# A REVIEW ON QUALITY CONTROL OF HERBAL RAW MATERIALS.

**Dr. G. Dharmamoorthy[1*], Dr. Anna Balaji [2.] A. Mounika[3], B. Anushna[4], K. SushmaChowdary[5,] C. Susmitha[6]**

Department of Pharmaceutical Analysis, Sree Vidyanikethan College of Pharmacy.
Sree Sainath Nagar.Rangampet, Tirupathi-517502. Andhrapradesh. India.
Corresponding Author.

Dr. G. Dharmamoorthy
Associate Professor & HOD
Department of Pharmaceutical Analysis, Sree Vidyanikethan College of Pharmacy,
Tirupathi-517501. Andhra pradesh. India.
E-mail: dharmamoorthy111@gmail.com
Mobile: 9603774847

REVEIWED BY
1)Dr.V.SHANMUGAM
M.pharm.PhD
Professor & HOD, Department of Pharmaceutics
Sree vidyanikethan college of pharmacy, Rangampeta, Tirupathi 517103
Mobile no; +91 9052155880 Emai id; shanmugam.v@vidyanikethan.edu

2)Dr. P. DHARANI PRASAD
M.pharm.ph.D
Professor & HOD, Department of Pharma.D,
Sree Vidyanikethan college of Pharmacy, Rangampeta. Tirupathi 517103
Mobile no; +91 9491201679 Emai id dharaniprasad@gmail.com

**ABSTRACT;**
Quality control for the safety and efficacy of herbal drug is essential. Herbal medicines have the potential for improving public health at low cost and comparison with modern medicines Medicinal plants constitute a source of raw material for both traditional system of medicine and modern medicine., the occurrence of undesirable side effects seems to be less frequent. In most countries herbal products are launched in to the market without proper scientific evaluation and any mandatory safety, toxicological studies. Each country or area should adopt a regulatory system to manage the appropriate use of herbal medicines. Adopting a regulatory mechanism will help ensure that herbal medicines have acceptable quality, safety and efficacy. The WHO guidelines for the assessment of herbal medicines should be consulted when assessment for herbal medicines are being prepared. The quality con-trol of phytopharmaceuticals may be defined as the status of a drug, which is determined either by identity, puri-ty, drug contents and other physico-chemical properties and biological parameters or by the manufacturing process. Quality assurance of herbal medicinal product is not only the responsibility of manufacturers but also the regulatory bodies. The assurance of the safety of an herbal drug requires monitoring of the quality of the consumer information on the herbal remedy.

## Introduction

Owing to long standing and time proven use of herbal drugs along with higher safety margins, world health organization (WHO) has taken necessary steps to ensure quality control with modern techniques and application of suitable standards for this purpose. The pharmamacopocia of different countries include monogeapols indicating quality parameters and standards for various herbal drugs, and also for some of their products.[1]
Plant materials are used throughout developed and developing countries as home remedies, over the counter drug products and raw materials for the pharmaceutical industry, and represent a substantial proportion of the global drug market. It is therefore essential to establish internationally recognized guidelines for assessing their quality.[2]

### QUALITY CONTROL

It is scientific or technical process to detect or correct or checking to demonstrate whether the anticipated results are complied with the standard limit or not.

### QUALITY ASSURANCE

It is a managerial function which prevents problem by heading of them.

### RAW MATERIALS

Raw materials are one which are purposely cultivated or collected from this original source as a crude form i.e. without altering any of their form or slightly making it into suitable form to sing their elegance for making value herbal drugs or pharmaceutical aids.[3-6]

**IMPORTANCE OF HERBAL RAW MATERIALS**

- They are extremely useful for various ailments including life threatening condition.
- They have higher safety margins (toxic effects)
- As a source of new lead compounds of novel chn structure
- As the active ingredient of useful treatment derived from traditional system of medicine.

**Example for some important herbal raw material and this therupentic application**

| Sl. No. | Raw Material | Therapentic Application |
|---|---|---|
| 1. | Vinca nosea (Periwinkle) | Cytotoxic, anticancer, antidibetic and antihypertensive |
| 2. | Cinchona Bark (Jesuits Bark) | Antimalarial |
| 3. | Digitalis (Foxglore leave's) | Antiarrythmic CHF (cardiotonic) |
| 4. | Alose (aloe vera) | Furgative, prepn of Alurgel |
| 5. | Senna (Sennae folium) | Purgative & Dying industry |
| 6. | Ranmolfia 9Sarpaghantha) | Transquillizer, Antihypertensive |
| 7. | Ginseng (Panax Ginseng) | Immuns modulatory |
| 8. | Gingko (Maiden hair tree) | Inhibitor of PAF Thrombocyto penlia |
| 9. | Silymarin (Marian thistle) | Hepato protective |
| 10. | Atropa (Deadly nigh shade leaves) | Parasympathilytic or Anticholinergic |
| 11. | Amla (Indian ghoose berry) | Vit-C |
| 12. | Gelatin | Capriale mfg |
| 13. | Agar (veg gelatin) | Phaim and suspending agent |

- In this ********** internationally recognized guidelines for their quality assessment and quality control are necessary.
- WHO has emphasized the need to ensure quality control of medicinal plant products by using modern techniques is by applying suitable standards.[7,8]

  They are broadly classified into 4 major categories.

**I. BOTANICAL**

- Sensory Evaluation: Visual microscopy / touch/C/O/T
- Foreign Matter: Foreign plants, Foriengs animals, Foreign minerals etc.
- Microscopy: Histological observation, Histochemical detection, linear measurements etc.

**II. PHYSICO-CHEMICAL**

- TLC
- Ash : Total, TA insole, W. sole.
- Extractive Matter : Hot $H_2O$, cold $H_2O$ & Ethanol
- Water contents
- Volalite matter : LOD, Azeotropic

- Volatile oils: By stem distillation, By hydrodistillation

**V. PHARMACOLOGICAL:**

- Bitterness Value : Units eq. to bitterness of standard soln of quinpic Hcl.
- Haemolytic activity: On ox blood by comparison with standard of saponin
- Astringency : In water
- Foaming Index : Foam height produced by 1 gm material under specified conditions.

**IV. TOXICOLOGICAL**

- Pesticide Residius : Total organic chloride, Total organic phosphorous
- Arsenic : Strain produced on $Hg\ Br_2$ paper in comparison to standard stain
- Heavy metals : Cadmium stead
- Microbial contamination : Total viable aerobic count (E.Coli, salmonella P. amo, S.auress) (By TLC using Standard).
- Radio active contamination: By following (IAE Agency) procedure.

