LAB. MANUAL 9

MANUAL OF METHODS

OF

ANALYSIS OF FOODS

METALS




FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA
MINISTRY OF HEALTH AND FAMILY WELFARE
GOVERNMENT OF INDIA
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MANUAL FOR ANALYSIS OF METALS

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MANUAL FOR ANALYSIS OF METALS

1.0 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.

1.1 Steps involved in Assay of Metals:

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

1.1.1 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.

1.1.2 Destruction of Organic Matter:

The commonly used methods of destruction of organic matter can be broadly grouped into Wet Oxidation, Dry Ashing and Microwave Digestion.

Wet Oxidation:

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.

Procedure:

Transfer suitable quantity of sample into a macro-kjeldahl digestion flask. Add 20 ml concentrated nitric acid and up to 20 ml water (depending on the water content of sample). Boil the contents of the flask to reduce the volume to 20 ml. Cool the solution, add 10 ml 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 10 ml 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.

(Ref:- Pearson's Composition and Analysis of Foods 9th edn, 1991 Page 143).

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.

(Ref:- Pearson's Composition and Analysis of Foods 9th edn, 1991 Page 142).

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 volatilise as much organic matter as possible, then transfer the basin to a temperature controlled muffle furnace. Keep the muffle at about 300ºC. Once the material is dry and charred, the temperature is allowed to rise to 450ºC 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 2 ml 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 50 ml of hydrochloric acid (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 10 ml of hydrochloric acid (1+1) and water to dissolve the salts. Filter into a 100 ml 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 20 ml of dilute sulphuric acid (1+1) (taking care to wet all the sample in the dish) and evaporate slowly at around 100ºC 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.

1.1.3 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 10 ml alcohol to 1000 ml of distillate.

(vi) Dithizone: Dissolve about 1 g of commercial reagent in 50 to 75 ml of $CHCl_3$ and filter, if insoluble materiel remains. Extract in separator with three 100 ml 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 20 ml 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 10 mg of $CuSO_4$ as co-precipitant. Filter, boil filtrate to expel excess H_2S completely and refilter, if necessary to obtain clear solution.

(Ref:- Manual Methods of Analysis for Adulterants and Contaminants in Foods I.C.M. R, 1990 Page137)

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.

Induction of Microwave digestion method will do the best digestion with loss of target metal concentration.

The Microwave digestion temperature needs to change according to food matrix. Advantage of the method is to avoid loss of volatile metal like Sn, As, Hg, Lead as well as less sample quantity. The lab can validate digestion method according to food matrix.

Sample amount: 0.5 g

Microwave equipment

Reagents :

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

Procedure:

1. Place a TFM vessel on the balance plate, tare it and weigh of the sample.
2. Introduce the TFM vessel into the HTC 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.

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*

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

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.

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 – 100 ml

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.

Reagents:

(a) Water – redistilled or deionised

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

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

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

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

(It is suggested to use NIST Tractable all solution of 1000mg/l. Prepare stock & working solution from this. For the better result, working solution should be prepared in the digestion solution).

(1) Lead Standard solution– 1mg / ml. Dissolve 1.000 gm Pb in 7 ml conc HNO_3 in 1 litre volumetric flask. Dilute to volume with water. Commercially available standard solutions for AAS may be used for all metal standards.

(2) Cadmium Standard solution – 1 mg / ml . Dissolve 1. 000 gm in 14 ml water and 7 ml conc HNO3 in 1 litre flask. Dilute to volume with water.

(3) Zinc Standard solution – 1mg / ml. Dissolve 1.000 gm Zinc in14 ml water + 7 ml conc HNO3 in 1 litre volumetric flask and dilute to volume with water.

(4) Copper Standard solution – 1mg / ml. Dissolve 1.000 gm Copper in 7 ml HNO_3 in 1 litre flask. Dilute to volume with water.

(5) Iron Standard solution – 1mg / ml. Dissolve1 .000 gm Iron in 14 ml water and 7 ml conc. HNO3 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 tractable metal standard, internal standard may be used for accuracy of test result.

Preparation of Sample:

Digestion by Microwave Method.

Weigh accurately about 25 g of well homogenised sample into a clean silica dish. Add 25 ml of 20% sulphuric acid (b). 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 110ºC. 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 250ºC. Slowly raise temperature to 500 ºC. 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 2 ml of HNO_3 and mix well. Dry thoroughly on hot plate. Return dish to furnace at 500ºC and ash for 30 minutes. Repeat nitric acid treatment using 1 ml 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 10 ml of water. Heat on hot plate till sample ash is dissolved. Quantitatively transfer the contents of the dish to a 50 ml volumetric flask, heat the dish with 10 ml 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 ample 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 470ºC.

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.

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

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

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.

