

**GUIDELINES SERIES-I**

**GENERAL GUIDELINES FOR  
DRUG DEVELOPMENT OF  
AYURVEDIC FORMULATIONS**



**CENTRAL COUNCIL FOR RESEARCH IN AYURVEDIC SCIENCES**

**Ministry of AYUSH, Government of India**

**New Delhi**



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# **GENERAL GUIDELINES FOR DRUG DEVELOPMENT OF AYURVEDIC FORMULATIONS**

**Volume - I**



**CENTRAL COUNCIL FOR RESEARCH IN AYURVEDIC SCIENCES**  
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## Prologue

Research & Development in the field of AYUSH system in different areas such as drug development including quality assurance, pre-clinical safety evaluation and clinical research are being conducted at different levels such as Research Council under AYUSH, Academic institutions (both AYUSH and non AYUSH institutes such as Medical Colleges, Universities etc.), other Research organization such as ICMR, CSIR etc. Further, research support is also being extended through grant under EMR vide Ministry of AYUSH, DST, DBT, ICMR etc. in the area of traditional medicine.

Lot of research is being conducted at different levels as above in the field of AYUSH adopting different guidelines, methods and protocols and ending up research outcomes with low or poor translational value. Only few of them have led to clinical trial and marketing level. This may be attributed to lack of awareness regarding AYUSH strategies for R&D and provisions of Drug & Cosmetic Act related to AYUSH.

In spite of availability of several guidelines such as GCP guidelines for ASU drugs, ICMR guidelines for biomedical research for human participants, GCP guidelines published by CDSCO Ministry of Health and Family Welfare, WHO guidelines for traditional medical research etc., there is no single comprehensive directive to conduct research in AYUSH sector is available.

This might be one among the major reasons that has led to R&D in AYUSH sector with diverse approaches with low translational value.

In the light of this preamble, it becomes imperative to develop directives on research practices for various components of AYUSH research sectors for uniform adoption across all stakeholders such as research councils, academic institutes, funding agencies engaged in AYUSH research.

Considering this, efforts have been made by CCRAS and developed three comprehensive and concise Guidelines and directives focusing on drug development (Standardization and quality assurance), safety and toxicity and clinical evaluation for ready reference of stack holders. These directive and Guidelines encompassed with research practices generally to be adopted and followed by researchers in the field of AYUSH system such as Research organizations, academic institutions and Researchers seeking grant from EMR/IMR schemes of different agencies funding for research on AYUSH system, would certainly help the researchers while designing and formulating the proposals and also planning academic industrial research in the field of AYUSH systems. The users may refer other two documents for having an overall idea concerning drug development and R & D in this field.

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## Background

Quest for healthy and long life are perhaps as old as human existence and efforts are unremitting to address the challenges and triumph over the bottlenecks across this journey. Ayurveda –the science of life, evolved as a comprehensive system of healthcare systematically through scientific experimentations of high order backed by sound and reproducible evidence base and stood the test of the time. Several strategies and road maps are being drawn to carry forward merits of this science so as to meet the current day health needs and mainstream its core strengths alongside through research & development in this country and across the globe. The fundamental aspects of holistic systems needs adequate positioning while designing clinical trials to examine the safety and efficacy of Ayurveda approaches. Furthermore, the other challenges and issues related to quality and safety *viz.* dosage forms/delivery system, diverse concepts and complex approaches in trial design, diagnosis and therapy, outcomes of clinical efficacy and drug interactions also pose certain limitations in research. A systems approach may be adopted to validate the therapies and approaches with integration of principles of Ayurveda and bio-medicine without losing the vital fundamentals of both systems. Such an approach with well designed research plans could possibly facilitate to generate tangible evidence.

Ayurveda and Siddha drugs are mainly based on the plants and plant products besides animal products metal/minerals and products of marine origin. About 90% products of Ayurveda are purely herbal. These plant drugs have different chemical constituents which may vary in same species of plant due to influence of climatic condition under which they grow, nature and properties of soil and fertilizer, geographical distribution, age of the plant, altitude and period of harvesting and storage conditions. Hence to control the quality of raw plant material with reference to chemical constituents is a tedious and difficult job. Due to these reason plant drugs differ from that of the conventional drugs and some innovative methods are in practices for the quality assessment of herbal drugs. It also includes a composite study of Ayurveda and Siddha dosage forms like churnas, bhasma, kwatha, taila, tablets and ointments etc.

Plant products and their derivatives constitute about 50% of modern drugs. There has been a quest to develop new drugs /formulation despite of being a costly and low success rate process. In recent years the research have focused on drug discovery from herbal medicines or botanical source by gaining leads from traditional literatures, folklore claims, database etc. As Traditional Systems have long history of herbal usage in management of disease, the success rate of their development as therapeutic approach is comparatively higher than that of the synthetic counterpart. Examples of plant products and derivatives used by the pharmaceutical industry include paclitaxel, vincristine, vinblastine, artemesinin, camptothecin, podophyllotoxin, etc. (Patwardhan, et. al. 2004)

Although Drug Development has been driven by various technology platforms which are also helpful in development of therapeutic agents from herbals medicines, drug development remains a lengthy process with lot of investment and low success rate. Normally, it takes about 10-15 years for a new synthesized compound to become a

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marketable therapeutic agent and cost in 2006 was approximately €1 billion (Barden & Weaver, 2010). About half of all drugs fail in the late stages of clinical trials. Sometimes soon after their approval some new drugs have to be withdrawn from the market due to severe side effects and clinical risks that were not detected in Phase III trials. For example rofecoxib (vioxx), which was launched in 1999, was withdrawn in 2004 due to an increased risk of heart attack in users.

Following modern approach drug development is a costly and time consuming process, whereas the approach leasend on the time-tested traditional medicine following a reverse pharmacology is cost-effective and less time consuming.

New approaches to improve and accelerate the joint drug discovery and development process are expected to take place mainly from innovation in drug target elucidation and lead structure discovery. Powerful new technologies such as automated separation techniques like flash chromatography, high-throughput screening are revolutionizing drug discovery. Traditional knowledge will serve as a powerful search engine for the drug discovery process (Bhushan, et. al., 2004). The objective of this book is to provide a protocol for Drug development to the industries, academic institutions and researchers and other stakeholders.

Despite existence of several guidelines such as quality control of Ayurveda, Siddha and Unani Drugs, Quality control methods for medicinal plant materials,(WHO, 1998), Quality control methods for herbal materials, WHO 2011, Laboratory Manual for the Analysis of Ayurveda and Siddha formulations, (CCRAS, 2009), Quality Control Manual for Ayurvedic, Siddha & Unani Medicine, (PLIM, 2008), Protocol for Testing of ASU Medicines, (PLIM, 2007), ASU Pharmacopoeias etc. there is a need to evolve a comprehensive guideline to address system specific issues for drug development of ASU drugs. The present document would certainly serve as a ready reference for researchers engaged in Research and Drug development in ASU systems.

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## CHAPTER-1

### PROCESS FOR DRUG DEVELOPMENT

#### Classification of Ayurvedic Drugs

As per Rule 3a of Drugs and Cosmetics Act, 1940 “Ayurvedic, Siddha or Unani drug” includes all medicines intended for internal or external use for in the diagnosis, treatment, mitigation or prevention of [disease or disorder in human beings or animals, and manufactured] exclusively in accordance with the formulae described in, the authoritative books of [Ayurveda, Siddha, Unani Tibb systems of medicine]., specified in the First Schedule;].

The following dosage forms of Ayurvedic drugs are commonly encountered:

Classical drugs	Dosage form	Patent and proprietary drug
Raw /crude drugs	Asava and arista	Syrup
Extracts	Arka	Ointment
Compound formulations	Avaleha/Leha/Paka	Capsule
Herbo mineral formulations	Kvatha Churna	Granules
	Guggulu	Confectioneries
	Churna	Dusting powder
	Ghrita/taila	Tablet
	Lavana ksara	Suppositories
	Lepa	
	Vati and gutika/pills	
	Netra bindu and anjana	
	Parpati	
	Pisti	
	Mandura	
	Rasayoga	
	Lauha	
	Dhupa	
	Bhasma	



## Plant Parts Uses in Ayurveda

In Ayurveda the raw drugs are generally procured from wild/cultivated source. Usually the content of active constituent varies between different parts of a plant. The following plants parts are used in preparation of Ayurvedic Drugs.

1. Aerial Root (A. Rt.)
2. Aril (Ar.)
3. Bulb (Bl.)
4. Dry Fruit (Dr. Fr.)
5. Dry Seed (Dr. Sd.)
6. Endosperm (Beeja Majja) (Enm.)
7. Exudate (Exd.)
8. Extract (Ext.)
9. Flower Bud (Fl. Bd.)
10. Flower Petal (Fl. Petal)
11. Flower (Fl.)
12. Fruit Exudate (Fr. Exd.)
13. Fruit Pulp (Phala majja) (Fr. Pp.)
14. Fruit Rind (Fr. R.)
15. Fruit (Fr.)
16. Glands & Hairs on Fruit (G.H.F.)
17. Gall (Gl.)
18. Heart Wood Extract (Ht. Wd. Ext.)
19. Heart Wood (Ht. Wd.)
20. Inflorescence (If.)
21. Latex (L.)
22. Leaf Pulp (Lf. Pp.)
23. Leaf (Lf.)
24. Oleoresin (O.R.)
25. Pericarp (P.)
26. Plant (Whole) (Pl.)
27. Root Bark (Rt. Bk.)
28. Root Stock (Rt. Stk.)
29. Root (Rt.)
30. Rhizome (Rz.)
31. Silicacious Concretion (S.C.)
32. Seed (Sd.)
33. Stem (St.)
34. Stem Bark (St. Bk.)
35. Stem Extract (St. Ext.)
36. Style & Stigma (Stl./Stg.)
37. Stamen (Stm.)
38. Sublimated Extract (Subl. Ext.)
39. Tender Leaf (T. Lf.)
40. Wood Extract (Wd. Ext.)

## Good Agricultural Practices

Good Agriculture Practices (GAP) is defined, in the context of medicinal plants, as a cultivation programme designed to ensure optimal yield in terms of both quality and quantity of any crop intended for health purposes. As the demand of the herbal drugs is increasing worldwide, their safety and quality of the crude drugs and finished products have become a major concern. The WHO (World Health Organization) has published guidelines for GACPs (good agriculture and collection practices) for medicinal plants. The development of WHO guidelines on GACPs for medicinal plants is an important step to ensure quality of herbal medicines and ecologically sound cultivation practices.

The GACPs cover a wide spectrum of cultivation and collection activities, including site selection, climate and soil considerations, seed identification, main post-harvest operations, and legal aspects. It is necessary to concentrate on standardizing the cultivation practices, collection practices, and post-harvest technologies for these plants adhering to GACPs.



WHO while recommending Good Agriculture and Collection Practices (GACP) has also advocated that medicinal plants should be harvested during the optimal season or time period to ensure the production of medicinal plant materials and finished herbal products of the best possible quality. The time of harvest depends on the plant part to be used (Anonymous, 2003). In brief, the time factors influencing the quality of crude drugs can be classified into three sub-types *viz.* Seasonal variation, lunar influence and diurnal variation.

### **Components of Drug Development**

The pharmaceutical Industry is one of the biggest industries for economy development world wide. In 2006 global spending on prescription drugs topped US \$ 643 billion and the USA accounted for almost half of the Global Pharmaceutical Market with US \$ 289 billion in annual sales. Life is not easy for humans without medicine, particularly in developed countries. Drug Development has been a lengthy process with low rate of success and huge capital investment. On an average it take about 10 to 15 years for a newly synthesize compound to become a marketable therapeutic agent and cost in 2006 was approximately Euro one billion. In 1996 only 22 new drugs were approved by Food and Drug Administration (FDA) in USA, whereas 53 drugs reached the market. Last year turned out to be a disappointing one for new drug approvals with the U.S. Food and Drug Administration clearing just 22 new medicines for sale, the lowest number since 2010 and sharply down on 2015's tally of 45.” Source; <http://www.reuters.com/article/us-pharmaceuticals-approvals-idUSKBN14M08R>.

Half of all drug candidates failed in the late stages of clinical trials. And soon after their approval, some new drugs have to be withdrawn from the market due to their severe side effects and clinical risk that are not detected in Phase-III trials, as per the pharmacovigilance studies. Given that the development of synthetic chemical for therapeutic use is, by and large, a random process that might result in serendipitous discovery, many Pharmaceutical Companies are now focusing on the development of plant- derived drugs.

Since human beings have evolved from simple herbivorous animals to omnivorous animals, the plant derived herbs may interact favourably with human body and hence produce beneficial effect in terms of health promotion. (Pan,*at.el* 2013)

### **Literature Survey:**

Literature survey extends opportunities in finding leads for proposed or targeted disease. For example official books like Ayurvedic Formulary of India, National Formulary of Unani Medicine, Siddha Formulary of India etc. list thousands of formulations which can be used as a lead in drug development.

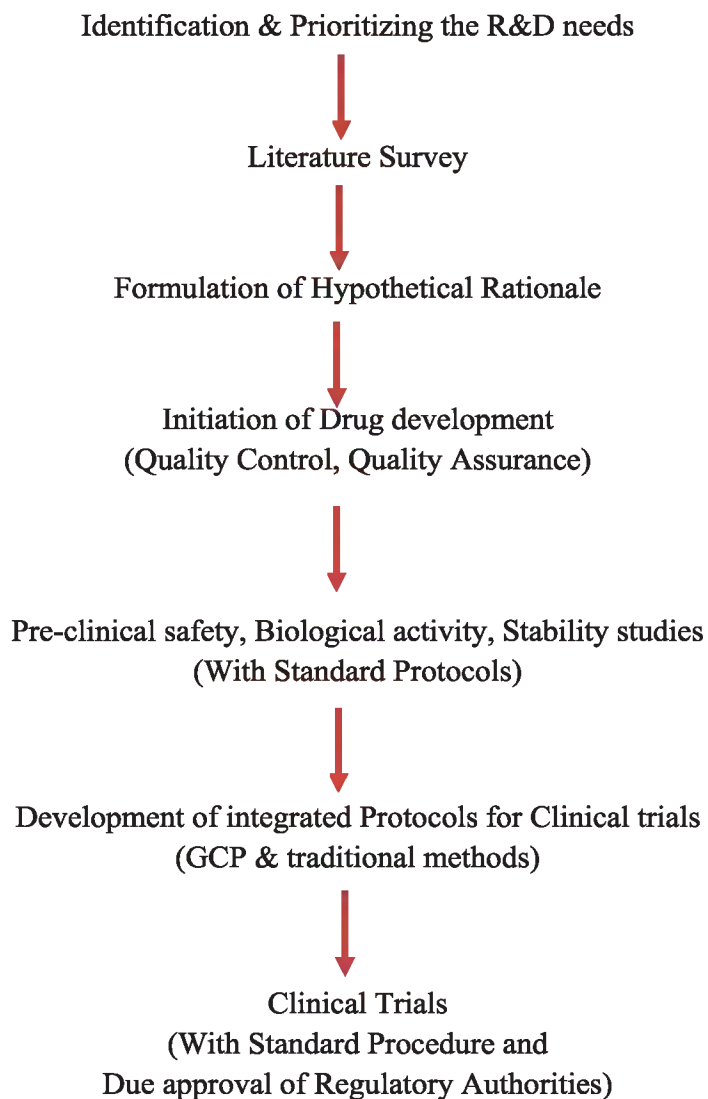
A typical drug development process from herbal medicine broadly includes the following aspects:

1. Isolation of bioactive ingredients and synthetic modifications of it.
2. Evaluation of safety and efficacy.



3. Regulatory approval of the therapeutic agent in case of new drug.
4. Clinical Trials.

The drug should be subjected to drug standardization followed by biological activity, preclinical studies and safety studies etc. The standardization protocols of various single and compounds formulations are appended at appropriate places.



**Figure 1: COMPONENTS OF DRUG DEVELOPMENT**





**PREPARATORY PHASE (1)**

Prevalence survey and

Formulation of drug /combination for

Specific targeted indication /activity (1)

(Appropriate basis of literary survey, Previous clinical data of ingredients /any other data of folk lore claims, classical Evidences)

Formulation of SOPs and

Standardization, stability studies

Quality assurance drug (4)

(Considering the classical methods and current available physical/chemical, Biological parameters for standardization).

Design of study and Formulation of

Clinical protocols (7) (considering

(As per current guidelines and adopting Classical methodology)

Finalization with task force of experts

**Bulk preparation of quality assured Drug for clinical trial, packing labeling etc. as per need at appropriate time.**

**DRUG DEVELOPMENT PHASES (2-8)**

Collection of raw drugs (2)

(considering current good agricultural practices good field collection practices and Ayurvedic textual methods)

Botanical identification/

Pharmacognostic/Chemical studies of ingredients. (3)

(based on available guidelines and classical methodology.)

Pre clinical safety studies (5)

(acute/subacute/chronic studies as per the clinical use of the drug) efficacy (specific/mechanism of action (with appropriate animal ethical clearances as per available guidelines )

Execution of Clinical Trial (8)

Approval of IEC/IRB and CTRI registration

Trial conduct

Trial monitoring

Trial coordination

Data analysis

Publication

Animal Studies for biological activity/ efficacy (specific/mechanism of action activity for clinical co relation) (6)

**Note : IPR Protection and issues of filing of patent to be addressed at suitable stage**



## CHAPTER-2

### STANDARDIZATION OF RAW DRUGS

Standardization is a process resulting from a consensus based on scientific finding obtained by parties most affected by it. It may refer to a manufacturing process, an analytical test, operational procedure, calibration of an instrument or any set of conditions required for a purpose. It deals with formulating and applying rules for an orderly approach to a scientific activity for the benefit of, and with the co-operation of all concerned. The various components of Standardization are:

1. Authentication- The starting raw material for preparation of a medicament should be authenticated on the basis of Botanical (Pharmacognostic) characters.
2. Foreign Matter- The medicinal plant should be free from visible signs of contamination by insects, moulds and other animal contamination. Foreign matter refers to the material, other than desired part of the drug. For example in case of flowers being used as drug, leaves, stalks, branches, debris of insects, fungi, sand particle, stones etc. would be considered as foreign matter and should remain within prescribed Pharmacopoeial limit. Macroscopic examination with magnifying glass (6X or 10X) or with the help of suitable sieve can be conveniently used for determination of foreign matter in whole or cut plant material.
3. Organoleptic Characters- Visual characters like size, colour, surface characteristics like texture and fracture characteristics and other characters like odour and taste. The size of the plant material may be used as an identification character. The colour of the material may be compared with an authentic material for genuineness. The odour of the plants may be a characteristic feature for example peppermint should give smell of menthol and similarly cloves should give an odour similar to that of eugenol.
4. Macroscopic and Microscopic Characters. These should match with Pharmacopoeial standards.
5. Loss on Drying
6. Total Ash
7. Acid-insoluble ash
8. Extractable value (Alcohol and Water)
9. Heavy Metals (Lead, Cadmium, Mercury & Arsenic) - The limits as prescribed in ASU Pharmacopoeias i.e. Lead-10ppm, Cadmium- 0.3ppm, Mercury-1ppm and Arsenic- 3ppm, may be observed.
10. Microbial Load - The limits as prescribed in ASU Pharmacopoeias i.e. *Staphylococcus aureus*/g.- absent, *Salmonella* sp./g.- absent, *Pseudomonas aeruginosa*/g.- absent, *Escherichia coli*- absent, Total Microbial plate count (TPC)- $10^5$ /g,  $10^7$ /g (for topical use), Total Yeast & Mould-  $10^3$ /g may be observed.

