

UINADS:UPI-2/2020/SJ/1.0

**PHARMACOPOEIAL MONOGRAPH  
OF  
AYUSH SAFŪF-i-JOSHĀNDA**

**THE UNANI PHARMACOPOEIA OF INDIA  
PART-II (FORMULATIONS)**



सत्यमेव जयते

**Government of India  
Ministry of AYUSH  
Pharmacopoeia Commission for Indian Medicine & Homoeopathy  
2020**

PDH.....

**UINADS:** UPI-2/2020/SJ/1.0

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Ministry of AYUSH, Government of India

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**FOREWORD**



**PREFACE**



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## **LEGAL NOTICES**

1. In India, there are laws dealing with drugs for which monographs with quality standards and certain other requirements are prescribed. This Monograph should be read subject to the restrictions imposed by these laws wherever they are applicable.
2. It is expedient that enquiry be made in each case in order to ensure that the provisions of any law are being complied with.
3. In general, the Drugs and Cosmetics Act, 1940; the Dangerous Drugs Act, 1930; the Poisons Act, 1919; Drugs and Magic Remedies (Objectionable Advertisement) Act, 1954; the Narcotic Drugs and Psychotropic Substances Act, 1985 and the Biodiversity Act, 2002; all as amended from time to time, alongwith the Rules framed thereunder, should be consulted to ensure that the provisions of such laws are being complied with.
4. The Class of Formulation and the Formulation itself published herein have been introduced as a *sui generis* provision in wake of outbreak of COVID-19 pandemic and shall not to be generalised or replicated in any other context.
5. Under the Drugs and Cosmetics Act, the Unani Pharmacopoeia of India, represented by its Parts and Volumes is the book of standards for substances included therein and such standards are official. If considered necessary, these standards can be amended and only the Pharmacopoeia Commission for Indian Medicine & Homoeopathy on behalf of Ministry of AYUSH, Government of India is authorised to issue such amendments. Whenever such amendments are issued, the specific Unani Pharmacopoeia of India intended thereby would be deemed to have been amended accordingly.



## GENERAL NOTICES

**Title:** The title of the document is “Pharmacopoeial Monograph of Ayush Safūf-i-Joshānda” with Unique Identification Number for AYUSH Drug Standards (UINADS):UPI-2/2020/SJ/1.0. Wherever the UINADS:UPI-2/2020/SJ/1.0 and/or its subsequent version(s) are referred, it stands for “Pharmacopoeial Monograph of Ayush Safūf-i-Joshānda” and for the Supplements or Amendments thereto.

**UINADS:** Unique Identification Number for AYUSH Drug Standards is the specific identity assigned to each Pharmacopoeial monograph or Formulary specification published by PCIM&H. In case of Pharmacopoeial monograph of Formulation, the first fragment of the UINADS is the acronym of the pharmacopoeia under concern i.e. Unani Pharmacopoeia of India (UPI) in given case. Second fragment separated by a hyphen (-) and denoted in Arabic numeral, specifies the part of Pharmacopoeia, where “1” stands for first part comprising of Single drugs and “2” for second part comprising of Formulations. Third fragment separated by a slash (/) and denoted in Arabic numeral, specifies the four-digit calendar year in Christian Era in which this solitary monograph is published for first time. Fourth fragment separated by a slash (/) denotes the acronym of the Class of Formulation i.e. Safūf-i-Joshānda (SJ) in given case. Fifth fragment separated by a slash (/) and denoted in Arabic numeral denotes the serial number assigned to the given monograph while last fragment separated by a period (.) and denoted in Arabic numeral denotes the version of the document under concern. With amendments as made time to time, the version number in the UINADS i.e. last Arabic numeral succeeding the period (.) shall go on increasing progressively.

**Name of the Formulation:** The name given on top of each monograph is as mentioned in the National Formulary of Unani Medicine (NFUM) and will be considered *Official*.

**Ingredients and Processes:** Formulations are to be prepared from individual ingredients that comply with the requirements for those individual ingredients for which monographs are provided in the volumes of Unani Pharmacopoeia of India (UPI), Part-I. Where Water is used as an ingredient, it should meet the requirements of *water* as specified in the appendix. In general, all the ingredients used are required to be free from insects, other foreign matter, from animal excreta, and to show no abnormal odour, colour, sliminess, mould or other evidence of deterioration.