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

Aluminium 309.3 Air – acetylene / Nitrous oxide - Ac

Nickel 232.0 Air – acetylene

(Ref:- A.O.A.C 17th edn , 2000 Official Method 999.11 Determination of Lead , Cadmium , Copper , Iron and Zinc in Foods Atomic Absorption Spectrophotometry after dry ashing) / Manual Methods of Analysis for Adulterants and Contaminants in Foods I.C.M.R 1990 Page 138)

NOTE:

1. Prepare spike standard in the same kind of sample.
2. Use of QC NIST tractable 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)

ICP-OES Method:

Sample Digestion: See Microwave Digestion Method

Instruments Parameter:-

RF power (emission intensity)	1200 W
Nebulizer type	Concentric
View height	8 mm
Gas (as 600 kpa)	Argon
Auxiliary gas (250 kpa)	N2or Argon
Plasma gas flow	10 L/min
Auxillary gas flow	0.5 L/min
Nebulizer flow 0.5L/min	
PMT volts	600 V
Sample flow	0.9 ml/min

Pump speed	15 rmp
Stabilization time	15 s
Flush time	15 s
Auto integration	10 s
Rinse time	5

Preparation of Calibration Curve:

Calibrate the ICP-OES. Then Read the emission for sample Blank of a series of respective metal solutions of working range in the ICP-OES. Please use best emission line (for optimum accuracy) given by supplier of equipment. Plot absorbance against µg of metal/ml solution (0mg/l (Sample Blank) 0.1 mg/l, 0.2mg/l, 0.5mg/l,1mg/l & 2mg/L).

Internal Standard: Use Yttrium as an Internal Standard

Measurement of Metals in Sample: Read concentration against prepared calibration curve. If concentration of measured sample is going out of calibration range, then dilute further into the calibration range.

QC Check Standard: Run QC check standards (NIST/BAM/IRRRM certified) after 20 sample

Spike: Add known concentration of metals in sample and follow, the microwave digestion method. Analyze spiked sample and analyze recovery of added metal standards from spiked sample. Use Recover factor in final reporting.

Calculation: (mg/L) =

$$\frac{\text{Concentration (mg) X Volume (V) X Dilution Factor (DF)}}{\text{Weight of sample}}$$

Uncertainty: - Lab will use Uncertainty of measurement

Note: Use Hydride generator technique for Analysis of Mercury, Arsenic, Selenium & Antimony.

ICP-MS: - Method:

Sample Preparation: Follow Microwave Digestion Method

ICP-MS Parameter : -

Gas Flow Parameters (L/min) Plasma flow 18

Auxiliary flow 1.8

Sheath gas 0.15

Nebulizer flow 1.0

RF Setting RF power (kW) 1.45

Sample Introduction

Sampling depth (mm) 6.5

Pump rate (rpm) 4

Stabilization time (s) 30

Quadrupole Scan

Scan mode Peak Hopping

Dwell time (ms) 30

Acquisition Points per peak 1

Scans/Replicated 10

Replicates/Sample 5

Detector Settings

Attenuation mode Automatic: Mn, Co, Cu, Fe, Zn, and Pb

High: Na, K, Ca, Mg, and P

Nebulizer Quartz MicroMist-concentric (0.4 mL/min)

Spray chamber

Peltier-cooled (3°C), double-pass Scott type

PumpTubing Sample and internal

standard lines Black/Black

(0.030 in. ID)

Spray chamber waste line

Blue/Blue

(0.065 in. ID)

CRI Skimmer gas type No gas H_2 He

Skimmer flow (mL/min) 0 80 120

Ion Optics (volts)

First extraction lens −1 −35 −25

Second extraction lens −150 −150 −150

Third extraction lens −200 −240 −240

Corner lens −210 −230 −230

Mirror lens left 38 28 28

Mirror lens right 26 16 16

Mirror lens bottom 32 26 26

Entrance lens 1 1 1

Fringe bias −2.9 −2.9 −2.9

Entrance plate −40 −40 −40

Pole bias 0 0 0

Preparation of Calibration Curve:

Calibrate the ICP-OES.

Then read the emission for sample Blank of a series of respective metal solutions of working range in the ICP-OES. Please use best emission line (for optimum accuracy) given by supplier of equipment. Plot absorbance against µg of metal/ml solution (0mg/l (Sample Blank) 0.1 mg/l, 0.2mg/l, 0.5mg/l,1mg/l & 2mg/L).

Internal Standard: Use Yttrium as an Internal Standard

Measurement of Metals in Sample: Read concentration against prepared calibration curve. If concentration of measured sample is going out of calibration range, then dilute further into the calibration range.

QC Check Standard: Run QC check standards (NIST/BAM/IRRRM certified) after 20 sample

Spike: Add known concentration of metals in sample and follow, the microwave digestion method. Analyze spiked sample and analyze recovery of added metal standards from spiked sample. Use Recover factor in final reporting.

Calculation: (mg/L) =

$$\frac{\text{Concentration (mg) X Volume (V) X Dilution Factor (DF)}}{\text{Weight of sample}}$$

Uncertainty: - Lab will use Uncertainty of measurement

Note: Use Hydride generator technique for Analysis of Mercury, Arsenic, Selenium & Antimony.

(**Ref:** ICP-MS for Detecting Heavy Metals in Foodstuffs, by Shona McSheehy Ducos, *et al.* Food Quality magazine, February/March 2010)

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

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.

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.

