calibration by running 0.3- and/or 1.0 µg/L standards if analyte concentration are less than 5 µg/L. Ensure that all vessels and reagents are free from contamination . During analytical run include quality control analyses. Internal standard recoveries must be between 70% and 125% of internal standard response in the laboratory-fortified blank: otherwise, dilute sample, add internal standard mix, and reanalyze. Make known-addition analyses for each case separate matrix in a digestion or filtration batch.

For every new or unusual matrix, it is highly recommended that a semi- quantitative analysis be carried out to screen the sample for elements at high concentration. Information gained from this may be used to prevent potential damage to the detector during sample analysis and to identify elements which may be higher than the linear range should be diluted into range and reanalyzed. Select abundant masses for the metals as given below in the table

Element of Interest	**Masses**
Aluminum	27
Antimony	123
Arsenic	75
Barium	137
Beryllium	9
Cadmium	111
Chromium	52
Cobalt	59
Copper	63
Lead	206, 207, and 208
Manganese	55
Mercury	202
Molybdenum	98
Nickel	60
Selenium	82
Silver	107
Thallium	205
Thorium	232

	<table><tr><td>Uranium</td><td>238</td></tr><tr><td>Vanadium</td><td>51</td></tr><tr><td>Zinc</td><td>66</td></tr></table>
Calculation with units of expression	Metal conc. in sample (mg/L) = Sample conc. from instrument (mg/L) X Dilution factor (if any)
Interference	Several interference sources may cause inaccuracies in the determination of trace elements by ICP-MS. These are: 1. **Isobaric elemental interferences**: Are caused by isotopes of different elements which form singly or doubly charged ions of the same nominal mass-to-charge ratio and which cannot be resolved by the mass spectrometer in use. All elements determined by this method have, at a minimum, one isotope free of isobaric elemental interference. of the analytical isotopes only molybdenum-98 (ruthenium) and selenium82 (krypton) have isobaric elemental interferences. If alternative analytical isotopes having higher natural abundance are selected in order to achieve greater sensitivity, an isobaric interference may occur. All data obtained under such conditions must be corrected by measuring the signal from another isotope of the interfering element and subtracting the appropriate signal ratio from the isotope of interest. A record of this correction process should be included with the report of the data. It should be noted that such corrections will only be as accurate as the accuracy of the isotope ratio used in the elemental equation for data calculations. Relevant isotope ratios should be established prior to the application of any corrections. 2. **Abundance sensitivity**: Is a property defining the degree to which the wings of a mass peak contribute to adjacent masses. The abundance sensitivity is affected by ion energy and quadrupole operating pressure. Wing overlap interferences may result when a small ion peak is being measured adjacent to a large one. The potential for these interferences should be recognized and the spectrometer resolution adjusted to minimize them. 3. **Isobaric polyatomic ion interferences** : Are caused by ions consisting of more than one atom which have the same nominal mass-to-charge ratio as the isotope of interest, and which cannot be resolved by the mass spectrometer in use. These ions are commonly formed in the plasma or interface system from support gases or sample components. Most of the common interferences have been identified3, and these are listed in Table2

	together with the method elements affected. Such interferences must be recognized, and when they cannot be avoided by the selection of alternative analytical isotopes, appropriate corrections must be made to the data. Equations for the correction of data should be established at the time of the analytical run sequence as the polyatomic ion interferences will be highly dependent on the sample matrix and chosen instrument conditions. In particular, the common 82Kr interference that affects the determination of both arsenic and selenium, can be greatly reduced with the use of high purity krypton free argon. 4. **Physical interferences** ; Are associated with the physical processes which govern the transport of sample into the plasma, sample conversion processes in the plasma, and the transmission of ions through the plasma mass spectrometer interface. These interferences may result in differences between instrument responses for the sample and the calibration standards. Physical interferences may occur in the transfer of solution to the nebulizer (e.g., viscosity effects), at the point of aerosol formation and transport to the plasma (e.g., surface tension), or during excitation and ionization processes within the plasma itself. High levels of dissolved solids in the sample may contribute deposits of material on the extraction and/or skimmer cones reducing the effective diameter of the orifices and therefore ion transmission. Dissolved solids levels not exceeding 0.2% (w/v) have been recommended3 to reduce such effects. Internal standardization may be effectively used to compensate for many physical interference effects. Four Internal standards ideally should have similar analytical behavior to the elements being determined. 5. **Memory interferences**: Result when isotopes of elements in a previous sample contribute to the signals measured in a new sample. Memory effects can result from sample deposition on the sampler and skimmer cones, and from the buildup of sample material in the plasma torch and spray chamber. The site where these effects occur is dependent on the element and can be minimized by flushing the system with a rinse blank between samples. The possibility of memory interferences should be recognized within an analytical run and suitable rinse times should be used to reduce them. The rinse times necessary for a particular element should be estimated prior to analysis. This may be achieved by aspirating a standard containing elements corresponding to 10 times the upper end of the linear range for a normal sample analysis period, followed by analysis of the rinse blank at designated intervals. The length of time required to reduce analyte signals to within a factor of 10 of the method detection limit, should be noted. Memory interferences may also be assessed within

