# Standardization Parameter of Churn

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## ABSTRACT

Churna preparations are an important and widely used form of Ayurvedic herbal formulations in India. These are prepared by mixing powdered form of single or mixture of several crude drugs meant to be dispensed as such.

Churna is defined as a fine powder of drug or drugs in ayurvedic system of medicine. Drugs mentioned in patha are cleaned properly, dried thoroughly, pulverized and then sieved. The churna is free flowing and retains its potency for one year, if preserved in airtight containers. Churna formulations are similar to powder formulations in allopathic system of medicine. In recent days churna is formulated into tablets in order to fix the dose easily. These forms of medicament are prescribed generally because of their particle size. Smaller the particle size greater is the absorption rate from g.i.t and hence the greater is bioavailability. Aim of this article to give a detail account on some parameter use for standardization of churna.

**Keywords: -** Churn, Bioavailability, Medicine, Parameter

## INTRODUCTION

Safety and efficacy of herbal medicines are two main issues of a drug therapy to which, the source and quality of raw materials plays an important role . There is wide awareness among the scientific community regarding the quality control of herbal drugs and formulations in the last decade. Associated factors such as the use of fresh plants, temperature, light exposure, water availability, nutrients, period and time of collection, method of collecting, drying, packing, storage and transportation of raw material, age and part of the plant collected, etc., can greatly affect the quality and consequently the therapeutic value of herbal medicines . The World Health Organization, in a number of resolutions, has also emphasized the need to ensure the quality control of plant products by using modern techniques, suitable analytical methods and by applying suitable standards In order to have a good coordination between the quality of raw materials, in process materials and the final products, it has become essential to develop reliable, specific & sensitive quality control methods using a combination of classical and modern instrumental method of analysis. Standardization is an essential measurement for ensuring the quality control of herbal drugs. “Standardization” expression is used to describe all measures, which are taken during the manufacturing process and quality control leading to a reproducible quality. It also encompasses the entire field of study from birth of a plant to its clinical application. It also means adjusting the herbal drug preparation to a defined content of a constituent or a group of substances with known therapeutic activity respectively by adding excipients or by mixing herbal drugs or herbal drug preparations. “Evaluation” of a drug means confirmation of its identity and determination of its quality and purity and detection of its nature of adulteration

Parameters uses for standardization are as follow:

1. Physical parameters include color, odor, appearance, Fluorescence Analysis, Ash Value, Crude Fiber, Moisture Content, Extractive Value, Swelling Index, Foaming Index, pH Value, Angle of repose and flow properties, calculation of bulk and tap density, Compressibility Index and Determination of Tannins.
2. Chemical parameters include limit tests, chemical tests etc.
3. Chromatographic analysis done using TLC, HPLC, HPTLC, GC, UV, GC-MS, fluorimetry etc.

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4. Microbiological parameters include total viable content, total mold count, total enterobacterial and their count, Detection of Aflatoxin.

**Physical Parameters**

**Fluorescence Analysis: -** the original molecules absorb light usually over a specific range of wavelength, get

excited to a high level and many of them emit such radiation while coming back to the original state. Such a phenomenon of re-emission of absorbed light that occurs only when the substance is receiving the excited rays is known as fluorescence. It can use to check the adulteration in powder drug.

**Method**:-About 1 gm of powder drug was taken in a petridish and treated separately with different regent viz, methanol, 1 N Methanolic sodium hydroxide, Ethanol,1 N Ethanolic sodium hydroxide, 1N HCL, 1N NaOH, 50 % H2S04, 50 % HNO3, and 5 % potassium hydroxide, acetone. These observed under lights of wave length 254 nm, 365 nm and visible light. Various color radiations emitted were observed in comparison with standard color chart. Before fluorescence analysis powdered drug was sieved through 60 ≠ mesh and observations made following Chase and Pratt.

**Ash Value Determination**

Total ash: - total ash is designated to measure the total amount of material produced after complete incarnation of the crude drug. It uses to detect the contamination and adulteration like sand or earth, unwanted part mixed in crude drug.