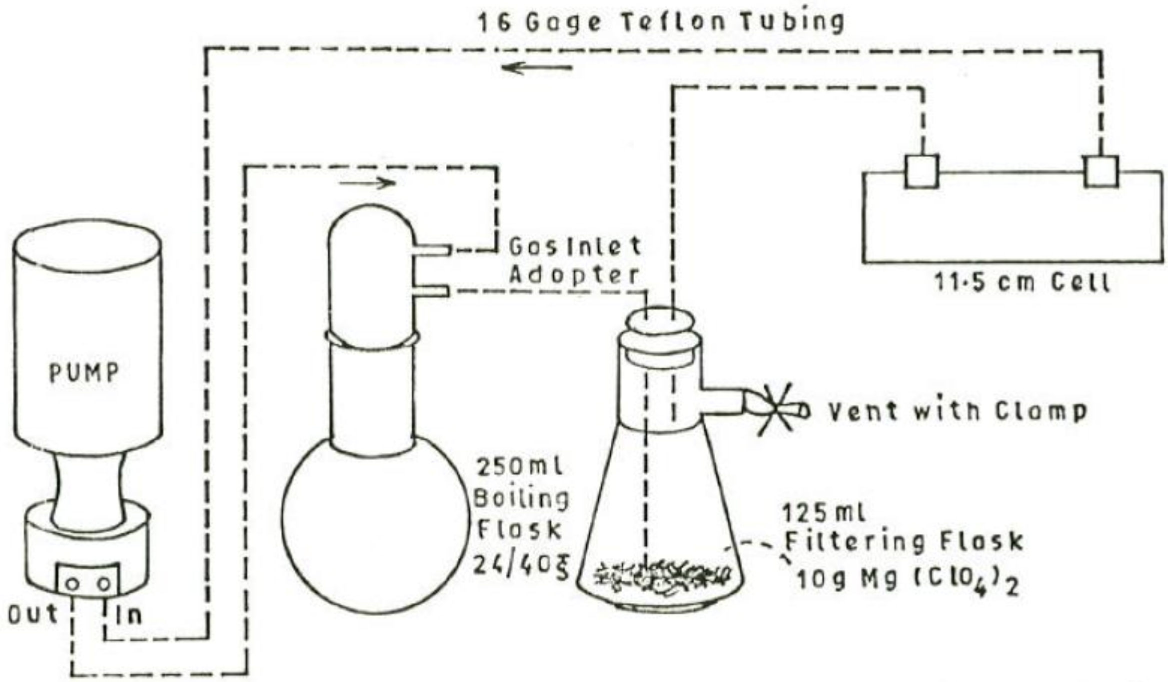

Fig.1-1. Apparatus for flameless atomic absorption analysis of mercury.

Fig. 1.

Reagents:

(a) Reducing solution:

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

(b) Diluting solution:

To 1000 ml volumetric flask containing 300 to 500 ml water add 58ml HNO_3 and 67 ml 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 tractable standard)
(i) Stock Solution (1000 µg/ml): Dissolve 0.1354 g HgCl2 in 100ml water.
(ii) Working solution (1 µg/ml): Dilute 1 ml stock solution to 1000 ml with 1N H_2SO_4. Prepare fresh daily.

Preparation of Sample:

Digest the sample using microwave method.

Weigh 5.0 g of sample into digestion flask, add 25 ml of 18N H_2SO_4, 20 ml of 7N HNO_3, 1 ml 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 20 ml 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 10 ml water through condenser while swirling liquid in flask. Again boil solution for 10 minutes. Remove heat and wash condenser with three 15 ml potions of water.

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

Determination:

Transfer 25.0 ml of aliquot from digest solution of each sample to another digestion flask. Adjust volume to about 100 ml 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 20 ml 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), disconnects pressure on 'out' side of pump, and open vent of filter flask to flush system.

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 100 ml 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: - A.O.A.C 17th edn, 2000 Official Method 971.21 Mercury in Food Flameless Atomic Absorption Method) / Manual Methods of Analysis for Adulterants and Contaminants in Foods I.C.M.R 1990 Page 141)

2.2 DETERMINATION OF MERCURY IN FOOD USING MERCURY ANALYSER

I. Method I--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 Model MA 5800 C/D of Electronics Corporation of India or equivalent equipment.

(Many brands are offering low range detection system for mercury in Food matrix. ICP-OES/ ICP-MS with the VGA give perfect result for mercury. Method for ICP-MS is given under method no. 2.0)

Instrument:

Mercury Analyzer MA 5800 C/D: It is basically a cold vapour atomic absorption spectrophotometer based on the principle that mercury vapour (atoms) absorbs resonance radiation at 253.7 nm. The analyser consists of a low pressure mercury lamp emitting the 253.7 nm 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.

Reagents: (AR grade reagents shall be used)

(a) 1.0 % w/v $KMnO_4$ in 10% of sulphuric acid:

Dissolve 1.0 g of $KMnO_4$ in water and carefully add to it 10 ml of sulphuric acid. Make up to a volume of 100 ml 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 250 ml.