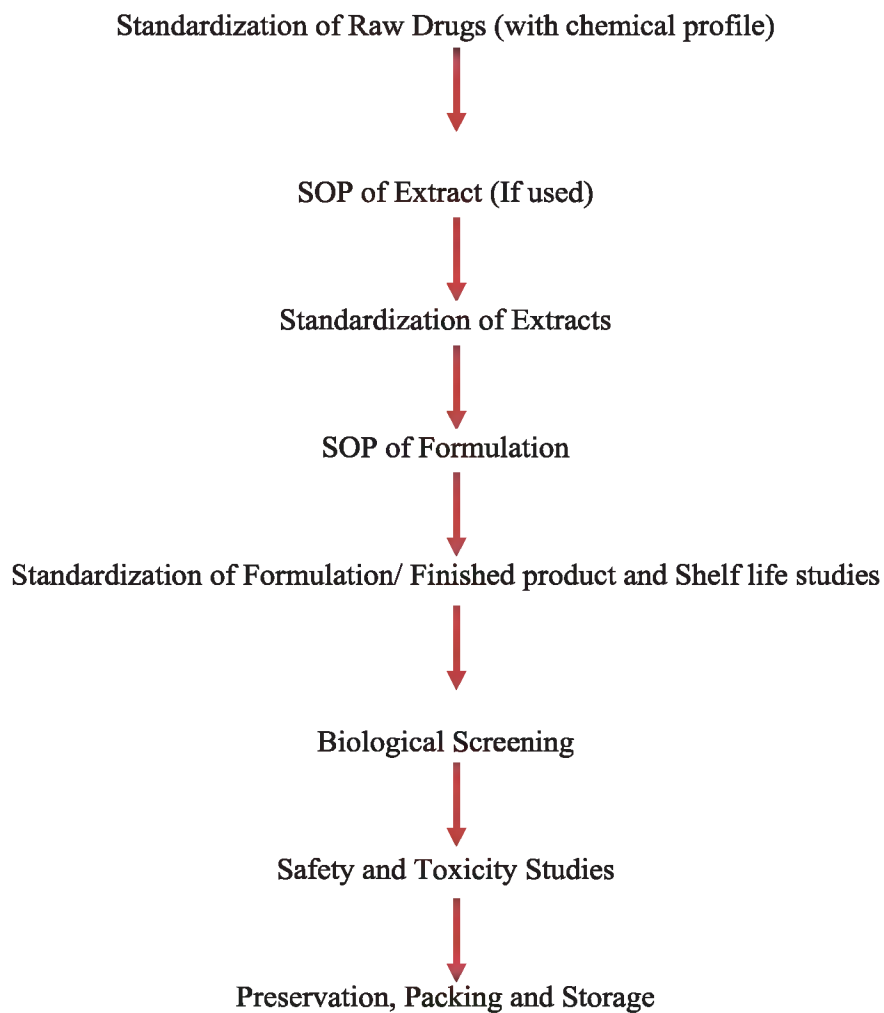




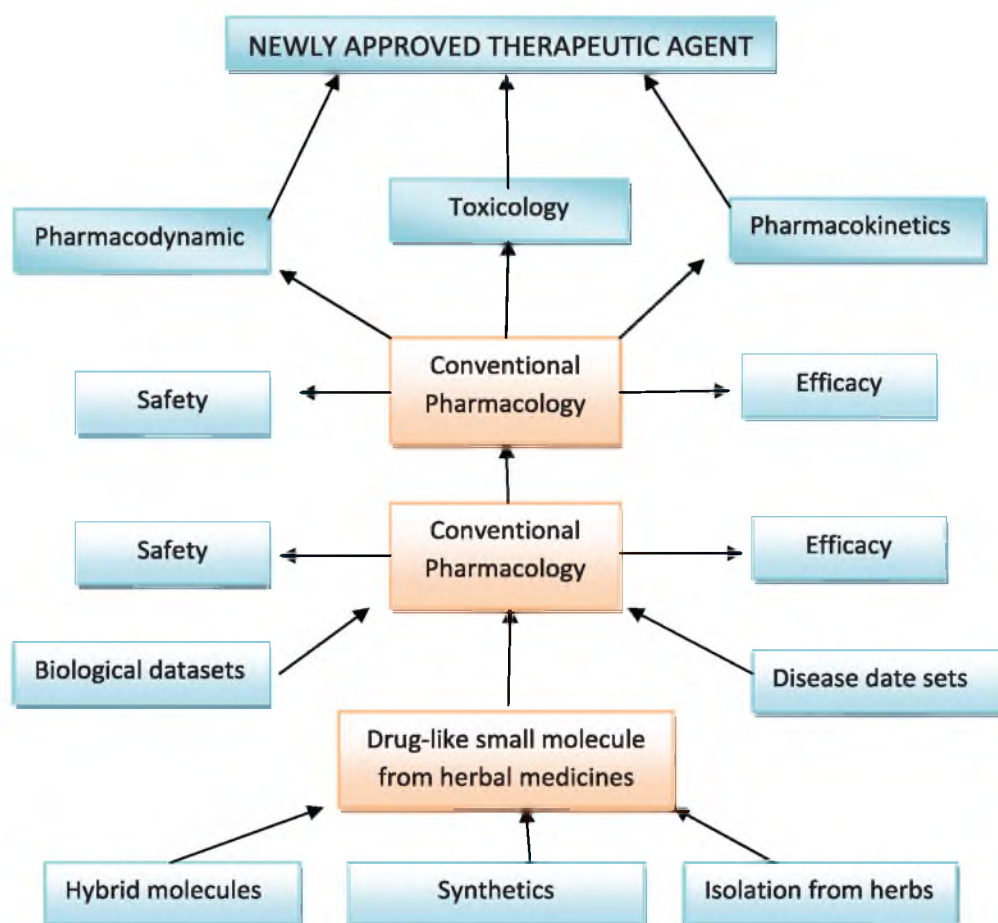
11. Aflatoxins (B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, G<sub>2</sub>) - The limits as prescribed in ASU Pharmacopoeias i.e. B<sub>1</sub> - <2 ppb; B<sub>1</sub> +B<sub>2</sub> +G<sub>1</sub> +G<sub>2</sub> <5 ppb may be observed.
12. Chromatographic profiles (TLC, HPLC or GC)
13. Assay of Marker Compounds
14. Pesticide Residue (Organo Chlorine and Organo phosphorous)- 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 limits as prescribed in ASU Pharmacopoeias as under may be observed:

**Permissible limits for Pesticide Residue:**

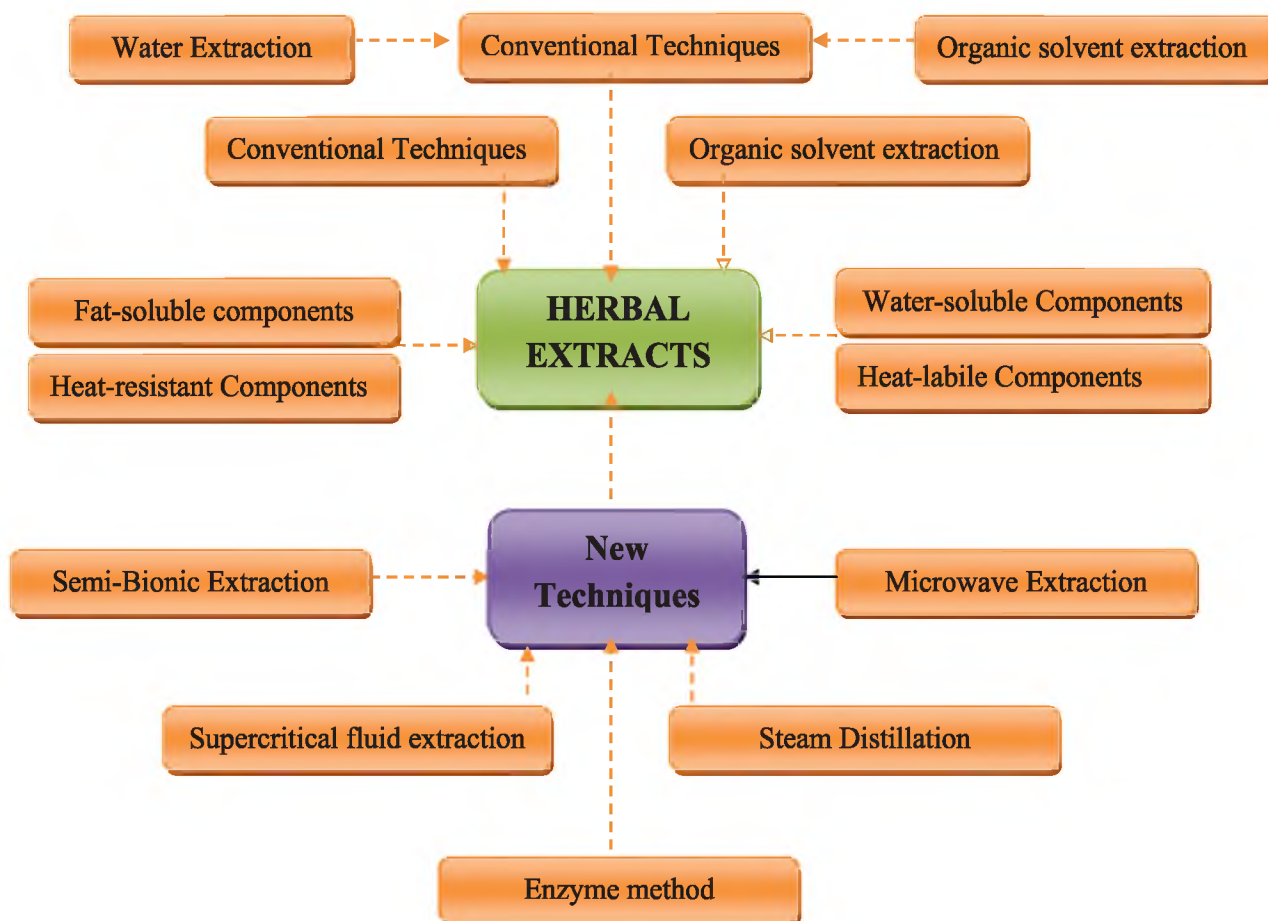
Substance	Limit (mg/kg)
Alachlor	0.02
Aldrin and Dieldrin (sum of )	0.05
Azinphos-methyl	1.0
Bromopropylate	3.0
Chlordane (sum of <i>cis</i> -, <i>trans</i> - and Oxythlordane)	0.05
Chlorfenvinphos	0.5
Chlorpyrifos	0.2
Chlorpyrifos-methyl	0.1
Cypermethrin (and isomers)	1.0
DDT (sum of p,p'-DDT, o,p'-DDT, p,p-DDE and p,p'-TDE)	1.0
Deltamethrin	0.5
Diazinon	0.5
Dichlorvos	1.0
Dithiocarbamates (as CS <sub>2</sub> )	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 Heptachlor epoxide)	0.05
Hexachlorobenzene	0.1
Hexachlorocyclohexane isomers (other than $\gamma$ )	0.3
Lindane ( $\gamma$ -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



**Figure 2: ASU DRUG DEVELOPMENT, STANDARDIZATION & QUALITY PARAMETERS**



**Figure 3: STANDARD OPERATING PROCEDURE (SOPs) FOR DRUG DEVELOPMENT (Pan, et al 2013)**



**Figure 4: EXTRACTION TECHNIQUES FOR HERBAL DRUGS (Pan, et al 2013)**



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## Glossary

### ***Herbal medicines***

These include herbs, herbal materials, herbal preparations and finished herbal products:

**Herbs.** Herbs include crude plant material such as leaves, flowers, fruit, seeds, stems, wood, bark, roots, rhizomes or other plant parts, which may be entire, fragmented or powdered.

### **Herbal materials**

Herbal materials are either whole plants or parts of medicinal plants in the crude state. They include herbs, fresh juices, gums, fixed oils, essential oils, resins and dry powders of herbs. In some countries, these materials may be processed by various local procedures, such as steaming, roasting, or stir baking with honey, alcoholic beverages or other materials.

### **Herbal preparations**

Herbal preparations are the basis for finished herbal products and may include comminuted or powdered herbal materials, or extracts, tinctures and fatty oils, expressed juices and processed exudates of herbal materials. They are produced with the aid of extraction, distillation, expression, fractionation, purification, concentration, fermentation or other physical or biological processes. They also include preparations made by steeping or heating herbal materials in alcoholic beverages and/or honey, or in other materials.

### **Finished herbal products or herbal medicinal products**

Medicinal products containing as bioactive substances exclusively are herbal drugs or herbal drug preparations. They may consist of herbal preparations made from one or more herbs. If more than one herb is used, the term *mixed herbal product* can also be used. They may contain excipients in addition to the active ingredients. In some countries herbal medicines may contain, by tradition, natural organic or inorganic active ingredients, which are not of plant origin (e.g. animal materials and mineral materials). Generally, however, finished products or mixed products to which chemically defined active substances have been added, including synthetic compounds and/ or isolated constituents from herbal materials, are not considered to be herbal.

### **Medicinal plant**

A plant, either growing wild or cultivated used for its medicinal purposes.

### **References**

Anon. 2007- WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues, pg. 4-5.

# ANNEXURES





## ANNEXURE-I

### MINISTRY OF AYURVEDA, YOGA AND NATUROPATHY, UNANI, SIDDHA AND HOMOEOPATHY (AYUSH)

#### NOTIFICATION

New Delhi, the 12th August, 2016

**G.S.R. 789(E).**—Whereas the draft of certain rules further to amend the Drugs and Cosmetics Rules, 1945 was published as required by sub-section (1) of section 33N of the Drugs and Cosmetics Act, 1940 (23 of 1940) in Part II, Section 3, Sub-section (i) of the Gazette of India, Extraordinary, vide number G.S.R 897(E), dated the 24<sup>th</sup> November, 2015 inviting objections and suggestions from persons likely to be affected thereby before the expiry of a period of forty five days from the date on which copies of the Official Gazette containing the said notification were made available to the public; And whereas, the said Gazette was made available to the public on the 24<sup>th</sup> November, 2015; And where as, objections or suggestions received from the public on the said draft rules have been considered by the Central Government; Now, therefore, in exercise of the powers conferred by sub-section (1) of section 33-N read with clause (e) of sub-section (2) of the said section of the Drugs and Cosmetics Act, 1940 (23 of 1940), the Central Government, after consultation with the Ayurvedic, Siddha and Unani Drugs Technical Advisory Board, hereby makes the following rules further to amend the Drugs and Cosmetics Rules, 1945, namely:-

#### RULES

- (1) These Rules may be called the Drugs and Cosmetics (5th Amendment) Rules, 2016.
- (2) They shall come into force on the date of their publication in the Official Gazette.
2. In the Drugs and Cosmetics Rules, 1945, for rule 161-B the following rule shall be substituted, namely:-

#### “161-B. Shelf life or date of expiry of medicines.—

- 1) The date of expiry of Ayurvedic, Siddha or Unani medicines shall be conspicuously displayed on the label of container or package of Ayurvedic, Siddha or Unani medicine, as the case may be, and after the said date of expiry, no medicine shall be marketed, sold, distributed or consumable; Provided that this rule shall apply to Ayurvedic, Siddha and Unani medicines seeking licence or renewal of licence for manufacturing after the date of notification of the rules. Provided also that this rule shall not be applicable to the Ayurvedic, Siddha or Unani medicines manufactured and marketed prior to the date of this notification.
- 2) Every person applying for licence or renewal of licence for the manufacturing of Ayurveda, Siddha or Unani medicines defined under clause (h) of section 3 of the Act shall submit to the State Licensing Authority scientific data based shelf life or date of



expiry of the medicine based on the Real time stability studies of medicines in accordance with the guidelines prescribed in the Ayurvedic Pharmacopoeia of India. Provided that this sub-rule shall be applicable after three years from the date of notification of the rules.