**IP, BP, USP and International Pharmacopolia**

Several Pharmacopolias including Indian Pharmacology British Pharmacopolia, Pharmacopolias of Republic of china Japanese Pharmacopoeia, United States pharmacopolia, to name some of them do cover monographs and quality control tests for few of the interest in natural raw materials is evidenced by the inclusion of some 35 monogeaph of natural substances in the 1980 BP. But, basically these pharmacoporias are designed necessities by giving their standards, test methods etc.

**British Herbal Pharmacopoeia (BHP)**

Looking at the increased interest in the natural raw materials the UK founded British Herbal Medical Association (BHMA) in 1964 to advance the science and practice of herbal medicines in order to ensure its statutory recognize first British Herbal Pharmacopoeia (BHP) was published by BHMA in 1971 given monographs and quality control tests for some of the common herbs and herbal products in the UK. BHP published in 1983 contains 233 monographs on single herbal drugs. However it is not comprehensive to cover all the medicinal plants that are used in UK. The general format of monographs of BHP 1983 is shown below.

**BHP Monographs**

- Monograph title
- Synonyms
- Definition
- Description
- Macroscopic, Microscopic
- Physico - Chemical Parameters
- Moisture content
- Total Ash / Acid instate Ash / water sol. Ash
- Foreign organic matter / contents of stalk, fruit & seeds / others
- Content of Alkalads / volatite oil
- TLC /GLC/PC/ Electrophoresis Ref.
- Extractive : water Sol/ Alchol. Sop
- Identification Test (colour reactions)
- Biological Activity Related Parameters
- Bitterness / absorbency (swelling index)
- Therapeutics
- Action
- Indication
- Specific Indication
- Combination used
- Precautions
- Preparation and max concentrations
- Toxicity
- Incompatibilities
- Contra indications
- Direction for use
- Side effects
- Storage

**WHO's guidelines on quality control methods for medicinal plant materials**

Who has offered a small document with an objective to provide recommended general test methods and general limit for contaminants in medicinal plant materials. Who has suggested that for non pharmacopocial material where official norms are not available, national inter (house) specifications may be developed on the basis of local experience by using simple statistical methods or "general average and three stigma limits" by pooling to gather analytical results of 20 or so successive batches.[9]

## WHO MONOGRAPH TITLE

**Botanical**

| | | |
|---|---|---|
| Sensory Evaluation | : | Visual / touch / Odeon / taste |
| Foreign matter | : | Foreign plants, animals & minerals etc. |
| Microscopy | : | Histological observation<br>Histo chemical detection<br>Measurements |

**Physico – Chemical parametrs**

Thin Layer Chromatography.
Ash : total, acid insole, water soluble
Extractable matter: In hot water, cold water and ethanol
Water content & volatile matter: LOD, Azeatrapic
Volatile oils : by steam distillation

**Pharmacological parameters**

Bitterness value
Hemolytic activity
Astringency
Swelling index
Foaming index

**Toxicological parameters**

Pesticide residences : Total organic chloride & total organic phosphorous
Arsenic
Heavy metals : cadmium & lead
Microbial contamination
Total viable Aerobic count
Pathogens : Enterobacteria, E, odi
Salmonella, P alogenisa, S aurcus
Aftatoxins : By TLC using std. aflatoxins
(B1, B2, G1, G2) Mixture.
Radioactive contamination

**Quality Central of Raw Material Based on their Botanical Identity**

**Sampling of raw material**

Samples must be truly respective of the material undergoing analysis. Confirming the uniformity of the batch samples are taken in the following manner.

**5 container / units –** Samples to be taken from all the units 6-50 containers / units – samples from 5 packages to be taken more than 50 containers – 10% of sample to be taken.

After checking the package, there original samples from the upper, middle and lower parts are taken and the average samples are obtained by quartering repeatedly till the required quantity is selected for testing final scimga are tested for the following characters.

- Degree of fragmentation (sieve test)
- Identity and level of impunities
- Determination of incisture and ash content
- Assay of active ingredients where possible
- A portion of the final sample should be retained to serve as reference standard.

**Sensory Evaluation**

This states only the external features, for sample for a

i. **Leafy drug :** Colour, shape, size, margin, base , presence or absence of trichomes / thorns, surface marking, venation, petiole / sessile / texture. For eg. Senna leaflet has mucnonate apex, unequal base pressed and shows markings, warty trichome, papery texture.

ii. **Stem drug :** Shape, external markings, presence of hairs / thorns, arrangement of leaf. For eg. Tinospora has warty surface with circular lenticels.

iii. **Bark :** Shape, external markings, colour of the inner and outer surface, fracture and centrals and quelled or not. For eg. Cinnamon bark has glossy surface, compound quill, paler outer surface and splintery fracture.

**iv.** **Root:** Shape, colour, external markings like wrinkles; annular rings, fracture. For eg. Withania is cylindrical with tapering ends and uniform surface and Ipecac not has annular rings.

**v.** **Flower :** Colour, inflorescence, shape and arrangement corolla, pedical or sessile, hair in calyse, type of androceum and gynoecium, ovules and florets.

For eg. Clove has dome shaped corolla, four sided hypanthium or stalk and pyrethrum has ray and disc florets.

**vi.** **Fruit :** Shape, color, texture marking of pericarp, presence or absence of spines and types of fruit.

For eg. Embica offinails has furrous like markings on the epicarp and has a glossy surface. Foiniculum vulgare is a schizo carp with ridges on the surface.

**vii.** **Seed :** Size, shape, colour, external marking like rap, aril, nature of endosperm, embryo.

For eg. Pysllium seed has glossy surface with pinkish spot in the centre of dersal surface where as whitish membrane in the ventral surface.

**viii.** **Unorganized drug:** Shape, color surface appearance solubility, viscosity and density in case of liquid or semisolid material.

**MICROSCOPICAL EVALUATION**

This refers to sectional view or powder analysis raw material.

Sectional view refers to T.S. R.L.S and T.L.S. of the material besides quantitative microscopy and histochemical studies. This is applicable only for the material either in entire form or in fragments.

Powder analysis is applicable to material in entire or fragment or in powder form. The diagnostic characters to be observed are,

i. Leafy drug : Type of stemata, trichome, staich grain, calcium oxalate crystals, quantitative, microscopy.

ii. Root : Type of cork, xylem vessels, fibres, shape and size of starch grains, presence co observed of crystal sheath, growth rings.

iii. Flower : Oil gland, papillose type of corolla, vascular system, trichomes, nature of pollen grains.

iv. Fruits : Parquetry arrangement, endosperm cells, aleurone grains, calcium oxalate crystals selerenchyma type of oil glands, raphe etc.

v. Seed : Endosperm, embryo, aleurone grains, presence or obsence of trichomes.

vi. Bark : Phloem fibres, pericyclic fibres, ca.oxalate crystals, presence or absence of oil cells, muila cells, nature of cook cells.

