MANUAL OF METHODS

OF

ANALYSIS OF FOODS

METALS




FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA
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MANUAL FOR ANALYSIS OF METALS

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

MANUAL FOR ANALYSIS OF METALS

General The term "Trace Metals" refers to metals which may be present in foods in amounts well below 50 mg/kg and which have some toxicological or nutritional significance While some inorganic elements such as, sodium, potassium calcium, phosphorous are essential for man, elements like lead, cadmium, mercury, arsenic are found to cause deleterious effects even in low levels of 10 – 50 mg/kg. Although iron, copper, zinc, etc., are found to be necessary in certain quantities in foods, the same elements can cause ill effects when consumed at higher levels. Hence, determination of both major and trace levels of metal contents in food is important for both food safety and nutritional considerations

There are four major steps involved in the analysis of foods for the metal contents, viz.

(a) Obtaining a representative sample from the bulk received for testing.

(b) Destruction of organic matter.

(c) Separation and concentration of the element of interest and

(d) Determination

1.0 SAMPLING:

The object of this step is to obtain a small and representative portion from the large sample in such a way that any subsequent test on the sample will give a reproducible value.

For fresh foods, the homogenization process is like macerating in a blender whereas dry products are normally ground mechanically and then mixed and the powder is sieved before analysis. Contamination during this step can be avoided with the use of stainless steel equipment. Hard foods, such as, chocolates are sampled by grating/chopping finely by

hand. Meat and meat products are thoroughly minced and then ground in a mortar and in this case, too small quantities should not be taken for analysis.

Fats are melted before analysis. Wet foods such as pickles, etc should be homogenized in high-speed blender. Liquids are normally sampled after they have been thoroughly mixed by repeated slow inversion of container.

After the sample is properly homogenized and reduced to usable form, it should be stored in an air tight container.

If the sample received for analysis is too large, it has to be reduced to a more convenient size (for homogenization purpose) by repeated quartering in which the sample is arranged in a flat heap, opposite two quarters are rejected and remaining two quarters are mixed and again subjected to quartering. This process is continued till a convenient quantity of sample remains for homogenization by grinding etc. The edible portion of the sample of food has to be taken for preparation of sample for analysis. e.g. fish, etc.

2.0 SAMPLE PREPARATION:

The commonly used methods of destruction of organic matter can be broadly grouped into Wet Oxidation Open & Wet Digestion closed using Microwave Digestion System and Dry Ashing.

2.1 Wet Oxidation

2.1.1 Wet Digestion Open

This procedure is applicable to a wide variety of samples and elements. The general procedure given below is suitable for most determinations. The Experiment should be done in a fume hood with proper exhaust system.

2.1.1.1 Procedure:

Transfer suitable quantity of sample into a macro-kjeldahl digestion flask. Add 20mL concentrated nitric acid and up to 20mL water (depending on the water content of sample). Boil the contents of the flask to reduce the volume to 20mL. Cool the solution, add 10mL of conc. sulphuric acid and boil again. Add further small quantities of nitric acid whenever the contents begin to blacken. When the addition of nitric acid is no longer necessary (i.e. when the liquid no longer blackens) continue heating till white fumes are evolved.

At this stage, cool the solution and add 10mL of saturated ammonium oxalate solution and again boil until copious white fumes are again produced. The oxalate treatment assists in removing yellow coloration due to nitro compounds, fats etc so that the final solution is colourless. Every trace of Nitric acid must be removed before proceeding for assay of metals.

A blank should be prepared at the same time.

The commonly used oxidants are nitric acid, sulphuric acid, perchloric acid and hydrogen peroxide. As each of them possesses inherent advantages, use of mixtures containing two or more of the above reagents is recommended.

The procedure can be shortened considerably by the use of mixed oxidizing agents. The main advantage is that by oxidizing at less than 350°C, the nitric acid is used more economically and there is little likelihood of loss of element by volatilization and the process does not require constant Supervision.

2.1.2 Wet Digestion Closed using Microwave Digestion

The method provides for the acid digestion of the samples in a closed vessel device using temperature control microwave heating for the metal determination by spectroscopic methods.

The Microwave digestion temperature needs to be changed according to food matrix. Advantage of the method is to avoid loss of volatile metal like Tin, Arsenic, Mercury, Lead as well as less sample quantity is used. The lab can validate digestion method according to food matrix.

2.1.2.1 Sample preparation:

In case of dry samples, homogenize the sample by grinding as finely as possible, weigh 0.2-0.5gm test portion into digestion vessel. In case of infant cereals and fortified milk powders or any other such matrix, prepare 10% slurry with water and accurately weigh the sample mass on dry weight basis equivalent to 0.5gm, transfer it into MDS vessel. In case of water containing samples, maximum weight is restricted to 2.0gm, dry matter content must never exceed 0.5gm.

2.1.2.2 Equipment: Microwave digestion system

2.1.2.3 Reagents:

7mL of HNO_3 65%, 1mL of H_2O_2 30%

2.1.2.4 Procedure:

1. Place a Teflon material vessel (TFM) vessel on the balance plate, tare it and weigh of the sample.

2. Introduce the Teflon material vessel (TFM) into the safety shield.

3. Add the acids; if part of the sample stays on the inner wall of the TFM vessel, wet it by adding acids drop by drop, then gently swirl the solution to homogenize the sample with the acids.

4. Close the vessel and introduce it into the rotor segment, then tighten by using the torque wrench.

5. Insert the segment into the microwave cavity and connect the temperature sensor

6. Run the microwave program to completion.

7. Cool the rotor by air or by water until the solution reaches room temperature.

8. Open the vessel and transfer the solution to a marked flask.

2.1.2.5 Microwave program:

Step	Time	Temperature	Microwave power
1	10 minutes	200°C	Up to 1000 Watt*
2	20 minutes	200°C	Up to 1000 Watt*

2.2 Dry Ashing and Preparation of Solution

This procedure is also used for destruction of organic matter. Precautions are to be taken to avoid losses by volatilization of elements, retention of element on the surface of vessel used or incomplete extraction of ash. These problems can be avoided by using controlled muffle furnace, by adding ash aid wherever necessary (Magnesium nitrate, sodium carbonate sulphuric acid etc) to the food before ashing and by using a suitable acid for extraction. Silica or platinum vessels are to be preferred.

2.2.1 Procedure:

Weigh accurately a suitable quantity of the well mixed sample in a tared silica or platinum dish. Heat first by means of a soft flame to volatilize as much organic matter as possible, then transfer the basin to a temperature controlled muffle furnace. Keep the muffle at about 300°C. Once the material is dry and charred, the temperature is allowed to rise to 450°C and ash at this temperature till no carbon remains. If it is suspected that all carbon has not been removed, cool the ash, add about 1 to 2mL of conc. nitric acid, evaporate to dryness and again heat in muffle furnace. After ashing is complete, remove the dish from muffle furnace, cool, cover the dish with watch glass, and add gently 40 to 50mL of hydrochloric acid and water (1 +1). Rinse down watch glass with water and heat on steam bath for 30 minutes, remove the cover and rinse. Continue heating for another 30 minutes. Add 10mL of hydrochloric acid and water (1 +1) to dissolve the salts. Filter into a 100mL volumetric flask using Whatman No. 44 filter paper. Wash the residue and basin twice using dilute HCl. Make up to volume with water.

For food stuffs of low ash or high chloride content and where the loss of heavy metals by volatilization is suspected, add about 20mL of dilute sulphuric acid and water (1+1) (taking care to wet all the sample in the dish) and evaporate slowly at around 100°C and then ash in the normal way. The commonly used ashing aids are nitric acid, dilute sulphuric acid, magnesium nitrate, magnesium acetate, sodium carbonate, etc.

2.2.2 Reagents:

It is necessary to use reagents and distilled water of suitably low metal content taking into consideration that the concentrated mineral acids are generally used in amounts several times more than the sample. Even when these reagents are used, reagent blank determination shall be necessary.

Blanks must be prepared with the same quantities of the reagents as are used in the test.

In expressions like (1+2), (1+3) etc, used with the name of a reagent, the first numeral indicates (volume/weight) of (liquid/solid) reagent and second numeral indicates volume of water. For example HCl (1+2) means reagent prepared by mixing one volume of HCl with two volumes of water.

All chemicals that are used in these procedures should be of highest purity i.e. AR grade. The chemicals should not be transferred to other bottles if any chemical to be used has any kind of impurity, then it should be purified.

Procedures for purification of some frequently used chemicals are given below:

(i) Adsorbent cotton (metal free): If traces of metals are present, remove them by digesting cotton several hours with 0.2N HCl, filtering on Buchner and finally washing with water until acid free.
(ii) Ammonium hydroxide: Distil ordinary reagent into ice cold redistilled water. Concentration of re-distilled NH_4OH can be determined by specific gravity or titration.
(iii) Conc. HCl, HNO_3, Bromine: Distill the reagents in an all glass apparatus.
(iv)Carbon tetrachloride: Reflux the ordinary CCl_4 vigorously on steam bath for 1 hour with 1/20 volumes of 20% KOH in methanol. Cool, add water, drain off CCl_4 layer and wash with water until alkali free. Dry over anhydrous calcium chloride, filter and distill on hot water bath.
(v) Chloroform: Distil ordinary reagent, from hot water bath, collecting distillate in absolute alcohol in proportion of 10mL alcohol to 1000mL of distillate.
(vi)Dithizone: Dissolve about 1 gm of commercial reagent in 50 to 75mL of $CHCl_3$ and filter, if insoluble materiel remains. Extract in separator with three 100mL portions of NH_4OH (1+99). Dithizone passes into aqueous phase to give orange solution. Filter aqueous

extracts into large separator through cotton pledget inserted in stem of funnel. Acidify slightly with dilute HCl and extract the precipitated dithizone with three 20mL portions of $CHCl_3$. Combine extracts in a separator and wash, 2 or 3 times with water. Repeat the above process again.

Drain the final water washed solution of dithizone in $CHCl_3$ into a beaker and evaporate major portion of $CHCl_3$ spontaneously and complete drying under vacuum. Store dry reagent in dark, in tightly stoppered bottle. Prepare solutions for extraction in pure $CHCl_3$/ CCl_4.

(vii) Citric Acid, Sodium/Ammonium acetate, Aluminium nitrate, Calcium nitrate, Sodium Sulphate, etc. (in assay of lead).

Adjust pH of their aqueous solution to 3.0 to 3.5 (using bromophenol blue indicator) with NH_4OH. Precipitate lead and some other metals from the solution as sulphides, with H_2S using 5 to 10mg of $CuSO_4$ as co-precipitant. Filter, boil filtrate to expel excess H_2S completely and refilter, if necessary to obtain clear solution.

3.0 ANALYSIS OF METALS BY AAS

3.1 DETERMINATION OF LEAD, CADMIUM, COPPER, IRON AND ZINC IN FOODS BY ATOMIC ABSORPTION SPECTROPHOTOMETER

3.1.1 Scope:

This method is applicable for determination of Lead, Copper, Cadmium, Iron, and Zinc in food by Atomic Absorption Spectrophotometer using Flame and Furnace Technique.

3.1.2 Principle:

Test portions are dried and then ashed at 450°C under a gradual increase (about 50°C/hr) in temperature, 6 N HCl (1+1) is added and the solution is evaporated to dryness.

The residue is dissolved in 0.1N HNO_3 and the analytes are determined by flame and graphite procedures.

3.1.3 Apparatus:

(a) Atomic absorption Spectrophotometer – with an air – acetylene burner or nitrous oxide- acetylene burner for flame and a graphite furnace for electro-thermal determinations with appropriate background (non atomic correction). Instrument parameters are usually given by the manufacturer in the manual provided with the instrument

(b) Hollow cathode or electrode less discharge lamps for all elements.

(c) Furnace – Programmable or muffle furnace with thermostat maintaining 450 ±25°C

(d) Hot plate – with heating control to heat upto 300°C

(e) Quartz or platinum dishes

(f) Polystyrene bottles – with leak proof closures – 100mL

Carefully clean and rinse all glassware and plastic ware with HNO_3 or HCL to avoid metal contamination – First wash with water and detergent, rinse with tap water, followed by distilled water, then with dilute acid (1 + 9) and finally 3-4 times with distilled water.

NOTE: Microwave Digestion can be done since there is a chance of loss of target metals i.e. Lead, during ashing.

3.1.4 Reagents:

(a) Water – redistilled or deionised

(b) Hydrochloric acid A.R (6N) – Dilute 500mL HCl to 1 litre with water

(c) Nitric Acid A.R 0.1M – dilute 7mL conc. acid to 1 litre

(d) Nitric acid concentrated (Sp. Gravity 1.40)

(e) Standard solutions of cadmium, copper, lead and zinc prepared as follows:

(It is suggested to use NIST traceable all solution of 1000mg/L. Prepare stock & working solution from this. For the better result, working solution should be prepared in the digestion solution. Commercially available standard solutions for AAS may be used for all metal standards).

(1) Lead Standard solution– 1mg/mL. Dissolve 1.000 gm Pb in 7 mL conc. HNO_3 in 1litre volumetric flask. Dilute to volume with water.
(2) Cadmium Standard solution – 1 mg/mL. Dissolve 1.000gm in 14mL water and 7mL conc. HNO_3 in 1 litre flask. Dilute to volume with water.
(3) Zinc Standard solution – 1mg/mL. Dissolve 1.000gm Zinc in14mL water + 7mL conc. HNO_3 in 1 litre volumetric flask and dilute to volume with water.
(4) Copper Standard solution – 1mg/mL. Dissolve 1.000gm Copper in 7mL HNO_3 in 1 litre flask. Dilute to volume with water.
(5) Iron Standard solution – 1mg/mL. Dissolve1.000gm Iron in 14mL water and 7mL conc. HNO_3 in 1 litre volumetric flask. Dilute to volume with water.

(e) Working Standard solution – For graphite furnace analysis dilute standard solutions with 0.1 M HNO_3 to a range of standards that cover the linear range of the elements to be determined. For Flame analysis dilute standard solutions with 0. 1 M HNO_3 to a range of standards that covers the concentration of the elements to be determined.

NOTE: Apart from the NIST traceable metal standard, internal standard may be used for accuracy of test result.

3.1.5 Preparation of Sample:

Sample may be prepared by wet digestion using microwave digestion system or by dry ashing as given below. Weigh accurately about 25 gm of well homogenized sample into a clean silica dish. Add 25mL of 20% sulphuric acid. Mix thoroughly with a glass stirring

rod ensuring all sample material is wetted by the acid. Rinse stirring rod with water into silica dish. Dry the contents of the dish thoroughly on a steam bath or in an oven around 110°C. When the sample is thoroughly dry, heat the contents of the dish with a soft flame (such as that of a Bunsen burner) until all volatile or readily combustible matter has been removed.