- 3) The guidelines regarding stability studies as prescribed in the Ayurvedic Pharmacopoeia of India, Part-I, Volume-VIII shall be applicable to all the medicines of Ayurvedic, Siddha and Unani.
- 4) The State Licensing Authority shall, before granting license or renewal of license for an Ayurvedic, Siddha or Unani medicine, ensure validity of the data submitted by the manufacturer in support of the claimed shelf-life of that medicine.
- 5) The State Licensing Authority may at any time direct the manufacturer to provide the samples of the medicine and any other related information; and may share it with the Pharmacopoeial Laboratory for Indian Medicine, Ghaziabad for analysis or independent validation. Where the manufacturer fails to comply with direction of the State Licensing Authority under subrule (5), the license for the manufacturing of the medicine shall be liable for suspension after giving a reasonable opportunity of being heard.
- 6) Any person aggrieved by an order passed by the State Licensing Authority under sub-rule (6), may within sixty days of such order, appeal to the Central Government, and the Central Government may, after such enquiry into the matter as is considered necessary, pass such order in relation thereto as it deems fit. The decision of the Central Government shall be final and binding.
- 7) The shelf life or date of expiry of an Ayurveda, Siddha or Unani medicine defined under clause (a) of section 3 of the Act shall, unless otherwise determined on the basis of scientific data, be as follows:-



(Ayurvedic Medicines)

S. No.	Dosage form	Shelf life or date of expiry with effect from the date of manufacture
(1)	(2)	(3)
(i)	Anjana	
	a) Anjana made from Kasthaushadhi	1 year
	b) Anjana made from Kasthaushadhi along with Rasa/Uprasa/Bhasma	2 years
	c) Anjana made only from Rasa/Uprasa/Bhasma	3 years
(ii)	Arka	1 year
(iii)	Asava Arista	10 years
(iv)	Avaleha, Khanda, Paka, Guda	3 years
(v)	Churna, Kwatha Churna, Lepa Churna, Danta Manjan (Churna)	2 years
(vi)	Dhoopan	2 years
(vii)	Dravaka, Lavana, Kshara	5 years
(viii)	Ghrita	2 years
(ix)	Guggulu	5 years
(x)	Gutika/Vati	
	(I) Gutika or Vati containing Kasthaushadhi along with Rasa / Uprasa/ Bhasma/ Guggulu (including Lepa Gutika and GhanVati)	5 years
	(II) Gutika or Vati containing only Kasthaushadhi (including Lepa Gutika and Ghan Vati)	3 years
	(III) Gutika / Vati containing only Ras / Uprasa / Bhasma	10 years



	except Naga, Vanga and Tamra Bhasma	
(xi)	Karna/ Nasabindu	2 years
(xii)	Kupipakva Rasayana	10 years
(xiii)	Malahar	3 years
(xiv)	Mandura-Lauha	10 years
(xv)	Naga Bhasma, Vanga Bhasma and Tamra Bhasma	5 years
(xvi)	Netrabindu	1 year
(xvii)	Parpati	10 years
(xviii)	Pishti and Bhasma except Naga, Vanga and Tamra Bhasma	10 years
(xix)	Pravahi Kwatha	3 years
(xx)	Rasayoga	
	(I) Rasayoga Containing only Rasa / Uprasa / Bhasma except Naga, Vanga and Tamra Bhasma	10 years
	(II) Rasayoga Containing Rasa / Uprasa/ Bhasma along with Kasthanushadhi/Guggulu	5 years
(xxi)	Sattva (derived from medicinal plant)	2 years
(xxii)	Sharkar / Panak/Sharbat	3 years
(xxiii)	Shveta parpati	2 years
(xxiv)	Taila	3 years
(xxv)	Varti	2 years



### (Siddha Medicines)

S. No.	Dosage form	Shelf life or date of expiry with effect from the date of manufacture
	<b>Curanam</b>	
(i)	Kutinir Curanam/Adai Curanam/Kanchi Curanam/Utkali Curanam/Pittu Curanam/ Podithimirthal Curanam/ Podi/ Pattru Curanam/ Pottanam or Kizhi Curanam/ Ottratam Curanam/ Vethu Curanam/ Pugai Curanam/Kali Curanam / Thuvalai Curanam	2 years
(ii)	<b>Mattirai/Vatakam</b>	
	I. Containing only Mooligai ingredients (including Kudineer Curanam Mattirai) (eg. Nilavembu kutinir Mattirai)	2 years
	II. Containing Mooligai ingredients along with Thathu Porutkal/ Jeeva Porutkal /Parpam/Centuram /Cunnam. (including kutinir Curanam Mattirai )	5 years
	III. Containing only Thathu Porutkal Parpam/Centuram/Cunnam/ Kattu/Kalanku.	10 years
(iii)	<b>Rasa-Paadana Marunthugal (All Mercurial Preparation)</b>	
	I. Containing Mooligai ingredients along with Thathu Porutkal/Parpam/ Centuram/Cunnam /Kattu/Kalanku	2 years
	II. Containing only Thathu Porutkal / Parpam/Centuram/Cunnam Kattu/ Kalanku	10 years
(iv)	<b>Parpam / Centuram</b>	
	I. Containing only Mooligai ingredients (e.g. KungiliyaParpam)	2 years
	II. Containing Mooligai ingredients with Thathu Porutkal / Parpam/Centuram/Cunnam/ Kattu/Kalanku (eg. Aya Centuram)	10 years
	III. Containing Mooligai ingredients with Jeeva Porutkal (e.g. Sangu Parpam )	10 years



(v)	<b>Karuppu</b>	
	I. Containing only Mooligai ingredients (e.g. Vasambu Sutta Kari)	2 years
	II. Containing Mooligai ingredients with Thathu Porutkal (e.g. Sivanar Amirtham, Thalaga Karuppu)	5 years
	III. Containing Mooligai ingredients with Jeeva Porutkal (e.g. Kasthuri Karuppu, Pattu Karuppu)	5 years
(vi)	<b>Patankam</b>	
	I. Mooligai based Patankam (e.g. Sambirani Patankam)	5 years
	II. Rasa based Patankam (e.g. Rasa Centuram)	10 years
(vii)	<b>Kulampu</b>	
	Based on process-	
	Araippu Kulampu (eg. Agathiyar Kulampu)	5 years
	Erippu Kulampu (eg. Kumatti Kulampu)	3 years
(viii)	<b>Meluku</b>	
	Based on process-	
	I. Araippu Meluku (eg. Linga Meluku)	5 years
	Based on process- II. Idippu Meluku (eg. Rasa Gandhi Meluku / Idi Vallthi Meluku)	3 years
	Based on raw materials- III. Mooligai Meluku (eg. Malaikudara Meluku)	3 years
(ix)	<b>Karpam</b>	
	Based on raw materials- I. Mooligai Karpam (eg. Karisalai Karpam , Thiripala Karpam)	2 years
	Based on raw materials- II. Mooligai Thathu Karpam (eg. Aya Bringaraja Karpam)	5 years
	Based on process-	3 years



	III. Araippu Karpam (eg. Irunelli Karpam)	
(x)	<b>Satthu</b>	
	I. Satthu derived from Mooligai (eg. Seenthil Satthu)	2 years
	II. Satthu derived from Thathu Porutkal (eg. Aya Satthu, Eya Satthu, Thurusu Satthu)	10 years
	III. Satthu derived from Jeeva Porutkal (eg. Sembu Satthu derived from Poonagam, Mayiliragu)	5 years
(xi)	Ilakam / Legiyam/ Iracayanaam	3 years
(xii)	Kallikkam/ Mai/ Kalimbu/ Neer/ Venney	1 year
(xiii)	Karam (Karanool)	2 years
(xiv)	Kattu (Medicated bandage cloth)/Seelai/Varthy/ Thiri	1 year
(xv)	Kattu / Kalanku/ Cunnam	10 years
(xvi)	Kutinir / Kiyazham(with preservatives)	3 years
(xvii)	Manappaku/ Panagam	3 years
(xviii)	Nasiyathuli/Kanthuli /Sevithuli	1 year
(xix)	Ney / Ghirutham/Kadugu	2 years
(xx)	Oothal/Nasigaparanam/ Thoopasarakku	1 year
(xxi)	Pakkuvam , Thennoral	1 year
(xxii)	Panda Vaippu	10 years
(xxiii)	Peechu	2 years
(xxiv)	Sutigai	2 years
(xxv)	Tailam / Ennai/ Poochu 3 years	3 years
(xxvi)	Tinir	1 year
(xxvii)	Tiravakam (derived from ThathuPorutkal )	2 years



### (Unani Medicines)

S. No.	Dosage form	Shelf life or date of expiry with effect from the date of manufacture
(1)	(2)	(3)
(i)	Arq (except Arq-e-Ajeeb)	1 year
(ii)	Arq -e-Ajeeb	5 years
(iii)	Ayarij / Sunoon/ Zuroor/Ghazah	2 years
(iv)	Burood	1 year
(v)	Shiyaf	2 years
(vi)	Surma / Kohal	3 years
(vii)	Habb	3 years
(viii)	Halwa	3 years
(ix)	Itrifal	3 years
(x)	Jauhar/ Jawahir	5 years
(xi)	Jawarish	4 years
(xii)	Khamira	3 years
(xiii)	Kushta	10 years
(xiv)	Laboob	3 years
(xv)	Laooq	3 years
(xvi)	Majoon / Dawa	3 years
(xvii)	Marham / Zimad / Qairooti	2 years
(xviii)	Mufarreh	2 years
(xix)	Murabba	1 year
(xx)	Nabeez	10 years
(xxi)	Qurs	3 years
(xxii)	Qutoor	1 year
(xxiii)	Raughaniyat/ Tila	3 years
(xxiv)	Sharbat/ Sikajabeen	3 years
(xxv)	Sufoof (Without Salt)	2 years
	Sufoof (Containing salt)	1 year
(xxvi)	Tiryaq	3 years

[F. No. K. 11024/5/2013-DCC (AYUSH)]  
ANIL KUMAR GANERIWALA, Jt. Secy.

**Note :** The principal rules were published in the Gazette of India *vide* notification No. F. 28-10/45H(I), dated 21st December, 1945 and were last amended *vide* notification G.S.R 640(E) dated 29.06.2016.





## ANNEXURE-II

### API guidelines for stability testing and shelf life determination for new and existing Ayurvedic drugs

The guidance on the evaluation and statistical analysis of stability data provided in the parent guideline is brief in nature and limited in scope. The parent guideline states that regression analysis is an appropriate approach to analyze quantitative stability data for retest period or shelf life estimation and recommends that a statistical test for batch pool ability be performed using a level of significance of 0.25. However, the parent guideline includes few details and does not cover situations where multiple factors are involved in a full- or reduced-design study.

#### Scope and Objective

The objective of this guideline is to specify the method of arriving at shelf life by stability testing. The shelf life determined by the process mentioned in this guideline can be used to decide the expiry date, in case a manufacturer wishes to assign a shelf life longer than one specified by the notification GSR 764(E) dated October 15, 2009.

The guideline can be used for all patented and proprietary Ayurvedic medicines, both new and existing products.

#### General Information on Stability

Information of shelf life (expiry date) is mandatory requirement for all licensed Ayurvedic medicines. The stability depends on various factors like the nature of the product, the ingredients of the products, the packaging material, etc. Stability studies are carried out to demonstrate that the medicine will remain suitable for consumption during shelf period when stored under the condition(s) mentioned on the packaging. On the product label, if there is no mention about any specific storage condition, then it is assumed that the product can be stored at room temperature (below 30°). For a suitable drug substance, retest period is more appropriate than expiry date.

The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of variety of environmental factors such as temperature, humidity, and light, to establish a retest period for drug substance or a shelf life for drug products.

Two approaches can be followed to monitor the stability of the product. The first approach is to store the samples of same batch material at standard storage and accelerated storage conditions and test them periodically. Based on the evaluation of the results, the expiry date or shelf life may be determined.

The second approach is to select samples from batches manufactured over a period of last



five years spanning six months and evaluate them simultaneously. Based on the result obtained the expiry date or shelf life may be determined. This approach is applicable for existing products which do not have yet a declared shelf life. This approach has been referred in scientific literature as the “cross sectional approach”.

### **Selection of batches**

Formal stability studies should be conducted on at least three primary batches. The primary batches should be of the same formulation as proposed for marketing. For new products, the batches should be manufactured to a minimum of pilot scale by the same route as, and using a method of manufacture and procedure that simulates the final process to be used for production batches. Pilot batches which are at least 1/10 of the commercial batch size can be used. The overall quality of the batches of drug placed in formal stability studies should be representative of the quality of the material made on production scale. Where possible, batches of drug product should be manufactured by using different batches of drug substance. Stability to be performed on each individual strength and container size of the product unless bracketing and matrixing is applied.

For cross sectional approach at least two batches per year to be selected. For example if stability to be evaluated for four years eight batches should be selected.

### **Container and closure system**

The stability studies should be conducted on the dosage form packaged in the container and closure system proposed for marketing (including as appropriate, any secondary packaging and container label). If the container is too large for drug substances the stability studies should be conducted in a container and closure system that is the same as or simulates the packaging proposed for storage and distribution.

### **Specification**

Specification is a list of tests, reference to analytical procedures and proposed acceptance criteria.

Stability study should include testing of those attributes of the drug that are susceptible to change during storage and are likely to influence quality, safety, and/or efficacy. The testing should cover as appropriate, the physical, chemical, biological, and microbiological attributes. Validated stability-indicating analytical procedures should be applied. Whether and to what extent replication should be performed will depend on the results from validation studies.

The physical parameters included in the specification need not be limited to colour, odour, appearance, shape and taste only. The chemical parameters should include colour reaction, pH value, weight variation, disintegration, bulk density, extractive values, estimation of



active or marker or category compound by suitable methods and chromatographic profiling. A suitable bioassay may be employed wherever possible.

The limits of acceptance for the products should be those specified in pharmacopoeia. If limits are not available these should be derived from release specification. Shelf life acceptance criteria should be derived from consideration of all available stability information. It may be appropriate to have justifiable differences between the shelf life and release acceptance criteria based on the stability evaluation and the changes observed on storage. Any differences between the release and shelf life acceptance criteria for antimicrobial preservative content should be supported by a validated correlation of chemical content and preservative effectiveness demonstrated during development of the product in its final formulation (except for preservative concentration) intended for marketing.

### Testing frequency

For long term studies frequency of testing should be sufficient to establish the stability profile of the drug. For a drug with proposed shelf life of at least 12 months, the frequency of testing at long term storage condition should normally be every 6 months over first year, and the second year and annually thereafter through the proposed re-test period or shelf life.

At the accelerated storage condition, a minimum of three time points including the initial and final time points (*e.g.* 0, 3 and 6 months) from a 6 month study is recommended.

Reduced designs *i.e.*, matrixing or bracketing, where the testing frequency is reduced or certain factor combinations are not tested at all, can be applied if justified.

### Storage condition

The world can be divided into four climatic zones I - IV. This guideline addresses zone IV. The choice of test conditions defined in this guideline is based on an analysis of the effects of climatic conditions in the zone. Recommended storage conditions are

S. No	Study	Storage condition	Minimum time
1	Accelerated	40° ± 2°/ 75 % RH ± 5 %	6 months
2	Long term	30° ± 2°/ 60 % RH ± 5 %	12 months

Other storage conditions are allowable if suitably justified. For products which are temperature sensitive, to be stored in lower temperature which will then become the condition designated long term storage temperature. The accelerated testing should be then carried out at least 10° more than the long term storage condition along with appropriate relative humidity condition for that temperature.



The reference samples for the above study should be stored in a temperature less than 10°.

### **Evaluation**

The purpose of stability is to establish, based on testing a minimum of at least three batches of the drug, a retest period applicable to all future batches for the drug substance, or a shelf life and label storage instructions applicable for all future batches of the drug product manufactured and packed under similar circumstances.

An Ayurvedic drug can be considered to be stable if “no significant change” occurs during at any time of testing at accelerated storage condition or at real time storage condition.

“Significant change” for a drug is defined as

1. A + or - 20 per cent change from the initial assay value (If the drug is analysed for its marker). A + or - 15 per cent change from the initial assay value (If the drug is analysed for its active compound).
2. Appearance of new spots in Identification by TLC (when compared with the sample stored in less than 10°) or completely disappearance of existing spot.
3. The physico-chemical parameters (moisture, ash, particle size) shall not vary beyond 25 per cent of the initial value.
4. Failure to meet the acceptance criteria as per individual monographs or specification.
5. Failure to meet acceptance criteria for appearance (Physical attributes, and functionality tests *e.g.*, colour, phase separation, caking, hardness).



## ANNEXURE -III

### TEST PARAMETERS

#### RAW PLANT MATERIAL

S. No.	TEST Parameters
1.	Passport data of plant material (place and date of collection) Part of plant used, botanical description, Adulteration (if any) reported in literature Substitution (if any) reported in literature
2.	Foreign matter (if any)
3.	Organoleptic character (colour, odor, taste and texture etc)
4.	Macroscopic and microscopic characters, powder microscopy
5.	Loss on drying at 105°C /Moisture content
6.	pH value (10% aqueous extract)
7.	Total ash
8.	Acid- insoluble ash
9.	Water- soluble extractive
10.	Alcohol- soluble extractive
11.	Volatile oil (if oil bearing plants) Generally plants belonging to Asteraceae and Lamiaceae family
12.	TLC/ HPTLC/GLC/HPLC (As per requirement)
13.	Assay for active constituents (total tannins/total alkaloids/resins etc. or of individual constituents.)
14.	Test for heavy/toxic metals Lead, Cadmium, Mercury, Arsenic (Limits as per ASU Pharmacopoeia)
15.	Pesticide residue Organo chlorine pesticides, organophosphorus pesticides, pyrethroids (Limits as per ASU Pharmacopoeia)
16.	Microbial contamination Total viable aerobic count Enterobacteriaceae Total fungal count (Limits as per ASU Pharmacopoeia)
17.	Test for specific pathogen <i>Escherichia coli</i> , <i>Salmonella</i> spp., <i>Staphylococcus aureus</i> , <i>Pseudomonas aeruginosa</i> (Limits as per ASU Pharmacopoeia)
18.	Aflatoxins ( B <sub>1</sub> ,B <sub>2</sub> ,G <sub>1</sub> ,G <sub>2</sub> ) (Limits as per ASU Pharmacopoeia)
19.	Shelf life



## PLANT EXTRACT

S. No	TEST Parameters
1.	Description
2.	Colour
3.	Odour
4.	Taste (if necessary)
5.	Loss on drying at 105 °C /Moisture content
6.	Total ash
7.	Acid -insoluble ash
8.	Water- soluble extractives
9.	Alcohol- soluble extractives
10.	pH
11.	Bulk density
12.	Tap density
13.	Volatile oil content (if present)
14.	Residual solvent
15.	Chemical test for secondary metabolites e.g. alkaloids, terpenoids, flavonoids, glycosides, saponins etc.
16.	TLC/HPTLC/HPLC with marker(s)
17.	Test for heavy/toxic metals (Lead, Cadmium, Mercury, Arsenic) (Limits as per ASU Pharmacopoeia)
18.	Pesticide residue Organo chlorine pesticides, organophosphorus pesticides, pyrethroids (Limits as per ASU Pharmacopoeia)
19.	Microbial contamination Total viable aerobic count Enterobacteriaceae Total fungal count (Limits as per ASU Pharmacopoeia)
20.	Test for specific pathogen <i>Escherichia coli</i> , <i>Salmonella</i> spp. , <i>Staphylococcus aureus</i> , <i>Pseudomonas aeruginosa</i> (Limits as per ASU Pharmacopoeia)
21.	Aflatoxins (B <sub>1</sub> ,B <sub>2</sub> ,G <sub>1</sub> ,G <sub>2</sub> ) (Limits as per ASU Pharmacopoeia)
22.	Shelf life



## ARKA/ DISTILLATES

S. No.	Tests Parameters
1.	Description
2.	Colour
3.	Odour
4.	pH
5.	Specific gravity at 25°C
6.	Boiling point
7.	Assay for essential oil (percentage of essential oil)
8.	Refractive index
9.	Optical rotation
10.	Viscosity
11.	TLC/HPTLC/GLC/GC-MS (any one or all)
12.	Total acidity
13.	Test for heavy/toxic metals Lead, Cadmium, Mercury, Arsenic (Limits as per ASU Pharmacopoeia)
14.	Pesticide residue Organo chlorine pesticides, organ phosphorus pesticides, pyrethroids (Limits as per ASU Pharmacopoeia)
15.	Microbial contamination Total viable aerobic count Enterobacteriaceae Total fungal count (Limits as per ASU Pharmacopoeia)
16.	Test for specific pathogen <i>Escherichia coli</i> , <i>Salmonella</i> spp., <i>Staphylococcus aureus</i> , <i>Pseudomonas aeruginosa</i> (Limits as per ASU Pharmacopoeia)
17.	Aflatoxins ( B <sub>1</sub> ,B <sub>2</sub> ,G <sub>1</sub> ,G <sub>2</sub> )(Limits as per ASU Pharmacopoeia)
18.	Shelf life