**Histochemical Detection**

To the section of the material or fragments the following reagents are added.

| **Sl.No.** | **Treatment** | **Colour observed** | **Chemical nature** |
|---|---|---|---|
| 1 | $I_2$ Water | Blue or Black | Starch |
| 2. | Sudan Red | Red | Suberin |
| 3. | Dragendroff’s reagent | Orange red | Alkaloids |
| 4. | 10% NaoH | Red | Quinones |
| 5. | Alc. $Fecl_3$ (5%) | Persian Blue | Phenolic compd |
| 6. | Neutral $Fecl_3$ | Bluish Black | Tannin |
| 7. | Tinc. of alkana | Red | Oils fats, suberin, cuticle |
| 8. | Picric acid | Yellow | Aleurone grains, animal fibres |
| 9. | Sudan red III | Red | Secretary cells, ducts |
| 10. | Ruthenium red | Pink | Mucilage, gums & pectin |
| 11. | Corallin soda | Pink | Gums, mucilage callose of sieve plates |
| 12. | 50% NaoH Acetic aud | Yellow needle dissotes effervescence | Flavores, calcium oxalate calcium carbonate |
| 13. | Chilor. Zinc- 10 dine | Blue or violet yellow blue | Cellulose fignise starch |

**Linear Measurements**

**Equipment**

Use a microscope with an ocular micrometer to measure the size of small objects. The scale should be calibrated using a stage micrometer, consisting of a glass slide of usual size upon which a scale is engraved or photographed, usually upon 1 or 2mm long in 0.1 and 0.01 mm divisions. The ocular micrometer consists of a small disc of glass, across the diameter of which a 100 line scale is engraved. The disc is placed into the eyepiece.

**Calibration of the ocular micrometer**

Place the ocular micrometer scale in the eyepiece of the microscope. Put the stage micrometer on the microscope stage and focus on the scale divisions. Turn the eyepiece to place the scales in a parallel position and if necessary. Move the stage micrometer until the starting lines of both scales Coincide. Now find another point as far along the scale as possible. These two other division lines are exactly suycer imposed.

If it is 1mm scale, the ocular micrometer can be calculated by using the formula.

Length of the scale in stage micrometer = 1 mm

1 mm is equally divided onto 100 division = 1000 μm

$$\therefore \text{ 1 division of stage micrometer} = \frac{1000}{100}$$

= 10 μm

1 divisional of use (Ocular) micrometer

$$= \frac{\text{Stage micrometer division (CP)}}{\text{Eye piece micrometer division (P)}} \times 10 \mu m \text{ s}$$

By this determination of length and width of

a) phloem fibre
b) Trichome
c) Calcium oxalate
d) Starch grains
e) Can be done

Stomatal index and stomatal numbers of these two parameter especially for leaf drug (raw materials) have been determined by measuring this characters in a particular square mm area drawn by using camma lieder.

$$\text{SI} = \frac{\text{S}}{\text{E+S}} \times 100$$

**Determination of foreign matter**

Medicinal plant materials should be entirely free from visible sign of contamination by moulds or insects and their animal contamination, including animal excreta. Vo abnormal odour, discoloration, slime or signs of letirioration should be detected. It is seldom possible to obtain marketed plant materials that are entirely. Fee from some form of innocuous foreign matter. However, is poisonous, dangerous or otherwise harmful foreign matter or residue should be allowed.

**Definition**

Foreign matter is material consisting of any or all of the following

- Parts of the medicinal plant material (or) material other than those named with the limits specified for the plant material concerned.

- Any organism, part or product of an organism, other than that named in the specification and description of the plant material concerned.
- Mineral admixture not adhering to the medicinal plant materials, such as soil, stones, sand and dust.

**Sample Size**

The following quantities constitute a sample, unless otherwise specified in the rest procedure for the plant material concerned.

| Plant Material | Sample Size |
|---|---|
| Rcots, the zomes and bark | 500 gm |
| Leaves, flowers , seeds and fruit | 250 gm |
| Cut med plant materials (average wt. of each fragment less than 0.5g) | 50 gm |

**Recommended procedures**

Leigh a sample of plant material, taking the qty indicated above unless otherwise specified in the test procedure for the plant material concerned. Spread it in a thin layer and sort the foreign matter into groups either by visual inspection, using a magnifying lens (6 x (or) 10 X) or with the help of a suitable sieve, according to the requirements for the specific plant material. Sift the remained of the sample through a No. 250 sieve ; dust is regarded as mineral admixture, weigh the portions of this sorted foreign matter to within 0.05g. Calculate the content of each group in grams per 100g of oil dried sample.

**Utility control of Raw materials Based on their Physlco – Chemical Characters**
**Thin Layer Chromatography (TLC)**

**Significance:**

1. TLC is particularly valuable for the qualitative determination of small amounts of impurities.
2. As it is effective and easy to perform.
3. The equipment required is inexpensive
4. The technique is frequently used for evaluating medicinal plant materials and their preparations.
5. Quantitative extracts of Crde drugs are prepared and compared chromotographically with standard reference solution of the known constituents.
6. Intersities of the visuialized chromatographic spots can be visually compared and the method used to eliminate inferior or adulterated drug.
7. Semi – Quantitative tests for the principles of drugs (peppermint, saffron, chamomid, digitalis) not rapidly evaluated by other means have been developed by TLC.
8. Identification and quantitation of specific chemical marker compound representative of specific herb.
9. As a mean for assessing quality and purity.
10. It is a rapid method to eliminate inferior or adulterated drug.

**Factors to be considered**

- Type of adsorbent and method of activation if no information the latter can be obtained, heat at $110^0$c for 30 min.
- Method of preparation and concentration of the test and reference solutions.
- Volume of the solutions to be applied on the plate.
- Mobile phase and the distance of migration
- Drying conditions (including temperature) and method of detection.
- For the spot obtained.
  - No and approximate position, or the Rf values if necessary.
  - Fluoresunce and colour.

**Methods adopted**

1. Classical method
2. Micro method

**1. Classical Method**

**Apparatus:**

The equipment consists of

1. Glass plates of uniform thickness throughout their entire are 15-20cm long and wide enough to accommodate the required number of test and reference solution.
2. a device for spreading a uniform layer of coating material of desired thickness onto the glass plates.
3. A rack to hold the prepared plates during the drying period or for transportation and storage. The rack should be small enough to fit in a drying oven and desicator.
4. A chromatographic chamber of transparent material usually glass, with a tightly fithing lid of suitable size to accommodate the test plates.
5. A suitable spraying implement with a fine spray nozzle made of a material resistant to the redgents to be used
6. An ultraviolet light source emithing short (254nm) and long (365 nm) wavelengths,

**Preparation of the adsorbent**

Unless otherwise specified in the test procedure for the plant material concerned, prepare a slurry of the coating material and water or an aqueons solution and using the spreading device, coat the cleared plates with a layer about 0.25 mm thick. Dry the coated plates in air heat to activate at $110^0$c for 30 min and then allow to cool.