Monograph for each Formulation includes its full composition together with directions for its preparation. Such composition and directions are intended for preparation of small quantities for short-term supply and use. When so prepared, no deviation from the stated composition and directions is permitted. However, if such a preparation is manufactured on a large scale with the intention of sale or distribution, deviations from the directions given are permitted, provided that the same ratio is maintained as stated in the monographs, with the ingredients complying with its compendial requirements, and also ensuring that the final product complies with all of the requirements stated in the Formulation Composition for the specific formulation.

If a preparation is intended to be stored over a period of time, deterioration due to microbial contamination may be inhibited by the addition to the formulation of a permitted preservative.

In such circumstances, the label should state the name of the preservative and the appropriate storage conditions.

**Monograph:** Each monograph begins with a Definition in an introductory paragraph followed by the Formulation Composition giving the scientific names of the drugs and respective form of the ingredient

intended to be entered to the formulation alongwith a brief account of the Method of Preparation. For drugs of plant origin, the part used has also been specified.

The form and quantity/proportion of each ingredient mentioned in the Formulation composition are as intended to be entered to the formulation after whatever processing intended. It is the onus of manufacturer to ensure addition of exact quantity/proportion of each ingredient to the Formulation and shall not be mistaken with the quantity/proportion of the raw material as such.

The requirements given in the monographs are not framed to provide against all impurities, contaminants or adulterants; they provide appropriate limits only for possible impurities that may be permitted to a certain extent. Material found to contain an impurity, contaminant or adulterant which is not detected by means of the prescribed tests in the Appendix-2 to 4 are also to be considered as impurity, should rational consideration require its absence.

**Standards:** For statutory purposes, unless otherwise specified, the following shall be considered *Official Standards*: Definition, Formulation Composition, Identification, Physico-chemical parameters, Assay and Other requirements. Under Formulation Composition, each ingredient for which a monograph has been given in the UPI, the pharmacopoeial claim is indicated by the letters UPI; where the ingredient has currently no monograph or it is under process, the letter UPI is given within brackets.

**Added Substances:** A Formulation contains no added substances/excipients, except when specifically permitted in the individual monograph. Unless otherwise specified in the individual monograph, or elsewhere in the General Notices, such added substances/excipients shall be from the approved list of Drugs and Cosmetics Rules, under Rule 169 to a formulation to enhance its stability, usefulness, elegance, or to facilitate its preparation. Such added substances shall comply with the quality indicated for it, shall be harmless in the amounts used, shall not exceed the minimum quantity required to provide their intended effect, shall not impair the therapeutic efficacy or the bioavailability and safety of the preparation. Particular care should be taken to ensure that such substances are free from harmful organisms. Though the manufacturer of a formulation is given the freedom to use an added substance, the manufacturer must guarantee the innocuousness of the added substance. The manufacturer shall also be responsible to explain to the appropriate authority, if needed, regarding the purpose of the added substance(s).

**Description:** Statement given under this title is not to be interpreted in a strict sense although it may help in the evaluation of an article. However, substantial departure from the requirement will not be acceptable.

**Capital Letters in the Text:** The names of the Pharmacopoeial substances, preparations and other materials in the text are printed in capital initial letters and these infer that materials of Pharmacopoeial quality have been used.

**Italics:** Italic types are used for Scientific names of the plant drugs and microorganisms, and for some sub-headings and certain notations of the chemical names. Italic types have also been used for words which refer to solvent system in TLC procedure, reagents and substances, processes covered under Appendices. Chemicals and Reagents and Substances of Processes in Appendices have also been printed in Italics.

**Odour and Taste:** Wherever a specific odour has been observed, it has been mentioned as characteristic for that Formulation, but the description as 'odourless' or 'no odour' has generally been avoided in the Description where a substance has no odour. Where an 'odour' is said to be present, it is examined by smelling the drug directly after opening the container. If an odour is discernible, the contents are rapidly

transferred to an open vessel and reexamined after 15 minutes. If odour persists to be discernible, the sample complies with the description for 'odour', as a characteristic for that Formulation.