(c) 20 % $SnCl_2$ (w/v) in 10% HCl:

Take 20 g of $SnCl_2$ in a clean beaker. Add 10 ml concentrated HCl and dissolve while warming it over a burner. Boil for 1 min, cool and dilute with distilled water to make 100 ml. Add 1 to 2 g 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 125 ml of sulphuric acid to water and make up to 250 ml.

(e) 10% Nitric acid:

Add 20 ml nitric acid to water and make up to 200 ml.

(f) Mercury standard (stock solution):

Dissolve 0.1354 g of $HgCl_2$ in water and add 1 ml of 1% potassium dichromate and make up to a volume of 100 ml with 2.0% HNO_3.

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

Preparation of sample:

As given for preparation of sample under determination of mercury by Dithiozone method (see clause 3.7)

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 10 ml. Add 2 ml 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 t through a trap containing 20 ml 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 4 ml of 20% w/v NaOH and then through a trap containing 4 ml 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 20 ml of 1.0% $KMnO_4$ in 10% H_2SO_4 to absorb the mercury vapour and thereby avoid contaminating the surrounding with mercury vapour.

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.

(Ref: - Manual Methods of Analysis for Adulterants and Contaminants in Foods I.C.M.R 1990 Page 142).

II. Method II---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.

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: Neptune Dyna Pump or equivalent.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 (Kontes Glass Co., No. K- 181000 or equivalent.

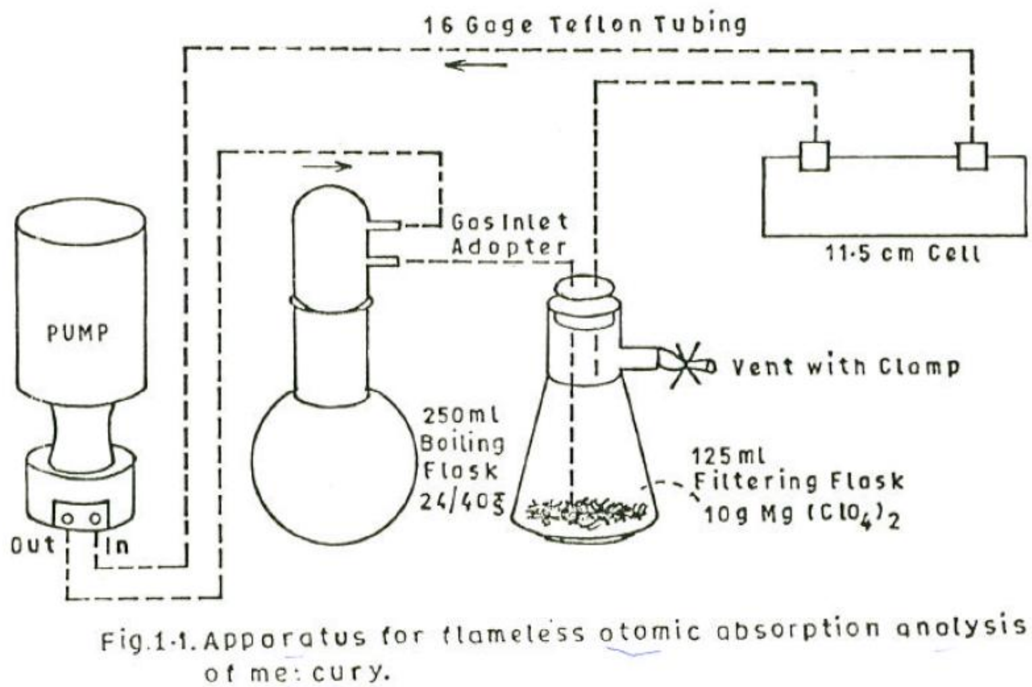

Fig. 1

Reagents:

(a) Reducing solution:

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

(b) Diluting solution:

To 1000 ml volumetric flask containing 300 to 500 ml water add 58ml HNO_3 and 67 ml 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 tractable standard)

(i) Stock Solution (1000 µg/ml): Dissolve 0.1354 g HgCl2 in 100ml water.

(ii) Working solution (1 µg/ml): Dilute 1 ml stock solution to 1000 ml with 1N H_2SO_4. Prepare fresh daily.

Preparation of Sample:

Digest the sample using microwave method.

Weigh 5.0 g of sample into digestion flask; add 25 ml of 18N H_2SO_4, 20 ml of 7N HNO_3, 1 ml 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 20 ml 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 10 ml water through condenser while swirling liquid in flask. Again boil solution for 10 minutes. Remove heat and wash condenser with three 15 ml potions of water.

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

Determination:

Transfer 25.0 ml of aliquot from digest solution of each sample to another digestion flask. Adjust volume to about 100 ml 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 20 ml 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), disconnects pressure on 'out' side of pump, and open vent of filter flask to flush system.

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 100 ml 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: - A.O.A.C 17th edn, 2000 Official Method 971.21 Mercury in Food Flameless Atomic Absorption Method) / Manual Methods of Analysis for Adulterants and Contaminants in Foods I.C.M.R 1990 Page 141)

3.0 SINGLE ELEMENT METHODS

3.1 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).

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.

