THE AYURVEDIC PHARMACOPOEIA OF INDIA

PART - II (FORMULATIONS)
VOLUME - I

First Edition

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Note: Page numbers as per the original published book

APPENDIX-1

APPARATUS FOR TESTS AND ASSAYS

1.1. -Nessler Cylinders

Nessler cylinders which are used for comparative tests are matched tubes of clear colourless glass with a uniform internal diameter and flat, transparent base. They comply with Indian Standard 4161-1967. They are of transparent glass with a nominal capacity of 50 ml. The overall height is about 150 mm, the external height to the 50 ml mark 110 to 124 mm, the thickness of the wall 1.0 to 1.5 mm and the thickness of the base 1.5 to 3.0 mm. The external height to the 50 ml mark of the cylinder used for a test must not vary by more than 1 mm.

1.2. -Sieves

Sieves for pharmacopoeial testing are constructed from wire cloth with square meshes, woven from wire of brass, bronze, stainless steel or any other suitable material. The wires should be of uniform circular cross-section and should not be coated or plated. There must be no reaction between the material of the sieve and the substance being sifted.

Sieves conform to the following specifications –

Table 1

Approximate sieve number*	Nominal mesh aperture size mm	Tolerance average aperture size ± mm
4	4.0	0.13
6	2.8	0.09
8	2.0	0.07
10	1.7	0.06
12	1.4	0.05
16	1.0	0.03
--	µm	±µm
22	710	25
25	600	21
30	500	18
36	425	15
44	355	13
60	250	3(9.9) **

85	180	11(7.6)
100	150	9.4(6.6)
120	125	8.1(5.8)
150	106	7.4(5.2)
170	90	6.6(4.6)
200	75	6.1(4.1)
240	63	5.3(3.7)
300	53	4.8(3.4)
350	45	4.8(3.1)

* Sieve number is the number of meshes in a length of 2.54 cm. in each transverse direction parallel to the wires.

** Figures in brackets refer to close tolerances, those without brackets relate to full tolerances.

1.3. -Thermometers

Unless otherwise specified, thermometers suitable for pharmacopoeial tests conform to Indian Standard 4825-1968 and are standardised in accordance with the 'Indian Standard Method of Calibrating Liquid-in-Glass Thermometers', 6274-1971.

The thermometers are of the mercury-in-glass type and are filled with a dried inert gas, preferably nitrogen. They may be standardised for total immersion or for partial immersion. Each thermometer should be employed according to the condition of immersion under which it was standardised. In the selection of the thermometer it is essential to consider the conditions under which it is to be used.

1.4. –Ultra-violet Lamp (For general purposes and for chromatography work)

An instrument consisting of mercury vapour lamp and a filter which gives an emission band with maximum intensity at about 254 nm (near UV rays) and 366 nm (far UV rays) is used. To ensure that the required emission is being given by the lamp, carry out the following test periodically.

Apply to a plate coated with *silica gel* G, 5 µl of a 0.04 per cent w/v solution of *sodium salicylate* in *ethanol* (95%) for lamps of maximum output at 254 nm and 5 µl of a 0.2 per cent w/v solution in *ethanol* (95%) for lamps of maximum output at 365 nm. Examine the spot in a position normal to the radiation. The distance between the lamp and the plate under examination used in a pharmacopoeial test should not exceed the distance used to carry out the above test.

1.5. -Volumetric Glassware

Volumetric apparatus is normally calibrated at 27^0. However, the temperature generally specified for measurements of volume in the analytical operations of the pharmacopoeia, unless otherwise stated, is 25^0. The discrepancy is inconsequential as long as the room temperature in the laboratory is reasonably constant and is around 27^0.

Pharmacopoeial assays involving volumetric measurements require the use of accurately calibrated glassware. Volumetric apparatus must be suitably designed to assure accuracy. The design, construction and capacity of volumetric glassware should be in accordance with those laid down by the Bureau of Indian Standards. The tolerances on capacity for volumetric flasks, pipettes and burettes, as laid down in the relevant Indian Standards, are permisibile.

1.6. -Weights and Balances

Pharmacopoeial tests and assays require the use of analytical balances that vary in capacity, sensitivity and reproducibility. The accuracy needed for a weighing should dictate the type of balance. Where substances are to be "accurately weighed", the weighing is to be performed so as to limit the error to not more than 0.1 per cent. For example, a quantity of 50 mg is to be weighed to the nearest 0.05 mg; a quantity of 0.1 g is to be weighed to the nearest 0.1 mg; and quantity of 10 g is to be weighed to the nearest 10 mg. A balance should be chosen such that the value of three times the standard deviation of the reproducibility of the balance, divided by the amount to be weighed, does not exceed 0.001.

1.7. -Muslin Cloth

Muslin cloth is a cotton fabric where warp is 22 per cm±1 and weft is 18 ±1 per centimeter.

Method: Take a cardboard or an aluminium plate with a centimeter square opening. Keep the plate on the cloth to be used, so that the edges on the X or Y axis coincides with a warp or weft yarn in the fabric.Count the number of the threads of both warp and weft within the opening.

APPENDIX - 2

TESTS AND DETERMINATIONS

2.1. - Microscopic identification:

Microscopic identification of the botanical ingredients is a standard for statutory purposes in several solid and semi-solid compound formulations. Microscopic identification tests are confined to those formulations where the botanical ingredients are **not more than ten**, and where they are added *'in situ'* in powder form as '*Praksepa Dravyās'*. Such comminuted ingredients lend themselves for microscopic identification, as they are not drastically changed in cell structure or contents while processing, and appear intact in microscopic slide preparations, after proper treatment.

Appropriate processing for separation and isolation of botanical debris from a formulation without loss of debris, by hand picking, shifting, washing, sedimentation, density separation or by floatation etc., are the preliminary steps. This is followed by clearing the debris in chemical reagents, reacting it with suitable reagents and stains and finally mounting a little part on a slide in a medium of suitable refractive index (see later part) that helps to show the unit structures in good relief. Identification of the discrete, but disoriented units from the botanical ingredients in a formulation will not be possible without proper isolation, and should not be attempted.

Monographs where the test is prescribed give both a relevant method of isolation and diagnostic features specific to the expected ingredients in that formulation. Only a brief method and a few of the characteristics for each ingredient are given, but an analyst may use other methods of isolation and choose more characteristics to draw a correct conclusion.

Although monographs prescribe standards only for the '*Praksepa Dravyas',* characteristics from other ingredients that are processed into extracts or decoctions prior to their addition to a formulation may also be seen in a slide preparation, giving rise to recognisable unique characteristics. In addition, cell or tissue structures common to several ingredients added to a formulation, and therefore not specific to any one of them, would also be present. Caution should therefore be exercised so that such features are not construed as parts from adulterants or substitutes or foreign parts. Proper study of the individual ingredients using authentic material and reference to their monographs in the Ayurvedic Pharmacopoeia for Single Drugs would help avoid errors of this nature. Skill in the recognition of discrete and disoriented tissue components and the knowledge required to ascribe them to their correct source should be acquired by the analyst.

A. Stains and Reagents for Microchemical Reactions:

The Ayurvedic Pharmacopoeia volumes on single drugs already include microchemical reactions for ergastic substances and may be consulted in addition to the following for use on isolated debris:

Acetic acid: Dilute 6 ml of glacial acetic acid with 100 ml of distilled water; *used for identification of cystoliths, which dissolve with effervescence.*

Aniline Chloride Solution: Dissolve 2 g in a mixture of 65 ml of 30 per cent ethyl alcohol and 15 ml distilled water and add 2 ml of conc. Hydrochloric acid. *Lignified tissues are stained bright yellow.*

Bismarck Brown: Dissolve 1 g in 100 ml of 95 per cent of ethyl alcohol; *used as a general stain for macerated material (with Schultze's).*

Chlorinated Soda Solution (Bleaching Solution): Dissolve 75 g of sodium carbonate in 125 ml of distilled water; triturate 50 g of chlorinated lime (bleaching powder) in a mortar with 75 ml of distilled water, adding it little by little. Mix the two liquids and shake occasionally for three or four hours. Filter and store, protected from light. *Used for lighting highly coloured material, by warming in it and washing the tissues thoroughly.*

Breamer's reagent: Dissolve 1 g of sodium tungstate and 2 g of sodium acetate in sufficient quantity of water to make 10 ml yellowish to brown precipitates; *indicate the presence of tannin.*

Canada Balsam (as a Mountant): Heat Canada balsam on a water bath until volatile matter is removed and the residue sets to a hard mass on cooling. Dissolve residue in xylene to form a thin syrupy liquid. *Used for making permanent mounts of reference slides of selected debris.*

Chloral Hydrate Solution: Dissolve 50 g of chloral hydrate in 20 ml of distilled water. *A valuable clarifying agent for rendering tissues transparent and clear, by freeing them from most of the ergastic substances, but leaving calcium oxalate crystals unaffected.*

Chloral Iodine: Saturate chloral hydrate solution with iodine, leaving a few crystals undissolved; useful *for detecting minute grains of starch otherwise undetectable.*

Chlorziniciodine (Iodinated Zinc Chloride solution): Dissolve 20 g of zinc chloride and 6.5 g of potassium iodide in 10 ml of distilled water. Add 0.5 g of iodine and shake for about fifteen minutes before filtering. Dilute if needed prior to use. *Renders cellulosic walls bluish violet and lignified walls yellowish brown to brown.*

Chromic acid Solution: 10 g of dissolved in 90 ml of dilute sulphuric acid: *macerating agent similar to Schultze's.*

Corallin Soda: Dissolve 5 g of corallin in 100 ml of 90 per cent ethyl alcohol. Dissolve 25 g of sodium carbonate in 100 ml distilled water; keep the solutions separate and mix when required, by adding 1 ml of the corallin solution to 20 ml of the aqueous sodium carbonate solution. Prepare fresh each time, as the mixture will not keep for long. *Used for staining sieve plates and callus bright pink and imparts a reddish tinge to starch grains and lignified tissues.*

Ammoniacal solution of Copper oxide (Cuoxam): Triturate 0.5 g of copper carbonate in a mortar with 10 ml of distilled water and gradually add 10 ml of strong solution of ammonia (sp. gr. 0.880) with continued stirring; *used for dissolving cellulosic materials.*

Eosin: 1 per cent solution in 90 per cent ethyl alcohol; *stains cellulose and aleurone grains red.*

Ferric Chloride solution: A per cent solution ferric chloride in distilled water. *Taninn containing tissues coloured bluish or greenish black.*

Glycerin: Pure or diluted as required with one or two volumes of distilled water. *Used as a general mountant.*

Haematoxylin, Delafield's: Prepare a saturated solution of ammonia alum. To 100 ml of this add a solution of 1 g of Haematoxylin in 6 ml of ethyl alcohol (97 per cent). Leave the mixed solution exposed to air and light in an unstopped bottle for three or four days. Filter and add to the filtrate 25 ml of glycerin and 25 ml of methyl alcohol. Allow the solution to stand exposed to light, till it acquires a dark colour (about two months). Refilter and store as a stock solution. Dilute it 3 or 4 times volumes with distilled water. *Stains cellulosic fibers blue; used only on water washed material.*

Iodine Water: Mix 1 volume of decinormal iodine with 4 volumes of distilled water. *Stains starch blue, and reveals crystalloids and globoids when present in aleurone grains.*

Iodine and Potassium iodide solution: Dissolve 1 g of *potassium iodide* in 200 ml of distilled water and 2 g of iodine; *stains lignified walls yellow and cellulosic walls blue.*

Lactophenol (Amman's Fluid): *Phenol* 20 g, *lactic acid* 20 g, *glycerin* 40 g, *distilled water* 20 ml dissolve; *reveals starch grains in polarised light with a well marked cross at hilum, and also minute crystals of calcium oxalate as brightly polarising points of light.*

Methylene blue: A solution in 25 ml of *ethyl alcohol* (95 per cent). *A general stain for nucleus and bacteria.*

Millon''s Reagent: Dissolve 1 volume of mercury in 9 volumes of fuming nitric acid (sp. Gr. 1.52), keeping the mixture well cooled during reaction. Add equal volume distilled water when cool. *Stains proteins red.*

Naphthol Solution: Dissolve 10 g of Naphthol in 100 ml of *ethyl alcohol*; *a specific stain for detection of inulin; cells containing inulin turn deep reddish violet.*

Pholorglucinol: 1 g of *phloroglucinol* dissolved in 100 ml of 90 per cent *ethyl alcohol*; mount debris in a few drops, allow to react for a minute, draw off excess of reagent with a filter paper strip, and add a drop of conc. hydrochloric acid to the slide; *lignified tissues acquire a deep purplish red colour; very effective on water washed material but not in chloral hydrate washed debris.*

Picric acid Solution (Trinitrophenol Solution): A saturated aqueous solution made by dissolving 1 g of picric acid in 95 ml of distilled water; *stains animal and insect tissues, a light to deep yellow; in a solution with ethyl alcohol, aleurone grains and fungal hyphae are stained yellow.*

Potash, Caustic: A 5 per cent aqueous solution*; used to separate tenacious tissues of epidermis and also laticiferous elements and vittae, both of which are stained brown.*
Ruthenium Red: Dissolve 0.008 g of ruthenium red in 10 ml of a 10 per cent solution of lead acetate; (to be freshly prepared) *used for identification of most kinds of mucilage containing tissues, which turn pink. A 0.0008 g ruthenium red dissolved in 10 ml of distilled water and used immediately stains cuticular tissues in debris to a light pink*.

Safranin: A 1 per cent solution in ethyl alcohol 50 per cent; *used to stain lignified cell walls deep red, even after clearing with choral hydrate.*

Schultze's Maceration Fluid: Add isolated debris to 50 per cent conc. *nitric acid* in a test tube and warm over water bath: add a few crystals of *potassium chlorate* while warming, till tissues soften; cool, wash with water thoroughly and tease out for mounting hard tissues; *isolated cell structures are clearly revealed, but the structures are not useful for measurement of dimensions*.

Sudan Red III: Dissolve 0.01 g of sudan red III in 5 ml of *ethyl alcohol* (90 per cent) and 5 ml of pure *glycerin*; *suberised walls of cork cells, and fatty material in cells are stained bright red.*

Sulphovanadic Acid (Mandelin's Reagent)**:** Triturate 1 g of ammonium vandate with 100 ml conc. s*ulphuric acid*. Allow the deposit to subside and use the clear liquid. *This is to be prepared fresh; useful for identification of alkaloids, particularly strychnine which turns violet in the cells containing it.*

Table 3 - Refractive Indices of Certain Mountants

Water	1.333
Lactophenol	1.444
Chloral Hydrate solution	1.44 to 1.48
Olive oil	1.46 to 1.47
Glycerol	1.473
Castor oil	1.48
Clove oil	1.53
Cresol	1.53
Cassia oil	1.6
Xylol	1.49
Alcohol	1.36
Chloroform	1.44

2.2. -Determination of Quantitative Data:

2.2.1. - Net Content: The content of the final or retail pack shall not be less than 98 percent of the declared net content.

2.2.2. - Foreign Matter: The sample shall be free from visible signs of mold growth, sliminess, stones, rodent excreta, insects or any other noxious foreign matter when examined as given below.

Take a representative portion from a large container, or remove the entire contents of the packing if 100 g or less, and spread in a thin layer in a suitable dish or tray. Examine in daylight with unaided eye.Transfer suspected particles, if any, to a petri dish, and examine with 10x lens in daylight.

2.2.3. - Determination of Total Ash:

Incinerate about 2 to 3 g accurately weighed, of the ground drug in a tared platinum or silica dish at a temperature not exceeding 450^0 until free from carbon, cool and weigh. If a carbon free ash cannot be obtained in this way, exhaust the charred mass with hot water, collect the residue on an ashless filter paper, incinerate the residue and filter paper, add the filtrate, evaporate to dryness, and ignite at a temperature not exceeding 450^0. Calculate the percentage of ash with reference to the air-dried drug.

2.2.4. - Determination of Acid-Insoluble Ash:

To the crucible containing total ash, add 25 ml of *dilute hydrochloric acid*. Collect the insoluble matter on an ashless filter paper (Whatman 41) and wash with hot water until the filtrate is neutral. Transfer the filter paper containing the insoluble matter to the original crucible, dry on a hot-plate and ignite to constant weight. Allow the residue to cool in a suitable desiccator for 30 minutes and weigh without delay. Calculate the content of acid-insoluble ash with reference to the air-dried drug.

2.2.5. - Determination of Water Soluble Ash:

Boil the ash for 5 minutes with 25 ml of water; collect insoluble matter in a Gooch crucible or on an ashless filter paper, wash with hot water, and ignite for 15 minutes at a temperature not exceeding 450^0. Subtract the weight of the insoluble matter from the weight of the ash; the difference in weight represents the water-soluble ash. Calculate the percentage of water-soluble ash with reference to the air-dried drug.

2.2.6. - Determination of Sulphated Ash:

Heat a silica or platinum crucible to redness for 10 minutes, allow to cool in a desiccator and weigh. Put 1 to 2 g of the substance, accurately weighed, into the crucible, ignite gently at first, until the substance is thoroughly charred. Cool, moisten the residue with 1 ml of *sulphuric acid,* heat gently until white fumes are no longer evolved and ignite at $800^0 \pm 25^0$ until all black particles have disappeared. Conduct the ignition in a place protected from air currents. Allow the crucible to cool, add a few drops of *sulphuric acid* and heat. Ignite as before, allow to cool

and weigh. Repeat the operation until two successive weighing do not differ by more than 0.5 mg.

2.2.7. - Determination of Alcohol Soluble Extractive:

Macerate 5 g of the air dried drug, coarsely powdered, with 100 ml of alcohol the specified strength in a closed flask for twenty-four hours, shaking frequently during six hours and allowing to stand for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105^0, to constant weight and weigh. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug.

2.2.8. - Determination of Water Soluble Extractive:

Proceed as directed for the determination of alcohol-soluble extractive, using *chloroform-water* instead of ethanol.

2.2.9. - Determination of Ether Soluble Extractive (Fixed Oil Content):

Transfer a suitably weighed quantity (depending on the fixed oil content) of the air-dried, crushed drug to an extraction thimble, extract with *solvent ether* (or *petroleum ether,* b.p. 40^0 to 60^0) in a continuous extraction apparatus (Soxhlet extractor) for 6 hours. Filter the extract quantitatively into a tared evaporating dish and evaporate off the solvent on a water bath. Dry the residue at 105^0 to constant weight. Calculate the percentage of ether-soluble extractive with reference to the air-dried drug.

2.2.10. - Determination of Moisture Content (Loss on Drying):

Procedure set forth here determines the amount of volatile matter (i.e., water drying off from the drug). For substances appearing to contain water as the only volatile constituent, the procedure given below, is appropriately used.

Place about 10 g of drug (without preliminary drying) after accurately weighing (accurately weighed to within 0.01 g) it in a tared evaporating dish. For example, for unground or unpowderd drug, prepare about 10 g of the sample by cutting shredding so that the parts are about 3 mm in thickness.

Seeds and fruits, smaller than 3 mm should be cracked. Avoid the use of high speed mills in preparing the samples, and exercise care that no appreciable amount of moisture is lost during preparation and that the portion taken is representative of the official sample. After placing the above said amount of the drug in the tared evaporating dish, dry at 105^0 for 5 hours, and weigh. Continue the drying and weighing at one hour interval until difference between two successive weighing corresponds to not more than 0.25 per cent. Constant weight is reached when two consecutive weighing after drying for 30 minutes and cooling for 30 minutes in a desiccator, show not more than 0.01 g difference.

2.2.11. - Determination of Volatile Oil in Drugs

The determination of volatile oil in a drug is made by distilling the drug with a mixture of *water* and *glycerin,* collecting the distillate in a graduated tube in which the aqueous portion of the distillate is automatically separated and returned to the distilling flask, and measuring the volume of the oil. The content of the volatile oil is expressed as a percentage v/w.

The apparatus consists of the following parts (see Fig. 1). The clevenger's apparatus described below is recommended but any similar apparatus may be used provided that it permits complete distillation of the volatile oil. All glass parts of the apparatus should be made of good quality resistance glass.

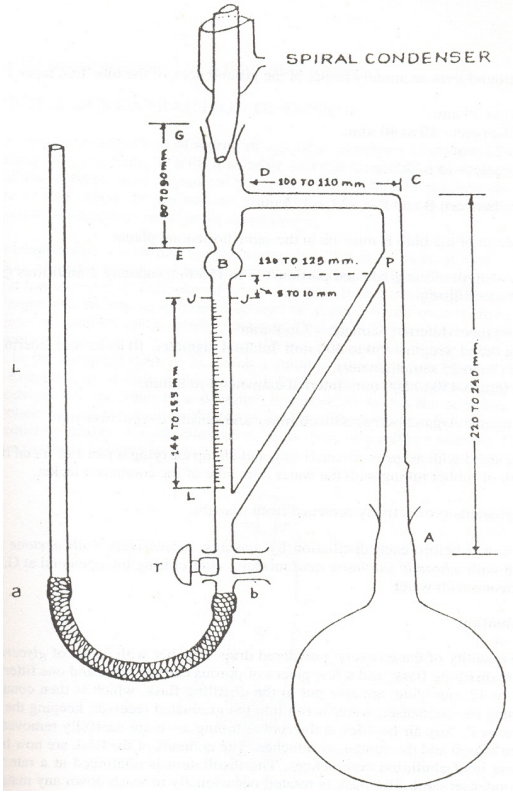

Fig. 1 Apparatus for volatile oil determination

The apparatus is cleaned before each distillation by washing successively with *acetone* and *water,* then inverting it, filling it with *chromic sulphuric acid* mixture, after closing the open end at G, and allowing to stand, and finally rinsing with water.

Method of determination:

A suitable quantity of the coarsely powdered drug together with 75 ml of *glycerin* and 175 ml of *water* in the one litre distilling flask, and a few pieces of porous earthen ware and one filter paper 15 cm cut into small strips, 7 to 12 mm wide, are also put in the distilling flask, which is then connected to the still head. Before attaching the condenser, water is run into the

graduated receiver, keeping the tap T open until the water overflows, at P. Any air bubbles in the rubber tubing a—b are carefully removed by pressing the tube. The tap is then closed and the condenser attached. The contents of the flask are now heated and stirred by frequent agitation until ebullition commences. The distillation is continued at a rate, which keeps the lower end of the condenser cool. The flask is rotated occasionally to wash down any material that adheres to its sides.

At the end of the specified time (3 to 4 hours) heating is discontinued, the apparatus is allowed to cool for 10 minutes and the tap T is opened and the tube L_1 lowered slowly; as soon as the layer of the oil completely enters into the graduated part of the receiver the tap is closed and the volume is read.

The tube L_1 is then raised till the level of water in it is above the level of B, when the tap T is slowly opened to return the oil to the bulb. The distillation is again continued for another hour and the volume of oil is again read, after cooling the apparatus as before. If necessary, the distillation is again continued until successive readings of the volatile oil do not differ.

The measured yield of volatile oil is taken to be the content of volatile oil in the drug. The dimensions of the apparatus may be suitably modified in case of necessity.

2.2.12. - Special Processes Used in Alkaloidal Assays:

A-Continuous extraction of drug:

Where continuous extraction of a drug of any other substance is recommended in the monograph, the process consists of percolating it with suitable solvent at a temperature approximately that of the boiling point of the solvent. Any apparatus that permits the uniform percolation of the drug and the continuous flow of the vapour of the solvent around the percolator may be used. The type commonly known as the Soxhlet apparatus is suitable for this purpose.

B -Tests for complete extraction of alkaloids: Complete extraction is indicated by the following tests:

When extracting with an aqueous or alcoholic liquid: After extracting at least three times with the liquid, add to a few drops of the next portion, after acidifying with 2 *N hydrochloric acid* if necessary, 0.05 ml of *potassium mercuri-iodide solution* or for solanaceous alkaloids 0.05 ml of *potassium iodobismuthate solution;* no precipitate or turbidity, is produced.

When extracting with an immiscible solvent: After extracting at least three times with the solvent, add to 1 to 2 ml of the next portion 1 to 2 ml of 0.1 *N hydrochloric acid,* remove the organic solvent by evaporation, transfer the aqueous residue to a test tube, and add 0.05 ml of *potassium mercuri-iodide solution* for solanaceous alkaloids 0.05 ml of *potassium iodobismuthate solution* or for emetine, 0.05 ml of *iodine solution;* not more than a very faint opalescenece is produced.

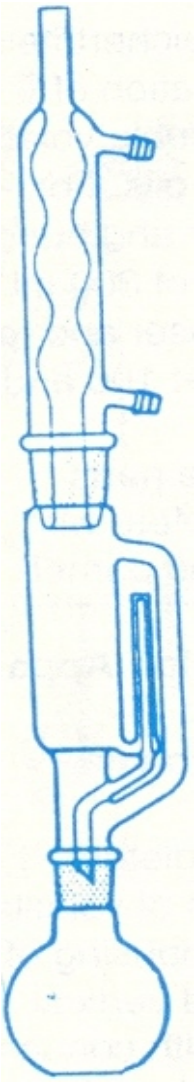

Fig. 2 - Apparatus for the continuous extraction of Drugs (Soxhlet apparatus)

2.2.13. - Thin-Layer Chromatography (TLC):

Thin-layer chromatography is a technique in which a solute undergoes distribution between two phases, stationary phase acting through adsorption and a mobile phase in the form of a liquid. The adsorbent is a relatively thin, uniform layer of dry finely powdered material applied to a glass, plastic or metal sheet or plate. Precoated plates are most commonly used. Separation may also be achieved on the basis of partition or a combination of partition and adsorption, depending on the particular type of support, its preparation and its use with different solvent.

Identification can be effected by observation of spots of identical R_f value and about equal magnitude obtained, respectively, with an unknown and a reference sample chromatographed on the same plate. A visual comparison of the size and intensity of the spots usually serves for semi-quantitative estimation.

Apparatus:

(a) Flat glass plates of appropriate dimensions which allow the application at specified points of the necessary quantities of the solution being examined and appropriate reference solutions and which allow accommodation of the specified migration path-length. The plates are prepared as described below; alternatively, commercially prepared plates may be used.

(b) An aligning tray or a flat surface on which the plates can be aligned and rested when the coating substance is applied.

(c) The adsorbent or coating substance consisting of finely divided adsorbent materials, normally 5 µm to 40 µm in diameter is suitable for chromatography. It can be applied directly to the plate or can be bonded to the plate by means of plaster of paris (Hydrated Calcium Sulphate) or with any other suitable binders. The adsorbent may contain fluorescing material to help in visualising spots that absorb ultra-violet light.

(d) A spreader which, when moved over the glass plate, will apply a uniform layer of adsorbent of desired thickness over the entire surface of the plate.

(e) A storage rack to support the plates during drying and transportation.

(f) A developing chamber that can accommodate one or more plates and can be properly closed and sealed. The chamber is fitted with a plate support rack that supports the plates, back to back, with lid of the chamber in place.

(g) Graduated micro-pipettes capable of delivering microlitre quantities say 10 µl and less.

(h) A reagent sprayer that will emit a fine spray and will not itself be attacked by the reagent.

(i) An ultra-violet light, suitable for observation at short (254 nm) and long (365 nm) ultra-violet wavelengths.

Preparation of plates: Unless otherwise specified in the monograph, the plates are prepared in the following manner. Prepare a suspension of the coating substance in accordance with the instructions of the supplier and, using the spreading device designed for the purpose, spread a uniform layer of the suspension, 0.20 to 0.30 mm thick, on a flat glass plate 20 cm long. Allow the coated plates to dry in air, heat at 100^0 to 105^0 for at least 1 hour (except in the case of plates prepared with cellulose when heating for 10 minutes is normally sufficient) and allow to cool, protected from moisture. Store the plates protected from moisture and use within 3 days of preparation. At the time of use, dry the plates again, if necessary, as prescribed in the monographs. Now a days pre coated plates of silica gel on glass/aluminium/ plastic sheets are also available.

Method:

Unless unsaturated conditions are prescribed, prepare the tank by lining the walls with sheets of filter paper; pour into the tank, saturating the filter paper in the process, sufficient of the mobile phase to form a layer of solvent 5 to 10 mm deep, close the tank and allow to stand for 1 hour at room temperature. Remove a narrow strip of the coating substance, about 5 mm wide, from the vertical sides of the plate. Apply the solutions being examined in the form of circular spots about 2 to 6 mm in diameter, or in the form of bands (10 to 20 mm x 2 to 6 mm unless otherwise specified) on a line parallel with, and 20 mm from, one end of the plate, and not nearer than 20 mm to the sides; the spots should be 15 mm apart. If necessary, the solutions may be applied in portions, drying between applications. Mark the sides of the plate 15 cm, or the distance specified in the monograph, from the starting line. Allow the solvent to evaporate and place the plate in the tank, ensuring that it is as nearly vertical as possible and that the spots or bands are above the level of the mobile phase. Close the tank and allow to stand at room temperature, until the mobile phase has ascended to the marked line. Remove the plate and dry and visualise as directed in the monograph; where a spraying technique is prescribed it is essential that the reagent be evenly applied as a fine spray.

For two-dimensional chromatography dry the plate after the first development and carry out the second development in a direction perpendicular to the first.

When the method prescribed in the monograph specifies 'protected from light' or 'in subdued light' it is intended that the entire procedure is carried out under these conditions.

Visualisation:

The phrases *ultra-violet light (254 nm)* and *ultra-violet light (365 nm)* indicate that the plate should be examined under an ultra-violet light having a maximum output at about 254 or at about 365 nm, as the case may be.

The term *secondary spot* means any spot other than the principal spot. Similarly, a *secondary band* is any band other than the principal band.

Rf Value :

Measure and record the distance of each spot from the point of its application and calculate the R_f value by dividing the distance travelled by the spots by the distance travelled by the front of the mobile phase.

2.2.14. - Starch estimation (Mont Gomery, 1957) [Spectrophotometric method]:

Prepare 10 per cent homogenate of the plant tissue in 80 per cent *ethanol*. Centrifuge at 2000 rpm for 15 minutes. To the residue thus obtained, add 4 ml of *distilled water*, heat on a water bath for 15 minutes and macerate with the help of glass rod. To each of the samples, add 3 ml of *52* per cent *perchloric acid* and centrifuge at 2000 rpm for 15 minutes. The supernatant thus obtained is made upto known volume (generally upto 10 ml or depending on the expected

concentration of starch). Take 0.1 ml aliquot, add 0.1 ml of 80 per cent *phenol* and 5 ml conc. sulphuric acid, cool and then read the absorbance at 490 nm.

2.2.15. - Sugar estimation (Mont Gomery, 1957) [Spectrophotometric method]:

Prepare 10 per cent homogenate of the plant tissue in 80 per cent *ethanol*. Centrifuge at 2000 rpm for 15 minutes. The supernatant obtained is made upto known volume (generally upto 10 ml or depending on the expected concentration of sugar). Take 0.1 ml aliquot, add 0.1 ml of 80 per cent phenol and 5 ml conc. sulphuric acid, cool and then read the absorbance at 490 nm.

2.2.16. - Fatty oil estimation:

To estimate fatty oils, extract accurately weighed air-dried powdered plant material with *petroleum ether* (40-60^0) in a Soxhlet apparatus. Dry the extract over *anhydrous sodium sulphate* and remove the solvent under vacuum at 40^0. Weigh the residue and calculate the percentage with reference to the weight of plant material used.

2.2.17. –Protein estimation (Lowry et. al 1951):

Homogenise 100 mg plant metarial with 3 ml of 10% *trichloroacetic acid*. Centrifuge the homogenate at 10,000 rpm. Discard the supernatant. Treat the pallets obtained after centrifugation with 3 ml *1N sodium hydroxide*, heat on water bath for 7 minutes and cool. Centrifuge the solution again for five to ten minutes at 5000 rpm. To 0.5 ml of supernatant thus obtained after centrifugation, add 5 ml reagent containing 100 parts of 2% solution of sodium carbonate and one part of 2% solution of *sodium potassium* tartrate. Allow it so stand for ten to fifteen minutes. Then add 5 ml *Folin and Ciocalteu's Phenol reagent* (diluted with distilled water in ratio of 1:1) and allow to stand for half-hour for development of colour and then finally measure the absorbance at 700 nm.

2.2.18. - Method for Alkaloid estimation:

Macerate the plant material with 2 per cent acetic acid in water, filter and concentrate the filtrate under reduced pressure at 45^0 to one third of the original volume. Adjust the pH to 2 by *4 M hydrochloric acid*. The yellow precipitate will be separated from the solution (A). Dissolve in it 0.1 M to give solution (B). Add *Mayer's reagent* to the solution A and B to give precipitate of alkaloid-Mayers reagent complex. Dissolve it again in *acetone - methanol - water* (6 : 2 : 10) to give solution. Pass this complex finally through Amberlite IRA 400 anion exchange resin (500 g) to give an aqueous solution of alkaloid chlorides.

2.3. - Limit Tests:

Table 4- Permissible Limits of Heavy Metals

S.No.	Heavy Metal contents	Permissible limits
1.	Lead	10 ppm
2	Arsenic	3 ppm
3.	Cadmium	0.3 ppm
4.	Mercury	1 ppm

2.3.1. - Limit Test for Arsenic

In the limit test for arsenic, the amount of arsenic is expressed as arsenic, As ppm

Apparatus –

A wide-mouthed bottle capable of holding about 120 ml is fitted with a rubber bung through which passes a glass tube. The latter, made from ordinary glass tubing, has a total length of 200 mm and an internal diameter of exactly 6.5 mm (external diameter about 8 mm). It is drawn out at one end to a diameter of about 1 mm and a hole not less than 2 mm in diameter is blown in the side of the tube, near the constricted part. When the bung is inserted in the bottle containing 70 ml of liquid, the constricted end of the tube is above the surface of the liquid, and the hole in the side is below the bottom of the bung. The upper end of the tube is cut off square, and is either slightly rounded or ground smooth.

Two rubber bungs (about 25 mm x 25 mm), each with a hole bored centrally and true, exactly 6.5 mm in diameter, are fitted with a rubber band or spring clip for holding them tightly together. Alternatively the two bungs may be replaced by any suitable contrivance satisfying the conditions described under *the General Test.*

Reagents:

Ammonium oxalate AsT: *Ammonium oxalate* which complies with the following additional test:

Heat 5 g with 15 ml of *water,* 5 ml of *nitric acid AsT,* and 10 ml of *sulphuric acid AsT* in narrow necked, round-bottomed flask until frothing ceases, cool, and apply the General Test; no visible stain is produced.

Arsenic solution, dilute, AsT:

Strong Arsenic solution AsT	1 ml
Water sufficient to produce	100 ml

Dilute arsenic solution, AsT must be freshly prepared.
1 ml contains 0.01 mg of arsenic, as.

Arsenic solution, strong, AsT:

Arsenic trioxide	0.132 g
Hydrochloric acid	50 ml
Water sufficient to produce	100 ml

Brominated hydrochloric acid AsT:

Bromine solution AsT	1 ml
Hydrochloric acid AsT	100 ml

Bromine solution AsT:

Bromine	30 g
Potassium bromide	30 g
Water sufficient to produce	100 ml

It complies with the following test:

Evaporate 10 ml on a water-bath nearly to dryness, add 50 ml of purified water, 10 ml of *hydrochloric acid AsT* and sufficient *stannous chloride solution AsT* to reduce the remaining bromine and apply the General Test; the stain produced is not deeper than 1 ml *standard stain,* showing that the proportion of arsenic present does not exceed 1 part per million.

Citric acid AsT: *Citric acid* which complies with the following additional tests: Dissolve 10 g in 50 ml of water add 10 ml of *stannated hydrochloric acid AsT* and apply the General Test; no visible stain is produced.

Hydrochloric acid AsT: *Hydrochloric acid* diluted with *water* to contain about 32 per cent w/w of *hydrochloride acid* and complying with the following additional tests:

(i) Dilute 10 ml with sufficient water to produce 50 ml, add 5 ml of *ammonium thiocyanate solution* and stir immediately; no colour is produced.

(ii) To 50 ml add 0.2 ml of *bromine solution AsT,* evaporate on a water-bath until reduced to 16 ml adding more *bromine solution AsT,* if necessary, in order that an excess, as indicated by the colour, may be present throughout the evaporation; add 50 ml of *water* and 5 drops of *stannous chloride solution AsT,* and apply the General Test; the stain produced is not deeper than a 0.2 ml *standard stain* prepared with the same acid, showing that the proportion of arsenic present does not exceed 0.05 part per million.

Hydrochloric acid (constant-boiling composition) AsT : Boil hydrochloric acid AsT to constant boiling Composition in the presence of hydrazine hydrate, using 1 ml of 10 per cent w/v solution in water per litre of the acid.

***Mercuric Chloride Paper:** Smooth white filter paper, not less than 25 mm in width, soaked in a saturated solution of *mercuric chloride,* pressed to remove superfluous solution, and dried at about 60^0, in the dark. The grade of the filter paper is such that the weight is between 65 and 120 g per sq. mm; the thickness in mm of 400 papers is approximately equal numerically, to the weight in g per sq. mm.

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*NOTE –*Mercuric chloride paper should be stored in a stoppered bottle in the dark. Paper which has been exposed to sunlight or to the vapour of ammonia affords a lighter stain or no stain at all when employed in the limit test for arsenic.*

Nitric acid AsT: *Nitric acid* which complies with the following additional test:

Heat 20 ml in a porcelain dish with 2 ml of *sulphuric acid AsT,* until white fumes are given off. Cool, add 2 ml of water, and again heat until white fumes are given off; cool, add 50 ml of water and 10 ml of *stannated hydrochloric acid AsT,* and apply the General Test; no visible stain is produced.