The taste of a drug is examined by taking a small quantity of drug by the tip of a moist glass rod and allowing it to remain on the tongue. ***This does not apply in the case of poisonous ingredients.***

**Powders:** Ingredients added to a formulation are often required to be comminuted to various sizes ranging from very coarse to very fine, depending on their use in a formulation. Where they are added to processed formulations, the size of sieve restricting the particle size is given in the monograph, but does not constitute an analytical standard. But where formulations are themselves powders, or where extracts are prepared either as solids (*Satt*) or liquids/*Joshānda*, particle size is an analytical standard and limits are recommended in the monographs, as follows:

**Safūf-i-Joshānda:** For preparing the *Safūf* for *Joshānda*, the drugs (roots, stems, seeds, etc.,) are crushed to small pieces in an iron mortar and softened in order to obtain the maximum efficacy. The standard is as follows: 'All particles shall pass through 710 µm IS Sieve (sieve number 22), and not more than 10 per cent through 355 µm IS Sieve (sieve number 44). The product will be in form of coarse powder from which extemporaneous preparations of *Joshānda* (decoction) by patients themselves can be recommended.

The particle sizes are given in terms of sieve sizes using the latest revision of the Bureau of Indian Standards (BIS) sieve sizes, and for the users' convenience, the equivalents or nearest equivalent number of the earlier BIS have also been given in the relevant Appendix.

**Weights and Measures:** The metric system of weights and measures is employed. Weights are given in multiples or fractions of a gram (g) or of a milligram (mg). Fluid measures are given in multiples of fraction of millilitre (ml). The amount stated is approximate but the quantity actually used must be accurately weighed and must not deviate by more than 10 per cent from the one stated.

When the term 'drop' is used, measurement is to be made by means of a tube which delivers 20 drops per gram of distilled water at 15°.

**Identity, Purity and Strength:** Under the heading 'Identification' tests are provided as an aid to identification and are described in the respective monographs. Microscopical characters are prescribed for the identification of individual ingredients where these do not exceed ten in number and are added '*in situ*' to the Formulation. Appendix 2 gives detailed procedure.

Herbal drugs/drugs of plant origin used in Formulation(s), should be duly identified and authenticated and be free from insects, pests, and other animal matter including animal excreta, and be within the permitted and specified limits for arsenic and heavy metals, microbial load, pesticides, aflatoxins and show no abnormal odour, colour, sliminess, mould or any sign of deterioration. Where any ingredient is to be subjected to a *Tadbīr-i-Adwiyah*, this shall be carried out as specified in the monograph or in the text referred to therein.

Quantitative tests namely loss on drying, total ash, acid-insoluble ash, alcohol-soluble extractive, water-soluble extractive, volatile oil content, pH and assays are the parameters upon which the standards of Pharmacopoeia depend. Methods of determination for these tests are given in Appendices, with a suitable reference in the monograph to the specific Appendix.

An analyst is not precluded from employing an alternate method in any instance if one is satisfied that the method, which one uses, will give the same result as the Pharmacopoeial method described under assay. However, in the event of doubt or dispute the methods of analysis of the Pharmacopoeia are alone

authoritative. Unless otherwise prescribed, the assays and tests are carried out at a temperature between 20° and 30°.

In the performance of an assay or any test procedure, *not less than* the specified number of dosage units or quantities should be taken for analysis. Proportionately larger or smaller quantities than the specified weights and volumes may be taken for substances under assay or test substances, Reference Standards or Standard Preparations, provided the measurement is made with at least equivalent accuracy and provided that any subsequent steps, such as dilutions, are adjusted accordingly to yield concentrations equivalent to those specified and are made in such a manner as to provide at least equivalent accuracy.

**Limits for Heavy metals, Contaminants, Microbial load, Pesticide residues and Aflatoxins:** Formulation under concern is required to comply with the limits for heavy metals, microbial load, pesticide residues and aflatoxins prescribed in the limits given in the respective Appendices. The methods for determination of these parameters are given in the Appendices.

The limit tests for heavy metals, microbial load and aflatoxins are exempted for *Kushta*, if packed and sold as such.

**Thin Layer Chromatography (TLC):** Under this title, wherever given, the  $R_f$  values given in the monographs are not absolute but only indicative. The analyst may use any other solvent system and detecting reagent to establish the identity of any particular chemical constituent reported to be present in the formulation. However, in case of dispute the Pharmacopoeial method would prevail. Unless specified in the individual monograph, all TLC have been carried out on pre-coated Silica gel 60F<sub>254</sub> of 0.2 mm thickness aluminium plates.

**Reference Standards:** Reference substance and standard preparation are authentic substances that have been verified for their suitability for use as standards for comparison in some assays, tests and TLC of the UPI.