**Saturation of the chromatographic chamber**

Unless otherwise specified in the test procedure, the chromatography is carried out in a saturated chamber. To achieve saturation, line at least half of the total area of the inside walls of the chamber with filter paper, pour into the chamber a sufficient quantity of mobile phase saturate the filter paper and form a layer about 5mm deep. Close the chamber and allow to stand for at least 1 hour at onom temp.

All operations during which the plate is exposed to the air should preferably be carried out at a relative humidity of 50-60% and the plants should be handled with care.

**Application of the test and reference solutions**

Using a micropipette or a syringe graduated in µl, place spots of the test and reference solution onto the starting line which should be parallel to and about 15 mm above the lower edge. The spot should be as small as possible, preferably not morethan 4mm in diameter. Mark the distance the mobile phase is intended to ascend as specified in the test procedure, usually 10 -15 cm from the starting line.

**Development of Chromatograms**

Allow the spots to dry, place the plate as nearly vertical as possible – into the chamber, ensuring that the points of application are above the surface of the mobile phase. Close the chamber. Develop the chromatogram at nom temp, unless otherwise specified in the test procedure, allowing the solvent to ascend the specified distance. Remove the plates mark the position of the solvent front and allow the solvent to eraporate at non temp or specified.

**Observation and interpretation of the chromatograms**

Observe the spots produced in daylight, then under short – wave and long wave UV light. Mark the centre of the spot with a needle. Measure and record the distance from the centre of each spot to the point of application and indicate for each spot the wavelength under which it was observed.

$$Rf = \frac{a}{c}, Rc = \frac{a}{c}; Rf = 100 \times Rf$$

Where,

Rf = Rate of flow

a = the distance between the point of application and the centre of spot of the material being examined.

b = The distance between the point of application and the centre of the spot of reference material.

c = The distance between the point of application and the centre of the spot of reference material.

Rf value vary from 0.0 to 1.00

Rf value vary from in the range 0 - 100

**Micro method**

Apparatus

The equipment consists of :Pre coated or specially prepared, not more than, 100 mm long and 100 mm wide, that permit development over at least 60 mm.

1ul or 2 ul micropipettes chamber with a tightly fitting lid and a flat base; the chamber must be or such a size to acolumn date the plates and the appropriate volume of mobiliphase.

**Method**

- ✓ Place a sufficient quantity of a previously m need and homogenol mobile phase into the chromatographic chamber to form a lay to mm deep.
- ✓ Close the chamber and allow to stand at constant rom temp / protected from draughts and direct sunlight for 15 min.
- ✓ Using a micropippete, apply spots of the solutions being examines into the starting line, which should be parallel to and about 10 mm above the lower edge of the plate.
- ✓ Mark the distance the mobile phase is intended to ascent as specified in the test procedure for the plant material concerned usually 60 mm from the starting.
- ✓ Develop the chromatogram at room temp, unless otherwise specified in the test procedure, allowing the solvent to ascent the specified distance.
- ✓ Remove the plate mark the position of the solvent front and allow the solvent to evament al room temp --- specified.

The residue remaining after ignition of plant material free of carbon is known as ash usually at consists of carbonates, phosphates, silica, sand and drit.

The ash remaining following ignition or med plant material is determined by three different methods which measures total ash, acid-insoluble ash and water soluble ash.

The total ash method is designed to measure the total amount of material remaining after ignition. This includes both “Physiological ash” which is the residue of the extraneous matter (eg Sand and soil) adhening to the plant surface.

Acid insoluble ash is the residue obtained after boiling the total ash with dilute hydrochloric acid and igniting the remaining insoluble matter. This measure the amount of silica resent especially as sand and silicones earth.

Water soluble ash is the difference in weight between the total ash and the residue after treatment of the total ash with water.

While determining the total ash very high temp (>600$^0$c) may result in the conversion of carbonates to oxides. In that cases ie. – carbonation may be done by treatment of the ash with a schlitic of ammonium carbonate and further re-drying to constant weight which gives the carbonated ash. The similar treatment with utility $So_4$, results in sulphated ash where the exides are converted sulphates when the same treatment is done by dil $HNO_3$ results in nitrated ash.

**RECOMMENDED PROCEDURE:**

Caution: This test should not be carried out until the identity of the plant material has been confirmed.

**Preparation of solutions:**

**Stock and diluted quinine hydrochloride solution**

Dissolve 0.100 g of quinine HCl in sufficient safe drinking water to produce 100 ml. Further dilute 5ml of this solution to 500 ml with safe drinking water. This stock solution of quinine HCl (Sa) contains 0.01 mg/ml. Use nine test-tubes for the serial dilution for the initial test as indicated in following Table.

| **Particular** | **Tube No** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** |
| Sq (ml) | 4.2 | 4.4 | 4.6 | 4.8 | 5. | 5.2 | 5.4 | 5.6 | 5.8 |
| Safe drinking water (ml) | 5.8 | 5.6 | 5.4 | 5.2 | 5.0 | 4.8 | 4.6 | 4.4 | 4.2 |
| Quinine Hcl in 10 ml soln (=c in mg) | 0.042 | 0.044 | 0.046 | 0.048 | 0.050 | 0.052 | 0.054 | 0.056 | 0.058 |

**Stock and diluted solutions of plant material**

Prepare the solution as specified in the test procedure for the given material (St). Use 10-test tubes for the serial dilution for the test as indicated in Table. 2.

| **Particular** | **Tube No** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** |
| St in ml | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Safe drinking water in ml | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 | - |

**Method**

After rinsing the mouth with safe drinking water, taste 10 ml of the most dilute solution swirling it in the month mounly near base of the tongue for 30 seconds. If the bither sensation is no longer felt in the mouth after 30 seconds. Spit out the solution and wait for 1 minute to ascertain whether this is due to delayed sensitisity. Then rinse with safe drinking waer. The next highest concentration should not be tasted until at least 10 minutes have passed. The threshold bitter concentration is the lowest concentration at which a material continues to provoke a bitter sensation after 30 seconds. After the first series of tests, rinse the mouth thoroughly with safe drinking water until no bitter sensation remains. Wait for at least 10 minutes before carrying out the second test.

In order to save time in the second test, it is advisable to ascertain first whether the solution in table No. 5 (containing 5 ml of st in 10 ml) gives a bitter sensation. If so, find the threshold bitter concentration of the material by tasting the dilutions in table 1-4.

If the solution in table no. 5 does not give a bitter sensation find the threshold bitter concentration by tasting the dilutions in tubes 6-10.

All solutions and the safe drinking water for mouth washing should be at 20-25$^0$C.