Potassium chlorate AsT: *Potassium chlorate* which complies with the following additional test:

Mix 5 g in the cold with 20 ml of *water* and 22 ml of *hydrochloric acid AsT;* when the first reaction has subsided, heat gently to expel chlorine, remove the last traces with a few drops of *stannous chloride solution AsT,* add 20 ml of water, and apply the General Test; no visible stain is produced.

Potassium iodide AsT: *Potassium iodide* which complies with the following additional test:

Dissolve 10 g in 25 ml of *hydrochloric acid AsT* and 35 ml of *water,* add 2 drops of *stannous chloride solution AsT* and apply the General Test; no visible stain is produced.

Potassium iodide AsT: *Potassium iodide* which complies with the following additional test:

Dissolve 10 g in 25 ml of *hydrochloric acid AsT* and 35 ml of *water,* add 2 drops of *stannous chloride solution AsT* and apply the General Test; no visible stain is produced.

Sodium carbonate, anhydrous AsT: *Anhydrous sodium carbonate* which complies with the following additional test:

Dissolve 5 g in 50 ml of *water,* add 20 ml of *brominated hydrochloric acid AsT,* remove the excess of bromine with a few drops of *stannous chloride solution AsT,* and apply the General Test; no visible stain is produced.

Sodium Salicylate: Of the Indian Pharmacopoeia.

Stannated hydrochloric acid AsT:

Stannous chloride solution AsT	1 ml
Hydrochloric Acid AsT	100 ml

Stannous Chloride solution AsT: Prepared from *stannous chloride solution* by adding an equal volume of *hydrochloric acid*, boiling down to the original volume, and filtering through a fine-grain filter paper.

It complies with the following test:

To 10 ml add 6 ml of water and 10 ml of *hydrochloric acid AsT,* distil and collect 16 ml. To the distillate and 50 ml of *water* and 2 drops of *stannuous chloride solution AsT* and apply the General Test; the stain produced is not deeper than a 1-ml *standard stain,* showing that the proportion of arsenic present does not exceed 1 part per million.

Sulphuric acid AsT: *Sulphuric acid* which complies with the following additional test:

Dilute 10 g with 50 ml of water, add 0.2 ml of *stannous chloride solution AsT,* and apply the General Test; no visible stain is produced.

Zinc AsT: *Granulated Zinc* which complies with following additional test:

Add 10 ml of *stannated hydrochloric acid AsT* to 50 ml of *water,* and apply the General Test, using 10 of the zinc and allowing the action to continue for one hour; no visible stain is produced (limit of arsenic). Repeat the test with the addition of 0.1 ml of *dilute arsenic solution AsT;* a faint but distinct yellow stain is produced (test for sensitivity).

General Method of Testing: By a variable method of procedure suitable to the particular needs of each substance, a solution is prepared from the substance being examined which may or may not contain that substance, but contains the whole of the arsenic (if any) originally present in that substance. This solution, referred to as the `test solution', is used in the actual test.

General Test: The glass tube is lightly packed with cotton wool, previously moistened with *lead acetate solution* and dried, so that the upper surface of the cotton wool is not less than 25 mm below the top of the tube. The upper end of the tube is then inserted into the narrow end of one of the pair of rubber bungs, either to a depth of about 10 mm when the tube has a rounded-off end, or so that the ground end of the tube is flush with the larger end of the bung. A piece of *mercuric chloride paper* is placed flat on the top of the bung and the other bung placed over it and secured by means of the rubber band or spring clip in such a manner that the borings of the two bungs (or the upper bung and the glass tube) meet to form a true tube 6.5 mm in diameter interrupted by a diaphragm of *mercuric chloride paper.*

Instead of this method of attaching the *mercuric chloride paper,* any other method may be used provided (1) that the whole of the evolved gas passes through the paper; (2) that the portion of the paper in contact with the gas is a circle 6.5 mm in diameter; and (3) that the paper is protected from sunlight during the test. The test solution prepared as specified, is placed in the wide-mouthed bottle, 1 g of *potassium iodide AsT* and 10 g of *zinc AsT* added, and the prepared glass tube is placed quickly in position. The action is allowed to proceed for 40 minutes. The yellow stain which is produced on the *mercuric chloride paper* if arsenic is present is compared by day light with the *standard stains* produced by operating in a similar manner with known quantities of *dilute arsenic solution AsT*. The comparison of the stains is made immediately at the completion of the test. The standard stains used for comparison are freshly prepared; they fade on keeping.

By matching the depth of colour with *standard stains*, the proportion of arsenic in the substance may be determined. A stain equivalent to the 1-ml standard stain, produced by operating on 10 g of substance indicates that the proportion of arsenic is 1 part per million.

NOTE: (1) The action may be accelerated by placing the apparatus on a warm surface, care being taken that the *mercuric chloride paper* remains dry throughout the test.

(2) The most suitable temperature for carrying out the test is generally about 40^0 but because the rate of the evolution of the gas varies somewhat with different batches

zinc AsT, the temperature may be adjusted to obtain a regular, but not violent, evolution of gas.

(3) The tube must be washed with *hydrochloric acid AsT*, rinsed with water and dried between successive tests.

Standard Stains: Solutions are prepared by adding to 50 ml of water, 10 ml of *stannated hydrochloric acid AsT* and quantities of *dilute arsenic solutions AsT* varying from 0.2 ml to 1 ml. The resulting solutions, when treated as described in the General Test, yield stains on the *mercuric chloride paper* referred to as the standard stains.

Preparation of the Test Solution:

In the various methods of preparing the test solution given below, the quantities are so arranged unless otherwise stated, that when the stain produced from the solution to be examined is not deeper than the 1-ml standard stain, the proportion of arsenic present does not exceed the permitted limit.

Ammonium Chloride: Dissolve 2.5 g in 50 ml of *water,* and 10 ml of *stannated hydrochloric acid AsT.*

Boric acid: Dissolve 10 g with 2 g of *citric acid AsT* in 50 ml water, and add 12 ml of *stannated hydrochloric acid AsT.*

Ferrous Sulphate: Dissolve 5 g in 10 ml of *water and 15 ml of stannated hydrochloric* acid *AsT and* disitil 20 ml; to the distillate add a few drops of *bromine solution AsT.* Add 2 ml of *stannated hydrochloric acid AsT,* heat under a reflux condenser for one hour, cool, and add 10 ml of *water* and 10 ml of *hydrochloric acid AsT.*

Glycerin: Dissolve 5 g in 50 ml of water, and add 10 ml of *stannated hydrochloric acid AsT.*

Hydrochloric acid: Mix 10 g with 40 ml of water and 1 ml of *stannous chloride solution AsT.*

Magnesium Sulphate: Dissolve 5 g in 50 ml of *water* and add 10 ml of stannated *hydrochloric acid AsT.*

Phosphoric acid: Dissolve 5 g in 50 ml of *water* and add 10 ml of *stannated hydrochloric acid AsT*

Potassium iodide: Dissolve 5 g in 50 ml of *water* and add 2 ml of *stannated hydrochloric acid AsT.*

Sodium bicarbonate: Dissolve 5 g in 50 ml of *water* and add 15 ml of *brominated hydrochloric acid AsT,* and remove the excess of bromine with a few drops of *stannous chloride solution AsT.*

Sodium hydroxide: Dissolve 2.5 g in 50 ml of *water,* add 16 ml of *brominated hydrochloric acid AsT,* and remove the excess of *bromine* with a few drops of *stannous chloride solution AsT.*

2.3.2. - Limit Test for Chlorides:

Dissolve the specified quantity of the substance in *water* or prepare a solution as directed in the text and transfer to a *Nessler cylinder.* Add 10 ml of *dilute nitric acid,* except when nitric acid is used in the preparation of the solution, dilute to 50 ml with water, and add 1 ml of *silver nitrate solution.* Stir immediately with a glass rod and allow to stand for 5 minutes. The opalescence produced is not greater than the *standard opalescence,* when viewed transversely.

Standard Opalescence:

Place 1.0 ml of a 0.05845 per cent w/v solution of *sodium chloride* and 10 ml of *dilute nitric acid* in a *Nessler cylinder*. Dilute to 50 ml with water and add 1 ml of *silver nitrate solution*. Stir immediately with a glass rod and allow to stand for five minutes.

2.3.3. - Limit Test for Heavy metals:

The test for heavy metals is designed to determine the content of metallic impurities that are coloured by sulphide ion, under specified conditions. The limit for heavy metals is indicated in the individual monographs in terms of the parts of lead per million parts of the substance (by weight), as determined by visual comparison of the colour produced by the substance with that of a control prepared from a standard lead solution.

Determine the amount of heavy metals by one of the following methods and as directed in the individual monographs. Method A is used for substances that yield clear colourless solutions under the specified test conditions. Method B is used for substances that do not yield clear, colourless solutions under the test conditions specified for method A, or for substances which, by virtue of their complex nature, interfere with the precipitation of metals by sulphide ion. Method C is used for substances that yield clear, colourless solutions with *sodium hydroxide solution.*

Special Reagents:

Acetic acid Sp.: *Acetic acid* which complies with the following additional test : Make 25 ml alkaline with *dilute ammonia solution Sp.,* add 1 ml of *potassium cyanide solution Sp.,* dilute to 50 ml with *water* and add two drops of *sodium sulphide* solution; no darkening is produced.

Dilute acetic acid Sp.: *Dilute acetic acid,* which complies with the following additional test – Evaporate 20 ml in a porcelain dish, nearly to dryness on a water-bath. Add to the residue 2 ml of the acid and dilute with water to 25 ml, add 10 ml of *hydrogen sulphide solution.* Any dark colour produced is not more than that of a control solution consisting of 2 ml of the acid and 4.0 ml of *standard lead solution* diluted to 25 ml with *water.*

Ammonia solution Sp.: *Strong ammonia solution* which complies with the following additional test : Evaporate 10 ml to dryness on a water-bath; to the residue add 1 ml of *dilute hydrochloric acid Sp. a*nd evaporate to dryness. Dissolve the residue in 2 ml of dilute acetic acid Sp. Add sufficient water to produce 25 ml.

Add 10 ml of *hydrogen sulphide solution.* Any darkening produced is not greater than in a blank solution containing 2 ml of dilute acetic acid Sp. 1.0 ml of *standard lead solution* and sufficient *water* to produce 25 ml.

Dilute ammonia solution Sp.: *Dilute ammonia solution* which complies with the following additional test: To 20 ml add 1 ml of *potassium cyanide solution Sp.,* dilute to 50 ml with *water,* and add two drops of *sodium sulphide solution;* no darkening is produced.

Hydrochloric acid: *Hydrochloric acid* which complies with the following additional test: Evaporate off the acid in a beaker to dryness on a water-bath. Dissolve the residue in 2 ml of *dilute acid Sp.,* dilute to 17 ml with water and add 10 ml of *hydrogen sulphide solution;* any darkening produced is not greater than in a blank solution containing 2.0 ml of *standard lead solution,* 2 ml of *dilute acetic acid Sp.* and dilute to 40 ml with water.

Dilute hydrochloric acid Sp.: *Dilute hydrochloric acid,* which complies with the following additional test: Treat 10 ml of the acid in the manner described under *Hydrochloric acid Sp.*

Lead nitrate stock solution: Dissolve 0.1598 g of *lead nitrate* in 100 ml of *water* to which has been added 1 ml of *nitric acid,* then dilute with *water* to 1000 ml. This solution must be prepared and stored in polyethylene or glass containers free from soluble lead salts.

Standard lead solution: On the day of use, dilute 10.0 ml of *lead nitrate* stock solution with *water* to 100.0 ml. Each ml of *standard lead solution* contains the equivalent of 10 µg of lead. A control comparison solution prepared with 2.0 ml of standard lead solution contains, when compared to a solution representing 1.0 g of the substance being tested, the equivalent of 20 parts per million of lead.

Nitric acid Sp.: *Nitric acid* which complies with the following additional test: Dilute 10 ml with 10 ml of *water,* make alkaline with *ammonia solution Sp.,* add 1 ml of *potassium cyanide solution Sp.,* dilute to 50 ml with water, and add two drops of *sodium sulphide solution;* no darkening is produced.

Potassium cyanide solution Sp.: See Appendix 2.3.5.

Sulphuric acid Sp.: Sulphuric acid which complies with following additional test: Add 5 g to 20 ml of *water* make alkaline with *ammonia solution Sp.,* add 1 ml of *potassium cyanide solution Sp.*, dilute to 50 ml with *water* and add two drops of *sodium sulphide solution;* no darkening is produced.

Method A

Standard solution: Into a 50 ml *Nessler cylinder,* pipette 2 ml of *standard lead solution* and dilute with *water* to 25 ml. Adjust with *dilute acetic acid Sp.* or *dilute ammonia solution Sp* to a pH between 3.0 and 4.0, dilute with *water* to about 35 ml, and mix.

Test solution: In to a 50 ml *Nessler cylinder,* place 25 ml of the solution prepared for the test as directed in the individual monograph, or using the stated volume of acid when specified in the individual monograph, dissolve and dilute with *water* to 25 ml the specified quantity of the substance being tested. Adjust with *dilute acetic acid Sp.* or *dilute ammonia solution Sp.* to a pH between 3.0 and 4.0, dilute with *water* to about 35 ml and mix.

Procedure: To each of the cylinders containing the *standard solution* and test solution, respectively, add 10 ml of freshly prepared *hydrogen sulphide solution,* mix, dilute with *water* to 50 ml, allow to stand for five minutes, and view downwards over a white surface; the colour produced in the *test solution* is not darker than that produced in the *standard solution.*

Method B

Standard solution: Proceed as directed under Method A.

Test solution: Weigh in a suitable crucible the quantity of the substance specified in individual monograph, add sufficient *sulphuric acid Sp.* to wet the sample, and ignite carefully at a low temperature until thoroughly charred. Add to the charred mass 2 ml of *nitric acid Sp.* and five drops of *sulphuric acid Sp.* and heat cautiously until white fumes are no longer evolved. Ignite, preferably in a muffle furnace, at 500^0 to 600^0 until the carbon is completely burnt off. Cool, add 4 ml of *hydrochloric acid Sp.,* cover, digest on a water bath for 15 minutes, uncover and slowly evaporate to dryness on a water-bath. Moisten the residue with one drop of *hydrochloric acid Sp.,* add 10 ml of hot water and digest for two minutes. Add *ammonia solution* sp*.,* dropwise, until the solution is just alkaline to *litmus paper,* dilute with *water* to 25 ml and adjust with dilute acetic acid Sp. to a pH between 3.0 and 4.0. Filter if necessary, rinse the crucible and the filter with 10 ml of water, combine the filtrate and washings in a 50 ml *Nessler cylinder,* dilute with *water,* to about 35 ml, and mix. Procedure: Proceed as directed under Method A.

Method C

Standard solution: Into a 50 ml *Nessler cylinder,* pipette 2 ml of *standard lead solution,* add 5 ml of *dilute sodium hydroxide solution.,* dilute with *water* to 50 ml and mix.

Test solution: Into a 50 ml *Nessler cylinder,* place 25 ml of the solution prepared for the test as directed in the individual monograph; or, if not specified otherwise in the individual monograph, dissolve the specified quantity in a mixture of 20 ml of *water* and 5 ml of *dilute sodium hydroxide solution.* Dilute 50 ml with water and mix.

Procedure: To each of the cylinders containing the *standard solution* and the *test solution,* respectively add 5 drops of *sodium sulphide solution,* mix, allow to stand for five minutes and

view downwards over a white surface; the colour produced in the *test solution* is not darker than that produced in the *standard solution.*

2.3.4. - Limit Test for Iron

Standard Iron solution: Weigh accurately 0.1726 g of *ferric ammonium sulphate* and dissolve in 10 ml of 0.1 *N sulphuric acid* and sufficient *water* to produce 1000.0 ml. Each ml of this solution contains 0.02 mg of Fe.

Method:

Dissolve the specified quantity of the substance being examined in 40 ml of *water,* or use 10 ml of the solution prescribed in the monograph, and transfer to a *Nessler cylinder.* Add 2 ml of a 20 per cent w/v solution of *iron-free citric acid* and 0.1 ml of *thioglycollic acid,* mix, make alkaline with *iron-free ammonia solution,* dilute to 50 ml with *water* and allow to stand for five minutes. Any colour produced is not more intense than the standard colour.

Standard colour: Dilute 2.0 ml of *standard iron solution* with 40 ml of *water* in a *Nessler cylinder.* Add 2 ml of a 20 per cent w/v solution of *iron-free citric acid* and 0.1 ml of *thioglycollic acid,* mix, make alkaline with *iron-free ammonia solution,* dilute to 50 ml with *water* and allow to stand for five minutes.

2.3.5. - Limit Test for Lead

The following method is based on the extraction of lead by solutions of *dithizone*. All reagents used for the test should have as low a content of lead as practicable. All reagent solutions should be stored in containers of borosilicate glass. Glassware should be rinsed thoroughly with warm *dilute nitric acid,* followed by *water.*

Special Reagents:

(1) **Ammonia-cyanide solution Sp.:** Dissolve 2 g of *potassium cyanide* in 15 ml of *strong ammonia solution* and dilute with *water* to 100 ml.

(2) **Ammonium citrate solution Sp.:** Dissolve 40 g of *citric acid* in 90 ml *water.* Add two drops of phenol *red solution* then add slowly *strong ammonia solution* until the solution acquires a reddish colour. Remove any lead present by extracting the solution with 20 ml quantities of *dithizone* extraction solution until the *dithizone* solution retains its orange-green colour.

(3) **Dilute standard lead solution:** Dilute 10.0 ml of *standard lead solution* with sufficient 1 per cent v/v solution of nitric acid to produce 100 ml. Each ml of this solution contains 1 µg of lead per ml.

(4) **Dithizone extraction solution:** Dissolve 30 mg of *diphenylthiocarbazone* in 1000 ml of *chloroform* and add 5 ml of *alcohol.* Store the solution in a refrigerator. Before use, shake a suitable volume of the solution with about half its volume of 1 per cent v/v solution of *nitric acid* and discard the acid.

(5) **Hydroxylamine hydrochloride solution Sp.:** Dissolve 20 g of *hydroxylamine hydrochloride* in sufficient *water* to produce about 65 ml. Transfer to separator, add five drops of *thymol blue solution*, add *strong ammonia solution* until the solution becomes yellow. Add 10 ml of a 4 per cent w/v solution of *sodium diethyldithiocarbamate* and allow to stand for five minutes. Extract with successive quantities, each of 10 ml, of *chloroform* until a 5 ml portion of the extract does not assume a yellow colour when shaken with dilute copper sulphate solution. Add *dilute hydrochloric acid* until the solution is pink and then dilute with sufficient water to produce 100 ml.

(6) **Potassium cyanide solution Sp.:** Dissolve 50 g of *potassium cyanide* in sufficient *water* to produce 100 ml. Remove the lead from this solution by extraction with successive quantities, each of 20 ml of *dithizone extraction solution* until the dithizone solution retains its orange-green colour. Extract any dithizone remaining in the cyanide solution by shaking with *chloroform.* Dilute this cyanide solution with sufficient *water* to produce a solution containing 10 g of *potassium cyanide* in each 100 ml.

(7) **Standard dithizone solution:** Dissolve 10 ml of *diphenylthiocarbazone* in 1000 ml of *chloroform.* Store the solution in a glass-stoppered, lead-free bottle, protected from light and in a refrigerator.

(8) **Citrate-cyanide wash solution:** To 50 ml of *water* add 50 ml of *ammonium citrate solution Sp.* and 4 ml of *potassium cyanide solution Sp.,* mix, and adjust the pH, if necessary, with strong *ammonia solution* to 9.0.

(9) **Buffer solution *p*H 2.5:** To 25.0 ml of 0.2 *M potassium hydrogen phthalate add* 37.0 ml of 0.1 N *hydrochloric acid,* and dilute with sufficient *water* to produce 100.0 ml.

(10) **Dithizone-carbon tetrachloride solution:–** Dissolve 10 mg of *diphenylthiocarbazone* in 1000 ml of carbon tetrachloride. Prepare this solution fresh for each determination.

(11) *p*H 2.5 wash solution: To 500 ml of a 1 per cent v/v *nitric acid* add *strong ammonia solution* until the pH of the mixture is 2.5, then add 10 ml of *buffer solution* pH 2.5 and mix.

(12) Ammonia-cyanide wash solution: To 35 ml of pH 2.5 *wash solution* add 4 ml of *ammonia-cyanide solution Sp.,* and mix.

Method

Transfer the volume of the prepared sample directed in the monograph to a separator and unless otherwise directed in monograph, add 6 ml of *ammonium citrate solution Sp.,* and 2 ml *hydroxylamine hydrochloride solution Sp.,* (For the determination of lead in iron salts use 10 ml of *ammonium citrate solution Sp.).* Add two drops of *phenol red solution* and make the solution just alkaline (red in colour) by the addition *of strong ammonnia solution.* Cool the solution if necessary, and add 2 ml of *potassium cyanide solution* Sp. Immediately extract the solution with several quantities each of 5 ml, of *dithizone extraction solution,* draining off each extract into another separating funnel, until the dithizone extraction solution retains its green colour. Shake the combine dithizone solutions for 30 seconds with 30 ml of a 1 per cent w/v solution of *nitric acid* and discard the chloroform layer. Add to the solution exactly 5 ml of

standard dithizone solution and 4 ml of *ammonia-cyanide solution* Sp. and shake for 30 seconds; the colour of the chloroform layer is of no deeper shade of violet than that of a control made with a volume of *dilute standard lead solution* equivalent to the amount of lead permitted in the sample under examination.

2.3.6. - Limit Test for Sulphates:

Reagents

Barium Sulphate reagent: Mix 15 ml of 0.5 *M barium chloride,* 55 ml of *water,* and 20 ml of *sulphate free alcohol,* add 5 ml of a 0.0181 per cent w/v solution of potassium sulphate, dilute to 100 ml with *water,* and mix. Barium sulphate reagent must be freshly prepared.

0.5 M Barium Chloride: *Barium chloride* dissolved in *water* to contain in 1000 ml 122.1 g of $BaCl_2$, $2H_2O$.

Method

Dissolve the specified quantity of the substance in *water,* or prepare a solution as directed in the text, transfer to a *Nessler cylinder,* and add 2 ml of *dilute hydrochloric acid,* except where *hydrochloric acid* is used in the preparation of the solution. Dilute to 45 ml with *water*, add 5 ml of barium sulphate reagent. Stir immediately with a glass rod, and allow to stand for five minutes. The turbidity produced is not greater than the *standard turbidity*, when viewed transversely. Standard turbidity : Place 1.0 ml of 0.1089 per cent w/v solution of potassium sulphate and 2 ml of *dilute hydrochloric acid* in a *Nessler cylinder*,dilute to 45 ml with *water*, add 5 ml of barium sulphate reagent, stir immediately with a glass rod and allow to stand for five minutes.

2.3.7. - Heavy Metals by Atomic absorption spectrophotometry:

Atomic absorption spectrophotometry is used in the determination of heavy metal elements and some nonmetal elements in the atomic state.

The light of characteristic wave length emitted from a cathodic discharge lamp is absorbed when it passes through the atomic vapor generated from sample containing the element being examined atomized to the ground state. The assay of the element being examined is tested by determining the decreased degree of light intensity of radiation. Atomic absorption obeys the general rule for absorption spectrophotometry. The assay is carried out by comparing the abosorbance of the test preparation with that of the reference preparation.

Apparatus

An atomic absorption spectrophotometer consists of a light source, an atomic generator, a monochromator and a detector system. Some are equipped with a background compensation system and automatic sampling system, etc.

1.Light Source: A hollow-cathode discharge lamp is usually used. The cathode is made of the element being examined.

2.Atomic Generator: There are four main types : flame atomizer, graphite furnace atomizer, hydride-generated atomizer, cold vapor atomizer.

(1) **Flame atomizer:** It mainly consists of a nebulizer and a burner. Its function is to nebulize the test solution into aerosol, which is mixed with combustion gas. And the mixture is introduced into the flame generated by the burner. So that the substance being examined is to be dried, evaporated to form the ground state atoms of the element being examined. The burning flame is generated by different mixtures of gases, acetylene-air is mostly used. By modifying the proportion of combustion gas, the temperature of the flame can be controlled and a better stability and a better sensitivity can be obtained.

(2) **Furnace atomizer:** It consists of electric furnace and a power supply. Its function is to dry and incinerate the substance being examined. During the stage of high temperature atomization, the ground state atoms of the element being examined are to be formed. Graphite is commonly used as the heater. Protection gas is introduced into the furnace to avoid oxidation and used to transfer the sample vapor.

(3) **Hydride-generated atomizer:** It consists of hydride generator and atomic absorption cell. It is used for the determination of the elements such as arsenic, selenium and antimony etc. Its function is to reduce the element to be examined in acidic medium to the low-boiling and easily pyrolyzed hydride. The hydride is then swept by a stream of carrier gas into the atomic absorption cell which consists of quartz tube and heater etc., in which the hydride is pyrolyzed by heating to form the ground-state atom.

(4) **Cold vapor atomizer:** It consists of a mercury vapor atomizer and an absorption cell. It is suitable for the determination of mercury. Its function is to reduce the mercuric ion into mercury vapor which is swept into the quartz absorption cell by carrier gas.

3. Monochromator: Its function is to separate the specified wavelength radiation from the electromagnetic radiations erradiated from the light source. The optical path of the apparatus should assure the good spectra resolution and has the ability to work well at the condition of narrow spectral band (0.2 nm). The commonly used wavelength region is 190.0 - 900.0 nm.

4. Detector system: It consists of a detector, a signal processor and a recording system. It should have relatively higher sensitivity and better stability and can follow the rapid change of the signal absorption.

5. Background compensation system: System employed for the correction of atmospheric effects on the measuring system. Four principles can be utilized for background compensation: continuous spectrum sources (a deuterium lamp is often used in the UV region), the Zeeman effect, the self inversion phenomenon and the non resonance spectrum. In the analysis using atomic absorption spectrophotometry, the interference to the determination caused by background and other reasons should be noticed. Changes of some experimental conditions, such as the wavelength, the slit width, the atomizing condition, etc., may affect the sensitivity, the stability and the interference. If it is flame, the suitable wavelength, slit width and flame temperature, the addition of complexing agents and releasing agents and the use of Standard addition method may eliminate interference. If it is furnace, system, the selection of suitable background compensation system and the addition of suitable matrix modifying agents, etc may

remove the interference. Background compensation method shall be selected as specified in the individual monograph.

Procedure

Method (direct calibration method)

Prepare not less than 3 reference solutions of the element being examined of different concentrations, covering the range recommended by the instrument manufacturer and add separately the corresponding reagents as that for the test solution and prepare the blank reference solution with the corresponding reagents. Measure the absorbances of the blank reference solution and each reference solution of different concentrations separately, record the readings and prepare a calibration curve with the average value of 3 readings of each concentration on the ordinate and the corresponding concentration on the abscissa.

Prepare a test solution of the substance being examined as specified in the monograph, adjust the concentration to fall within the concentration range of the reference solution. Measure the absorbance 3 times, record the readings and calculate the average value. Interpolate the mean value of the readings on the calibration curve to determine the concentration of the element.

When used in the test for impurities, prepare two test preparations of the same concentration as specified in the monograph. To one of the test preparation add an amount of the reference substance equivalent to the limit of the element specified in the monograph. Proceed as directed above and measure this solution to give an appropriate reading a; then measure the test preparation without the addition of the reference substance under the same condition and record the reading b; b is not greater than (a-b).

Determination of Lead, Cadmium, Arsenic, Mercury and Copper:

(1) Determination of lead (graphite oven method):

Determination conditions Reference condition: dry temperature: 100-120^0 , maintain 20 seconds; ash temperature: 400-750^0, maintain 20-25 seconds; atomic temperature: 1700-2100^0 , maintain 4-5 seconds; measurement wavelength: 283.3 nm; background calibration: deuterium lamp (D lamp) or Zeeman effect.

Preparation of lead standard stock solution: Measure accurately a quantity of lead single-element standard solution to prepare standard stock solution with 2 per cent nitric acid solution, which containing 1 μg per ml, stored at 0-5^0.

Preparation of calibration curve: Measure accurately a quantity of lead standard stock solutions respectively, diluted with 2 per cent nitric acid solution to the concentration of 0, 5, 20, 40, 60, 80 ng per ml, respectively. Measure respectively accurately 1 ml the above solution, add respectively 1 ml of 1 per cent ammonium dihydrogen phosphate and 0.2 per cent *magnesium nitrate* mix well, pipette accurately 20 μl to inject into the atomic generator of graphite oven and determine their absorbance, then draw the calibration curve with absorbance as vertical axis and concentration as horizontal ordinate.

Preparation of test solution:
Method: Weigh accurately 0.5 g of the coarse powder of the substance being examined, transfer into a casparian flask, add 5-10 ml of the mixture of *nitric acid* and *perchloric acid* (4 : 1), add a small hopper on the flask-top, macerate overnight, heat to slake on the electric hot plate, keep somewhat-boiling, if brownish-black, add again a quantity of the above mixture, continuously heat till the solution becomes clean and transparent, then raise temperature, heat continuously to thick smoke, till white smoke disperse, the slaked solution becomes colourless and transparent or a little yellow, cool, transfer it into a 50 ml volumetric flask, wash the container with 2 per cent *nitric acid solution* add the washing solution into the same volumetric flask and dilute with the same solvent to the volume, shake well. Prepare synchronously the reagent blank solution according to the above procedure.

Determination: Measure accurately 1 ml of the test solution and its corresponding reagent blank solution respectively, add 1 ml of solution containing 1per cent *ammonium dihydrogen phosphate* and 0.2 per cent *magnesium nitrate*, shake well, pipette accurately 10-20 μl to determine their absorbance according to the above method of "Preparation of calibration curve". Calculate the content of lead (Pd) in the test solution from the calibration curve.

(2) Determination of cadmium (Cd) (graphite oven method):

Determination conditions Reference condition: dry temperature: 100-120^0 , maintain 20 seconds; ash temperature: 300-500^0 , maintain 20-25 seconds; atomic temperature: 1500-1900^0, maintain 4-5 seconds; measurement wavelength: 228.8 nm; background calibration: deuterium lamp (D lamp) or Zeeman effect.

Preparation of Cd standard stock solution: Measure accurately a quantity of Cd single-element standard solution to prepare standard stock solution Cd with 2 per cent nitric acid, which containing 0.4 μg per ml Cd, stored at 0-5^0.

Preparation of calibration curve: Measure accurately a quantity of cadmium standard stock solutions, diluted to the concentration of 1.6, 3.2, 4.8, 6.4 and 8.0 ng per ml with 2 per cent nitric acid, respectively. Pipette accurately 10 μl the above solutions respectively, inject them into the graphite oven, determine their absorbance, and then draw the calibration curve with absorbance as vertical axis and concentration as horizontal ordinate.

Preparation of test solution: Reference to "Preparation of test solution" of Pb in the above.

Determination: Pipette accurately 10-20 μl of the test solution and its corresponding reagent blank solution respectively, determine their absorbance according to the above method of "Preparation of calibration curve. If interference occurs, weigh accurately respectively 1 ml of the standard solution, blank solution and test solution, add 1 ml of a solution containing 1per cent *ammonium dihydrogen phosphate* and 0.2 per cent *magnesium nitrate*, shake well, determine their absorbance according to the method above, calculate the content of Cd in the test solution from the calibration curve.

(3) Determination of Arsenic (As) (hydride method):

Determination conditions Apparatus: suitable hydride generator device, reducing agent: a solution containing 1 per cent *sodium borohydride* and 0.3 per cent *sodium hydroxide*; carrier liquid: 1 per cent *hydrochloric acid*; carrier gas: nitrogen; measurement wavelength: 193.7 nm; background calibration: deuterium lamp (D lamp) or Zeeman effect.

Preparation of As standard stock solution: Measure accurately a quantity of As single-element standard solution to prepare standard stock solution with 2 per cent *nitric acid* solution, which containis 1.0 μg per ml As, stored at $0\text{-}5^0$.

Preparation of calibration curve: Measure accurately proper quantity of arsenic standard stock solutions, diluted with 2 per cent *nitric acid* to the concentration of 2, 4, 8, 12 and 16 ng per ml respectively. Accurately transfer 10 ml of each into 25 ml volumetric flask respectively, add 1 ml of 25 per cent *potassium iodide solution* (prepared prior to use), shake well, add 1 ml of *ascorbic acid solution* (prepared prior to use), shake well, dilute with hydrochloric acid solution (20-100) to the volume, shake well, close the stopper and immerse the flask in a water bath at 80^0 for 3 minutes. Cool, transfer proper quantities of each solution respectively into the hydride generator device, determine the absorbance, then plot the calibration curve with peak area (absorbance) as vertical axis and concentration as horizontal ordinate.

Preparation of test solution: Reference to A or B method of "Preparation of test solution" of Pb in the above.

Determination: Pipette accurately 10 ml of the test solution and its corresponding reagent blank solution respectively, proceed as described under "Preparation of calibration curve" beginning at the words "add 1 ml of 25 per cent *potassium iodide solution*". Calculate the content of As in the test solution from the calibration curve.

(4) Determination of Mercury (Hg) (cold absorption method):

Determination conditions: Apparatus: suitable hydride generator device; reducing agent: a solution containing 0.5 per cent *sodium borohydride* and 0.1 per cent *sodium hydroxide*; carrier liquid: 1 per cent *hydrochloric acid*; carrier gas: nitrogen; measurement wavelength: 253.6 nm; background calibration: deuterium lamp (D lamp) or Zeeman effect.

Preparation of mercury standard stock solution: Measure accurately a proper quantity of mercury single-element standard solution to prepare standard stock solution with 2 per cent nitric acid solution, which containing 1.0 μg per ml Hg, stored at $0\text{-}5^0$.

Preparation of calibration curve: Measure accurately 0, 0.1, 0.3, 0.5, 0.7 and 0.9 ml of mercury standard stock solution, transfer into a 50 ml volumetric flask respectively, add 40 ml 4 per cent *sulphuric acid solution* and 0.5 ml of 5 per cent *potassium permanganate solution*, shake well, drop 5 per cent *hydroxylamine hydrochloride solution* until the violet red just disappears, dilute with 4 per cent *sulfuric acid solution* to the volume, shake well. A quantity of each solution is injected to the hydride generator device, determine the absorbance, then plot the calibration curve with peak area (absorbance) as vertical axis and concentration as horizontal ordinate.

Preparation of test solution:

Method : Transfer 1 g of the coarse powder of the substance being examined, accurately weighed, into a casparian flask, add 5-10 ml of the mixture solution of *nitric acid* and *perchloric acid* (4 : 1), mix well, fix a small hopper on the flask-top, immerse overnight, heat to slake on the electric hot plate at 120-140^0 for 4-8 hours until *slaking* completely, cool, add a quantity of 4 per cent *sulfuric acid solution* and 0.5 ml of 5 per cent *potassium permanganate solution*, shake well, drop 5 per cent *hydroxylamine hydrochloride solution* until the violet red colour just disappears, dilute with 4 per cent *sulphuric acid solution*s to 25 ml, shake well, centrifugate if necessary, the supernatant is used as the test solution. Prepare synchronally the reagent blank solute based on the same procedure.

Determination: Pipette accurately a quantity of the test solution and its corresponding reagent blank solution, respectively, proceed as described under "Preparation of calibration curve" beginning at the words "add 1 ml of 25 per cent *potassium iodide solution*". Calculate the content of mercury (Hg) in the test solution from the calibration curve.

(5) Determination of Copper (flame method):

Determination conditions: Measurement wavelength: 324.7 nm; flame: air -acetylene flame; background calibration: deuterium lamp or Zeeman effect.

Preparation of copper standard stock solution: Measure accurately a proper quantity of copper single-element standard solution, to prepare the standard stock solution with 2 per cent *nitric acid solution*, which containing 10 μg per ml Cu, stored at 0-5^0.

Preparation of calibration curve: Measure accurately a quantity of copper standard stock solutions, dilute with 2 per cent *nitric acid* to the concentrations of 0.05, 0.2, 0.4, 0.6 and 0.8 μg per ml, respectively. Inject each standard solution into the flame and determine the absorbance, respective, then plot the calibration curve with absorbance as vertical axis and concentration as horizontal ordinate.

Preparation of test solution: Reference to "Preparation of test solution" of Pb in the above.

Determination: Pipette accurately quantities of the test solution and its corresponding reagent blank solution respectively, proceed as described under "Preparation of calibration curve". Calculate the content of Cu in the test solution from the calibration curve.

2.4. - Microbial Limit Tests:

The following tests are designed for the estimation of the number of viable aerobic micro-organisms present and for detecting the presence of designated microbial species in pharmaceutical substances. The term 'growth' is used to designate the presence and presumed proliferation of viable micro-organisms.

Preliminary Testing

The methods given herein are invalid unless it is demonstrated that the test specimens to which they are applied do not, of themselves, inhibit the multiplication under the test conditions of micro-organisms that can be present. Therefore, prior to doing the tests, inoculate diluted specimens of the substance being examined with separate viable cultures of *Escherichia coli*, *Salmonella* species, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. This is done by adding 1 ml of not less than 10^{-3} dilutions of a 24 h broth culture of the micro-organisms to the first dilution (in buffer solution pH 7.2, fluid soyabean-casein digest medium or fluid lactose medium) of the test material and following the test procedure. If the organisms fail to grow in the relevant medium the procedure should be modified by (a) increasing the volume of diluent with the quantity of test material remaining the same, or (b) incorporating a sufficient quantity of a suitable inactivating agent in the diluents, or (c) combining the aforementioned modifications so as to permit growth of the organisms in the media. If inhibitory substances are present in the sample, 0.5 per cent of soya lecithin and 4 per cent of polysorbate 20 may be added to the culture medium. Alternatively, repeat the test as described in the previous paragraph, using fluid casein digest-soya lecithin-polysorbate 20 medium to demonstrate neutralization of preservatives or other antimicrobial agents in the test material. Where inhibitory substances are contained in the product and the latter is soluble, the Membrane filtration method described under Total Aerobic Microbial Count may be used.