	an analytical run by using a minimum of three replicate integrations for data acquisition. If the integrated signal values drop consecutively, the analyst should be alerted to the possibility of a memory effect, and should examine the analyte concentration in the previous sample to identify if this was high. If a memory interference is suspected, the sample should be reanalyzed after a long rinse period. In the determination of mercury, which suffers from severe memory effects, the addition of 100 µg/L gold will effectively rinse 5 µg/L mercury in approximately two minutes. Higher concentrations will require a longer rinse time.
Reference	APHA 24TH Edition 2023
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	**Determination of Gross Beta Activity Measurement**		
Method No.	**FSSAI 14.048:2024**	**Revision No. & Date**	0.0
Scope	This method is applicable for measurement of gross-beta activity in water and waste water.		
Caution	Each chemical should be treated as a potential health hazard, and exposure to these chemicals should be minimized. A reference file of safety data sheets (SDSs) should be made available to all personnel involved in the preparation of samples and their analyses.		
Principle	For measurement of beta activity of water, waste water, soil, gummed paper, air filters, ash of vegetation and biological samples, the samples taken are prepared with suitable treatment (digestion) and then measured for beta activity by a low background beta counter. For the purpose of this method the following terms shall apply. 1) Activity - The number of spontaneous nuclear transformations occurring in a given quantity of material during a suitably small interval of time divided by that interval of time. It is commonly expressed in Becquerel (Bq), formerly expressed in Curies. NOTE - Sometimes used to designate a quantity of radionuclide (also called disintegration rate) 2) Nuclide - A species of atom characterized by its mass number, atomic number and nuclear energy state, provided that the mean life in that state is long enough life time usually more than 10^{10} s to be observable. 3) Radionuclide - An unstable form of a chemical element that radioactively decays, resulting in the emission of nuclear radiation.		
Apparatus/Instruments	1) **Low Background Beta Counter** Low background beta counter of background less than 0.05 cps with about 30 percent efficiency for potassium-40 betas. The system shall be sensitive and capable to detect gross β activity below 0.2 mBq/m3 and 30 mBq/L, for air and for drinking water samples respectively. 2) **Shielding to Detector** Sufficient shielding to reduce the counting system background to 0.05 cps or less (generally, about 50.8 mm lead shield is in use). 3) **Sample Holder Assembly** Capable of taking planchets of minimum 25 mm diameter with 2 to 3 mm rims. 4) **Aluminium or Stainless Steel Planchet** Minimum 25 mm diameter and with 2 to 3 mm rims. 5) **Air Oven** 6) **Infrared Lamp** - Maximum 500 W.		