Type: - Total ash, Physiological ash, Non-physiological ash, carbonated ash, Sulphated ash, Nitrate ash, and Acid insoluble ash and Water soluble ash.

## METHOD

**Total ash: -** Place about 2 – 4 gm of grounded material (as mentioned in monograph of drug) in crucible and ignited it until it is white.

**Acid insoluble ash: -** Residue obtained after extracting the total ash with hydrochloric acid (HCL). Use to detect the contamination form sand or soil. Boil ash with 25 ml of 2M HCl. for 5min; collect the residue on ashless filter paper, wash with hot water, ignite, cool in desiccators and weigh.

**Water soluble ash: -** Water soluble as is that part of the total ash content which is soluble in water. It is good indicator of either previous extraction of the water soluble salt in the drug or incorrect preparation. Boil the total ash for 5 min in 25 ml distilled water. Collect the insoluble residue on ashless filter paper, wash with hot water, ignite, cool in desiccators and weigh. Subtract the weight of insoluble matter from the weight of total ash gives water soluble ash value.

**Crude Fiber Determination: -** Estimation of crude fiber denotes the measurement of the content of cellulose, lignin and cork cell in the plant tissue. The crude fiber consists of the material other than ash which cannot be dissolved in water and cannot be digested by boiling H2S04 or NaOH. Thus it represent the more resistant part of the plant cells as well as some less resistant cell wall component like cellulose and pectin. The presence of adulteration containing sclerenchyma or other resistant tissue than is permissible for the crude drug under examination may be determined by ascertaining the crude fiber of that sample.

**Method**

About 2 gm of the drug sample accurately weighed is extracted with ether. Hen 200 ml of 1.25 % H2S04 is added to the extracted drug and the whole mixture boiled for 30 mins under reflux in a 500 ml flask. The mixture is then filtered through a hardened filter and the residue washed with boiling water until free of acid. The entire residue is rinsed back into the flask with 200 ml of 1.25 % NaOH and gained boiled under reflux for 30 mins. The liquid is then quickly filtered and residue with boiling water until neutral, dried at 1100c to constant weight

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue V May 2026

and weigh (W1). After that it is incinerated in crucible and form ash (W2). The difference in weight indicated weight of crude fiber content of drug.

**Moisture Content Determination: -** Moisture is an inevitable component of crude drug, which must be eliminated as far as practicable. Drying of crude drug is important during collection of drug and is also important for preservation, preventing hydrolytic degradation ofactive constituents and for easy size reduction of crude drug. Excess moisture or insufficient drying is responsible for spoilage of drug due to growth of microbes. There for drying process should reduce the moisture content of drug below the critical level. Moisture content determination is use to check the total water content in given weight of crude drug. Excess moisture content in a crude drug sample suggest not only that the purchaser could be paying a high price due to unwanted water, but also that the drug has been incorrectly prepared or subsequently incorrectly stored.

**Method**: - Loss on drying, Azeotropic distillation method and Karl fisher method use for moisture content determination in crude drug. Loss on drying: - required quantity of sample taken as per mentioned in monograph of drug and dried in hot air oven at 1050C to a constant weight**.** Difference in weight indicates the moisture content of drug.

**Extractive Value Determination: -** Extraction of any crude drug with a particular solvent yields a solution containing different phyto-constituents. The composition of these phytoconstituents in that particular solvent depends upon the nature of the drug and solvent used.

Extractive value determined the amount of active constituents in a given amount of medicinal plant material when extracted with solvent. It is employed for that material for which no chemical or biological assay method exist. As mentioned in IP – 1996, BP- 1980, the determination of water soluble and alcohol soluble extractives is used as a means of evaluating crude drugs which are not readily estimated by other means.

**Water soluble extractive**: - 5 gm of the air dried drug, coarsely powdered have to be macerated with 100 ml of water closed flask for 24 hrs, shaking frequently during the first 6 hours and allowing standing for 18 hrs. Filter and evaporates 25 ml of the filtrate to dryness at 1050c and weigh.