If in spite of incorporation of suitable inactivating agents and a substantial increase in the volume of diluent it is still not possible to recover the viable cultures described above and where the article is not suitable for applying the membrane filtration method it can be assumed that the failure to isolate the inoculated organism may be due to the bactericidal activity of the product. This may indicate that the article is not likely to be contaminated with the given species of micro-organisms. However, monitoring should be continued to establish the spectrum of inhibition and bactericidal activity of the article.

Media

Culture media may be prepared as given below or dehydrated culture media may be used provided that, when reconstituted as directed by the manufacturer, they have similar ingredients and / or yield media comparable to those obtained from the formulae given below.

Where agar is specified in a formula, use agar that has a moisture content of not more than 15 per cent. Where water is called for in a formula, use purified water. Unless otherwise indicated, the media should be sterilized by heating in an autoclave at 115^0 for 30 minutes.

In preparing media by the formulas given below, dissolve the soluble solids in the water, using heat if necessary, to effect complete solution and add solutions of hydrochloric acid or sodium hydroxide in quantities sufficient to yield the required *p*H in the medium when it is ready for use. Determine the *p*H at $25^0 \pm 2^0$.

Baird-Parker Agar Medium

Pancreatic digest of casein	10.0	g
Beef extract	5.0	g

Yeast extract	1.0	g
Lithium chloride	5.0	g
Agar	20.0	g
Glycine	12.0	g
Sodium pyruvate	10.0	g
Water to	1000	ml

Heat with frequent agitation and boil for 1 minute. Sterilise, cool to between 45^0 and 50^0, and add 10 ml of a one per cent w/v solution of sterile *potassium tellurite* and 50 ml of egg-yolk emulsion. Mix intimately but gently and pour into plates. (Prepare the egg-yolk emulsion by disinfecting the surface of whole shell eggs, aseptically cracking the eggs, and separating out intact yolks into a sterile graduated cylinder. Add sterile saline solution, get a 3 to 7 ratio of egg-yolk to saline. Add to a sterile blender cup, and mix at high speed for 5 seconds). Adjust the *p*H after sterilization to 6.8 ± 0.2.

Bismuth Sulphite Agar Medium

Solution (1)

Beef extract	6	g
Peptone	10	g
Agar	24	g
Ferric citrate	0.4	g
Brilliant green	10	mg
Water to	1000	ml

Dissolve with the aid of heat and sterilise by maintaining at 115º for 30 minutes.

Solution (2)

Ammonium bismuth citrate	3	g
Sodium sulphite	10	g
Anhydrous disodium hydrogen Phosphate	5	g
Dextrose monohydrate	5	g
Water to	100	ml

Mix, heat to boiling, cool to room temperature, add 1 volume of solution (2) to 10 volumes of solution (1) previously melted and cooled to a temperature of 55^0 and pour.

Bismuth Sulphite Agar Medium should be stored at 2^0 to 8^0 for 5 days before use.

Brilliant Green Agar Medium

Peptone	10.0	g
Yeast extract	3.0	g
Lactose	10.0	g
Sucrose	10.0	g

Sodium chloride	5.0	g
Phenol red	80.0	g
Brilliant green	12.5	mg
Agar	12.0	g
Water to	1000	ml

Mix, allow to stand for 15 minutes, sterilise by maintaining at 115^0 for 30 minutes and mix before pouring.

Buffered Sodium Chloride-Peptone Solution pH 7.0

Potassium dihydrogen phosphate	3.56	g
Disodium hydrogen phosphate	7.23	g
Sodium chloride	4.30	g
Peptone (meat or casein)	1.0	g
Water to	1000	ml

0.1 to 1.0 per cent w/v polysorbate 20 or polysorbate 80 may be added. Sterilise by heating in an autoclave at 121^0 for 15 minutes.

Casein Soyabean Digest Agar Medium

Pancreatic digest of casein	15.0	g
Papaic digest of soyabean meal	5.0	g
Sodium chloride	5.0	g
Agar	15.0	g
Water to	1000	ml

Adjust the pH after sterilization to 7.3±0.2.

Cetrimide Agar Medium

Pancreatic digest of gelatin	20.0	g
Magnesium chloride	1.4	g
Potassium sulphate	10.0	g
Cetrimide	0.3	g
Agar	13.6	g
Glycerin	10.0	g
Water to	1000	ml

Heat to boiling for 1 minute with shaking. Adjust the *p*H so that after sterilization it is 7.0 to 7.4. Sterilise at 121^0 for 15 minutes.

Desoxycholate-Citrate Agar Medium

Beef extract	5.0	g
Peptone	5.0	g
Lactose	10.0	g
Trisodium citrate	8.5	g

Sodium thiosulphate	5.4	g
Ferric citrate	1.0	g
Sodium desoxycholate	5.0	g
Neutral red	0.02	g
Agar	12.0	g
Water to	1000	ml

Mix and allow to stand for 15 minutes. With continuous stirring, bring gently to the boil and maintain at boiling point until solution is complete. Cool to 80^0, mix, pour and cool rapidly.

Care should be taken not to overheat Desoxycholate Citrate Agar during preparation. It should not be remelted and the surface of the plates should be dried before use.

Fluid Casein Digest-Soya Lecithin-Polysorbate 20 Medium

Pancreatic digest of casein	20	g
Soya lecithin	5	g
Polysorbate 20	40	ml
Water to	1000	ml

Dissolve the pancreatic digest of casein and soya lecithin in water, heating in a water-bath at 48^0 to 50^0 for about 30 minutes to effect solution. Add polysorbate 20, mix and dispense as desired.

Fluid Lactose Medium

Beef extract	3.0	g
Pancreatic digest of gelatin	5.0	g
Lactose	5.0	g
Water to	1000	ml

Cool as quickly as possible after sterilization. Adjust the pH after sterilization to 6.9 ± 0.2.

Lactose Broth Medium

Beef extract	3.0	g
Pancreatic digest of gelatin	5.0	g
Lactose	5.0	g
Water to	1000	ml

Adjust the pH after sterilisation to 6.9±0.2.

Levine Eosin-Methylene Blue Agar Medium

Pancreatic digest of gelatin	10.0	g
Dibasic potassium phosphate	2.0	g
Agar	15.0	g

Lactose	10.0	g
Eosin Y	400	mg
Methylene blue	65	mg
Water to	1000	ml

Dissolve the pancreatic digest of gelatin, dibasic potassium phosphate and agar in water with warming and allow to cool. Just prior to use, liquefy the gelled agar solution and the remaining ingredients, as solutions, in the following amounts and mix. For each 100 ml of the liquefied agar solution use 5 ml of a 20 per cent w/v solution of lactose, and 2 ml of a 2 per cent w/v solution of eosin Y, and 2 ml of a 0.33 per cent w/v solution of methylene blue. The finished medium may not be clear. Adjust the *p*H after sterilisation to 7.1±0.2.

MacConkey Agar Medium

Pancreatic digest of gelatin	17.0	g
Peptone (meat and casein, equal parts)	3.0	g
Lactose	10.0	g
Sodium chloride	5.0	g
Bile salts	1.5	g
Agar	13.5	g
Neutral red	30	mg
Crystal violet	1	mg
Water to	1000	ml

Boil the mixture of solids and water for 1 minute to effect solution. Adjust the *p*H after sterilisation to 7.1 ± 0.2.

MacConkey Broth Medium

Pancreatic digest of gelatin	20.0	g
Lactose	10.0	g
Dehydrated ox bile	5.0	g
Bromocresol purple	10	mg
Water to	1000	ml

Adjust the *p*H after sterilisation to 7.3±0.2.

Mannitol-Salt Agar Medium

Pancreatic digest of gelatin	5.0	g
Peptic digest of animal tissue	5.0	g
Beef extract	1.0	g
D-Mannitol	10.0	g
Sodium chloride	75.0	g
Agar	15.0	g
Phenol red	25	mg
Water to	1000	ml

Mix, heat with frequent agitation and boil for 1 minute to effect solution. Adjust the *p*H after sterilisation to 7.4 ± 0.2.

Nutrient Agar Medium : Nutrient broth gelled by the addition of 1 to 2 per cent w/v of agar.

Nutrient Broth Medium

Beef extract	10.0	g
Peptone	10.0	g
Sodium chloride	5	mg
Water to	1000	ml

Dissolve with the aid of heat. Adjust the pH to 8.0 to 8.4 with *5M sodium hydroxide* and boil for 10 minutes. Filter, and sterilise by maintaining at 115^0 for 30 minutes and adjust the *p*H to 7.3±0.1.

Pseudomonas Agar Medium for Detection of Flourescein

Pancreatic digest of casein	10.0	g
Peptic digest of animal tissue	10.0	g
Anhydrous dibasic potassium phosphate	1.5	g
Magnesium sulphate hepta hydrate	1.5	g
Glycerin	10.0	ml
Agar	15.0	g
Water to	1000	ml

Dissolve the solid components in water before adding glycerin. Heat with frequent agitation and boil for 1 minute to effect solution. Adjust the *p*H after sterilisation to 7.2±0.2.

Pseudomonas Agar Medium for Detection of Pyocyanin

Pancreatic digest of gelatin	20.0	g
Anhydrous magnesium chloride	1.4	g
Anhydrous potassium sulphate	10.0	g
Agar	15.0	g
Glycerin	10.0	ml
Water to	1000	ml

Dissolve the solid components in water before adding glycerin. Heat with frequent agitation and boil for 1 minute to effect solution. Adjust the *p*H after sterilisation to 7.2±0.2.

Sabouraud Dextrose Agar Medium

Dextrose	40	g
Mixture of equal parts of peptic digest of animal tissue and Pancreatic digest of casein	10	g

Agar	15	g
Water to	1000	ml

Mix, and boil to effect solution. Adjust the *p*H after sterilisation to 5.6 ± 0.2.

Sabouraud Dextrose Agar Medium with Antibiotics

To 1 liter of Sabouraud Dextrose Agar Medium add 0.1 g of benzylpenicillin sodium and 0.1 g of tetracycline or alternatively add 50 mg of chloramphenicol immediately before use.

Selenite F Broth

Peptone	5	g
Lactose	4	g
Disodium hydrogen phosphate	10	g
Sodium hydrogen selenite	4	g
Water to	1000	ml

Dissolve, distribute in sterile containers and sterilise by maintaining at 100º for 30 minutes.

Fluid Selenite-Cystine Medium

Pancreatic digest of casein	5.0	g
Lactose	4.0	g
Sodium phosphate	10.0	g
Sodium hydrogen selenite	4.0	g
L-Cystine	10.0	mg
Water to	1000	ml

Mix and heat to effect solution. Heat in flowing steam for 15 minutes. Adjust the final *p*H to 7.0±0.2. Do not sterilise.

Tetrathionate Broth Medium

Beef extract	0.9	g
Peptone	4.5	g
Yeast extract	1.8	g
Sodium chloride	4.5	g
Calcium carbonate	25.0	g
Sodium thiosulphate	40.7	g
Water to	1000	ml

Dissolve the solids in water and heat the solution to boil. On the day of use, add a solution prepared by dissolving 5 g of potassium iodide and 6 g of iodine in 20 ml of water.

Tetrathionate-Bile-Brilliant Green Broth Medium

Peptone	8.6	g
Dehydrated ox bile	8.0	g
Sodium chloride	6.4	g
Calcium carbonate	20.0	g
Potassium tetrathionate	20.0	g
Brilliant green	70	mg
Water to	1000	ml

Heat just to boiling; do not reheat. Adjust the *p*H so that after heating it is 7.0±0.2.

Triple Sugar-Iron Agar Medium

Beef extract	3.0	g
Yeast extract	3.0	g
Peptone	20.0	g
Lactose	10.0	g
Sucrose	10.0	g
Dextrose monohydrate	1.0	g
Ferrous sulphate	0.2	g
Sodium chloride	5.0	g
Sodium thiosulphate	0.3	g
Phenol red	24	mg
Agar	12.0	g
Water to	1000	ml

Mix, allow standing for 15 minutes, bringing to boil and maintain at boiling point until solution is complete, mix, distributing in tubes and sterilising by maintaining at 115^0 for 30 minutes. Allow to stand in a sloped form with a butt about 2.5 cm long.

Urea Broth Medium

Potassium dihydrogen orthophosphate	9.1	g
Anhydrous disodium hydrogen phosphate	9.5	g
Urea	20.0	g
Yeast extract	0.1	g
Phenol red	10	mg
Water to	1000	ml

Mix, sterilise by filtration and distribute aseptically in sterile containers.

Vogel-Johnson Agar Medium

Pancreatic digest of casein	10.0	g
Yeast extract	5.0	g

Mannitol	10.0	g
Dibasic potassium phosphate	5.0	g
Lithium chloride	5.0	g
Glycerin	10.0	g
Agar	16.0	g
Phenol red	25.0	mg
Water to	1000	ml

Boil the solution of solids for 1 minute. Sterilise, cool to between 45^0 to 50^0 and add 20 ml of a 1 per cent w/v sterile solution of potassium tellurite. Adjust the *p*H after sterilisation to 7.0±0.2.

Xylose-Lysine-Desoxycholate Agar Medium

Xylose	3.5	g
L-Lysine	5.0	g
Lactose	7.5	g
Sucrose	7.5	g
Sodium chloride	5.0	g
Yeast extract	3.0	g
Phenol red	80	mg
Agar	13.5	g
Sodium desoxycholate	2.5	g
Sodium thiosulphate	6.8	g
Ferric ammonium citrate	800	mg
Water to	1000	ml

Heat the mixture of solids and water, with swirling, just to the boiling point. Do not overheat or sterilise. Transfer at once to a water-bath maintained at about 50^0 and pour into plates as soon as the medium has cooled. Adjust the final *p*H to 7.4 ± 0.2.

Sampling : Use 10 ml or 10 g specimens for each of the tests specified in the individual monograph.

Precautions : The microbial limit tests should be carried out under conditions designed to avoid accidental contamination during the test. The precautions taken to avoid contamination must be such that they do not adversely affect any micro-organisms that should be revealed in the test.

2.4.1. - Total Aerobic Microbial Count:

Pretreat the sample of the product being examined as described below.

Water-soluble products : Dissolve 10 g or dilute 10 ml of the preparation being examined, unless otherwise specified, in buffered sodium chloride-peptone solution *p*H 7.0 or any other suitable medium shown to have no antimicrobial activity under the conditions of test and adjust the volume to 100 ml with the same medium. If necessary, adjust the *p*H to about 7.

Products insoluble in Water (non-fatty): Suspend 10 g or 10 ml of the preparation being examined, unless otherwise specified, in buffered sodium chloride-peptone solution *p*H 7.0 or any other suitable medium shown not to have antimicrobial activity under the conditions of the test and dilute to 100 ml with the same medium. If necessary, divide the preparation being examined and homogenize the suspension mechanically.

A suitable surface-active agent such as 0.1 per cent w/v of polysorbate 80 may be added to assist the suspension of poorly wettable substances. If necessary, adjust the *p*H of the suspension to about 7.

Fatty products: Homogenise 10 g or 10 ml of the preparation being examined, unless otherwise specified, with 5 g of polysorbate 20 or polysorbate 80. If necessary, heat to not more than 40^0. Mix carefully while maintaining the temperature in the water-bath or in an oven. Add 85 ml of buffered sodium chloride-peptone solution *p*H 7.0 or any other suitable medium shown to have no antimicrobial activity under the conditions of the test, heated to not more than 40^0 if necessary. Maintain this temperature for the shortest time necessary for formation of an emulsion and in any case for not more than 30 minutes. If necessary, adjust the *p*H to about 7.

Examination of the sample: Determine the total aerobic microbial count in the substance being examined by any of the following methods.

Membrane filtration : Use membrane filters 50 mm in diameter and having a nominal pore size not greater than 0.45 µm the effectiveness of which in retaining bacteria has been established for the type of preparation being examined.

Transfer 10 ml or a quantity of each dilution containing 1 g of the preparation being examined to each of two membrane filters and filter immediately. If necessary, dilute the pretreated preparation so that a colony count of 10 to 100 may be expected. Wash each membrane by filtering through it three or more successive quantities, each of about 100 ml, of a suitable liquid such as *buffered sodium chloride-peptone solution pH 7.0.* For fatty substances add to the liquid *polysorbate 20* or *polysorbate 80.* Transfer one of the membrane filters, intended for the enumeration of bacteria, to the surface of a plate of *casein soyabean digest agar* and the other, intended for the enumeration of fungi, to the surface of a plate of *Sabouraud dextrose agar* with antibiotics.

Incubate the plates for 5 days, unless a more reliable count is obtained in shorter time, at 30^0 to 35^0 in the test for bacteria and 20^0 to 25^0 in the test for fungi. Count the number of colonies that are formed. Calculate the number of micro-organisms per g or per ml of the preparation being examined, if necessary counting bacteria and fungi separately.

Plate count for bacteria: Using Petri dishes 9 to 10 cm in diameter, add to each dish a mixture of 1 ml of the pretreated preparation and about 15 ml of liquefied *casein soyabean digest agar* at not more than 45^0. Alternatively, spread the pretreated preparation on the surface of the solidified medium in a Petri dish of the same diameter. If necessary, dilute the pretreated preparation as described above so that a colony count of not more than 300 may be expected. Prepare at least two such Petri dishes using the same dilution and incubate at 30^0 to 35^0 for 5 days, unless a more reliable count is obtained in a shorter time. Count the number of colonies that are formed. Calculate the results using plates with the greatest number of colonies but taking 300 colonies per plate as the maximum consistent with good evaluation.

Plate count for fungi: Proceed as described in the test for bacteria but use *Sabouraud dextrose agar with antibiotics* in place of *casein soyabean digest agar* and incubate the plates at 20^0 to 25^0 for 5 days, unless a more reliable count is obtained in a shorter time. Calculate the results using plates with not more than 100 colonies.

Multiple-tube or serial dilution method : In each of fourteen test-tubes of similar size place 9.0 ml of sterile *fluid soyabean casein digest medium.* Arrange twelve of the tubes in four sets of three tubes each. Put aside one set of three tubes to serve as controls. Into each of three tubes of one set ("100") and into fourth tube (A) pipette 1 ml of the solution of suspension of the test specimen and mix. From tube A pipette 1 ml of its contents into the one remaining tube (B) not included in the set and mix. These two tubes contain 100 mg (or 100 µl) and 10 mg (or 10 µl) of the specimen respectively. Into each of the second set ("10") of three tubes pipette 1 ml from tube

Table 5 – Most Probable Total Count by Multiple-Tube Or Serial Dilution Method

Observed combination of numbers of tubes showing growth in each set			Most probable number of micro-organisms per g or per ml
No.of mg (or ml) of specimen per tube			
100 (100 µl)	10 (10 µl)	1 (1 µl)	
3	3	3	>1100
3	3	2	1100
3	3	1	500
3	3	0	200
3	2	3	290
3	2	2	210
3	2	1	150
3	2	0	90
3	1	3	160
3	1	2	120
3	1	1	70
3	1	0	40

3	0	3	95
3	0	2	60
3	0	1	40
3	0	0	23

A, and into each tube of the third set ("1") pipette 1 ml from tube B. Discard the unused contents of tube A and B. Close well and incubate all of the tubes. Following the incubation period, examine the tubes for growth. The three control tubes remain clear. Observations in the tubes containing the test specimen, when interpreted by reference to Table 1, indicate the most probable number of micro-organisms per g or per ml of the test specimen.

2.4.2. - Tests for Specified Micro-organisms:

Pretreatment of the sample being examined: Proceed as described under the test for total aerobic microbial count but using lactose broth or any other suitable medium shown to have no antimicrobial activity under the conditions of test in place of buffered sodium chloride-peptone solution *p*H 7.0.

Escherichia coli : Place the prescribed quantity in a sterile screw-capped container, add 50 ml of nutrient broth, shake, allow to stand for 1 hour (4 hours for gelatin) and shake again. Loosen the cap and incubate at 37^0 for 18 to 24 hours.

Primary test: Add 1.0 ml of the enrichment culture to a tube containing 5 ml of MacConkey broth. Incubate in a water-bath at 36^0 to 38^0 for 48 hours. If the contents of the tube show acid and gas carry out the secondary test.

Secondary test: Add 0.1 ml of the contents of the tubes containing (a) 5 ml of MacConkey broth, and (b) 5 ml of peptone water. Incubate in a water-bath at 43.5^0 to 44.5^0 for 24 hours and examine tube (a) for acid and gas and tube (b) for indole. To test for indole, add 0.5 ml of Kovac's reagent, shake well and allow to stand for 1 minute; if a red colour is produced in the reagent layer indole is present. The presence of acid and gas and of indole in the secondary test indicates the presence of *Escherichia coli*.

Carry out a control test by repeating the primary and secondary tests adding 1.0 ml of the enrichment culture and a volume of broth containing 10 to 50 *Escherichia coli* (NCTC 9002) organisms, prepared from a 24-hour culture in nutrient broth, to 5 ml of MacConkey broth. The test is not valid unless the results indicate that the control contains *Escherichia coli*.

Alternative test: By means of an inoculating loop, streak a portion from the enrichment culture (obtained in the previous test) on the surface of MacConkey agar medium. Cover and invert the dishes and incubate. Upon examination, if none of the colonies are brick-red in colour and have a surrounding zone of precipitated bile the sample meets the requirements of the test for the absence of *Escherichia coli*.

If the colonies described above are found, transfer the suspect colonies individually to the surface of Levine eosin-methylene blue agar medium, plated on Petri dishes. Cover and invert the plates and incubate. Upon examination, if none of the colonies exhibits both a

characteristic metallic sheen under reflected light and a blue-black appearance under transmitted light, the sample meets the requirements of the test for the absence of *Escherichia coli*. The presence of *Escherichia coli* may be confirmed by further suitable cultural and biochemical tests.

Salmonella : Transfer a quantity of the pretreated preparation being examined containing 1 g or 1 ml of the product to 100 ml of nutrient broth in a sterile screw-capped jar, shake, allow to stand for 4 hours and shake again. Loosen the cap and incubate at 35^0 to 37^0 for 24 hours.

Primary test: Add 1.0 ml of the enrichment culture to each of the two tubes containing (a) 10 ml of selenite F broth and (b) tetrathionate-bile-brilliant green broth and incubate at 36^0 to 38^0 for 48 hours. From each of these two cultures subculture on at least two of the following four agar media: bismuth sulphate agar, brilliant green agar, deoxycholatecitrate agar and xylose-lysine-deoxycholate agar. Incubate the plates at 36^0 to 38^0 for 18 to 24 hours. Upon examination, if none of the colonies conforms to the description given in Table 2, the sample meets the requirements of the test for the absence of the genus *Salmonella*.

If any colonies conforming to the description in Table 2 are produced, carry out the secondary test.

Secondary test: Subculture any colonies showing the characteristics given in Table 2 in triple sugar-iron agar by first inoculating the surface of the slope and then making a stab culture with the same inoculating needle, and at the same time inoculate a tube of urea broth. Incubate at 36^0 to 38^0 for 18 to 24 hours. The formation of acid and gas in the stab culture (with or without concomitant blackening) and the absence of acidity from the surface growth in the triple sugar iron agar, together with the absence of a red colour in the urea broth, indicate the presence of *Salmonella*. If acid but no gas is produced in the stab culture, the identity of the organisms should be confirmed by agglutination tests.

Carry out the control test by repeating the primary and secondary tests using 1.0 ml of the enrichment culture and a volume of broth containing 10 to 50 *Salmonella abony* (NCTC 6017) organisms, prepared from a 24-hour culture in nutrient broth, for the inoculation of the tubes (a) and (b). The test is not valid unless the results indicate that the control contains *Salmonella.*

Table 6 – Test for *Salmonella*

Medium	***Description*** **of colony**
Bismuth sulphite agar	Black or green
Brilliant green agar	Small, transparent and colourless, or opaque, pinkish or white (frequently surrounded by a pink or red zone)
Deoxycholate-citrate agar	Colourless and opaque, with or without black centers
Xylose-lysine-desoxy-cholate agar	Red with or without black centres

Pseudomonas aeruginosa : Pretreat the preparation being examined as described above and inoculate 100 ml of fluid soyabean-casein digest medium with a quantity of the solution, suspension or emulsion thus obtained containing 1 g or 1 ml of the preparation being examined. Mix and incubate at 35^0 to 37^0 for 24 to 48 hours. Examine the medium for growth and if growth is present, streak a portion of the medium on the surface of cetrimide agar medium, each plated on Petri dishes. Cover and incubate at 35^0 to 37^0 for 18 to 24 hours.

If, upon examination, none of the plates contains colonies having the characteristics listed in Table 3 for the media used, the sample meets the requirement for freedom from *Pseudomonas aeruginosa*. If any colonies conforming to the description in Table 3 are produced, carry out the oxidase and pigent tests.

Streak representative suspect colonies from the agar surface of cetrimide agar on the surfaces of *Pseudomonas* agar medium for detection of fluorescein and *Pseudomonas* agar medium for detection of pyocyanin contained in Petri dishes. Cover and invert the inoculated media and incubate at 33^0 to 37^0 for not less than 3 days. Examine the streaked surfaces under ultra-violet light. Examine the plates to determine whether colonies conforming to the description in Table 3 are present.

If growth of suspect colonies occurs, place 2 or 3 drops of a freshly prepared 1per cent w/v solution of *N*,*N*,*N*^{1},*N*^{1}-tetramethyl-4-phenylenediamine dihydrochloride on filter paper and smear with the colony; if there is no development of a pink colour, changing to purple, the sample meets the requirements of the test for the absence of *Pseudomonas aeruginosa.*

Staphylococcus aureus : Proceed as described under ***Pseudomonas aeruginosa*.** If, upon examination of the incubated plates, none of them contains colonies having the characteristics listed in Table 4 for the media used, the sample meets the requirements for the absence of *Staphylococcus aureus*.

If growth occurs, carry out the coagulase test. Transfer representative suspect colonies from the agar surface of any of the media listed in Table 4 to individual tubes, each containing 0.5 ml of mammalian, preferably rabbit or horse, plasma with or without additives. Incubate in water-bath at 37^0 examining the tubes at 3 hours and subsequently at suitable intervals up to 24 hours. If no coagulation in any degree is observed, the sample meets the requirements of the test for the absence of *Staphylococcus aureus*.

Validity of the tests for total aerobic microbial count:

Grow the following test strains separately in tubes containing fluid soyabean-casein digest medium at 30^0 to 35^0 for 18 to 24 hours or, for *Candida albicans*, at 20^0 for 48 hours.

Table 7 – Tests for *Pseudomonas aeruginosa*

Medium	Characteristic colonial morphology	Fluorescence in UV light	Oxidase test	Gram stain
Cetrimide agar	Generally greenish	Greenish	Positive	Negative rods
Pseudomonas agar medium for detection of fluorescein	Generally colourless to yellowish	Yellowish	Positive	Negative rods
Pseudomonas agar medium for detection of pyocyanin	Generally greenish	Blue	Positive	Negative rods

Table 8 – Tests for *Staphylococcus aureus*

Selective medium	Characteristic colonial morphology	Gram stain
Vogel-Johnson agar	Black surrounded by yellow zones	Positive cocci (in clusters)
Mannitol-salt agar	Yellow colonies with yellow zones	Positive cocci (in clusters)
Baird-Parker agar	Black, shiny, surrounded by clear zones of 2 to 5 mm	Positive cocci (in clusters)

Staphylococcus aureus (ATCC 6538; NCTC 10788)
Bacillus subtilis (ATCC 6633; NCIB 8054)
Escherichia coli (ATCC 8739; NCIB 8545)
Candida albicans (ATCC 2091; ATCC 10231)

Dilute portions of each of the cultures using buffered sodium chloride-peptone solution pH 7.0 to make test suspensions containing about 100 viable micro-organisms per ml. Use the suspension of each of the micro-organisms separately as a control of the counting methods, in the presence and absence of the preparation being examined, if necessary.

A count for any of the test organisms differing by not more than a factor of 10 from the calculated value for the inoculum should be obtained. To test the sterility of the medium and of the diluent and the aseptic performance of the test, carry out the total aerobic microbial count method using sterile buffered sodium chloride-peptone solution *p*H 7.0 as the test preparation. There should be no growth of micro-organisms.

Validity of the tests for specified micro-organisms: Grow separately the test strains of *Staphylococcus aureus* and *Pseudomonas aeruginosa* in fluid soyabean-casein digest medium and *Escherichia coli* and *Salmonella typhimurium* at 30^0 to 35^0 for 18 to 24 hours. Dilute portions of each of the cultures using buffered sodium chloride-peptone solution *p*H 7.0 to make test suspensions containing about 10^3 viable micro-organisms per ml. Mix equal volume of each suspension and use 0.4 ml (approximately 10^2 micro-organisms of each strain) as an

inoculum in the test for *E. coli, Salmonella*, *P. aeruginosa* and *S. aureus*, in the presence and absence of the preparation being examined, if necessary. A positive result for the respective strain of micro-organism should be obtained.

Table 9- Microbial Contamination Limits

S.No.	Parameters	Permissible limits
1.	*Staphylococcus aureus*/g.	Absent
2.	*Salmonella* sp./g .	Absent
3.	*Pseudomonas aeruginosa*/g	Absent
4.	*Escherichia coli*	Absent
5.	Total microbial plate count (TPC)	10^5/g*
6.	Total Yeast & Mould	10^3/g

*For topical use, the limit shall be 10^7/g.

2.5 - Pesticide Residue:

Definition: For the purposes of the Pharmacopoeia, a pesticide is any substance or mixture of substances intended for preventing, destroying or controlling any pest, unwanted species of plants or animals causing harm during or otherwise interfering with the production, processing, storage, transport or marketing of vegetable drugs. The item includes substances intended for use as growth-regulators, defoliants or desiccants and any substance applied to crops either before or after harvest to protect the commodity from deterioration during storage and transport.

Limits: Unless otherwise indicated in the monograph, the drug to be examined at least complies with the limits indicated in Table -1, The limits applying to pesticides that are not listed in the table and whose presence is suspected for any reason comply with the limits set by European Community directives 76/895 and 90/642, including their annexes and successive updates. Limits for pesticides that are not listed in Table.-1 nor in EC directives are calculated using the following expression:

$$\frac{ADI \times M}{MDD \times 100}$$

ADI = Acceptable Daily Intake, as published by FAO-WHO, in milligrams per kilogram of body mass,

M = body mass in kilograms (60 kg),

MDD = daily dose of the drug, in kilograms.

If the drug is intended for the preparation of extracts, tinctures or other pharmaceutical forms whose preparation method modifies the content of pesticides in the finished product, the limits are calculated using the following expression:

$$\frac{ADI \times M \times E}{MDD \times 100}$$

E = Extraction factor of the method of preparation, determined experimentally.

Higher limits can also be authorised, in exceptional cases, especially when a plant requires a particular cultivation method or has a metabolism or a structure that gives rise to a higher than normal content of pesticides.

The competent authority may grant total or partial exemption from the test when the complete history (nature and quantity of the pesticides used, date of each treatment during cultivation and after the harvest) of the treatment of the batch is known and can be checked precisely.

Sampling

Method: For containers up to 1 kg, take one sample from the total content, thoroughly mixed, sufficient for the tests. For containers between 1 kg and 5 kg, take three samples, equal in volume, from the upper, middle and lower parts of the container, each being sufficient to carry out the tests. Thoroughly mix the samples and take from the mixture an amount sufficient to carry out the tests. For containers of more than 5 kg, take three samples, each of at least 250 g from the upper, middle and lower parts of the container. Thoroughly mix the samples and take from the mixture an amount sufficient to carry out the tests.

Size of sampling: If the number (n) of containers is three or fewer, take samples from each container as indicated above under Method. If the number of containers is more than three, take n+1 samples for containers as indicated under Method, rounding up to the nearest unit if necessary.

The samples are to be analysed immediately to avoid possible degradation of the residues. If this is not possible, the samples are stored in air-tight containers suitable for food contact, at a temperature below 0^0, protected from light.

Reagents: All reagents and solvents are free from any contaminants, especially pesticides, that might interfere with the analysis. It is often necessary to use special quality solvents or, if this is not possible, solvents that have recently been re-distilled in an apparatus made entirely of glass. In any case, suitable blank tests must be carried out.

Apparatus: Clean the apparatus and especially glassware to ensure that they are free from pesticides, for example, soak for at least 16 h in a solution of phosphate-free detergent, rinse with large quantities of *distilled water* and wash with *acetone* and *hexane* or *heptane*.

2.5.1 - Qualitative and Quantitative Analysis of Pesticide Residues:

The analytical procedures used are validated according to the regulations in force. In particular, they satisfy the following criteria :

the chosen method, especially the purification steps, are suitable for the combination pesticide residue/substance to be analysed and not susceptible to interference from co-extractives; the limits of detection and quantification are measured for each pesticide-matrix combination to be analysed.

between 70 per cent to 110 per cent of each pesticide is recovered.

the repeatability of the method is not less than the values indicated in Table 10

the reproducibility of the method is not less than the values indicated in Table 11

the concentration of test and reference solutions and the setting of the apparatus are such that a linear response is obtained from the analytical detector.

Table -10

Substance	**Limit (mg/kg)**
Alachlor	0.02
Aldrin and Dieldrin (sum of)	0.05
Azinphos-methyl	1.0
Bromopropylate	3.0
Chlordane (sum of cis-, trans – and Oxythlordane)	0.05
Chlorfenvinphos	0.5
Chlorpyrifos	0.2
Chlorpyrifos-methyl	0.1
Cypermethrin (and isomers)	1.0
DDT (sum of p,p-'DDT, o,p-'DDT, p,p-'DDE and p,p-'TDE	1.0
Deltamethrin	0.5
Diazinon	0.5
Dichlorvos	1.0
Dithiocarbamates (as CS2)	2.0
Endosulfan (sum of isomers and Endosulfan sulphate)	3.0
Endrin	0.05
Ethion	2.0
Fenitrothion	0.5
Fenvalerate	1.5
Fonofos	0.05
Heptachlor (sum of Heptachlor and Heptachlorepoxide)	0.05
Hexachlorobenzene	0.1
Hexachlorocyclohexane isomers (other than γ)	0.3
Lindane (γ-Hexachlorocyclohexane)	0.6
Malathion	1.0
Methidathion	0.2
Parathion	0.5
Parathion-methyl	0.2

Permethrin	1.0
Phosalone	0.1
Piperonyl butoxide	3.0
Pirimiphos-methyl	4.0
Pyrethrins (sum of)	3.0
Quintozene (sum of quintozene, pentachloroaniline and methyl pentachlorophenyl sulphide)	1.0

Table -11

Concentration of the pesticide (mg/kg)	Repeatability (difference, ± mg/kg)	Reproducibility (difference, ± mg/kg)
0.010	0.005	0.01
0.100	0.025	0.05
1.000	0.125	0.25

2.5.2. Test for Pesticides:
Organochlorine, Organophosphorus and Pyrethroid Insecticides.

The following methods may be used, in connection with the general method above, depending on the substance being examined, it may be necessary to modify, sometimes extensively, the procedure described hereafter. In any case, it may be necessary to use, in addition, another column with a different polarity or another detection method (mass spectrometry) or a different method (immunochemical methods) to confirm the results obtained.

This procedure is valid only for the analysis of samples of vegetable drugs containing less than 15 per cent of water. Samples with a higher content of water may be dried, provided it has been shown that the drying procedure does not affect significantly the pesticide content.

Extraction

To 10 g of the substance being examined, coarsely powdered, add 100 ml of *acetone* and allow to stand for 20 min. Add 1 ml of a solution containing 1.8 µg/ml of *carbophenothion* in *toluene* . Homogenise using a high-speed blender for 3 min. Filter and wash the filter cake with two quantities, each of 25 ml, of *acetone.* Combine the filtrate and the washings and heat using a rotary evaporator at a temperature not exceeding 40^0 C until the solvent has almost completely evaporated. To the residue add a few milliliters of *toluene* and heat again until the acetone is completely removed. Dissolve the residue in 8 ml of *toluene.* Filter through a membrane filter (45 µm), rinse the flask and the filter with *toluene* and dilute to 10.0 ml with the same solvent (solution A).

Purification

Organochlorine, organophosphorus and pyrethroid insecticides:

Examine by size-exclusion chromatography.
The chromatographic procedure may be carried out using:

a stainless steel column 0.30 m long and 7.8 mm in internal diameter packed with styre:e-divinylbenzene copolymer (5 µm).
as mobile phase *toluene* at a flow rate of 1 ml/min.

Performance of the column: Inject 100 µl of a solution containing 0.5 g/l of *methyl red* and 0.5 g/l of *oracet blue* in *toluene* and proceed with the chromatography. The column is not suitable unless the colour of the eluate changes from orange to blue at an elution volume of about 10.3 ml. If necessary calibrate the column, using a solution containing, in *toluene,* at a suitable concentration, the insecticide to be analysed with the lowest molecular mass (for example, dichlorvos) and that with the highest molecular mass (for example, deltamethrin). Determine which fraction of the eluate contains both insecticides.

Purification of the test solution: Inject a suitable volume of solution A (100 µl to 500 µl) and proceed with the chromatography. Collect the fraction as determined above (solution B). Organophosphorus insecticides are usually eluted between 8.8 ml and 10.9 ml. Organochlorine and pyrethroid insecticides are usually eluted between 8.5 ml and 10.3 ml.