Transfer the dish to a furnace set at 250°C. Slowly raise temperature to 500°C. Ash at this temperature for about 6 to 8 hours. Remove the dish and cool. Ash should now be white or brownish red and essentially be carbon free. If ash contains carbon particles, wash down sides of dish with water and add 2mL of HNO_3 and mix well. Dry thoroughly on hot plate. Return dish to furnace at 500°C and ash for 30 minutes. Repeat nitric acid treatment using 1mL increments of HNO_3 until white/brownish red, carbon free ash is obtained. When clean ash is obtained, remove the dish from furnace, cool and add 1mL HNO_3 and 10mL of water. Heat on hot plate till sample ash is dissolved. Quantitatively transfer the contents of the dish to a 50mL volumetric flask, heat the dish with 10mL of HCl (1+1) and transfer the solution again to the same volumetric flask to volume with water.

Prepare sample blank solution by following the same procedure as described for sample. Use same quantities of reagents including water for both sample and blank. Subject both sample and sample blank to identical treatment (even the length of time kept in furnace etc.)

NOTE:

1. Do not ash HNO_3 in furnace. Always dry HNO_3 (in the dish) on steam bath or hot plate and then ash in furnace.
2. Do not allow sample to ignite during any stage of ashing.
3. If the calcium content of the sample is high, then avoid the use of sulphuric acid (Ash aid) and ash at temperatures not exceeding 470°C.

3.1.6 Determination:

Atomic Absorption Spectrophotometry: - Lead and Cadmium in foods generally require graphite furnace AAS (GFAAS) for determination Zinc, Copper and Iron can be determined by flame AAS

(1) Set the instrument as per the previously established optimum conditions /as per the guide lines given in the Instruction Manual (provided along with the instrument). The standard conditions for Atomic absorption spectrophotometer are given below.

(2)Determine absorbance of sample solution(s) and blank.

(3)Calculate the heavy metal content from standard curve.

NOTE: calibrate AAS with copper solution (NIST traceable) before use, for absorption value (pre defined).

3.1.7 Preparation of Standard Curve:

Read the absorbance of a series of standard metal solutions in the Atomic Absorption Spectrophotometer after setting the instrument as per optimum conditions. Plot absorbance against µg of metal/mL solution.

3.1.8 Standard Conditions for Atomic Absorption Spectrophotometer:

Element	**Wavelength**	**Flame-Gases:**
Copper	324.8	Air – acetylene
Lead	217.0	Air – acetylene
Zinc	213.9	Air – acetylene
Cadmium	228.8	Air – acetylene
Iron	248.3	Air – acetylene

(Ref: - AOAC 19th edn, 2012 Official Method 999.11 Determination of Lead, Cadmium, Copper, Iron and Zinc in Foods Atomic Absorption Spectrophotometry after dry ashing)

NOTE:

1. Prepare spike standard in the same kind of sample.
2. Use of QC NIST traceable standard in the different kind of food matrix after interval of 20 sample. If any deviation observed, then equipment should be calibrated again
3. Always monitor current density of AAS lamp. Old lamp (after expiry of pre defined age of lamp), normally users increase lamp current, which creates a noise and chance of false positive result.
4. Use Spike method and analyze recovery of metals (see in ICP-OES Methods)

3.2 DETERMINATION OF MERCURY IN FOOD BY FLAMELESS ATOMIC ABSORPTION SPECTROPHOTOMETRIC METHOD

3.2.1 Principle:

The sample is digested with HNO_3 and H_2SO4 and HNO_3 – $HClO_4$ mixture in the presence of sodium molybdate solution. The mercury content is estimated by flameless atomic absorption method.

3.2.2 Apparatus:

(i) Atomic absorption spectrophotometer: equipped with Hg hollow cathode lamp and gas flow through cell (Fig. 1), {25 (id) x 115} mm with quartz windows commented in place.
Operating condition: Wavelength 253.7 nm, slit with 160 µm, lamp current 3mA, and sensitivity scale 2.5.
OR
Use Mercury Analyzer

(ii) Diaphragm pump: Coat diaphragm and internal parts of pump with acrylic type plastic spray.Use 16 gauge teflon tubing for all connections.

(iii) Water condenser: 12 to 18 (id) x 400 mm borosilicate, 24/40 standard taper joint, modified to hold 6 mm Raschig rings. Fill condenser with Raschig rings to a height of 100 mm, then place 20 mm layer of 4 mm diameter glass beads on top of rings.

(iv)Digestion flask: 250 mL fat bottom boiling flask with 24/40 standard taper joint.

(v) Gas inlet adapter: 24/40 standard taper.

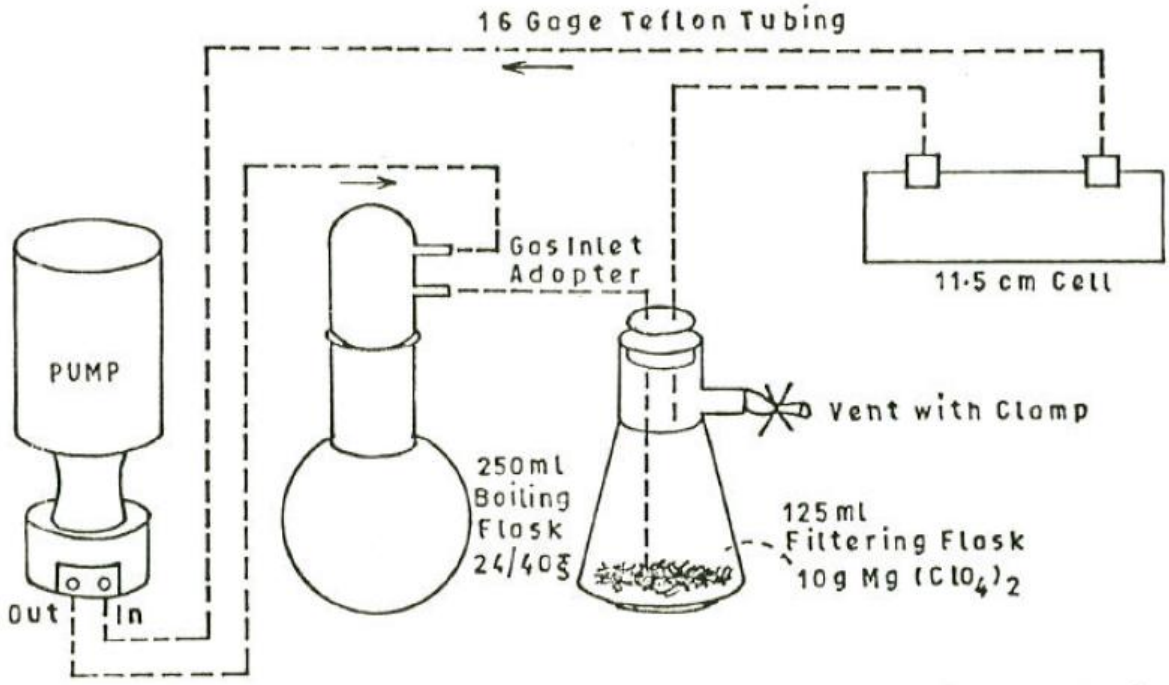

Fig.1-1. Apparatus for flameless atomic absorption analysis of mercury.

Fig. 1

3.2.3 Reagents:

(a) Reducing solution:

Mix 50mL H_2SO_4 with approximately 300mL water. Cool to room temperature and dissolve 15 gm NaCl, 15 gm hydroxylamine sulphate and 25 gm $SnCl_2$ in solution. Dilute to 500mL.

(b) Diluting solution:

To 1000mL volumetric flask containing 300 to 500mL water add 58mL HNO_3 and 67mL H_2SO_4. Dilute to volume with water.

(c) Magnesium perchlorate:

Drying agent placed in filter flask (Fig. 1.). Replace as needed
(Caution: $Mg(ClO_4)_2$ is explosive when in contact with organic substances).

(d) Mercury standard solution: (USE 1000mg/L NIST traceable standard)
(i) Stock Solution (1000 µg/mL): Dissolve 0.1354 gm $HgCl_2$ in 100mL water.
(ii) Working solution (1 µg/mL): Dilute 1 mL stock solution to 1000mL with 1N H_2SO_4. Prepare fresh daily.

3.2.4 Preparation of Sample:

Digest the sample using microwave method or by method given below:.

Weigh 5.0 gm of sample into digestion flask, add 25mL of 18N H_2SO_4, 20mL of 7N HNO_3, 1mL of 2% sodium molybdate solution and 5-6 boiling chips.

Connect condenser (with water circulating through it) and apply gentle heat for about 1 hour. Remove heat and let stand for 15 minutes. Add 20mL HNO_3 – $HClO_4$ (1+1)

through condenser; turn off water circulating through condenser and boil vigorously until white fumes appear in flask. Continue heating for 10 minutes.

Cool, cautiously add 10mL water through condenser while swirling liquid in flask. Again boil solution for 10 minutes. Remove heat and wash condenser with three 15mL potions of water.

Cool solution to room temperature. Completely transfer digested sample with water to 100mL volumetric flask and dilute to volume with water.

3.2.5 Determination:

Transfer 25.0mL of aliquot from digest solution of each sample to another digestion flask. Adjust volume to about 100mL with diluting solution.

Adjust output of pump to approximately 2 litre air/min by regulating speed of pump with variable transformer. Connect apparatus as in Fig. 1.1 except for gas inlet adapter with pump working and spectrophotometer zeroed; add 20mL of reducing solution to diluted aliquot. Immediately connect gas inlet adapter and aerate for about 3 minutes. (Adjust aeration time to obtain maximum absorbance) Record absorbance (A), disconnect pressure on 'out' side of pump, and open vent of filter flask to flush system.

3.2.6 Preparation of Standard Curve:

Prepare reagent blank and standard curve by adding 0, 0.2, 0.5, 1.0, 1.5 and 2.0 µg Hg to series of digestion flasks. To each flask add 100mL diluting solution. Finally add reducing solution and aerate standards as for samples.

Plot standard curve from least squares linear regression of absorbance against µg of Hg. Determine µg of Hg in aliquot from curve. If µg of Hg falls outside the range of calibration, repeat determination with smaller aliquot of sample solution to bring µg of Hg into region of standard curve.

From size of aliquot used, determine total mercury content in original sample.

Concentration Hg (µg/kg) = µg Hg/gm test portion

(Ref: - AOAC 19th edn, 2012 Official Method 971.21 Mercury in Food Flameless Atomic Absorption Method)

4.0 DETERMINATION OF MERCURY IN FOOD USING MERCURY ANALYSER

4.1 Method I

4.1.1 Principle:

Sample is digested with nitric acid and sulphuric acid under reflux in special apparatus. By reduction mercury vapour is generated which is measured using Mercury Analyser.

4.1.2 Instrument:

Mercury Analyzer: It is basically a cold vapour atomic absorption spectrophotometer based on the principle that mercury vapour (atoms) absorbs resonance radiation at 253.7nm. The analyser consists of a low pressure mercury lamp emitting the 253.7nm line, an absorption cell, a filter, a detector with associated electronics and a vapour generation system.

The carrier gas (air free from mercury) bubbles through the vapour generation system carries elemental mercury from the solution and then passes through the absorption cell.

4.1.3 Reagents: (AR grade reagents shall be used)

(a) 1.0 % w/v $KMnO_4$ in 10% of sulphuric acid: Dissolve 1.0 gm of $KMnO_4$ in water and carefully add to it 10mL of sulphuric acid. Make up to a volume of 100mL using distilled water.

(b) 20 % w/v sodium hydroxide: Dissolve 50 mg of NaOH pallets in distilled water and make up to a volume of 250mL.

(c) 20 % $SnCl_2$ (w/v) in 10% HCl: Take 20 gm of $SnCl_2$ in a clean beaker. Add 10mL concentrated HCl and dissolve while warming it over a burner. Boil for 1 min, cool and dilute with distilled water to make 100mL. Add 1 to 2 gm of tin metal (pellet) after the preparation of the solution. Check up the blank. If mercury is present, bubble pure N_2 for 30 min through the solution. This solution with metallic tin is likely to be stable for more than a month. However, it should be discarded in case it turns turbid.

(d) Dilute sulphuric acid (1: 1): Add 125mL of sulphuric acid to water and make up to 250mL.

(e) 10% Nitric acid: Add 20mL nitric acid to water and make up to 200mL.

(f) Mercury standard (stock solution): Dissolve 0.1354 gm of $HgCl_2$ in water and add 1mL of 1% potassium dichromate and make up to a volume of 100mL with 2.0% HNO_3.

Dilute standard solution (100µg/mL) can be prepared from the stock solution.

4.1.4 Preparation of sample:

As given for preparation of sample under determination of mercury by Dithiozone method (see clause 7.7).

4.1.5 Determination:

Take a suitable aliquot of the blank, standard or sample solution in the reaction vessel. Add the required amount of 10% nitric acid to maintain a volume of 10mL. Add 2mL of stannous chloride solution (20% w/v in 10% HCl) and stopper the reaction vessel immediately. Switch on the magnetic stirrer and stir vigorously for about 5 min. After adjusting '0' and 100% T, start the pump and allow the mercury free air to purge through the reaction vessel. The air is rendered mercury free by passing it through a trap containing 20mL of 1.0% permanganate solution in 10% H_2SO_4. Note the absorbance as early as possible (within one minute) in the 'Hold' mode of operation and switch back to 'Normal' mode. Switch off the pump and the magnetic stirrer. Adjust 0% and 100% T just before each measurement.

Before reaching the absorption cell the air along with the mercury vapour from the reaction vessel is first passed through a trap containing 4mL of 20% w/v NaOH and then through a trap containing 4mL of 1:1 H_2SO_4 to absorb acid vapour and moisture respectively. Air along with mercury vapour leaving the absorption cell is passed through a trap containing 20mL of 1.0% $KMnO_4$ in 10% H_2SO_4 to absorb the mercury vapour and thereby avoid contaminating the surrounding with mercury vapour.

4.1.6 Preparation of Standard Curve:

Repeat the measurement for standards 30, 60, 90, 120 and 150 µg Hg and draw calibration graph by plotting absorbance versus concentration of Hg.

Concentration of mercury in the sample is calculated from the calibration curve.

5.0 DETERMINATION OF ELEMENTS IN FOOD USING MICROWAVE ASSISTED DIGESTION BY INDUCTIVELY COUPLED PLASMA-OPTICAL EMISSION SPECTROMETER (ICP-OES)

5.1 Scope:

This method describes procedure for determining total acid-extractable concentrations of elements in food by microwave assisted acid decomposition and inductively coupled plasma - optical emission spectrometer (ICP-OES).

5.2 Principle:

An analytical portion of food is decomposed in acid inside a high-pressure digestion vessel using microwave heating. The analytical solution is then sprayed into the core of an inductively coupled argon plasma, which can reach temperatures of approximately 7000-8000 K. At such high temperatures, all analyte species are atomized, ionized and thermally excited, and they can then be detected and quantified with an optical emission spectrometer (OES). OES measures the light emitted at element-specific characteristic wavelengths from thermally excited analyte ions. This light emitted is separated and measured in a spectrometer, yielding an intensity measurement that can be converted to an elemental concentration by comparison with calibrated reference standards.