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 g 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

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 molybdatesulphuric acid solution:

Dissolve 5.0 g of $(NH_4)_6MO_7O_{24}.4H_2O$ in water and slowly add 42.8 ml H_2SO_4 (Sp. Grade 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 g 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 g of As2O3 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.

Preparation of Sample:

(a) 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.

(b) For dried fruit products:

Prepare sample by alternately grinding and mixing 4 to 5 times in food chopper. Place 35 to 70 g 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 (a).

(c) For small fruits and vegetables etc:

Use 70 to 140 g sample and digest as in (a) or (b).

(d) For materials other than (a), (b) or (c):

Digest 5 to 50 g according to moisture content and amount of as expected as in (a) or (b).

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 g 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 g 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.

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 /A.O.A.C 15th edn, Official Method 942.17 Arsenic in Food Molybdenum Blue Method)

3.2 DETERMINATION 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).

Preparation of silver diethyl dithiocarbamate reagent solution:

Chill 200 ml of 0.1M $AgNO_3$ solution (3.4 g /200 ml) and 200 ml 0.1M sodium diethyl dithiocarbamate solution (4.5g/200 ml) to 10ºC 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 g of salt, prepared as above, in colorless pyridine in 100 ml volumetric flask and dilute to 100 ml with pyridine. Mix and store in amber bottle. Reagent is stable for several months at room temperature.

Determination:

Transfer aliquot of sample digest, prepared as given in 'preparation of sample', and same volume of blank to generator bottles. Add water to 35 ml and then add 5 ml HCl, 2 ml 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 4.0 ml 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.

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 35 ml 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 / A.O.A.C 15th edn Official Method 952.13 Arsenic in Food, Silver diethyldithiocarbamate Method)

3.3 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).

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.

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 g of NaOH in water and dilute to 100 ml.

(h) Thymol blue indicator: Triturate 0.1 g of indicator in agate mortar with 4.3 ml of 0.05N NaOH. Dilute to 200 ml 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 25 ml HNO_3 (1+9), evaporate to dryness, add 5 ml 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 100 ml.

(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).

Preparation of the Sample:

Digest a suitable quantity of sample equivalent to 5 to 10 g of product, calculated on dry basis, with 10 ml of H_2SO_4 (1+1) and HNO_3 as needed. If sample tends to char rather than to oxidize evenly, add 5 or 10 ml of additional H_2SO_4. Continue digestion, adding HNO_3 as required, until digestion is complete and SO_3 is evolved. Cool, add 15 ml 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.

Determination:

Dilute digest with 25 ml water, filter free of excessive insoluble matter if present and transfer to separator marked 125 ml, using additional 10 ml portions of water for rinsing and completing transfer. Add 1 to 2 g of citrate reagent (a) and 1 ml 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 125 ml mark with water. Extract vigorously with 5 ml portions of dithizone in chloroform solution until chloroform layer remains green. Then extract with 3 ml of chloroform.

Transfer all chloroform extracts to second separator previously wetted with 2 to 3 ml $CHCl_3$. Add 40 ml of 0.2N HCl to combined extracts, shake vigorously for more than a minute and let layers separate. Carefully drainchloroform phase and discard. Remove remaining droplets of dithizone by extracting with 1 to 2 ml 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 10 ml of NaOH solution.

Extract cadmium with 25 ml of dithizone solution, by shaking vigorously for more than or equal to one min and transfer to third separator previously wetted with 2 to 3 ml of same dithizone solution. Repeat extraction with additional 10 ml 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 40 ml 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 2 ml 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 10 ml NaOH solution. Add exactly 25 ml 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 5 ml. 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.

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 40 ml with 0.2N HCl, and 10 ml of NaOH solution and 25 ml of dithizone solution. Shake exactly for 1 min. let stand exactly for 3 min and filter organic layer through a pledged of adsorbent cotton, discarding first 5 ml.

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

(Ref: - A.O.A.C 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)

3.4 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).

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.

Reagents:

(a) Sodium diethyldithiocarbamate solution: (1.0% w/v)

Dissolve 1.0 g of the salt in water and dilute to 100 ml with water and filter. Store in refrigerator and prepare weekly.

(b) Citrate-EDTA solution:

Dissolve 20 g of dibasic ammonium citrate and 5 g Na_2 EDTA in water and dilute to 100 ml. Remove traces of copper by adding 0.1 ml of carbamate solution and extracting with 10 ml CCl_4. Repeat extraction until CCl4 extract is colourless.

(c) Ammonium hydroxide – 6N:

Purify as in (b) above.

(d) Copper Standard solution:

(i) Stock solution:

Place 0.2500 g of Cu wire or foil in 125 ml Erlenmeyer flask.

Add 15 ml 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 10 ml of stock solution to 100 ml with water.

(iii) Working solution: (2 µg/ml)

Prepare daily by diluting 5 ml of intermediate standard solution to 250 ml with 2N H_2SO_4.

Preparation of sample:

Weigh sample containing not more than 20 g 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 5 ml. 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 25 ml water and heat to fumes. Repeat addition of 25 ml water and fuming. Cool, filter off any insoluble matter present and dilute to 100 ml with water. Prepare reagent blank similarly.