Organochlorine and pyrethroid insecticides: In a chromatography column, 0.10 m long and 5 mm in internal diameter, introduce a piece of defatted cotton and 0.5 g of silica gel treated as follows: heat *silica gel for chromatography* in an oven at 150^0 for at least 4 h. Allow to cool and add dropwise a quantity of *water* corresponding to 1.5 per cent of the mass of silica gel used; shake vigorously until agglomerates have disappeared and continue shaking for 2 h using a mechanical shaker. Condition the column using 1.5 ml of *hexane.* Prepacked columns containing about 0.50 g of a suitable silica gel may also be used provided they are previously validated.

Concentrate solution B in a current of helium for chromatography or oxygen-free nitrogen almost to dryness and dilute to a suitable volume with *toluene* (200 µl to 1 ml according to the volume injected in the preparation of solution B). Transfer quantitatively onto the column and proceed with the chromatography using 1.8 ml of *toluene* as the mobile phase. Collect the eluate (solution C).

2.5.3. - Quantitative Analysis:

A. Organophosphorus insecticides: Examine by gas chromatography, using *carbophenothion* as internal standard. It may be necessary to use a second internal standard to identify possible interference with the peak corresponding to carbophenothion.

Test solution: Concentrate solution B in a current of helium for chromatography almost to dryness and dilute to 100 µl with *toluene.*

Reference solution: Prepare at least three solutions in *toluene* containing the insecticides to be determined and *carbophenothion* at concentrations suitable for plotting a calibration curve.

The chromatographic procedure may be carried out using:

a fused-silica column 30 m long and 0.32 mm in internal diameter the internal wall of which is covered with a layer 0.25 µm thick of poly (dimethyl) siloxane.
hydrogen for chromatography as the carrier gas. Other gases such as helium for chromatography or nitrogen for chromatography may also be used provided the chromatography is suitably validated.
a phosphorus-nitrogen flame-ionisation detector or a atomic emission spectrometry detector.

Maintaining the temperature of the column at 80^0 for 1 min, then raising it at a rate of 30^0 /min to 150^0, maintaining at 150^0 for 3 min, then raising the temperature at a rate of 4^0/min to 280^0 and maintaining at this temperature for 1 min and maintaining the temperature of the injector port at 250^0 and that of the detector at 275^0. Inject the chosen volume of each solution. When the chromatograms are recorded in the prescribed conditions, the relative retention times are approximately those listed in Table 12 Calculate the content of each insecticide from the peak areas and the concentrations of the solutions.

B. Organochlorine and Pyrethroid Insecticides:

Examine by gas chromatography, using *carbophenothion* as the internal standard. It may be necessary to use a second internal standard to identify possible interference with the peak corresponding to *carbophenothion*.

Test solution: Concentrate solution C in a current of helium for chromatography or oxygen-free nitrogen almost to dryness and dilute to 500 µl with *toluene.*

Reference solution: Prepare at least three solutions in *toluene* containing the insecticides to be determined and *carbophenothion* at concentrations suitable for plotting a calibration curve.

Table 12- Relative Retention Times of Pesticides

Substance	**Relative retention times**
Dichlorvos	0.20
Fonofos	0.50
Diazinon	0.52
Parathion-methyl	0.59
Chlorpyrifos-methyl	0.60
Pirimiphos-methyl	0.66
Malathion	0.67
Parathion	0.69
Chlorpyrifos	0.70
Methidathion	0.78
Ethion	0.96
Carbophenothion	1.00
Azinphos-methyl	1.17

Phosalon	1.18

The chromatographic procedure may be carried out using:

a fused silica column 30 m long and 0.32 mm in internal diameter the internal wall of which is covered with a layer 0.25 µm thick of *poly (dimethyl diphenyl) siloxane.*
hydrogen for chromatography as the carrier gas. Other gases such as helium for chromatography or nitrogen for chromatography may also be used, provided the chromatography is suitably validated.
an electron-capture detector.
a device allowing direct cold on-column injection.

maintaining the temperature of the column at 80^0 for 1 min, then raising it at a rate of 30^0/min to 150^0, maintaining at 150^0 for 3 min, then raising the temperature at a rate of 4^0/min to 280^0 and maintaining at this temperature for 1 min and maintaining the temperature of the injector port at 250^0 and that of the detector at 275^0. Inject the chosen volume of each solution. When the chromatograms are recorded in the prescribed conditions, the relative retention times are approximately those listed in Table 13. Calculate the content of each insecticide from the peak areas and the concentrations of the solutions.

Table 13- Relative Retention Times of Insecticides

Substance	**Relative retention times**
α-Hexachlorocyclohexane	0.44
Hexachlorobenzene	0.45
β-Hexachlorocyclohexane	0.49
Lindane	0.49
δ-Hexachlorocyclohexane	0.54
ε-Hexachlorocyclohexane	0.56
Heptachlor	0.61
Aldrin	0.68
cis-Heptachlor-epoxide	0.76
o,p-'DDE	0.81
α-Endosulfan	0.82
Dieldrin	0.87
p,p-'DDE	0.87
o,p-'DDD	0.89
Endrin	0.91
β-Endosulfan	0.92
o,p-'DDT	0.95
Carbophenothion	1.00
p,p-'DDT	1.02
cis-Permethrin	1.29
trans-Permethrin	1.31
Cypermethrin*	1.40
Fenvalerate*	1.47 and 1.49
Deltamethrin	1.54

*The substance shows several peaks.

2.6. - Gas Chromatography:

Gas chromatography (GC) is a chromatographic separation technique based on the difference in the distribution of species between two non-miscible phases in which the mobile phase is a carrier gas moving through or passing the stationary phase contained in a column. It is applicable to substances or their derivatives, which are volatilized under the temperatures employed.

GC is based on mechanisms of adsorption, mass distribution or size exclusion.

Apparatus

The apparatus consists of an injector, a chromatographic column contained in an oven, a detector and a data acquisition system (or an integrator or a chart recorder). The carrier gas flows through the column at a controlled rate or pressure and then through the detector.

The chromatography is carried out either at a constant temperature or according to a given temperature programme.

Injectors

Direct injections of solutions are the usual mode of injection, unless otherwise prescribed in the monograph. Injection may be carried out either directly at the head of the column using a syringe or an injection valve, or into a vaporization chamber which may be equipped with a stream splitter.

Injections of vapour phase may be effected by static or dynamic head-space injection systems.

Dynamic head-space (purge and trap) injection systems include a sparging device by which volatile substances in solution are swept into an absorbent column maintained at a low temperature. Retained substances are then desorbed into the mobile phase by rapid heating of the absorbent column.

Static head-space injection systems include a thermostatically controlled sample heating chamber in which closed vials containing solid or liquid samples are placed for a fixed period of time to allow the volatile components of the sample to reach equilibrium between the non-gaseous phase and the vapour phase. After equilibrium has been established, a predetermined amount of the head-space of the vial is flushed into the gas chromatograph.

Stationary Phases

Stationary phases are contained in columns, which may be:

a capillary column of fused-silica close wall is coated with the stationary phase.
a column packed with inert particles impregnated with the stationary phase.
a column packed with solid stationary phase.

Capillary columns are 0.1 mm to 0.53 mm in internal diameter (Φ) and 5 to 6 m in length. The liquid or stationary phase, which may be chemically bonded to the inner surface, is a film 0.1 µm to 5.0 µm thick.

Packed columns, made of glass or metal, are usually 1 m to 3 m in length with an internal diameter (Φ) of 2 mm to 4 mm. Stationary phases usually consist of porous polymers or solid supports impregnated with liquid phase.

Supports for analysis of polar compounds on columns packed with low-capacity, low-polarity stationary phase must be inert to avoid peak tailing. The reactivity of support materials can be reduced by silanising prior to coating with liquid phase. Acid-washed, flux-calcinated diatomaceous earth is often used. Materials are available in various particle sizes, the most commonly used particles are in the ranges of 150 µm to 180 µm and 125 µm to 150 µm.

Mobile Phases

Retention time and peak efficiency depend on the carrier gas flow rate; retention time is directly proportional to column length and resolution is proportional to the square root of the column length. For packed columns, the carrier gas flow rate is usually expressed in milliliters per minute at atmospheric pressure and room temperature, flow rate is measured at the detector outlet, either with a calibrated mechanical device or with a bubble tube, while the column is at operating temperature. The linear velocity of the carrier gas through a packed column is inversely proportional to the square root of the internal diameter of the column for a given flow volume. Flow rates of 60 ml/min in a 4 mm internal diameter column and 15 ml/min in a 2 mm internal diameter column, give identical linear velocities and thus similar retention times.

Helium or nitrogen is usually employed as the carrier gas for packed columns, whereas commonly used carrier gases for capillary columns are nitrogen, helium and hydrogen.

Detectors

Flame-ionisation detectors are usually employed but additional detectors which may be used include: electron-capture, nitrogen-phosphorus, mass spectrometric, thermal conductivity, Fourier transform infrared spectrophotometric and others, depending on the purpose of the analysis.

Method

Equilibrate the column, the injector and the detector at the temperatures and the gas flow rates specified in the monograph until a stable baseline is achieved. Prepare the test solution (s) and the reference solutions (s) as prescribed. The solutions must be free from solid particles.

Criteria for assessing the suitability of the system are described in the chapter on *Chromatographic separation techniques*. The extent to which adjustments of parameters of the chromatographic system can be made to satisfy the criteria of system suitability are also given in this chapter.

2.7. - Test for Aflatoxins:

Caution: Aflatoxins are highly dangerous and extreme care should be exercised in handling aflatoxin materials.

This test is provided to detect the possible presence of aflatoxins B_1, B_2, G_1 and G_2 in any material of plant origin. Unless otherwise specified in the individual monograph, use the following method.

Zinc Acetate – Aluminum Chloride Reagent: Dissolve 20 g of *zinc acetate* and 5 g of *aluminum chloride* in sufficient water to make 100 ml.

Sodium Chloride Solution: Dissolve 5 g of *sodium chloride* in 50 ml of purified water.

Test Solution 1: Grind about 200 g of plant material to a fine powder. Transfer about 50 g of the powdered material, accurately weighed, to a glass-stoppered flask. Add 200 ml of a mixture of *methanol* and *water* (17: 3). Shake vigorously by mechanical means for not less than 30 minutes and filter. [Note – If the solution has interfering plant pigments, proceed as directed for Test Solution 2.] Discard the first 50 ml of the filtrate and collect the next 40 ml portion. Transfer the filtrate to a separatory funnel. Add 40 ml of sodium chloride solution and 25 ml of *hexane* and shake for 1 minute. Allow the layers to separate and transfer the lower aqueous layer to a second separatory funnel. Extract the aqueous layer in the separatory funnel twice, each time with 25 ml of *methylene chloride*, by shaking for 1 minute. Allow the layers to separate each time, separate the lower organic layer and collect the combined organic layers in a 125 ml conical flask. Evaporate the organic solvent to dryness on a water bath. Cool the residue. If interferences exist in the residue, proceed as directed for *Cleanup Procedure;* otherwise, dissolve the residue obtained above in 0.2 ml of a mixture of *chloroform* and *acetonitrile* (9.8 : 0.2) and shake by mechanical means if necessary.

Test Solution 2: Collect 100 ml of the filtrate from the start of the flow and transfer to a 250 ml beaker. Add 20 ml of *Zinc Acetate-Aluminum Chloride Reagent* and 80 ml of water. Stir and allow to stand for 5 minutes. Add 5 g of a suitable filtering aid, such as diatomaceous earth, mix and filter. Discard the first 50 ml of the filtrate, and collect the next 80 ml portion. Proceed as directed for *Test Solution 1,* beginning with "Transfer the filtrate to a separatory funnel."

Cleanup Procedure: Place a medium-porosity sintered-glass disk or a glass wool plug at the bottom of a 10 mm x 300 mm chromatographic tube. Prepare slurry of 2 g of silica gel with a mixture of *ethyl ether* and *hexane* (3: 1), pour the slurry into the column and wash with 5 ml of the same solvent mixture. Allow the absorbent to settle and add to the top of the column a layer of 1.5 g of *anhydrous sodium sulfate*. Dissolve the residue obtained above in 3 ml of *methylene chloride* and transfer it to the column. Rinse the flask twice with 1 ml portions of *methylene chloride*, transfer the rinses to the column and elute at a rate not greater than 1 ml per minute. Add successively to the column 3 ml of *hexane*, 3 ml of *diethyl ether* and 3 ml of *methylene chloride*; elute at a rate not greater than 3 ml per minute; and discard the eluates. Add to the column 6 mL of a mixture of *methylene chloride* and *acetone* (9 : 1) and elute at a rate not greater than 1 ml per minute, preferably without the aid of vacuum. Collect this eluate in a small vial, add a boiling chip if necessary and evaporate to dryness on a water bath. Dissolve

the residue in 0.2 ml of a mixture of *chloroform* and *acetonitrile* (9.8 : 0.2) and shake by mechanical means if necessary.

Aflatoxin Solution: Dissolve accurately weighed quantities of aflatoxin B_1, aflatoxin B_2, aflatoxin G_1 and aflatoxin G_2 in a mixture of *chloroform* and *acetonitrile* (9.8: 0.2) to obtain a solution having concentrations of 0.5 µg /per ml each for aflatoxin B_1 and G_1 and 0.1µg per ml each for aflatoxins for B2 and G_2.

Procedure: Separately apply 2.5 µl, 5 µl, 7.5 µl and 10 µl of the Aflatoxin Solution and three 10 µl applications of either *Test Solution 1* or *Test Solution 2* to a suitable thin-layer chromatographic plate coated with a 0.25-mm layer of chromatographic silica gel mixture. Superimpose 5 µl of the *Aflatoxin Solution* on one of the three 10 µl applications of the *Test Solution.* Allow the spots to dry and develop the chromatogram in an unsaturated chamber containing a solvent system consisting of a mixture of *chloroform*, *acetone* and *isopropyl alcohol* (85:10:5) until the solvent front has moved not less than 15 cm from the origin. Remove the plate from the developing chamber, mark the solvent front and allow the plate to air-dry. Locate the spots on the plate by examination under UV light at 365 nm: the four applications of the *Aflatoxin Solution* appear as four clearly separated blue fluorescent spots; the spot obtained from the *Test Solution* that was superimposed on the *Aflatoxin Solution* is no more intense than that of the corresponding *Aflatoxin Solution;* and no spot from any of the other *Test Solutions* corresponds to any of the spots obtained from the applications of the *Aflatoxin Solution.* If any spot of aflatoxins is obtained in the *Test Solution,* match the position of each fluorescent spot of the *Test Solution* with those of the *Aflatoxin Solution* to identify the type of aflatoxin present. The intensity of the aflatoxin spot, if present in the *Test Solution,* when compared with that of the corresponding aflatoxin in the *Aflatoxin Solution* will give an approximate concentration of aflatoxin in the *Test Solution.*

Table14 - Permissible Limit of Aflatoxins*

S.No	Aflatoxins	Permissible Limit
1.	B_1	0.5 ppm
2.	G_1	0.5 ppm
3.	B_2	0.1 ppm
4.	G_2.	0.1 ppm

*For Domestic use only

APPENDIX - 3

PHYSICAL TESTS AND DETERMINATIONS

3.1. - Refractive Index:

The refractive index (η) of a substance with reference to air is the ratio of the sine of the angle of incidence to the sine of the angle of refraction of a beam of light passing from air into the substance. It varies with the wavelength of the light used in its measurement.

Unless otherwise prescribed, the refractive index is measured at 25^0 (±0.5) with reference to the wavelength of the D line of sodium (λ 589.3 nm). The temperature should be carefully adjusted and maintained since the refractive index varies significantly with temperature.

The Abbe's refractometer is convenient for most measurements of refractive index but other refractometer of equal or greater accuracy may be used. Commercial refractometers are normally constructed for use with white light but are calibrated to give the refractive index in terms of the D line of sodium light.

To achieve accuracy, the apparatus should be calibrated against *distilled water* which has a refractive index of 1.3325 at 25^0 or against the reference liquids given in the following table.

Table 15

Reference Liquid	$\eta_D^{20^\circ}$	**Temperature Co-efficient Δn/Δt**
Carbon tetrachloride	1.4603	-0.00057
Toluene	1.4969	-0.00056
α-Methylnaphthalene	1.6176	-0.00048

***** Reference index value for the D line of sodium, measured at 20^0

The cleanliness of the instrument should be checked frequently by determining the refractive index of distilled water, which at 25^0 is 1.3325.

3.2. - Weight per Millilitre and Specific Gravity:

A. Weight per millilitre: The weight per millilitre of a liquid is the weight in g of 1 ml of a liquid when weighed in air at 25^0, unless otherwise specified.

Method

Select a thoroughly clean and dry pycnometer. Calibrate the pycnometer by filling it with recently boiled and cooled water at 250 and weighing the contents. Assuming that the weight of 1 ml of water at 250 when weighed in air of density 0.0012 g per ml, is 0.99602 g. Calculate the capacity of the pycnometer. (Ordinary deviations in the density of air from the value given do not affect the result of a determination significantly). Adjust the temperature of the substance to be examined, to about 200 and fill the pycnometer with it. Adjust the temperature of the filled pycnometer to 250, remove any excess of the substance and weigh. Substract the tare weight of the pycnometer from the filled weight of the pycnometer. Determine the weight per milliliter dividing the weight in air, expressed in g, of the quantity of liquid which fills the pycnometer at the specified temperature, by the capacity expressed in ml, of the pycnometer at the same temperature.

B. Specific gravity: The specific gravity of a liquid is the weight of a given volume of the liquid at 25^0 (unless otherwise specified) compared with the weight of an equal volume of water at the same temperature, all weighing being taken in air**.**

Method

Proceed as described under wt. per ml. Obtain the specific gravity of the liquid by dividing the weight of the liquid contained in the pycnometer by the weight of water contained, both determined at 25^0 unless otherwise directed in the individual monograph.

3.3. - Determination of *p*H Values:

The *p*H value of an aqueous liquid may be defined as the common logarithum of the reciprocal of the hydrogen ion concentration expressed in g per litre. Although this definition provides a useful practical means for the quantitative indication of the acidity or alkalinity of a solution, it is less satisfactory from a strictly theoretical point of view. No definition of *p*H as a measurable quantity can have a simple meaning, which is also fundamental and exact.

The *p*H value of a liquid can be determined potentiometrically by means of the glass electrode, a reference electrode and a pH meter either of the digital or analogue type.

3.4. - Determination of Melting Range and Congealing Range:

3.4.1. Determination of Melting Range:

The melting-range of a substance is the range between the corrected temperature at which the substance begins to form droplets and the corrected temperature at which it completely melts, as shown by formation of a meniscus.

Apparatus:

(a) A capillary tube of soft glass, closed at one end, and having the following dimensions:

(i) thickness of the wall, about 0.10 to 0.15 mm.

(ii) length about 10 cm or any length suitable for apparatus used.

(iii) internal diameter 0.9 to 1.1 mm for substances melting below 100^0 or 0.8 to 1.2 mm for substances melting above 100^0.

Thermometers:

Accurately standardized thermometers covering the range 10^0 to 300^0 the length of two degrees on the scale being not less than 0.8 mm. These thermometers are of the mercury-in-glass, solid-stem type; the bulb is cylindrical in shape, and made of approved thermometric glass suitable for the range of temperature covered; each thermometer is fitted with a safety chamber. The smallest division on the thermometer scale should vary between 0.1^0 to 1.5^0 according to the melting point of the substance under test.

The following form of heating apparatus is recommended.

A glass heating vessel of suitable, construction and capacity fitted with suitable stiring device, capable of rapidly mixing the liquids.

Suitable liquids for use in the heating vessel:

Glycerin	Upto 150^0
Sulphuric acid to which a small crystal of *potassium nitrate* or 4 Drops of *nitric acid* per 100 ml has been added	Upto 200^0
A liquid paraffin of sufficiently high boiling range	Upto 250^0
Seasame oil	Upto 300^0
30 parts of *potassium sulphate*, dissolved by heating in 70 parts of *sulphuric acid*	Upto 300^0

Any other apparatus or method, preferably, the electric method may be used subject to a check by means of pure substances having melting temperature covering the ranges from 0^0 to 300^0 and with suitable intervals.

The following substances are suitable for this purpose.

Substance	Melting range
Vanillin	81^0 to 83^0
Acetanilide	114^0 to 116^0
Phenacetin	134^0 to 136^0
Sulphanilamide	164^0 to 166.5^0
Sulphapyridine	191^0 to 193^0
Caffeine (Dried at 100^0)	234^0 to 237^0

Procedure

Method I: Transfer a suitable quantity of the powdered and thoroughly dried substance to a dry capillary tube and pack the powder by tapping the tube on a hard surface so as to form a tightly packed column of 2 to 4 mm in height. Attach the capillary tube and its contents to a

standardized thermometer so that the closed end is at the level of the middle of the bulb; heat in a suitable apparatus (preferably a round-bottom flask) fitted with an auxiliary thermometer regulating the rise of temperature in the beginning to 3^0 per minute. When the temperature reached is below the lowest figure of the range for the substance under examination, the heating of the apparatus is adjusted as desired; if no other directions are given, the rate of rise of temperature should be kept at 1^0 to 2^0 per minute. The statement 'determined by rapid heating' means that the rate of rise of temperature is 5^0 per minute during the entire period of heating.

Unless otherwise directed, the temperature at which the substance forms droplets against the side of the tube and the one at which it is completely melted as indicated by the formation of a definite meniscus, are read.

The following emergent stem corrections should be applied to the temperature readings.

Before starting the determination of the melting temperature the auxiliary thermometer is attached so that the bulb touches the standard thermometer at a point midway between the graduation for the expected melting temperature and the surface of the heating material. When the substance has melted, the temperature is read on the auxiliary thermometer. The correction figure to be added to the temperature reading of the standardized thermometer is calculated from the following formula

$$0.00015\ N\ (T—t)$$

Where 'T' is the temperature reading of the standardized thermometer.

't' is the temperature reading of the auxiliary thermometer.

'N' is the number of degrees of the scale of the standardized thermometer between the surface of the heating material and level of mercury.

The statement "melting range, a^0 to b^0" means that the corrected temperature at which the material forms droplets must be at least a^0, and that the material must be completely melted at the corrected temperature, b^0.

Method II: The apparatus employed for this test is the same as described for method I except for such details as are mentioned in the procedure given below

Procedure: A capillary tube open at both ends is used for this test. Melt the material under test at as low a temperature as possible. Draw into the capillary a column of the material about 10 mm high. Cool the charged tube in contact with ice for at least 2 hours. Attach the tube to the thermometer by means of rubber band and adjust it in the heating vessel containing water so that the upper edge of the material is 10 mm below the water level. Heat in the manner as prescribed in Method I until the temperature is about 5^0 below the expected melting point and then regulate the rate of rise of temperature to between 0.5^0 to 1^0 per minute. The temperature at which the material is observed to rise in the capillary tube is the melting temperature of the substance.

3.4.2. - Determination of Congealing Range:

The congealing temperature is that point at which there exists a mixture of the liquid (fused) phase of a substance and a small but increasing proportion of the solid phase. It is distinct from the freezing point which is the temperature at which the liquid and solid phase of a substance are in equilibrium. In certain cases, this may happen over a range of temperatures.

The temperature at which a substance solidifies upon cooling is a useful index of its purity if heat is liberated when solidification takes place.

The following method is applicable to substances that melt between -20^0 and 150^0.

Apparatus

A test-tube (About 150 mm × 25 mm) placed inside another test-tube (about 160 mm × 40 mm) the inner tube is closed by a stopper that carries a stirrer and a thermometer (About 175 mm long and with 0.2^0 graduations) fixed so that the bulb is about 15 mm above the bottom of the tube. The stirrer is made from a glass rod or other suitable material formed at one end into a loop of about 18 mm overall diameter at right angles to the rod. The inner tube with its jacket is supported centrally in a 1-litre baker containing a suitable cooling liquid to within 20 mm of the top. The thermometer is supported in the cooling bath.

Method

Melt the substance, if a solid, at a temperature not more than 20^0 above its expected congealing point, and pour it into the inner test-tube to a height of 50 to 57 mm. Assemble the apparatus with the bulb of the thermometer immersed half-way between the top and bottom of the sample in the test-tube. Fill the bath to almost 20 mm from the top of the tube with a suitable fluid at a temperature 4^0 to 5^0 below the expected congealing point. If the substance is a liquid at room temperature, carry out the determination using a bath temperature about 15^0 below the expected congealing point. When the sample has cooled to about 5^0 above its expected congealing point stir it continuously by moving the loop up and down between the top and bottom of the sample at a regular rate of 20 complete cycles per minute. If necessary, congelation may be induced by scratching the inner walls of the test-tube with the thermometer or by introducing a small amount of the previously congealed substance under examination. Pronounced supercooling may result in deviation from the normal pattern of temperature changes. If it happens, repeat the test introducing small fragments of the solid substance under examination at 1^0 intervals when the temperature approaches the expected congealing point.

Record the reading of the thermometer every 30 seconds and continue stirring only so long as the temperature is falling. Stop the stirring when the temperature is constant to starts to rise slightly. Continue recording the temperature for at least 3 minutes after the temperature again begins to fall after remaining constant.

The congealing point will be mean of not less than four consecutive readings that lie within a range of 0.2^0.

3.5. - Determination of Boiling Range:

The boiling-range of a substance is the range of temperature within which the whole or a specified portion of the substance distils.

Apparatus

The boiling-range is determined in a suitable apparatus, the salient features of which are described below:

(a) **Distillation flask:** The flask shall be made of colourless transparent heat-resistant glass and well annealed. It should have a spherical bulb having a capacity of about 130 ml. The side tube slopes downwards in the same plane as the axis of the neck at angle of between 72^0 to 78^0. Other important dimensional details are as under:

Internal diameter of neck	15 to 17 mm
Distance from top of neck to center of side tube	72 to 78 mm
Distance from the center of the side tube to surface of the Liquid when the flask contains 100 ml liquid	87 to 93 mm
Internal diameter of side tube	3.5 to 4.5 mm
Length of side tube	97 to 103 mm

(b) **Thermometer**: Standardised thermometers calibrated for 100 mm immersion and suitable for the purpose and covering the boiling range of the substance under examination shall be employed; the smallest division on the thermometer scale may vary between 0.2^0 to 1^0 according to requirement.

(c) **Draught Screen**: suitable draught screen, rectangular in cross section with a hard asbestos board about 6 mm thick closely fitting horizontally to the sides of the screen, should be used. The asbestos board shall have a centrally cut circular hole, 110 mm in diameter. The asbestos board is meant for ensuring that hot gases from the heat source do not come in contact with the sides or neck of the flask.

(d) **Asbestos Board:** A 150 mm square asbestos board 6 mm thick provided with a circular hole located centrally to hold the bottom of the flask, shall be used. For distillation of liquids boiling below 60^0 the hole shall be 30 mm in diameter; for other liquid it should be 50 mm in diameter. This board is to be placed on the hard asbestos board of the draught screen covering its 110 mm hole.

(e) **Condenser:** A straight water-cooled glass condenser about 50 cm long shall be used.

Procedure: 100 ml of the liquid to be examined is placed in the distillation flask, and a few glass beads or other suitable substance is added. The bulb of the flask is placed centrally over a circular hole varying from 3 to 5 cm in diameter (according to the boiling range of the

substance under examination), in a suitable asbestos board. The thermometer is held concentrically in the neck of the flask by means of a well fitting cork in such a manner that the bulb of the thermometer remains just below the level of the opening of the side-tube. Heat the flask slowly in the beginning and when distillation starts, adjust heating in such a manner that the liquid distils at a constant rate of 4 to 5 ml per minute. The temperature is read when the first drop runs from the condenser, and again when the last quantity of liquid in the flask is evaporated.

The boiling ranges indicated, apply at a barometric pressure of 760 mm of mercury. If the determination is made at some other barometric pressure, the following correction is added to the temperatures read:

K- (760—p)

Where p is the barometric pressure (in mm) read on a mercury barometer, without taking into account the temperature of the air;

K is the boiling temperature constant for different liquids having different boiling ranges as indicated below:—

Observed Boiling range	'K'
Below 100^0	0.04
100^0 to 140^0	0.045
141^0 to 190^0	0.05
191^0 to 240^0	0.055
above 240^0	0.06

If the barometric pressure is below 760 mm of mercury the correction is added to the observed boiling-range; if above, the correction is subtracted.

The statement 'distils between a^0 and $b^{0,}$, means that temperature at which the first drop runs from the condenser is not less than a^0 and that the temperature at which the liquid is completely evaporated is not greater than b^0.

Micro-methods of equal accuracy may be used.

3.6. - Determination of Optical Rotation and Specific Optical Rotation:

A. Optical Rotation :Certain substances, in a pure state, in solution and in tinctures posses the property of rotating the plane of polarized light, i.e., the incident light emerges in a plane forming an angle with the plane of the incident light. These substances are said to be optically active and the property of rotating the plane of polarized light is known as optical rotation. The optical rotation is defined as the angle through which the plane of polarized light is rotated when polarized light obtained from sodium or mercury vapour lamp passes through one decimeter thick layer of a liquid or a solution of a substance at a temperature of 25º unless as otherwise stated in the monograph. Substances are described as dextrorotatory or laevoretatory

according to the clockwise or anticlockwise rotation respectively of the plane of polarized light. Dextrorotation is designated by a plus (+) sign and laevorotation by a minus (-) sign before the number indicating the degrees of rotation.

Apparatus: A polarimeter on which angular rotation accurate 0.05^0 can be read may be used.

Calibration: The apparatus may be checked by using a solution of previously dried *sucrose* and measuring the optical rotation in a 2-din tube at 25^0 and using the concentrations indicated in Table.

Concentration (g/100 ml)	Angle of Rotation (+) at 25^0
10.0	13.33
20.0	26.61
30.0	39.86
40.0	53.06
50.0	66.23

Procedure: For liquid substances, take a minimum of five readings of the rotation of the liquid and also for an empty tube at the specified temperature. For a solid dissolve in a suitable solvent and take five readings of the rotation of the solution and the solvent used. Calculate the average of each set of five readings and find out the corrected optical rotation from the observed rotation and the reading with the blank (average).

B. Specific Rotation : The apparatus and the procedure for this determination are the same as those specified for optical rotation.

Specific rotation is denoted by the expression

$$[\alpha]_x^t$$

t denotes the temperature of rotation; α denotes the wave length of light used or the characteristic spectral line. Specific rotations are expressed in terms of sodium light of wave length 589.3 mw (D line) and at a temperature of 25^0, unless otherwise specified.

Specific rotation of a substance may be calculated from the following formulae:
For liquid substances

$$[\alpha]^t = \frac{a}{ld}$$

For solutions of substances

$$[\alpha]^t = \frac{a \times 100}{lc.}$$

Where a is the corrected observed rotation in degrees

1 is the length of the polarimeter tube in decimeters.

D is the specific gravity of the liquid C is the concentration of solution expressed as the number of g of the substance in 100 ml of solution.

3.7. - Determination of Viscosity:

Viscosity is a property of a liquid, which is closely related to the resistance to flow.

In C.G.S. system, the dynamic viscosity (n) of a liquid is the tangential force in dryness per square centimeter exerted in either of the two parallel planes placed, 1 cm apart when the space between them is filled with the fluid and one of the plane is moving in its own plane with a velocity of 1 cm per second relatively to the other. The unit of dynamic viscosity is the poise (abbreviated p). The centi poise (abbreviated cp) is $1/100^{th}$ of one poise.

While on the absolute scale, viscosity is measured in poise or centi poise, it is mot convenient to use the kinematic scale in which the units are stokes (abbreviated S) and centi-stokes (abbreviated CS). The centistokes is $1/100^{th}$ of one stoke. The kinematic viscosity of a liquid is equal to the quotient of the dynamic viscosity and the density of the liquid at the same temperature, thus :

$$\text{Kinematic Viscosity} = \frac{\text{Dynamic Viscosity}}{\text{Density}}$$

Viscosity of liquid may be determined by any method that will measure the resistance to shear offered by the liquid.

Absolute viscosity can be measured directly if accurate dimensions of the measuring instruments are known but it is more common practice to calibrate the instrument with a liquid of known viscosity and to determine the viscosity of the unknown fluid by comparison with that of the known.

Procedure: The liquid under test is filled in a U tube viscometer in accordance with the expected viscosity of the liquid so that the fluid level stands within 0.2 mm of the filling mark of the viscometer when the capillary is vertical and the specified temperature is attained by the test liquid. The liquid is sucked or blown to the specified weight of the viscometer and the time taken for the meniscus to pass the two specified marks is measured. The kinematic viscosity in centistokes is calculated from the following equation:

$$\text{Kinematic viscosity} = kt$$

Where k = the constant of the viscometer tube determined by observation on liquids of known kinematic viscosity; t = time in seconds for meniscus to pass through the two specified marks.

3.8. - Determination of Total Solids:

Determination of total solids in Asava/ Aristha is generally required. Asava/ Aristha containing sugar or honey should be examined by method 1, sugar or honey free Asava/ Aristha and other material should be examined by method 2.

Method 1: Transfer accurately 50 ml of the clear Asava/ Aristha an evaporable dish and evaporate to a thick extract on a water bath. Unless specified otherwise, extract the residue with 4 quantities, each of 10 ml, of dehydrated ethanol with stirring and filter. Combine the filtrates to another evaporating dish which have been dried to a constant weight and evaporate nearly to dryness on a water bath, add accurately 1 g of diatomite (dry at 105^0 for 3 hours and cooled in a desiccator for 30 min), stir thoroughly, dry at 105^0 for 3 hours, cool the dish in a desiccator for 30 min, and weigh immediately. Deduct the weight of diatomite added, the weight of residue should comply with the requirements stated under the individual monograph.

Method 2: Transfer accurately 50 ml of the clear Asava/ Aristha to an evaporable dish, which has been dried to a constant weight and evaporate to dryness on a water bath, then dry at 105^0 for 3 hours. After cooling the dish containing the residue in a desiccator for 30 min, weigh it immediately. The weight of residue should comply with the requirements stated under the individual monograph.

3.9. - Solubility in Water:

Take 100 ml of distil water in a *Nessler cylinder* and add air-dried and coarsely powdered drug up to saturation. Then stir the sample continuously by twirling the spatula (rounded end of a microspatula) rapidly. After 1 minute, filter the solution using Hirsch funnel, evaporate the filtrate to dryness in a tared flat bottomed shallow dish and dry at 105^0 to constant weight and calculate the solubility of the drug in water (wt. in mg/100ml).

3.10. - Determination of Saponification Value:

The saponification value is the number of mg of potassium hydroxide required to neutralize the fatty acids, resulting from the complete hydrolysis of 1 g of the oil or fat, when determined by the following method :

Dissolve 35 to 40 g of potassium hydroxide in 20 ml water, and add sufficient alcohol to make 1,000 ml. Allow it to stand overnight, and pour off the clear liquor.

Weigh accurately about 2 g of the substance in a tared 250 ml flask, add 25 ml of the alcoholic solution of potassium hydroxide, attach a reflux condenser and boil on a water-bath for one hour, frequently rotating the contents of the flask cool and add 1 ml of solution of phenolphthalein and titrate the excess of alkali with 0.5 N hydrochloric acid. Note the number of ml required (a). Repeat the experiment with the same quantities of the same reagents in the manner omitting the substance. Note the number of ml required (b) Calculate the saponification value from the following formula:—

$$\text{Saponification Value} = \frac{(b-a) \times 0.02805 \times 1.000}{W}$$

Where 'W' is the weight in g of the substance taken.

3.11. Determination of Iodine Value:

The Iodine value of a substance is the weight of iodine absorbed by 100 part by weight of the substance, when determined by one of the following methods:-

Iodine Flasks—The Iodine flasks have a nominal capacity of 250 ml.

A. Iodine Monochloride Method—Place the substance accurately weighed, in dry iodine flask, add 10 ml of *carbon tetrachloride*, and dissolve. Add 20 ml of iodine monochloride solution, insert the stopper, previously moistened with solution of potassium iodine and allow to stand in a dark place at a temperature of about 17^0 or thirty minutes. Add 15 ml of solution of potassium iodine and 100 ml water; shake, and titrate with 0.1 N sodium thiosulphate, using solution of starch as indicator. Note the number of ml required (a). At the same time carry out the operation in exactly the same manner, but without the substance being tested, and note the number of ml of *0.1 N sodium thiosulphate* required (b).

Calculate the iodine value from the formula:—

$$\text{Iodine value} = \frac{(b-a) \times 0.01269 \times 100}{W}$$

Where 'W' is the weight in g of the substance taken.

The approximate weight, in g, of the substance to be taken may be calculated by dividing 20 by the highest expected iodine value. If more than half the available halogen is absorbed, the test must be repeated, a smaller quantity of the substance being used.

Iodine Monochloride Solution: The solution may be prepared by either of the two following methods:

(1) Dissolve 13 g of iodine in a mixture of 300 ml of carbon tetrachloride and 700 ml of glacial acetic acid. To 20 ml of this solution, add 15 ml of *solution of potassium iodide* and 100 ml of *water*, and titrate the solution with *0.1 N sodium thiosulphate*. Pass chlorine, washed and dried, through the remainder of the iodine solution until the amount of 0.1 N sodium thiosulphate required for the titration is approximately, but more than, doubled.

(2)

Iodine trichloride	8 g
Iodine	9 g
Carbon tetrachloride	300 ml
Glacial acetic acid, sufficient to produce	1000 ml

Dissolve the iodine trichloride in about 200 ml of glacial acetic acid, dissolve the iodine in the carbon tetrachloride, mix the two solutions, and add sufficient glacial acetic acid to produce 1000 ml. Iodine Monochloride Solution should be kept in a stoppered bottle, protected from light and stored in a cool place.