5.3 Apparatus:

5.3.1 Inductively coupled plasma optical emission spectrometer (ICP-OES) — inductively coupled plasma optical emission spectrometer (ICP-OES)-Simultaneous or sequential ICP-AES with associated glassware, which uses a mass flow controller to regulate argon nebulizer flow rate supplied by a Dewar of liquid argon or tank of gaseous argon. A variable speed peristaltic pump to deliver all solutions to nebulizer. Pneumatic nebulizer which can aspirate high dissolved solids (e.g., V-groove, cross flow, etc.) or an

ultrasonic nebulizer. ***Safety Note: Inductively coupled plasmas should only be viewed with proper eye protection from ultraviolet emissions***.

5.3.2 Microwave digestion system—requires temperature control to at least 200°C and pressures ≥ 300 psi (~ 20 bar) with appropriate safety features to prevent over pressurization of vessels. Microwave must have multi-step programming with ramp to temperature capability. Digestion vessels must be PFA, TFM Teflon® lined or quartz.

5.3.3 Analytical balance—Capable of measuring to 0.1 mg.

5.4 Reagents:

1. Reagent water**—**Water meeting specifications for ASTM Type-I water.
2. Argon supply—High purity (99.99%) argon.
3. High purity nitric acid—Concentrated (67-70%, sp. Gr. 1.42), double distilled. The trade name for double distilled grade will vary by manufacturer.
4. High purity hydrochloric acid—Concentrated (30-35%, sp. Gr. 1.18), double distilled.
5. Hydrogen Peroxide—Concentrated (30%), high purity or trace metals grade.
6. Standard stock solution- Stock standards may be purchased from a reputable commercial source or prepared from ultra high-purity grade chemicals or metals (99.99 - 99.999% pure).
7. Preparation of working Standards -Dilute stock standard with 5% HNO3 – 0.5% HCl to prepare multi element working standards. Store in Teflon® FEP, PP or HDPE bottles.

Safety Notes: ***Reagents should be regarded as potential health hazards and exposure to these materials should be minimized. Follow universal precautions. Wear gloves, a lab coat, and safety glasses while handling reagents. Exercise caution when handling and dispensing concentrated acids. Always add acid to water. Acids are caustic chemicals that are capable of causing severe eye and skin damage. If acids or bases***

come in contact with any part of the body, quickly wash the affected area with copious quantities of water for at least 15 minutes.

5.5 Procedure:

5.5.1 Sample Preparation:

Food samples are prepared by microwave digestion system (As per procedure given in clause 2.1.2). The digested solution is diluted by water to a known volume (test solution). The concentration of nitric acid used in the calibration solutions should be similar to the final concentrations of nitric acid in the test solution. If hydrogen peroxide was added for the digestion, the calibration solutions need no addition of hydrogen peroxide.

5.5.2 Standard Preparation

For calibration of the instrument a set of at least three different concentrations are used. The concentration range should be chosen with respect to the concentrations expected and with respect to the linear dynamic range. It is important that the concentration of nitric acid in the sample solutions and the calibration solutions are approximately the same. Calibration solutions should be prepared freshly before use.

5.5.3 Analysis of Sample

5.5.3.1 Instrument Setup- Setup inductively coupled plasma Optical emission spectrometer according to the manufacturer's recommendations and with the following attributes:

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

- Use background correction.
- Configure instrument for 3 integrations of emission. Use integration time appropriate for the particular instrument and emission line. Allow at least 10 sec after the solution reaches the plasma before starting integration. Report each emission reading and the mean and RSD.
- Program instrument to use a linear, least squares calculated intercept, curve fit algorithm for converting emission values to mg/L concentration units. Do not subtract standard blank response from standard solution response. Use the mean of the emission integrations to calculate concentration of analyte.
- Follow manufacturer's recommendations for optimizing the emission spectrometer.
- After instrument warm-up, perform optical profiling. Optical profiling is performed either with a built-in mercury lamp, a 2 mg/L Mn solution, or procedure recommended by instrument manufacturer.
- Check instrument performance.
- Verify emission counts are within 80-100% of expected value with a mid-range standard.
- Verify short term precision is less than 5% relative standard deviation with a mid-range standard (n=5).

Instrument Conditions:-
Plasma
Incident RF powr: 1200 watts Reflected RF power: 10 watts Viewing height above work coil: 15 mm Argon pressure: 90 psi Injector tube orifice internal diameter: 1 mm Coolant argon flow rate: 12 L/min Auxiliary (plasma) argon flow rate: 1 L/min Aerosol carrier Ar flow rate: 0.85 L/mi

Pneumatic Nebulizer
ARL Maximum Dissolved Solids Nebulizer (V-groove): Sample uptake rate controlled to 2.5 mL/min
Ultrasonic Nebulizer
Heating Temperature: 140 °C Cooling Temperature: 0.5 °C Sample uptake rate controlled to 1 mL/min
Data Acquisition Parameters
Integration Time: 10 sec Number of Integrations: 3

Element	**Wavelength (nm) × Order[a]**	**Element**	**Wavelength (nm) × Order[a]**
Aluminium	308.22×2	Magnesium	383.83×1[b]
Arsenic	189.04×3	Manganese	257.61×3
Barium	493.41×1	Molybdenum	202.03×3
Boron	249.68×3	Nickel	231.60×3
Cadmium	226.50×3	Phosphorus	178.29×3[b]
Calcium	317.93×2[b]	Potassium	766.49×1[b]
Chromium	267.72×3	Sodium	589.59×1[b]
Cobalt	228.62×3	Strontium	407.77×1[b]
Copper	324.75×2	Thallium	190.86×3
Iron	259.94×2	Vanadium	292.40×2
Lead	220.35×3	Zinc	213.86×2

5.5.3.2 Determination of Analyte Concentration Using Standard Curve

5.5.3.2.1 Standardize the instrument using the standard blank and at least 3 standard solution concentration levels. Allow at least 10 sec after the standard solution reaches the plasma before starting integration. Flush system with standard blank for at least 60 sec between each standard solution.

5.5.3.2.2 Check Standardization Performance

- Correlation coefficient (r) of linear regression (emission intensity verses concentration) is ≥0.998.
- Independent standard check solution recovery within 100 ± 5% (initial calibration verification).

5.5.3.2.3 Analyze analytical solutions and quality control solutions. Interpolate analyte concentration from standard curve. Rinse sample introduction system by aspirating standard blank for a minimum of 60 sec between all analyses (or longer if necessary)

- Check Instrument Measurement Performance
- Check solution analyzed at a frequency of 10% and at the end of the analytical run has a recovery of 100 ± 10% (continuing calibration verification).
- Standard blank analyzed at a frequency of 10% and at the end of the analytical run (continuing calibration blank).
- Measurements are below highest standard solution. Dilute analytical solution with standard blank if necessary to comply with criteria.
- Wavelength scan indicates absence of spectral interference that is not corrected for by background correction or inter-element correction factors.
- Inter-element Correction Factors

- If analytical solution has or is expected to have Al, Ca, Fe, Cr, Cu, Mn, Ti or V at concentrations >20 mg/L then inter-element correction factors must be determined as outlined in manufacturer's Instructions. Program instrument to use these factors.
- Analyze the solution(s) used to determine the inter-element correction factors as a sample to demonstrate proper correction for interference. *Note: Each analytical solution must be checked for spectral interference by performing a wavelength scan. An intensity (emission counts) verses wavelength scan must be recorded for each element for each analytical solution. Depending on ICP-OES instrument software, these scans can be incorporated into the ICP-OES analytical run or performed in a separate "scan" run. An appropriate standard solution must be scanned and the result overlaid with the scan of the analytical solution. A standard solution close in element concentration to the analytical solution should be chosen. A broad or double peak indicates an unresolved peak that may result in a positive bias. Interfering peaks could be from elements not being quantified. Peaks in the area of the background correction point(s) may result in a negative bias. Background correction points should be chosen in an area(s) free from other peaks.*

5.5.4 Calculation:

Calculate the concentration (mass fraction) of the analyte in the analytical portion according to the formula.

Calculate the content, w, as mass fraction, of the element to be determined in milligrams per kilogram of sample, using the following formula:

$$w = \frac{a \times V \times F}{m \times 1000}$$

Where;

a is the mass fraction of the element in the test solution, in microgram per litre (µg/L);

V is the volume of the digestion solution after being made up, in milliliters (mL);

F is the dilution factor of the test solution;

m is the initial sample mass, in grams (gm).

5.5.5 Analytical quality control: For analytical quality control, blank solutions and reference samples of comparable matrix having reliably known contents of the elements to be determined shall be analysed in parallel with all the series of samples analysed. The reference samples shall be subjected to all the steps in the method, starting from the digestion.

5.6 References:

U S Food and Drug Administration, Elemental Analysis Manual, Inductively Coupled Plasma--Atomic Emission Spectrometric Determination of Elements in Food Using Microwave Assisted Digestion. Version 1.1 (August 2010)

6.0 DETERMINATION OF ARSENIC, CADMIUM, CHROMIUM, LEAD, MERCURY, AND OTHER ELEMENTS IN FOOD USING MICROWAVE ASSISTED DIGESTION BY INDUCTIVELY COUPLED PLASMA-MASS SPECTROMETER (ICP-MS)

6.1 Scope:

This method describes procedure for determining total acid-extractable concentrations of arsenic, cadmium, and chromium, copper, lead, manganese, mercury, molybdenum, nickel, selenium and zinc in food by microwave assisted acid decomposition and inductively coupled plasma mass spectrometry (ICP-MS).

6.2 Principle:

An analytical portion of food is decomposed in acid inside a high-pressure digestion vessel using microwave heating. The analytical solution is nebulised and the aerosol transferred to a high frequency inductively coupled argon plasma. The high temperature of the plasma is used to dry the aerosol and to atomise and ionise the elements. The ions are extracted from the plasma by a set of sampler and skimmer cones and transferred to a mass spectrometer where the ions are separated by their mass/charge ratio and determined by a pulse-count and/or analogue detector against calibrated reference standards.

6.3 Apparatus:

6.3.1 Inductively coupled plasma mass spectrometer (ICP-MS)—Capable of scanning mass to-charge (m/z) range 5 – 240 amu with a minimum resolution of 0.9 amu at 10% peak height. Must have collision/reaction cell that can be pressurized with helium and kinetic energy discrimination for polyatomic interference attenuation.

6.3.2 Microwave digestion system—Requires temperature control to at least 200°C and pressures ≥ 300 psi (~ 20 bar) with appropriate safety features to prevent over pressurization of vessels. Microwave must have multi-step programming with ramp to temperature capability. Digestion vessels must be PFA, TFM Teflon® lined or quartz.

6.3.3 Analytical balance—Capable of measuring to 0.1 mg.

6.4 Reagents:

6.4.1 Reagent water—Water meeting specifications for ASTM Type-I water.

6.4.2 Argon supply—High purity (99.99%) argon.

6.4.3 Helium for collision cell—Ultra high purity (99.999%).

6.4.4 **High purity nitric acid**—Concentrated (67-70%, sp. Gr. 1.42), double distilled. The trade name for double distilled grade will vary by manufacturer.

6.4.5 **High purity hydrochloric acid**—Concentrated (30-35%, sp. Gr. 1.18), double distilled.

6.4.6 **Hydrogen Peroxide**—Concentrated (30%), high purity or trace metals grade.

6.4.7 **High purity isopropanol**—Electronic grade or equivalent.

6.4.8 **Recommended Tuning Solution**—1 µg/L Li, Co, Y, Ce, and Tl solution in 5% HNO3 –0.5% HCl used to tune ICP-MS.

6.4.9 **Standard stock solution**- Stock standards , tuning solution and Internal standard may be purchased from a reputable commercial source or prepared from ultra high-purity grade chemicals or metals (99.99 - 99.999% pure).

6.4.9.1 Preparation of working Standards -Dilute intermediate standard with 5% HNO3 – 0.5% HCl to prepare multi element working standards. Store in Teflon® FEP, PP or HDPE bottles.

6.4.9.2 Preparation working internal standard- Multi-element solution prepared by diluting an appropriate volume of stock standard. Internal standard solution contains 1% HNO3, 0.5% HCl and 4% isopropanol. The presence of isopropanol will help equalize arsenic and selenium sensitivities due to residual carbon post digestion.

Safety Notes: ***Reagents should be regarded as potential health hazards and exposure to these materials should be minimized. Follow universal precautions. Wear gloves, a lab coat, and safety glasses while handling reagents. Exercise caution when handling and dispensing concentrated acids. Always add acid to water. Acids are caustic chemicals that are capable of causing severe eye and skin damage. If acids or bases come in contact with any part of the body, quickly wash the affected area with copious quantities of water for at least 15 minutes.***

6.5 Procedure:

6.5.1 Sample Preparation:

Food samples are prepared by microwave digestion system (As per procedure given in clause 2.1.2). The digested solution is diluted by water to a known volume (test solution). The concentration of nitric acid used in the calibration solutions should be similar to the final concentrations of nitric acid in the test solution. If hydrogen peroxide was added for the digestion, the calibration solutions need no addition of hydrogen peroxide.

6.5.2 Standard Preparation:

For calibration of the instrument a set of at least three different concentrations are used. The concentration range should be chosen with respect to the concentrations expected and with respect to the linear dynamic range. It is important that the concentration of nitric acid in the sample solutions and the calibration solutions are approximately the same. Calibration solutions should be prepared freshly before use.

6.5.3 Analysis of Sample:

6.5.3.1 Instrument Setup- The instrument parameters described in the manufacturer's operating manual should be used. Generally, a plasma power of 1 100 W to 1 500 W should be chosen. By use of shorter or longer integration times on the isotope, the sensitivity may be influenced in some extend. Generally, three repeated measurements of each solution should be done. An example of instrument settings is given below in the Table.

Parameter	Setting
RF-Power (W)	1500
Carrier gas flow (l/min)	1.2
Plasma gas flow (l/min)	15
Auxiliary gas flow (l/min)	1.0
Spray chamber	Water cooled double pass

Spray chamber temperature (°C)	2.0
Lens voltage	4.5
Mass resolution	0.8
Integration time points/ms	3
Points per peak	3
Replicates	3

6.5.3.2 Set up procedure for the ICP-MS- Before starting routine measurements the following set up procedure should be run: The ICP-MS should warm up in full running mode for a minimum 20 min to 30 min. Mass resolution, mass calibration, sensitivity and stability of the system are checked by the use of a suitable optimising solution (Tuning solution). With an optimising solution the ICP-MS is adjusted daily to achieve maximum ion signals and both low oxide rates (e.g. < 2 %) and low rates of doubly charged ions (e.g. < 2 %). If a collision or reaction cell instrument is used, the flow rate of the cell gas (es) should be optimised, in order to ensure sufficient reduction of polyatomic interferences. If a high resolution mass spectrometer is used, mass calibration and sensitivity shall be checked for every range of resolution used. Check the sample feed and washout times with respect to the length of the tubing. If large differences in concentration of the test solutions are expected, the sample feed and washout times should be prolonged.