Calculate the bitterness value in units per of using the following formula;

$$\frac{2000 \times C}{a \times b}$$

Where a = the concentration of the stock soln (St) (mg/ml)

b = the volume of st (in ml) in the tube with the threshold bitter concentration

c = the quality of quinine HCl (in mg) in the tube with the threshold bitter concentration

**Determination of Haemolytic activity**

- Many medicinal plant materials, especially those derived from the families caryophyllaceae, Araliaceae, Sapindaceal, Primulaceae and Discoreaceae contain saponins.
- The most characteristic property of saponins is their ability of cause haemolysis; when added to a suspension of blood, saponins produce changes in erythrocyte membranes, causing haemoglobin to diffuse into the surrounding medium.
- The haemolytic activity of plant materials or a preparation containing saponins, is determined by comparison with that of a reference materials, which has a haemocytic activity of 1000 units per g.

**Recommended Procedure:**

**i) Preparation of citrated blood:**

- To prepare the erythrocyte suspension fill a glass, stopped flusk to $1/10^{th}$ of its volume with rod. Citrate (36.5 g/lit); swirling to ensure that the inside of the flusk is thoroughly moistened.
- Introduce a sufficient volume of blood freshly collected from a healthy ox and shake immediately.
- Citrated blood prepared in this way can be stored for about 8 days at 2-$4^0$C.
- Place 1 ml of citrated blood in a 50 ml volumetric flask with phosphate buffer pH 7.4 and carefully dilute to volume.
- This diluted blood suspension (2% soln) can be used as ling as the supernatant fluid remains clear and colourless. It must be stored in a cool temperature.

**Preparation of Reference solution:**

- Transfer about 10 mg of saponin, accurately weighed, to a volumetric flask and add sufficient phosphate buffer pH 7.4 to make 100 ml. This solution should be prepared freshly.

- **Preparation of plant material extract:**
- The external of plant material and dilutions should be prepared as specified in the test procedure for the plant material concerned, using phosphate buffer pH 7.4.

**Preliminary test:**

- Prepare a serial dilution of the plant material extract with phosphate buffer pH 7.4 and blood suspension (2%) using 4 test tubes as shown in the following 3table

| **Particular** | **Tube No** | | | |
|---|---|---|---|---|
| | **1** | **2** | **3** | **4** |
| Plant Material (ml) | 0.1 | 0.2 | 0.5 | 1.0 |
| Phosphate buffer pH 7.4 (ml) | 0.9 | 0.8 | 0.5 | - |
| Blood suspension 2% (ml) | 1.0 | 1.0 | 1. | 1.0 |

As soon as the tubes have been prepared, gently invert them to mize avoiding the formation of foam. Shake again after 30 minute interval and allow to stand for 6 hours at room temperature. Examine the tubes and record the dilution at which total haemolysis has occurred, indicated by a clear, red solution without any deposit of erythrocytes.

- If total haemolysis is observed only in tube No.4. Use the original plant material extract directly for the main test.
- If the total haemolysis is observed in tube no 3 and 4, prepare a two fold dilution of the original plant material extract with phosphate buffer pH 7.4.
- If total haemolysis is observed in tube no 2, 3 and 4, prepare a five fold dilution of the original plant material extract with phosphate buffer pH 7.4.
- If, after 6 hours, all four tubes contain a clear, red solution, prepare a ten – fold dilution of the original plant material extract with phosphate buffer pH 7.4 and carryout the preliminary test again as described above.
- If total haemolysis is not observed in any of the tubes, Repeat the preliminary test, using a more concentrated plant material extract.

**Main test**

- Prepare a serial dilution of the plant material extract, undiluted or diluted as determined by the preliminary test, with phosphate buffer pH 7.4 and blood suspension (2%) using 13 test tubes as shown in table.4

| **Particulars** | **Tube No** | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** |
| Plant material ext/diluted in necessary in ml) | 0.40 | 0.45 | 0.50 | 0.55 | 0.60 | 0.65 | 0.70 | 0.75 | 0.80 | 0.85 | 0.90 | 0.95 | 1.00 |
| Phosphate buffer pH 7.4 in ml | 0.60 | 0.55 | 0.50 | 0.45 | 0.40 | 0.35 | 0.30 | 0.25 | 0.20 | 0.15 | 0.10 | 0.05 | - |
| Blood suspension (2%) in ml | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |

Carry out the dilution and evaluations as in the preliminary test but observe the results after 24 hours. Calculate the amount of medicinal plant material in g, or of the preparation in g or ml, that produces total haemolysis.

Calculate the haemolytic activity of the medicinal plant material using the following formula.

$$\frac{10000 \times a}{b}$$

Where 1000 = the defined haemolytic activity saponin in relation to Ox blood

a = Quantity of saponin that produces total haemolysis in g

b = Quantity of plant material that produces total haemolysis in g

## TANNINS AND ASTRINGENLY

Tannins or tanning substances are substances capable of turning animal hides into leather by binding proteins to form water insoluble substances that are resistant to proteolytic enzes. This process, when applied to living tissue, is known as an “astringent” action and is the reason for the therapeutic application of tannins.

Chemically, tannins are complex substances; they usually occurs as mixture of polyphenols that are difficult to separate and crystallize. They are easily oxidized and polymerized in solution; if this happens they lose much of their astringent effect and are therefore of little therapeutic value.

**Determination of tannins:**

**Recommended procedure:**

**To prepare the plant material extract:**

Specified quantity in the test procedure for the plant material concerned, previously pondered to a known fineness and weighed accurately is taken 250 ml volumetric flask. Add 150 ml water and heat over a boiling water bath for 30 m cool, transfer the mixture and dilute to volume will water. Allow the solid material to settle and filter the liquid through a filter – paper, diameter 12 cm, discardi the first 50 ml of the filtrate.

To determine the total amount of material, that is extra into water, evaporate 50 ml of the plant material extract to dryness, dry the residue in an oven at $105^0$C for 4 hours.

**Plant material not bound to hide provider:**

To determine the amount of plant material not bound to hide powder that is extractable into water, take 80 ml of the plant material extract, add 6 g of hide powder and shake well for 60 minutes. Filter and evaporate 50 ml of the clear filtrate to dryness. Dry the residue in an oven at $105^0$C and weight (T2).

**Solubility of hide powder:**

To determine the solubility of hide powder, take bg of hide powder, add 80 ml of water and shake well for 60 minutes. Filter and evaporate 50 ml of the clear filtrate to dryness. Dry the residue in an oven at $105^0$C and weight (To).

Calculate the quantity of tannins as a percentage the following formula,

$$= \frac{[T_1 - (T_2 - T_0)}{\omega} \times 500$$

Where w = the weight of the plant material grams.

## SWELLING INDEX

Many medicinal plant materials are of specific therapeutic or pharmaceutical utility because of their swelling properties, especially gums, murilage, pestin and Hemicellulose.