B. Pyridine Bromide Method—Place the substance, accurately weighed, in a dry iodine flask, add 10 ml of *carbon tetrachloride* and dissolve. Add 25 ml of pyridine bromide solution, allow to stand for ten minutes in a dark place and complete the determination described under iodine monochloride method, beginning with the words. Add 15 ml.

The approximate weight in gram, of the substance to be taken may be calculated by dividing 12.5 by the highest expected iodine value. If more than half the available halogen is absorbed the test must be repeated, a small quantity of the substance being used.

Pyridine bromide Solution: Dissolve 8 g pyridine and 10 g of *sulphuric acid* in 20 ml of *glacial acetic acid*, keeping the mixture cool. Add 8 g of *bromine* dissolved in 20 ml of *glacial acetic acid* and dilute to 100 ml with *glacial acetic acid*.

Pyridine bromide Solution should be freshly prepared.

3.12. - Determination of Acid Value:

The acid value is the number of mg of *potassium hydroxide* required to neutralize the free acids in 1 g of the substance, when determined by the following method:

Weigh accurately about 10 g of the substance (1 to 5) in the case of a resin into a 250 ml flask and add 50 ml of a mixture of equal volumes of alcohol and solvent ether, which has been neutralized after the addition of 1 ml of solution of phenolphthalein. Heat gently on a water-bath, if necessary until the substance has completely melted, titrate with *0.1 N potassium hydroxide*, shaking constantly until a pink colour which persists for fifteen seconds is obtained. Note the number of ml required. Calculate the acid value from the following formula:

$$\text{Acid Value} = \frac{a \times 0.00561 \times 1000}{W}$$

Where 'a' is the number of ml of *0.1 N potassium hydroxide* required and 'W' is the weight in g of the substance taken.

3.13. - Determination of Peroxide Value:

The peroxide value is the number of milliequivalents of active oxygen that expresses the amount of peroxide contained in 1000 g of the substance.

Method

Unless otherwise specified in the individual monograph, weigh 5 g of the substance being examined, accurately weighed, into a 250-ml glass-stoppered conical flask, add 30 ml of a mixture of 3 volumes of *glacial acetic acid* and 2 volumes of *chloroform,* swirl until dissolved and add 0.5ml volumes of saturated *potassium iodide soluton.* Allow to stand for exactly 1 minute, with occasional shaking, add 30 ml of *water* and titrate gradually, with continuous and vigorous shaking, with *0.01M sodium thiosulphate* until the yellow colour almost disappears. Add 0.5 ml of *starch solution* and continue the titration, shaking vigorously

until the blue colour just disappears (a ml). Repeat the operation omitting the substance being examined (b ml). The volume of *0.01M sodium thiosulphate* in the blank determination must not exceed 0.1 ml.

Calculate the peroxide value from the expression

Peroxide value = 10 (a – b)/*W*

Where *W* = weight, in g, of the substance.

3.14. - Determination of Unsaponifiable Matter:

The unsaponifiable matter consists of substances present in oils and fats, which are not saponifiable by alkali hydroxides and are determined by extraction with an organic solvent of a solution of the saponified substance being examined.

Method

Unless otherwise specified in the individual monograph, introduce about 5 g of the substance being examined, accurately weighed, into a 250-ml flask fitted with a reflux condenser. Add a solution of 2 g of *potassium hydroxide* in 40 ml of *ethanol (95per cent)* and heat on a water-bath for 1 hour, shaking frequently. Transfer the contents of the flask to a separating funnel with the aid of 100 ml of hot water and, while the liquid is still warm, shake very carefully with three quantities, each of 100 ml, of *peroxide-free ether.* Combine the ether extracts in a second separating funnel containing 40 ml of water, swirl gently for a few minute, allow to separate and reject the lower layer. Wash the ether extract with two quantities, each of 40 ml, of water and with three quantities, each of 40 ml, of a 3 per cent w/v solution of *potassium hydroxide,* each treatment being followed by a washing with 40 ml of water. Finally, wash the ether layer with successive quantities, each of 40 ml, of water until the aqueous layer is not alkaline to *phenolphthalein solution.* Transfer the ether layer to a weighed flask, washing out the separating funnel with *peroxide-free ether.* Distil off the ether and add to the residue 6 ml of *acetone.* Remove the solvent completely from the flask with the aid of a gentle current of air. Dry at 100^0 to 105^0 for 30 minutes. Cool in a desiccator and weigh the residue. Calculate the unsaponifiable matter as per cent w/w.

Dissolve the residue in 20 ml of *ethanol (95per cent)*, previously neutralised to *phenolphthalein solution* and titrate with *0.1M ethanolic potassium hydroxide.* If the volume of *0.1M ethanolic potassium hydroxide* exceeds 0.2 ml, the amount weighed cannot be taken as the unsaponifiable matter and the test must be repeated.

3.15. - Detection of Mineral Oil (Holde's Test):

Take 22 ml of the alcoholic potassium hydroxide solution in a conical flask and add 1ml of the sample of the oil to be tested. Boil in a water bath using an air or water cooled condenser till the solution becomes clear and no oily drops are found on the sides of the flask. Take out the flask from the water bath, transfer the contents to a wide mouthed warm test tube and carefully add 25ml of boiling distilled water along the side of the test tube. Continue shaking the tube

lightly from side to side during the addition. The turbidity indicates presence of mineral oil, the depth of turbidity depends on the percentage of mineral oil present.

3.16. - Rancidity Test (Kreis Test):

The test depends upon the formation of a red colour when oxidized fat is treated with conc. *hydrochloric acid* and a solution of phloroglucinol in ether. The compound in rancid fats responsible for the colour reaction is epihydrin aldehyde. All oxidized fats respond to the Kreis test and the intensity of the colour produced is roughly proportional to the degree of oxidative rancidity.

Procedure

Mix 1 ml of melted fat and 1 ml of conc. *hydrochloric acid* in a test tube. Add 1 ml of a 1 per cent solution of phloroglucinol in *diethyl ether* and mix thoroughly with the fat-acid mixture. A pink colour formation indicates that the fat is slightly oxidized while a red colour indicates that the fat is definitely oxidized.

3.17. - Determination of Alcohol Content:

The ethanol content of a liquid is expressed as the number of volumes of ethanol contained in 100 volumes of the liquid, the volumes being measured at 24.9^0 to 25.1^0. This is known as the "percentage of ethanol by volume". The content may also be expressed in g of ethanol per 100 g of the liquid. This is known as the 'percentage of ethanol by weight".

Use Method I or Method II, as appropriate, unless otherwise specified in the individual monograph.

Method I

Carry out the method for gas chromatography, using the following solutions. Solution (1) contains 5.0 per cent v/v of ethanol and 5.0 per cent v/v of 1-propanol (internal standard). For solution (2) dilute a volume of the preparation being examined with water to contain between 4.0 and 6.0 per cent v/v of ethanol. Prepare solution (3) in the same manner as solution (2) but adding sufficient of the internal standard to produce a final concentration of 5.0 per cent v/v.

The chromatographic procedure may be carried out using a column (1.5 m x 4 mm) packed with porous polymer beads (100 to 120 mesh) and maintained at 150^0, with both the inlet port and the detector at 170^0, and nitrogen as the carrier gas.

Calculate the percentage content of ethanol from the areas of the peaks due to ethanol in the chromatogram obtained with solutions (1) and (3).

Method II

For preparations where the use of Industrial Methylated Spirit is permitted in the monograph, determine the content of ethanol as described in Method I but using as solution (2)

a volume of the preparation being examined diluted with water to contain between 4.0 and 6.0 per cent v/v of total ethanol and methanol.

Determine the concentration of methanol in the following manner. Carry out the chromatographic procedure described under Method I but using the following solutions. Solution (1) contains 0.25 per cent v/v of methanol and 0.25 per cent v/v of 1-propanol (internal standard). For solution (2) dilute a volume of the preparation being examined with water to contain between 0.2 per cent and 0.3 per cent v/v of methanol. Prepare solution (3) in the same manner as solution (2) but adding sufficient of the internal standard to produce a final concentration of 0.25 per cent v/v.

The sum of the contents of ethanol and methanol is within the range specified in the individual monograph and the ration of the content of methanol to that of ethanol is commensurate with Industrial Methylated Spirit having been used.

Method III

This method is intended only for certain liquid preparations containing ethanol. Where the preparation contains dissolved substances that may distil along with ethanol Method III B or III C must be followed.

Apparatus

The apparatus (see Fig. 3) consists of a round-bottomed flask (A) fitted with a distillation head (B) with a steam trap and attached to a vertical condenser (C). A tube is fitted to the lower part of the condenser and carries the distillate into the lower part of a 100-ml or 250-ml volumetric flask (D). The volumetric flask is immersed in a beaker (E) containing a mixture of ice and water during the distillation. A disc with a circular aperture, 6 cm in diameter, is placed under the distillation flask (A) to reduce the risk of charring of any dissolved substances.

Method III A

Transfer 25 ml of the preparation being examined, accurately measured at 24.9^0 to 25.1^0, to the distillation flask. Dilute with 150 ml of water and add a little pumice powder. Attach the distillation head and condenser. Distil and collect not less than 90 ml of the distillate into a 100-ml volumetric flask. Adjust the temperature to 24.9^0 to 25.1^0 and dilute to volume with distilled water at 24.9^0 to 25.1^0. Determine the relative density at 24.9^0 to 25.1^0. The values indicated in column 2 of Table 17 are multiplied by 4 in order to obtain the percentage of ethanol by volume contained in the preparation. If the specific gravity is found to be between two values, the percentage of ethanol should be obtained by interpolation. After calculation of the ethanol content, report the result to one decimal place.

NOTE – (1) If excessive frothing is encountered during distillation, render the solution strongly acid with phosphoric acid or treat with a small amount of liquid paraffin or silicone oil.

(2) The distillate should be clear or not more than slightly cloudy. If it is turbid or contains oily drops, follow Method IIIC. When steam-volatile acids are present, make the solution just alkaline with *1M sodium hydroxide* using solid *phenolphthalein* as indicator before distillation.

Method III B

Follow this method or the following one if the preparation being examined contains appreciable proportions of volatile materials other than ethanol and water.

Mix 25 ml of the preparation, accurately measured at 24^0 to 25.1^0, with about 100 ml of water in a separating funnel. Saturate this mixture with sodium chloride, add about 100 ml of *hexane* and shake vigorously for 2 to 3 minutes. Allow the mixture to stand for 15 to 20 minutes. Run the lower layer into the distillation flask, wash the *hexane* layer in the separating funnel by shaking vigorously with about 25 ml of *sodium chloride* solution, allow to separate and run the wash liquor into the first saline solution. Make the mixed solutions just alkaline with 1M sodium hydroxide using solid phenolphthalein as indicator, add a little pumice powder and 100 ml of water, distil 90 ml and determine the percentage v/v of ethanol by Method IIIA beginning at the words “Adjust the temperature…”.

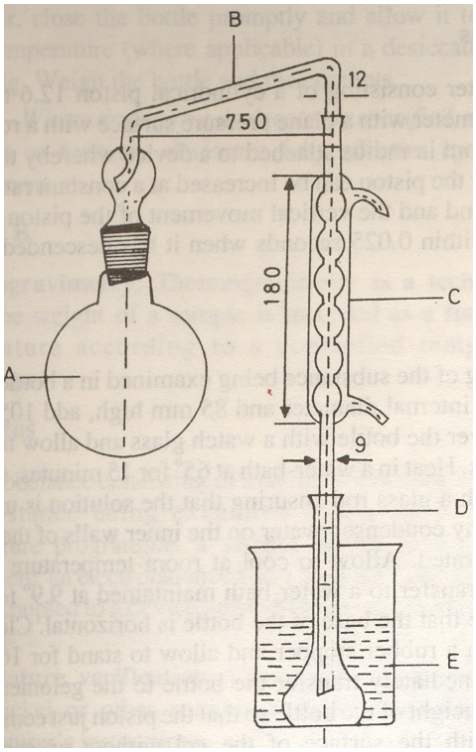

Fig.3 Apparatus for Determination of Ethanol by Distillation Method

Table 17

Specific gravity at 25^0	Ethanol content*
1.0000	0
0.9985	1
0.9970	2
0.9956	3
0.9941	4
0.9927	5
0.9914	6
0.9901	7
0.9888	8
0.9875	9
0.9862	10
0.9850	11
0.9838	12
0.9826	13
0.9814	14
0.9802	15
0.9790	16
0.9778	17
0.9767	18
0.9756	19
0.9744	20
0.9733	21
0.9721	22
0.9710	23
0.9698	24
0.9685	25

* per cent v/v at 15.56^0.

Method III C

Transfer 25 ml of the preparation, accurately measured at 24.9^0 to 25.1^0, to the distillation flask. Dilute with 150 ml of water and add a little pumice powder. Attach the distillation head and condenser. Distil and collect about 100 ml. Transfer to a separating funnel and determine the percentage v/v of ethanol by Method III B beginning at the words “Saturate this mixture…”.

APPENDIX –4

REAGENTS AND SOLUTIONS

Acetic Acid – Contains approximately 33 per cent w/v of C2H4O2. Dilute 315 ml of glacial acetic acid to 1000 ml with water.

Acetic Acid, Glacial – CH_3COOH =60.05.

Contains not less than 99.0 per cent w/w of $C_2H_4O_2$. About 17.5 N in strength.

Description **–** At temperature above its freezing point a clear colourless liquid, odour, pungent and characteristic; crystallises when cooled to about 10^0 and does not completely re-melt until warmed to about 15^0.

Solubility – Miscible with *water,* with *glycerin* and most fixed and volatile oils.

Boiling range –Between 117^0 and 119^0.

Congealing temperature –Not lower than 14.8^0.

Wt. per ml –At 25^0about 1.047 g.

Heavy metals –Evaporate 5 ml to dryness in a porcelain dish on water-bath, warm the residue with 2 ml of 0.1 *N hydrochloric acid* and water to make 25 ml; the limit of heavy metals is 10 parts per million, Appendix 2.3.3.

Chloride –5 ml complies with the limit test for chlorides, Appendix 2.3.2.

Sulphate –5 ml complies with the limit test for sulphates,

Certain aldehydic substances – To 5 ml add 10 ml of *mercuric chloride solution* and make alkaline with *sodium hydroxide solution,* allow to stand for five minutes and acidify with dilute *sulphuric acid*; the solution does not show more than a faint turbidity.

Formic acid and oxidisable impurities – Dilute 5 ml with 10 ml of water, to 5 ml of this solution add 2.0 ml of 0.1 *N* potassium dichromate and 6 ml of sulphuric acid, and allow to stand for one minute, add 25 ml of water, cool to 15^0, and add 1 ml of freshly prepared potassium iodide solution and titrate the liberated iodine with 0.1 *N* sodium thiosulphate, using starch solution as indicator. Not less than 1 ml of 0.*N sodium thiosulphate* is required.

Odorous impurities –Neutralise 1.5 ml with *sodium hydroxide solution*; the solution has no odour other than a faint acetous odour.

Readily oxidisable impurities – To 5 ml of the solution prepared for the test for Formic Acid and Oxidisable Impurities, add 20 ml of water and 0.5 ml of 0.1 *N potassium permanganate;* the pink colour does not entirely disappear within half a minute.

Non-volatile matter – Leaves not more than 0.01 per cent w/w of residue when evaporated to dryness and dried to constant weight at 105^0.

Assay –Weigh accurately about 1 g into a stoppered flask containing 50 ml of *water* and titrate with *N sodium hydroxide,* using *phenolphthalein solution* as indicator. Each ml of *sodium hydroxide* is equivalent to 0.06005 g of $C_2H_4O_2$.

Acetic Acid, Lead-Free –Acetic acid which complies with following additional test, boil 25 ml until the volume is reduced to about 15 ml, cool make alkaline with lead-free ammonia solution, add 1 ml of lead free *potassium cyanide solution, dilute* to 50 ml with water, add 2 drops of *sodium sulphide solution;* no darkening is produced.

Acetone – Propan-2-one; $(CH_3)_2CO$ = 58.08

Description – Clear, colourless, mobile and volatile liquid; taste, pungent and sweetish; odour characteristic; flammable.

Solubility –Miscible with *water*, with alcohol, with *solvent ether,* and with *chloroform,* forming clear solutions.

Distillation range – Not less than 96.0 per cent distils between 55.5^0 and 57^0.

Acidity– 10 ml diluted with 10 ml of freshly boiled and cooled water; does not require for neutralisation more than 0.2 ml of 0.1 *N sodium hydroxide*, using phenolphthalein solution as indicator.

Alkalinty – 10 ml diluted with 10 ml of freshly boiled and cooled water, is not alkaline to litmus solution.

Methyl alcohol –Dilute 10 ml with water to 100 ml. To 1 ml of the solution add 1 ml of *water* and 2 ml of *potassium permanganate* and *phosphoric acid solution*. Allow to stand for ten minutes and add 2 ml of *oxalic acid* and *sulphuric acid solution;* to the colourless solution add 5 ml of *decolorised magenta solution* and set aside for thirty minutes between 15^0 and 30^0; no colour is produced.

Oxidisable substances –To 20 ml add 0.1 ml of 0.1 *N potassium permanganate,* and allow to stand for fifteen minutes; the solution is not completely decolorised.

Water – Shake 10 ml with 40 ml of *carbon disulphide*; a clear solution is produced.

Non-volatile matter –When evaporated on a water-bath and dried to constant weight at 105^0, leaves not more than 0.01 per cent w/v residue.

Acetone Solution, Standard – A 0.05 per cent v/v solution of acetone in water.

Alcohol –

Description – Clear, colourless, mobile, volatile liquid, odour, characteristic and spirituous; taste, burning, readily volatilised even at low temperature, and boils at about 78^0, flammable. Alcohol containing not less than 94.85 per cent v/v and not more than 95.2 per cent v/v of C_2H_5OH at 15.56^0.

Solubility –Miscible in all proportions with *water,* with *chloroform* and with *solvent ether.*

Acidity or alkalinity – To 20 ml add five drops of *phenolphthalein solution*; the solution remains colourless and requires not more than 2.0 ml of 0.1*N sodium hydroxide* to produce a pink colour.

Specific gravity –Between 0.8084 and 0.8104 at 25^0.

Clarity of solution –Dilute 5 ml to 100 ml with *water* in glass cylinder; the solution remains clear when examined against a black background. Cool to 10^0 for thirty minutes; the solution remains clear.

Methanol – To one drop add one of water, one drop of *dilute phosphoric acid,* and one drop of *potassium permanganate solution.* Mix, allow to stand for one minute and add sodium bisulphite solution dropwise, until the permanganate colour is discharged. If a brown colour remains, add one drop of *dilute phosphoric* acid. To the colourless solution add 5 ml of freshly prepared *chromotropic acid* solution and heat on a water-bath at 60^0 for ten minutes; no violet colour is produced.

Foreign organic substances – Clean a glass-stoppered cylinder thoroughly with *hydrochloric acid,* rinse with water and finally rinse with the alcohol under examination. Put 20 ml in the cylinder, cool to about 15^0 and then add from a carefully cleaned pipette 0.1 ml 0.1 *N potassium permanganate.* Mix at once by inverting the stoppered cylinder and allow to stand at 15^0 for five minutes; the pink colour does not entirely disappear.

Isopropyl alcohol and t-butyl alcohol – To 1 ml add 2 ml of water and 10 ml of *mercuric sulphate solution* and heat in a boiling water-bath; no precipitate is formed within three minutes.

Aldehydes and ketones – Heat 100 ml of *hydroxylamine hydrochloride solution* in a loosely stoppered flask on a water-bath for thirty minutes, cool, and if necessary, add sufficient 0.05 *N sodium hydroxide* to restore the green colour. To 50 ml of this solution add 25 ml of the alcohol and heat on a water bath for ten minutes in a loosely stoppered flask. Cool, transfer to a *Nesseler cylinder*, and titrate with 0.05 *N sodium hydroxide* until the colour matches that of the remainder of the *hydroxylamine hydrochloride solution* contained in a similar cylinder, both solutions being viewed down the axis of the cylinder. Not more than 0.9 ml of 0.05 *N sodium hydroxide* is required.

Fusel oil constituents – Mix 10 ml with 5 ml of *water* and 1 ml of *glycerin* and allow the mixture to evaporate spontaneously from clean, odourless absorbent paper; no foreign odour is perceptible at any stage of the evaporation.

Non-volatile matter – Evaporate 40 ml in a tared dish on a water-bath and dry the residue at 105º for one hour; the weight of the residue does not exceed 1 mg.

Storage – Store in tightly-closed containers, away from fire.

Labelling – The label on the container states "Flammable".

Alcohol, Aldehyde-free. –Alcohol which complies with the following additional test :

Aldehyde – To 25 ml, contained in 300 ml flask, add 75 ml of *dinitrophenyl hydrazine solution,* heat on a water bath under a reflux condenser for twenty four hours, remove the alcohol by distillation, dilute to 200 ml with a 2 per cent v/v solution of sulphuric acid, and set aside for twenty four hours; no crystals are produced.

Alcohol, Sulphate-free. –Shake alcohol with an excess of anion exchange resin for thirty minutes and filter.

Ammonia, XN. –Solutions of any normality xN may be prepared by diluting 75 x ml of strong ammonia solution to 1000 ml with water.

Ammonia Solution, Iron-free –Dilute ammonia solution which complies with the following additional test :-

Evaporate 5 ml nearly to dryness on a water-bath add 40 ml of water, 2 ml of 20 per cent w/v *solution of iron free citric acid* and 2 drops of *thioglycollic acid*, mix, make alkaline with *iron-free ammonia solution and dilute* to 50 ml with *water*, no pink colour is produced.

Ammonium Chloride Solution –A 10.0 per cent w/v solution of *ammonium chloride* in water.

Ammonium molybdate- $NH_4Mo_7O_{24}.4H_2O$=1235.86
Analytical reagent grade of commerce.
White crystal or crystalline masses, sometimes with a yellowish or green tint.

Ammonium Thiocyanate – NH_4SCN = 76.12.

Description –Colourless crystals.

Solubility – Very soluble in water, forming a clear solution, readily soluble in alcohol.

Chloride –Dissolve 1 g in 30 ml of solution of hydrogen peroxide, add 1 g of *sodium hydroxide*, warm gently, rotate the flask until a vigorous reaction commences and allow to stand until the reaction is complete; add a further 30 ml of *hydrogen peroxide solution* boil for two minutes, cool, and add 10 ml of *dilute nitric acid* and 1 ml of *silver nitrate solution*; any opalescence produced is not greater than that obtained by treating 0.2 ml of 0.01 *N hydrochloric acid* in the same manner.

Sulphated ash –Moisten 1 g with *sulphuric acid* and ignite gently, again moisten with sulphuric acid and ignite; the residue weighs not more than 2.0 mg.

Ammonium Thiocyanate, 0.1N – NH_4SCN = 76.12; 7.612 in 1000 ml. Dissolve about 8 g of *ammonium thiocyanate* in 100o ml of water and standardise the solution as follows :

Pipette 30 ml of standardised 0.1 *N silver nitrate* into a glass stoppered flask, dilute with 50 ml of *water* then add 2 ml of *nitric acid* and 2 ml of *ferric ammonium sulphate solution* and titrate with the *ammonium thiocyanate solution* to the first appearance of a red brown colour. Each ml of 0.1N *silver nitrate* is equivalent to 0.007612 g of NH_4SCN.

Ammonium Thiocyanate Solution – A 10.0 per cent w/v solution of *ammonium thiocyanate solution*.

Anisaldehyde-Sulphuric Acid Reagent – 0.5 ml *anisaldehyde* is mixed with 10 ml *glacial acetic acid*, followed by 85 ml methanol and 5 ml concentrated *sulphuric acid* in that order.

The reagent has only limited stability and is no longer usable when the colour has turned to redviolet.

Arsenomolybdic Acid Reagent- 250 mg of ammonium molybdate was dissolved in 45 ml of distilled water. To this, 2.1 ml of concentrated H_2SO_4 was added and mixed well. To this solution, 3mg of $Na_2ASO_4.7\ H_2O$ dissolved in 25 ml of distilled water, mixed well and placed in incubator maintained at 37^0 C for 24 h.

Borax - Sodium Tetraborate, $Na_2B_4O_7.\ 10H_2O$ = 381.37.
Contains not less than 99.0 per cent and not more than the equivalent of 103.0 per cent of $Na_2B_4O_7.\ 10H_2O$.

Description –Transparent, colourless crystals, or a white, crystalline powder; odourless, taste, saline and alkaline. Effloresces in dry air, and on ignition, loses all its water of crystallisation.

Solubility –Soluble in water, practically insoluble in alcohol.

Alkalinity –A solution is alkaline to litmus solution.

Heavy metals – Dissolve 1 g in 16 ml of water and 6 ml of *N hydrochloric acid* and add *water* to make 25 ml; the limit of heavy metals is 20 parts per million, Appendix 2.3.3.

Iron –0.5 g complies with the *limit test for iron*, Appendix 2.3.4.

Chlorides –1 g complies with the *limit test for chlorides,* Appendix 2.3.2.

Sulphates –1g complies with the *limit test for sulphates,* Appendix 2.3.6.

Assay –Weigh accurately about 3 g and dissolve in 75 ml of *water* and titrate with 0.5 *N hydrochloric acid*, using *methyl red solution* as indicator. Each ml of 0.5 *N* hydrochloric acid is equivalent to 0.09534 g of $Na_2B_4O_7.10H_2O$.

Storage – Preserve Borax in well-closed container.

Bromine – Br_2 =159.80.

Description –Reddish-brown, fuming, corrosive liquid.

Solubility –Slightly soluble in *water*, soluble in most organic solvents.

Iodine –Boil 0.2 ml with 20 ml of *water*, 0.2 ml of *N sulphuric acid* and a small piece of marble until the liquid is almost colourless. Cool, add one drop of *liquefied phenol*, allow to stand for two minutes, and then add 0.2 g of *potassium iodide* and 1 ml of *starch solution*; no blue colour is produced.

Sulphate –Shake 3 ml with 30 ml of *dilute ammonia solution* and evaporate to dryness on a water bath, the residue complies with the *limit test for sulphates*, Appendix 2.3.6.

Bromine Solution – Dissolve 9.6 ml of *bromine* and 30 g of *potassium bromide* in sufficient *water* to produce 100 ml.

Canada Balsam Reagent –General reagent grade of commerce.

Carbon Tetrachloride – CCl_4 = 153.82

Description –Clear, colourless, volatile, liquid; odour, characteristic.

Solubility –Practically insoluble in water; miscible with ethyl alcohol, and with solvent ether.

Distillation range –Not less than 95 per cent distils between 76^0 and 77^0.

Wt. per ml – At 20^0, 1.592 to 1.595 g.

Chloride, *free acid* –Shake 20 ml with 20 ml of freshly boiled and cooled water for three minutes and allow separation to take place; the aqueous layer complies with the following test :

Chloride – To 10 ml add one drop of nitric acid and 0.2 ml of *silver nitrate solution*; no opalescence is produced.

Free acid –To 10 ml add a few drops of *bromocresol purple solution;* the colour produced does not indicate more acidity than that indicated by the addition of the same quantity of the indicator to 10 ml of freshly boiled and cooled *water.*

Free chlorine –Shake 10 ml with 5 ml of *cadmium iodide solution* and 1 ml *of starch solution*, no blue colour is produced.

Oxidisable impurities –Shake 20 ml for five minutes with a cold mixture of 10 ml of *sulphuric acid* and 10 ml of 0.1 *N potassium dichromate*, dilute with 100 ml of water and add 3 g of *potassium iodide* : the liberated iodine requires for decolourisation not less than 9 ml of 0.1 *N sodium thiosulphate*.

Non-volatile matter –Leaves on evaporation on a water-bath and drying to constant weight at 105^0 not more than 0.002 per cent w/v of residue.

Caustic Alkali Solution, 5 per cent – Dissolve 5 g of *potassium or sodium hydroxide* in *water* and dilute to 100 ml.

Charcoal, Decolourising –General purpose grade complying with the following test.

Decolourising powder –Add 0.10 g to 50 ml of 0.006 per cent w/v solution of *bromophenol blue* in ethanol (20 per cent) contained in a 250 ml flask, and mix. Allow to stand for five minutes, and filter; the colour of the filtrate is not deeper than that of a solution prepared by diluting 1 ml of the *bromophenol blue solution* with *ethanol* (20 per cent) to 50 ml.

Chloral Hydrate –$CCl_3.CH(OH)_2$ = 165.40.

Description –Colourless, transparent crystals, odour, pungent but not acrid; taste, pungent and slightly bitter, volatilises slowly on exposure to air.

Solubility –Very soluble in *water,* freely soluble in *alcohol*, in chloroform and in *solvent ether*.

Chloral alcoholate – Warm 1 g with 6 ml of *water* and 0.5 ml of *sodium hydroxide solution* : filter, add sufficient 0*.1 N iodine* to impart a deep brown colour, and set aside for one hour; no yellow crystalline precipitate is produced and no smell of iodoform is perceptible.

Chloride – 3 g complies with the limit test for chlorides, Appendix 2.3.2.

Assay – Weigh accurately about 4 g and dissolve in 10 ml of *water* and add 30 ml of *N sodium hydroxide.* Allow the mixture to stand for two minutes, and then titrate with *N sulphuric acid* using *phenolphthalein solution* as indicator. Titrate the neutralised liquid with 0.1 *N silver nitrate* using solution of *potassium chromate* as indicator. Add two-fifteenth of the amount of 0.1 *N silver nitrate* used to the amount of *N sulphuric acid* used in the first titration and deduct the figure so obtained from the amount of *N sodium hydroxide* added. Each ml of *N sodium hydroxide,* obtained as difference; is equivalent to 0.1654 g of $C_2H_3Cl_3O_2$.

Storage – Store in tightly closed, light resistant containers in a cool place.

Chloral Hydrate Solution –Dissolve 20 g of *chloral hydrate* in 5 ml of water with warming and add 5 ml of *glycerin*.

Chloral Iodine Solution –Add an excess of crystalline *iodine with* shaking to the *chloral hydrate solution*, so that crystals of undissolved iodine remain on the bottom of bottle. Shake before use as the iodine dissolves, and crystals of the iodine to the solution. Store in a bottle of amber glass in a place protected from light.

Chloroform – $CHCl_3$ = 119.38

Description –Colourles, volatile liquid; odour, characteristic. Taste, sweet and burning.

Solubility –Slightly soluble in water; freely miscible with ethyl alcohol and with solvent ether.

Wt. per ml. : Between 1.474 and 1.478 g.

Boiling range – A variable fraction, not exceeding 5 per cent v/v, distils below 60^0 and the remainder distils between 50^0 to 62^0.

Acidity –Shake 10 ml with 20 ml of freshly boiled and cooled water for three minutes, and allow to separate. To a 5 ml portion of the aqueous layer add 0.1 ml of *litmus solution*; the colour produced is not different from that produced on adding 0.1 ml of *litmus solution* to 5 ml of freshly boiled and cooled water.

Chloride –To another 5 ml portion of the aqueous layer obtained in the test for Acidity, add 5 ml of water and 0.2 ml of *silver nitrate solution*; no opalescence is produced.

Free chlorine –To another 10 ml portion of the aqueous layer, obtained in the test for Acidity, add 1 ml of *cadmium iodide solution* and two drops of starch solution; no blue colour is produced.

Aldehyde –Shake 5 ml with 5 ml of water and 0.2 ml of *alkaline potassium mercuri-iodide solution* in a stoppered bottle and set aside in the dark for fifteen minutes; not more than a pale yellow colour is produced.
Decomposition products – Place 20 ml of the *chloroform* in a glass-stoppered flask, previously rinsed with *sulphuric acid,* add 15 ml of *sulphuric acid* and four drops of *formaldehyde solution*, and shake the mixture frequently during half an hour and set aside for further half an hour, the flask being protected from light during the test; the acid layer is not more than slightly coloured.

Foreign organic matter – Shake 20 ml with 10 ml of *sulphuric* acid in a stoppered vessel previously rinsed with *sulphuric acid* for five minutes and set aside in the dark for thirty minutes, both the acid and chloroform layers remain colourless. To 2 ml of the acid layer add 5 ml of water; the liquid remains colourless and clear, and has no unpleasent odour. Add a further 10 ml of water and 0.2 ml of *silver nitrate solution*; no opalescence is produced.

Foreign odour –Allow 10 ml to evaporate from a large piece of filter paper placed on a warm plate; no foreign odour is detectable at any stage of the evaporation.

Non volatile matter – Not more than 0.004 per cent w/v determined on 25 ml by evaporation and drying at 105^0.

Storage : Store in tightly-closed, glass-stoppered, light-resistant bottles.

Copper Sulphate – CuSO4.5H2O = 249.68

Contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of $CuSO_4.5H_2O$.

Description –Blue triclinic prisms or a blue, crystalline powder.

Solubility –Soluble in *water*, very solube in boiling water, almost insoluble in *alcohol*; very slowly soluble in glycerin.

Acidity and clarity of solution – 1 g, dissolved in 20 ml of water, forms a clear blue solution, which becomes green on the addition of 0.1 ml of *methyl orange solution*.

Iron – To 5 g, add 25 ml of water, and 2 ml of nitric acid, boil and cool. Add excess of *strong ammonia solution*, filter, and wash the residue with *dilute ammonia solution* mixed with four times its volumes of water. Dissolve the residue, if any, on the filter with 2 ml of *hydrochloric acid*, diluted with 10 ml of water; to the acid solutions add *dilute ammonia solution* till the precipitation is complete; filter and wash; the residue after ignition weighs not more than 7 mg.

Copper Sulphate, Anhydrous – $CuSO_4$ =159.6

Prepared by heating copper sulphate to constant weight at about 230^0.

Copper Sulphate Solution –A10.0 per cent w/v solution of *copper sulphate* in *water*.
Cresol Red – 4,4/, –(3H-2, 1-Benzoxathiol-3 ylidene) di-O-cresol SS-dioxide; $C_{12}H_8O_5S$ = 382.4.

Gives a red colour in very strongly acid solutions, a yellow colour in less strongly acid and neutral solutions, and a red colour in moderately alkaline solutions (*p*H ranges, 0.2 to 1.8, and 7.2 to 8.8).

Cresol Red Solution –Warm 50 ml of cresol red with 2.65 ml of 0.05 M sodium hydroxide and 5 ml of ethanol (90 per cent); after solution is effected, add sufficient ethanol (20 per cent) to produce 250 ml.

Sensitivity –A mixitue of 0.1 ml of the solution and 100 ml of *carbon dioxide-free water* to which 0.15 ml of 0.02 M s*odium hydroxide* has been added is purplish-red. Not more than 0.15 ml of 0.02 *M hydrochloric acid* is required to change the colour to yellow.

Disodium Ethylenediamine tetraacetate – (Disodium Acetate) C10H14N2Na2O8.2H2O = 372.2 , Analytical reagent grade.

Dragendorff Reagent –

Solution 1 –Dissolve 0.85 g of *bismuth oxy nitrate* in 40 ml of water and 10 ml of acetic acid.

Solution 2 –Dissolve 8 g of *potassium iodide* in 20 ml of water.

Mix equal volumes of solution 1 and 2, and to 10 ml of the resultant mixture add 100 ml of water and 20 ml of acetic acid.

Dithizone;1,5-Diphenylthiocarbazone;Diphenylthiocarbazone;C_6H_5N:NCSNHNHC_6H_5= 56.32
Analytical Reagent grade of commerce.
Almost black powder; mp, about 168^0, with decomposition.
Store in light-resistant containers.

Eosin – Acid Red 87; Tetrabromofluorescein disodium salt; $C_{20}H_6O_5Br_4Na_2$ =691.86.

Description – Red powder, dissolves in water to yield a yellow to *purplish-red* solution with a greenish-yellow fluorescence.

Solubility –Soluble in *water* and in alcohol.

Chloride –Dissolve 50 mg in 25 ml of *water*, add 1 ml of *nitric acid*, and filter; the filtrate complies with *the limit test for chlorides*, Appendix 2.3.2.

*Sulphate*d ash –Not more than 24.0 per cent, calculated with reference to the substance dried at 110^0 for two hours, Appendix 2.2.6.

Eosin Solution –A 0.5 per cent w/v solution of eosin in water.

Eriochrome Black T –Mordant Black 11; Sodium 2(1-hydroxy-2-naphthylazo) 5-nitro-2-naphtol-4-sulphonate; $C_{20}H_{12}N_3NaO_7S$ = 461.38.

Brownish black powder having a faint, metallic sheen, soluble in alcohol, in *methyl alcohol* and in hot water.

Ethyl Acetate –$CH_3.CO_2C_2H_5$ = 88.11.

Analytical reagent grade.

A colourless liquid with a fruity odour; boiling point, about 77^0; weight per ml about 0.90g.

Ethyl Alcohol –C_2H_5OH = 46.07.

Absolute Alcohol; Dehydrated Alcohol.

Description –Clear, colourless, mobile, volatile liquid; odour, characteristic and spirituous; taste, burning; hygroscopic. Readily volatilisable even at low temperature and boils at 78^0 and is flammable.

Solubility –Miscible with water, with solvent ether and with chloroform.

Contains not less than 99.5 per cent w/w or 99.7 per cent v/v of C_2H_5OH.

Identification –Acidity or Alkalinity: Clarity of Solution; Methanol; Foreign organic substances; Isopropyl alcohol and butyl alcohol; Aldehydes and ketones; fusel oil constituents; Non-volatile matter; complies with the requirements described under Alcohol.

Specific gravity –Between 0.7871 and 0.7902, at 25^0.

Storage –Store in tightly closed containers in a cool place away from fire and protected from moisture.