Different types of interferences can influence the results obtained by ICP-MS measurements. Non-spectral interferences are caused by e.g. viscosity and the amount of matrix of the test solution. High amounts of salt can lead to deposition effects especially in the cone system. Generally the amount of salt in the test solution should not exceed 0.2 % (mass fraction). By the use of internal standards some of the non-spectral interference effects can be corrected for. Memory effects in the sample delivery system can influence the results of samples analysed after measurement of high concentrations. Especially high concentrations of Hg need prolonged washout times and control runs of blank solutions.

Method parameters, recommended isotopes & internal standards and analysis mode are listed below in the table. Internal standards help compensate for matrix effects and instrumental drift.

Element	Monitored isotopes	Recommended Internal standard	Recommended Reporting isotopes	Minimum integration time (sec)	Analysis mode
Chromium	$^{52, 53}$Cr	^{103}Rh	^{52}Cr	0.3	Helium
Manganese	^{55}Mn	^{103}Rh	^{55}Mn	0.1	Helium
Nickel	$^{60, 62}$Ni	^{103}Rh	^{60}Ni	0.3	Helium
Copper	$^{63, 65}$Cu	^{103}Rh	^{65}Cu	0.1	Helium
Zinc	$^{66, 68}$Zn	^{103}Rh	^{66}Zn	0.1	Helium
Arsenic	^{75}As	^{74}Ge	^{75}As	0.5	Helium
Selenium	$^{78, 82}$Se	^{103}Rh	^{78}Se	0.3	Helium
Molybdenum	$^{95, 98}$Mo	^{103}Rh	^{95}Mo	0.1	Helium
Cadmium	$^{111, 114}$Cd	^{103}Rh	^{111}Cd	0.3	Helium
Lead	$^{206,207, 208}$Pb	^{209}Bi	Sum isotopes	0.1	Helium
Mercury	$^{201, 202}$Hg	^{193}Ir	^{201}Hg	0.5	Helium

6.5.3.3 Preparation of calibration solutions and test solutions for ICP-MS measurement - Every solution to be measured in the ICP-MS during routine runs should contain an internal standard. The concentration of the internal standard(s) shall be equal in all of the solutions. For the determination of mercury, gold shall be added, in order to stabilise the mercury. The test sample obtained by pressure digestion should be analysed after dilution. EXAMPLE Pipette 10 mL of zero member or calibration solution to a sample vessel, add 0.1 mL of diluted internal standard solution (conc. 5 mg/L) and mix. Pipette

2mL of test sample to a sample vessel; add 8 mL of water and 0.1 mL of diluted internal standard solution and mix. Every solution contains approximately 10 µg/L of the internal standards. The internal standard solutions may also be added on-line by a different channel on the peristaltic pump used for the analyses. Adjust the concentration of the internal standard solution and the pump flow rate in order to achieve a mass concentration of the internal standard of approximately 50 µg/L.

Measure the blank solution and then the calibration solutions. According to the instrument manual calculate the calibration function. Different isotope ratios between calibration solutions and test solutions should be taken into account if necessary.

After calibration of the instrument, the test solutions can be analysed. The samples obtained by pressure digestion should be diluted before measurement in order to avoid interference by high concentrations of matrix elements. If the final volume of the digested solution is 20 mL to 30 mL, a dilution by a factor of 10 is recommended for the ICP-MS measurement. Within suitable short intervals (e.g. after five or ten samples) the blank solution and one calibration solution shall be checked. The response of that calibration solution should range within ± 10% of the response of the previous calibration/recalibration. For high concentrations of Hg prolonged washout times shall be applied. To apply appropriate (prolonged) wash-out times, the system should be tested for the duration of the wash-out time, using the highest calibration standard. Blank control measurements are recommended after high count rates of these elements to check the memory effect.

6.5.4 Calculation:

Calculation of the concentration is generally done automatically by the software of the ICP-MS instrument. The following steps are performed for each element: The count rates are corrected according to the correction functions chosen. The count rates measured in the zero members, calibration and test solutions are normalised on the count rates of the internal standard. The calibration function is calculated. By the use of the count rates, the

calibration function and the dilution factor the concentrations of the elements are calculated.

Calculate the content, w, as mass fraction, of the element to be determined in milligrams per kilogram of sample, using the following formula:

$$w = \frac{a \times V \times F}{m \times 1000}$$

where

a is the mass fraction of the element in the test solution, in microgram per litre (μg/L);

V is the volume of the digestion solution after being made up, in milliliters (mL);

F is the dilution factor of the test solution;

m is the initial sample mass, in grams (gm).

6.5.5 Analytical quality control:

For analytical quality control, blank solutions and reference samples of comparable matrix having reliably known contents of the elements to be determined shall be analysed in parallel with all the series of samples analysed. The reference samples shall be subjected to all the steps in the method, starting from the digestion.

6.6 References:

- BRITISH STANDARD, BS EN 15763: 2009 Foodstuffs — Determination of trace elements — Determination of arsenic, cadmium, mercury and lead in foodstuffs by inductively coupled plasma mass spectrometry (ICPMS) after pressure digestion.
- U S Food and Drug Administration , Elemental Analysis Manual, Inductively Coupled Plasma-Mass Spectrometric Determination of Arsenic, Cadmium, Chromium, Lead, Mercury, and Other Elements in Food Using Microwave Assisted Digestion. Version 1.1 (March 2015)

7.0 ANALYSIS OF METALS BY SPECTROPHOTOMETER

7.1 DETERMINATION OF ARSENIC IN FOODS BY COLORIMETRIC MOLYBDENUM BLUE METHOD

NOTE: Method is colorimetric, hence chance of cross contamination will in higher side. The method may be used for nutrient metals not trace (defined as contaminants).

7.1.1 Principle:

The sample is digested with nitric acid and sulphuric acid. After the digestion/oxidation is complete, the digest is treated with saturated ammonium oxalate solution to remove yellow coloration due to nitro compounds, fats, etc. Arsine is generated from digest using zinc and HCl and trapped in NaOBr solution and is treated with ammonium molybdate to form a blue compound which absorbs as 845 nm.

7.1.2 Apparatus:

(i) Generators and absorption tubes (Fig. 2.1):

Use about 100 mL wide mouth bottles of uniform capacity and design as generators. Fit each of them by means of perforated stopper with glass tube of 1 cm diameter and 6 to 7 cm long, with additional constricted end to facilitate connection. Place small pad of glass wool in constricted bottom end of tube and add 3.5 to 4.0 gm sand (same amount should be added in each tube) Moisten sand with 10% lead acetate solution and remove excess by light suction. Clean sand when necessary by treatment with nitric acid followed by water rinse and suction and treat with lead acetate solution (for cleaning sand, the sand should not be removed from tube). If sand has dried, clean and remoisten it as mentioned above. Connect tube by means of a rubber stopper, glass tube and rubber sleeve to bent capillary

tubing (7 mm outer diameter, 2 mm inner diameter) tapered at the end to slide easily into connecting tube and later into neck of 25 mL volumetric flask.

Other end of capillary is sealed to pyrex standard taper 19/38 female joint. To transfer contents of trap, attach bulb aspirator to male standard taper 19/38 joint and place it in the tip of the trap. Clean traps between determinations without removing beads by flushing with water followed by nitric acid until nitric acid becomes colourless.

Remove every trace of acid with water, rinse with acetone and dry with air current applied by suction to tip of traps use.

(ii) Spectrophotometer to read absorbance at 845 nm.

Fig: 2.1

Apparatus for generation and absorption of arsenic

7.1.3 Reagents:

(a) Bromine water (half saturated): Dilute 75 mL of saturated bromine water with equal volume of water.

(b) Sodium hypobromite solution: Place 25 mL of 0.5N NaOH in a 100 mL volumetric flask and dilute to volume with half saturated bromine water.

(c) Ammonium molybdate sulphuric acid solution: Dissolve 5.0 gm of $(NH_4)_6MO_7O_{24}.4H_2O$ in water and slowly add 42.8 mL H_2SO_4 (Sp. Gravity 1.84). Dilute to 100 mL with water.

(d) Hydrazine sulphate solution: 1.5% (w/v) $N_2H_4.H_2SO_4$ in water.

(e) Potassium iodide solution: 15% w/v in water. Keep in dark. Discard when solution turns yellow.

(f) Stannous chloride solution: Dissolve 40 gm as free $SnCl_2$ in conc. HCl and dilute to 100 mL with conc. HCl.

(g) Dilute hydrochloric acid solution: Dilute 180 mL of conc. HCl (Sp. Grade 1.18) to 250 mL with water.

(h) Lead acetate solution: 10% (w/v) Pb (OAC) $2.3H_2O$ in water.

(i) Zinc metal

(j) Sea sand: To clean sand (30 mesh) before use and between determinations, mount piece of 3 mm inner diameter glass tubing through rubber stopper in suction flask. Fit a piece of rubber tubing over top to take bottom of sulphide absorption tube easily and to maintain it upright.

Add, in turn with suction aqua regia, water, nitric acid and water to remove all traces of acid. Wet sand with lead acetate solution and remove the excess with suction.

(k) Arsenious oxide standard solution:

(i) Stock solution: (1 mg/mL): Dissolve 1.0 gm of As_2O_3 in 25 mL of 20% NaOH solution and dilute to 1000mL.

(ii) Intermediate solution: (10µg/mL): Dilute 10 mL of stock solution to 1000 mL.

(iii) Working solution: (2µg/mL): Dilute 20 mL of intermediate solution to 100 mL.

7.1.4 Preparation of Sample:

7.1.4.1 Fresh fruits:

Weigh and peel representative sample (0.5 to 2 kg). At blossom and steam end cut out all flesh thought to be contaminated with as compounds and include with peelings, if

desired. Place peelings in one of more 800 mL kjeldahl flasks. Add 25 to 50 mL HNO_3 and cautiously add 40 mL of H_2SO_4. Place each flask on asbestos mat with 5 cm hole. Warm slightly and discontinue when foaming becomes excessive. When reaction had reduced, heat flask cautiously and rotate occasionally to prevent caking of sample. Continue adding small amounts of HNO_3 whenever mixture turns brown or darkens. Continue digestion until organic matter is destroyed and SO_3 fumes are copiously evolved. At this stage, the solution should be colourless or at most light straw colour. Cool slightly and add 75 mL water and 25 mL saturated ammonium oxalate solution. Evaporate again to point where fumes of SO_3 appear in the neck of the flask. Cool and dilute with water to a known volume.

7.1.4.2 For dried fruit products:

Prepare sample by alternately grinding and mixing 4 to 5 times in food chopper. Place 35 to 70 gm portion in 800 mL kjeldahl flask, add 10 to 25 mL water, 25 to 50 mL HNO_3 and 20 mL H_2SO_4 and continue digestion as in **7.1.4.1**.

7.1.4.3 For small fruits and vegetables etc:

Use 70 to 140 g sample and digest as in **7.1.4.1** or **7.1.4.2**.

7.1.4.4 For materials other than **7.1.4.1**, **7.1.4.2** or **7.1.4.3**:

Digest 5 to 50 gm according to moisture content and amount of as expected as in 7.1.4.1 or **7.1.4.2**.

7.1.5 Isolation and determination:

When interfering substances are present in digest (like pyridine from tobacco etc) or when samples containing excessive amounts of salts or H_2SO_4 from digestions, isolation

of arsenic is to be done. The arsenic is either isolated after digestion or isolated by $AsCl_3$ distillation method.

Transfer 20 mL aliquots of sample and blank digest solutions to generator bottles. Add, while swirling after each addition, 10 mL water, 5 mL dil. HCl (g), 5 mL of KI solution (e) and 4 drops of $SnCl_2$ solution (f). Let stand for more than 15 min.

Place 4 gm of sea sand over small glass wool wad in sulphide absorption tube and cap with glass wool. Place 3 mm diameter solid glass beads in trap over small glass wool pad until ¼ full and add 3.0 mL of sodium hypobromite solution (h). Assemble apparatus except for generator bottle. Add 4 gm of zinc (i) to generator bottle, attach immediately and let react for 30 min.

Disconnect trap and transfer contents of 25 mL volumetric flask with the help of aspirator assembly. Rinse trap with six 2 mL portions of water and aspirate into flask. Add, with swirling, 0.5 mL ammonium molybdate-sulphuric acid solution (c) and 1.0 mL hydrazine sulphate solution (d), dilute to volume, mix and let stand for 75 min and mix. Read absorbance at 845 nm against blank prepared similarly.

7.1.6 Preparation of Standard Curve:

Place 0.1, 1.0, 2.0, 3.0, 5.0, 6.0 and 10.0 mL of standard solution containing 2 µg As_2O_3/mL, in 25 mL volumetric flask. Add 3.0 mL of sodium hypobromite solution and water to 15 mL. Add with swirling 0.5 mL of ammonium molybdate sulphuric acid solution and 1.0 mL of hydrazine sulphate solution. Dilute to volume, mix and let stand 75 min. Mix and read absorbance at 845 nm. Plot absorbance against µg of As_2O_3.

(Ref:- Manual Methods of Analysis for Adulterants and Contaminants in Foods I.C.M.R 1990 Page 144 /AOAC 15th edn, Official Method 942.17 Arsenic in Food Molybdenum Blue Method)

7.2DETERMINATION OF ARSENIC BY COLORIMETRIC SILVER DIETHYL DITHIO CARBAMATE METHOD

NOTE: Method is colorimetric, hence chance of cross contamination will in higher side. The method may be used for nutrient metals not trace (defined as contaminants).

7.2.1 Preparation of silver diethyl dithiocarbamate reagent solution:

Chill 200mL of 0.1M $AgNO_3$ solution (3.4 gm/200mL) and 200mL 0.1M sodium diethyl dithiocarbamate solution (4.5gm/200 mL) to 10°C or lower.

Add carbamate solution to $AgNO_3$ solution slowly with stirring. Filter through buchner, wash with chilled water and dry at room temperature under reduced pressure. Dissolve this salt in pyridine (reagent grade) with stirring, chill and add cold water slowly until precipitated completely. Filter through buchner and wash with water to remove all pyridine. Dry the pale yellow crystals under reduced pressure and store in amber bottle in refrigerator.

Dissolve 0.5 gm of salt, prepared as above, in colorless pyridine in 100mL volumetric flask and dilute to 100mL with pyridine. Mix and store in amber bottle. Reagent is stable for several months at room temperature.

7.2.2 Determination:

Transfer aliquot of sample digest, prepared as given in 'preparation of sample', and same volume of blank to generator bottles. Add water to 35mL and then add 5mL HCl, 2mL KI solution and 8 drops of $SnCl_2$ solution and let stand for more than 15 min. Evolve AsH_3 as mentioned in para 3 of 'Isolation and Determination', except add 4.0mL of silver diethyl dithio-carbamate solution to trap.

Disconnect trap and mix solution in trap by gently drawing back and forth five times with aspirator assembly. Transfer this solution directly to spectrophotometer cell (glass stoppered preferred) and read absorbance at 522 nm. Determine As_2O_3 in aliquot from standard curve.

7.2.3 Preparation of Standard Curve:

Place 0.1, 1.0, 2.0, 3.0, 5.0, 6.0 and 8.0 mL of standard solution containing 2 µg As_2O_3/mL, in generator bottles. Add water to 35mL and proceed as in 'Determination' Plot absorbance against µg of As_2O_3.