**Alcohol soluble extractive**: - 5 gm of the air dried drug, coarsely powdered have to be macerated with 100 ml of ethanol of specified strength in a closed flask for 24 hrs, shaking frequently during the first 6 hours and allowing standing for 18 hrs. Filter and evaporates 25 ml of the filtrate to dryness at 1050c and weigh.

**Determination of Swelling Index: -** Many medicinal plants materials are of specific therapeutic properties or pharmaceutical utility because of their swelling properties, especially gums and those containing an appreciable amount of mucilage, pectin or hemicelluloses. Swelling index is defined as the volume in ml of taken up by the swelling of 1 g of plant material under specified condition. Its determination is based on the addition of water or a swelling agent as specified in the test procedure for each individual plant materials (either whole, cut or pulverized). Using a glass stopper measuring cylinder, theplant 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.

**Method**: - The specified quantity of the plant material concerned is introduced previously reduced to the required fineness and accurately weighed, into a 25 ml glass stoppered measuring cylinder. 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. Unless otherwise indicated in the test procedure, 25 ml of water is to be added. The mixture is shaken thoroughly every 10 minutes for 1 hour. Allow to stand for 3 hours at room temperature or as specified. The volume (in ml) has be measured which is occupied by the plant materials, including any sticky mucilage. The man value of the individual determination is calculated related to 1 g of plant materials

**Determination of Foaming Index**

The saponins are high molecular weight containing phytoconstituents having the detergent activity. Saponins are mostly characterized based on their frothing property. Medicinal plants of different groups, those derived

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from the families Caryophyllaceae, Arailiaceae, Diosscoreaceae contain saponins. Many medical plant materials contain saponins that can cause persistent foam when an aqueous decoction is shaken. The foaming ability of an aqueous decoction of plant materials and their extracts is measured in terms of foaming index.

**Method**: - Reduced about 1 g of the plant materials to a coarse powder (Sieve size No. 1250),weigh accurately and transfer to a 500 ml conical flask containing 100 ml of boiling 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 dilute. Pour the decoction in 10 ml stoppered test tube (height 16 cm, diameter 16 mm) in successive portion in 1 ml, 2 ml, 3 ml and adjust the volume of the liquid in each tube with water to 10 ml. stopper the tubes and shaken them in a length wise motion for 15 seconds, two shakes per second. Allow standing for 15 minutes and measured the height of the foam. The result are assessed as follows

**Determination of Tannins: -** Tannins are widely distributed in plants and occur in solution in cell sap, often in the distinct vacuoles. They are chemical substance that can betested by gold beaters test. Tannins are substance cable of turning animal's hides into leather by binding proteins to form water insoluble substances that are resistant to proteolytic enzyme. When tannins applied to living tissues known as an "astringent" action and are thereason for the therapeutic applications of tannins. Chemically tannins are complex substance, they usually occur as mixture of poly-phenols that are difficult to separates and crystallize. The tannins are having very high molecular weight from 1000- 5000. Tannins with low molecular weight are known as pseudo – tannins.

**Method**: - To prepare the plant materials extract, introduced the quantity specified in the test procedure for the plant material concerned, previously powdered to a known fineness and weighted accurately into a conical flask. Add 150 ml of water and heated over a boiling water bath for 30 mins. Cool transfer the mixture to a 250 ml volumetric flask and dilute to volume with water. Allow the solid materials to settle and filter the liquid through a filter paper, discard first 50 ml of the filtrate.

To determine the total amount of materials that is extractable into water, evaporate 50 ml of the plant materials extract to dryness, dry the residue in an oven for 4 hours at 1050 C and weigh (T1). 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 shaken well for 60 minutes. Filter and evaporate 50 ml of the clear filtrate to dryness. Dry the reside in an oven at 1050 C and weight (T2). To determine the soluble of hide powder, take 6 gm of the powder and add 80 ml of water and shaken well for 60 minutes. Filter and evaporate 50 ml of the clear filtrate to dryness. Dry the residue in an oven at 1050 C and weigh (T0).