The swelling index is the volume in ml taken up by the swelling of 1g of plant material under specified conditions. Its determination is based on the addition of water or a swelling agent as specified in the test procedure for each individual plant material (either whole, cut or pulverized).

Using a glass – stoppered measuring cylinder, the material is shaken repeatedly for 1 hour and then allowed to stand for a required period of time. The volume of the mixture (in ml) is then read.

**Recommended procedure:**

It should be done atleast 3 times and the average height occupied by the plant material is taken.

1. Introduce a specified quantity of the plant material concerned, previously reduced to the required fineness and accurately weighed into a 25-ml glass stoppers measuring cylinder.
2. Unless otherwise indicated in the test procedure, add 25 ml of water and shake the mixture thoroughly every 10 minutes for 1 hour.
3. Allow to stand for 3 hours at room temperature.
4. Measure the volume in ml occupied by the plant material, including any sticky mucilage.
5. Calculate the mean value of the determinations related to 1 g of the plant material concerned.

**Note:** The internal diameter of the cylinder should be about 16 mm, the length of the graduated portion about 125 mm, marked in 0.2 ml division from 0 to 25 ml in an upwards direction.

The diagrammatic representation of the apparatus is given below.

Figure:

**FOAMING INDEX**

- The foaming ability of an aqueous decoction of plant materials and their extracts is measured in terms of a foaming index.
- Many medicinal plant materials contain saponins that can cause a persistent foam when an aqueous decoction is shaken.

**Recommended Procedure:**

- Weight accurately about 1 g of plant material reduced into coarse powder (Sieve Size No: 1250) and transfer to a 500 ml conical flask containing 100 ml of boiler water.
- Maintain at moderate boiling for 30 minutes.
- Cool and filter into a 100 ml volumetric flask and add sufficient water through the filter to makeup the volume.
- Pour the decoction into 10stoppered test-tubes in successive portion of 1ml, 2ml… up 10 ml and adjust the volume of the liquid in each tube with water to 10 ml.

- Stopper the tubes and shake them in a lengthwise motion for 15 seconds, two shakes per second.
- Allow to stand for 15 minutes and measure the height of the foam.
- The results are as follows,
- If the foam height in every tube is less than 1 cm, the foaming index is <100.
- If the foam height in any tube is 1 cm, the volume of the decoction of the tube (a) is used to determine the index, if this tube is 1 or 2 tube in a series, prepare an intermediate dilution in a similar manner to obtain a more precise result.

- If the foam height is more than 1 cm, the foaming index is > 1000.
  Calculate the foaming index using the following formula.
  Formula$1000 \times 10/a$
  Where a = the volume ml of the decoction used for preparing the dilution in the tube where foaming to a height of 1 cm is observed.

**TOXICOLOGICAL ASPECTS OF QUALITY CONTROL OF RAW MATERIALS**

**Pesticide Residues:**

Medicinal plant materials are liable to contain pesticide residues which accumulate from,

i. Agricultural practices.
ii. Spraying the pesticides during cultivation & storage.
iii. Treatment of soil during cultivation.
iv. Administration of fumigants during storage.

Since many medicinal preparation of plant origin are taken over long periods of time, limits for pesticide residues should be established following the recommendation of,

FAO = Food and Agricultural organization of united nations
WHO = World Health Organization.

**Classification of Pesticides:**

A classification based on the chemical composition or structure of pesticide is must useful for analytical chemists,

1. Chlorinated hydrocarbon and related products;
- Aldrin
- HCH (Hexachlorocyclohexane)
- BHC (Benzene hexachloride)
- HCB (Hexa chlorobenzene)
- DDT (Dichloro diphenyl trichloro ethane)
2. Chlorinated Phenoxy alkanoic acids
- 2, 4 D (2,4 dichlorophenoxy acetic acid)
- 2,4, 5 T (2,4,5 trichloro phenoxy acetic acid)
3. Organophosphorous pesticides
- Malathion
- Parathion
- Methyl parathion
4. Carbamate insecticides
- Carbaryl
5. Dithiocarbamate fungicides
- Ferbam
- Zineb
6. Inorganic pesticides
- Aluminium phosphate
- Calcium arsenate

- Lead arsenate
7. Natural pesticides (or) pesticides of plant origin
- Pyrethroids
- Rotencids
- Nicotine
8. Miscellaneous
- Bromo propylate
- Etheyline dibromide
- Ethylene oxide
- Methyl bromide

**Maximum limit for pesticide residues for Medicinal plant materials:**

- In general, the intake of residues from medicinal plant materio should account for no more than 1% of total intake from all sources, including food and drinking water.
- Certain plant materials after extraction are usually much lower because of the law solubility in water or alcohol. It is therefore important to determine the actual quantity of residues consumed in the final dosage forms.
- ARL (Acceptable Residual level) in mg of pesticides per kg of plant material can be calculated on the basis of the maximum acceptable daily intake of the pesticide for human (ADI), as recommended by FAO and WHO, and the mean daily intake (MDI) of the medicinal plant material.

$$ARL = \frac{ADI \times E \times 60}{MDI \times 100}$$

Where, ADI =Max. acceptable daily intake of pesticide (mg/kg of body weight)

E = Extraction factor (transition rate of pesticide from the plant plant material into the dosage foam)

MDI = Mean daily intake of medicinal plant product

60 = numerater represents mean adult body weight

100 = Consumption factor (>1% of total pesticide)

**Recommended methods:**

- Column chromatography
- Thin layer chromatography
- HPTLC & HPLC
- Determination of total chlorine & phosphorous
- Determination of chlorides & phosphates
- Gas chromatography
- Determination specific pesticide compounds by their individual procedure specified in the monograph.

They are mentioned above are various methods to detect and determine the presence of pesticides residues in the medicinal plant materials and their products.

**HEAVY METALS AND ARSENIC**

Contamination of medicinal plant materials with heavy metals and arsenic can be attributed by

i. Due to environmental pollution.
ii. Due to pesticide residues
iii. Due to contamination during cultivation, collection, processing, storage and formulation practices.

**Limit test for Arsenic;**

The amount of arsenic in the medicinal plant material is estimated by matching the depth of colour with that of a standard stain.

**Recommended Procedure:**

**i) Preparation of the sample by and digestion**

Place about 35-70g of coarsely powdered, accurate weighed medicinal plant material in a 800-1000 ml capacity Kjeldahl/flask

↓

Add 10-25 ml of water and 25-50 mlf of nitric acid and
20 ml Sulphuric acid carefully

↓

Heat cautiously so that no excessive foaming takes place

↓

Gradually add nitric acid drop by drop, until all the organic matter is destroyed

↓

Cool, and add 75 ml of water and 25 ml of ammonium oxalate

↓

Heat again until sulphur trioxide vapour develops.