Labelling –The label on the container states "Flammable".

Fehlings Solution –

A. Dissolve 69.278 g of CuSO4. $5H_2O$ in water and make the volume up to 1 litre
B. Dissolve 100 g of sodium hydroxide and 340 g of Sodium potassium tartarate in water and make the volume to 1 litre.

Mix equal volumes of A and B before the experiment.

Formaldehyde Solution –Formalin; HCHO =30.03

Formaldehyde Solution is a solution of formaldehyde in water with *methyl alcohol* added to prevent polymerisation. It contains not less than 34.0 per cent w/w and not more than 38.0 per cent w/w of CH_2O.

Description – Colourless liquid; odour, characteristic, pungent and irritating; taste, burning. A slight white cloudy deposit is formed on long standing, especially in the cold, due to the separation of paraformaldehyde. This white deposit disappears on warming the solution.
Solubility –Miscible with *water*, and with *alcohol*.

Acidity –To 10 ml add 10 ml of *carbon dioxide free water* and titrate with 0.1 *N sodium hydroxide* using *bromothymol blue solutions* as indicator; not more than 5 ml of 0.1 *N sodium hydroxide* is required.

Wt. per ml – At 20^0, 1.079 to 1.094 g.

Assay –Weigh accurately about 3 g and add to a mixture of 50 ml of *hydrogen peroxide solution* and 50 ml of *N sodium hydroxide*, warm on a water-bath until effervescence ceases and titrate the excess of alkali with *N sulphuric acid* using *phenolphthalein solution* as indicator. Repeat the experiment with the same quantities of the same reagents in the same manner omitting the formaldehyde solution. The difference between the titrations represents the sodium hydroxide required to neutralise the formic acid produced by the oxidation of the formaldehyde. Each ml of N sodium hydroxide is equivalent to 0.03003 g of CH_2O.

Storage–Preserve Formaldehyde Solution in well-closed container preferably at a temperature not below 15^0.

Formaldehyde Solution, Dilute –

Dilute 34 ml of *formaldehyde solution* with sufficient water to produce 100 ml.

Folin Ciocalteu reagent- Dilute commercially available Folin-Ciocalteu reagent (2N) with an equal volume of distilled water. Transfer it in a brown bottle and store in a refrigerator (4^0). It should be goldern in colour. Do not use it if it turns olive green.

Formic acid- HCOOH = 46.03

Description:-Colourless liquid, odour, very pungent, highly corrosive; wt per ml. about 1.20 g, contains about 90.0 per cent of HCOOH and is about 23.6 M in strength.

Assay:- Weigh accurately, a conical flask containing 10ml of water, quickly add about 1ml of the reagent being examined and weigh again. Add 50ml of water and titrate with *1M sodium hydroxide* using 0.5 ml of *phenolphthalein solution* as indicator. Each ml of 1*M sodium hydroxide* is equivalent to 0.04603 g of HCOOH.

Glycerine –$C_3H_8O_3$ = 82.09.

Description – Clear, colorless, liquid of syrupy consistency; odourless, taste sweet followed by a sensation of warmth. It is hygroscopic.

Solubility –Miscible with water and with *alcohol*; practically insoluble in chloroform, in solvent ether and in fixed oils.

Acidity –To 50 ml of a 50 per cent w/v solution add 0.2 ml of *dilute phenolphthalein solution*; not more than 0.2 ml of 0.1 *N sodium hydroxide* is required to produce a pink colour.

Wt. per ml –Between 1.252 g and 1.257 g, corresponding to between 98.0 per cent and 100.0 per cent w/w of $C_3H_8O_3$.

Refractive index –Between 1.470 and 1.475 determined at 20^0.

Arsenic –Not more than 2 parts per million, Appendix 2.3.1.

Copper –To 10 ml add 30 ml of *water*, and 1 ml of *dilute hydrochloric acid*, and 10 ml of *hydrogen sulphide solution*; no colour is produced.

Iron – 10 g complies with the *limit test* for iron, Appendix 2.3.4.

Heavy metals – Not more than 5 parts per million, determined by Method A on a solution of 4 g in 2 ml of 0.1 *N hydrochloric acid* and sufficient water to produce 25 ml, Appendix 2.3.3.

Sulphate –1 ml complies with the *limit test* for sulphates, Appendix 2.3.6.

Chloride –1 ml complies with the *limit test* for chloride, Appendix 2.3.2.

Acraldehyde and glucose –Heat strongly; it assumes not more than a faint yellow, and not a pink colour. Heat further; it burns with little or no charring and with no odour of burnt sugar.

Aldehydes and related substances – To 12.5 ml of a 50 per cent w/v solution in a glass-stoppered flask add 2.5 ml of *water* and 1 ml of *decolorised magenta solution*. Close the flask and allow to stand for one hour. Any violet colour produced is not more intense than that produced by mixing 1.6 ml of 0.1 *N potassium permanganate* and 250 ml of *water*.

Sugar –Heat 5 g with 1 ml of *dilute sulphuric acid* for five minutes on a water-bath. Add 2 ml of *dilute sodium hydroxide solution* and 1 ml of *copper sulphate solution*. A clear, blue coloured solution is produced. Continue heating on the water-bath for five minutes. The solution remains blue and no precipitate is formed.

Fatty acids and esters –Mix 50 ml with 50 ml of freshly boiled *water* and 50.0 ml of 0.5N *sodium hydroxide,* boil the mixture for five minutes. Cool, add a few drops of *phenolphthalein solution* and titrate the excess alkali with 0.5 *N hydrochloric acid*. Perform a blank determination, not more than 1 ml of 0.5 *N sodium hydroxide* is consumed.

*Sulphate*d ash –Not more than 0.01 per cent, Appendix 2.2.6.

Storage –Store in tightly-closed containers.

Glycerin Solution –Dilute 33 ml of *glycerin* to 100 ml with water and add a small piece of camphor or liquid phenol.

***n*- Hexane:-** C_6H_{14},= 86.18
Analytical reagent grade of commerce containing not less than 90.05 of *n*-Hexane.

Colourless, mobile, highly flammable liquid, bp 68^0; wt per ml, about 0.674 g.

Hydrochloric Acid –HCl = 36.46

Concentrated Hydrochloric Acid

Description –Clear, colourless, fuming liquid; odour, pungent.

Arsenic –Not more than 1 part per million, Appendix 2.3.1.

Heavy metals –Not more than 5 parts per million, determined by Method A on a solution prepared in the following manner : Evaporate 3.5 ml to dryness on a water-bath, add 2 ml of *dilute acetic acid* to the residue, and add water to make 25 ml, Appendix 2.3.3.

Bromide and iodide –Dilute 5 ml with 10 ml of *water*, add 1 ml of *chloroform*, and add drop by drop, with constant shaking, *chlorinated lime solution*; the chloroform layer does not become brown or violet.

Sulphite –Dilute 1 ml with 10 ml of water, and add 5 drops of *barium chloride solution* and 0.5 ml of 0.001 *N iodine*; the colour of the iodine is not completely discharged.

Sulphate –To 5 ml add 10 mg of sodium bicarbonate and evaporate to dryness on a water bath; the residue, dissolved in *water*; complies with the *limit test for sulphates*, Appendix. 2.3.7.

Free chlorine –Dilute 5 ml with 10 ml of freshly boiled and cooled *water*, add 1 ml of cadmium *iodide solution*, and shake with 1 ml of *chloroform*; the chloroform layer does not become violet within one minute.

*Sulphate*d ash –Not more than 0.01 per cent, Appendix 2.2.6.

Assay –Weigh accurately about 4 g into a stoppered flask containing 40 ml of water, and titrare with *N sodium hydroxide*, using *methyl orange solution* as indicator. Each ml of *N sodium hydroxide* is equivalent to 0.03646 g of HCl.

Storage –Store in glass-stoppered containers at a temperature not exceeding 30^0.

Hydrochloric Acid, x N –Solution of any normality x N may be prepared by diluting 84 x ml of hydrochloric acid to 1000 ml with water.

Hydrochloric Acid –(1 per cent w/v) Dilute 1 g of hydrochloric *acid* to 100 ml with *water.*

Dilute Hydrochloric Acid –

Description –Colourless liquid.

Arsenic, Heavy metals bromoide and iodide, Sulphate, free chlorine –Complies with the tests described under Hydrochloric Acid, when three times the quantity is taken for each test.

Assay –Weigh accurately about 10 g and carry out the Assay described under Hydrochloric Acid.

Storage –Store in stoppered containers of glass or other inert material, at temperature below 30^0.

Hydrochloric Acid, N – HCl = 36.460

36.46 g in 1000 ml

Dilute 85 ml of hydrochloric acid with water to 1000 ml and standardise the solution as follows :

Weigh accurately about 1.5 g of anhydrous sodium carbonate, previously heated at about 270^0 for one hour. Dissolve it in 100 ml of *water* and add two drops of *methyl red solution*. Add the acid slowly from a burette with constant stirring, until the solution becomes faintly pink. Heat again to boiling and titrate further as necessary until the faint pink colour no longer affected by continued boiling. Each 0.5299 g of *anhydrous* sodium carbonate is equivalent to 1 ml of N hydrochloric acid.

Hydrochloric Acid, Iron-Free –Hydrochloric acid, which complies with the following additional test. Evaporate 5 ml on a water-bath nearly to dryness, add 40 ml of water, 2 ml of a 20 per cent w/v solution of citric acid and two drops of thioglycollic acid, mix, make alkaline with *dilute ammonia solution*, and dilute to 50 ml with water; no pink colour is produced.

Hydrogen Peroxide Solution – (20 Vol.) H2O2 = 34.02

Analytical reagent grade of commerce or *hydrogen peroxide solution* (100 Vol.) diluted with 4 volumes of water.

A colourless liquid containing about 6 per cent w/v of H_2O_2; weight per ml, about 1.02 g.

Hydroxylamine Hydrochloride; Hydroxylammonium Chloride – $NH_2OH.HCl = 69.49$.

Contains not less than 97.0 per cent w/w of NH_2OH. HCI.

Description –Colourless crystals, or a white, crystalline powder.

Solubility –Very soluble in water; soluble in alcohol.

Free acid –Dissolve 1.0 g in 50 ml of *alcohol*, add 3 drops of *dimethyl yellow solution* and titrate to the full yellow colour with *N sodium hydroxide*; not more than 0.5 ml of *N sodium hydroxide* is required.

Sulphated ash –Not more than 0.2 per cent, Appendix 2.2.6.

Assay –Weigh accurately about 0.1 g and dissolve in 20 ml of water, add 5 g of ferric ammonium sulphate dissolve in 20 ml of water, and 15 ml of *dilute sulphuric acid*, boil for five minutes, dilute with 200 ml of water, and titrate with 0.1 *N potassium permanganate*. Each ml of 0.1 *N potassium permanganate* is equivalent to 0.003475 g of NH_2OH. HCl.

Hydroxylamine Hydrochloride Solution –Dissolve 1 g of *hydroxylamine hydrochloride* in 50 ml of *water* and add 50 ml of *alcohol,* 1 ml of *bromophenol blue solution* and 0.1 *N sodium hydroxide* until the solution becomes green.

Mercuric Chloride –$HgCl_2$ =271.50.

Contains not less than 99.5 per cent of $HgCl_2$;

Description –Heavy, colourless or white, crystalline masses, or a white crystalline powder.

Solubility –Soluble in *water*; freely soluble in *alcohol*.

Non-volatile matter –When volatilised, leaves not more than 0.1 per cent of residue.

Assay –Weigh accurately about 0.3 g and dissolve in 85 ml of *water* in a stoppered-flask, add 10 ml of *calcium chloride solution*, 10 ml of *potassium iodide solution*, 3 ml of *formaldehyde solution and* 15 ml of *sodium hydroxide solution*, and shake continuously for two minutes. Add 20 ml of acetic acid and 35 ml of 0.1 *N iodine.* Shake continuously for about ten minutes, or until the precipitated mercury is completely redissolved, and titrate the excess of iodine with 0.1 *N sodium thiosulphate*. Each ml of 0.1 *N iodine is* equivalent to 0.01357 g of $HgCl_2$.

Mercuric Chloride, 0.2 M – Dissolve 54.30 g of *mercuric chloride* in sufficient water to produce 1000 ml.

Mercuric Chloride Solution –A 5.0 per cent w/v solution of *mercuric chloride* in water.

Mercuric Potassium Iodide Solution – See Potassium - Mercuric Iodide solution.

Methyl Alcohol : Methanol : CH_3OH = 32.04.

Description –Clear, Colourless liquid with a characteristic odour.

Solubility –Miscible with water, forming a clear colourless liquid.

Specific Gravity – At 25^0, not more than 0.791.

Distillation range – Not less than 95 per cent distils between 64.5^0 and 65.5^0.

Refractive Index –At 20^0, 1.328 to 1.329.

Acetone –Place 1 ml in a *Nessler cylinder*, add 19 ml of water, 2 ml of a 1 per cent w/v solution of *2-nitrobenzaldehyde* in *alcohol (50 per cent)*, 1 ml of 30 per cent w/v *solution of sodium hydroxide* and allow to stand in the dark for fifteen minutes. The colour developed does not exceed that produced by mixing 1 ml of standard acetone solution, 19 ml of water, 2 ml of the solution of *2-nitrobenzaldehyde* and 1 ml of the *solution of sodium hydroxide* and allowing to stand in the dark for fifteen minutes.

Acidity –To 5 ml add 5 ml of *carbon dioxide-free water*, and titrate with 0.1 *N sodium hydroxide*, using *bromothymol blue solution* as indicator; not more than 0.1 ml is required.

Non-volatile matter – When evaporated on a water-bath and dried to constant weight at 105º, leaves not more than 0.005 per cent w/v of residue.

Methyl Alcohol, Dehydrated –Methyl alcohol, which complies with the following additional requirement.

Water –Not more than 0.1 per cent w/w.

Methyl Orange – Sodium-*p*-di methylamineazobenzene sulphate, $C_{14}H_{14}O_3N_3SNa$.

An orange-yellow powder or crystalline scales, slightly soluble in cold water; insoluble in alcohol; readily soluble in hot water.

Methyl Orange Solution –Dissolve 0.1 g of methyl orange in 80 ml of water and dilute to 100 ml with alcohol.

Test for sensitivity –A mixture of 0.1 ml of the methyl orange solution and 100 ml freshly boiled and cooled water is yellow. Not more than 0.1 ml of 0.1 N hydrochloric acid is required to change the colour to red.

Colour change – *p*H 3.0 (red) to pH 4.4 (yellow).

Methyl Red – *p*-Dimethylaminoazobenzene-O-carboxylic acid, $C_{15}H_{15}O_2N_3$.

A dark red powder or violet crystals, sparingly soluble in *water*; soluble in alcohol.

Methyl red solution –Dissolve 100 mg in 1.86 ml of 0.1 *N sodium hydroxide* and 50 ml of *alcohol* and dilute to 100 ml with water.

Test for sensitivity –A mixture of 0.1 ml of the *methyl red solution* and 100 ml of freshly boiled and cooled *water* to which 0.05 ml of 0.02 *N hydrochloric acid* has been added is red. Not more than 0.01 ml of 0.02 *N sodium hydroxide* is required to change the colour to yellow.

Colour change – *p*H 4.4 (red) to *p*H 6.0 (yellow).

Molish's Reagent –Prepare two solutions in separate bottles, with ground glass stoppers:

(a) Dissolve 2 g of α-naphthol in 95 per cent alcohol and make upto 10 ml with alcohol (*α*-naphthol can be replaced by thymol or resorcinol). Store in a place protected from light. The solution can be used for only a short period.

(b) *Concentrated sulphuric acid*.

Nitric Acid –Contains 70.0 per cent w/w of HNO_3 (limits, 69.0 to 71.0). About 16 N in strength.

Description –Clear, colourless, fuming liquid.

Wt. per ml. – At 20^0, 1.41 to 1.42 g.

Copper and Zinc –Dilute 1 ml with 20 ml of water, and add a slight excess of dilute ammonia solution; the mixture does not become blue. Pass hydrogen sulphide; a precipitate is not produced.

Iron –0.5 ml of complies with the limit test for iron, Appendix 2.3.4.

Lead –Not more than 2 parts per million, Appendix 2.3.5.

Chloride –5 ml neutralised with dilute ammonia solution, complies with the limit test for chlorides, Appendix 2.3.2.

Sulphates –To 2.5 ml add 10 mg of sodium bicarbonate and evaporate to dryness on a water-bath, the residue dissolved in water, complies with the limit test for sulphates, Appendix 2.3.7.

*Sulphate*d ash – Not more than 0.01 per cent w/w, Appendix 2.2.6.

Assay –Weigh accurately about 4 g into a stoppered flask containing 40 ml of water, and titrate with N Sodium hydroxide, using methyl orange solution as indicator. Each ml of N sodium hydroxide is equivalent to 0.06301 g of HNO_3.

Nitric Acid, xN –Solutions of any normality XN may be prepared by diluting 63x ml of nitric acid to 1000 ml with water.

Nitric Acid, Dilute –Contains approximately 10 per cent w/w of HNO_3. Dilute 106 ml of nitric acid to 1000 ml with water.

Petroleum Light – Petroleum Spirit.

Description – Colourless, very volatile, highly flammable liquid obtained from petroleum, consisting of a mixture of the lower members of the paraffin series of hydrocarbons and complying with one or other of the following definitions :

Light Petroleum –(Boiling range, 30^0 to 40^0).

Wt. per ml. –At 20^0, 0.620 to 0.630 g.

Light Petroleum –(Boiling range, 40^0 to 60^0).

Wt. per ml –At 20^0, 0.630 to 0.650 g.

Light Petroleum –(Boiling range, 60^0 to 80^0).

Wt. per ml. –At 20^0, 0.670 to 0.690.

Light Petroleum –(Boiling range, 80^0 to 100^0).

Wt. per ml. –At 20^0, 0.700 to 0.720

Light Petroleum –(Boiling range, 100^0 to 120^0).

Wt. per ml –At 20^0, 0.720 to 0.740 g.

Light Petroleum –(Boiling range, 120^0 to 160^0).

Wt. per ml –At 20^0, about 0.75 g.

Non-volatile matter –When evaporated on a water-bath and dried at 105^0, leaves not more than 0.002 per cent w/v of residue.

Phenolphthalein –$C_{20}H_{14}O_4$.

A white to yellowish-white powder, practically insoluble in water, soluble in alcohol.

Phenolphthalein Solution –Dissolve 0.10 g in 80 ml of *alcohol* and dilute to 100 ml with water.

Test for sensitivity –To 0.1 ml of the *phenolphthalein solution* add 100 ml of freshly boiled and cooled water, the solution is colourless. Not more than 0.2 ml of 0.02 *N sodium hydroxide* is required to change the colour to pink.

*Colour change – p*H 8.2 (colourless) to pH 10.0 (red)

Phloroglucinol – 1 , 3 , 5 – Trihydroxybenzene , $C_6H_3(OH)_3 . 2H_2O$.

Description – White or yellowish crystals or a crystalline powder.

Solubility –Slightly soluble in water; soluble in *alcohol*, and in *solvent ether*.

Melting range –After drying at 110^0 for one hour, 215^0 to 219^0.

*Sulphate*d ash – Not more than 0.1 per cent, Appendix 2.2.6.

Phloroglucinol should be kept protected from light.

Phosphoric Acid – H_3PO_4 = 98.00.

(Orthophosphoric Acid; Concentrated Phosphoric Acid).

Description –Clear and colourless syrupy liquid, corrosive.

Solubility –Miscible with water and with alcohol.

Phosphoric Acid, x N –

Solutions of any normality, x N may be prepared by diluting 49 x g of *phosphoric acid* with water to 1000 ml.

Phosphoric Acid, Dilute –

Contains approximately 10 per cent w/v of H_3PO_4.

Dilute 69 ml of *phosphoric acid* to 1000 ml with water.
Potassium Chloride –KCl = 74.55

Analytical reagent grade

Potassium Chromate – K_2CrO_4 = 194.2

Analytical reagent grade

Potassium Chromate Solution –A 5.0 per cent w/v solution of potassium chromate.

Gives a red precipitate with *silver nitrate* in neutral solutions.

Potassium Cupri-Tartrate Solution –Cupric Tatrate Alkaline Solution: Fehling's Solution.

(1) *Copper Solution* – Dissolve 34.66 g of carefully selected small crystals of *copper sulphate,* showing no trace of efflorescence or of adhering moisture, in sufficient water to make 500 ml. Keep this solution in small, well-stoppered bottles.

(2) *Alkaline Tartrate Solution* – Dissolve 176 g of sodium *potassium tartrate* and 77 g of *sodium hydroxide* in sufficient *water* to produce 500 ml.

Mix equal volumes of the solutions No. 1 and No. 2 at the time of using.

Potassium Dichromate – $K_2Cr_2O_7$ =294.18.

Contains not less than 99.8 per cent of $K_2Cr_2O_7$.

Description – Orange-red crystals or a crystalline powder.

Solubility – Soluble in *water*

Chloride –To 20 ml of a 5 per cent w/v solution in *water* and 10 ml *nitric acid,* warm to about $50^0$and add a few drops of *silver nitrate solution*; not more than a faint opalescence is produced.

Assay –Carry out the assay described under Potassium Chromate, using 2 g. Each ml of 0.1 *N sodium thiosulphate* is equivalent to 0.004904 g of $K_2Cr_2O_7$.

Potassium Dichromate Solution – A 7.0 per cent w/v solution of *potassium dichromate* in *water.*

Potassium Dichromate, Solution 0.1N – $K_2Cr_2O_7$ = 294.18, 4.903 g in 1000 ml.

Weigh accurately 4.903 g of *potassium dichromate* and dissolve in sufficient *water* to produce 1000 ml.

Potassium Dihydrogen Phosphate - KH_2PO_4 = 136.1

Analytical reagent grade of commerce.

Potassium Ferrocyanide – $K_4Fe(CN)_6.3H_2O$ =422.39.

Contains not less than 99.0 per cent of $K_4Fe(CN)_6.3H_2O$.

Description –Yellow, crystalline powder.

Solubility –Soluble in water.

Acidity or Alkalinity –A 10 per cent w/v solution in water is neutral to litmus paper.

Assay –Weigh accurately about 1g and dissolve in 200 ml of *water,* add 10 ml of *sulphuric acid and* titrate with 0.1 *N potassium permanganate.* Each ml of 0.1 *N potassium permanganate* is equivalent to 0.04224 g of $K_4Fe(CN)_6 . 3H_2O$.

Potassium Ferrocyanide Solution –A 5.0 per cent w/v solution of *potassium ferrocyanide in water.*

Potassium Hydrogen Phthalate – $CO_2H . C_6H_4 . CO_2K$ =204.22.

Contains not less than 99.9 per cent and not more than the equivalent of 100.1 per cent of $C_8H_5O_4K$ calculated with reference to the substance dried at 110^0 for one hour.

Description –White, crystalline powder.

Solubility –Slowly soluble in *water,* forming clear, colourless solution.

Acidity –A 2.0 per cent w/v solution in carbon dioxide free water gives with *bromophenol blue solution* the grey colour indicative of *p*H 4.0.

Assay –Weigh accurately about 9 g, dissolve in 100 ml of *water* and titrate with *N sodium hydroxide* using *phenolphthalein solution* as indicator. Each ml of *N Sodium hydroxide* is equivalent to 0.2042 g of $C_8H_5O_4K$.

Potassium Hydrogen Phthalate, 0.02 M – Dissolve 4.084 g of *Potassium hydrogen phthalate* in sufficient *water* to produce 1000 ml.

Potassium Hydrogen Phthalate, 0.2 M – Dissolve 40.84 g of *potassium hydrogen phthalate* in sufficient *water* to produce 1000 ml.

Potassium Hydroxide –Caustic Potash : KOH = 56.11

Contains not less than 85.0 per cent of total alkali, calculated as KOH and not more than 4.0 per cent of K_2CO_3.

Description – Dry white sticks, pellets or fused mass; hard, brittle and showing a crystalline fracture; very deliquescent; strongly alkaline and corrosive.

Solubility –Freely soluble in water, in alcohol and in glycerin; very soluble in boiling *ethyl alcohol*.

Aluminium, iron and matter insoluble in *hydrochloric acid* –Boil 5 g with 40 ml of dilute *hydrochloric acid*, cool, make alkaline with dilute ammonia solution, boil, filter and wash the residue with a 2.5 per cent w/v solution of ammonium nitrate; the insoluble residue, after ignition to constant weight, weighs not more than 5 mg.

Chloride –0.5 g dissolved in water with the additon of 1.6 ml of nitric acid, complies with the limit test for chlorides, Appendix 2.3.2.

Heavy metals –Dissolve 1 g in a mixture of 5 ml of water and 7 ml of dilute hydrochloric acid. Heat to boiling, add 1 drop of phenolphthalein solution and dilute ammonia solution dropwise to produce a faint pink colour. Add 2 ml of acetic acid and water to make 25 ml; the limit of heavy metals is 30 parts per million, Appendix 2.3.3.

Sulphate –Dissolve 1 g in water with the addition of 4.5 ml of hydrochloric acid; the solution complies with the limit test for sulphates, Appendix 2.3.6.

Sodium –To 3 ml of a 10 per cent w/v solution add 1 ml of water, 1.5 ml of alcohol, and 3 ml of potassium antimonate solution and allow to stand; no white crystalline precipitate or sediment is visible to the naked eye within fifteen minutes.

Assay –Weigh accurately about 2 g, and dissolve in 25 ml of water, add 5 ml of barium chloride solution, and titrate with N hydrochloric acid, using phenolphthalein solution as indicator. To the solution in the flask add bromophenol blue solution, and continue the titration with N hydrochloric acid. Each ml of N hydrochloric acid, used in the second titration in equivalent to 0.06911 g of K_2CO_3 . Each ml of N hydrochloric acid, used in the combined titration is equivalent to 0.05611 g of total alkali, calculated as KOH.

Storage –Potassium Hydroxide should be kept in a well-closed container.

Potassium Hydroxide, xN – Solution of any normality, x N, may be prepared by dissolving 56.11x g of potassium hydroxide in water and diluting to 1000 ml.

Potassium Hydroxide Solution –Solution of Potash.

An aqueous solution of potassium hydroxide containing 5.0 per cent w/v of total alkali, calculated as KOH (limits, 4.75 to 5.25).

Assay –Titrate 20 ml with N sulphuric acid, using solution of methyl orange as indicator. Each ml of N sulphuric acid is equivalent to 0.05611 g of total alkali, calculated as KOH.

Storage –Potassium hydroxide solution should be kept in a well-closed container of lead-free glass or of a suitable plastic.

Potassium Iodide –KI = 166.00

Description – Colourless crystals or white powder; odourless, taste, saline and slightly bitter.

Solubility –Very soluble in water and in glycerin; soluble in alcohol.

Arsenic –Not more than 2 parts per million, Appendix 2.3.1.

Heavy metals -Not more than 10 parts per million, determined on 2.0 g by Method A, Appendix 2.3.3.

Barium –Dissolve 0.5 g in 10 ml of water and add 1 ml of dilute sulphuric acid; no turbidity develops within one minute.

Cyanides –Dissolve 0.5 g in 5 ml of warm water, add one drop of ferrous sulphate solution and 0.5 ml of sodium hydroxide solution and acidify with hydrochloric acid; no blue colour is produced.

Iodates –Dissolve 0.5 g in 10 ml of freshly boiled and cooled water, and add 2 drops of dilute sulphuric acid and a drop of starch solution; no blue colour is produced within two minutes.

Assay –Weigh accurately about 0.5 g, dissolve in about 10 ml of water and add 35 ml of hydrochloric acid and 5 ml of chloroform. Titrate with 0.05 M potassium iodate until the purple colour of iodine disappears from the chloroform. Add the last portion of the iodate solution drop-wise and agitate vigorously and continuously. Allow to stand for five minutes. If any colour develops in the chloroform layer continue the titration. Each ml of 0.05 M potassium iodate is equivalent to 0.0166 mg of KI.

Storage –Store in well-closed containers.

Potassium Iodide, M –Dissolve 166.00 g of potassium iodide in sufficient water to produce 1000 ml.

Potassium Iodide and Starch Solution –Dissolve 10 g of potassium iodide in sufficient water to produce 95 ml and add 5 ml of starch solution.

Potassium Iodide and Starch solution must be recently prepared.

Potassium Iodide Solution –A 10 per cent w/v solution of potassium iodide in water.

Potassium Iodobismuthate Solution –Dissolve 100 g of tartaric acid in 400 ml of water and 8.5 g of bismuth oxynitrate. Shake during one hour, add 200 ml of a 40 per cent w/v

Potassium Iodobismuthate Solution, Dilute –Dissolve 100 g of tartaric acid in 500 ml of water and add 50 ml of potassium iodobismuthate solution.

Potassium Mercuric-Iodide Solution –Mayer's Reagent.

Add 1.36 g of mercuric chloride dissolved in 60 ml of water to a solution of 5 g of potassium iodide in 20 ml of water, mix and add sufficient water to produce 100 ml.

Potassium Mercuri-Iodide Solution, Alkaline (Nessler's Reagent)

To 3.5 g of potassium iodide add 1.25 g of mercuric chloride dissolved in 80 ml of water, add a cold saturated solution of mercuric chloride in water, with constant stirring until a slight red precipitate remains. Dissolve 12 g of sodium hydroxide in the solution, add a little more of the cold saturated solution of mercuric chloride and sufficient water to produce 100 ml. Allow to stand and decant the clear liquid.

Potassium Permanganate – $KMnO_4$ = 158.03

Description –Dark purple, slender, prismatic crystals, having a metallic lustre, odourless; taste, sweet and astringent.

Solubility –Soluble in *water*; freely soluble in *boiling water.*

Chloride and *Sulphate* –Dissolve 1 g in 50 ml of boiling *water*, heat on a water-bath, and add gradually 4 ml or a sufficient quantity of *alcohol* until the meniscus is colour-less; filter. A 20 ml portion of the filtrate complies with the limit test for *chloride,* Appendix 2.3.2., and another 20 ml portion of the filtrate complies with the limit test for *sulphates,* Appendix 2.3.7.

Assay –Weigh accurately about 0.8 g, dissolve in water and dilute to 250 ml. Titrate with this solution 25.0 ml of 0.1 *N oxalic acid* mixed with 25 ml of *water* and 5 ml of *sulphuric acid.* Keep the temperature at about 70^0 throughout the entire titration. Each ml of 0.1 *N oxalic acid* is equivalent to 0.00316 g of $KMnO_4$.

Storage –Store in well-closed containers.

Caution –Great care should be observed in handling *potassium permanganate*, as dangerous explosions are liable to occur if it is brought into contact with organic or other readily oxidisable substance, either in solution or in the dry condition.

Potassium Permanganate Solution – A 1.0 per cent w/v solution of *potassium permanganate* in water.

Potassium Permanganate, 0.1 N Solution –158.03. 3.161 g in 1000 ml

Dissolve about 3.3. g of *potassium permanganate* in 1000 ml of *water*, heat on a water-bath for one hour and allow to stand for two days. Filter through glass wool and standardise the solution as follows :

To an accurately measured volume of about 25 ml of the solution in a glass stoppered flask add 2 g of *potassium iodide* followed by 10 ml of *N sulphuric acid.* Titrate the liberated *iodine* with standardised 0.1 *N sodium thiosulphate,* adding 3 ml of *starch solution* as the end point is approached. Correct for a blank run on the same quantities of the same reagents. Each ml of 0.1 *N sodium thiosulphate* is equivalent to 0.003161 g of $KMnO_4$.

Potassium Tellurite: $K_2\ TeO_3$ (approx)
General reagent grade of commerce.

Purified Water –H_2O = 18.02.

Description –Clear, colourless liquid, odourless, tasteless.

Purified water is prepareed from potable water by distillation, ion-exchange treatment, reverse osmosis or any other suitable process. It contains no added substances.

*p*H – Between 4.5 and 7.0 determined in a solution prepared by adding 0.3 ml of a saturated solution of *potassium chloride* to 100 ml of the liquid being examined.

Carbon dioxide –To 25 ml add 25 ml of *calcium hydroxide solution*, no turbidity is produced.

Chloride –To 10 ml add 1 ml of *dilute nitric acid* and 0.2 ml of *silver nitrate solution;* no opalescence is produced, Appendix 2.3.2.
Sulphate –To 10 ml add 0.1 ml of *dilute hydrochloric acid* and 0.1 ml of *barium chloride,* Appendix 2.3.6.

Solution : the solution remains clear for an hour.

Nitrates and Nitrites –To 50 ml add 18 ml of *acetic acid* and 2 ml of *naphthylamine-sulphanilic acid* reagent. Add 0.12 g of *zinc reducing mixture* and shake several times. No pink colour develops within fifteen minutes.

Ammonium – To 20 ml add 1 ml of *alkaline potassium mercuric-iodide solution* and after five minutes view in a Nessler cylinder placed on a white tile; the colour is not more intense than that given on adding 1 ml of *alkaline potassium mercuric-iodide solution* to a solution containing 2.5 ml of *dilute ammonium chloride solution (Nessler's)* 7.5 ml of the liquid being examined.

Calcium –To 10 ml add 0.2 ml of *dilute ammonia solution* and 0.2 ml of *ammonium oxalate solution;* the solution remains clear for an hour.

Heavy metals –Adjust the *p*H of 40 ml to between 3.0 and 4.0 with *dilute acetic acid,* add 10 ml of freshly prepared *hydrogen sulphide solution* and allow to stand for ten minutes; the colour of the solution is not more than that of a mixture of 50 ml of the liquid being examined and the same amount of *dilute acetic acid* added to the sample, Appendix 2.3.3.

Oxidisable matter –To 100 ml add 10 ml of *dilute sulphuric acid* and 0.1 ml of 0.1 *N potassium permanganate* and boil for five minutes. The solution remains faintly pink.

Total Solids –Not more than 0.001 per cent w/v determined on 100 ml by evaporating on a water bath and drying in an oven at 105^0 for one hour.

Storage –Store in tightly closed containers.

Silver Nitrate Solution –

A freshly prepared 5.0 per cent w/v solution of silver nitrate in water.

Silver Nitrate, 0.1 N– $AgNO_3$ = 169. 87; 16.99 g in 1000 ml. Dissolve about 17 g in sufficient *water* to produce 1000 ml and standardise the solution as follows:

Weigh accurately about 0.1 g of *sodium chloride* previously dried at 110^0 for two hours and dissolve in 5 ml of *water*. Add 5 ml of *acetic acid*, 50 ml of *methyl alcohol* and three drops of *eosin solution is* equivalent to 1 ml of 0.1 *N silver nitrate.*

Sodium Bicarbonate – $NaHCO_3$ =84.01

Description –White, crystalline powder or small, opaque, monoclinic crystals; odourless; taste, saline.

Solubility –Freely soluble in *water*; practically insoluble in *alcohol.*
*Carbonate –p*H of a freshly prepared 5.0 per cent w/v solution in *carbon dioxide-free water,* not more than 8.6.

Aluminium, calcium and insoluble matter –Boil 10 g with 50 ml of *water* and 20 ml of *dilute ammonia solution,* filter, and wash the residue with water; the residue, after ignition to constant weight, not more than 1 mg.

Arsenic –Not more than 2 parts per million, Appendix 2.3.1.

Iron –Dissolve 2.5 g in 20 ml of *water* and 4 ml of *iron-free hydrochloric acid,* and *dilute* to 40 ml with *water*; the solution complies with the *limit test for iron,* Appendix 2.3.4.

Heavy metals –Not more than 5 parts per million, determined by Method A on a solution prepared in the following manner:

Mix 4.0 g with 5 ml of *water* and 10 ml of *dilute hydrochloric acid,* heat to boiling, and maintain the temperature for one minute. Add one drop of *phenolphthalein solution* and sufficient *ammonia solution* drop wise to give the solution a faint pink colour. Cool and dilute to 25 ml with *water,* Appendix 2.3.3.

Chlorides –Dissolve 1.0 g in *water* with the addition of 2 ml of *nitric acid;* the solution complies with the *limit test for chlorides,* Appendix 2.3.2.

Sulphates –Dissolve 2 g in *water* with the addition of 2 ml of *hydrochloric acid;* the solution complies with the limit test for *sulphates,* Appendix 2.3.6.

Ammonium compounds –1 g warmed with 10 ml of *sodium hydroxide solution* does not evolve ammonia.

Assay –Weigh accurately about 1 g, dissolve in 20 ml of *water,* and titrate with 0.5 *N sulphuric acid* using *methyl orange solutions* as indicator. Each ml of 0.5 *N sulphuric acid* is equivalent to 0.042 g of $NaHCO_3$.

Storage –Store in well-closed containers.

Sodium Bicarbonate Solution –A 5 per cnet w/v solution of *sodium bicarbonate* in *water.*

Sodium Carbonate – Na_2CO_3. $10H_2O$ =286.2.

Analytical reagent grade.

Sodium Chloride – NaCl = 58.44

Analytical reagent grade.

Sodium Hydroxide –NaOH = 40.00

Description –White sticks, pellets, fused masses, or scales; dry, hard brittle and showing a crystalline fracture, very deliquescent; strongly alkaline and corrosive.

Solubility –Freely soluble in *water* and in *alcohol.*

Aluminium, iron and matter insoluble in hydrochloric acid –Boil 5 g with 50 ml of dilute hydrochloric acid, cool, make alkaline with *dilute ammonia solution,* boil, filter, and wash with a 2.5 per cent w/v solution of *ammonium nitrate;* the insoluble residue after ignition to constant weight weighs not more than 5 mg.

Arsenic –Not more than 4 parts per million, Appendix 2.3.1.