**Constant Weight:** The term “constant weight” when it refers to drying or ignition means that two consecutive weighings do not differ by more than 1.0 mg per gram of the substance taken for the determination, the second weighing following an additional hour of drying or further ignition.

**Percentage of Solutions:** In defining standards, the expression per cent (%), is used, according to circumstances, with one of the four meanings given below:

Per cent w/w (percentage weight in weight) expresses the number of grams of active substance in 100 grams of product.

Per cent w/v (percentage weight in volume) expresses the number of grams of active substance in 100 millilitres of product.

Per cent v/v (percentage volume in volume) expresses the number of millilitres of active substance in 100 millilitres of product.

Per cent v/w (percentage volume in weight) expresses the number of millilitres of active substance in 100 grams of product.

**Percentage of Alcohol:** All statements of percentage of alcohol C<sub>2</sub>H<sub>5</sub>OH refer to percentage by volumes at 15.56°.

**Temperature:** Unless otherwise specified, all temperatures refer to centigrade (Celsius) thermometric scale and all measurements are made at 25°.

**Solutions:** Unless otherwise specified, all solutions are prepared with *Purified Water*.

**Reagents and Solutions:** Reagents required for the assay and tests of the Pharmacopoeia are defined in the Appendix showing the nature, degree of the purity and strength of solutions to be made from them.

**Filtration:** Where it is directed to filter, without further qualification, it is intended that the liquid be filtered through suitable filter paper or equivalent device until the filtrate is clear.

**Therapeutic use(s):** Therapeutic uses of the Formulation mentioned in this monograph are as given in the National Formulary of Unani Medicine.

**Dose(s):** The doses mentioned in monograph are in the metric system. Doses mentioned in the UPI are intended merely for general guidance and represent, unless otherwise stated, the average range of quantities per dose which is generally regarded suitable by clinicians for adults only when administered orally. They are not to be regarded as binding upon the prescribers.

The medical practitioner will exercise his own judgment and act on his own responsibility in respect of the amount of the formulation he/she may prescribe or administer or on the frequency of its administration. If it is usual to administer a medicine by a method other than by mouth, the single dose suitable for that method of administration is mentioned.

**Storage:** Statement under the heading 'Storage' constitutes non-mandatory advice. The substances and preparations are to be stored under conditions that prevent contamination and, as far as possible, deterioration. The container and its closure must not interact physically or chemically with the substance which it holds so as to alter the strength, quality or purity of the substance. If interaction is unavoidable, the alteration must not be so significant as to bring the substance below the prescribed requirements. Precautions that should be taken in relation to the effects of the atmosphere, moisture, heat and light are indicated, where appropriate, in the individual monographs.

Specific directions are given in the monograph with respect to the temperatures at which Pharmacopoeial articles should be stored, where it is considered that storage at a lower or higher temperature may produce undesirable results. The conditions are defined by the following terms.

*Cold:* Any temperature not exceeding 8° and usually between 2° and 8°. A refrigerator provides a cold place in which the temperature is maintained thermostatically between 2° and 8°.

*Cool:* Any temperature between 8° and 25°. An article for which storage in a cool place is directed may, alternately, be stored in a refrigerator, unless otherwise specified in the individual monograph.

*Room temperature:* The temperature prevailing in a working area

*Warm:* Any temperature between 30° and 40°

*Excessive heat:* Any temperature above 40°

*Protection from freezing:* Where, in addition to the risk of breaking of the container, freezing results in loss of strength or potency or in destructive alteration of the characteristics of an article, the label on the container bears an appropriate instruction to protect from freezing.

*Storage under non-specific conditions:* Where no specific storage directions or limitations are given in the individual monograph, it is to be understood that the storage conditions include protection from moisture, freezing and excessive heat.

**Containers:** The container is the device that holds the article. The immediate container is that which is in direct contact with the article at all times. The closure is a part of the container.

The container is designed so that the contents may be taken out for the intended purpose in a convenient manner.

It provides the required degree of protection to the contents from environmental hazards.

The container should not interact physically or chemically with the article placed in it so as to alter the strength, quality or purity of the article beyond the official requirements.

Prior to its being filled, the container should be clean. Special precautions and cleaning procedures may be necessary to ensure that each container is clean and that extraneous matter is not introduced into or onto the container.