The quantity of tannins as a percentage can be determined using formula = [T1 – (T2 - T0)] x 500/W

Where W = the weight of the plant materials in grams

**Determination of pH Value: -** The pH value conventionally represents the acidity or alkalinity of an aqueous solution. In pharmacopoeia standers and limits of pH have been provided for those pharmacopeia substances in which pH as a measure of the hydrogen – ion activity is important from the standpoint of stability of physiological suitability. The determination is carried out at a temperature of 250c ± 20-, unless otherwise specified in the individual monograph.

**Apparatus: -** The pH value of a solution s determined potentiometrically by means of a glass electrode and a pH meter either of the digital or analogue type. Calibrate the apparatus using buffer solution D as the primary standard, adjusting meter to read the appropriate pH value given in table corresponding to the temperature of solution. To set the scales use a second reference buffer solution. To set the scale use a second reference buffer solution eitherbuffer solution A or E or G or carry out a check with a third buffer solution of intermediate pH. The pH reading of the intermediate solution must not differ by more than 0.05 from the corresponding value indicated . Preparation of reference buffer solution: -

Prepare the following buffer solution using carbon dioxide free water. Buffer solution should be stored in bottles made of alkali free glass and must not be used later than 3 months after preparations.

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue V May 2026

**Method: -** Immerse the electrode in the solution being examined and measure the pH at the same temperature as for the standard solutions. At the end of set of measurements record the pH of the solution used to standardize the meter and the electrodes. If the difference between this reading and the original value is greater than 0.05 the set of measurements must be prepared. All solution and suspension of substance being examined must be prepared using carbon dioxide free water.

**Determinations of angle of repose and flow rate: -** Good flow properties are critical for the development of any pharmaceutical product including powder formulations. It is essential that an accurate assessment of flow porters to be made as early in the development process so that an optimum formulation can be quickly identified. Interparticle forces between particles as well as flow characteristics of powders are evaluated by angle of repose.

Angle of repose is defined as the maximum angle possible between the surface of pile of sample and horizontal plane. Angle of repose is affected by presence of moisture, size and shape of sample, percent fines, and amount of glidant and lubricant. More the moisture in sample, more it becomes sticky. Similarly smaller the size more is the flow of powder. Angle of repose is a helpful parameter to monitor quality of powdered of granular pharmaceutical formulations. For good flowing powders angle of repose should be less than 300

**Method**

1. Take a clean and dry funnel with a round stem of 20 to 30 mm diameter with flat tip and attached it to the burette stand.
2. Place a graph paper sheet below the funnel, on clean and dry platform.
3. Adjust the distance between lower tip of the funnel and sheet to some specified height.
4. Gentles pour sample in funnel from top till a heap of powder forms and touches of lower tip of the funnel.
5. Repeat the procedure four times to obtain average reading.
6. Find out average diameter and radium of the each drawn circle

**Angle of repose Calculation**

1. Radius determination - r = diameter of heap (d) / height of heap (h)
2. Average radius determination – r = r1 + r1 + r1 + r1/4
3. Angle of repose (θ) - tan-1 (h/r)
4. Flow rate calculation = mass of powder / time required for flow = W2/t (gm/sec)

**Determination of Bulk and Tap Density and Compressibility Index**

Requirement: - Graduated measuring cylinder and bulk density apparatus and weighing balance.

**Method**

1. Weigh accurately 25 g of powder (W1) .
2. Place it in dried graduated measuring cylinder and note the volume as V1 ml.
3. Place the cylinder containing sample in bulk density apparatus for 100 tapping and operate it. Record the volume occupied by the powder as V2 ml.

**Bulk and tap density Calculation**

1. Bulk density = Mass / Bulk volume = W1 / V1 (g/ml)
2. Tap density = Mass / Tap volume = W1 / V2 (g/ml)
3. Compressibility index = tap density – Bulk density / tap density Lower the compressibility index value better flow rate of powder.