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 25 ml and add 10 ml 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 1 ml of carbamate solution and 15 ml of CCl_4. Shake vigorously for 2 min. 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 10 ml 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 10 ml of 1N NaOH and shake for 1 min. Let layers separate and drain CCl_4 into third separator. Again wash CCl_4 extract with 10 ml of 1N NaOH.

Determine absorbance of CCl4 layer and convert to µg of Cu.

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

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 25 ml. Add 10 ml 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 / A.O.A.C 15th edn, Official Method 960.40 Copper in Food)

3.5 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).

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.

Reagents:

(a) Magnesium nitrate solution: (50% w/v)

Dissolve 50 g of Mg $(NO_3)_2.6H_2O$ in water and dilute to 100 ml with water.

(b) Concentrated hydrochloric acid

(c) Hydroxylamine hydrochloride solution: (10% w/v)

Dissolve 10 g H_2 N OH. HCl in water and dilute to 100 ml.

(d) Acetate buffer solution:

Dissolve 8.3 g of anhydrous NaOAC (previously dried at 100ºC) in water, add 12 ml of glacial acetic acid and dilute to 100 ml.

(e) Alpha, alpha-dipyridyl solution: (0.1% w/v)

Dissolve 0.1 g of alpha, alpha-dipyridyl in water and dilute to 100 ml.

Keep this reagent in cool and dark place.

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

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

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

(g) Orthophenanthroline solution: (0.1% w/v)

Dissolve 0.1 g of O-Phenanthroline in 80 ml of water at 80ºC, cool and dilute to 100 ml with water. Keep in cool and dark place.

Apparatus:

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

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 450ºC. Continue ashing at 450ºC 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 450ºC). After the ash is carbon-free remove the dish from furnace and cool.

Add 5 ml 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 2.0 ml of conc. HCl, heat for 5 min on steam bath with watch glass covering the dish. Rinse watch glass with water, filter into a 100 ml volumetric flask, cool and dilute to volume.

Determination of Iron:

Pipette 10 ml aliquot of ash solution into 25 ml volumetric flask, and add 1 ml hydroxylamine hydrochloride solution. After 5 min, add 5 ml buffer solution and 1 ml O -

phenanthroline solution or 2 ml 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.

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 25 ml volumetric flasks and add to each of them exactly 0.2 ml of conc. HCl. Dilute each of them to exactly 10 ml with water, and then add reagents in the same way as for the sample, Plot the quantity of Fe (in mg) against the absorbance.

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)

3.6 DETERMINATION OF LEAD IN FOOD

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

Reagents:

(a) Lead standard solution:

(i) Stock solution: (1 mg Pb/ml in 1% HNO3)

Dissolve 1.5985 g 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 10 ml of fresh, colourless HNO3 (sp. Gr. 1.40) to 1000 ml with redistilled water. If acid is redistilled, boil off nitrous fumes before diluting.

(c) “Acid-aid” solution:

Dissolve 40 g Al $(NO_3)_3.9H_2O$ and 20 g 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 g 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 g 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 g 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.

Preparation of sample:

Accurately weigh representative sample of 5 to 100 g, 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 5 ml 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 350ºC. 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 500ºC 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.

Isolation and Determination of Lead:

(i) Transfer suitable aliquot of ash solution of sample to a 250 ml separator and add citric acid reagent equivalent to 10 g 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 g of citric acid, make ammonical and proceed from step (iii) onwards.

(iii) To the clear solution obtained after step (ii), add 5 ml 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 20 ml 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 25 ml 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 25 ml 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 50 ml 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 50 ml of 1.0% HNO_3 solution containing lead (obtained in step (vii) and enough 1.0% HNO_3 reagent to make total volume to 50 ml (add acid solution first and later lead extract).

(ix) Add 10 ml 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.

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 50 ml. Add the required volume of acid solution first and then the proposed volume of standard solution.

(2) Add 10 ml 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)

3.7 DETERMINATION OF MERCURY IN FOOD

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).

Principle :

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.

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, 200 ml 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 500 ml capacity. Necks are 3 cm apart. Second neck is used for attaching 75 ml dropping funnel 'B'.

Reagents:

(a) Mercury Standard Solution:

(i) Stock solution (1 mg/ml):

Prepare from dry, recrystallized $HgCl_2$ (135.4 mg $HgCl_2$ in 100 ml solution)

(ii) Working solution (2 μg/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

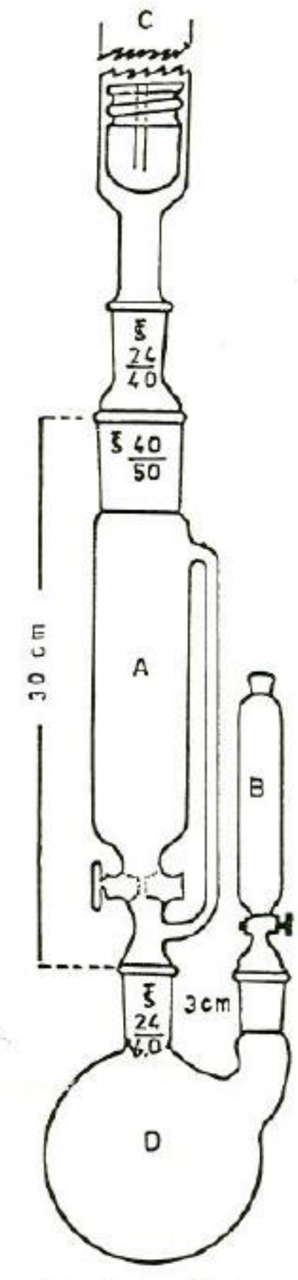

Fig. 2.2. Special digestion apparatus tor mercury residues.