## ASAVA AND ARISHTA (FERMENTED LIQUIDS)

S. No.	TEST Parameters
1.	Description
2.	Colour
3.	Odour
4.	pH
5.	Specific gravity at 25°C
6.	Boiling point
7.	Refractive index
8.	Optical rotation
9.	Viscosity
10.	Total solids
11.	Alcohol content
12.	Reducing sugar
13.	Non- reducing sugar
14.	TLC/HPTLC/ GC-MS (any one or all)
15.	Test for methanol
16.	Total acidity
17.	Test for heavy/toxic metals Lead, Cadmium, Mercury, Arsenic (Limits as per ASU Pharmacopoeia)
18.	Pesticide residue Organo chlorine pesticides, organ phosphorus pesticides, pyrethroids (Limits as per ASU Pharmacopoeia)
19.	Microbial contamination Total viable aerobic count Enterobacteriaceae Total fungal count (Limits as per ASU Pharmacopoeia)
20.	Test for specific pathogen <i>Escherichia coli</i> , <i>Salmonella</i> spp., <i>Staphylococcus aureus</i> , <i>Pseudomonas aeruginosa</i> (Limits as per ASU Pharmacopoeia)
21.	Aflatoxins ( B <sub>1</sub> ,B <sub>2</sub> ,G <sub>1</sub> ,G <sub>2</sub> ) (Limits as per ASU Pharmacopoeia)
22.	Shelf life





## AVACHURNAM YOGA (AYURVEDIC DUSTING POWDER)

S. No.	TEST Parameters
1.	Description
2.	Colour
3.	Odour
4.	Microscopic characters
5.	Particle size
6.	Total ash
7.	Acid- insoluble ash
8.	pH (5% aqueous extract)
9.	Water- soluble extractive
10.	Alcohol -soluble extractive
11.	Loss on drying at 105°C/ Moisture content
12.	Tap Density Flow Density (angle of repose)
13.	TLC/HPTLC/HPLC/LC-MS /Moisture content
14.	Test for heavy / toxic metals Lead, Cadmium, Mercury, Arsenic, (Limits as per ASU Pharmacopoeia)
15.	Test for metals/non metals Magnesium, Carbonate, Aluminium, Iron and Chloride
16.	Pesticide residue Organochlorine pesticides, organophosphorus pesticides, Pyrethroids (Limits as per ASU Pharmacopoeia)
17.	Microbial contamination Total bacterial count Total fungal count (Limits as per ASU Pharmacopoeia)
18.	Test for specific pathogen <i>Escherichia coli</i> , <i>Salmonella</i> spp., <i>Staphylococcus aureus</i> , <i>Pseudomonas aeruginosa</i> (Limits as per ASU Pharmacopoeia)
19.	Shelf life



## AVALEHA/LEHAM/MODAK/ILAGAM (CONFECTIONARY/SEMI SOLID)

S. No.	TEST Parameters
1.	Description
2.	Colour
3.	Odour
4.	Taste
5.	Consistency
6.	Loss on drying at 105°C / Moisture content
7.	Total ash
8.	Acid- insoluble ash
9.	Alcohol- soluble extractive
10.	Water -soluble extractive
11.	pH (10% aqueous extract)
12.	Total acidity
13.	Specific gravity at 25°C
14.	Total solid content
15.	Fat content
16.	Reducing sugar/ Non-reducing sugar
17.	Total sugars
18.	Assay for major ingredients/Major constituents of main ingredients
19.	TLC/HPTLC/HPLC/LC-MS (any one or all)
20.	Test for heavy/toxic metals Lead, Cadmium, Mercury, Arsenic (Limits as per ASU Pharmacopoeia)
21.	Pesticide residue Organo chlorine pesticides, organophosphorus pesticides, pyrethroids (Limits as per ASU Pharmacopoeia)
22.	Microbial contamination Total viable aerobic count, <i>Enterobacteriaceae</i> , Total fungal count (Limits as per ASU Pharmacopoeia)
23.	Test for specific pathogen <i>Escherichia coli</i> , <i>Salmonella</i> spp., <i>Staphylococcus aureus</i> , <i>Pseudomonas aeruginosa</i> (Limits as per ASU Pharmacopoeia)
24.	Aflatoxins (Limits as per ASU Pharmacopoeia) ( B <sub>1</sub> ,B <sub>2</sub> ,G <sub>1</sub> ,G <sub>2</sub> )
25.	Shelf life



## CHEWING CANDY

S. No	TEST Parameters
1.	Description
2.	Colour
3.	Taste
4.	Odour
5.	Identification Microscopic(If active ingredients are added in biological form)
6.	Loss on drying at 105°C /Moisture content
7.	Friability
8.	Hardness
9.	Uniformity of weight (single dose does not exceed more than 5g)
10.	Disintegration time (More than 30 minutes)
11.	pH
12.	Total ash
13.	Acid -insoluble ash
14.	Water- soluble extractives
15.	Alcohol- soluble extractives
16.	Salt content
17.	Total sugars
18.	Reducing sugars
19.	TLC/HPTLC with markers
20.	Test for heavy/toxic metals Lead, Cadmium, Mercury, Arsenic (Limits as per ASU Pharmacopoeia)
21.	Microbial contamination Total bacterial count Total fungal count Enterobacteriaceae <i>Salmonella</i> spp. (Limits as per ASU Pharmacopoeia)
22.	Aflatoxins (Limits as per ASU Pharmacopoeia) ( B <sub>1</sub> ,B <sub>2</sub> ,G <sub>1</sub> ,G <sub>2</sub> )
23.	Shelf life



**CHURNA/CHOORNAM (FINE POWDER)/KVATHA CHURNA/  
KUDINIR CHOORNAM (CORASE POWDER FOR DECOCTION)**

S. No.	TEST Parameters
1.	Description
2.	Colour
3.	Odour
4.	Foreign matters
5.	Powder microscopy
6.	Particle size (80-100 mesh for Curna ; 40 –60 mesh for Kvatha curna)
7.	Loss on drying at 105° C / Moisture content
8.	Total ash
9.	Acid - insoluble ash
10.	pH (5% aqueous extract)
11.	Water- soluble extractive
12.	Alcohol- soluble extractive
13.	Bulk density and Tap density
14.	Assay for specific ingredients/Constituents group of compounds (if possible)
15.	TLC/HPTLC/HPLC with markers (any one or all)
16.	Test for heavy/toxic metals Lead, Cadmium, Mercury, Arsenic (Limits as per ASU Pharmacopoeia)
17.	Pesticide residue Organo chlorine pesticides, organophosphorus pesticides, pyrethroids (Limits as per ASU Pharmacopoeia)
18.	Microbial contamination Total viable aerobic count Enterobacteriaceae Total fungal count (Limits as per ASU Pharmacopoeia)
19.	Test for specific pathogen <i>Escherichia coli</i> , <i>Salmonella</i> spp., <i>Staphylococcus aureus</i> , <i>Pseudomonas aeruginosa</i> (Limits as per ASU Pharmacopoeia)
20.	Aflatoxins (Limits as per ASU Pharmacopoeia) ( B <sub>1</sub> ,B <sub>2</sub> ,G <sub>1</sub> ,G <sub>2</sub> )
21.	Shelf life



## LEPA/MALHARA/KALIMBU/PASAI/MEDICATED WAX/CREAM/POULTICE

S. No.	TEST Parameters
1.	Description
2.	Colour
3.	Odour
4.	Viscosity (If in the form of flowing material)
5.	Rancidity test
6.	Microscopy (if powdered drugs incorporated)
7.	Particle size(if powdered drugs incorporated)of the ingredients
8.	Total acidity
9.	TLC/HPTLC/GC/GC-MS/HPLC (any one or all)
10.	Assay (Wherever possible)
11.	Uniformity of content
12.	pH
13.	Thermal stability
14.	Total fat content
15.	Loss on drying at 105°C/Moisture content
16.	Spreadibility
17.	Test for heavy/toxic metals Lead, Cadmium, Mercury, Arsenic (Limits as per ASU Pharmacopoeia)
18.	Pesticide residue Organo chlorine pesticides, organophosphorus pesticides, pyrethroids (Limits as per ASU Pharmacopoeia)
19.	Microbial contamination Total viable aerobic count Enterobacteriaceae Total fungal count (Limits as per ASU Pharmacopoeia)
20.	Test for specific pathogen <i>Escherichia coli</i> , <i>Salmonella</i> spp., <i>Staphylococcus aureus</i> , <i>Pseudomonas aeruginosa</i> (Limits as per ASU Pharmacopoeia)
21.	Aflatoxins (Limits as per ASU Pharmacopoeia) ( B <sub>1</sub> ,B <sub>2</sub> ,G <sub>1</sub> ,G <sub>2</sub> )
22.	Shelf life



## NETRABINDU/KAN CHOTTU MARUNTHU (EYE DROPS)

S. No.	TEST Parameters
1.	Description
2.	Colour
3.	Odour
4.	pH
5.	Clarity test (suspended particles)
6.	Assay for active ingredients (if available)
7.	TLC/HPTLC /GLC/HPLC (any one or all)
8.	Test for heavy/toxic metals Lead, Cadmium, Mercury, Arsenic
9.	Pesticide residue Organo chlorine pesticides, organophosphorus pesticides, pyrethroids (Limits as per ASU Pharmacopoeia)
10.	Microbial contamination Total viable aerobic count Enterobacteriaceae Total fungal count (Limits as per ASU Pharmacopoeia)
11.	Test for specific pathogen <i>Escherichia coli</i> , <i>Salmonella</i> spp., <i>Staphylococcus aureus</i> , <i>Pseudomonas aeruginosa</i> (Limits as per ASU Pharmacopoeia)
12.	Aflatoxins (Limits as per ASU Pharmacopoeia) ( B <sub>1</sub> ,B <sub>2</sub> ,G <sub>1</sub> ,G <sub>2</sub> )
13.	Shelf life



## PISHTI/BHASMA/PARPAM/SINDUR (PROCESSED FINE POWDER )

S. No.	TEST Parameters
1.	Description
2.	Colour
3.	Odour
4.	Taste
5.	pH ( 1% aqueous extract)
6.	Loss on drying at 105°C /Moisture content
7.	Particle size
8.	Total ash
9.	Acid -insoluble ash
10.	Sulphated ash
11.	Water- soluble extractive
12.	Alcohol- soluble extractive
13.	Assay for element (s) (if available)
14.	IR/XRF/XPS/XRD/SEM/EDX/AFM (As per requirement)
15.	Nishchandrica (Lusterless)
16.	Rekha purnatva (Fine enough to enter within lines of finger)
17.	Varitara (Floats on water)
18.	Nirthoom (Smokeless)
19.	Niswadu (Tasteless)
20.	Apurnar Bhav (Irreversible)
21.	Shelf life



## SHARKARA SIKTA/SIRUTHUGALGAL (GRANULES)

S. No.	TEST Parameters
1.	Description
2.	Colour
3.	Taste
4.	Odour
5.	Microscopic
6.	Bulk density
7.	Tap density
8.	Compressibility
9.	Flow property
10.	Total ash
11.	Acid- insoluble ash
12.	Alcohol- soluble extractive
13.	Water -soluble extractive
14.	pH
15.	Total sugars
16.	Reducing sugars
17.	Non-reducing sugars
18.	TLC/HPTLC/HPLC/LC-MS (any one or all)
19.	Test for heavy/toxic metals Lead, Cadmium, Mercury, Arsenic (Limits as per ASU Pharmacopoeia)
20.	Pesticide residue Organo chlorine pesticides, organophosphorus pesticides, pyrethroids (Limits as per ASU Pharmacopoeia)
21.	Microbial contamination Total viable aerobic count Enterobacteriaceae Total fungal count (Limits as per ASU Pharmacopoeia)
22.	Test for specific pathogen <i>Escherichia coli</i> , <i>Salmonella</i> spp., <i>Staphylococcus aureus</i> , <i>Pseudomonas aeruginosa</i> (Limits as per ASU Pharmacopoeia)
23.	Aflatoxins (Limits as per ASU Pharmacopoeia) ( B <sub>1</sub> ,B <sub>2</sub> ,G <sub>1</sub> ,G <sub>2</sub> )
24.	Shelf life





## SHARBAT/MANAPPAGU (SYRUP)

S. No.	TEST Parameters
1.	Description
2.	Colour
3.	Odour
4.	Taste
5.	Viscosity
6.	pH
7.	Total solids
8.	Reducing sugars/ Non-reducing sugars
9.	Total sugars
10.	Specific gravity at 25°C
11.	TLC/HPTLC/HPLC/LC-MS (any one or all)
12.	Test for heavy/toxic metals Lead, Cadmium, Mercury, Arsenic (Limits as per ASU Pharmacopoeia)
13.	Pesticide residue Organo chlorine pesticides, organophosphorus pesticides, pyrethroids (Limits as per ASU Pharmacopoeia)
14.	Microbial contamination Total viable aerobic count Enterobacteriaceae Total fungal count (Limits as per ASU Pharmacopoeia)
15.	Test for specific pathogen <i>Escherichia coli</i> , <i>Salmonella</i> spp., <i>Staphylococcus aureus</i> , <i>Pseudomonas aeruginosa</i> (Limits as per ASU Pharmacopoeia)
16.	Aflatoxins (Limits as per ASU Pharmacopoeia) ( B <sub>1</sub> ,B <sub>2</sub> ,G <sub>1</sub> ,G <sub>2</sub> )
17.	Shelf life



## TAILAS/ GHRITAS/THYLAM/NEI (MEDICATED OILS AND GHEE)

S. No.	TEST Parameters
1.	Description
2.	Colour
3.	Odour
4.	Rancidity
5.	Specific gravity/ Weight/ml.
6.	pH value
7.	Loss on drying at 105°C/ Moisture content
8.	Congearing point
9.	Refractive index
10.	Viscosity
11.	Iodine value
12.	Saponification value
13.	Unsaponifiable matter (percentage of)
14.	Acid value
15.	Peroxide value
16.	Free fatty acids (percentage of)
17.	Total fatty matter (percentage of)
18.	Mineral oil test (it should be negative)
19.	GLC/TLC/HPTLC/HPLC/GC-MS (any one of all)
20.	Test for heavy/toxic metals Lead, Cadmium, Mercury, Arsenic (Limits as per ASU Pharmacopoeia)
21.	Pesticide residue Organo chlorine pesticides, organophosphorus pesticides, pyrethroids (Limits as per ASU Pharmacopoeia)
22.	Microbial contamination Total viable aerobic count, <i>Enterobacteriaceae</i> , Total fungal count (Limits as per ASU Pharmacopoeia)
23.	Test for specific Pathogen <i>Escherichia coli</i> , <i>Salmonella</i> spp., <i>Staphylococcus aureus</i> , <i>Pseudomonas aeruginosa</i> (Limits as per ASU Pharmacopoeia)
24.	Aflatoxins, (B <sub>1</sub> , B <sub>2</sub> , G <sub>1</sub> , G <sub>2</sub> ) (Limits as per ASU Pharmacopoeia)
25.	Shelf life



## VATI/ GUTIKA/KULIGAI/MARTHIRAI/VADAGAM (TABLET/ PILLS)

S. No.	TEST Parameters
1.	Description
2.	Colour
3.	Taste
4.	Odour
5.	Microscopic characters (if vegetable parts are used as ingredients)
6.	pH (5% aqueous extract)
7.	TLC/HPTLC/HPLC/LC-MS (any one of all)
8.	Loss on drying at 105°C/ Moisture content
9.	Friability
10.	Hardness
11.	Uniformity of weight
12.	Disintegration time(not more than 35 minutes except guggulu )
13.	Total ash
14.	Acid- insoluble ash
15.	Alcohol- soluble extractive
16.	Water -soluble extractive
17.	Total sugar(if added)
18.	Reducing sugar/Non-reducing sugar (if added)
19.	Assay (active constituents of major ingredients) (if available)
20.	Test for heavy/toxic metals Lead, Cadmium, Mercury, Arsenic (Limits as per ASU Pharmacopoeia)
21.	Pesticide residue Organo chlorine pesticides, organophosphorus pesticides, pyrethroids (Limits as per ASU Pharmacopoeia)
22.	Microbial contamination Total viable aerobic count, <i>Enterobacteriaceae</i> , Total fungal count (Limits as per ASU Pharmacopoeia)
23.	Test for specific Pathogen <i>Escherichia coli</i> , <i>Salmonella</i> spp., <i>Staphylococcus aureus</i> , <i>Pseudomonas aeruginosa</i> (Limits as per ASU Pharmacopoeia)
24.	Aflatoxins (Limits as per ASU Pharmacopoeia) (B <sub>1</sub> , B <sub>2</sub> , G <sub>1</sub> , G <sub>2</sub> )



## VARTTI, NETRA VARTTI

S. No.	TEST Parameters
1.	Description
2.	Colour
3.	Odour
4.	Microscopy
5.	pH (5% of aqueous solution)
6.	Friability
7.	Hardness
8.	Disintegration time
9.	Dissolution time
10.	Uniformity of weight
11.	Loss on drying at 105°C/Moisture content
12.	Total ash
13.	Acid-insoluble ash
14.	Alcohol- soluble extractives
15.	Water-soluble extractives
16.	Volatile oil (percentage) if present
17.	TLC/HPTLC/HPLC/LC-MS (any one or all)
18.	Assay (if possible) as IP/BP/USP/API
19.	Test for heavy/toxic metals Lead, Cadmium, Mercury, Arsenic (Limits as per ASU Pharmacopoeia)
20.	Pesticide residue Organo chlorine pesticides, organophosphorus pesticides, pyrethroids (Limits as per ASU Pharmacopoeia)
21.	Microbial contamination Total viable aerobic count Enterobacteriaceae Total fungal count (Limits as per ASU Pharmacopoeia)
22.	Test for specific pathogen <i>Escherichia coli</i> , <i>Salmonella</i> spp., <i>Staphylococcus aureus</i> , <i>Pseudomonas aeruginosa</i> (Limits as per ASU Pharmacopoeia)
23.	Aflatoxins (B <sub>1</sub> , B <sub>2</sub> , G <sub>1</sub> , G <sub>2</sub> ) (Limits as per ASU Pharmacopoeia)
24.	Shelf life



## TEST PARAMETERS FOR SUPPOSITORIES

S. No.	TEST Parameters
1.	Description
2.	Colour
3.	Melting range(°C)
4.	Specific Gravity/ Weight/mL
5.	Refractive index at 25°C
6.	Viscosity
7.	Iodine value
8.	Saponification value
9.	Unsaponifiable Matter (percentage of )
10.	pH (5% of aqueous solution)
11.	Identification: HPTLC/GC/HPLC/GC-MS (any one or all)
12.	Free fatty acids (percentage of )
13.	Total fatty matter (percentage of )
14.	Liquification/Softening time
15.	Microbial test
16.	Pesticide residue
17.	Uniformity of content
18.	Dissolution test
19.	Test for heavy metals: Lead, Cadmium, Mercury & Arsenic (Limits as per ASU Pharmacopoeia)
20.	Shelf life



## ANNEXURE-IV

### SCHEDULE- E1

In Drugs and Cosmetic Act, 1940, the following drugs are included in Schedule E1 List of Poisonous Substances under the Ayurvedic (including Siddha) and Unani Systems of Medicine

#### A. AYRVEDIC SYSTEM

##### I. *Drugs of vegetable origin*

1. Ahipena	:	<i>Papaver somniferum</i> Linn.
2. Arka*	:	<i>Colotropis gigantean</i> (Linn.) R. Br. Ex. Ait.
3. Bhallataka	:	<i>Semecarpus anacardium</i> Linn. f.
4. Bhang (except seeds)	:	<i>Cannabis sativa</i> Linn.
5. Danti	:	<i>Baliospermum montanum</i> Mall. Arg.
6. Dhatura	:	<i>Datura metel</i> Linn.
7. Gunja (Seed)	:	<i>Abrus precatorius</i> Linn.
8. Jaipala (Seed)	:	<i>Croton tiglium</i> Linn.
9. Karaveera	:	<i>Nerium indicum</i> Mill.
10. Langali	:	<i>Gloriosa superba</i> Linn.
11. Parasilka Yavani	:	<i>Hyocyamus inibar</i> Linn.
12. Vatsanabha/Shringivisha	:	<i>Acontium chasmanthum</i> Stapfex Holm.
13. Vishmushti	:	<i>Strychnos nuxvomica</i> Linn.