(Ref: - Manual Methods of Analysis for Adulterants and Contaminants in Foods I.C.M.R 1990 Page 147 / AOAC 15th edn Official Method 952.13 Arsenic in Food, Silver diethyldithiocarbamate Method)

7.3 DETERMINATION OF CADMIUM IN FOOD BY COLORIMETRIC DITHZONE METHOD

NOTE: Method is colorimetric, hence chance of cross contamination will in higher side. The method may be used for nutrient metals not trace (defined as contaminants).

7.3.1 Principle:

The sample is digested with H_2SO_4 and HNO_3. The pH of the solution is adjusted to 9.0. The dithizone metals along with cadmium are extracted from aqueous solution with dithizone chloroform solution. Cadmium is separated from Cu, Hg and most of any Ni or Co present, by stripping $CHCl_3$ solution with dil. HCl solution leaving Cu, Hg, Ni and Co in organic phase. Aqueous layer is adjusted to 5% NaOH and is extracted with dithizone – CCl_4 solution. At this alkalinity Zn, Bi and Pb do not extract whereas cadmium dithizonate is relatively stable. Cadmium is finally estimated photometrically at 510 nm.

7.3.2 Reagents:

(a) Citrate – Diammonium salt or citric acid.
(b) Chloroform
(c) Carbon tetrachloride
(d) Dithizone
(e) Dithizone in carbon tetrachloride: 20 mg/ 1 CCl_4. Prepare daily
(f) Dithizone in chloroform – 1000 mg / 1 $CHCl_3$ Prepare when needed
(g) Sodium hydroxide solution: 28% w/v. Dissolve 28 gm of NaOH in water and dilute to 100 mL.
(h) Thymol blue indicator: Triturate 0.1 gm of indicator in agate mortar with 4.3mL of 0.05N NaOH. Dilute to 200mL in glass stoppered flask with water.
(i) Adsorbent cotton
(j) Cadmium standard solution:
(i) Stock solution: (1000 µg Cd/mL)
Dissolve 1.000 g of pure cadmium in 20 to 25mL HNO_3 (1+9), evaporate to dryness, add 5mL HCl (1+1), and evaporate to dryness.
Dilute to 1.0 litre with water.
(ii) Intermediate solution: (100 µg /mL)
Dilute 100 ml of stock solution to 100mL.
(iii) Working solution: (2.0 µg /mL)

Transfer 20 mL intermediate solution to 1000 mL volumetric flask, add about 15 mL HCl and dilute to volume with water (the acidity of final solution should be approximately 0.2N).

7.3.3 Preparation of the Sample:

Digest a suitable quantity of sample equivalent to 5 to 10 gm of product, calculated on dry basis, with 10mL of H_2SO_4 (1+1) and HNO_3 as needed. If sample tends to char rather

than to oxidize evenly, add 5 or 10mL of additional H_2SO_4. Continue digestion, adding HNO_3 as required, until digestion is complete and SO_3 is evolved. Cool, add 15mL saturated ammonium oxalate solution and again heat to fumes.

Fat in biological material such as kidney and liver may cause bumping and frothing during digestion. If comparatively large sample of such materials are available, make partial digestion with warm HNO_3 until only fat remains undissolved. Cool, filter free of solid fat, wash residue with water, make combined filtrate to suitable volume and digest appropriate aliquot as above.

7.3.4 Determination:

Dilute digest with 25mL water, filter free of excessive insoluble matter if present and transfer to separator marked 125mL, using additional 10mL portions of water for rinsing and completing transfer. Add 1 to 2 gm of citrate reagent (a) and 1mL of thymol blue indicator and adjust pH to approximately 8.8 by adding NH_4OH slowly while cooling intermittently, until solution changes from yellowish green to greenish blue. Dilute to 125mL mark with water. Extract vigorously with 5mL portions of dithizone in chloroform solution until chloroform layer remains green. Then extract with 3mL of chloroform.

Transfer all chloroform extracts to second separator previously wetted with 2 to 3mL $CHCl_3$. Add 40mL of 0.2N HCl to combined extracts, shake vigorously for more than a minute and let layers separate. Carefully drain chloroform phase and discard. Remove remaining droplets of dithizone by extracting with 1 to 2mL CCl_4 layer. Conduct the draining operations so that no acid enters bore or stem of separator. Discard CCl_4 layer.

Adjust aqueous phase to 5.0% alkalinity by adding 10mL of NaOH solution.

Extract cadmium with 25mL of dithizone solution, by shaking vigorously for more than or equal to one min and transfer to third separator previously wetted with 2 to 3mL of same dithizone solution. Repeat extraction with additional 10mL portions of dithizone solution until CCl_4 layer becomes colourless. Amounts of Cd upto 100 µg are completely removed by third extraction.

To verify assumption that pale pink persisting after third extraction is due to Zn, transfer a questionable extract to another separator containing 5.0% NaOH solution. Add several mL of dithizone solution and shake vigorously.

If CCl_4 layer becomes colourless, original pink was due to Zn and no further extractions are necessary. If however, pink persists, indicating the presence of Cd, and extract to contents of third separator and continue extraction.

Convert Cd and Zn dithizonates in third separator to chlorides by adding 40mL of 0.2N HCl and shaking vigorously for one minute. Carefully drain CCl_4 layer and discard. Remove droplets of dithizone from aqueous phase by rinsing with 1 to 2mL of CCl_4 and drain off CCl_4 as completely as possible without allowing acid layer to pass bore of separator. Again adjust alkalinity to 5% by adding 10mL NaOH solution. Add exactly 25mL of dithizone solution and shake vigorously exactly for 1 min. Allow layers to separate exactly for 3 min. Wipe separator stems dry with cotton. Filter organic layer through pledget of cotton, discarding first 5mL. Read the absorbance of the cadmium dithizone complex solution at 510 nm. Calculate cadmium in µg from standard curve or by substituting absorbance in linear equation.

7.3.5 Preparation of Standard Curve:

Prepare in duplicate 6 standards containing 0, 5, 10, 15, 20, 25 µg of cadmium as follows:

Add appropriate volumes of standard solution to separator, adjust to 40mL with 0.2N HCl, and 10mL of NaOH solution and 25mL of dithizone solution. Shake exactly for 1min. let stand exactly for 3 min and filter organic layer through a pledged of adsorbent cotton, discarding first 5mL.

Determine absorbance 'A' at 510 nm and plot standard curve.

(Ref: - AOAC 15th edn Official Method 945.58 Cadmium in Food, Dithiozone Method/Manual Methods of Analysis for Adulterants and Contaminants in Foods I.C.M.R, 1990 Page 148)

7.4 DETERMINATION OF COPPER IN FOOD BY COLORIMETRIC CARBAMATE METHOD (IUPAC METHOD)

NOTE: Method is colorimetric, hence chance of cross contamination will in higher side. The method may be used for nutrient metals not trace (defined as contaminants).

7.4.1 Principle:

The sample is digested with HNO_3 and H_2SO_4. Copper is isolated and determined calorimetrically at pH 8.5 as diethyl dithiocarbamate in presence of chelating agent EDTA. Bi and Te also give coloured carbamates at pH 8.5, but are decomposed with 1N NaOH. Range of colour measurement is 0 to 50 µg.

7.4.2 Reagents:

(a) Sodium diethyldithiocarbamate solution: (1.0% w/v) - Dissolve 1.0 gm of the salt in water and dilute to 100mL with water and filter. Store in refrigerator and prepare weekly.

(b) Citrate-EDTA solution: Dissolve 20 gm of dibasic ammonium citrate and 5 gm Na_2 EDTA in water and dilute to 100mL. Remove traces of copper by adding 0.1mL of carbamate solution and extracting with 10mL CCl_4. Repeat extraction until CCl_4 extract is colourless.
(c) Ammonium hydroxide – 6N: Purify as in (b) above.
(d) Copper Standard solution:
(i) Stock solution: Place 0.2500 gm of Cu wire or foil in 125mL Erlenmeyer flask. Add 15mL of HNO_3 (1+4) cover the flask with watch glass and let copper dissolve, warming to complete solution. Boil to expel fumes, cool and dilute to 250mL.
(ii) Intermediate solution: (100 µg/mL) - Dilute 10mL of stock solution to 100mL with water.
(iii) Working solution: (2 µg/mL) - Prepare daily by diluting 5mL of intermediate standard solution to 250mL with 2N H_2SO_4.

7.4.3 Preparation of sample:

Weigh sample containing not more than 20 gm of solids depending on the expected copper content. If sample contains less than 75% water, add water to obtain this dilution. Add initial volume of HNO_3 equal to about two times dry sample weight and volume of H_2SO_4 equal to as many grams of dry sample but not less than 5mL. Warm the contents of flask slightly and discontinue heating if frothing becomes excessive. When reaction has quietened, heat flask cautiously and rotate occasionally to prevent caking of sample. Maintain oxidizing conditions in flask at all times during digestion by cautiously adding small amount of HNO_3 whenever mixture turns brown.

Continue digestion till organic matter is destroyed and SO_3 fumes are evolved. When sample contain large amounts of fat, make partial digestion with HNO_3 till only fat is undissolved. Cool, filter free of solid fat, wash residue with water, add H_2SO_4 to filtrate and carry out digestion.

After digestion, cool, add 25mL water and heat to fumes. Repeat addition of 25mL water and fuming. Cool, filter off any insoluble matter present and dilute to 100mL with water. Prepare reagent blank similarly.

7.4.4 Determination:

Pipette suitable aliquot of sample digest (containing not more than 50 µg of copper) into a short stem separator, add 2N H_2SO_4 to make total volume of 25mL and add 10mL of citrate EDTA reagent. Add two drops of thymol blue indicator and 6N NH_4OH dropwise until solution turns green or blue-green.

Cool and add 1mL of carbamate solution and 15mL of CCl_4. Shake vigorously for 2min. Let layers separate and drain CCl_4 through cotton pledget into glass stoppered tube. Determine absorbance 'A' at 400 nm.

To test for Bi and Te, return CCl_4 solution to separator, add 10mL 5.0% KCN solution and shake for 1 min. If CCl_4 layer becomes colourless Bi and Te are absent. If test is positive, develop colour in another aliquot of digest solution as above (without KCN). Drain CCl_4 layer into second separator add 10mL of 1N NaOH and shake for 1 min. Let layers separate and drain CCl_4 into third separator. Again wash CCl_4 extract with 10mL of 1N NaOH.

Determine absorbance of CCl_4 layer and convert to µg of Cu.

NOTE: KCN extraction method is not recommended due to environmental hazard.

7.4.5 Preparation of Standard Curve:

Transfer 0, 1, 2.5, 5, 10 and 25 mL of copper standard solution (2µg/mL) to separators and add 2N H_2SO_4 to make total volume of 25mL. Add 10mL citrate EDTA

reagent and proceed as for sample as given in 'Determination' beginning' Add two drops of thymol blue indicator. Plot absorbance 'A' against µg of copper. Since there is usually some deviation from linearity read sample values from smoothened curve.

(Ref: - Manual Methods of Analysis for Adulterants and Contaminants in Foods I.C.M.R 1990 Page 150 / AOAC 15th edn, Official Method 960.40 Copper in Food)

7.5 DETERMINATION OF IRON IN FOODS

NOTE: Method is colorimetric, hence chance of cross contamination will in higher side. The method may be used for nutrient metals not trace (defined as contaminants).

7.5.1 Principle:

Organic matter in the sample is destroyed by ashing and the resulting ash is dissolved in hydrochloric acid and diluted to a known volume with water.

Whole of the iron present in the aliquot of ash solution is reduced with hydroxylamine hydrochloride and the Fe (II) is determined spectrophotmetically as its coloured complex with, α- α-dipyridyl, the solution being buffered with acetate buffer solution. Absorption of the resulting complex is read at 510 nm.

7.5.2 Reagents:

(a) Magnesium nitrate solution: (50% w/v): Dissolve 50 gm of Mg $(NO_3)_2.6H_2O$ in water and dilute to 100mL with water.
(b) Concentrated hydrochloric acid
(c) Hydroxylamine hydrochloride solution: (10% w/v) - Dissolve 10 gm H_2NOH. HCl in water and dilute to 100mL.

(d) Acetate buffer solution: Dissolve 8.3 gm of anhydrous NaOAC (previously dried at 100°C) in water, add 12mL of glacial acetic acid and dilute to 100mL.

(e) Alpha, alpha-dipyridyl solution: (0.1% w/v) - Dissolve 0.1 gm of alpha, alpha-dipyridyl in water and dilute to 100mL. Keep this reagent in cool and dark place.

(f) Iron standard solution: (0.01 mg Fe/mL)

(i) Dissolve 0.3512 gm Fe $(NH_4)_2$ $(SO_4)_2.6H_2O$ in water, add 2 drops of conc. HCl and dilute to 100mL.

(ii) Dilute 5mL of solution (f) to 250mL.

(g) Orthophenanthroline solution: (0.1% w/v)- Dissolve 0.1 gm of O-Phenanthroline in 80mL of water at 80°C, cool and dilute to 100mL with water. Keep in cool and dark place.

7.5.3 Apparatus:

(1) Spectrophotometer/Colorimeter to read absorption at 510 nm.

7.5.4 Preparation of sample:

Weigh accurately, a suitable quantity of well homogenised sample, into a cleaned and tared silica dish. If sample contains more water, dry on a water bath. Char the sample (in the dish) on low flame of a burner till all the volatile matter escapes and smoking ceases. Transfer the dish to a cold muffle furnace and raise the temperature slowly to 450°C. Continue ashing at 450°C till practically carbon-free ash is obtained. (If carbon is present in ash even after 4 to 5 hour of ashing, remove the dish from furnace, cool and moisten the ash with 1 mL of magnesium nitrate solution (a), dry on water bath/hot place and ash in furnace at 450°C). After the ash is carbon-free remove the dish from furnace and cool.

Add 5mL of conc. HCl letting acid rinse the upper portion of the dish and evaporate to dryness on a water bath. Dissolve residue by adding exactly 2.0mL of conc. HCl, heat for

5 min on steam bath with watch glass covering the dish. Rinse watch glass with water, filter into a 100mL volumetric flask, cool and dilute to volume.

7.5.5 Determination of Iron:

Pipette 10mL aliquot of ash solution into 25mL volumetric flask, and add 1mL hydroxylamine hydrochloride solution. After 5 min, add 5mL buffer solution and 1 mL O - phenanthroline solution or 2mL of dipyridyl solution and dilute to volume. Determine absorbance of solution at 510 nm. From absorbance reading, determine Fe content present in aliquot of ash solution taken by referring to standard curve.

7.5.6 Preparation of Standard Curve:

Pipette 0.0, 0.5, 1.0, 1.5, 2.0, 3.0 and 4.0 mL of Fe standard solution F (II) into a series of 25mL volumetric flasks and add to each of them exactly 0.2mL of conc. HCl. Dilute each of them to exactly 10mL with water, and then add reagents in the same way as for the sample, Plot the quantity of Fe (in mg) against the absorbance.