↓

Cool and transfer with the help of water to a 250 ml volumetric flask and makeup the volume with water

**ii) Preparation of standard stain:**

Add 10 ml of stannated hydrochloric acid and 1ml of dilute arsenic to 50ml of water. The resulting solution which contain 10 µg of arsenic, when treated as described in the general limit test for arsenic, yields a stain on mercuric bromide paper.

**iii) Apparatus:**

A wide mouthed bottle of about 120 ml capacity filled with a rubber bung, throught which a glass tube passes. The late made up of ordinary glass tubing, have a total height of about 200mm and an internal diameter of exactly 6.5 mm (external diameter about 8 mm). The lower end of the tube is drawn out an internal diameter of about 1mm and there is hole not less than 2mm in diameter blown in the side of the tube.

The tube is positioned so that when the bottle contains 70ml of liquid the constricted end 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 has a flat, ground surface at right, angles to the axis of the tube, with slightly rounded – off edges.

One of the two rubber burgs (25mm x 25 mm) each with a central hole of exactly 6.5 mm diameter, is fitted at the upper end of the tube. The other bung is fitted with a piece of glass tube about 3 mm long and with an internal diameter of exactly 6.5 mm and with a similar ground surface.

One end of each of the tube is flush with the larger end of the bungs, so that when ends are held tightly together with a rubber bund or a spring clip, the opening of the tube meet to form a true tube. Alternatively, the two bungs may be replaced by any suitable construction satisfying the conditions described in the test.

**iv) Method:**

Moisten some cotton wool with lead acetate (80 g/l), allow to dry and lightly pack into the tube which fits into the wide mouthed bottle to not less than 25 mm from the top. Between the flat surfaces of the tubes, place a piece of mercuric bromide paper that is large enough to cover their openings. The mercuric bromide paper can be fitted by any other means provided that;

- The whole of the evolved gas passes through the paper
- The portion of the paper in contact with the gas is a circle 6.5 mm
- The paper is protected foam syndicate lurina the test.
-

**Limits test for a cadmium and lead**

**Materials required:**

**a) Digestion vessel**

Consisting of a vitreous silica crucible, "tall form", height 62 mm, diameter 50 mm, capacity 75 ml, with a vitreous silica cover.

**b) Digestion mixture**

2 parts by weight of nitric acid and 1 part by weight of perchloric acid.

**c) Reference material**

Olive leaves and hay powder

**Preparation of the sample**

In a clean silica crucible, place 200-250 mg of air dried, accurately weighed, finely cut and homogenized plant material.

↓

Add 1 ml of digestion mixture, place it in an oven with a controlled temperature and time regulator

↓

Heat slowly at $100^0$C and maintain at this
temperature for upto 3 hours.

↓

Then heat to $120^0$C and maintain at this temperature for 2 hours

↓
Raise the temperature very slowly to $240^0$C, avoiding losses due to violent reactions especially in temperature of 160-$200^0$C.
↓
Maintain this temperature ($240^0$C) for 4 hours
↓
Dissolve the remaining inorganic residue in 2.5 ml of nitric acid and use for the determination of heavy metals

**Method**

- The content of the lead and cadmium may be determined by a) inverse voltametry (or) b) Atomic adsorption spectrophotometry
- Every sample should be tested parallel with blank
- The max. amounts in dried plant material which are base on ADI values, are proposed.
- Lead 10 mg / kg
- Cadmium 0.3 mg/kg

**MICRO – ORGANISMS**

- Medicinal plant materials carry a great number of bacteria and moulds, often originating in soil .While a large range of bacteria and fungi form the naturally occurring microflora of herbs, aerobic spere forming bacteria frequently predominate.
- Current practices of harvesting, handling and production may cause additional contamination and microbial growth.
- In addition, the presence of aflatoxins in plant material can be hazardous to health if absorbed even in very small amounts. They should therefore be determined after using a suitable clean-up procedure.

**Test for specific micro-organisms**

**Pre – treatment of the material being examined**

Depending on the nature of the crude medicinal plant material, grind, dissolve, dilute, suspend or emulsify the material being examined using a suitable method and eliminate any antimicrobial properties by dilution, neutralization or filtration.

**Klates – soluble materials**

Dissolve or dilute 10 g or 10ml of plant material, unless otherwise specified in the test procedure, in lactose broth or another suitable medium proven to have no antibacterial activity under the conditions of the test, adjust the volume to 100ml with the same medium. If necessary adjust the pH of the suspension to about 7.

**Non-fatty materials insoluble in water:**

Suspended 10g or 10ml of material, unless otherwise specified in the monograph, in lactose broth or another suitable medium proven to have no antibacterial activity under the conditions the test, dilute to 100 ml with same medium. If necessary divide the material being

examined and homogenize the suspension mechanically. A suitable surfactant, such as a solution of polysorbate SOR containing 1 mg/ml may be added. If necessary adjust the pH of the suspension to about 7.

**Fatty materials:**

Homogenise 10g or 10ml of material, unless otherwise specified in the monograph, with 5g of polyorbate 20R Cr polysorbate SOR if necessary heat NMT $40^0$C. Mix carefully while maintaining the temperature in a water bath or oven. Add 85 ml of lactose broth or another suitable medium proven to have no antibacterial activity in the conditions of the test. Maintain this ($45^0$C) temperature for the shortest time if necessary until an emulsion is formed an in any case not morethan 30 minutes. If necessary adjust the pH of the emulsion to about 7.

**Detection of bacteria:**

Homogenize the pretreated material approximately and incubate at 30-$37^0$C for a length of time sufficient for revi/vififcation of the bacteria but not sufficient for multiplication of the organisms (usually 2-5 hours). Shake the container, transfer 1g or 1ml of the lumogenized mixture or material to 100 ml. Enterobacteriaceae enrichment broth- Mossel and incubate at 35-$37^0$C for 18-48 hrs. Prepare a subculture on a plate with violet – red bile agar with glucose and lactose. Incubate at 35-$37^0$C for 18-48 hrs. The material passes the test if no growth of colonies of grain negative bacteria is detected in the plate.

**Quantitative evaluation:**

Inoculate a suitable amount of Interobacterianceae enrichment broth-Mossel with qualities of homogenized material prepared as described under "Detection of bacteria" above. Approximately diluted as necessary, containing 1g, 0.1g cr 10 µg or 1 ml, 0.1 ml and 10µl of the material being examined. Incubate at 35-$37^0$C for 24-48 hours. Prepare a subculture of each of the cultures on a plate with violet – red bile gar with glucose and lactose in order to obtain selective isolation. Incubate at $37^0$C for 18-24 hrs. The growth of well-developed colonies, generally red or reddish in colour of gram-negative bacteria constitute a positive result. Note the smallest quantity of material that gives a positive result. Deterine the probable no bacteria using the table below.