##### II. *Drugs of Animal Origin*

14. Sarpa Visha	:	Snake poison.
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##### III. *Drugs of Mineral Origin*

15. Gauripashna	:	Arsenic
16. Hartala	:	Arseno sulphide
17. Manahashila	:	Arseno sulphide
18. Parada	:	Mercury
19. Rasa Karpura	:	Hydragyri subchloridum
20. Tuttha	:	Copper Sulphate
21. Hingula	:	Cinnabar

#### B. SIDDHA SYSTEM

1. Abini (except seed)	:	<i>Papaver somniferum</i> Linn.
2. Alari	:	<i>Nerium indicum</i> Mill.
3. Attru Thumatti	:	<i>Citrullus colocynthis</i> Scharad.
4. Umathai	:	<i>Datura stramonium</i> Linn.
5. Etti	:	<i>Strychnos nuxvomica</i> Linn.
6. Ganja (except seed)	:	<i>Cannabis sativa</i> Linn.
7. Kalappaik Kizhangu	:	<i>Gloriosa superba</i> Linn.
8. Kodikkalli (exempted)	:	<i>Euphorbia tituqalli</i> Linn.



- for external use)
9. Chadurakkalli (exempted for external use) : *Euphorbia antiquorium* Linn.
10. Kattu Thumatti : *Cucumis trigonus* Roxb.
11. Kunri (Except Root) : *Arbus precatorius* Linn.
12. Cheramkottai : *Semicarpits anacardium* Linn.
13. Thillai : *Enoecaria agallocha* Linn.
14. Nabi : *Aconitum ferox* Wall.
15. Nervalam : *Croton tiglium* Linn
16. Pugaielai : *Nicotiana tobacum* Linn.
17. Mancevikkalli (exempted for external use) : *Euphorbia* sp.

### C. UNANI SYSTEM

#### I. Drugs of Vegetable Origin

1. Afyun (except seed) : *Papaver somniferum* Linn.
2. Bazrul-banj : *Hyoscyamus niger* Linn.
3. Bish : *Aconitum chasmanthum* Stapfex Holmes.
4. Bhang (except seed) : *Cannabis sativa* Linn.
5. Charas (resin) (except seed) : *Cannabis sativa* Linn.
6. Dhatura seeds : *Datura metal* Linn.
7. Kuchla : *Strychnos nuxvomica* Linn.
8. Shokran : *Conium maculatum* Linn.

#### II. Drugs of Animal Origin

9. Sanp (head) : Snake (head)
10. Telni makkhi : *Mylabaris cichorii* Linn.  
*Mylaboris pustulata* Thumb  
*Mylabaris rnaeilenta*

#### III. Drugs of Mineral Origin

- Darachikna : Hydrargyri perchloridum
- Hira : Diamond
- Ras Kapoor : Hydrargyri Subchloridum (calomel)
- Shingruf : Hydrargyri bisulphuratum
- Zangar : Cupri subaccetas
- Sammul-Far : Abyaz, Asfar, Aswad and Ahmar (white, yellow, black and red Arsenic, respectively)
- Tootiya : Copper Sulphate
- Para : Hydrargyrum
- Hartal : Arsenic trisulphide (yellow).

\*Arka used for Bhawna before making Bhasma is exempted.



### SCHEDULE T (The Good Manufacturing Practices)

Govt. of India notified 'Schedule T' vide GSR No. 560(E) on 23<sup>rd</sup> June 2000 under Rule, 157 of Drugs & Cosmetic Rules and further amended Good Manufacturing Practices (GMP) vide GSR No. 198(E), dated 07<sup>th</sup> March 2003. These Rules became mandatory to all new ASU drug manufacturing units from 23<sup>rd</sup> June, 2000 and for existing units from 23<sup>rd</sup> June, 2002 respectively (2 years grace period was given for existing ASU units to obtain GMP certification). Schedule T specify the requirements of factory premises and hygienic conditions. The main aim of introducing Schedule-T was to maintain a uniform standard of hygiene for the manufacturers. Compliance to Good manufacturing Practices is mandatory for all the manufacturers of ASU drugs.

The Good Manufacturing Practices (GMP) are prescribed as follows in Part I and Part II to ensure: -

- i) Raw materials used in the manufacture of drugs are authentic, of prescribed quality and are free from contamination;
- ii) The manufacturing process is as has been prescribed to maintain the standards;
- iii) Adequate quality control measures are adopted;
- iv) The manufactured drug which is released for sale is of acceptable quality;
- v) To achieve the objectives listed above, each licensee shall evolve methodology and procedures for following the prescribed process of manufacture of drugs which should be documented as a manual and kept for reference and inspection.

However, under IMCC Act 1970 registered Vaidyas, Siddhas and Hakeems who prepare medicines on their own to dispense to their patients and not selling such drugs in the market are exempted from the purview of Good Manufacturing Practices (GMP).

#### PART I

##### ***Factory Premises:***

The manufacturing plant should have adequate space for: -

- (i) Receiving and storing raw material;
- (ii) Manufacturing process areas;
- (iii) Quality control section;
- (iv) Finished goods store;
- (v) Office;
- (vi) Rejected goods/drugs store.

##### ***1.1. General Requirements:***

1.1 (A) *Location and surroundings.* - The factory building for manufacture of Ayurveda, Siddha and Unani medicines shall be so situated and shall have such construction as to avoid contamination from open sewerage, drain, public lavatory for any factory which produces disagreeable or obnoxious odour or fumes or excessive soot, dust and smoke.





1.1(B) *Buildings*. - The buildings used for factory shall be such as to permit production of drugs under hygienic conditions and should be free from cobwebs and insects/rodents. It should have adequate provision of light and ventilation. The floor and the walls should not be damp or moist. The premises used for manufacturing, processing, packaging and labeling will be in conformity with the provisions of the Factory Act. It shall be located so as to be:

(I) Compatible with other manufacturing operations that may be carried out in the same or adjacent premises.

(II) Adequately provided with working space to allow orderly and logical placement of equipment and materials to avoid the risk of mix up between different drugs or components thereof and control the possibility of cross contamination by other drugs or substances and avoid the risk of omission of any manufacturing or control step.

(III) Designed, constructed and maintained to prevent entry of insects and rodents. Interior surface (walls, floors and ceilings) shall be smooth and free from cracks and permit easy cleaning and disinfection. The walls of the room in which the manufacturing operations are carried out shall be impervious to and be capable of being kept clean. The flooring shall be smooth and even and shall be such as not to permit retention or accumulation of dust or waste products.

(IV) Provided with proper drainage system in the processing area. The sanitary fittings and electrical fixtures in the manufacturing area shall be proper and safe.

(V) Furnace/Bhatti section could be covered with tin roof and proper ventilation, but sufficient care should be taken to prevent flies and dust.

(VI) There should be fire safety measures and proper exits should be there.

(VII) *Drying Space*: - There should be separate space for drying of raw materials, in process medicine or medicines require drying before packing. This space will be protected from flies/ insects/ dust etc., by proper flooring, wire mesh window, glass panels or other material.

1.1(C) *Water Supply* - The water used in manufacture shall be pure and of potable quality. Adequate provision of water for washing the premises shall be made.

1.1(D) *Disposable of Waste* - From the manufacturing section and laboratories the waste water and the residues which might be prejudicial to the workers or public health shall be disposed off.

1.1(E) *Container's Cleaning* - In factories where operations involving the use of containers such as glass bottles, vials and jars are conducted, there shall be adequate arrangements separated from the manufacturing operations for washing, cleaning and drying of such containers.

1 Ins. by G.O.I. Notification No. GSR 673(E) dt 27.10.1993.

2 Ins. by G.O.I. Notification No. GSR 553(E) dt 20.7.1995.

3 Sub. By G.O.I. Notification No. GSR 198(E) dt 07.3.2003



1. Raw material of metallic origin.
2. Raw material of mineral origin
3. Raw material from animal source
4. Fresh Herbs
5. Dry Herbs or Plant parts
6. Excipients etc
7. Volatile oils/perfumes and flavours
8. Plant concentrates/ extracts and exudates/resins.

1.1(F) *Stores* - Storage should have proper ventilation and shall be free from dampness. It should provide independent adequate space for storage of different types of material, such as raw material, packaging material and finished products.

1.1. (F) (A) *Raw Materials*. - All raw materials procured for manufacturing will be stored in the raw materials store. The manufacture based on the experience and the characteristics of the particular raw material used in Ayurveda, Siddha and Unani system shall decide the use of appropriate containers which would protect the quality of raw materials as well as prevent it from damage due to dampness, microbiological contamination or rodent and insect infestation, etc. If certain raw materials require such controlled environmental conditions, the raw materials stores may be sub-divided with proper enclosures to provide such conditions by suitable cabinization. While designing such containers, cupboard or areas in the raw materials store, care may be taken to handle the following different categories of raw materials:-

Each container used for raw material storage shall be properly identified with the label which indicates name of the raw material, source of supply and will also clearly state the status of raw material such as 'UNDER TEST' or 'APPROVED' or 'REJECTED'. The labels shall further indicate the identity of the particular supply in the form of Batch No. or Lot No. and the date of receipt of the consignment.

All the raw materials shall be sampled and got tested either by the in-house Ayurvedic, Siddha and Unani experts (Quality control technical person) or by the laboratories approved by the Government and shall be used only on approval after verifying. The rejected raw material should be removed from other raw material store and should be kept in separate room. Procedure of 'First in first out' should be adopted for raw materials wherever necessary. Records of the receipt, testing and approval or rejection and use of raw material shall be maintained.

1.1. (F)(B) *Packing Materials*. - All packaging materials such as bottles, jars, capsules etc. shall be stored properly. All containers and closure shall be adequately cleaned and dried before packing the products.

1.1. (F)(C) *Finished Goods Stores*. - The finished goods transferred from the production area after proper packaging shall be stored in the finished goods stores within an area marked "Quarantine". After the quality control laboratory and the experts have checked the correctness of finished goods with reference to its packing/labeling as



well as finished product quality as prescribed, then it will be moved to “Approved Finished Goods Stock” area. Only approved finished goods shall be dispatched as per marketing requirements. Distribution records shall be maintained as required. If any Ayurvedic, Siddha and Unani drug needs special storage conditions, finished goods store shall provide necessary environmental requirements.

1.1(G) *Working space.* - The manufacturing area shall provide adequate space (manufacture and quality control) for orderly placement of equipment and material used in any of the operations for which these employed so as to facilitate easy and safe working and to minimize or to eliminate any risk of mix-up between different drugs, raw materials and to prevent the possibility of cross contamination of one drug by another drug that is manufactured, stored or handled in the same premises.

1.1(H) *Health, Clothing, Sanitation and Hygiene of Workers.*- All workers employed in the Factory shall be free from contagious diseases. The clothing of the workers shall consist of proper uniform suitable to the nature of work and the climate and shall be clean. The uniform shall also include cloth or synthetic covering for hands, feet and head wherever required. Adequate facilities for personal cleanliness such as clean towels, soap and scrubbing brushes shall be provided. Separate provision shall be made for lavatories to be used by men and women, and such lavatories shall be located at places separated from the processing rooms. Workers will also be provided facilities for changing their clothes and to keep their personal belongings.

1.1. (I) *Medical Services:* The manufacturer shall also provide:-

(a) Adequate facilities for first aid;

(b) Medical examination of workers at the time of employment and periodical check up thereafter by a physician once a year, with particular attention being devoted to freedom from infections. Records thereof shall be maintained.

1.1(J) *Machinery and Equipments.* - For carrying out manufacturing depending on the size of operation and the nature of product manufactured, suitable equipment either manually operated or operated semi-automatically (Electrical or steam based) or fully automatic machinery shall be made available. These may include machines for use in the process of manufacture such as crushing, grinding powdering, boiling, mashing, burning, roasting, filtering, drying, filling, labeling and packing etc. to ensure ease in movement of workers and orderliness in operation a suitably adequate space will be ensured between two machines or rows of machines. These equipments have to be properly installed and maintained with proper cleaning.

List of equipments and machinery recommended is indicated in Part II A.

Proper Standard Operational Procedures (SOPs) for cleaning, maintaining and performance of every machine should be laid down.

1.1(K) *Batch Manufacturing Records.* - The licensee shall maintain batch manufacturing record of each batch of Ayurvedic, Siddha and Unani drugs manufactured irrespective of the type of product manufactured (classical preparation or patent and proprietary medicines).



Manufacturing records are required to provide an account of the list of raw materials and their quantities obtained from the store, tests conducted during the various stages of manufacture like taste, colour, physical characteristics and chemical tests as may be necessary or indicated in the approved books of Ayurveda, Siddha and Unani mentioned in the First Schedule of the Drugs and Cosmetics Act, 1940 (23 of 1940). These tests may include any in-house or pharmacopoeial test adopted by the manufacturer in the raw material or in the process material and in the finished product. These records shall be duly signed by Production and Quality Control Personnel respectively. Details of transfer of manufactured drug to the finished products store including dates and quantity of drugs transferred along with record of testing of the finished product, if any, and packaging, records shall be maintained. Only after the manufactured drugs have been verified and of accepted quality shall be allowed to be cleared for sale. It should be essential to maintain the record of date, manpower, machine and equipments used and to keep in process record of various shodhana, bhavana, burning and fire and specific grindings in terms of internal use.

1.1(L) *Distribution Records*. - Records of sale and distribution of each batch of Ayurveda, Siddha and Unani Drugs shall be maintained in order to facilitate prompt and complete recall of the batch, if necessary. The duration of record keeping should be the date of expiry of the batch.

Certain category of Ayurvedic, siddha and Unani medicines like Bhasma, Rasa, Kupi-pakva, Parpati, Sindura, Karpu/uppu/puram, Kushta, Asava-arishta etc. do not have expiry date in, contrast their efficiency increases with the passage of time. Hence, records need be maintained up to five years of the exhausting of stock.

1.1(M) *Record of Market Complaints*. - Manufacturers shall maintain a register to record all reports of market complaints received regarding the products sold in the market. The manufacturer shall enter all data received on such market complaints, investigations carried out by the manufacturers regarding the complaint as well as any corrective action initiated to prevent recurrence of such market complaints shall also be recorded. Once in a period of six months the manufacturer shall submit the record of such complaints to the licensing authority. The Register shall also be available for inspection during any inspection of the premises.

Records of any adverse reaction resulting from the use of Ayurvedic, Siddha and Unani drugs shall also be maintained in a separate register by each manufacturer. The manufacturer shall investigate any of the adverse reaction to find if the same is due to any defect in the product, and whether such reactions are already reported in the literature or it is a new observation.

1.1(N) *Quality Control*. - Every licensee is required to provide facility for quality control section in his own premises or through Government approved testing laboratory. The test shall be as per the Ayurveda, Siddha and Unani pharmacopoeial standard. Where the tests are not available, the test should be performed according to the manufacturers specification or other information available. The quality control section shall verify all the raw materials, monitor in process, quality checks and control the quality of finished product being released





to finished goods store/warehouse. Preferably for such Quality control there will be a separate expert. The quality control section shall have the following facilities: -

- 1) There should be 150 sq. feet area for quality control section.
- 2) For identification of raw drugs, reference books and reference samples should be maintained.
- 3) Manufacturing record should be maintained for the various processes.
- 4) To verify the finished products, controlled samples of finished products of each batch will be kept for 3 years.
- 5) To supervise and monitor adequacy of conditions under which raw materials, semifinished products and finished products are stored.
- 6) Keep record in establishing shelf life and storage requirements for the drugs.
- 7) Manufacturers who are manufacturing patent proprietary Ayurveda Siddha, and Unani medicines shall provide their own specification and control reference in respect of such formulated drugs.
- 8) The record of specific method and procedure of preparation, that is, “Bhavana”, “Mardana” and “Putra” and the record of every process carried out by the manufacturer shall be maintained.
- 9) The standards for identity, purity and strength as given in respective pharmacopoeias of Ayurveda, Siddha and Unani systems of medicines published by Government of India shall be complied with.
- 10) All raw materials will be monitored for fungal, bacterial contamination with a view to minimize such contamination.
- 11) Quality control section will have a minimum of:
  - (i) One person with Ayurveda /Siddha/ Unani qualification recognized under Schedule II of Indian Medicine Central Council Act, 1970 (84 of 1970). Two other persons one each with Bachelor qualification in Botany/ Chemistry/ Pharmacy could be on part time or contractual basis.
  - (ii) The manufacturing unit shall have a quality control section as explained under Section 35(ii). Alternatively, these quality control provisions will be met by getting testing etc., from a recognized laboratory for Ayurveda, Siddha and Unani drugs; under Rule 160-A of the drugs and Cosmetics Act. The manufacturing company will maintain all the record of various tests got done from outside recognized laboratory.
  - (iii) List of equipments recommended is indicated in Part II C.

### 1.2. Requirement for Sterile Product:

1.2(A) *Manufacturing Areas*: For the manufacture of sterile Ayurvedic, Unani and Siddha drugs, separate enclosed areas specifically designated for the purpose shall be provided. These areas shall be provided with air locks for entry and shall be essentially dust free and ventilated with an air supply. For all areas where aseptic manufacture has to be carried out, air supply shall be filtered through bacteria retaining filters (HEPA Filters) and shall be at a pressure higher than in the adjacent areas. The filters shall be checked for performance on installation and periodically thereafter the record of checks shall be maintained. All the



surfaces in sterile manufacturing areas shall be designed to facilitate cleaning and disinfection. For sterile manufacturing routine microbial counts of all Ayurvedic, Siddha and Unani drug manufacturing areas shall be carried out during operations. Results of such count shall be checked against established in-house standards and record maintained.

Access to manufacturing areas shall be restricted to minimum number of authorized personnel. Special procedure to be followed for entering and leaving the manufacturing areas shall be written down and displayed. For the manufacturing of Ayurvedic, Siddha and Unani drug that can be sterilized in their final containers, the design of the areas shall preclude the possibility of the products intended for sterilization being mixed with or taken to be products already sterilized. In case of terminally sterilized products, the design of the areas shall preclude the possibility of mix up between nonsterile products.