7.5.7 Calculations:

Iron content of sample
(Mg Fe/100gm sample) =

$$= \frac{\text{Quantity of Fe in aliquot of ash solution (From calibration curve)}}{\text{Aliquot of ash solution taken for determination}} \times \frac{\text{Total volume of ash solution}}{\text{Wt. of the sample taken for ashing}} \times 100$$

(Ref: - Manual Methods of Analysis for Adulterants and Contaminants in Foods I.C.M.R 1990, Page 152)

7.6 DETERMINATION OF LEAD IN FOOD

7.6.1 Principle:

The sample is ashed and the acid solution of ash is neutralized with ammonia in the presence of citrate. Several other interfering elements are complexed with cyanide and lead is isolated as lead dithizonate into $CHCl_3$.

The chloroform layer is shaken again with dilute nitric acid and chloroform layer is discarded. The aqueous phase is buffered to pH 9.5 to 10.0 and lead is re-extracted with dithizone in chloroform. The colour produced is read at 510 nm and is compared with known standard.

NOTE: It is suggested to go for Microwave digestion followed by AAS/ICP/ICP-MS. Microwave digestion will save chemical, time and sequential loss of target metal during digestion. Considering toxicity of the metals, highly precise and accurate method and equipment should be used for determination. The changes of cross contamination/false result are also very higher for lead. Hence proper QC and spike should be used during analysis

7.6.2 Reagents:

(a) Lead standard solution:
(i) Stock solution: (1 mg Pb/mL in 1% HNO_3) - Dissolve 1.5985 gm of pure $Pb(NO)_2$ crystal in 1% HNO_3 and dilute to 1000mL.
(ii) Working solution: Prepare as needed by diluting stock solution suitably with 1% HNO_3 solution.
(b) Nitric acid: (1%) Dilute 10mL of fresh, colourless HNO_3 (sp. Gr. 1.40) to 1000mL with redistilled water. If acid is redistilled, boil off nitrous fumes before diluting.

(c) "Acid-aid" solution: Dissolve 40 gm Al $(NO_3)_3.9H_2O$ and 20 gm Ca $(NO_3)_2.4H_2O$ in 100 mL water.

(d) Citric acid solution: Concentrated lead free solution. Prepare the solution so that 1 mL of solution contains about 0.5 gm of citric acid.

(e) Dithizone solution: (1 mg/ml in $CHCl_3$) - Dissolve 100 mg of purified dithizone in chloroform and dilute to 100 mL. Prepare dilute solutions as needed by diluting stock solution suitably with chloroform.

(f) Potassium cyanide solution: (10% w/v) - Dissolve 25 gm of recrystallised, phosphate free KCN in water and dilute to 250 mL.

(g) Ammonia-cyanide mixture: To 100 mL of 10% potassium cyanide solution in a 500 mL volumetric flask, add enough NH_4OH solution to introduce 19.1 gm of NH_3 and dilute to volume with water.

(h) Washed filter paper: Soak 9 cm quantitative papers overnight in 1% HNO_3. Wash with large volumes of water on buchner to remove acid and any traces of lead.

7.6.3 Preparation of sample:

Accurately weigh representative sample of 5 to 100 gm, depending upon amount of sample available and expected lead content, into a clean silica dish. Dry wet sample on steam bath or in oven. If sample is difficult to ash (meats) or has low ash content (candies, gellies etc) add 2 to 5mL of "Ash aid" solution, mix well and dry.

Char the sample carefully over burner. Do not let material ignite. Samples like milk, candies etc. may be charred without ignition by adding little at time to dish heated over burner or hot plate. Charring of sample is carried out by means of a soft flame (like that of Argand burner) to volatilise as much as possible of the organic matter. Oils and fats must be "smoked" away by heating at about 350°C. Materials being ashed must not be allowed to ignite during any phase of ashing.

When sample is dry and charred, place it in a temperature controlled muffle furnace and raise temperature slowly without ignition. Cover floor of furnace with piece of asbestos board or SiO_2 plate so that sample receives most of its heat by radiation and not by conduction. Ash the sample at temperatures not exceeding 500°C for about 8 to 10 hours. If ashing is not complete, remove dish from furnace, cool and moisten the char with 2 mL of ash aid solution. Dry contents of dish thoroughly and replace in furnace. If ashing is not complete after 30 minutes, remove dish, cool and cautiously add 2 to 3 mL HNO_3. Dry again and place in furnace and continue ashing until practically carbon-free ash is obtained.

When clean ash is obtained, cool, cover the dish with watch glass and cautiously add 15 to 20 mL conc. HCl. Rinse down watch glass with water and heat on steam bath. If clear solution is not obtained evaporate to dryness and repeat addition of HCl. Dilute the clear solution with water to a definite volume (filters solution if necessary, wash the insoluble material on filter successively with few ml of hot HCl, hot hydrochloric acid citric acid solution and hot 40% NH_4 OAC solution and make up to 100 mL).

Prepare a Sample Blank Solution using exactly the same amounts of reagents including water and exposing the sample blank in furnace or on steam bath for the same length of time and giving identical treatment as given in case of sample.

7.6.4 Isolation and Determination of Lead:

(i) Transfer suitable aliquot of ash solution of sample to a 250mL separator and add citric acid reagent equivalent to 10 gm of citric acid and mix well.

(ii) Make slightly alkaline to litmus with NH_4OH, keeping the solution cool, swirl gently and let stand 1 to 2 min. At this stage solution should be clear without any precipitate. (If precipitate forms, redissolve with HCl transfer solution to a stoppered conical flask and adjust to pH 3.0 to 3.4 (bromophenol blue) with NH_4OH. If enough Fe is present to colour

solution strongly, make adjust with the help of spot plate. Dissolve any precipitate, if formed, by shaking and cooling.

If amount of lead expected is small, add 5 to 10 mg of pure $CuSO_4$. $5H_2O$ to solution. Pass in H_2S until solution is saturated (3 to 5 min), immediately filter with suction. Dissolve the residue of precipitated sulphides, without previous washing, with 5 mL of hot HNO_3 drawing solution through into original flask, wash with hot water. Stopper the flask, shake the HNO_3 and water washes well and boil the solution to remove H_2S. Transfer this solution to separator add citrate solution equivalent to 5 gm of citric acid, make ammonical and proceed from step (iii) onwards.

(iii) To the clear solution obtained after step (ii), add 5mL of 10% KCN solution (more may be necessary if large amount of Zn, Cu, Cd etc are present), shake and check the pH of the solution by adding a drop of thymol blue indicator solution (pH should preferably be 8.5 to 9.5 blue green to blue colour with thymol blue).

(iv)Immediately add 20mL of dithizone solution (in this step usually solution of 8 mg dithizone /L of $CHCl_3$ is adequate), shake for 20 to 320 sec and let layers separate. Transfer the $CHCl_3$ layer to small separator containing 25mL of 1% HNO_3.

(v) Repeat step (iv) and continue extractions till the $CHCl_3$ layer is distinctly green. Drain all the extracts into the small separator containing 1.0% HNO_3.

(vi)Shake well combined extracts in smaller separator well and drain green dithizone layer into another separator containing additional 25mL portion of 1.0% HNO_3. Shake the contents of the separator, let layers separate and discard the organic layer.

(vii) Filter acid extracts, containing lead, in succession through small pledget of wet cotton inserted in stem of small funnel, into a 50mL flask using second acid extract to rinse

separator in which first extraction was made. Make up any slight deficiency in volume with 1.0% HNO_3 and mix.

(viii) Take suitable aliquot (or entire volume, if needed) of the 50mL of 1.0% HNO_3 solution containing lead (obtained in step (vii) and enough 1.0% HNO_3 reagent to make total volume to 50mL (add acid solution first and later lead extract).

(ix) Add 10mL of ammonia-cyanide mixture and mix (pH is about 9.7).

(x) Immediately add appropriate volume of dithizone solution of suitable concentration, and shake for 1 min and let layers separate. The appropriate volume and concentration of dithizone solution can be chosen as per information provided in the table below:

Pb range (µg)	Concentration of dithizone in $CHCl_3$ (mg of dithizone/1 solution) soln.	Vol. of dithizone to be taken (mL)
0 to 10	8	5
0 to 50	10	20
0 to 200	20	25

(xi) Drain the lower dithizone layer into a clean and dry tube and read absorbance at 510 nm against a reagent blank by following all the steps from Step (i) to Step (xi) as was done for sample but substituting the aliquot of ash solution taken with same volume of 2N HCl. Carry out isolation and determination of lead content in sample blank solutions in identical manner to that of sample solution.

Convert absorbance 'A' to µg of Pb from the calibration curve or preferably calculate with the help of the equation obtained by least squares method.

Subtract the lead content obtained for sample blank solution from that of sample solution to get actual lead content of sample solution and calculate the lead content of sample.

7.6.5 Preparation of Standard Curve:

Prepare working curve of required range (to be decided basing based on lead content of samples), starting with blank to final standard of range with four intermediate increments.

(1) Pipette appropriate amounts of lead solution into series of separators and add 1.0% HNO_3 solution so that total volume in each separator is always 50mL. Add the required volume of acid solution first and then the proposed volume of standard solution.
(2) Add 10mL of ammonia-cyanide mixture and mix (resultant pH will be 9.7).
(3) Immediately develop colour by shaking for 1 min with proper quantity of dithizone solution from the table. Let layers separate.
(4) Drain lower chloroform layer into a clean and dry tube. Read absorbance 'A' of each standard extract against the extract of zero lead content (i.e. reagent blank of standards). Plot absorbance 'A' against μg of Pb or calculate reference equation by method of least squares.

Standard lead and 1.0% HNO_3 solutions used in preparation of standard curve should be saturated with $CHCl_3$, before use, by shaking those solutions with clear $CHCl_3$ and discarding the organic layer.

(Ref: - Manual Methods of Analysis for Adulterants and Contaminants in Foods, I.C.M.R 1990 Page 153)

7.7 DETERMINATION OF MERCURY IN FOOD BY COLORIMETRIC METHOD

NOTE: Method is colorimetric, hence chance of cross contamination will in higher side. The method may be used for nutrient metals not trace (defined as contaminants).

7.7.1Principle:

The sample is digested with HNO_3 and H_2SO_4 under reflux in a special apparatus, mercury is isolated by dithizone extraction. Copper is removed and mercury is estimated by photometric measurement of mercury dithizonate at 490 nm.

7.7.2 Apparatus:

Special digestion apparatus:

Apparatus is made from pyrex glass with standard taper joints throughout as shown in Fig. below. Unit A is modified Soxhlet Extractor, 5 cm outer diameter, 200mL capacity to overflow. This unit is without inner siphon tube but is equipped with stopcock on tube leading to digestion flask 'D'. With stopcock open, the apparatus is in reflux position and when the stopcock is closed, the unit serves as trap for condensed water and acids. Top of 'A' is attached to Friedrichs condenser (c), 35 cm long. Bottom of 'A' is attached through center neck of 2 neck standard taper 24/40 joint, round bottom flask (D) Of 500mL capacity. Necks are 3 cm apart. Second neck is used for attaching 75 mL dropping funnel 'B'.

7.7.3 Reagents:

(a) Mercury Standard Solution:

(i) Stock solution (1 mg/mL): Prepare from dry, recrystallized $HgCl_2$ (135.4 mg $HgCl_2$ in 100mL solution)

(ii) Working solution (2µg/mL): Prepare from stock solution with suitable dilutions and store in pyrex bottles. Add conc. HCl in proportions of 8 mL/L to all standards before diluting to final volume.

(b) Chloroform

(c) Dithizone solution: Prepare stock solution in redistilled chloroform (100 mg/L is convenient) and store in refrigerator. Prepare dilutions as needed.

(d) Sodium thiosulphate solution: - 1.5 % in water. Prepare daily.

(e) Dilute acetic acid: 30 % by volume

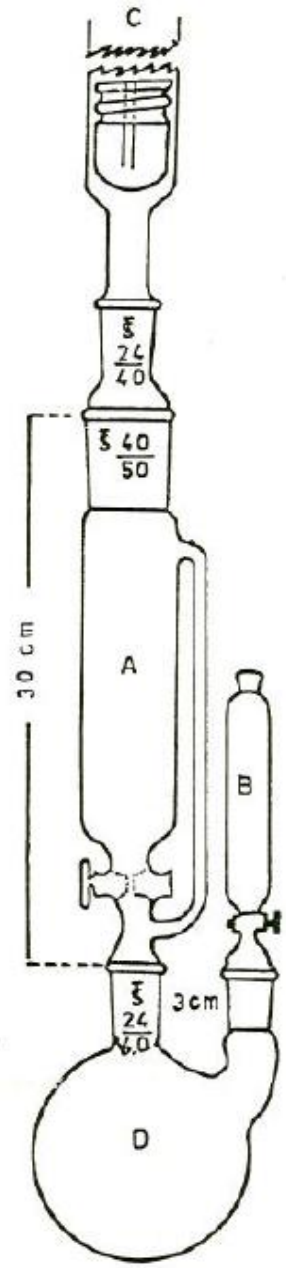

Fig. 2.2. Special digestion apparatus for mercury residues.

(f) Hydroxylamine hydrochloride solution: 20 % w/v in water. Extract with dilute dithizone solution until chloroform layer remains green, remove excess dithizone with chloroform and filter.

(g) Hydrochloric acid: 0.1N

(h) Sodium hypochlorite solution: Preferably 5% available chlorine reagent. Determine strength of reagent and store in refrigerator and determine strength monthly.

Reagents with more than 0.1 µg Hg/mL should not be used.

7.7.4 Determination of strength of Hypochlorite solution:

A. Reagents needed:

(i) Arseneous oxide standard solution (0.1N): Accurately weigh about 2.473 gm of pure As_2O_3 (dried for 1 hour at 105°C before use), dissolve in 25mL of 1N NaOH while heating on steam bath. Add approximately the same quantity of 1N H_2SO_4. Cool and transfer quantitatively to 500mL volumetric flask and dilute to volume with water. The final solution should be just neutral to litmus but not alkaline.

$$As_2O_3 = \frac{\text{gm of } As_2O_3 \text{ weighed} \times 4000}{\text{mL of final volume made} \times 197.84}$$

(ii) Iodine solution (0.1N): Dissolve about 13 gm of iodine and 20 gm of KI in about 50mL water, transfer to a 1000mL volumetric flask and dilute to volume with water and mix thoroughly.

(iii) Starch indicator (1% w/v):- Prepare in water

B. Procedure:

Transfer 20mL of hypochlorite reagent solution (Solution A) to 1000mL volumetric flask and dilute to volume with water (Solution B). Pipette 50mL aliquot of solution B into a 250mL Erlenmeyer. Add excess As_2O_3 solution and then a decided excess of $NaHCO_3$ (solid). Titrate excess As_2O_3 with iodine solution (0.1N) using starch solution as indicator, and note the titre volume (T_1). Carry out another titration using same volume of As_2O_3 solution and decided excess of $NaHCO_3$ (Solid) and note the titre volume with same iodine solution (T_2) (but without aliquot of solution 'B').