(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.

Determination of strength of Hyprochorite solution:

A. Reagents needed:

(i) Arseneous oxide standard solution (0.1N)

Accurately weigh about 2.473 g of pure As_2O_3 (dried for 1 hour at 105º before use), dissolve in 25 ml of 1N NaOH while heating on steam bath. Add approximately the same quantity of 1N H_2SO_4. Cool and transfer quantitatively to 500 ml

Volumetric flask and dilute to volume with water. The final solution should be just neutral to litmus but not alkaline.

$$As_2O_3 = \frac{\text{g of } As_2O_3 \text{ weighed x } 4000}{\text{ml of final volume made x } 197.84}$$

(ii) Iodine solution (0.1N):

Dissolve about 13 g of iodine and 20 g of KI in about 50 ml water, transfer to a 1000 ml volumetric flask and dilute to volume with water and mix thoroughly.

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

B. Procedure:

Transfer 20 ml of hypochlorite reagent solution (Solution A) to 1000 ml volumetric flask and dilute to volume with water (Solution B). Pipette 50 ml aliquot of solution B into a

250 ml 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 (T1). 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
(g of chlorine per 100 ml of hypochlorite for dilution reagent solution)

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

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

Preparation of Sample:

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

(a) Fresh fruits or vegetables and beverages:

Place weighed sample in digestion flask with 6 glass beads, connect assembly and add, through dropping funnel, 20 ml 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 20 ml cool HNO_3 – H_2SO_4 mixture (1+1). {Use 10 ml acid mixture for 5 g (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. isapparently 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 25 ml 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 10 ml 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 50 ml portions of water through condenser. Reflux solution until all NO_2 is expelled from apparatus. Add 5 ml of 40% w/v urea solution and reflux 15 min. Digest should be colourless or pale yellow.

(b) Dried fruit, cereal, seeds and grains:

Dilute sample with 50 ml water before adding HNO_3 and proceed with sample preparation as in (a).

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

Isolation and Determination of Mercury:

Titrate 1 ml 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 500 ml separator. Add 10 ml 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 25 ml of 0.1N HCl and 5 ml hydroxyl amine hydrochloride solution. Repeat extraction of sample solution with two 5 ml 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 50 ml of 0.1N HCl. Extract solution in second separator with 1 to 2 ml of $CHCl_3$ and transfer organic layer to third separator.

To contents of third separator add 2 ml of $Na_2S_2O_3$ solution, shake vigorously for 1 min. Let layers separate drain off $CHCl_3$ as completely as possible and discard. Extract again with 1 to 2 ml 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 5 ml 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 3 ml 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 3 ml of 30% HOAC and appropriate volume and concentration of dithizone solution as indicated in Table 3. 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.

Preparation of Standard Curve:

Table 3 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 50 ml of 0.1N HCl in separator. Add 5 ml $H_2NOH.HCl$ reagent (f) and 5 ml 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 3 ml 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.

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)

3.8 DETERMINATION OF TIN IN FOOD

Spectrophotometric Catechol Violet Method (IUPAC Method)

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.

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 107 ml of HCl (Sp. Gr. 1.18) to 250 ml with water.

(f) Sulphuric acid solution: (Approximately 4.5M)

Dilute 250 ml of H_2SO_4 (a) to 1000 ml with water.

(g) Potassium iodide solution: (Approximately 5M):

Dissolve 83 g KI in 100 ml water. Prepare fresh daily.

(h) Sodium hydroxide solutions:

Prepare fresh each fortnight:

(i) Approximately 5M solution:

Dissolve 100 g of NaOH in water and dilute to 500 ml.

(ii) Approximately 0.1M solution:

Dissolve 2 g of NaOH in water and dilute to 500 ml.

(i) Ascorbic acid solution: (5% w / v)

Dissolve 1 g of ascorbic acid in 20 ml 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 100 ml and mix. Prepare fresh daily.

(k) Sodium acetate solution: (20% w/v)

Dissolve 50 g of $CH_3COONa.3H_2O$ in water and dilute to 250 ml and mix.

(l) Ammonia solution: (Approximately 5M)

Dilute 184 ml of ammonia solution {(25% m/m) Sp. Gr. 0.91} to 500 ml with water.