1.2(B) *Precautions against contamination and mix:*

(a) Carrying out manufacturing operations in a separate block of adequately isolated building or operating in an isolated enclosure within the building,

(b) Using appropriate pressure differential in the process area.

(c) Providing a suitable exhaust system.

(d) Designing laminar flow sterile air system for sterile products.

(e) The germicidal efficiency of UV lamps shall be checked and recorded indicating the burning hours or checked using intensity.

(f) Individual containers of liquids and ophthalmic solutions shall be examined against black-white background fitted with diffused light after filling to ensure freedom from contamination with foreign suspended matter.

(g) Expert technical staff approved by the Licensing Authority shall check and compare actual yield against theoretical yield before final distribution of the batch.

All process controls as required under master formula including room temperature, relative humidity, volume filled, leakage and clarify shall be checked and recorded.



## PART II

### A. LIST OF MACHINERY, EQUIPMENT AND MINIMUM MANUFACTURING PREMISES REQUIRED FOR THE MANUFACTURE OF VARIOUS CATEGORIES OF AYURVEDIC, SIDDHA SYSTEM OF MEDICINES.

Sl.No.	Category of Medicine	Minimum manufacturing space required	Machinery/equipment recommended
(1)	(2)	(3)	(4)
		1200 Square feet covered area with separate cabins partitions for each activity. If Unani medicines are manufactured in same premises an additional area of 400 sq. feet will be required.	
1.	Anjana/Pisti	100 sq. feet	Karel/mechanized/motorized, kharel. End runner/Ball-Mill Sieves/Shifter
2.	Churna / Nasya Manjan/Lepa Kwath Churn	200 Sq feet	Grinder / disintegrator / Pulverisar / Powder mixer / Sieves / Shifter.
3.	Pills / Vatti / Gutika Matrica and tablets	100 sq. feet	Ball Mill, Mass mixer, powder mixer, granulator, drier, tablet compressing machine, pill/vati cutting machine, stainless steel trays/container for storage and sugar coating, polishing pan in the case of sugar coated tablets, mechanized chattoo (for mixing guggulu) where required.
4.	Kupi pakava / Ksara / Parpati / Lavana Bhasma Satva / Sindura Karpu / Uppu / Param	150 Sq. feet	Bhatti, Karahi/Stainless Steel Vessels/Patila Flask, Multani Matti/Plaster of Paris, copper Rod, Earthen container, GajPut Bhatti, Muffle furnace(Electrically operated) End/Edge Runner, Exhaust Fan, Wooden/Stainless Steel Spatula.



5.	Kajal	100 Sq. feet	Earthen lamps for Collection of Kajal, Triple Roller Mill, End Roller, Sieves, Stainless Steel Patila, filling / packing and manufacturing room should be provided with exhaust fan & ultra violet lamps
6.	Capsules	100 Sq. feet	Air Conditioner, Dehumidifier, hygrometer, thermometer, Capsule filling machine and chemical balance.
7.	Ointment /Marham / Pasai	100 Sq. feet	Tube filling machine, Crimping machine/Ointment mixer, End Runner/ Mill (Where required) Stainless Steel Storage Container, Stainless Steel Patila.
8.	Pak /Avalesh / Khand / Modak /Lakayam	100 Sq. feet	Bhatti section fitted with exhaust fan and should be fly proof, iron kadahi / Stainless Steel Patila and Stainless Steel Storage container.
9.	. Panak / Syrup / Pravahi Kwath Manapaku	150 Sq, feet	Tincture press, exhaust fan fitted and fly proof, Bhatti section, Bottle washing machine, filter press / Gravity filter liquid filling machine, P.P. Capping Machine
10.	. Asava / Arishta	200 Sq. ft	Same as mentioned above. Fermentation tanks containers and distillation plant where necessary, Filter Press.
11.	Sura	100 Sq. ft	Same as mentioned above plus distillation plant and transfer pump
12.	Ark/ Tinir	100 Sq. ft	Maceration tank, Distillation plant, Liquid filling tank with tap / Gravity filter/Filter press, Visual inspection box.
13.	Tail/Ghrit Ney	100 Sq. ft	Bhatti, Kadahi/ Stainless Steel





			Patila S.S.Storage Containers, Filtration equipment, filling tank with tap/Liquid filling machine.
14.	Aschyotan / Netra Malham Panir	100 Sq. ft	Hot air oven electrically heated with thermostatic control, kettle gas or electrically heated with suitable mixing arrangements collation mill, or ointment mill, tube filling equipment, mixing and storage tanks of stainless steel or of other suitable material sintered glass funnel, seitz filter or filter candle, liquid filling equipment, autoclave.
15.	Each manufacturing unit will have a separate area for Bhatti, furnace, boilers, puta, etc. This will have proper ventilation, removal of smoke, prevention of flies, insets, dust etc. The furnace section could have tin roof.	200 Sq. ft	



## B. LIST OF MACHINERY, EQUIPMENT AND MINIMUM MANUFACTURING PREMISES REQUIRED FOR THE MANUFACTURE OF VARIOUS CATEGORIES OF UNANI SYSTEM OF MEDICINES.

One machine indicated for one category of medicine could be used for the manufacturing of other category of machine also. Similarly some of the manufacturing areas like powdering, furnace, packing of liquids could also be shared for these items.

Sl. No.	Category of Medicine	Minimum manufacturing space required	Machinery/equipment recommended
1	2	3	4
		1200 square feet covered area with separate cabins, partitions for each activity. If Ayurveda / Siddha, Medicines are also manufactured in same premises an addition area of 400 square feet will be required.	
1.	Itrifal Tiryao / majoon / Looq / Jawarish Khamiras	100 Sq. feet	Grinder/ Pulveriser, Sieves, powder mixer (if required), Stainless Steel Patilas, Bhatti and other accessories, Planter mixer for Khamiras.
2.	Araq.	100 Sq. feet	Distillation Plant (garembic) Stainless Steel storage tank, boiling vessel, gravity filter, bottle filling machine, bottle washing machine, bottle drier.
3.	Habb (Pills) and tablets.	100 Sq. feet	Ball Mill, Mass Mixer/Powder mixer, Granulator drier, tablet compressing machine, pill/vati cutting machine, stainless steel trays/ container for storage and in the case of sugar coated tablets, mechanized chattoo, (for mixing guggulu) where required



4.	Sufoof (Powder)	100 Sq. feet	Grinder / Pulveriser, Sieves, Trays, Scoops, Powder mixer (where required).
5.	Raughan (oils) (Crushing and boiling)	100 Sq. feet	Oil expeller, Stainless Steel Patilas oil filter bottle, filling machine, bottle drier, bhatti.
6.	Shiyaf, Surma, Kajal	100 Sq. feet	End runner, mixing Stainless Steel Vessel
7.	Marham, Zimad (Ointment)	100 Sq. feet	Karal, Bhatti, End runner, Grinder, Pulveriser, Triple Roller Mill (if needed).
8.	Qurs (Tab)	100 Sq. feet	Grinder / Pulveriser, Sieves, Powder mixer (where needed), Granulator, Drier, Tablet Compressing Machine, Die punches Trays, Disintegration apparatus, Balance with weights, Scoops, Sugar Coating Pan, polishing pan, Heater.
9.	Kushta	100 Sq. feet	Bhatti, Kharal, Sil Batta, Earthen pots.
10.	Murabba	100 Sq. feet.	Aluminium Vessels 50-100 kgs. Capacity, Gendna, Bhatti.
11.	Capsule	100 Sq. feet	Pulveriser, Powder mixer (where needed), capsule filling machine, Air conditioner, Dehumidifier Balance with weights, storage containers, glass.



12.	Sharbat & Jushanda	100 Sq. feet	Tinctum Press, exhaust fan fitted, Bhatti section, Bottle washing machine, Filter Press, Gravity filter, Liquid filling tank with tap/liquid filling machine, PP capping machine, air over electrically heated with thermostatic control, kettle.
13.	Qutoor Chasm and Marham (Eye drops eye ointment)	100 Sq. feet	Hot air oven electrically heated with thermostatic control, kettle.
14.	Each Manufacturing unit will have a separate area for Bhatti, furnances, boiler, putta, etc. This will have proper ventilation, removal of smoke, prevention of files, insects, dust, etc.	200 sq. feet	



### C. LIST OF EQUIPMENT RECOMMENDED FOR IN HOUSE QUALITY CONTROL SECTION

(Alternatively unit can get testing done from a government approved laboratory.)

(A)	CHEMISTRY SECTION	(B)	PHARMACOGNOSY SECTION
1.	Alcohol determination Apparatus (complete set)	1.	Microscope Binocular
2.	Volatile Oil Determination Apparatus.	2.	Dissecting Microscope.
3.	Boiling Point Determination Apparatus	3.	Microtome.
4.	Melting Point Determination Apparatus	4.	Physical Balance.
5.	Refractometer	5.	Aluminium Slide Trays.
6.	Polarimeter	6.	Stage Micrometer.
7.	Viscometer	7.	Camera Lucida (Prism and Mirror Type.)
8.	Tablet Disintegration Apparatus.	8.	Chemicals, Glass-ware etc.
9.	Moisture Meter.		
10.	Muffle Furnace.		
11.	Electronic Balance.		
12.	Magnetic Stirrer.		
13.	Hot Air Oven.		
14.	Refrigerator.		
15.	Glass/Steel Distillation apparatus.		
16.	LPG Gas Cylinders with Burners.		
17.	Water Bath(Temperature controlled.)		
18.	Heating Mantles/ Hot Plates.		
19.	TLC Apparatus with all accessories (Manual)		
20.	Paper Chromatography apparatus with accessories.		
21.	Sieve size 10 to120 with Sieve shaker.		
22.	Centrifuge Machine		
23.	Dehumidifier		
24.	pH Meter.		
25.	Limit Test Apparatus.		

**Note:** - The above requirements of machinery, equipments, space, qualifications are made subject to the modification at the discretion of the Licensing Authority, if he is of the opinion that having regard to the nature and extent of the manufacturing operations it is necessary to relax or alter then in the circumstances in a particular case.



## ANNEXURE-V

### Rule 158 (B) - Guidelines for issue of license with respect to Ayurveda, Siddha or Unani drugs.

#### I. (A). Ayurveda, Siddha Unani Medicines under, section 3 (a):-

Ayurveda, Siddha or Unani drugs includes all medicines intended for internal or external use for or in the diagnosis, treatment, mitigation or prevention of disease  
or  
disorder in human beings or animals, and manufactured exclusively in accordance with the formulae described in the authoritative books of Ayurvedic, Siddha and Unani Tibb system of medicine, as specified in the First Schedule;

#### (B). Patent or Proprietary medicine under section 3(h);

- (i) In relation to Ayurvedic, Siddha and Unani Tibb system of medicine of all formulations containing only such ingredients mentioned in the formulae described in the authoritative books of Ayurveda, Siddha or Unani Tibb system of medicines specified in the First Schedule, but does not include a medicine which is administered by parenteral route and also a formulation included in the authoritative books as specified in clause (a);
- (ii) **Balya/Poshak/Muqawi/Unavuporutkal/positive health Promoter** formulations having ingredients mentioned in books of First Schedule of the Drugs and Cosmetics Act and recommended for promotional and preventive health.
- (iii) **Saundarya Prasadak (Husane afza)/Azhagh-sadhan formulation** having ingredients mentioned in Books of First Schedule of the Drugs and Cosmetics Act and recommended for oral, skin, hair and body care.
- (iv) **Aushadh Ghana (Medicinal plant extracts – dry/wet) extract** obtained from plant mentioned in books of First Schedule of the Act including Aqueous or hydro-alcohol.



**II. (A) For issue of licence to the medicine with respect to Ayurvedic, Siddha and Unani, the conditions relating to safety study and the experience or evidence of effectiveness shall be such as specified in columns (5) and (6) of the Table given below:-**

S. No.	Category	Ingredient (S)	Indication (s)	Safety study	Experience/Evidence of Effectiveness	
(1)	(2)	(3)	(4)	(5)	(6)	
					Published Literature	Proof of Effectiveness
1.	(A) Ayurveda, Siddha and Unani drugs given in 158.B as referred in 3(a)	As per text	As per text	Not Required	Required	Not Required
2.	(B) Any change in dosage form of Ayurveda Siddha and Unani drugs as described in section 3(a) of the Drugs and Cosmetics Act, 1940	As per text	As per text	Not Required	Required	Not Required
3.	(C) Ayurveda, Siddha and Unani drugs referred in 3(a) to be used for new indication	As per text	New	Not Required	If Required	Required

**II. B. For issue of license with respect to patent or proprietary medicine. The conditions relating to Safety studies and experience or evidence of effectiveness shall be specified as follows:-**

S. No.	Category	Ingredient (S)	Indication (s)	Safety study	Experience/Evidence of Effectiveness	
(1)	(2)	(3)	(4)	(5)	(6)	
					Published Literature	Proof of Effectiveness
1.	Patent or Proprietary medicine	As per text	Textual rationale	Not required	Of Ingredients	* Pilot study as per relevant protocol for Ayurveda and Siddha and Unani drugs.
2.	Ayurveda Siddha, Unani	As per text	Existing	Required	Required	Required



drug with any of the ingredients of Schedule E (1) of the Drugs and Cosmetics Act, 1940						
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**(V). For issue of license with respect to medicine Aushadh Ghana [extract of medicinal plant (dry/wet)]**

S. No.	Category	Ingredient (S)	Indication (s)	Safety study	Experience/Evidence of Effectiveness	
					Published Literature	Proof of Effectiveness
(1)	(2)	(3)	(4)	(5)	(6)	
					Published Literature	Proof of Effectiveness
1.	(A) Aqueous	As per text	As per text	Not Required	Not Required	Not Required
2.	(A1) Aqueous	As per text	New Indication	Not Required	Not Required	Required
3.	(B) Hydro-Alcohol	As per text	As per text	Not Required	If Required	Not Required
4.	(B1) Hydro-Alcohol	As specified	New Indication**	Required	If Required	Required
5.	Other than Hydro/Hydro-Alcohol	As specified	As specified	Required Acute, Chronic, Mutagenicity and Teratogenicity	If Required	Required

\* The standard protocol with also include concept of Anupan, Prakriti & Tridosh etc. published by Central Research Councils Ayurveda, Siddha, Unani and other Government/Research Bodies.

\*\* New Indication means which is other than mentioned in 1<sup>st</sup> schedule books of Drugs & Cosmetics Act, 1940





## ANNEXURE-VI

### MINISTRY OF HEALTH AND FAMILY WELFARE (Department of Health and Family Welfare)

#### NOTIFICATION

New Delhi, the 30th November, 2015.

**G.S.R. 918(E).**—Whereas a draft of the rules further to amend the Drugs and Cosmetics Rules, 1945, was published, as required by section 12 and section 33 of the Drugs and Cosmetics Act, 1940 (23 of 1940), vide notification of the Government of India in the Ministry of Health and Family Welfare (Department of Health and Family Welfare), number G.S.R. 702(E), dated the 24th October, 2013, in the Gazette of India, Extraordinary, Part II, Section 3, Sub-section (i), dated the 24th October, 2013, inviting objections and suggestions from all persons likely to be affected thereby before the expiry of a period of forty-five days from the date on which the copies of the Official Gazette of the said notification were made available to the public;

And whereas copies of the Gazette were made available to the public on the 29th October, 2013;

And whereas, the objections and suggestions received from the public on the said draft rules have been considered by the Central Government.

Now, therefore, in exercise of the powers conferred by section 12 and section 33 of the Drugs and Cosmetics Act, 1940 (23 of 1940), the Central Government, after consultation with the Drugs Technical Advisory Board, hereby makes the following rules further to amend the Drugs and Cosmetics Rules, 1945, namely:-

1. (1) These rules may be called the Drugs and Cosmetics (Eighth Amendment) Rules, 2015.  
  
(2) They shall come into force on the date of their publication in the Official Gazette.
2. In rule 2 of the Drugs and Cosmetics Rules, 1945 (hereinafter referred to as the said rules), after clause (ea) the following clause shall be inserted, namely:—  
  
‘(eb). “Phytopharmaceutical drug” includes purified and standardised fraction with defined minimum four bio-active or phyto-chemical compounds (qualitatively and quantitatively assessed) of an extract of a medicinal plant or its part, for internal or external use of human beings or animals for diagnosis, treatment, mitigation or prevention of any disease or disorder but does not include administration by parenteral route.’
3. In rule 122-A of the said rules,-



- (i) in sub-rule (1), in clause (b), in the second proviso, for the words, figures and letter “Appendix I or Appendix IA”, the words, figures and letters, “Appendix I or Appendix IA or Appendix IB”, shall be substituted;
  - (ii) in sub-rule (2), for the words, figures and letter “Appendix I or Appendix IA”, the words, figures and letters, “Appendix I or Appendix IA or Appendix IB”, shall be substituted.
4. In rule 122-B of the said rules,-
- (i) in sub-rule (1), in clause (b), in the second proviso, for the words, figures and letter “Appendix I or Appendix I A”, the words, figures and letters, “Appendix I or Appendix IA or Appendix I B”, shall be substituted;
  - (ii) in sub-rule (2), for the words, figures and letter “Appendix I or Appendix IA”, the words, figures and letters, “Appendix I or Appendix IA or Appendix IB”, shall be substituted.
5. In rule 122-E of the said rules, in clause (a), after the words “bulk drugs substance,” the words “or phytopharmaceutical drug” shall be inserted.
6. In Schedule Y of the said rules, after APPENDIX IA, the following Appendix shall be inserted, namely:-

“APPENDIX I B”

**DATA TO BE SUBMITTED ALONG WITH APPLICATION TO CONDUCT CLINICAL TRIAL OR IMPORT OR MANUFACTURE OF A PHYTOPHARMACEUTICAL DRUG IN THE COUNTRY**

**PART - I**

**1. Data to be submitted by the applicant:**

- 1.1. A brief description or summary of the phytopharmaceutical drug giving the botanical name of the plant (including vernacular or scriptural name, wherever applicable), formulation and route of administration, dosages, therapeutic class for which it is indicated and the claims to be made for the phytopharmaceutical product.
- 1.2. Published literature including information on plant or product or phytopharmaceutical drug, as a traditional medicine or as an ethno medicine and provide reference to books and other documents, regarding composition, process prescribed, dose or method of usage, proportion of the active ingredients in such traditional preparations per dose or per day’s consumption and uses.
- 1.3. Information on any contraindications, side effects mentioned in traditional medicine or ethno medicine literature or reports on current usage of the formulation.
- 1.4. Published scientific reports in respect of safety and pharmacological studies relevant for the phytopharmaceutical drug intended to be marketed,-
  - (a) where the process and usages are similar or same to the product known in traditional medicine or ethno medicine; and



- (b) where process or usage is different from that known in traditional medicine or ethno medicine.
- 1.5. Information on any contraindications, side effects mentioned or reported in any of the studies, information on side effects and adverse reactions reported during current usage of the phytopharmaceutical in the last three years, wherever applicable.
- 1.6. Present usage of the phytopharmaceutical drug, – to establish history of usages, provide details of the product, manufacturer, quantum sold, extent of exposure on human population and number of years for which the product is being sold.