Calculate the available chlorine as follows:

Available chlorine
(gm of chlorine per 100 mL of hypochlorite reagent solution)

$$= \frac{\text{Aliquot of } As_2O_3 \text{ Soln. taken for titration} \times (T_2 - T_1) \times 3.545 \times \text{Total volume of solution 'B'}}{\text{Aliquot of solution 'B' taken for titration} \times T_2 \times \text{Volume of solution 'A' taken for dilution}}$$

(i) **Urea solution:** (40% w/v in water)

7.7.5 Preparation of Sample:

In all determinations use sample equivalent to less than or equal to 10 gm on dry weight basis

7.7.5.1 Fresh fruits or vegetables and beverages:

Place weighed sample in digestion flask with 6 glass beads, connect assembly and add, through dropping funnel, 20mL of HNO_3. Pass rapid stream of water through

condenser, adjust stopcock of Soxhlet unit to reflux position and apply small flame to flask. Original reaction must not proceed vigorously or violently. After initial reaction is complete, apply heat so that digest just refluxes. If mixture darkens, add HNO_3 drop wise through funnel (B) as needed. Continue refluxing 0.5 hr or until digest does not change consistency, and cool.

Slowly add 20mL cool HNO_3 – H_2SO_4 mixture (1+1). {Use 10mL acid mixture for 5gm (dry weight) of sample}. Heat with small flame, subsequently adding HNO_3 dropwise, as needed to dispel darkening of digest. Continue heating until fibrous material like cellulose etc. is apparently digested. Turn stopcock of Soxhlet unit to trap water and acids, and continue heating. Let digest become dark brown (NOT BLACK) before adding further increments of HNO_3. When all except fat is in solution, let digest cool, and cautiously drain water and acids into the main digest. Cool and pour two 25mL portions of water through condenser and intermediate unit. Remove digestion flask, chill by surrounding with ice to solidify fats and waxes. Filter off insoluble matter on small pledget of glass wool. Rinse reaction flask and filter pad successively with two 10mL portions of water.

Remove Soxhlet unit and wash it and flask with hot water to remove insoluble matter. Pour hot water through condenser and discard all washings.

Connect digestion flask containing filtered sample solution (filtrate) to assembled apparatus, heat and collect water and acids in trap.

Complete digestion, using small additions of HNO_3 as needed. In final stage of digestion, adjust flame until digest reaches incipient boiling (Solution simmers) and acid vapours do not rise beyond lower half of condenser. Continue heating 15 min after last addition of HNO_3.

Digest should now be colourless or pale yellow. Let digest cool, drain trapped liquids carefully into reaction flask and add two 50mL portions of water through condenser. Reflux solution until all NO_2 is expelled from apparatus. Add 5mL of 40% w/v urea solution and reflux 15 min. Digest should be colourless or pale yellow.

7.7.5.2 Dried fruit, cereal, seeds and grains:

Dilute sample with 50mL water before adding HNO_3 and proceed with sample preparation as in **7.7.5.1**.

7.7.5.3 Meats, fish and biological material:

Because of high fat and protein content of these materials, conduct initial digestion carefully to avoid foaming of digest into condenser.

Add 20mL HNO_3 to sample, swirl flask, and let stand 0.5 hr indigestion assembly before heating. Add 25mL water and heat cautiously with small rotating flame until initial vigorous reaction is over and foaming ceases. Proceed as in **7.7.5.1**.

7.7.6 Isolation and Determination of Mercury:

Titrate 1mL of prepared sample solution, with standard alkali. Add calculated amount of conc. NH_4OH to reduce acidity of digest solution to 1.0 N. Swirl flask during addition of NH_4OH to avoid local excess. Solution should never be ammonical.

Transfer sample solution to 500mL separator. Add 10mL of 4 mg/L dithizone solution and shake vigorously for 1 min. (if characteristic green of dithizone is visible in $CHCl_3$ layer, indicating the excess of dithizone, amount of mercury is within 0 to 5 µg). Let layers separate and drain $CHCl_3$ layer quickly to second separator containing 25mL of 0.1N

HCl and 5mL hydroxyl amine hydrochloride solution. Repeat extraction of sample solution with two 5mL portions of dithizone solution, transferring $CHCl_3$ layer successively to second separator.

If first extraction indicates more than 5 µg of mercury, add stronger concentrations of dithizone as indicated in Table given below until after 1 min vigrous shaking, $CHCl_3$ layer contains dithizone in marked excess.

Drain the $CHCl_3$ layer into second separator containing 0.1N HCl and again extract sample solution with two 10 mL portions of 4 mg/L dithizone solution, draining each successive extract into second separator.

Hg range µg	Dithizone Concentration (mg/L)	Column of dithizone solution (mL)
0 to 10	6	5
0 to 50	10	25
0 to 100	10	40

Shake contents of second separator vigorously for 1 min and drain $CHCl_3$ layer into third separator containing 50mL of 0.1N HCl. Extract solution in second separator with 1 to 2mL of $CHCl_3$ and transfer organic layer to third separator.

To contents of third separator add 2mL of $Na_2S_2O_3$ solution, shake vigorously for 1min. Let layers separate drain off $CHCl_3$ as completely as possible and discard. Extract again with 1 to 2mL of $CHCl_3$ drain carefully and discard organic layer. Add enough solution of NaOCl reagent to furnish 175 mg available chlorine and shake vigorously for 1 min. Add 5mL of hydroxylamine hydrochloride reagent from pipette taking care to wet both stopper and neck of separator. Shake vigorously for 1 min. Hold mouth of separator in front of air vent and blow out any remaining gaseous chlorine.

Stopper separator and shake vigorously for 1 min. All Hypochlorite should be reduced. Extract solution with 2 to 3mL of $CHCl_3$, drain off organic layer carefully and discard. Final aqueous solution should now be colourless.

Now, to the solution in the third separator, add 3mL of 30% HOAC and appropriate volume and concentration of dithizone solution as indicated in Table. Shake vigorously for 1 min and let layers separate. Insert cotton pledget into the stem of the separator and collect dithizone extract (Discarding first ml) in test tube. Read absorbance 'A' of the extract at 490 nm. Convert 'A' to µg of Hg from working standard curve.

7.7.7 Preparation of Standard Curve:

Table shown above is useful in preparing standard curve and for establishing approximate Hg range in sample.

Prepare working standard curve of required range, starting with blank and extending to final standard of range with 4 intermediate increments.

Add appropriate amounts of Hg to 50mL of 0.1N HCl in separator. Add 5mL $H_2NOH.HCl$ reagent (f) and 5mL of $CHCl_3$. Shake vigorously for 1 min let layers separate; drain off $CHCl_3$ and discard, being careful to remove as completely as possible all droplets of $CHCl_3$. Add 3mL of 30% HOAC and appropriate volume of dithizone solution. Shake vigorously for 1 min and let layers separate. Insert cotton pledged into stem of separator and collect dithizone extract (discarding first mL) in test tube and read absorbance at 400 nm. Plot 'A' against µg of Hg.

7.7.8 Precautions:

(i) Digestion must be almost complete.

(ii) Oxidizing material in digest must also be destroyed.

(iii) Careful heating of digest during preparation of sample is required.

(iv)Acidity of final sample solution before extraction should be about 1N but not more than 1.2N.

(v) Do not use silicone grease in stop cocks.

(Ref: - Manual Methods of Analysis for Adulterants and Contaminants in Foods, I.C. M.R 1990, Page156)

7.8 DETERMINATION OF TIN IN FOOD

7.8.1 Spectrophotometric Catechol Violet Method (IUPAC Method)

7.8.1.1 Principle:

The sample is wet digested with a mixture of nitric and sulphuric acids followed by subsequent treatment with perchloric acid and hydrogen peroxide and the residue is diluted with water to give an approximately 4.5M concentration of the acid. Potassium iodide is added Tin (IV) iodide is selectively extracted into cyclohexane. Tin (IV) is returned to aqueous solution by shaking organic layer with sodium hydroxide solution which is subsequently acidified. After removal of free iodine, Tin (IV) is determined spectrophotometrically as its coloured complex with catechol violet, the Solution being buffered to pH 3.8. The absorption maximum of the resulting complex is 555 nm.

7.8.1.2 Reagents:

(a) Sulphuric acid (Sp. Gr. 1.84)

(b) Nitric acid (Sp. Gr. 1.40)

(c) Perchloric acid (Sp. Gr. 1.67)

(d) Hydrogen peroxide (30%)

(e) Hydrochloric acid solution: (Approximately 5M) - Dilute 107mL of HCl (Sp. Gr. 1.18) to 250mL with water.

(f) Sulphuric acid solution: (Approximately 4.5M) - Dilute 250mL of H_2SO_4 (a) to 1000mL with water.

(g) Potassium iodide solution: (Approximately 5M) - Dissolve 83 gm KI in 100mL water. Prepare fresh daily.

(h) Sodium hydroxide solutions: Prepare fresh each fortnight:

(i) Approximately 5M solution: Dissolve 100 gm of NaOH in water and dilute to 500mL.

(ii) Approximately 0.1M solution: Dissolve 2 gm of NaOH in water and dilute to 500mL.

(i) Ascorbic acid solution: (5% w / v) - Dissolve 1 gm of ascorbic acid in 20mL of water. Prepare fresh daily.

(j) Catechol violet solution: (0.025% w/v) - Dissolve 25 mg of catechol violet in a mixture of equal part of ethanol and water to produce 100mL and mix. Prepare fresh daily.

(k) Sodium acetate solution: (20% w/v) - Dissolve 50 gm of $CH_3COONa.3H_2O$ in water and dilute to 250mL and mix.

(l) Ammonia solution: (Approximately 5M) - Dilute 184mL of ammonia solution {(25% m/m) Sp. Gr. 0.91} to 500mL with water.

(m) Cyclohexane

(n) Tin Standard Solution:

(i) Stock Solution: (200 µg/mL) - Dissolve 0.1 gm pure granular Sn in 20mL of sulphuric acid (a) by heating until fumes appear. Cool, dilute cautiously with 50mL of water and cool again. Add 65mL of sulphuric acid (c) and transfer the solution to a 500mL volumetric flask and dilute to volume with water and mix.

(ii) Working solution: (5µg/mL)

Pipette, immediately before use, 2.5mL of stock solution into a 100mL volumetric flask dilute to volume with water and mix.

7.8.1.3 Preparation of Sample:

(i) Weigh accurately a suitable portion of homogenised sample containing 2 to 25 µg of Sn into a 500mL long necked, Kjeldahl flask.

(If tin concentration of sample is more, e.g. 50 mg/kg, the H_2SO_4 residue resulting after digestion should be properly diluted and an aliquot of the sample solution containing between 2 and 25 µg of tin should be taken for determination step). All dilutions should be carried out such that the final solution for determination of tin is approximately 4.5M in H_2SO_4.

(ii) Add successively 50mL HNO_3 (b), 12.5mL of H_2SO_4 (a) and three glass beads and mix thoroughly.

(iii) Heat the contents of the flask to boiling on soft flame of burner and keep contents boiling during digestion. Rotate the flask occasionally to prevent caking of sample on glass exposed to flame.

(iv)Maintain oxidizing conditions during digestion by adding small amounts of HNO_3 (b) whenever contents turn brown or darkens.

(v) Continue step (v) till all organic matter is destroyed. After digestion is complete, heat the contents of the flask till copious fumes of sulphuric acid are evolved and continue heating for 5 more minutes. Now the solution remains colourless or pale straw coloured.

(vi)Cool the solution to room temperature, add 1mL of $HClO_4$ (c) and reheat till copious white fumes appear. Continue heating for 5 minutes.

(vii) Cool the solution again, adds 1mL of H_2O_2 (d). Heat to fuming and continue heating for 5 minutes more.

(viii) Repeat H_2O_2 treatment twice, each time-re-heating to fuming which is continued for 5 min.

(ix) Cool, rinse the neck of the flask with approximately 5 mL of water and re-heat t fuming.

(x) Cool the solution.

7.8.1.4 Determination:

(1) Quantitatively transfer the cooled digest solution to 100mL separating funnel with 37.5 mL of water and mix by rotating the funnel by hand.

The test solution thus obtained is approximately 4.5M in sulphuric acid. (If the sample taken for digestion contains more than 25µg of Sn, quantitatively transfer the cooled digests into a 100mL volumetric flask with 37.5mL of water and dilute to volume with 4.5M sulphuric acid solution. Take a suitable aliquot of this solution into 100mL separatory funnel and bring the volume of solution in separator to 50mL with 4.5M sulphuric acid and proceed as follows.

(2) Add 5mL KI solution (g) mix and add 10mL of cyclohexane. Shake the funnel vigorously for 2 min and let layers separate. Transfer the aqueous layer into a second separator and retain the pink coloured cyclohexane layer.

(3) Add 10mL of cyclohexane to the contents of second separator, shake vigorously for 1 min let layers separate and discard aqueous phase.

(4) Combine cyclohexane extracts and discard any aqueous phase present after combining the extracts.

(5) To the combined extracts add successively, 5mL of water and 1.5mL of 5M NaOH solution (h - i). Shake the contents vigorously for 2 min. Let layers separate and transfer aqueous layer into a 50mL beaker containing 2.5mL of HCl (e).

(6) Repeat extraction by shaking cyclohexane layer with 3mL of sodium hydroxide solution (h – ii) and add the aqueous layer into the content of the 50mL beaker mentioned in step 5.

(7) Wash the cyclohexane layer retained in the separatory funnel with 5mL of NaOAC solution (k) by cautiously tilting the funnel and forcing the contents to flow forth and back six times.

(8) Decolourise the iodine present in the acidified aqueous solution (step 6) By dropwise addition of ascorbic acid solution (i). Add 2mL of catechol violet solution (j) and mix. Add to this solution, the sodium acetate washing solution (obtained in step 7) and adjust the pH of solution to 3.8 ± 0.1 with ammonia solution (1) and/or HCl solution (e) using pH meter.

(9) Quantitatively transfer the solution (after adjusting the pH to 3.8 ± 0.1 into a 25mL volumetric flask, add 2mL of absolute ethanol, make up to the mark with water and mix. Set the flask aside for 45 min at room temperature. Measure the absorbance 'A' of the solution using water as reference.

Carry out a blank determination starting with 50mL of 4.5M sulphuric acid solution (f) and carrying out steps from 2 to 9, the optical density of the blank should not exceed 0.070.

Convert the absorbance 'A' to µg of Tin from calibration graph and calculate Tin content of sample as follows:

$$\text{Tin content of sample (mg/kg)} = \frac{\text{µg of Sn present in test solution X Dilution factor}}{\text{Weight in grams of sample taken}}$$

7.8.1.5 Preparation of Standard Curve:

Pipette 0, 1, 2, 3, 4 and 5 mL of the Tin working standard solution (n – ii) Into six separatory funnels and add sufficient 4.5M sulphuric acid solution to bring the total volume to 50 mL. Follow the steps 2 to 9 under "Determination" and note the absorbance of each standard.

Calculate the linear expression (Y = a + bX) relating the Tin content of the standard (X) to the measured absorbance (Y). Plot the calibration graph corresponding to the calculated linear regression.