	7) **Muffle Furnace**
Materials and Reagents	1) Nitric acid (IS 264: 2005) 2) Hydrochloric acid (IS 265:1993) 3) Reagent grade water (IS 1070 : 1992)
Method of analysis	1) **Sample Preparation** For the gross activity estimation, preparation of sample should be simple and fast. The methods of sampling are prescribed in 1.1 and 1.2. 1.1. **Water with Low Dissolved Solids (< 100 mg/l)** 1.1.1.Total dissolved solid in water sample is measured with TDS meter. 1.1.2.Collect the water sample in a pre-cleaned polythene bottle after rinsing with same water. 300 to 500 ml of the sample should be sufficient for gross activity determination. To preserve the samples, use hydrochloric acid (HCl) or nitric acid (HNO_3) to obtain a pH < 2 at the time of sample collection. 1.1.3.Take a sample of 300 ml in a clean glass beaker and evaporate on a hot plate near dryness to about 5 ml. Avoid baking solid on evaporation container. 1.1.4.Cool the completely dried sample planchet. Add 2 drops of 1 percent collodion in acetone. Allow the acetone to evaporate. This sample should be counted in the beta counter. 1.2. **Water with High Dissolved Solids (≥ 100 mg/l)** 1.2.1.Take 500 ml from the 1 000 ml of water sample. Add 2 ml concentrated hydrochloric acid, stir and then add 5 mg ferric chloride or ferric nitrate as iron carrier, 2mg of calcium nitrate or calcium chloride as Ca-carrier. Warm on a hot plate and add 25 ml sulkowitch reagent (see Note) and 8 N ammonia with stirring until precipitate forms. Add 5 mg of ammonium molybdenum phosphate (AMP) and stir it using a magnetic stirrer for about 1 h till complete precipitation take place or alternatively stir it well with a glass rod and keep it overnight to settle the precipitate. NOTE - Sulkowitch reagent is 2.5 g of oxalic acid, 2.5 g of ammonium oxalate, 5ml of acetic acid and distilled water to make 150 m 1.2.2.Separate ferric hydroxide by decanting and centrifuging. Wash the precipitate with 10 ml distilled water twice and discard washings 1.2.3.Slurry the ferric hydroxide precipitates with 1 ml distilled

water and transfer to a 25 mm aluminium planchet using a glass dropper with attached rubber teat. Dry the slurry under an infra red lamp and transfer the precipitate completely using further small amount of distilled water.

1.2.4. Dry the planchet under infrared lamp. Ferric hydroxide when dried, flakes off (use a pointed glass rod to spread the sample uniformly in the planchet). After cooling the planchet, add a drop or two of 1 percent collodion in acetone and dry. The planchet is ready for counting.

2) **Calibration :**

2.1. For calibration, the normal practice is to use the radiation from potassium chloride of minimum purity level 99 percent (100 mg in an aluminium planchet) as a standard for the beta activity of mixed fission products. The average energy of potassium-40 is 0.40 MeV (E_{max} = 1.31 MeV). This is the best approximation of the energy for mixed fission products among the available long-lived isotopes. Alternatively electroplated Chlorine-36 beta sources with average energy of 0.24 MeV and E_{max}= 0.71MeV can be used.

NOTE —The potassium activity is taken from the nuclear data sheets of the National Academy of Sciences. The values are 28 betas per second per gram of potassium, and 3.5 gamma photons/s/g of potassium

2.2. The mass of the actual samples can usually be approximated quite well by a suitable mass of potassium chloride. In some cases where a more active standard is desired, pressed pellets are made up with several grams of potassium chloride (with a few percent of sodium stearate). These standards are first calibrated against the proper weight of potassium chloride and then are used merely to check reproducibility of the counters.

3) **Activity measurements :**

3.1. Keep the low background beta counter 'ON' for at least an hour to stabilize in case the counter is not already 'ON
3.2. Take 3 600 s background counts with a blank aluminium planchet.
3.3. Determine the efficiency of the counter using a standard potassium-40 source (100 mg KCl).
3.4. Keep the sample planchet in the counting system and count for 3 600 s.

Calculation with units of expression	$$\text{Sample count rate (Cs)} = C_S - C_b \pm \frac{\sqrt{S+b}}{3\,600}$$ $$\text{Background countrate } (C_b) = \frac{b}{3\,600} \pm \frac{\sqrt{b}}{3\,600}$$ $$\text{Net count rate } (C_{sb}) = C_S - C_b \pm \frac{\sqrt{S+b}}{3\,600}$$ $$\text{Activity (Bq/unit)} = \frac{C_{Sb}}{E} \times \frac{100}{V} \pm \frac{\sqrt{S+b}}{3\,600} \times \frac{100}{V}$$ Where, S = sample counts for 3600 s b = background counts for 3600 s; E = efficiency of counter using a standard K-40 source, in percentage; and V = size of the sample taken for counting; Normally expressed in **g** or in ml except in case of air filter, for which sample is expressed in m^3 . In case of fallout deposition sampler, it is expressed in m^2
Reference	IS 14194 (Part 1) : 2020
Approved by	Scientific Panel on Methods of Sampling and Analysis