## **2. Human or clinical pharmacology information:**

- 2.1. Published scientific reports in respect of pharmacological studies including human studies or clinical studies or epidemiological studies, relevant for the phytopharmaceutical drug intended to be marketed,-
  - (a) where the process and usages are similar or same to the product known in traditional medicine or ethno medicine; and
  - (b) where process or usage is different from that known in traditional medicine or ethno medicine.
- 2.2. Pharmacodynamic information (if available).
- 2.3. Monographs, if any, published on the plant or product or extract or phytopharmaceutical. (Copies of all publications, along with english translation to be attached.)

## **PART – II**

### **Data generated by applicant**

## **3. Identification, authentication and source of plant used for extraction and fractionation:**

- 3.1. Taxonomical identity of the plant used as a source of the phytopharmaceutical drug giving botanical name of genus, species and family, followed by the authority citation (taxonomist's name who named the species), the variety or the cultivar (if any) needs to be mentioned.
- 3.2. Morphological and anatomical description giving diagnostic features and a photograph of the plant or plant part for further confirmation of identity and authenticity. (Furnish certificate of confirmation of botanical identity by a qualified taxonomist).
- 3.3. Natural habitat and geographical distribution of the plant and also mention whether the part of the plant used is renewable or destructive and the source whether cultivated or wild.
- 3.4. Season or time of collection.



- 3.5 Source of the plant including its geographical location and season or time of collection.
- 3.6 A statement indicating whether the species is any of the following, namely:-
- (a) determined to be endangered or threatened under the Endangered Species Act or the Convention on International Trade in Endangered species (CITES) of wild Fauna and Flora;
  - (b) entitled to special protection under the Biological Diversity Act, 2002 (18 of 2003);
  - (c) any known genotypic, chemotypic and ecotypic variability of species.
- 3.7. A list of grower or supplier (including names and addresses) and information on the following items for each grower or supplier, if available or identified already, including information of primary processing, namely:-
- (a) harvest location;
  - (b) growth conditions;
  - (c) stage of plant growth at harvest;
  - (d) harvesting time;
  - (e) collection, washing, drying and storage conditions;
  - (f) handling, garbling and transportation;
  - (g) grinding, pulverising of the plant material; and
  - (h) sieving for getting uniform particle size of powdered plant material.
- 3.8 Quality specifications, namely:-
- (a) foreign matter;
  - (b) total ash;
  - (c) acid insoluble ash;
  - (d) pesticide residue;
  - (e) heavy metal contamination;
  - (f) microbial load;
  - (g) chromatographic fingerprint profile with phytochemical reference marker;
  - (h) assay for bio-active or photochemical compounds;
  - (i) chromatographic fingerprint of a sample as per test method given under quality control of the phytopharmaceutical drug (photo documentation).
- 3.9 . An undertaking to supply specimen sample of plant duly labeled and photocopy of the certificate of identity confirmation issued by a qualified taxonomist along with drawings or photographs of the diagnostic morphological and histological features of the botanical raw material used for the confirmation of authenticity.

#### **4. Process for extraction and subsequent fractionation and purification:**

- 4.1. Quality specifications and test methods for starting material.



#### 4.2. Steps involved in processing.

- (a) details of solvent used, extractive values, solvent residue tests or limits, physico-chemical tests, microbial loads, heavy metal contaminants, chromatographic finger print profile with phytochemical reference markers, assay for active constituents or characteristic markers, if active constituents are not known;
- (b) characterisation of final purified fraction;
- (c) data on bio-active constituent of final purified fraction;
- (d) information on any excipients or diluents or stabiliser or preservative used, if any.

#### 4.3. Details of packaging of the purified and characterised final product, storage conditions and labeling.

### 5. Formulation of phytopharmaceutical drug applied for:

- 5.1. Details of the composition, proportion of the final purified fraction with defined markers of phytopharmaceutical drug per unit dose, name and proportions of all excipients, stabilisers and any other agent used and packaging materials.
- 5.2. Test for identification for the phytopharmaceutical drug.
- 5.3. Quality specifications for active and inactive phytopharmaceutical chromatographic finger print profile with phytochemical reference marker and assay of active constituent or characteristic chemical marker.

### 6. Manufacturing process of formulation:

- 6.1. The outline of the method of manufacture of the dosage form, along with environmental controls, in-process quality control tests and limits for acceptance.
- 6.2. Details of all packaging materials used, packing steps and description of the final packs.
- 6.3. Finished product's quality specifications, including tests specific for the dosage form, quality and chromatographic finger print profile with phytochemical reference marker and assay for active constituent or characteristic marker, if active constituents are not known.

### 7. Stability data:

- 7.1. Stability data of the phytopharmaceutical drug described at 4 above, stored at room temperature at 40 +/- 2 deg. C and humidity at 75%RH +/- 5%RH for 0, 1, 2, 3 and 6 months.



- 7.2 Stability data of the phytopharmaceutical drug in dosage form or formulation stored at room temperature at  $40 \pm 2$  deg. C and humidity at 75%RH  $\pm$  5%RH for 0, 1, 2, 3 and 6 months, in the pack intended for marketing.

## **8. Safety and pharmacological information:**

- 8.1. Data on safety and pharmacological studies to be provided.
- 8.2. Animal toxicity and safety data:
- (a) 28 to 90 days repeat dose oral toxicity on two species of animals;
  - (b) In-vitro genotoxicity data (Ame's test and Chromosomal aberration test as per Schedule Y);
  - (c) dermal toxicity tests for topical use products;
  - (d) teratogenicity study (only if phytopharmaceutical drug is intended for use during pregnancy).

## **9. Human studies:**

- 9.1. Clinical trials for phytopharmaceutical drugs to be conducted as per applicable rules and guidelines for new drugs.
- 9.2. For all phytopharmaceutical drugs data from phase I (to determine maximum tolerated dose and associated toxicities) and the protocols shall be submitted prior to performing the studies.
- 9.3. Data of results of dose finding studies performed and the protocols shall be submitted prior to performing the studies:

Provided that in the case of phytopharmaceutical drug already marketed for more than five years or where there is adequate published evidence regarding the safety of the phytopharmaceutical drug, the studies may be abbreviated, modified or relaxed.

## **10. Confirmatory clinical trials:**

- 10.1. Submit protocols for approval for any specific or special safety and efficacy study proposed specific to the phytopharmaceutical drug.
- 10.2. Submit proposed protocol for approval for human clinical studies appropriate to generate or validate safety and efficacy data for the phytopharmaceutical dosage form or product as per applicable rules and guidelines.
- 10.3. Submit information on how the quality of the formulation would be maintained during the above studies.





### **11. Regulatory status:**

- 11.1 Status of the phytopharmaceutical drug marketed in any country under any category like functional food or dietary supplement or as traditional medicine or as an approved drug.

### **12. Marketing information:**

- 12.1. Details of package insert or patient information sheet of the phytopharmaceutical drug to be marketed.
- 12.2. Draft of the text for label and carton.

### **13. Post marketing surveillance (PMS):**

- 13.1. The applicant shall furnish periodic safety update reports every six months for the first two years after approval the drug is granted.
- 13.2. For subsequent two years the periodic safety update reports need to be submitted annually.

### **14. Any other relevant information:**

Any other relevant information which the applicant considers that it will help in scientific evaluation of the application.”.

[F. No. X. 11014/2/2012-DFQC]  
K. L. SHARMA, Jt. Secy.

**Note:** The principal rules were published in the Gazette of India vide notification No. F.28-10/45-H (1) dated the 21st December, 1945 and was last amended vide notification number GSR 826 (E), dated the 30th October, 2015.

**TEST PROTOCOLS****Identification of Single Drugs****Name of the Drugs**

The name given on the top of each monograph of the drug is in Sanskrit as mentioned in the Ayurvedic classics and/or in the Ayurvedic Formulary of India, Part-I and Part-II will be considered official. These names have been arranged in English alphabetical order. The Latin name (taxonomical nomenclature) of each drug as found in authentic scientific literature has been provided in the monograph in the introductory paragraph. The official name will be the main title of the drug and its scientific name will also be considered as legal name.

**1. Systematic Study of Crude Drugs**

In the Indian Systems of Medicine comprising of Ayurveda, Unani and Siddha, drugs of plant, animal and mineral origin, are used in their natural or so called “Crude” forms singly or in their mixture or in combination, to make a compound preparation or formulation. Nearly 90 per cent of the Crude Drugs are obtained from the plant sources while about 10 per cent of the drugs are derived from animal and mineral sources. The drugs of plant origin especially of herbaceous nature are frequently used as whole plant; otherwise their parts such as Root, Stem, Leaf, Flower, Seed, Fruit modifications of Stem and Root, Bark of a Stem or Root, Wood, and their Exudates or Gums etc. constitute single drugs in the Indian Systems of Medicine. These vegetable drugs are either used in dried forms or sometimes as whole fresh or their juice. The study of these crude drugs with respect to medicinal properties, uses, cultivation etc. are covered in Pharmacognosy (pharmakon = Drug; gignosis = to acquire knowledge of), meaning the knowledge or science of Drugs.

In Pharmacognosy a complete and systematic study of a drug is performed, which comprises of (i) origin, common names, scientific nomenclature and family, (ii) geographical source (and history), (iii) cultivation, collection, preservation and storage, (iv) Macroscopical, Microscopical and sensory (organoleptic) characters, (v) Chemical composition wherever possible, (vi) Identity, Purity, Strength and Assay, (vii) substitute and adulterants etc. Such systematic study of a drug as complete as possible is claimed to be the scientific or pharmacognositical evaluation.

As mentioned above each crude drug derived from the vegetable kingdom consists of a definite part of plant e.g., leaf, stem, fruit, seed, wood, bark, root etc. Morphological or macroscopical details of the respective part are given by observing it with a naked eye or with the aid of a magnifying lens. In this description general conditions of the drug, size, shape, outer surface, inner surface etc are referred to. Drugs can be identified with the aid of the above, only if they are available in entire condition. Sensory or Organoleptic characters describe colour, odour, taste, consistency etc. The microscopic examination of different





parts of the drug provides several diagnostic characters. In case of leaves, surface preparation and transverse section, preferably through midrib, are made and nature of epidermis, trichomes, stomata, arrangement of tissues like palisade cells, vascular bundles and nature of cell content are studied. Similarly in case of bark, root, rhizome and wood, transverse and longitudinal sections are made and from characteristic arrangements of tissues of each drug and from diagnostic elements like stone cells, fibres, vessels etc. as also from the study of the cell deposits like crystals, starch etc., the drugs are identified. The studies of diagnostic elements are helpful especially when the drugs are in powdered condition and give clues in the identification of drugs. Linear measurements and other methods of quantitative microscopy give further aid in the identification of the drugs. The sections or the powdered drugs samples are cleared by clearing agents, mostly chloralhydrate solution, before mounting on the slide. The basic chemical nature of cell-wall of almost all the plants is cellulosic, However, lignin, suberin, cutin or mucilage are deposited on the cellulose. Cellulose gives blue colour with chlorzinc-iodine solution or with cuoxam. (Copper-oxide-ammonia) reagent. Lignin present in the middle lamella and secondary cell-wall of many vessels, fibres and sclerieds gives red colour with phloroglucinol and concentrated hydrochloric acid. Suberin is present in cork and endodermis cells while cutin in the cuticle of leaf. Both suberin and cutin are fatty in nature and when heated with Sudan Red-III give red colour.

Mucilage gives red colour with ruthenium red. The chemical constituents present in the drugs can be identified by chemical or microchemical tests e.g., Rhubarb rhizomes give with 5% potassium hydroxide red colour because of anthraquinone derivatives, strychnine present in Nux-vomica gives purplish-red colour with ammonium vanadate and concentrated sulphuric acid.

## 2. Microscopical Methods of Examining Crude Drugs

Methods of preparing specimens of crude materials of drugs for microscopical studies vary, depending on the morphological groups of drugs to be examined and also on the natures of the material i.e., entire, cut or powdered.

### I. LEAVES, HERBS AND FLOWERS

For examining leaves, herbs and flowers (entire or cut) under microscope, following methods are employed for clarification:

#### A. Entire and cut materials

(i) Entire materials – When examining entire leaves, herbs and flowers, take pieces of leaf (margin and vein of leaves only), herbs (only leaf) and flowers (only calyx and corolla) in test tube. Add a solution of caustic alkali or nitric acid to the test tube and boil for 1-2 minutes, pour the contents into a porcelain dish, drain off the liquid, wash the material with water and leave for sometimes. Remove the pieces of the material from the water with a spatula and put on the slide, add a few drops of the solution of glycerol or chloral hydrate. Crush the material with scalpel and cover with cover slip before examining.



(ii) Cut materials – For examining cut leaves, herbs and flowers, take several pieces in a test tube and employ the same methods as described for entire materials. Other methods employed for clarification of the material (leaf and stem) are described below:-

(a) Leaf – Boil pieces of leaves in a test tube with chloral hydrate for several minutes until completely clarified and then examine them in chloral hydrate solution. After clarification, leaf pieces are divided into two parts with the help of a scalpel or needle, and carefully turn one part. The leaf can be examined from both the dorsal and ventral surfaces.

(b) Stem – To examine stem material (without leaf) boil pieces in a solution of caustic alkali or in nitric acid. Remove the epidermis with a scalpel or a needle for examining the surface. For examining pressed specimen of stem, take separate tissue and press them with a scalpel on the slide.

### **B. Powder**

For examining characters of the powder take sufficient amount of powder in Chloral-hydrate solution on a slide and cover it with a cover slip, warm over a low flame for a short time.

## **II. FRUITS AND SEEDS**

### **A. Entire materials**

For microscopical examination of fruit and seed take the specimens or outer coat of seed or fruit and examine as described below:

(i) Outer Coat – For examining the outer coat boil 3 or 4 seeds or fruits in caustic alkali solution in a test tube for 1-2 minutes (outer coat specimens with intensive pigmentation are boiled for longer period). After boiling, place the pieces on slide, remove the layers of the coat and examine them after mounting in glycerol solution.

(ii) Section – If fruits or seeds are too hard to cut then boil them for 15-30 minutes or more depending on their hardness or keep them in moistening chamber or absorb in water and chloroform solution or soften them with steam and then cut the specimen for examining purpose. For cutting small, flat seeds (which are difficult to hold) place them in a pith or potato slit for section cutting. Small, round or smooth seeds cannot be cut into section in the pith, then in such cases, they may be embedded in paraffin wax blocks for section cutting. For this, a block of paraffin (0.6 × 0.5 × 1.5 cms. in size) is made and the seed is embedded in the block by making a cavity or a pit in the block with a hot teasing needle. Cut the section with a sharp razor (through the object) together with the paraffin, place them on to the slide, remove paraffin with a needle or wash it with xylene and examine the section in chloral-hydrate solution.

### **B. Powder**

For examining the structure of the cells of the seed coat and the cells of the embryo take a small amount of powder of the material on a slide in glycerol and cover it with a cover slip and examine.



1. Starch – For examining the presence of starch in the seed, take two specimens, one in iodine solution and the other in water. With iodine solution starch turns blue. Shape and the structure of starch grains can be seen in water and their size is measured. When examining objects containing starch, prepare specimen by slightly warming in chloral-hydrate solution.

2. Fixed Oil – For examining the presence of fixed oil, prepare a specimen in a solution of Sudan III droplets of fixed oil are coloured orange pink. When examining objects containing small amount of fixed oil, prepare a specimen by slightly warming in chloral-hydrate solution, and when examining objects containing large amount of fixed oil, then the powder is de-fatted and clarified as follows :

Place 0.5 g. of the powder in a porcelain dish, add 5-10 ml. of dilute nitric acid and boil for 1 minute, then strain off the liquid through a cloth, wash the residue with hot water and return it to the porcelain dish with a spatula, boil it with 5-10 ml of caustic alkali solution for 1 minute and again strain it through the cloth and wash with water. Examine the residue in a glycerol solution, after the treatment the structure of the layers of the coat and their cells can be seen very distinctly.

3. Mucilage – Prepare a specimen in Ruthenium Red and examine it under a low power microscope or under dissecting microscope. Mucilage appears as pinkish-red or yellow coloured masses.

### III. BARKS

#### A. Entire material

Prepare transverse or longitudinal section of bark. To soften bark break it into pieces of about 1-2 cm long and 0.5-1 cm wide and boil with in a test tube for 1-3 minutes. Soft pieces are then straightened with a scalpel so as to have a exact transverse or longitudinal direction. Cut the section with razor, moisten the surface of the bark with glycerol solution. Remove the sections with a brush and place them on the slide. Thin pieces of the bark are cut by placing them in the pith (potato or carrot). The sections are treated with various reagents before examining.

1. Lignified elements – For testing lignin add several drops of phloroglucinol and a drop of concentrated hydrochloric acid to the section on a slide then draw off the liquid, immerse the section in chloral hydrate solution and cover with a cover slip (the specimen should not be heated); the lignified elements are coloured crimson. Phloroglucinol can be substituted by saffranine, and the lignified elements are coloured pink. The excessive stain can be washed out with acidified alcohol.

2. Starch – Starch is detected by treating with iodine solution.

3. Tannins –Tannins are detected by treating with ferric ammonium sulphate solution (blue-black or green black colour shows the presence of Tannin) or with potassium-dichromate solution brown colour indicates the presence of tannins).



4. Anthraquinone derivatives –Anthraquinone derivatives are detected by treating with alkali solution (blood-red colour shows the presence of anthraquinone derivatives).

### **B. Cut materials**

Prepare small pieces or scraping of bark and boil them for 3-5 minutes in a solution of caustic alkali or potassium hydroxide or in nitric acid solution and then mount in glycerin for examination on a slide covered with a cover slip.

### **C. Powder**

Prepare specimen for examination by placing a little amount of powder on a slide, add 1-2 drops of phloroglucinol and a drop of concentrated hydrochloric acid, cover it with a cover slip, draw off the liquid from one side of the slide with filter paper, and then apply 1-2 drops of chloral-hydrate solution from the other side of the slide, lignified elements are stained crimson-red. Specimen may also be prepared with caustic alkali or ferric ammonium sulphate for this purpose.