Note: Whenever the absorbance measured for the blank/standard zero exceed 0.070, reagent solutions should be renewed.

(Ref: - Manual Methods of Analysis for Adulterants and Contaminants in Foods, I.C.M.R 1990 Page 160)

NOTE: An alternative method -- Microwave digestion followed by AAS/ICP/ICP-MS analysis may also be used.

7.9 DETERMINATION OF ZINC IN FOOD (Colorimetric Dithizone Method)

NOTE: Method is colorimetric, hence chance of cross contamination will in higher side. The method may be used for nutrient metals not trace (defined as contaminants).

7.9.1 Principle:

The sample is wet or dry ashed. Lead, copper, cadmium, bismuth, antimony, tin, mercury and silver are eliminated as sulphides with added copper as scavenger agent. Cobalt and nickel are eliminated by extracting metal complexes of α-nitroso-β- naphthol and dimethyl glyoxime respectively. Zinc is extracted as zinc dithizonate with CCl_4, for colour measurement.

7.9.2 Reagents:

(a) Copper sulphate solution: 2 mg Cu/mL - Dissolve 8 gm of $CuSO_4.5H_2O$ in water and dilute to 1000mL.
(b) Ammonium citrate solution: Dissolve 225 gm of $(NH_4)2HC_6H_5O_7$ in water, make alkaline to phenol red with NH_4OH (pH 7.4, first distinct colour change), and add 75mL in excess. Dilute to 2000mL.

Extract this solution immediately before use as follows. Add slight excess of dithizone and extract with CCl_4 until solvent layer is clear bright green. Remove excess dithizone by repeated extraction with $CHCl_3$, and finally with CCl_4. Excess dithizone must be entirely removed.

(c) Dimethyl glyoxime solution: Dissolve 2 gm of reagent in 10mL of NH_4OH and 200-300 mL water, filter, and dilute to 1000mL with water.
(d) α-nitroso-β- naphthol solution: Dissolve 0.25 gm in $CHCl_3$ and dilute to 500mL.

(e) Chloroform.

(f) Diphenylthiocarbazone solution: Dissolve 0.05 gm of dithizone in 2mL of NH_4OH and 100mL of water and extract repeatedly with CCl_4 until solvent layer and extract repeatedly with CCl_4 until solvent layer is clear bright green. Discard solvent layer and filter aqueous portion through washed ashless filter paper. (Prepare this solution as needed since this solution is only moderately stable even in refrigerated conditions)

(g) Carbon tetrachloride.

(h) Dilute hydrochloric acid: (0.04N) - Dilute required amount of HCl with water.

(i) Zinc standard solution:

(i) Stock solution: (500 µg Zn/mL)- Dissolve 0.500 gm of pure Zinc in slight excess of dil. HCl and dilute to 1000mL.

(ii) Working solution: (5µg/mL) - Dilute 10mL of stock solution to 1000mL with 0.04N HCl.

7.9.3 Preparation of sample:

Sample preparation is done by following any one of the two procedures given below:

7.9.3.1 Wet Ashing:

Accurately weigh, into 300 or 500 mL Kjeldahl flask, representative sample of about 25 gm (containing about 100 µg of Zinc). Evaporate the liquid sample to small volume. Add conc. HNO_3 and heat cautiously until first vigorous reaction subsides and then add 2 to 5 mL conc. H_2SO_4. Continue heating, adding more HNO_3 in small portions ad needed to prevent charring, until solution is clear and almost colourless. Continue heating until dense fumes of H_2SO_4 are evolved and all HNO_3 has been removed. Cool, dilute with approximately 25 mL of water filter, if necessary through pre-washed fast filter paper and dilute the filtrate to 100 mL with water.

7.9.3.1 Dry Ashing:

Accurately weigh, into a clean platinum or silica dish, a representative portion of sample (about 25 gm). Char the sample and ash at temperatures not exceeding 500°C. Raise the temperature of the muffle furnace slowly to avoid ignition. When ash is carbon-free, dissolve ash under watch glass, in minimum volume of HCl (1+1).

Add about 20 mL of water and evaporate to near dryness on team bath.

Add 20 mL 0.1N HCl and continue heating for 5 minutes. Filter through pre-washed fast filter paper into 100 mL volumetric flask. Wash Dish and filter with several 5 to 10 mL portions of 0.1N HCl, cool and dilute to volume with 0.1N HCl.

7.9.4 Isolation and determination:

To a suitable aliquot of ash solution of sample, add 2 drops of methyl red indicator and 1 mL of $CuSO_4$ solution and neutralize with NH_4OH. Add enough HCl to make solution about 0.15N with respect to HCl (Approximately 0.5 mL excess in 50 mL solution is satisfactory). Adjust the pH of this solution, as measured with glass electrode, to 1.9 to 2.1. Pass stream of H_2S into solution until precipitation is Complete. Filter through fine paper {Whatman No. 42 or equivalent, previously washed with HCl (1+6) followed by water}. Receive the filtrate in 250 mL beaker, wash flask and filter with 3 or 4 small portions of water. Gently boil filtrate until odour of H_2S can no longer be detected. Add 5 mL of saturated bromine-water and continue boiling until Br-free. Cool, neutralize to phenol red with NH_4OH and make slightly acid with HCl {excess of 0.2 mL of HCl (1+1)}. Dilute resultant solution to definite volume. At this stage, for optimum conditions of measurement, the solution should contain 0.2 to 1.0 µg of Zinc/mL.

To 20 mL aliquot of this prepared solution, in a 125 mL separator, add 5 mL of ammonium citrate solution, 2 ml dimethylglyoxime solution and 10 mL of α-nitroso-β-naphthol solution and shake for 2 min. Discard solvent layer and extract with 10 mL of $CHCl_3$. Discard solvent layer. (The extraction procedure in this para eliminates nickel and cobalt present, if any, in the solution).

To aqueous phase following removal of nickel and cobalt, which at this point has pH 8.0 to 8.2, add 2 mL of dithizone solution (f) and 10 mL of CCl_4 and shake for 2 min. Let phases separate and remove aqueous layer as completely as possible, withdrawing liquid with pipette attached to vacuum line. Wash down sides of separator with about 25 mL water and without shaking again draw off aqueous layer. Add 25 mL of 0.04N HCl and shake 1 min. Drain and discard solvent, being careful to dislodge and remove drop of solvent that floats on surface. To acid solution, add 5.0 mL of ammonium citrate solution and 10.0 mL of CCl_4 (pH of solution at this point should be 8.8 to 9.0). Determine the volume of dithizone solution (f) and shake for 2 min. Pipette exactly 5.0 mL of solvent layer into clean, dry test tube, dilute with 10.0 mL of CCl_4, mix well and determine absorbance 'A' at 540 nm.

Convert absorbance 'A' to µg of Zn from standard curve and calculate Zn content of sample.

7.9.5 Determination of volume of Dithizone to be added:

To separator containing Zinc standard solution equivalent to 20 µg Zn and diluted to 25 mL with 0.04N HCl add 5.0 mL citrate solution, 10.0 mL of CCl_4 and add dithizone reagent in 0.1 mL increments, shaking briefly after each addition until faint yellow in aqueous phase indicates bare excess of reagent.

Multiply the volume of dithizone solution required by 1.5 and adds this volume (to nearest 0.05 mL) to all samples.

7.9.6 Preparation of Standard Curve:

Prepare series of separators containing 0, 5, 10, 15 and 20 µg of Zinc diluted to 25 mL with 0.04N HCl. Add to each separator, 5.0 mL of citrate solution, 10 mL of CCl_4 and determined volume of dithizone solution (determined for Sample as given above) and shake for 2 min. Pipette exactly 5.0 mL solvent layer into clean, dry test tube. Dilute with 10.0 mL of CCl_4. Mix well andm read absorbance 'A' at 540 nm.

Plot 'A' against concentration and draw smooth curve through points.

(Ref: - Manual Methods of Analysis for Adulterants and Contaminants in Foods, I.C.M.R 1990 Page 163)

8.0 ANALYSIS OF METALS BY TITRIMETRIC MEHTOD

8.1 DETERMINATION OF TIN IN FOOD (Volumetric Method)

NOTE: Method is colorimetric, hence chance of cross contamination will in higher side. The method may be used for nutrient metals not trace (defined as contaminants).

8.1.1 Principle:

The sample is ashed along with a mixture of potassium dihydrogen phosphate and magnesium nitrate as ash. The ash is dissolved in hydrochloric acid. Tin present in solution is reduced with aluminium (foil) and dilute hydrochloric acid, and titrated against KIO_3 solution (in carbon dioxide atmosphere) in presence of KI using starch as indicator.

8.1.2 Reagents:

(a) Potassium dihydrogen phosphate
(b) Magnesium nitrate
(c) Hydrochloric acid (Sp. Gr. 1.18)
(d) Aluminum metal
(e) Hydrogen peroxide (30%)
(f) Potassium iodate solution:
(i) Stock solution: Dissolve 0.3566 gm of KIO_3 in water and dilute to 100mL with water.
(ii) Working solution: Dilute 5mL of stock solution to 100mL with water. Prepare fresh daily.
(g) Potassium iodide-sodium bicarbonate solution: Dissolve 0.2 gm of KI and 3 gm $NaHCO_3$ in water and dilute to 100mL with water. Prepare fresh daily.
(h) Starch indicator: 1 gm of soluble starch and 20 gm of NaCl dissolved in 100mL water.

Note: Use boiled and cooled distilled water for preparation of reagents (f and g).

8.1.3 Preparation of Sample:

Weigh accurately about 50 gm of the homogenized sample into a clean silica dish, add 2 gm each of potassium dihydrogen phosphate and magnesium nitrate and mix well. Add a few milliliters of water if necessary for mixing. Evaporate the contents to dryness on a steam bath and char the dry product on a burner by means of a soft flame. Transfer the dish to a muffle furnace and ash the sample at about 500°C for 6 to 8 hours and cool. If ash is not carbon free moisten the sample with few milliliters of water, mix well, and dry over a steam bath and ash at 500°C for 1 hour.

When clean ash is obtained, boil the contents of the dish cautiously with about 5 to 10mL of H_2O_2 over a burner with controlled flame, to remove the nitrous fumes. This step

has to be continued till no nitrous fumes are evolved. The ash should essentially be free from every trace of nitrates.

After removing nitrates, cover dish with watch glass, add cautiously 5mL of conc. HCl, rinse down with water and heat on steam bath and evaporate to near dryness. Add 20mL of HCl (1+1); heat to dissolve the ash, transfer the contents of the dish to a 100mL volumetric flask and dilute to volume with water.

8.1.4 Determination of Tin:

Transfer on aliquot of the ash solution to a reduction flask. Add 30mL of HCl (1+3) and about 0.3 gm of aluminium foil. Fit the flask with delivery tube dipping the other end of the tube into bicarbonate solution. Heat gently until evolution of hydrogen has started and discontinues heating. Heat again when the aluminium has nearly dissolved and finally boil until the liquid is perfectly clear. Remove by gentle rotation/agitation, any metal particles remaining at liquid gas interface. Cool flask in ice to below room temperature. After cooling, wash down the inner walls of the flaks with approximately 5mL of iodide-carbonate solution (g), run in quickly, from a 5 mL pipette with the tip removed. Add a few drops of starch indicator and titrate with KIO_3 working solution (f - ii) to the first appearance of blue colour.

Calculate the Tin content of sample as follows:

$$\text{ppm of Tin} = \frac{297 \times \text{Titre volume (mL)} \times \text{Volume of ash solution made (mL)}}{\text{Aliquot of ash soln. taken (mL)} \times \text{Wt. of sample taken for ashing (gm)}}$$

8.1.5 Preparation of Standard Solution:

Stock Solution: (1mg/mL): Dissolve 0.19 gm of AR grade $SnCl_2.2H_2O$ in 5mL of conc. HCl and make upto 100mL with distilled water.

NOTE: Tin solution need to obtain with NIST traceability

Working Solution: (0.1 mg/mL) - Dilute 10mL of stock solution to 100mL with water.

Standard stock solution can also be prepared by dissolving a known weight of pure Tin metal in conc. HCl and diluting to a known volume.

Pipette 20mL of working solution to reduction flask and follow the method as given for sample solution and find out the factor as follows:

$$\text{Factor} = \frac{\mu g \text{ of Tin in the aliquot of standard solution taken for reduction}}{\text{Titre volume (mL) of } KIO_3 \text{ working solution (f – ii)}}$$

If this factor is differing from 297, then use that factor (instead of 297) for calculating Tin content of sample.

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REFERENCES

1. AOAC 19th edn, 2012 Official Method 999.11 Determination of Lead, Cadmium, Copper, Iron and Zinc in Foods Atomic Absorption Spectrophotometry after dry ashing
2. AOAC 19th edn, 2012 Official Method 971.21 Mercury in Food Flameless Atomic Absorption Method
3. U S Food and Drug Administration, Elemental Analysis Manual, Inductively Coupled Plasma--Atomic Emission Spectrometric Determination of Elements in Food Using Microwave Assisted Digestion. Version 1.1 (August 2010)
4. BRITISH STANDARD, BS EN 15763: 2009 Foodstuffs — Determination of trace elements — Determination of arsenic, cadmium, mercury and lead in foodstuffs by inductively coupled plasma mass spectrometry (ICPMS) after pressure digestion.
5. U S Food and Drug Administration , Elemental Analysis Manual, Inductively Coupled Plasma-Mass Spectrometric Determination of Arsenic, Cadmium, Chromium, Lead, Mercury, and Other Elements in Food Using Microwave Assisted Digestion. Version 1.1 (March 2015)
6. Manual Methods of Analysis for Adulterants and Contaminants in Foods I.C.M.R 1990 Page 144
7. AOAC 15th edn, Official Method 942.17 Arsenic in Food Molybdenum Blue Method
8. Manual Methods of Analysis for Adulterants and Contaminants in Foods I.C.M.R 1990 Page 147
9. AOAC 15th edn Official Method 952.13 Arsenic in Food, Silver diethyldithiocarbamate Method
10. AOAC 15th edn Official Method 945.58 Cadmium in Food, Dithiozone Method
11. Manual Methods of Analysis for Adulterants and Contaminants in Foods I.C.M.R, 1990 Page 148
12. Manual Methods of Analysis for Adulterants and Contaminants in Foods I.C.M.R 1990 Page 150
13. AOAC 15th edn, Official Method 960.40 Copper in Food
14. Manual Methods of Analysis for Adulterants and Contaminants in Foods I.C.M.R 1990, Page 152
15. Manual Methods of Analysis for Adulterants and Contaminants in Foods, I.C.M.R 1990 Page 153
16. Manual Methods of Analysis for Adulterants and Contaminants in Foods, I.C. M.R 1990, Page156
17. Manual Methods of Analysis for Adulterants and Contaminants in Foods, I.C.M.R 1990 Page 160

18. Manual Methods of Analysis for Adulterants and Contaminants in Foods, I.C.M.R 1990 Page 163

Food Safety and Standards Authority of India
(Ministry of Health and Family Welfare)
FDA Bhawan, Kotla Road,
New Delhi-110002
www.fssai.gov.in