*Light-resistant Container:* A light resistant container protects the contents from the effects of actinic light by virtue of the specific properties of the material of which it is made. Alternatively, a clear and colourless or a translucent container may be made light-resistant by means of an opaque (light-resistant) covering and/or stored in a dark place: in such cases, the label on the container should bear a statement that an opaque covering or storage in dark place is needed until the contents have been used up.

*Well-closed Container:* A well-closed container protects the contents from extraneous contamination and from loss of contents under normal conditions of handling, shipment, storage and distribution.

*Tightly-closed Container:* A tightly-closed container protects the contents from contamination by extraneous liquids solids or vapours, and from loss or deterioration of contents from effervescence, deliquescence or evaporation under normal conditions of handling, shipment, storage and distribution.

*Single Unit Container:* A single unit container is one that is designed to hold a quantity of the drug product intended for administration as a single finished device intended for use promptly after the container is opened. The immediate container and/or outer container or protective packaging is so designed as to reveal evidence of tampering, if any.

*Multiple Unit Container:* A multiple unit container is a container that permits withdrawals of successive portions of the contents without changing the strength, quality or purity of the remaining portion.

*Tamper-evident Container:* A tamper-evident container is fitted with a device or mechanism that reveals irreversibly whether the container has been opened.

**Labelling:** In general, the labelling of drugs and pharmaceuticals is governed by the Drugs and Cosmetics Act, 1940 and Rules thereunder.

## INDO-ROMANIC EQUIVALENTS FOR ARABIC AND PERSIAN ALPHABETS

The following Arabic letters have been transliterated with diacritical marks as mentioned against each:

ا	A	a/a	ص	Ṣ	ṣ
ب	B	b	ض	Ḍ	ḍ
ت	T	t	ط	Ṭ	ṭ
ث	TH	th	ظ	Ẓ	ẓ
ج	J	j	غ	GH	gh
ح	H	ḥ	ف	F	f
خ	KH	kh	ق	Q	q
د	D	d	ك	K	k
ذ	DH	dh	ل	L	l
ر	R	r	م	M	m
ز	Z	z	ن	N	n
س	S	s	ه	H	h
ش	SH	sh	ی	Y	y

The following Persian letters have been transliterated with diacritical marks as expressed against each:

پ	P	p	پھ	<u>PH</u>	<u>ph</u>
ٹ	<u>T</u>	<u>t</u>	تھ	TH	th
چ	CH	ch	چھ	<u>TH</u>	<u>th</u>
ڈ	<u>D</u>	<u>d</u>	ڈھ	<u>CH</u>	<u>ch</u>
ڑ	<u>R</u>	<u>r</u>	دھ	<u>DH</u>	<u>dh</u>
گ	G	g	گھ	<u>KH</u>	<u>kh</u>
ں	<u>N</u>	<u>n</u>	گھ	<u>GH</u>	<u>gh</u>
بھ	<u>BH</u>	<u>bh</u>			

- اُ / ء has been transliterated with elevated coma (ˆ) if used in the mid or end of word followed by relevant vowel and this elevated coma is not expressed at the beginning and only related vowel has been used directly.
- Letter ع is transliterated as elevated inverted coma (ˆ).
- Letter و as Arabic letter is transliterated as wand as Persian/Urdu letter is transliterated as v.
- ة And ه are not expressed in both pause and construct forms.
- Article ال is transliterated as al- ('l- in construct form) whether followed by a moon or a sun letter.
- و as a Persian/Urdu conjunction is transliterated as (-o-) and as an Arabic conjunction is transliterated as wa.
- Short vowel (ـ) in Persian/Urdu passive or in conjunction form is transliterated as (-i-).
- Doubled alphabet has been expressed in the following form:

وُ = uww

ئ = iyy

- Short and long vowels and diphthongs are used in the following form:

Short vowels

اَ	A	a
اِ	I	i
اُ	U	u

Long vowels

اَ	A	a/a
آ	Ā	ā/ā
و	Ū	ū
ى	Ī	ī

Diphthongs

اَـو	AW	aw
اَـى	AY	ay

## ABBREVIATIONS FOR TECHNICAL TERMS

°C	-	-	°
Analytical reagent	-	-	AR
concentrated	-	-	con.
gram(s)	-	-	g
hour(s)	-	-	h
kilogram(s)	-	-	kg
Safūf-i-Joshānda			Sa. Jo.
litre(s)	-	-	l
micron	-	-	μ
milligram(s)	-	-	mg
millilitre(s)	-	-	ml
Minute(s)	-	-	min
ortho	-	-	<i>o</i>
para	-	-	<i>p</i>
parts per billion	-	-	ppb
parts per million	-	-	ppm
quantity sufficient	-	-	Q.S.
Reference standard	-	-	RS
Second(s)	-	-	sec
volume	-	-	vol.
volume in volume	-	-	v/v
weight	-	-	wt.
weight in volume	-	-	w/v
weight in weight	-	-	w/w

## ABBREVIATIONS FOR PLANT PARTS

Fruit	Fr.
Leaf	Lf.
Rhizome	Rz.
Stem Bark	St. Bk.

**CONTRIBUTING LABORATORIES**

<b>Name of Laboratory</b>	<b>Personnel involved</b>
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Efforts of scientific staff of the Commission Dr. G. V. R. Joseph, Joint Director I/c, Dr. S. C. Verma, Principal Scientific Officer (Phyto-chem.), Dr. Jayanthi A., Principal Scientific Officer (Pharmacognosy), Dr. Vijay Gupta, Principal Scientific Officer (Ayurveda), Dr. V. Vijayakumar, Scientific Officer (Siddha), Dr. Nikhil M. Jirankalgikar, Scientific Officer (Ayurveda) and Dr. Farah Naaz, Scientific Officer (Unani) are appreciated.

Involvement of Dr. Anupam Maurya, Scientific Officer (Phyto-chem.), Dr. (Ms.) Nitin Rai, Scientific Officer (Pharmacognosy), Dr. Sweta Mohan, Scientific Officer (Inorganic chem.) and Sh. Ashish Kumar, Pharmacopoeial Associate (Pub.) is also placed on record.

In last, thanks are due to all those who have directly or indirectly contributed in bringing out this publication.

Sd/-  
Dr. D. C. Katoch  
Director I/c, PCIM&H

## SAFŪF-i-JOSHĀNDA

### Definition

Certain drugs or combination of drugs are made into coarse powder (Niyam kuwftah) and kept for preparation of Safūf-i-Joshānda.

### Method of preparation

Drugs are cleaned and dried. They are coarsely powdered to completely pass through 710 µm IS sieve (sieve number 22) and not more than 10 per cent pass through 355 µm IS sieve (sieve number 44), weighed as per formula, and then mixed well.

### Characteristics

The characteristic features are dried and coarsely powdered.

### Directions for Preparation of Ayush Joshānda as an Instant Hot Infusion:

Add 3 g of Ayush Safūf-i-Joshānda to 150 ml (approx. 1 tea-cup) of boiling water, mix well and allow to rest for 5 min. Strain through muslin cloth / tea strainer.

**Note:** The specific directions for preparation of Ayush Joshānda are exclusive to the given formulation and shall not be applicable to other classical *Joshānda* formulations.

### Mode of administration:

Ghur (jaggery) / Mawiz Munaqqa (black raisins) and/or Āb-i-Liyuwn (lemon juice) may also be added as per taste, while consuming.

### Storage

It should be stored in suitable air-tight container protected from light and moisture.



**AYUSH SAFŪF-i-JOSHĀNDA**  
(NFUM: UINADS: NFUM/2020/SJ/1.0 )

**Definition:**

Ayush Safūf-i-Joshānda is a powder preparation made with the ingredients in the formulation composition given below.

**Formulation composition:**

1. Tulsi (Rehan UPI)	<i>Ocimum tenuiflorum</i>	Lf.	Kv.Cū.	4 parts
2. Dalchini (Darchini UPI)	<i>Cinnamomum verum</i>	St. Bk	Kv.Cū.	2 parts
3. Sunthi (Zanjabeel UPI)	<i>Zingiber officinale</i>	Rz.	Kv.Cū.	2 parts
4. Krishna Marich (Filfil Siyah UPI)	<i>Piper nigrum</i>	Fr.	Kv.Cū.	1 part

**Method of Preparation:**

- a. Take all the ingredients of pharmacopoeial quality.
- b. Clean, wash and dry, as suitable, all the ingredients separately, powder and pass through 710 µm IS Sieve (sieve number 22).
- c. Weigh each powdered ingredient separately and mix them together thoroughly to obtain a homogenous blend.
- d. Store in air-tight containers to protect from light and moisture.