(m) Cyclohexane

(n) Tin Standard Solution:

(i) Stock Solution: (200 µg/ml)

Dissolve 0.1 g pure granular Sn in 20 ml of sulphuric acid (a) by heating until fumes appear. Cool, dilute cautiously with 50 ml of water and cool again. Add 65 ml of sulphuric acid (c) and transfer the solution to a 500-ml volumetric flask and dilute to volume with water and mix.

(ii) Working solution: (5 µg/ml)

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

Preparation of Sample:

(i) Weigh accurately a suitable portion of homogenised sample containing 2 to 25 µg of Sn into a 500 ml 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 50 ml HNO3 (b), 12.5 ml 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 1 ml of $HClO_4$ (c) and reheat till copious white fumes appear. Continue heating for 5 minutes.

(vii) Cool the solution again, adds 1 ml 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.

Determination:

(1) Quantitatively transfer the cooled digest solution to 100 ml 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 25 µg of Sn, quantitatively transfer the cooled digests into a 100 ml volumetric flask with 37.5 ml of water and dilute to volume with 4.5M sulphuric acid solution. Take a suitable aliquot of this solution into 100 ml separatory funnel and bring the volume of solution in separator to 50 ml with 4.5M sulphuric acid and proceed as follows.

(2) Add 5 ml KI solution (g) mix and add 10 ml 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 10 ml 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, 5 ml of water and 1.5 ml of 5M NaOH solution (h - i). Shake the contents vigorously for 2 min.
Let layers separate and transfer aqueous layer into a 50 ml beaker containing 2.5 ml of HCl (e).

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

(7) Wash the cyclohexane layer retained in the separatory funnel with 5 ml 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 2 ml 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 25 ml volumetric flask, add 2 ml 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 50 ml 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:

$$\text{Tin content of sample} = \frac{\mu g \text{ of Sn present in test solution X Dilution factor}}{\text{mgm/ kg Weight in grams of sample taken}}$$

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.

3.9 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).

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.

Reagents:

(a) Copper sulphate solution: 2 mg Cu/ml

Dissolve 8 g of $CuSO_4.5H_2O$ in water and dilute to 1000 ml.

(b) Ammonium citrate solution:

Dissolve 225 g 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 75 ml in excess. Dilute to 2000 ml.

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 g of reagent in 10 ml of NH_4OH and 200-300 ml water, filter, and dilute to 1000 ml with water.

(d) α-nitroso-β- naphthol solution:

Dissolve 0.25 g in $CHCl_3$ and dilute to 500 ml.

(e) Chloroform.

(f) Diphenylthiocarbazone solution:

Dissolve 0.05 f of dithizone in 2 ml of NH_4OH and 100 ml 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 g of pure Zinc in slight excess of dil. HCl and dilute to 1000 ml.

(ii) Working solution: (5 µg/ml)

Dilute 10 ml of stock solution to 1000 ml with 0.04N HCl.

Preparation of sample:

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

(A) Wet Ashing:

Accurately weigh, into 300 or 500 ml Kjeldahl flask, representative sample of about 25 g (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.

(B) Dry Ashing:

Accurately weigh, into a clean platinum or silica dish, a representative portion of sample (about 25 g). Char the sample and ash at temperatures not exceeding 500ºC. 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.

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.

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.

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)

4.0 MISCELLANEOUS METHODS

4.1 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).

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.

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 g of KIO_3 in water and dilute to 100 ml with water.
(ii) Working solution:
Dilute 5 ml of stock solution to 100 ml with water. Prepare fresh daily.
(g) Potassium iodide-sodium bicarbonate solution:
Dissolve 0.2 g of KI and 3 g $NaHCO_3$ in water and dilute to 100 ml with water. Prepare fresh daily.

(h) Starch indicator:

1 g of soluble starch and 20 g of NaCl dissolved in 100 ml water.

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

Preparation of Sample:

Weigh accurately about 50 g of the homogenized sample into a clean silica dish, add 2 g 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 500ºC 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 º C for 1 hour.

When clean ash is obtained, boil the contents of the dish cautiously with about 5 to 10 ml 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 5 ml of conc. HCl, rinse down with water and heat on steam bath and evaporate to near dryness. Add 20 ml of HCl (1+1); heat to dissolve the ash, transfer the contents of the dish to a 100 ml volumetric flask and dilute to volume with water.

Determination of Tin:

Transfer on aliquot of the ash solution to a reduction flask. Add 30 ml of HCl (1+3) and about 0.3 g 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 5 ml 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:

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

Preparation of Standard Solution:

Stock Solution: (1mg/ml):

Dissolve 0.19 g of AR grade $SnCl_2.2H_2O$ in 5 ml of conc. HCl and make upto 100 ml with distilled water.

NOTE: Tin solution need to obtain with NIST traceability

Working Solution: (0.1 mg/ml)

Dilute 10 ml of stock solution to 100 ml 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 20 ml 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.

Test method: Digestion of sample with Microwave and use AAS/ICP-OES/ICP-MS (for ppt level) to determine Tin (Sn) content may also be used.

(Ref: - Manual Methods of Analysis for Adulterants and Contaminants in Foods, I.C.M.R 1990 Page 165).

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*The methods mentioned in the manual needs to be verified/ validated before they are put in use by the laboratory.

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