Heavy metals –Not more than 30 parts per million, determined by Method A, Appendix 2.3.3. in a solution prepared by dissolving 0.67 g in 5 ml of water and 7 ml of 3 *N hydrochloric acid.* Heat to boiling, cool and dilute to 25 ml with water.

Potassium –Acidify 5 ml of a 5 per cent w/v solution with *acetic acid* and add 3 drops of *sodium cobaltnitrite solution;* no preciptitate is formed.

Chloride – 0.5 g dissolved in *water* with the addition of 1.8 ml of *nitric acid,* complies with the limit test for *chlorides,* Appendix 2.3.2.

Sulphates –1 g dissolved in *water* with the addition of 3.5 ml of *hydrochloric acid* complies with the limit test for *sulphates,* Appendix 2.3.6.

Assay –Weigh accurately about 1.5 g and dissolve in about 40 ml of *carbon dioxide-free water.* Cool and titrate with *N sulphuric acid* using *phenolphthalein solution* as indicator. When the pink colour of the solution is discharged, record the volume of acid solution required, add *methyl orange solution* and continue the titration until a persistent pink colour is produced. Each ml of *N sulphuric acid* is equivalent to 0.040 g of total alkali calculated as NaOH and each ml of acid consumed in the titration with *methyl orange* is equivalent to 0.106 g of Na_2CO_3.

Storage –Store in tightly closed containers.

Sodium Hydroxide, xN – Solutions of any normality, xN may be prepared by dissolving 40 x g of *sodium hydroxide* in *water* and diluting to 1000 ml.

Sodium Hydroxide Solution – A 20.0 per cent w/v solution of *sodium hydroxide* in *water.*

Sodium Hydroxide Solution, Dilute –

A 5.0 per cent w/v solution of *sodium hydroxide* in water.

Sodium Potassium Tartrate –Rochelle Salt COONa.CH(OH). CH(OH), COOK. $4H_2O$ = 282.17

Contains not less than 99.0 per cent and not more than the equivalent of 104.0 per cent of $C_4H_4O_6KNa.\ 4H_2O$.

Description –Colourless crystals or a white, crystalline powder; odourless; taste saline and cooling. It effloresces slightly in warm, dry air, the crystals are often coated with a white powder.

Solubility –Soluble in *water;* practically insoluble in alcohol.

Acidity or Alkalinity –Dissolve 1 g in 10 ml of recently boiled and cooled *water,* the solution requires for neutralisation not more than 0.1 ml of 0.1 *N sodium hydroxide* or of 0.1 *N hydrochloric acid,* using *phenolphthalein solution* as indicator.

Iron –0.5 g complies with the *limit test for iron,* Appendix 2.3.4.

Chloride –0.5 g complies with the *limit test for chlorides,* Appendix 2.3.2.

Sulphate –0.5 g complies with the *limit test for sulphate,* Appendix 2.3.6.

Assay –Weigh accurately about 2 g and heat until carbonised, cool, and boil the residue with 50 ml of *water* and 50 ml of 0.5 *N sulphuric acid;* filter, and wash the filter with *water;* titrate the excess of acid in the filtrate and washings with 0.5 N *sodium hydroxide,* using *methyl orange solution* as indicator. Each ml of 0.5 *N sulphuric acid* is equivalent to 0.07056 g of $C_4H_4O_6KNa.\ 4H_2O$.

Sodium Sulphate (anhydrous) – Na_2SO_4 = 142.04

Analytical reagent grade of commerce.
White, crystalline powder of granules; hygroscopic.

Sodium Thiosulphate – $Na_2S_2O_3.\ 5H_2O$ =248.17.

Description – Large colourless crystals or coarse, crystalline powder; odourless; taste, saline, deliquescent in moist air and effloresces in dry air at temperature above 33^0.

Solubility – Very soluble in *water*; insoluble in *alcohol.*

pH –Between 6.0 and 8.4, determined in a 10 per cent w/v solution.

Arsenic –Not more than 2 parts per million, Appendix 2.3.1.

Heavy metals –Not more than 20 parts per million, determined by Method A, Appendix 2.3.3. in a solution prepared in the following manner : Dissolve 1 g in 10 ml of *water,* slowly add 5 ml of *dilute hydrochloric acid* and evaporate the mixture to dryness on a water-bath. Gently boil the residue with 15 ml of *water* for two minutes, and filter. Heat the filtrate to boiling, and add

sufficient *bromine solution* to the hot filtrate to produce a clear solution and add a slight excess of *bromine solution.* Boil the solution to expel the *bromine* completely, cool to room temperature, then add a drop of *phenolphthalein solution* and *sodium hydroxide solution* until a slight pink colour is produced. Add 2 ml of *dilute acetic acid* and dilute with *water* to 25 ml.

Calcium –Dissolve 1 g in 20 ml of *water,* and add a few ml of *ammonium oxalate solution;* no turbidity is produced.

Chloride –Dissolve 0.25 g in 15 ml of *2N nitric acid* and boil gently for three to four minutes, cool and filter; the filtrate complies with the *limit test for chlorides,* Appendix 2.3.2.

Sulphate and Sulphite –Dissolve 0.25 g in 10 ml of *water*, to 3 ml of this solution add 2 ml of *iodine solution,* and gradually add more *iodine solution*, dropwise until a very faint-persistant yellow colour is procduced; the resulting solution complies with the limit test for sulphates, Appendix 2.3.7.

Sulphide –Dissolve 1 g in 10 ml of *water* and 10.00 ml of a freshly prepared 5 per cent w/v solution of *sodium nitroprusside;* the solution does not become violet.

Assay –Weigh accurately about 0.8 g and dissolve in 30 ml of *water.* Titrate with 0.1 *N iodine,* using 3 ml of *starch solution* as indicator as the end-point is approached. Each ml of 0.1 iodine is equivalent to 0.02482 g of $Na_2S_2O_3.5H_2O$.

Storage –Store in tightly-closed containers.

Sodium Thiosulphate 0.1 N – $Na_2S_2O_3.5H_2O$. = 248.17, 24.82 g in 1000 ml.

Dissolve about 26 g of *sodium thiosulphate* and 0.2 g of *sodium carbonate* in *carbon dioxide-free water* and dilute to 1000 ml with the same solvent. Standardise the solution as follows :

Dissolve 0.300 g of *potassium bromate* in sufficient *water* to produce 250 ml. To 50 ml of this solution, add 2 g of *potassium iodide* and 3 ml of 2 *N hydrochloric acid* and titrate with the *sodium-thiosulphate solution* using *starch solution,* added towards the end of the titration, as indicator until the blue colour is discharged. Each 0.002784 g of *potassium bromate* is equivalent to 1 ml of 0.1*N sodium thiosulphate.* Note: –Re-standardise 0.1 *N sodium thiosulphate* frequently.

Stannous Chloride – $SnCl_2.\ 2H_2O$ =225.63.

Contains not less than 97.0 per cent of $SnCl_2.\ 2H_2O$.

Description –Colourless crystals.

Solubility –Soluble in *dilute hydrochloric acid.*

Arsenic- Dissolve 5.0 g in 10 ml of *hydrochloric acid,* heat to boiling and allow to stand for one hour; the solution shows no darkening when compared with a freshly prepared solution of 5.0 g in 10 ml of *hydrochloric acid.*

Sulphate –5.0 g with the addition of 2 ml of *dilute hydrochloric acid*, complies with the *limit test for sulphates,* Appendix 2.3.7.

Assay –Weigh accurately about 1.0 g and dissolve in 30 ml of *hydrochloric acid* in a stoppered flask. Add 20 ml of *water* and 5 ml of *chloroform* and titrate rapidly with 0.05 *M potassium iodate until* the *chloroform* layer is colourless. Each ml of 0.05 M potassium iodate is equivalent to 0.02256 g of $SnCl_2$. $2H_2O$.

Stannous Chloride Solution – May be prepared by either of the two methods given below :

Dissolve 330 g of stannous *chloride* in 100 ml of *hydrochloric acid* and add sufficient *water* to produce 1000 ml.

Dilute 60 ml of *hydrochloric acid* with 20 ml of *water*, add 20 g of tin and heat gently until gas ceases to be evolved; add sufficient *water* to produce 100 ml, allowing the undissolved tin to remain in the solution.

Starch Soluble – Starch, which has been treated with *hydrochloric acid* until after being washed, it forms an almost clear liquid solution in hot water.

Description –Fine, white powder.

Solubility –Soluble in hot *water,* usually forming a slightly turbid *solution.*

Acidity or Alkalinity –Shake 2 g with 20 ml of *water* for three minutes and filter; the filtrate is not alkaline or more than fainthy acid to litmus paper.

Sensitivity –Mix 1 g with a little cold *water* and add 200 ml *boiling water.* Add 5 ml of this solution to 100 ml of *water* and add 0.05 ml of 0.1 *N iodine.* The deep blue colour is discharged by 0.05 ml of 0.1 *N sodium thiosulphate.*

Ash – Not more than 0.3 per cent, Appendix 2.3.

Starch Solution –Triturate 0.5 g of *soluble starch,* with 5 ml of *water,* and add this, with constant stirring, to sufficient water to produce about 100 ml. Boil for a few minutes, cool, and filter.

Solution of *starch must be recently prepared.*

Sulphamic Acid –NH_2SO_3H =97.09.

Contains not less than 98.0 per cent of H_3NO_3S.

Description -White crystals or a white crystalline powder.

Solubility –Readily soluble in water. Melting Range –203^0 to 205^0, with decomposition.

Sulphuric Acid – H_2SO_4 = 98.08.

When no molarity is indicated use analytical reagent grade of commerce containing about 98 per cent w/w of *sulphuric acid.* An oily, corrosive liquid weighing about 1.84 g per ml and about 18 M in strength.

When solutions of molarity xM are required, they should be prepared by carefully adding 54 ml of sulphuric acid to an equal volume of water and diluting with water to 1000 ml.

Solutions of sulphuric acid contain about 10 per cent w/v of H_2SO_4 per g mol.

Sulphuric Acid, Dilute –Contains approximately 10 per cent w/w of H_2SO_4.

Dilute 57 ml of sulphuric acid to 1000 ml with water.

Sulphuric Acid, Chlorine-free –Sulphuric acid which complies with the following additional test:

Chloride –Mix 2 ml with 50 ml of water and add 1 ml of solution of *silver nitrate,* no opalescence is produced.

Sulphuric Acid, Nitrogen-free-Sulphuric acid which contains not less than 98.0 per cent w/w of H_2SO_4 and complies with the following additional test :

Nitrate –Mix 45 ml with 5 ml of *water*, cool and add 8 mg of *diphenyl benezidine;* the solution is colourless or not more than very pale blue.

Tartaric Acid –$(CHOH.\ COOH)_2$ =150.1

Analytical reagent grade.

Thioglycollic Acid – Mercapto acetic acid, – HS. CH_2COOH =92.11.

Contains not less than 89.0 per cent w/w of $C_2H_4O_2S$, as determined by both parts of the Assay described below :

Description –Colourless or nearly colourless liquid; odour strong and upleasant.

Iron –Mix 0.1 ml with 50 ml of water and render alkaline with *strong ammonia solution;* no pink colour is produced.

Assay – Weigh accurately about 0.4 g and dissolve in 20 ml of *water* and titrate with 0.1 *N sodium hydroxide* using *cresol red solution* as indicator. Each ml of 0.1 *N sodium hydroxide is equivalent to* 0.009212 g of $C_2H_4O_2S$.

To the above neutralised solution and 2 g of *sodium bicarbonate* and titrate with 0.1 *N iodine.* Each ml of 0.1 N iodine is equivalent to 0.009212 g of $C_2H_4O_2S$.

Triethanolamine -

Toluene :-Methyl benzene,$C_6H_5.CH_3$ =102.14.

Analytical grade reagent of commerce.

Clear, colourless liquid, odour, characteristic; bp about 110^0, wt per ml, about 0.870 g.

Water –See purified water.

Water, Ammonia-free –Water, which has been boiled vigorously for a few minutes and protected from the atomosphere during cooling and storage.

Xylenol Orange – [3H-2,1-Benzoxathiol–3-ylidene bis – (6-hydroxy-5-methyl-m-phenylene) methylenenitrilo] tetra acetic acid SS-dioxide or its tetra sodium salt.

Gives a reddish-purple colour with mercury, lead, zinc and contain other metal ions in acid solution. When metal ions are absent, for example, in the presence of an excess of *disodium ethylenediamine tetraacetate*, this solution is yellow.

Xylenol Orange Solution –Dissolve 0.1 g of *xylenol orange* with 100 ml of *water* and filter, if necessary.

Zinc, Acetate – analytical grade reagent of commerce.

APPENDIX- 5

CHEMICAL TESTS AND ASSAYS

5.1.1. - Estimation of Total Phenolics

Prepare a stock solution (1 mg/ml) of the extract in *methanol*. From the stock solution, take suitable quantity of the extract into 25-ml volumetric flask and add 10 ml of water and 1.5 ml of *Folin Ciocalteau reagent*. Keep the mixture for 5 min, and then add 4 ml of 20 per cent *sodium carbonate solution* and make up to 25 ml with *double distilled water.*

Keep the mixture for 30 min and record absorbance at 765 nm. Calculate percentage of total phenolics from calibration curve of gallic acid prepared by using the above procedure and express total phenolics as percentage of gallic acid.

5.1.2. - Estimation of Total Tannins

Defat 2 g of sample with 25 ml *petroleum ether* for 12 h. Boil the marc for 2 h with 300 ml of *double distilled water*. Cool, dilute up to 500 ml and filter. Measure 25 ml of this infusion into 2-litre porcelain dish; add 20 ml *Indigo solution* and 750 ml *double distilled water*. Titrate it with *0.1N potassium permanganate solution,* 1 ml at a time, until blue solution changes to green. Thereafter add drops wise until solution becomes golden yellow in colour.

Similarly, titrate mixture of 20-ml *Indigo solution* and 750 ml of *double distilled water*. Calculate the difference between two titrations in ml.

Each ml of 0.1*N potassium permanganate solution* is equivalent to 0.004157 g of total tannins.

5.1.3. - Estimation of Sugars

Method A:

Estimate total soluble and reducing sugars according to Nelson – Somogyi photometric method for the determination of glucose.

Preparation of calibration curve for *d*-glucose (Dextrose)

Dissolve accurately weighed 500 mg of dextrose in a 100-ml volumetric flask (5 mg / ml). From the above stock solution pipette out aliquots of 0.05 ml to 0.3 ml in to 10- ml volumetric flask and makeup the volume with *double distilled water*. Add 1 ml of alkaline reagent to each tube (25 parts of Reagent I + 1 part of Reagent II).

Reagent I: Dissolve 25 g of anhydrous *sodium carbonate* 25 g of Rochelle salt or sodium potassium tartrate, 20 g of *sodium bicarbonate* and 200 g of anhydrous *sodium sulphate* in about 800 ml of water and dilute to 1 L.

Reagent II : Add 15 per cent *copper sulphate* containing concentrated *sulphuric acid* per 100 ml to the tube. Mix the contents and heat for 20 min in a boiling water-bath. Then cool the tubes

and add the solution 1 ml of *arsenomolybdic acid reagent* (dissolve 250 mg of *ammonium molybdate* in 45 ml of *purified water*. To this, add 2.1 ml of *concentrated sulphuric acid* and mix well. To this solution, dissolve 3 g of *sodium arsenate* in 25 ml of *purified water*, mix well and place in incubator maintained at 37 º C for 24 hr). Dilute the contents of the test tube to 10 ml by adding *purified water* mix well and then read color intensity at 520 nm using a *ultra violet* visible spectrophotometer. Record the absorbance and plot a standard curve of absorbance *vs*. concentration.

5.1.3.1. - Reducing sugars

For reducing sugars, weigh accurately 500 mg of the sample, dissolve in 100 ml of *double distilled water* and make up the volume to 100 ml in a volumetric flask. Then follow method as mentioned for the preparation of calibration curve.

5.1.3.2. - Total sugars

Place 25 ml of the solution from the 100 ml stock solution prepared for the reducing sugars in a 100 ml beaker. To this, add 5 ml of hydrochloric acid: *purified water* (1:1 v/v), mix well and allow to stand at room temperature for 24 hr for inversion. Neutralize the sample with 5 N *sodium hydroxide* and make up to 50 ml with *purified water*. From this diluted sample, use 1 ml of aliquot for the estimation of total soluble sugars using the method described in preparation of calibration curve for dextrose.

5.1.3.3. - Non -reducing sugars

Non-reducing sugars are determined by subtracting the content of reducing sugars from the amount of total sugars.

Preparation of reagent:

Fehling's solution:

A) Dissolve 69.278 g of *copper sulphate* in water and make the volume up to 1 liter.

B) Dissolve 100 g of *sodium hydroxide* and 340 g *sodium potassium tartarate* in purified water and make the volume to 1 liter.

Mix equal volumes of A and B before the experiment.

Clarifying reagent:

Solution 1: Dissolve 21.9 g of *zinc acetate* and 3 ml of *glacial acetic acid* in *purified water* and make the volume to 100 ml.

Solution II: Dissolve 10.6 g of *potassium ferrocyanide* in water and make up to 100 ml.

Reducing sugars: Take suitable amount of the sample and neutralize with *sodium hydroxide solution* (10per cent in water). Evaporate the neutralized solution to half the volume on a water

bath at 50^0 to remove the alcohol. Cool the solution add 10 ml of the clarifying solution I followed by 10 ml of the clarifying solution II. Mix, filter through a dry filter paper and make up the volume to 100 ml. Take 10 ml of the *Fehling's solution* and from a burette and add sugar solution (above prepared sample) in a drop wise manner and heat to boiling over the hot plate (maintained at 80^0) until the mixture of Copper (*Fehling's solution*) appears to be nearly reduced. Add 3-5 drops of 1per cent *methylene blue* and continue the titration till the blue colour is discharged. Note down the readings and calculate the percentage of glucose.

Non-reducing sugars: Take suitable amount of the sample and neutralize with *sodium hydroxide solution* (10per cent in water). Evaporate the neutralized solution to half the volume on a water bath at 50^0C to remove the alcohol. Cool the solution add 10 ml of the clarifying solution I followed by 10 ml of the clarifying solution II. Mix, filter through a dry filter paper. To the Filter add 15 ml of 0.1 N *hydrochloric acid*. Cover with stopper and heat to boiling for two minutes. Add *phenolpthlein* and neutralize with *sodium hydroxide* solution (10per cent). Transfer to 100 ml volumetric flask and make the volume to 100 ml and perform the titration as done for the reducing sugars. Calculate the percentage of the total sugars. Subtract the percentage of the reducing sugars from the sugars to obtain non reducing sugars.

5.1.4.- Estimation of Curcumin by TLC Densitometer:

Sample solution - Extract 5 g of avaleha with *methanol* (25 ml x 4), filter, pool, concentrate and make up the volume to 25 ml with *methanol.*

Standard solution - Prepare a stock solution of *curcumin* (160 µg/ml) by dissolving 4 mg of accurately weighed curcumin in methanol and making up the volume to 25 ml with methanol. Transfer the aliquots (0.4 – 1.4 ml) of stock solution to 10 ml volumetric flasks and make up the volume with methanol to obtain standard solutions containing 6.4 to 22.4 µg/ml curcumin, respectively.

Calibration curve - Apply 10 µl of the standard solutions (64 to 224 ng) on a precoated TLC plate of uniform thickness. Develop the plate in the solvent system *toluene: ethyl acetate: methanol* (5 : 0.5 : 1) to a distance of 10 cm. Scan the plate densitometrically at 429 nm. Record the peak area and prepare the calibration curve by plotting peak area *vs* concentration of *curcumin* applied.

Estimation of curcumin in the drug - Apply 5 µl of the test solution on a precoated silica gel 60 F_{254} TLC plate. Develop the plate in the solvent system *toluene: ethyl acetate: methanol* (5: 0.5: 1) and record the chromatogram as described above for the calibration curve. Calculate the amount of curcumin present in the sample from the calibration curve of curcumin.

5.2.1 -Determination of Aluminum:
Solutions:

10 per cent sodium hydroxide solution – Dissolve 10 g *sodium hydroxide* in 100 ml purified water.

EDTA solution 0.05 M – Dissolve 18.6120 g of sodium salt of EDTA in purified water and make up to 1000 ml.

Zinc acetate solution 0.05M:- Dissolve 10.9690 g of z*inc acetate* in 50 ml *purified water* and few drops of *glacial acetic acid* and dilute to 1000 ml.

Acetate buffer 5.5 pH – Dissolve 21.5 g of *sodium acetate* (AR) in 300 ml *purified water* containing 2 ml *glacial acetic acid* and dilute to 1000 ml

Xylenol orange indicator –Dissolve 0.2 g of *xylenol orange indicator* in 100 ml *purified water* with 2 ml *acetic acid*.

Procedure:

Take suitable aliquot from the stock solution in 250 ml beaker. Take 50 ml of 10 per cent *sodium hydroxide solution* in another beaker. Neutralize the aliquot with *sodium hydroxide solution*. Transfer the 10 per cent *sodium hydroxide solution* to aliquot with constant stirring. Add a pinch of *sodium carbonate* into the solution. Boil the content on burner. Cool and filter through Whatman 40 No. filter paper with pulp in 600 ml beaker. Wash the precipitate with hot water 6-8 times. Acidify the filtrate with *dil. hydrochloric acid* and adjust pH 5.5. Add, in excess normally 25 ml 0.05M EDTA solution. Add 25 ml *acetate buffer solution*. Boil the solution; cool and again adjust pH 5 – 5.5. Add 5-6 drops of *xylenol orange indicator*. The colour changes from golden yellow to orange red at the end point. Take 25 m l0.05 M EDTA solution and run a blank. Each of 1M EDTA is equivalent to 0.05098 g of Al_2O_3.

5.2.2 - Determination of Borax:

Powder 5-6 g of drug and incinerated at 450^0 for 3 hours to get it ash. Dissolve the ash in 20 ml. of *purified water* and left for 15 minutes, filter, wash the residue with 80 ml of *purified* water for 4-5 washings. If necessary, shake the contents and titrate with *0.5N hydrochloric acid* using solution of m*ethyl orange* as an indicator. Each ml of *0.5N hydrochloric acid* is equivalent to 0.09536 g of $Na_2\ B_4O_7.10H_2O$.

5.2.3 - Determination of Calcium:

Solutions:
20 per cent Potassium hydroxide solution – Dissolve 200 g *potassium hydroxide* in *purified water* and make up to 1000 ml.
Ammonia buffer solutions 9.5 pH – Dissolve 67.5 g *ammonium chloride* in 300 ml *purified water*, add 570 ml *ammonia solution* and dilute to 1000 ml.

EDTA (Ethylene Diethyl Tetra Acetic acid) solution 0.05 M – Dissolve 18.6120 g of solution salt of EDTA and in water and make up to 1000 ml.

Triethanolamine 20per cent Solution – 200 ml of triethanolamine, adds 800 ml water and make up to 1000 ml.

Eriochrome Black T indicator 0.1per cent solution – Dissolve 0.10 g indicator in 100 ml of Methanol.

Patterns & Reeders indicators 0.1per cent solution – Dissolve 0.01g indicator in 100 ml of Methanol.

Procedure:

Take one part of filtrate reserved from Iron (Fe) estimation. Add 5 ml Triethanolamine 20 per cent solution. Add a pinch of *Hydroxylamine hydrochloride*. Add 25-30 ml *potassium hydroxide* 20 per cent solution. Add 4-5 drops of Patterns and Reeders indicator, which imparts rose red colour. Titrate the solution against standard EDTA solution. The colour changes from rose red to Prussian blue mark end point.

Each ml of 1M EDTA solution is equivalent to 0.04008 g Calcium.

5.2.4 - Determination of Copper:

Solutions:

Standard 0.1 N sodium thiosulphate solutions.

Potassium iodide.

Starch 1per cent solution – Dissolve 1 g in water, boil and make up 100ml.

Procedure:

Take suitable aliquot from the stock solution in a beaker. Add approx. 1.0 g sodium fluoride. Add *ammonia solution* and precipitate solution. Add *acetic acid* to dissolve the precipitate. Boil and cool in water bath. Add approx 1.0 g *potassium iodide*. Titrate the liberated iodine against *0.1 N sodium thoisulphate* (hypo) solutions by adding *starch solution* as indicator. The liberated iodine colour blackish brown changes to white at the end point. Calculate copper value against 1 ml of hypo solution titrating against standard 1000 ppm copper solution.

Each ml of 1N $Na_2S_2O_3$ solution is equivalent to 0.06357 g of Copper

5.2.5.- Determination of Iron (Fe)

Preparation of sample solution:

Ignite a suitable quantity of the sample (in the presence of organic matter) in a crucible in a muffle furnace at 500-550^0 until the residue is free from organic matter. Moisten with 5-10 ml of hydrochloric acid, boil for two min, add 30 ml of water, heat on the water bath for few min, filter and wash thoroughly the residue with water and make up to volume in a volumentric flask.

Solutions:

Stannous chloride solution – Dissolve 5 g *stannous chloride* (A.R) in 25 ml Conc. *hydrochloric acid* and dilute to 100 ml (5 per cent solution).

Mercuric chloride – saturated solution in water.

Sulphuric acid + orthophosphoric acid mixture – take 60 ml water, add 15 ml conc. *sulphuric acid* and 15 ml H_3PO_4 cool and dilute to 1000ml.

Diphenylamine barium sulphonate – Dissolve 0.25 g in 100 ml water.

0.1 N *Standard potassium dichromate solution*. Dissolve 4.9035 g AR grade in water and dilute to 1000 ml.

Procedure:

Take /withdraw a suitable aliquot from the stock solution in 250 ml in duplicate. Dilute to about 100 ml with distilled water. Add 1-2 drops of *methyl red* indicator. Add 1-2 g *ammonium chloride*. Add dil. Ammonium solution till brown precipitate appears. Boil the solution with ppt. for 4-5 minutes. Cool the content and filter through Whatman 41 no. filter paper. Wash the residue with hot water 4-6 times. Dissolve the residue in dil. HCl in 250 ml beaker. Wash with hot water and make the volume to 100 ml approx. Boil the solution on burner. Reduce the Fe^{3+} to Fe^{2+} by adding *stannous chloride solution* drop wise till solution becomes colourless.

Add 1-2 drops of *stannous chloride solution* in excess. Cool the content in water. Add 10-15 ml 10per cent solution of *mercuric chloride*. Add 25 ml acid mixture. Add 2-3 drops of *diphenylamine barium sulphonate indicator*. Add distilled water, if required. Titrate against standard *potassium dichromate solution*. Appearance of violet colour show end point.

Each ml of 1N $K_2Cr_2O_7$ solution is equivalent to 0.05585 g Iron

Each ml of 1N $K_2Cr_2O_7$ solution is equivalent to 0.7985 g Fe_2O_3

5.2.6.- Determination of Magnesium:

Take another part of filtrate reserved from Fe estimation. Add 5 ml *triethanolamine 20 per cent solution*. Add a pinch of *hydroxylamine hydrochloride*. Add 25-30 ml *ammonia buffer 9.5 pH*. Add 4-5 drops of *eriochrome black* T indicator. The colour changes from rose red to blue marks the end point.

Each ml of 1M EDTA solution is equivalent to 0.0409 g of MgO.

5.2.7.- Determination of Mercury:

Powder 0.5 g drug and treat with 7 ml of conc. *nitric acid* and 15 ml of conc. *sulphuric acid* in a kjeldahl flask; heat under reflux gently at first then strongly for 30 minutes. Cool and add 50 ml conc. *nitric acid* boil so as to remove the brown fumes. Continue the addition of *nitric acid* and boiling until the liquid is colourless; cool, wash the condenser with 100 ml of water, remove the flask and add 1.0 per cent *potassium permangnate* solution drop wise until pink colour persists. Decolourize it by adding 6.0 per cent *hydrogen peroxide* drop wise to

remove excess of *potassium permangnate* followed by 3.0 ml of conc. *nitric acid* and titrate with *0.1N ammonium thiocyanate solutions* using *ferric alum* as indicator.

Each ml. of 0.1N NH_4SCN solution is equivalent to 0.01003 g Mercury.

5.2.8. - Determination of Silica (SiO_2)

Weigh 0.5 g (in case of high silica) or 1.0 g (low silica) finely powdered and dried sample in a platinum crucible (W_1). Add 4-5 g *anhydrous sodium carbonate* into the crucible. Mix thoroughly and cover the crucible with lid, if necessary. Place the crucible in muffle furnace. Allow the temperature to rise gradually to reach 900-950^0 and keep on this temp. for about ½ hour to complete the fusion. Take out the crucible and allow cool at room temperature. Extract the cooled mass in 25-30 ml dil *hydrochloric acid* in 250 ml beaker. Heat on hot plate/burner to dissolve the contents. Wash the crucible with distilled water. Keep the beaker on water bath and allow dry the mass. Dehydrate back and powder the mass. Take out the beaker and allow cooling at room temperature. Add 25-30 ml *hydrochloric acid* dilute to 100 ml distilled water. Boil the content and allow cool. Filter through Whatman no 40. filter paper. Wash the residue with hot water 6-8 times. Place the residue along with filter paper in platinum crucible. Ignite at 900-950^0 for 2-3 min. Allow to cool and weigh as SiO_2.

5.2.9. - Estimation of Sodium and Potassium by Flame Photometer:

Preparation of Standard solutions

Weigh 2.542 g of AR *sodium chloride* and dissolve in *purified water* and make upto 1000 ml in a volumetric flask. Dilute 1 ml of the stock solution to 100 ml. This gives standard of 1mg of sodium per 100 ml (10 ppm). Prepare 20, 30, 40 and 50 ppm standard solution.

Weigh 1.9115g of AR *potassium chloride* and dissolve in *purified water* and make up to 1000 ml in a volumetric flask. Dilute 1ml of the stock solution to 100ml. This gives standard of 1mg of sodium per 100 ml (10 ppm). Prepare 20, 30, 40 and 50 ppm standard solution.

Preparation of Sample solution

Weigh 10 g of sample in a preweighed silica dish and heat in a muffle furnace for 1hr at 600º. Cool and dissolve the ash in purified water and make up to 100ml in a volumetric flask.

Switch on the instrument first and then the pump. Keep distilled water for aspiration and allow it to stand for 15 min (warming time). Open the glass cylinder and ignite the flame. Adjust the instrument to zero.

Introduce the maximum concentration solution and adjust it to 100. Again introduce the purified water so that instrument shows zero. Then introduce the standard solution in ascending concentration. Note down the reading each time. Introduce the purified water for aspiration in between the standard solutions. Introduce the sample solution and if it is within the range take the reading. If it exceeds limit 100 then dilute the solution till the reading is within the range. Plot the curve with concentration in ppm against reading obtained. Find out the concentration of the sample solution. Take two or three readings and find out the average. Find out the concentration of sodium and potassium.

5.2.10. - Determination of Sodium Chloride:

Dissolve about 2-3g accurately weighed drug in 25 ml of *purified water* and left for 30 minutes, filter. Wash the filter paper completely with *purified water* and the filtrate is made 100 ml in volumetric flask, make the solution homogeneous, titrate 25 ml of this solution with 0.1 *N silver nitrate solution* using *potassium chromate* as indicator. The end point shows the light brick red colour.

Each ml. of 0.1 *N* Ag NO_3 solution is equivalent to 0.005845 g of NaCl.

5.2.11. - Determination of Sulphur:

Solution:

Carbon tetrachloride saturated with *Bromine*

Barium chloride – 10 per cent solution in water.

Procedure:

Take 0.5 – 1 g powdered sample in 250 ml beaker. Add 10 ml *carbon tetrachloride* saturated with bromine. Keep in cold condition in fume chamber over night. Add 10 – 15 ml conc. *nitric acid*. Digest on water bath. Add 10 ml conc. *hydrochloric acid*, digest it to expel nitrate fumes till syrupy mass. Cool and extract with *hydrochloric acid*, make volume to 100 ml. Boil and filter through Whatman No 40. filter paper. Wash the residue with hot water. Filter through Whatman 41 No. paper in 600 ml beaker. Acidify the filtrate with *hydrochloric acid*. Add 20 ml of 10 per cent *Barium chloride* solution. Stir the solution and digest on burner. Allow to settle $BaSO_4$ precipitate over night. Filter the precipitate through Whatman No. 42 filter paper. Wash the precipitate with water. Ignite the precipitate in muffle furnace in pre weighed platinum crucible up to 850^0. Allow to cool and weigh.

Each g of weight of precipitate is equivalent to 0.13734 g of Sulphur.

5.2.12.- Qualitative Reactions of Some Radicals:

Sodium

Sodium compounds, moistened with hydrochloric acid and introduced on a platinum wire into the flame of a Bunsen burner, give a yellow colour to the flame.

Solutions of sodium salts yield, with solution of uranyl zinc acetate, a yellow crystalline precipitate.

Potassium

Potassium compounds moistened with hydrochloric acid and introduced on platinum wire into the flame of a Bunsen burner, give a violde colour to the flame.

Moderately strong solutions of potassium salts, which have been previously ignited to remove ammonium salts, give a white, crystalline precipitate with perchloric acid.

Solutions of potassium salts, which have been previously ignited to free them from ammonium salts and from which iodine has been removed, give a yellow precipitate with solution os sodium cobaltinitrte and acetic acid.

Magnesium

Solution of magnesium salts yield a white precipitate with solution of ammonium carbonate, especially on boiling, but yield no precipitate in the presence of solution of ammonium chloride.

Solution of magnesium salts yield a white crystalline precipitate with solution of sodium phosphate in the presence of ammonium salts and dilute ammonia solution.

Solution of magnesium salts yield with solution of sodium hydroxide a white precipitate insoluble in excess of the reagent, but soluble in solution of ammonium chloride.

Carbonates and Bicarbonates

Carbonates and bicarbonates effervesce with dilute acids, liberating carbon doxide; the gas is colourless and produces a wihte precipitate in solution of calcium hydroxide.
Solutions of carbonates produce a brownish-red precipitate with solution of mercuric chloride; Solutions of bicarbonates produce a white precipitate.

Solutions of carbonates yield, with solution of silver nitrate, a with precipitate which becomes yellow on the addition of an excess of the reagent and brown on boiling the mixture. The precipitate is soluble in dilute ammonia solution and in dilute nitric acid.

Solutions of carbonates produce, at room temperature, a white precipitate with solution of magnesium sulphate. Solutions of bicarbonates yield no precipitate with the reagent at room temperature, but on boiling the mixture a white precipitate is formed.

Solutions of bicarbonates, on boiling, liberate carbon dioxide which produces a white precipitate in solution of calcium hydroxide.

Sulphates

Solutions of sulphates yield, with solution of barium chloride, a white precipitate insoluble in hydrochloric acid.

Solutions of sulphates yield, with solution of lead acetate, a white precipitate soluble in solution of ammonium acetate and in solution of sodium hydroxide.

Chlorides

Chlorides, heated with manganese dioxide and sulphuric acid, yield chlorine, recognisable by its odour and by giving a blue colour with potassium iodide and solution of starch.

Calcium

Solutions of calcium salts yield, with solution of ammonium carbonate, a white precipitate which after boiling and cooling the mixture, is insoluble in solution of ammonium chloride.

APPENDIX-6

AYURVEDIC DEFINITIONS AND METHODS

6.1. - Kalpanā Paribhāṣā:

1	Kalka	Charakasaṁhitā Sūtra sthāna, 4/7
2.	Kvātha / Kaṣāya	Śārṅgadhara saṁhitā - II - 9/3
3.	Cūrṇa	Śārṅgadhara saṁhitā - II - 6/1
4.	Puṭapāka Svarasa	Śārṅgadhara saṁhitā - II - 1/21-23
5.	Svarasa	Śārṅgadhara saṁhitā - II - 1/2
6.	Hima Kaṣāya	Śārṅgadhara saṁhitā - II - 4/1

6.1.1.-Kalka: *Kalka* is the fine paste of macerated fresh plant material.

(*Paribh*āṣā *Prabandha***)**

6.1.2.- Kvātha / Kaṣāya: *Kvātha* or *Kaṣāya* is the filtered liquid obtained by boiling coarse powder of drug(s) in proportion of 4, 8 or 16 [*Mṛdu Dravya* - 4, *Madhyama Dravya* - 8 and *Kaṭhina Dravya* - 16 respectively] times of water and reduced to one-fourth.

(*Śārṅgadhara saṁhitā* - II - 9/3**)**

6.1.3.- Cūrṇa: The fine sieved powder of well dried drug(s) is called *Cūrṇa*

(*Śārṅgadhara saṁhitā* - II - 6/1**)**

6.1.4.- Puṭapāka Svarasa: It is a kind of procedure, where juice of fresh green herb will be obtained by the process of *Puṭapāka*. Bundle the *Kalka* of green plant material in leaves of *Kāśmarī, Vaṭa, Jambu* etc., and cover with clay in layers of about 2 cm. thickness. Dry and place amidst fire till becomes reddish. Open the bundle and strain the juice from *Kalka* through a clean cloth.

(*Śārṅgadhara saṁhitā* - II - 1/21-23**)**

6.1.5.-Svarasa: The liquid part of fresh macerated plant material obtained by pressing through a fresh, clean cloth is called as *Svarasa.*

(*Śārṅgadhara saṁhitā* - II - 1/2**)**

6.1.6.- Hima Kaṣāya: *Hima Kaṣāya* is the extractive obtained by straining of 48 g. [1 part] of powdered drug(s) soaked in 288 ml. [6 parts] of water overnight.