**Description:**

Greyish brown coarse powder with pleasant aromatic odour, pungent and sweet taste; the powder completely passes through 710 µm IS Sieve (sieve number 22) and not more than 10 per cent passes through 355 µm IS Sieve (sieve number 44)

**Identification:**

*Microscopy* (Appendix 2):

Take about 2 g of coarse powder of formulation and grind it thoroughly using mortar and pestle; mount a few mg in *glycerin*; take a few mg of ground powder on a slide, warm it with *chloral hydrate* and mount in *glycerin*; take another few mg and treat it with *phloroglucinol* and con. *hydrochloric acid* and mount it in *pure glycerin*; treat another few mg of powder with *Sudan IV* and mount it; take a few mg on a slide and add *iodine in potassium iodide solution* and mount in *glycerin*. Observe following characters in various mounts:

Fragments of upper and lower epidermis in surface view with diacytic stomata and trichomes, entire or fragments of uniseriate multicellular covering trichomes, sessile, multicellular glandular trichomes and stalked glandular trichomes with unicellular head, fragments of epidermis with palisade cells (**Rehan**); entire or fragments of thick-walled narrow-lumened phloem fibres with pointed ends, stone cells with wall thickening on three sides, groups of parenchyma associated with or without oil cell and pericyclic fibres, parenchyma cells with acicular crystals of calcium oxalate and compound starch grains with 2-4 components (**Darchini**); cork cells in cross-sectional and surface view, groups of parenchyma cells with starch grains and associated oil cells, fragments of septate fibres associated with reticulate vessel fragments, simple round to oblong and elliptical starch grains with eccentric hilum (**Zanjabeel**); groups of beak-shaped stone cells, groups of perisperm cells with minute starch grains, fragments of pigment layer associated with stone cells, perisperm cells associated with oil cells (**Filfil Siyah**)

*Thin-layer Chromatography:*

Carry out *Thin-layer chromatography* on a pre-coated Silica gel 60F<sub>254</sub>TLC plate (Appendix 3.2). *Test Solution:* Extract 2 g of formulation by refluxing with 10 ml of *ethanol* on water-bath for 30 min. Filter and concentrate the extract to 5 ml. Procedure: Apply 7 µl of the extract as a 10-mm band at a height of 10 mm from the base of a 10×10 cm TLC plate and develop upto 8 cm from the base of the plate using the mobile phase *toluene: ethyl acetate: formic acid* (7: 3: 0.5). Air dry the plate and examine under UV 254 nm and UV 366 nm. Spray the plate with *vanillin - sulphuric acid reagent* and heat at temperature of 105-110° till the colour of the spots/bands appear without charring. TLC profile of the test solution should match with the TLC profile of Ayush Safūf-i-Joshānda RS (obtained in a similar way) with respect to the position and fluorescence/colour of the bands (Fig. 1-3).

**Physico-chemical parameters:**

*Loss on drying at 105°:* Not more than 10 per cent (Appendix 3.1.1); *Total ash:* Not more than 9 per cent (Appendix 3.1.2); *Acid-insoluble ash:* Not more than 2 per cent (Appendix 3.1.3); *Alcohol-soluble extractive:* Not less than 6 per cent (Appendix 3.1.4);

*Water-soluble extractive*: Not less than 12 per cent (Appendix 3.1.5), *Volatile oil*: Not less than 0.8 per cent (Appendix 3.1.6); *pH (10 % aqueous suspension)*: 5.5 - 6.8 (Appendix 3.1.7).

**Other requirements:**

*Microbial limits*: Complies with Appendix 4.1; *Aflatoxins*: Complies with Appendix 4.2; *Pesticide residues*: Complies with Appendix 4.3; *Heavy metals limits*: Complies with Appendix 4.4.

**Storage:**

Store in a cool place in tightly-closed suitable container/package, protected from light and moisture.

**Important Therapeutic uses:**

Su‘āl, Nazla-o-Zukām, ‘Usr al-Tanaffus, Su‘al-Haḍm, Butlan al-Dhawq / Fasad al-Dhawq, Şihḥat Afzā

**Dose:**

3 g once or twice a day in form of instant hot infusion as specified

**Directions for Preparation:**

Add 3 g of Ayush Safūf-i-Joshānda to 150 ml (approx.1 tea-cup) of boiling water, mix well and allow to rest for 5 min. Strain through muslin cloth / tea strainer.