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue V May 2026

**Chemical Parameters [12-13]**

**Limit tests**

**Arsenic Content**

Preparation of Standard Solution (10PPM)**:-** 0.33gms of arsenic trioxide was dissolved in 5ml of 2M Sodium hydroxide solution and then diluted to 250ml with water. One volume of this was then diluted to 100 volumes with water.

**Preparation of Sample**

**Preparation of Churna solution: -** The churna solution was prepared by means of diluting 1gm of churna to 100ml using distilled water. This is used to carry out limit test for iron and lead and also to perform qualitative test for mercury. 10ml of churna solution was pipette out into a flask and about 10ml of concentrated nitric acid was added and evaporated to dryness on a water bath. The residue was then dried at 130° C for 30minutes then about 10ml of hydrazine molybdate reagent was added and refluxed for 20minutes. The solution was then cooled and absorbance of both standard and test solution was measured at 800nm using Perkin Elmer UV spectrophotometer.

**Limit test for Iron**

**Preparation of Standard Solution (20 PPM):-** One volume of 0.1726% w/v solution of ferric ammonium sulphate solution was diluted in 0.05 M sulphuric acid to ten volumes using distilled water.

**Procedure**

Limit test was performed in Nessler's cylinder. 2ml of test and standard solutions were taken in separate cylinders and then 2ml of 20% solution of citric acid and 0.1 ml thioglycollic acid were added. The solution was then mixed and made alkaline with iron free ammonia, diluted to 50ml with distilled water. It was then allowed to stand for minutes and colour obtained in sample was compared with that of standard colour. If the colour produced in test is more when compared to that of standard solution then the sample was said to fail the limit test and said to pass the test if vice versa occurs

**Limit Test For Lead**

**Preparation of Standard (20 PPM):- 0.4** gm of lead nitrate was dissolved in water containing 2ml of nitric acid and sufficient water to produce 250ml. About 1volume of above solution was diluted to 10 volumes using distilled water.

**Procedure**

Limit test was performed in Nessler's cylinder. 1ml of standard lead solution and test solution were taken in separate cylinders and were diluted to 25ml using distilled water and then pH was adjusted to value 3-4 by adding dilute acetic acid or dilute ammonia solution and then diluted to 35ml using distilled water. To both the solutions 10ml freshly prepared hydrogen sulphide solution was added, mixed and diluted with water to 50ml. It was then allowed to stand for 5minutes and viewed downwards over white surface. The colour produced in test solution should not be more intense than that of standard solution, if so then the sample is said to pass the limit test for lead.

**Test for Mercury**

To 10 drops of test solution 6M HCL was added to get a white precipitate. The precipitate was then treated with 6M ammonia solution. If the colour of precipitate changes to grey or black colour then it indicates the presence of mercury.

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue V May 2026

**Chemical testing**

Phytochemical testing on extracts of churna carried out as per the test and procedure mentioned in Dr. Khandelwal K.R, Practical Pharmacognosy Book for both primary and secondary metabolite.

**Chromatographic Analysis[14-]**

Chromatographic analysis of churna was done using TLC, HPLC, HPTLC, GC, UV, GC-MS,fluorimetry etc. Some example of chromatographic analysis is mentioned in blow table