(*Śārṅgadhara saṁhitā* - II - 4/1**)**

6.2.-Sāmānyaparibhāsa:

1	Kajjalī	Rasataraṅgiṇī - 2/27
2.	Kāñjika	Paribhāṣā prabandha
3.	Kṣāra	
4.	Cūrṇodaka	Rasataraṅgiṇī - 11
5.	Prakṣepa	
6.	Bhāvanā	Rasataraṅgiṇī - 2/49
7.	Śodhana	
8.	Mūrchana	Bhaiṣajya ratnāvalī - Jvara

6.2.1.- Kajjalī: *Kajjaḥī* is the fine black coloured powder obtained by triturating Sulphur [*Gandhaka*] and Mercury [*Pārada*] without adding any liquid.

[*Rasa Taraṅgiṇī* - 2/27]

6.2.2. - Kāñjika: Sour liquid prepared with of rice grain etc. is called as *Kāñjika.* Take *Ṣaṣ ṭika sālī* in an earthen vessel, add five parts of water and boil. Shift the preparation in to another earthen vessel, add three parts of water and seal the mouth of the vessel tightly. Place the vessel aside for two to three weeks of period at regulated temperature during which the liquid becomes sour.

[*Paribhāṣā prabandha*]

6.2.3. - Kṣāra: *Kṣāra* is alkaline substance obtained from the ashes of different drugs. Cut the drug in to small pieces, dry and place in an earthen pot, burn to ashes. Allow the ash to cool down to room temperature and add 6 parts of water, mix well. Allow to settle down and decant the supernatant layers through a piece of clean cloth. Repeat the process of staining two or three times till a clear liquid is obtained. Heat the liquid over a moderate fire till the water evaporates completely, leaving a solid salty white substance at the bottom, which is known as *Kṣāra.*

6.2.4. - Cūrṇodaka:

i]	Cūrṇa [Lime powder]	250 mg.
ii]	Water	60 ml.

Take 250 mg. of lime powder in a stainless steel vessel, add 60 ml. of water, and keep aside for 9 hours. Decant the supernatant layers through a filter paper. The filtrate is known as Cūrṇodaka.

[Rasataraṅgiṇī - 11]

6.2.5. - Prakṣepa: Fine powder form of the drug(s), which is added to a *kalpa* such as *Leha, āsavāriṣṭa* etc. before administration is known as *Prakṣepa.*

6.2.6. - Bhāvanā: *Bhāvanā* is the process by which powders of drugs are levigated to a soft mass with specified liquids and allowed to dry.

[*Rasataraṅgiṇī* - 2/49]

6.2.7. - Śodhana: Ś*odhana* is the process which removes the impurities to some extent and helps in increasing the therapeutic values of the drugs.

1	Godantī Śodhana	Rasataraṅgiṇī - 11/239
2.	Gairika Śodhana	Rasa ratna samuccaya - 3/49 Āyurveda prakāśa - 2/272
3.	Gandhaka Śodhana	Rasāmṛtam - 2/3
4.	Guggulu Śodhana	
5.	Ṭaṅkaṇa Śodhana	Āyurveda prakāśa -2/ 244
6.	Tuttha Śodhana	Rasāmṛtam - 3/74
7.	Bhallātaka Śodhana	Rasāmṛtam - Parisiṣṭa
8.	Manaḥśilā Śodhana	Rasa ratna samuccaya - 3/93
9.	Vatsanābha Śodhana	Rasāmṛtam - Parisiṣṭa
10.	Śilājatu Śodhana	Rasataraṅgiṇī - 22/69-78
11.	Haratāla Śodhana	Rasa ratna samuccaya - 3/93
12.	Hiṅgu Śodhana	Rasāmṛtam
13.	Pārada samānya Śodhana	Rasataraṅgiṇī - 5/27-30
14.	Aṣṭa saṅskāra of Pārada	

6.2.7.1. - Godanti Śodhana:

i]	Godantī		1 Part
ii]	Nimbu Svarasa	Fr.	Q.S.
	or		
	Droṇapuṣpī Svarasa	Pl.	Q.S.

Bundle small pieces of *Godantī* in a cloth, suspend in *Nimbu* or *Droṇapuspī svarasa* in a *Dol*ā *yantra*, and boil for 3 hours.

[*Rasataraṅgiṇī* - 11/239]

6.2.7.2. - Gairika Śodhana:

i]	Gairika	1 Part
ii]	Godugdha	Q.S.

Fine powder of *Gairika* is to be levigated with Cow's milk.

[*Rasa Ratna Samuccaya* - 3/49]

or

i]	Gairika	1 Part
ii]	Goghrta	Q.S.

Fry the fine powder of *Gairika* in little amount of Ghṛta.

[*Āyurveda Prakāśa* - 2/272]

6.2.7.3. - Gandhaka Śodhana:

i]	Gandhaka		1 Part
ii]	Godugdha		Q.S.
	or		
	Bhṛṅgarāja svarasa	Pl.	Q.S.

Melt small pieces of *Gandhaka* in an iron pan smeared with *Ghṛta* and pour in to a pot containing Godugdha or Bhṛṅgarāja svarasa. Collect after cooling. Repeat the process for seven times. At the end of the seventh process, wash and dry the material.

[*Rasāmṛtam* - 2/3]

6.2.7.4. - Guggulu Śodhana:

Remove manually the big pieces of Sandstone, Glass, wood etc. if any from the *Guggulu*. Cut *Guggulu* in to small pieces, bundle in a cloth and immerse in *Dol*ā *yantra* containing any one of the following liquids.

Gomūtra
Godugdha
Triphalā kaṣāya
Vāsā kaṣāya / svarasa or
Nirguṇḍi svarasa with Haridrā cūrṇa

Boil till the whole amount of *Guggulu* passes in to the liquid through the cloth. Discard the residue present in the bundle if any.
Filter the liquid through muslin cloth and heat the mixture till a semi solid mass is obtained.
Dry in sun and store until further use.

6.2.7.5. - Ṭaṅkaṇa Śodhana: Take small pieces of *Ṭaṅkaṇa* into an iron pan, fry till complete dehydration.

[*Āyurveda prakāśa* - 2/244]

6.2.7.6. - Tuttha Śodhana:

i]	Tuttha		1 Part
ii]	Raktacandana kvātha	Ht. Wd.	Q.S.
iii]	Maňjiṣṭhā kvātha	Rt.	Q.S.
iv]	Triphalā kvathā	P.	Q.S.

Prepare fine powder of *Tuttha* and levigate with the individual liquid media number (ii) to (iv) mentioned above seven times each.

[*Rasāmṛtam* - 3/74]

6.2.7.7. - Bhallātaka Śodhana:

i]	Bhallātaka	Fr.	1 Part
ii]	Gomūtra		Q.S.
iii]	Godugdha		Q.S.
iv]	Iṣṭika Cūrṇa		Q.S.
v]	Water		Q.S.

Method of Preparation:-Take Bhallātaka, remove the attached thalamus and soak in Gomutra for 7 days. Replace *Gomūtra* every 24 h with fresh Gomūtra. After 7 days, rinse the Bhallataka twice with water, to wash off the Gomūtra. Soak *Bhallātaka* in *godugdha* for 7 days, replacing *godugdha* every 24 h with fresh *godugdha*. After 7 days, rinse the Bhallātaka 2 or 3 times with water to wash off the *godugdha*. Put the Bhallātaka in a thick jute bag containing coarse brick powder and rub carefully, with a view to reduce the oil content in *Bhallātaka*.

[*Rasāmṛtam - Pariśiṣṭa*]

6.2.7.8. - Manaḥśilā Śodhana:

i]	Manaḥśilā		1 Part
ii]	Agastya patra svarasa	Lf.	Q.S.
	or		
	Śṛṅgavera [Ārdraka] svarasa	Rz.	Q.S.

Prepare fine powder of *Manaḥśilā* and levigate with any one of the above specified liquid media for seven times.

[*Rasa Ratna Samuccaya* - 3/93]

6.2.7.9. - Vatsanābha Śodhana:

i]	Viṣa [Vatsanābha]	Rt.Tr.	1 Part
ii]	Gomūtra		Q.S.

Take small pieces of *Vatsanābha*, bundle in clean muslin cloth, and soak in *Gomūtra* for three days, replacing the later every day. Wash and dry to obtain *Sodhita Vatsanābha*.

[*Rasāmṛtam- Parisiṣṭa*]

6.2.7.10. - Śilājatu Śodhana:

i]	Śilājatu		2 Parts
ii]	Hot water		4 Parts
iii]	Triphalā kaṣāya	P.	1 Part

Take powder of Ś*ilājatu* add specified amounts of hot water and *Triphalā kaṣā*y*a* so as to disengage the soluble matter. Allow to settle down and decant the supernatant layers.
Repeat the process till a clear liquid is obtained.
Concentrate the decanted material to thick paste over moderate heat.
Dry in sun rays and preserve for further purposes.

[*Rasataraṅgiṇī* - 22/69-78]

6.2.7.11. - Haritāla Śodhana:

i]	Haritāla		1 Part
ii]	Kūṣmāṇḍa Toya	Fr.	Q.S.
	or		
	Tila Kṣāra Jala	Pl.	Q.S.

or

Cūrṇodaka Q.S.

Take small pieces of *Haratāla*, bundle in clean muslin cloth, suspend in a *dolā yantra* containing any one of the above liquid media. Boil for three hours, dry in sun rays and preserve for further purposes.

[*Rasa Ratna Samuccaya* - 3/70]

6.2.7.12. - Hiṅgu Śodhana:

i]	Rāmaṭha [Hiṅgu]	Exd.	1 Part
ii]	Ājya [Ghṛta]		Q.S.

Prepare fine powder of *Hiṅgu* and fry it in sufficient amounts of Ghṛta, till it becomes crisp.

[*Rasataraṅgiṇī,* 24/578]

6.2.7.13. - Pārada Sāmānya Śodhana:

i]	Pārada [Mercury]	2 Parts
ii]	Sudhāraja [Lime powder]	2 Parts
iii]	Rasona [Laśuna]	2 Parts
iv]	Saindhava Lavaṇa [Rock Salt]	1 Part

Take equal parts of *Pārada* and *Sudhāraja*, triturate for three days, and filter carefully through a clean cloth.

Add dehusked *Rasona* and *Saindhava lavaṇa* to the *Pārada,* triturate till the paste of *Rasona* becomes black.

Wash with warm water and separate the *Pārada* with caution.

[*Rasataraṅgiṇī* - 5/ 27-30]

6.2.7.14. - Aṣṭasaṁskāra of Pārada

Aṣṭasaṁskāra of *Pārada* was prescribed in Ayurvedic classics for purification and to increase the therapeutic activities.

The *Aṣṭasaṁskāra* are:

1) Svedana
2) Mardana
3) Mūrchana
4) Utthāpana
5) Pātana [Ūrdhva, Adhaḥ and Tiryak]
6) Rodhana / Bodhana
7) Niyāmana
8) Dīpana / Sandīpana

6.2.7.14. a. Svedana:

[*Rasahṛdayatantra* - 2/3]

	Pārada [Mercury]		1 Part
ii]	Āsurī [Rājikā]	Sd.	1/16th Part
iii]	Paṭu [Saindhava lavaṇa]		1/16th Part
iv]	Śuṇṭhī	Rz.	1/16th Part
v]	Marica	Fr.	1/16th Part
vi]	Pippalī	Fr	1/16th Part
vii]	Citraka	Rt.	1/16th Part
viii]	Ārdraka	Rz.	1/16th Part
ix]	Mūlaka	Rt. Tr.	1/16th Part
x]	Kāñjika		Q.S.

Method:

Take the ingredients numbered [ii] to [ix] in to wet grinder and grind with sufficient quantity of water to prepare *kalka* (homogeneous blend). Take leaf of *Bhūrja* [*Betula utilis*] or *Kadalī* [*Musa paradisiaca*], place it over four folded cloth, smear with the prepared *kalka*, and gently place *Pārada* over it. Place the remaining part of *kalka* if any, over the *Parada.* Suspend the *poṭṭalī* in a *Dolā yantra* containing *Kāñji.* Boil for three days. Remove *Pārada* and *kalka,* wash carefully with warm water and collect *Pārada.*

6.2.7.14.b. Mardana:

[*Rasahṛdayatantra* - 2/4]

	Pārada [Mercury]	1 Part
ii]	Guḍa	1/16th Part
iii]	Dagdhorṇa	1/16th Part
iv]	Lavaṇa [Saindhava lavaṇa]	1/16th Part
v]	Mandira dhūma	1/16th Part
vi]	Iṣṭika cūrṇa	1/16th Part
vii]	Āsurī [Rājikā]	1/16th Part
viii]	Kāñjika	Q.S.

Method:

Take the ingredients numbered [i] to [vii] in to *Khalva* yantra add with required amounts of *Kāñji* and levigate for three days. Remove *Parada* and *kalka,* wash carefully with warm water and collect *Pārada.*

6.2.7.14.c. Mūrchana:

[*Rasahṛdayatantra* - 2/6]

	Pārada [Mercury]		1 Part
ii]	Gṛhakanyā [Kumārī]	Lf.	1/16th Part
iii]	Harītakī	P.	1/16th Part
iv]	Bibhītaka	P.	1/16th Part
v]	Āmalakī	P.	1/16th Part
vi]	Citraka	Rt.	1/16th Part

Method:

Take the ingredients numbered [iii] to [vi], dry, powder and pass through sieve number 85. Add ingredient number [ii] and grind with sufficient quantity of water to prepare *kalka.* Add *Pārada* to the *kalka* and triturate for three days.

6.2.7.14. d. Utthāpana:

[*Rasahṛdayatantra* - 2/7]

i]	Pārada [Mercury]	1 Part
ii]	Kāñjika	Q.S.

Method:

Collect the *Pārada* at the end of *Mūrchana* process and subject it to Utthāpana, and wash with *Kāñjika* and collect the *Pārada* carefully.

6.2.7.14. e. Pātana:

The process of *Pātana* is again of three types, viz. *Ūrdhvapātana, Adhaḥpātana* and *Tiryakpātana.*

Ūrdhvapātana:

[*Āyurveda prakāśa* - 1/68-71]

	Pārada [Mercury]	3 Parts
ii]	Ravi [Tāmra]	1 Part
iii]	Jambīra [Nimbu] rasa	Q.S.

Method:

Take *Pārada* and *Tāmra* in the specified ratio and levigate with *Jambīra svarasa* to prepare thick paste. Apply the paste over the lower pot of *Ḍamarū yantra* and apply heat for 12 hours. Collect the *Pārada* settled at the upper pot gently.

Adhaḥpātana:

[*Āyurveda prakāśa* - 1/75-77]

	Pārada [Mercury]		1 Part
ii]	Harītakī	P.	1/16th Part
iii]	Bibhītaka	P.	1/16th Part
iv]	Āmalakī	P.	1/16th Part
v]	Śigru	St. Bk.	1/16th Part
vi]	Citraka	Rt.	1/16th Part
vii]	Saindhava lavaṇa		1/16th Part
viii]	Āsurī [Rājikā]	Sd.	1/16th Part
ix]	Nimbu rasa		Q.S.

Method: Take the ingredients numbered [ii] to [vi], dry, powder and pass through sieve number 85. Add the powders to *Pārada* and levigate by adding ingredients numbered [vii] to [ix] to prepare fine paste. Apply the paste in the *Adhaḥpātana yantra*, subject to heat and collect *Pārada.*

Tiryakpātana: [*Āyurveda prakāśa* - 1/79-81]

	Pārada [Mercury]	3 Parts
ii]	Ravi [Tamra]	1 Part
iii]	Jambīra [Nimbu] rasa	Q.S.

Method: Take *Pārada* obtained at the end of *Adhaḥpātana* process, add with *Tāmra* and levigate with *Jambīra svarasa* to prepare thick paste. Apply the paste in the *Tiryakpātana yantra*, subject to heat and collect *Pārada.*

6.2.7.14. f. Rodhana / Bodhana: [*Rasendracūḍāmaṇi* - 4/88]

	Pārada [Mercury]	3 Parts
ii]	Saindhava lavaṇa jala	Q.S.

Method: Place the *Pārada* in a pot containing *Saindhava lavaṇa jala* and seal the mouth of the pot tightly. Place the pot undisturbed for three days. Decant the water on the fourth day to collect the *Pārada.*

6.2.7.14. g. Niyāmana: [*Rasahṛdayatantra* - 2/10]

	Pārada [Mercury]		1 Part
ii]	Phaṇi [Nāgavallī]	Lf.	1/16th Part
iii]	Laśuna	Bl.	1/16th Part
iv]	Ambujā		1/16th Part
v]	Karkoṭi		1/16th Part
vi]	Mārkava [Bhṛṅgarāja]	Pl.	1/16th Part
vii]	Ciñcikā [Ciñcā]	Lf.	1/16th Part
viii]	Kāñji		Q.S.

Method: Prepare *Kalka* of the ingredients numbered [ii] to [vii], add with *Pārada* and prepare a *poṭṭalī.* Suspend the *poṭṭalī* in a *Dolā yantra* containing *Kāñji* and boil. Remove *Pārada* and *kalka,* wash carefully with warm water and collect *Pārada.*

6.2.7.14. h. Dīpana / Sandīpana: [*Rasahrdayatantra* - 2/11]

	Pārada [Mercury]	1 Part
ii]	Bhū [Sphaṭikā]	1/16th Part
iii]	Khaga [Kāsīsa]	1/16th Part

iv]	Ṭaṅkaṇa		1/16th Part
v]	Marica	Fr.	1/16th Part
vi]	Lavaṇa [Saindhava lavaṇa]		1/16th Part
vii]	āsurī [Rajika]	Sd.	1/16th Part
viii]	Śigru		1/16th Part
ix]	Kāñjika		Q.S.

Method: Prepare *Kalka* of the ingredients numbered [ii] to [viii], add with *Pārada* and prepare a *poṭṭalī*. Suspend the *poṭṭalī* in a *Dolā yantra* containing *Kānji* and boil for three days. Remove *Pārada* and *kalka,* wash carefully with warm water and collect *Pārada.*

6.2.8. - Mūrchana: *Mūrchana* is the process which removes *āma doṣa* of *Taila / Ghṛta* and provides good color and fragrance. *Mūrchana* process is to be followed before any *Sneha* preparation.

[*Bhaiṣajya ratnā vaḷī*]

Mūrchana

Eraṇḍa taila Mūrchana	Bhaiṣajya ratnāvalī - Jvara
Ghṛta Mūrchana	Bhaiṣajya ratnāvalī - Jvara
Taila Mūrchana	Bhaiṣajya ratnāvalī - Jvara

6.2.8.1. - Mūrchana of Eraṇḍa Taila (*Bhaiṣajya ratnāvalī, Jvarā dhikāra.*)

Ingredients:

1.	Mañjiṣṭha API	*Rubia cordifolia*	P.	12 g
2.	Mustā API	*Cyperus rotundus*	Rz.	12 g
3.	Dhānyaka API	*Coriandrum sativum*	Sd.	12 g
4.	Āmalakī API	*Emblica officinalis*	P.	12 g
5.	Harītakī API	*Terminalia chebula*	P.	12 g
6.	Bibhītaka API	*Terminalia belerica*	P.	12 g
7.	Agnimantha API	*Clerodendron phlomidis* (Official substitute)	Rt.	12 g
8.	Hrīverā API	*Coleus vettiveroides*	Rt.	12 g
9.	Kharjūra API	*Phoenix sylvestris*	Fr.	12 g
10.	Vaṭa API	*Ficus religiosa*	Lf. Bud.	12 g
11.	Haridrā API	*Curcuma longa*	Rz.	12 g
12.	Dāruharidrā API	*Berberis aristata*	St.	12 g.
13.	Nalikā API	*Cinnamomum tamala*	St. Bk.	12 g.
14.	Śuṇṭhī API	*Zingiber officinale*		12 g.
15.	Ketakī API	*Pandanus odoratissimus*	Rt.	12 g.
16.	Dadhi API	Curd		1.536 l
17.	Kāñjī API			1.536 l
18.	Eraṇḍa taila API	*Ricinus communis*	Ol.	768 ml

Method of preparation: Take the ingredients (*Kalka dravyas*) numbered 1 to 15 in the composition, dry, powder and pass through sieve number 85. Transfer the powdered ingredients to wet grinder, grind with sufficient quantity of water to prepare *kalka* (homogeneous blend). Take *Eraṇḍa taila* in a stainless steel vessel and heat it mildly. Add increments of *Kalka*. Add *Dadhi* and Kāñjī in the specified ratio and stir thoroughly. Continue heating till the mixture becomes moisture free. Filter while hot through a muslin cloth and allow to cool.

6.2.8.2. - Mūrchana of Ghṛta

(*Bhaisajya ratnāvalī , Jvarādhikāra.*)

Ingredients:

1.	Harītakī	*Terminalia chebula*	P.	48 g
2.	Dhātrī	*Emblica officinalis*	P.	48 g
3.	Bibhītaka	*Terminalia belerica*	P.	48 g

4.	Mustā	*Cyperus rotundus*	Rz.	48 g
5.	Haridrā	*Curcuma longa*	Rz.	48 g
6.	Mātuluṅga	*Citrus medica*	Ft.	48 g
7.	Ghṛta	*Clarified butter of cow's milk*		768 g
8.	Water			3.072 l

Method of preparation: Take the ingredients (*Kalka dravyas*) numbered 1 to 5 in the composition, dry, powder and pass through sieve number 85. Transfer the powdered ingredients to wet grinder, add the ingredient number 6 and grind with sufficient quantity of water to prepare *kalka* (homogeneous blend).

Take *Ghṛta* in a stainless steel vessel and heat it mildly. Add increments of *Kalka*. Stir thoroughly while adding water in the ratio of 1:4. Start heating and observe the boiling mixture for subsidence of froth (*Pheṇa Śānti*) and constantly check the *kalka* for formation of *varti* (*Madhyama Pāka Lakṣaṇa*). Stop heating when the *kalka* forms a *varti* and the froth subsides. Filter while hot through a muslin cloth and allow to cool.

6.2.8.3. - Mūrchana of Taila

(*Bhaisajya ratnāvalī , Jvarādhikāra.*)

Ingredients:

1.	Mañjiṣṭa API	*Rubia cordifolia*	Rt.	96 gs.
2.	Harītakī API	*Terminalia chebula*	P.	24 gs.
3.	Bibhītaka API	*Terminalia belerica*	P.	24 gs.
4.	Āmalakī API	*Emblica officinalis*	P.	24 gs.
5.	Hrīverā API	*Coleus vettiveroides*	Rt.	24 gs.
6.	Haridrā API	*Curcuma longa*	Rz.	24 gs.
7.	Jaladhara [Mustā API]	*Cyperus rotundus*	Rz.	24 gs.
8.	Lodhra API	*Symplocos racemosa*	St. Bk.	24 gs.
9.	Sūcīpuṣpa [Ketakī API]	*Pandanus odoratissimus*	Rt.	24 gs.
10.	Vaṭāṅkura [Nyagrodha API]	*Ficus bengalensis*	*Lt. Bd.*	24 gs.
11.	Nalikā [Nālukā API]	*Cinnamomum tamala*	St. Bk.	24 gs.
12.	Taila (Tila API)	*Sesamum indicum*	Ol.	1.536 L.
13.	Water API			6.144 L.

Method of preparation: Take the ingredients (*Kalka dravyas*) numbered 1 to 11 in the composition, dry, powder and pass through sieve number 85. Transfer the powdered ingredients to wet grinder and grind with sufficient quantity of water to prepare *kalka*

(homogeneous blend). Take *Tila Taila* in a stainless steel vessel and heat it mildly. Add increments of *Kalka*. Stir thoroughly while adding water in the ratio of 1:4. Start heating and constantly check the *kalka* for formation of *varti* (*Madhyama Pāka Lakṣaṇa*) and observe the boiling mixture for appearance of froth. Stop heating when the *kalka* forms a *varti* and the froth emerges. Filter while hot through a muslin cloth and allow to cool.

6.3. - Yantra Paribhāṣā:

1	Khalva yantra	Rasataraṅgiṇī - 4/53
2.	Tiryak pātana yantra	Āyurveda Prakāśa - 1/79
3.	Ḍamarū yantra	Rasataraṅgiṇī - 4/41
4.	Dolā yantra	Rasa ratna samuccaya - 9/3-4

6.3.1.- Khalva yantra: It is an instrument made up of good quality of stone in different sizes and shapes, useful for trituration and levigation processes. It resembles with mortar and pestle.

[Rasataraṅgiṇī - 4/53]

6.3.2.- Tiryak pātana yantra: *Tiryak pātanā yantra* is an instrument prepared for distillation of *Parada* with the delivery tank weld approximately at an angle of 45^0

[Āyurveda Prakāśa - 1/79]

6.3.3.- Ḍamarū yantra: Ḍ*amarūyantra* is a contravenes of shape resembling *Ḍamarū* for sublimation prepared by sealing two pots with their mouths one telescoping the other sealing joint securely.

[*Rasa taraṅgiṇī* - 4/41]

6.3.4.- Dolā Yantra: *Dolā Yantra* consists of a pot half filled with specified liquid with a horizontal rod placed on the rim from which the bundle of material to be treated will be immersed and heated.

[*Rasa Ratna Samuccaya* - 9/3-4]

APPENDIX-7

WEIGHTS AND MEASURES

7.1. - METRIC EQUIVALENTS OF CLASSICAL WEIGHTS AND MEASURES

Weights and measures described in Ayurvedic classics and their metric equivalents adopted by the Ayurvedic Pharmacopoeia Committee

The following table of metric equivalents of weights and measures, linear measures and measurement of time used in the Ayurvedic classics have been approved by the Ayurvedic Pharmacopoeia committee in consultation with Indian Standards Institution.

I. WEIGHTS AND MEASURES

Classical Unit			Metric Equivalent
1 Ratti or Guñjā			= 125 mg
8 Ratti or Guñjās	=1 Māṣa	= 1 g	
12 Māṣas		=1 Karṣa (Tola)	= 12 g
2 Karṣas (Tolas)	=1 Śukti	= 24 g	
2 Śuktis (4 Karṣas or Tolas)		=1 Palam	= 48 g
2 Palams		=1 Prasrti	= 96 g
2 Prasṛtis		=1 Kuḍava	= 192 g
2 Kuḍavas		=1 Mānika	= 384 g
2 Mānikas		=1 Prastha	= 768 g
4 Prasthas		=1 Āḍhaka	= 3 kg 73 g
4 Āḍhakas		=1 Droṇa	= 12kg 228 g
2 Droṇas		=1 Śūrpa	= 24kg 576 g
2 Śūrpas		=1 Droṇi (Vahi)	= 49kg 152 g
4 Droṇis		=1 Khāri	= 196kg 608g
1 Palam			= 48 g
100 Palams		=1 Tula	= 4 kg 800 g
20 Tulas	=1 Bhāra	= 96 kg	

In case of liquids, the metric equivalents would be the corresponding litre and milliliter.

II. LINEAR MEASURES

Classical Unit	Inches	Metric Equivalents
1. Yavodara	1/8 of ¾"	0.24 cm
2. Aṅgula	¾"	1.95 cm
3. Bitahasti	9"	22.86 cm
4. Aratni	10 ½"	41.91 cm
5. Hasta	18"	45.72 cm
6. Nṛpahasta (Rājahasta)	22"	55.88 cm
7. Vyama	72"	182.88 cm

III. MEASUREMENT OF TIME

Unit		Equivalent (in hours, minutes & seconds)
2 Kṣaṇa	=1 Lava	
2 Lavas	=1 Nimeṣa	
3 Nimeṣas	=1 Kaṣ°ha	= 4.66 seconds
1 Ghati		= 24 Minutes
30 Kas°has	=1 Kalā	= 2 Minutes 20 seconds
20 Kalā + 3 Kaṣṭhas	=1 Muhūrta	= 48 Minutes
30 Muhūrtas	=1 Ahorātra	= 24 Hrs.
15 Ahorātras	=1 Pakṣa	= 15 Days
2 Pakṣas	=1 Māsa	= 30 Days/ One Month
2 Māsa	=1 Ṛtu	= 60 Days/ Two Months
3 Ṛtus	=1 Ayana	= 6 Months
2 Ayanas	=1 Samvatsara	= 12 Months/ One Year
5 Samvatsara	=1Yuga	= 5 Years
1 Ahorātra of Devas		= 1 Year
1 Ahorātra of Pitaras		= 1 Month

7.2. - METRIC SYSTEM :

Measure of Mass (Weights)

1 Kilogram (Kg) – is the mass of the International Prototype Kilogram.
1 Gramme (g) – the 1000th part of 1 Kilogram.
1Milligram (mg) – the 1000th part of 1 gramme.
1 Microgram (μg) – the 1000th part of 1 milligram.

Measures of capacity (Volumes)

1 Litre (1) is the volume occupied at its temperature of maximum density by a quantity of water having a mass of 1 Kilogram.
1 Millilitre (ml) the 1000^{th} part of 1 litre.

The accepted relation between the litre and the cubic centimetre is 1 litre –1000.027 cubic centimeters.

Relation of capacity of Weight (Metric)

One litre of water at 20º weighs 997.18 grammes when weighed in air of density 0.0012 gramme per millilitre against brass weights of density 84 grammes per millilitre.

Measures of Length

1 Metre (m) is the length of the International Prototype Metre at 0.
1 Centimetre (cm) – the 100^{th} part of 1 metre.
1 Millimetre (mm) – the 1000^{th} part of 1 metre.
1 Micron (μ) – the 1000^{th} part of 1 millimetre
1 Milliimicron (mμ) – the 1000^{th} part of micron.

APPENDIX-9

LIST OF SINGLE DRUGS OF PLANT ORIGIN USED IN FORMULATIONS, WITH LATIN NOMENCLATURE

Name of the Drug	Botanical Name
Agaru	*Aquilaria agallocha* Roxb.
Agnimantha	*Clerodendrum phlomidis* Linn.
Ājamodā	*Apium leptophyllum* (Pers.) F.V.M. ex Benth.
Amalakī	*Phyllanthus embilca* (*Emblica officinalis* Gaertn.)
Apāmārga	*Achyranthes aspera* Linn.
Ārdraka	*Zingiber officinale* Rosc.
Arka	*Calotropis procera* (Ait.) R. Br.
Asana	*Pterocarpus marsupium* Roxb.
Aśvagandhā	*Withania somnifera* Dunal.
Ativisā	*Aconitum heterophyllum* Wall. Ex Royle
Balā	*Sida cordifolia* Linn.
Bākucī	*Psoralea corylifolia* Linn.
Bhallātaka	*Semecarpus anacardium* Linn.
Bh¨¬garāja	*Eclipta alba* Hassk
Bibhītaka	*Terminalia bellirica* Roxb.
Bilva	*Aegle marmelos* Corr.
Brāhmī	*Bacopa monnieri* (Linn.) Wettst.
B¨hatī	*Solanum indicum* Linn.
Bhūmyāmalkī	*Phyllanthus amarus* Schun. & Th.
Cakramarda	*Cassia tora* Linn.
Cavya	*Piper retrofractum* Vahl.
Ciñca	*Tamarindus indica* Linn.
Citraka	*Plumbago zeylanica* Linn.
Darabh	*Imperata cylindrica* (L.) Beauv
Dāḍima	*Punica granatum* Linn.
Dantī	*Baliospermum montanum* Muell-Arg.
Dāruharidrā	*Berberis aristata* DC
Devadāru	*Cedrus deodara* (Roxb.) Loud.
Dhānyaka	*Coriandrum sativum* Linn.
Drāk¾ā	*Vitis vinifera* Linn.
Elāvāluka	*Prunus* avium
Eraṇḍa	*Ricinus communis* Linn.
Gambhārī	*Gmelina arborea* Linn.
Goksura	*Tribulus terrestris* Linn.
Guḍūcī	*Tinospora cordifolia* (Willd.) Miers.
Guggulu	*Commiphora wightii* (Arn.) Bhand.
Haridrā	*Curcuma longa* Linn.
Harītakī	*Terminalia chebula* Retz.

Hiṁsrā	*Capparis spinosa* Linn.
Hi¬gu	*Ferula foetida* Regel.
Hrīvera	*Coleus vettiveroides* K.C. Jacob
Iksu	*Saccharum officinarum* Linn.
Īśvarī	*Aristolochia indica* Linn.
Indravārunī	*Citrullus colocynthis* Schrad.
Jambu	*Syzygium cuminii* (Linn.) Skeels
Jatāma¬sī	*Nardostachys jatamansi* DC.
Jātī	*Jasminum officinale* Linn.
Jātiphala	*Myristica fragrans* Houtt.
Jivantī	*Leptadenia* reticulata Weight & Arn.
Jīvaka	*Malaxis acuminata* D.Don
Kākanāśikā	*Martynia annua* L.
Kākolī	*Lilium polyphyllum* D.Don
Kamala	*Nelumbo nucifera* Gaertn.
Ka¬kola	*Piper cubeba* Linn. f.
Kaṇṭakārī	*Solanum surattense* Burm.f.
Karañja	*Pongamia pinnata* (Linn.)
Karkaṭaś¨¬gī	*Pistacia lentiscus* Linn.
Kāsa	*Saccharum spontaneum* Linn.
Kaṭphala	*Myrica esculenta* Buch._Ham. Ex D.Don
Kaṭukā	*Picrorhiza kurroa* Royle ex Benth.
Khadira	*Acacia catechu* (Linn. f.) Willd.
Kola	*Zizyphus jujuba* Lam.
K¨¾ṇajīraka	*Carum carvi* Linn.
K¨¾ṇasārivā	*Cryptolepis buchanani* Roem. & Schult.
K¾īravidāri	*Ipomoea digitata* Linn.
Kulañjan	*Alpinia galanga* Willd.
Kulattha	*Vigna unguiculata* (Linn.) Walp.
Kunduru	*Boswellia serrata* Roxb.
Ku¬kuma(Vāhlīka)	*Crocus sativws* L.
Kūsmāṇḍa	*Benincasa hispida* (Thunb.) Cogn.
Ku¾ṭha	*Saussurea lappa* C.B. Clarke
Laśuna	*Allium sativum* Linn.
Lava¬ga	*Syzygium aromaticum* (Linn.) Merr. & L.M. Perry
Marica	*Piper nigrum* Linn.
Matsyāksī	*Alternanthera sessilis* (Linn.) R.Br.
Meda	*Polygonatum cirrhifolium* Royal
Mocarasa (Śālmalī)	*Salmalia malabarica* (DC) Schott & Endl.
Mūlaka	*Raphanus sativus* Linn.

Mañji¾ṭhā	*Rubia cordifolia* Linn.
Mustā	*Cyperus rotundus* Linn.
Nāgakeśara	*Mesua ferrea* Linn.
Nimba	*Azadirachta indica* A.Juss.
Padmaka	*Prunus cerasoides* D.Don
Palāśa	*Butea monosperma* (Lam.) Kuntze
Pāṭolā	*Trichosanthes dioica* Roxb.
Pā¾āṇa Bheda	*Bergenia ciliata* (Haw.) Sternb.
Pāṭalā	*Stereospermum suaveolens* DC.
Pāṭhā	*Cissampelos pareira* Linn.
Pippalī	*Piper longum* Linn.
Priyāla	*Buchanania lanzan* Spreng.
Priyaṇgu	*Callicarpa macrophylla* Vahl.
P¨śniparṇī	*Uraria picta* Desv.
Pūga	*Areca catechu* Linn.
Punarnavā (Rakta)	*Boerhaavia diffusa* Linn.
Pu¾kara	*Inula racemosa* Hook.f.
Raktacandana	*Pterocarpus santalinus* Linn.
Ṛ¾abhaka	*Malaxis muscifera* (Lindley) Kuntze.
Ṛddhī	*Habenaria intermedia* D.Don
Śaileya	*Parmelia perlata* (Huds.) Ach.
Sālaparnī	*Desmodium gangeticum* DC.
Śāli	*Oryza sativa* Linn.
Sama¬ga (Lajjālu)	*Mimosa pudica* L.
Sa¬khapu¾pī	*Convolvulus pluricaulis* Choisy
Saptalā	*Euphorbia dracunculoides* Lam.
Sarala	*Pinus roxburghii* Sargent.
Sarsapa	*Brassica campestris* Linn.
Śatāvarī	*Asparagus recemosus* Willd.
Śatī	*Hedychium spicatum* Ham. Ex Smith
Śigru	*Moringa oleifera* Lam.
Śṛṇgataka	*Trapa natans* Linn.
Śthūlailā	*Amomum subulatum* Roxb.
Śūksmailā	*Elettaria cardamomum* (Linn.) Maton
Śuṇṭhī	*Zingiber officinale* Roxb.
Śūraṇa	*Amorphophallus campanulatus* (Roxb.) Blume
Śveta sārivā	*Hemidesmus indicus* (Linn.) R.Br.
Śvetacandana	*Santalum album* Linn.
Śvetajīraka	*Cuminum cyminum* Linn.
Śyonāka	*Oroxylum indicum* vent.
Tagara	*Valeriana wallichii* DC.
Tālīsa	*Abies webbiana* Lindl.

Tila	*Sesamum indicum* Linn.
Triv¨t	*Operculina turpethum* (Linn.) Silva Manso
Tugāk¾īrī	*Bambusa bambos* Druce.
Tvak	*Cinnamomum zeylanicum* Blume
Tvakapatra	*Cinnamomum tamala* (Buch.-Ham) Nees & Eberm.
Uśīra	*Vetiveria zizanioides* (Linn.) Nash
Utpala	*Nymphaea stellata* Willd.
Vacā	*Acorus calamus* Linn.
Vāsā	*Adhatoda vasica* Nees
Viḍa¬ga	*Embelia ribes* Burm.f.
Vidārī	*Pueraria tuberosa* DC.
Ya¾ṭī	*Glycyrrhiza glabra* Linn.
Yava	*Hordeum vulgare* Linn.
Yavānī	*Trachyspermum ammi* (Linn.) Sprague ex Turril

APPENDIX-10

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