A.P- II

| **Results for each quantity or volume** | | | **Probable number of bacteria per gm of material** |
|---|---|---|---|
| 10 g (or) 1.0 ml | 0.1 g (or) 0.1 ml | 0.01 g (or) 0.01 ml | |
| + | + | + | More than $10^2$ |
| + | + | - | Less than $10^2$ but more than 10 |
| + | - | - | Less than 10 but more than 1 |
| - | - | - | Less than 1 |

**Microbial contamination limits in Medicinal plant materials:**

Different limits are set according to the use of the material or the material itself.

**I. Untreated plant material harvested under acceptable hygienic condition**

- Escherichia coli, max $10^4$ per gram
- Mould propaguiles, max $10^5$ per gram

**II. Pretreated plant materials (or) used as topical dosage forms**

- Aerobic bacteria, max $10^2$ per gram
- Yeast and Moulds, max $10^4$ per gram
- Escherichia coli, max $10^2$ per gram
- Other enterobactericeae, max $10^4$ per gram
- Salmonella, None

**III. For other plant materials for internal use,**

- Aerobic bacteria, max $10^5$ per gram
- Yeast and Moulds, max $10^3$ per gram
- Escherichia Coli, max 10 per gram
- Other enterobacteria, max max $10^3$ per gram
- Salmonella, None

**TEST FOR AFLATOXINS**

This test is designed to detect the possible presence of aflatoxins $B_1$, $B_2$, G, and $G_2$ which are highly dangerous contaminants in any materials of the plant origin.

**Recommended Procedure:**

**Preparation of the sample:**

Grind or reduce not lessthan 100g of crude medicinal plant Material to a moderately five powder (Siere no 355/180)

↓

Kleigh 50g of the powdered material, transfer to a conical glass stoppered flask and add 170 ml of methanol and 30 ml of water

↓

Using a mechanical device, shake vigorously for 30 minutes

↓

Filter through a porosity filter paper

↓

Collect 100ml of the filtrate (A) – from the start of flow, otherwise discard the first 50ml and collect 40 ml of fitrate CB

↓

In order to eliminate interfering plant pigments use a special
clean-up procedure

↓

Transfer 100ml of filtrate A to a 250 ml beaker and add 20 ml of Zinc acetate / aluminum chloride and 80 ml of water
↓
Stir and allow to stand for 5 minutes, add 5g of a filter – as such as diatomaceous earth, mix and filter through a medium porosity filter paper
↓
Discard 50 ml of first and collect 80 ml of the filtrate (C)

**Method:**

To either of the residue obtained above, add 0.2 ml of mixture of 98 volumes of chloroform and 2 volumes of acetonitrile, close the vial and shake vigorously until the residues are dissolved, preferably using a vortex mixture.

Carryout the "Thin layer chromatography" using silicaget as the coating substance and a mixture of 85 volumes of $CHCl_3$ and 10 volumes of acetone and 5 volumes of 2-propnaol as the mormile phase.

Apply separately to the plate 2.5µl, 5µl, 7.5µl & 10 µl of aflatoxin mixtures, then apply three volumes, each of 10µl of the sample residues.

Further super impose on one of these spots 5 µl aftotoxin mixture phase the plates in an unsaturated sample and develop. After removing the plate from the chromatographic chamber, allow it to dry in air and examine the chromatogram in a dark room under ultraviolet light (365 nm).

**Interpretation of Results:**

No spots corresponding to aflotoxin should be obtained from any of the sample residues. If any such spot is obtained compare its position with the spots obtained from the aflatoxin mixture to identify the type of alfatoxin present.

An approximate estimation of the concentration of alatoxin in the sample may be obtained by comparing the intensity of the spots with those of the aflatoxin mixture.

## RADIO ACTIVE CONTAMINATION

- A certain amount of exposure to ionizing radiation cannot be avoided since they are many sources, including radionuclides occurring naturally in the ground and the atmosphere.
- The range of radionuclides that may be released into the environment as the result of a nuclear accident might include long lives and short-lived fission products, activities and activation products.
- The nature and the intensity of radionuclides released may different markedly and depend on the source (reactor, reprocessing plant, fuel fabrication plant, isotope production unit, etc).
- The amount of exposure to radiation depends on the intake of radio nuclides and other variables such as age, metabolic kinetics and weight of the individual.
- Even at maximum observed levels of radioactive contamination with the more dangerous radio nuclides, significant risk is associate only with consumption of quantities of over 20 kg of plant material per year so that a risk to health is most unlikely to be encountered given the amount of medicinal plant materials that would need to be ingested. Additionally, the level of

contamination might be reduced during the manufacturing process. Therefore, no limit for radioactive contamination are proposed.

**Methods of Measurements**

Since radionuclides from accidental discharges vary with the type of facility involved, a generalized method of measures me is so for not available. However, should such contamination be of concern, suspect samples can be analysed by a competent laboratory. Details of laboratory techniques of available from the International Atomic Energy Agency (IAE) address.

**IAEA,( International Atomic Energy Agency )**

Analytical quality control Services,
Laboratory Seibersdorf,
PO Box 100, Vienna, Asutria

## Conclusion

These techniques can be used as quality control tool in assessing the quality of herbal materials and herbal pharmaceuticals. This article will be useful in bio-prospecting of natural products and traditional medicine-inspired drug discovery and development.This review highlights the current advances in knowledge about the safety, efficacy and quality control of herbal medicines.

**References**

**1**) Archana Gautam et.al., “identification, evaluation & standardization of herbal drugs: a review”, Pub in scholars research library, 2010, Vol 2(6), Page no. 302-315

2) Bhattacharjee Supriya Kumar, Dr. De L.C, “Medicinal Herbs and Flowers” 1st published in 2005, Published by Aavishkar Publishing and Distributers, Jaipur, Page no. 1,8
3)Calixto J.B, ‘Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines’ Online available on - scielo.br/scielo.php?pid=S0100-879X2000000200004&script=sci_arttext

4) http://www.pharmatutor.org/pharmacognosy/quality-control-of-phytomedicines.html

5) Kunle et.al., “Standardization of herbal medicines- A review”, Pun in International journal of biodiversity & conservation, 2012, Vol 4(3), Page no. 101-112

6) Mukherjee Pulok K., “Quality control of herbal drugs” First edition 2002, Published by Business horizons, New Delhi, Page no. 21, 57-59,70-82
.
7) Nandna Khurana et.al., “Microbiological quality control assessment of some commercial herbal drugs”, Pub in International journal of pharmaceutical quality assurance, 2011, Vol 3(4), Page no. 15-17

8)WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues, 2007

9) Wyk Ben-Erik Van, Wink Michael, “Medicinal Plants of The World” 1st Published in 2004, Published by Times Editions, Singapore, Page no. 24-26.