## **IV. ROOTS AND RHIZOMES**

### **A. Entire materials**

For anatomical examination of entire roots and rhizomes cut transverse and longitudinal sections. For this, soften small pieces of roots without heating in glycerol solution for 1-3 days, depending on their hardness. The softened roots are straightened with the help of a scalpel in the right direction and then cut a section with the razor. First, cut thicker entire slices and then make thin, smaller sections. Stain the entire slices with phloroglucinol and concentrated hydrochloric acid or with safranin examine the specimen under a dissecting microscope. For micro-chemical test the small and thin sections are examined under microscope, as follows:

1. Starch – Starch is detected with iodine solution. For this, prepare specimen with water to measure the granule of starch with an ocular micrometer.
2. Inulin –Inulin is detected with Molish's reagent. For this place a pinch of powder on a slide and apply 1-2 drops of naphthol and a drop of concentrated sulphuric acid, if inulin is present, the powder will appear reddish-violet coloured. Starch also gives this test, so the test for inulin can be done in the absence of starch.
3. Lignified elements –Lignified elements (fibrovascular bundles, mechanical tissue etc.) are detected with phloroglucinol and concentrated hydrochloric acid or safranin solution as mentioned above for barks.
4. Fixed oil –For fixed oil detection use Sudan IV, as mentioned above for fruits and seeds.If required for tannin, anthraquinone derivatives test as mentioned above.

### **B. Cut material**

Make small pieces or scraping of roots or rhizomes and boil them for 3-5 minutes in caustic alkali, or in nitric acid and then make pressed specimen and immerse them in





glycerol. Microchemical tests can be performed with scrapings for various chemicals as mentioned above.

### C. Powder

Prepare several specimens of the powder on slides in chloral hydrate solution and perform the above mentioned standard tests for detection of starch, fixed oil, inulin, lignified elements, anthraquinone derivatives, tannins, mucilage, etc.

### Types of Stomata

There are several types of stomata, distinguished by the form and arrangement of the surrounding cells. The following descriptions apply to mature stomata.

1. Anomocytic (irregular-celled) –Previously known as ranunculaceous. The stoma is surrounded by a varying number of cells in no way differing from those of the epidermis generally.
2. Anisocytic (unequal-celled) –Previously known as cruciferous or solanaceous. The stoma is usually surrounded by three subsidiary cells, of which one is markedly smaller than the others.
3. Diacytic (cross-celled) –previously known as caryophyllaceous. The stoma is accompanied by two subsidiary cells whose common wall is at right angles to the guard cells.
4. Paracytic (parallel-celled) –Previously known as rubiaceous. The stoma has one each side one or more subsidiary cells parallel to the long axis of the pore and guard cells.

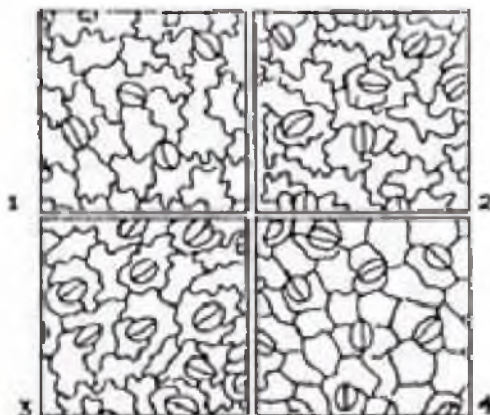


Fig. 1 Various types of stomata

### Determination of Stomatal Index

The stomatal index is the percentage of the number of stomata formed by the total number of epidermal cells, including the stomata, each stoma being counted as one cell. Place leaf fragments of about  $5 \times 5$  mm in size in a test tube containing about 5 ml of chloral hydrate solution and heat in a boiling water-bath for about 15 minutes or until the fragments become



transparent. Transfer a fragment to a microscopic slide and prepare the mount, the lower epidermis uppermost, in chloral hydrate solution and put a small drop of glycerol-ethanol solution on one side of the cover-glass to prevent the preparation from drying. Examine with a 40x objective and a 6x eye piece, to which a microscopical drawing apparatus is attached. Mark on the drawing paper a cross (x) for each epidermal cell and a circle (o) for each stoma. Calculate the result as follows:

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

Where S = the number of stomata in a given area of leaf; and E = the number of epidermal cells (including trichomes) in the same area of leaf. For each sample of leaf make not fewer than ten determinations and calculate the average index.

### Determination of Palisade Ratio

Palisade ratio is the average number of palisade cells beneath one epidermal cell. Place leaf fragments of about 5 × 5 mm in size in a test-tube containing about 5 ml of chloral hydrate solution and heat in a boiling water-bath for about 15 minutes or until the fragments become transparent. Transfer a fragment to a microscopical slide and prepare the mount of the upper epidermis in chloral hydrate solution and put a small drop of glycerol solution on one side of the cover-glass to prevent the preparation from drying. Examine with a 40x objective and a 6x eye piece, to which a microscopical drawing apparatus is attached. Trace four adjacent epidermal cells on paper; focus gently downward to bring the palisade into view and trace sufficient palisade cells to cover the area of the outlines of the four epidermal cells. Count the palisade cells under the four epidermal cells. Where a cell is intersected, include it in the count only when more than half of it is within the area of the epidermal cells. Calculate the average number of palisade cells beneath one epidermal cell, dividing the count by 4; this is the "Palisade ratio" (See Fig. 2). For each sample of leaf make not fewer than ten determinations and calculate the average number.



18.4

Fig. 2 Palisade ratio  $\frac{18.4}{4} = 4.6$

4



### **Determination of Vein-Islet Number**

The mesophyll of a leaf is divided into small portions of photosynthetic tissue by anastomosis of the veins and veinlets; such small portions or areas are termed “Vein- Islets”. The number of vein-islets per square millimeter is termed the “Vein-Islet number”. This value has been shown to be constant for any given species and, for full-grown leaves, to be unaffected by the age of the plant or the size of the leaves. The vein-islet number has proved useful for the critical distinction of certain nearly related species.

The determination is carried out as follows:

For Whole or Cut leaves —Take pieces of leaf lamina with an area of not less than 4 square millimeters from the central portion of the lamina and excluding the midrib and the margin of the leaf. Clear the pieces of lamina by heating in a test tube containing chloral hydrate solution on a boiling water-bath for 30 to 60 minutes or until clear and prepare a mount in glycerol-solution or, if desired, stain with safranin solution and prepare the mount in Canada Balsam. Place the stage micrometer on the microscope stage and examine with 4x objective and a 6x eye piece. Draw a line representing 2 mm on a sheet of paper by means of a microscopical drawing apparatus and construct a square on the line representing an area of 4 square millimeters. Move the paper so that the square is seen in the centre of the field of the eyepiece. Place the slide with the cleared leaf piece on the microscope stage and draw in the veins and veinlets included within the square, completing the outlines of those vein-islets which overlap two adjacent sides of the square. Count the number of vein-islets within the square including those overlapping on two adjacent sides and excluding those intersected by the other two sides. The result obtained is the number of vein-islets in 4 square millimeters. For each sample of leaf make no fewer than three determinations and calculate the average number of vein-islets per square millimeter.

For Leaf Fragments having an area less than 4 square millimeters – Take fragments of leaf lamina each with an area of not less than 1 square millimeter, excluding the midrib and the margin of the leaf. Clear and prepare a mount as stated above. Use a 10x objective and a 6x eyepiece and draw a line representing 1 mm on a sheet of paper by means of a microscopical drawing apparatus and construct a square on this line representing an area of 1 square millimetre. Carry out the rest of the procedure as stated above. The result obtained is the number of vein-islets in 1 square millimetre. For each sample of leaf make no less than 12 determinations and calculate the average number.

### **Determination of Stomatal Number**

Place leaf fragments of about 5x5 mm in size in a test tube containing about 5 ml of chloral hydrate solution and heat in a boiling water-bath for about 15 minutes or until the fragments become transparent. Transfer fragments to a microscopic slide and prepare the mount the lower epidermis uppermost, in chloral hydrate solution and put a small drop of glycerol-ethanol solution on one side of the cover glass to prevent the preparation from drying. Examine with a 40 x objective and a 6x eye piece, to which a microscopical drawing



apparatus is attached. Mark on the drawing paper a cross (x) for each stomata and calculate the average number of stomata per square millimetre for each surface of the leaf.

## 2.2. -DETERMINATION OF QUANTITATIVE DATA

### 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°C 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°C. 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°C. 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 18M *sulphuric acid*, heat gently until white fumes are no longer evolved and ignite at 800°C ± 25°C 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 of the specified strength in a closed flask for twenty-four hours, shaking frequently during six hours and allow 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°C, 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°C to 60°C) in a continuous extraction apparatus (Soxhlet extractor) for 6 hours. Filter the extract quantitatively into a tared evaporating dish and evaporate of the solvent on a water bath. Dry the residue at 105°C 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 unpowdered 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°C 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 Water-insoluble Matter**

Take 10gm of the sample, add 200ml hot distilled water and bring to boiling. Allow to cool to room temperature. Filter through a tared gooch crucible having a bed of asbestos or sintered glass filter. Wash the residue with hot water till the filtrate is sugar-free (perform Molisch test). Dry the gooch crucible or sintered glass filter and weigh. Express as percentage insoluble matter.



### 2.2.12. - 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.

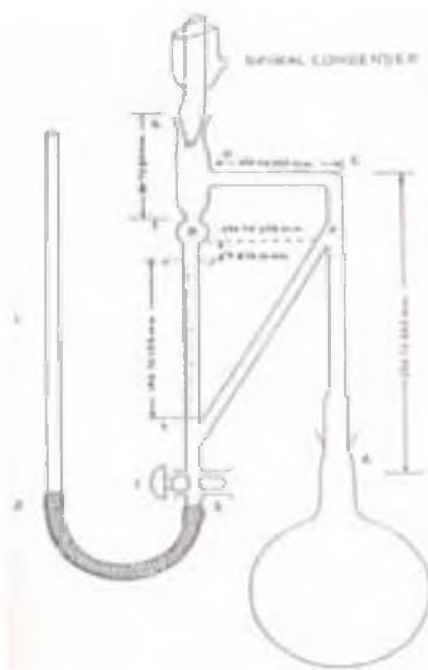


Fig.1 Apparatus for volatile oil determination

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<sub>1</sub> 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<sub>1</sub> 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.

### **2.2.13. - 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<sub>f</sub> 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.

#### **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.



### 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.

### R<sub>f</sub> Value

Measure and record the distance of each spot from the point of its application and calculate the R<sub>f</sub> value by dividing the distance travelled by the spots by the distance travelled by the front of the mobile phase.

### 2.2.14.- Determination of Acidity

#### Reagents

- (1) Standard Sodium Hydroxide solution – 0.05 N
- (2) Phenolphthalein indicator – Dissolve 0.5 gm Phenolphthalein in 100 ml of 50% ethyl alcohol (v/v)

#### Procedure

Take 10 gm of the sample in a suitable titration flask and dissolve in 75 ml of carbon dioxide free water. Mix thoroughly. Titrate against standard sodium hydroxide solution using 4-6 drops of phenolphthalein indicator till pink colour persists for 10 seconds.

Determine blank on water and indicator and correct the volume of sodium hydroxide solution used.

#### Calculation

$$\text{Acidity as formic acid (\%)} \text{ by weight} = \frac{0.23 \times V}{M}$$

Where V = corrected volume of 0.05 N sodium hydroxide used

M = weight in gm of the sample taken for test

### 2.2.15. - 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°C 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.



### 3.1. - REFRACTIVE INDEX

The refractive index ( $\eta$ ) 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°C ( $\pm 0.5$ ) with reference to the wavelength of the D line of sodium ( $\lambda$  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°C or against the reference liquids given in the Table 3.1.

**Table 3.1**

Reference Liquid	$\eta_D^{20^\circ}$	Temperature Co-efficient $\Delta n/\Delta t$
Carbon tetrachloride	1.4603	-0.00057
Toluene	1.4969	-0.00056
$\alpha$ -Methylnaphthalene	1.6176	-0.00048

\* Reference index value for the D line of sodium, measured at 20°C

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

#### 3.1.2. - 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°C, unless otherwise specified.

##### *Method*

Select a thoroughly clean and dry pycnometer. Calibrate the pycnometer by filling it with recently boiled and cooled water at 25°C and weighing the contents. Assuming that the weight of 1 ml of water at 25°C 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 20°C and fill the pycnometer with it. Adjust the temperature of the filled pycnometer to 25°C, remove any excess of the substance and weigh. Subtract 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°C (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°C unless otherwise directed in the individual monograph.

### **3.1.3. - DETERMINATION OF pH VALUES**

The pH value of an aqueous liquid may be defined as the common logarithm 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 pH as a measurable quantity can have a simple meaning, which is also fundamental and exact.

The pH 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.

### **3.2. - DETERMINATION OF MELTING RANGE AND CONGEALING RANGE**

#### **3.2.1. 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°C or 0.8 to 1.2 mm for substances melting above 100°C.

#### **Thermometers**

Accurately standardized thermometers covering the range 10°C to 300°C 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°C to 1.5°C 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 stirring device, capable of rapidly mixing the liquids.

Suitable liquids for use in the heating vessel:

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

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°C to 300°C and with suitable intervals.

The following substances are suitable for this purpose.

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

### 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°C 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°C to 2°C per minute. The statement 'determined by rapid heating' means that the rate of rise of temperature is 5°C 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 °C to b °C" means that the corrected temperature at which the material forms droplets must be at least a °C, and that the material must be completely melted at the corrected temperature, b °C.

**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 °C below the expected melting point and then regulates the rate of rise of temperature to between 0.5 °C to 1 °C per minute. The temperature at which the material is observed to rise in the capillary tube is the melting temperature of the substance.

### 3.2.2. - 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 is 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  $-20^{\circ}\text{C}$  and  $150^{\circ}\text{C}$ .

#### **Apparatus**

A test-tube (About  $150\text{ mm} \times 25\text{ mm}$ ) placed inside another test-tube (about  $160\text{ mm} \times 40\text{ mm}$ ) the inner tube is closed by a stopper that carries a stirrer and a thermometer (About  $175\text{ mm}$  long and with  $0.2^{\circ}\text{C}$  graduations) fixed so that the bulb is about  $15\text{ mm}$  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  $18\text{ mm}$  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  $20\text{ mm}$  of the top. The thermometer is supported in the cooling bath.

#### **Method**

Melt the substance, if a solid, at a temperature not more than  $20^{\circ}\text{C}$  above its expected congealing point, and pour it into the inner test-tube to a height of  $50$  to  $57\text{ mm}$ . 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  $20\text{ mm}$  from the top of the tube with a suitable fluid at a temperature  $4^{\circ}\text{C}$  to  $5^{\circ}\text{C}$  below the expected congealing point. If the substance is a liquid at room temperature, carry out the determination using a bath temperature about  $15^{\circ}\text{C}$  below the expected congealing point. When the sample has cooled to about  $5^{\circ}\text{C}$  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  $1^{\circ}\text{C}$  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  $0.2^{\circ}\text{C}$ .

### **3.2.3. - 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°C to 78°C. 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. °C to 1°C 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°C 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°C	0.04
100°C to 140°C	0.045
141°C to 190°C	0.05
191°C to 240°C	0.055
above 240°C	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 'distills between a °C and b °C', means that temperature at which the first drop runs from the condenser is not less than a °C and that the temperature at which the liquid is completely evaporated is not greater than b °C.

Micro-methods of equal accuracy may be used.

### 3.3. - DETERMINATION OF OPTICAL ROTATION AND SPECIFIC OPTICAL ROTATION

**A. Optical Rotation:** Certain substances, in a pure state, in solution and in tinctures possess 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 25°C 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°C 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-dm tube at 25°C and using the concentrations indicated in Table.



Concentration (g/100 ml)	Angle of Rotation (+) at 25°C
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

$$[\alpha]_{\lambda}^t = \frac{\alpha}{x}$$

‘t’ denotes the temperature of rotation; ‘ $\lambda$ ’ 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°C, unless otherwise specified.

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

For liquid substances

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

For solutions of substances

$$[\alpha]_{\lambda}^t \leftrightarrow = \frac{a \times 100}{lc}$$

Where a is the corrected observed rotation in degrees

l 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.





### 3.4. - 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 ( $\eta$ ) 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<sup>th</sup> of one poise.

While on the absolute scale, viscosity is measured in poise or centi poise, it is most convenient to use the kinematic scale in which the units are stokes (abbreviated S) and centi-stokes (abbreviated CS). The centistokes is 1/100<sup>th</sup> 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.

### 3.5. - DETERMINATION OF TOTAL SOLIDS

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



**Method 1:** Transfer accurately 50 ml of the clear Asava/ Aristha an evaporating 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°C for 3 hours and cooled in a desiccator for 30 min), stir thoroughly, dry at 105°C 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 evaporating dish, which has been dried to a constant weight and evaporate to dryness on a water bath, then dry at 105°C 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.

### 3.6. - 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°C to constant weight and calculate the solubility of the drug in water (wt. in mg/100ml).

### 3.7. - 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.





### 3.8. - 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°C 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.

### 3.9. - 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.

### 3.10. - 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 solution*. 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

$$\text{Peroxide value} = 10 (a - b)/W$$

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

### 3.11. - 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°C to 105°C 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.

### 3.12. - 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.



### 3.13. - 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.

### 3.14. - 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°C to 25.1°C. 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°C, with both the inlet port and the detector at 170°C, 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°C to 25.1°C, 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°C to 25.1°C and dilute to volume with distilled water at 24.9°C to 25.1°C. Determine the relative density at 24.9°C to 25.1°C. The values indicated in column 2 of Table 3.2 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°C to 25.1°C, 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...”.

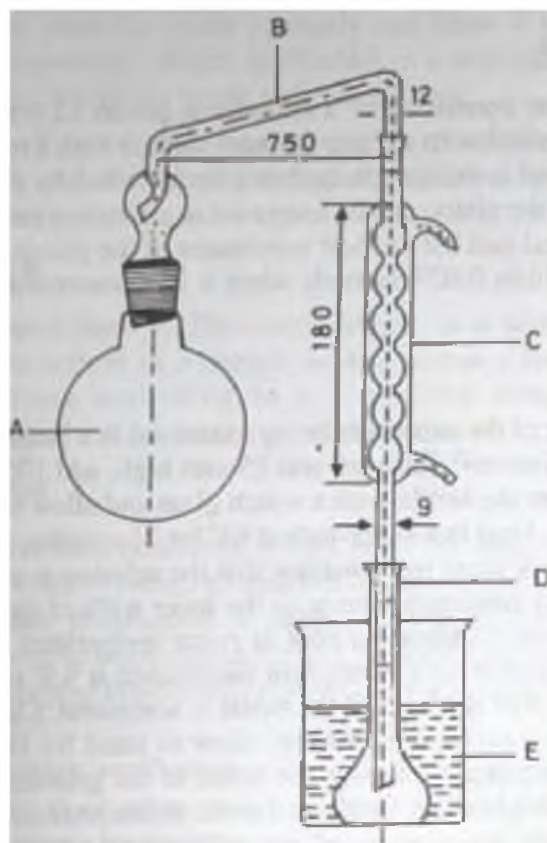


Fig.3. Apparatus for determination of ethanol by distillation method.



**Table 3.2**

Specific gravity at 25°C	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°C.

### **Method III C**

Transfer 25 ml of the preparation, accurately measured at 24.9°C to 25.1°C, 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...”.





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