**Chromatographic analysis of churna**

| No | Name of churn | Chromatographic method use | Method Details |
|---|---|---|---|
| **1** | Haritaki Churna | HPLC | HPLC system use: Waters chromatographic system consisting Waters 2695 separation module (quaternary pump) equipped with an auto injector and Waters 2998 photodiode array detector.<br>Colum use: Thermo Scientific BDS HYPERSIL Phenyl reversed phase column (100mm×4.6mm,3µm).<br>Mobile phase: 0.02% triethyl amine aqueous Ph 3.0 with ortho-phosphoric acid and acetonitrile.<br>Flow rate: - The flow rate was 1.0ml/min and liquots of 10µl were injected. |
| **2** | Pippali Churna | | Stationary Phase : Silica gel 60 F254 of 0.2 mm<br>2. Mobile Phase : Toluene : ethyl acetate: formic acid ( 5 : 15 : 0.5) Visualization : Under UV cabinet ( 366 nm) |
| **3** | Sitopaladi churna | UV-spectrophotometric | Instrument use : UV- spectrophotometric Std. Piperine solution preparation: Piperine (100mg) was dissolved in methanol and volume was made up to 100ml with methanol in volumetric flask. Two ml of this solution was diluted with methanol up to 100ml in volumetric flask to give 20µg/ml piperine solution.<br>Preparation of piperine extract of Sitopaladi churna: Reflux the powder Sitopaladi churna (1gm) with 60 ml ethanol for 1 hour. Filter the extract and reflux the marc left with 40 ml of methanol for another 1 hr. filter and combine the filtrate. Concentrate the methanol extract under vaccum till the semisolid mass is obtained. Dissolve the residue in 75 ml methanol and filter through sintered glass funnel (G-2) by vaccum filtration assembly. The filtrate was centrifuged at 2000 rpm for 20 minutes, the supernatant was collected in 100ml volumetric flask and volume was made with methanol. |

**Microbiological Parameters**

**Determination of Microbial Content**

1gm of churna was dissolved in lactose broth and volume adjusted to 100ml with the samemedium. About 10ml of sample was transferred into 100ml of Macconkey broth and incubated for 18-24 hours at 43-45°C. A subculture was prepared on a plate with Macconkey agar and incubated at 43-45°C for 18-24 hours. The growth of red, generally non-mucoid colonies of gram negative rods appearing as reddish zones indicates the presence of E.coli if not then it indicates the absence of E.coli.

**Detection of Aflatoxin: -** Aflatoxin is a toxin from Aspergillus flavus and Aspergillus parasiticus having the chemical formula C12H12O6 and may cause hepatica carcinoma in human being. The plant species may be

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue V May 2026

contaminated with this toxin. The test for aflatoxin asprescribed by WHO for the herbal drugs is designed to detect the presence of B1, B2, and G1, G2 which are the dangerous contaminants in any plant material of plant origin.

**Method**: - Not less than 100 gm of to crude drug of plant origin is grinded to a moderately fine powder (sieve 355/180). 50 g of the powder material is mixed with 170 ml of methanol and 30 ml of water in a conical stopper flask and shaken vigorously using a mechanical device for 30 minutes. Through a medium porosity filter paper it is filtered. 100 ml of filtrate from the start of flow (filtrate - A) is collected. Otherwise first 50 ml of the filtrate is discarded and next 40 ml of the filtrate is to be collected (Filtrate - B). 100 ml filtrate transfer into anther flask and 20 ml zinc acetate – aluminum chloride and 80 ml of water is added. It is stirred and allowed to stand for 5 minutes. 5 g of filter aids like diatomaceous earth are added, mixed and filtered through a medium porosity filter paper. First 50 ml of the filtrate is to be discarded and next 80 ml is o be collected (filtrate - C). Either filtrate B or C is transferred to a separating funnel and 40 ml of sodium chloride (100 g / lit) and 25 ml of light petroleum ether are added and shaken for one minute. Allow the layers to separate and lower layer is transferred to a second separating funnel. It is extracted twice with 25 ml of dichloromethane and shaken for 1 min. the layers are allowed to separate out and combined each of the lower layers in a 125 ml conical flask. Several boiling chips are added and is evaporated to dryness on a water bath. The residue obtained used for TLC.

To the residue obtained above add 0.2 ml mixture of chloroform and acetonitrile (98:2) is added and closed properly and shaken vigorously until the residue is dissolved. The chromatographic detail as follows

1. Stationary phase – Silica G
2. Mobile Phase – chloroform : acetone : 2-propanol(85:10:5)
3. Std :- 2.5, 5, 7.5 and 10 µl aflatoxin
4. Visualization: - UV light at 365 nm. [Blue spot for aflatoxin]

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ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue V May 2026

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