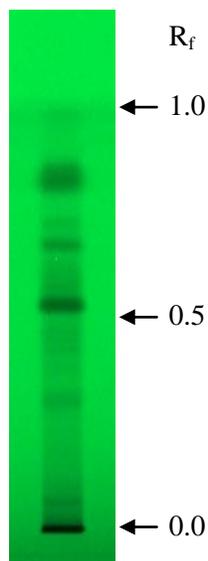
**Note:** The specific directions for preparation of Ayush Joshānda are exclusive to the given formulation and shall not be applicable to other classical *Joshānda* formulations.

**Mode of administration:**

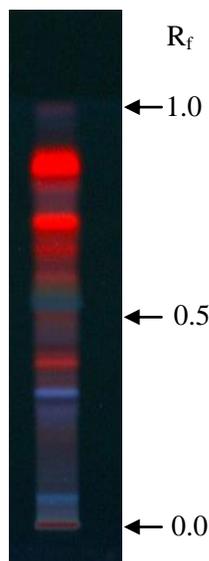
Consume while luke warm. Ghur (jaggery) / Mawiz Munaqqa (black raisins) and/or Āb-i-Liymwn (lemon juice) may also be added as per taste, while consuming.

**Precaution:**

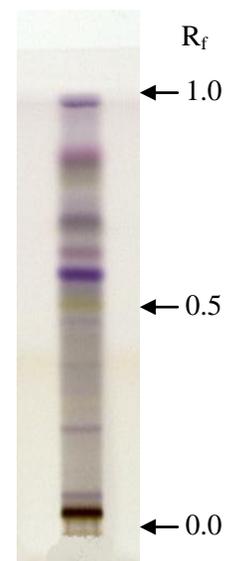
Pregnant women should take the formulation under medical supervision.



**Fig. 1:**TLC profile of Ayush Safūf-i-Joshānda RS under UV 254 nm



**Fig. 2:**TLC profile of Ayush Safūf-i-Joshānda RS under UV 366 nm



**Fig. 3:**TLC profile of Ayush Safūf-i-Joshānda RS after spraying with *vanillin - sulphuric acid reagent*

## APPENDIX - 1

### APPARATUS FOR TESTS AND ASSAYS

#### 1.1. Sieves

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

Sieves conform to the following specifications:

IS 460 (Pt I) 1985 (Reaffirmed 1998)	IS 460-1978
mm	--
4.0	4
2.8	6
2.0	8
1.7	10
1.4	12
1.0	16
µm	--
710	22
600	25
500	30
425	36
355	44
250	60
180	85
150	100
125	120
106	150
90	170
75	200
63	240
53	300
45	350

#### Designation

Test sieves of metal wire cloth are designated by the nominal size of aperture of the wire cloth, followed by the inscription 'IS Sieve'.

Examples:

- a. 5.60 mm IS Sieve
- b. 425 µm IS Sieve

Nominal aperture sizes of 1 mm and above, as well as their associated tolerances and wire diameters, are expressed in millimeters (mm) and for aperture sizes smaller than 1 mm, these are expressed in micrometers (µm).

#### 1.2. Thermometers

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

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

#### 1.3. Ultraviolet Lamp (For general purposes and for chromatography work)

An instrument consisting of mercury vapour lamp and a filter which gives an emission band with maximum intensity at about 254 nm (near UV rays) and 366 nm (far UV rays) is used. To ensure that the required emission is being given by the lamp, carry out the following test periodically. Apply to a plate coated with *silica gel G*, 5 µl of a 0.04 per cent w/v solution of *sodium salicylate* in *ethanol* (95 per cent) for lamps of maximum output at 254 nm and 5 µl of a 0.2 per cent w/v solution in *ethanol* (95 per cent) for lamps of maximum output at 365 nm. Examine the spot in a position normal to the radiation. The distance between the lamp and the plate under examination used in a pharmacopoeial test should not exceed the distance used to carry out the above test.

#### 1.4. Volumetric Glassware

Volumetric apparatus is normally calibrated at 27°. However, the temperature generally specified for

measurements of volume in the analytical operations of the pharmacopoeia, unless otherwise stated, is 25°. The discrepancy is inconsequential as long as the room temperature in the laboratory is reasonably constant and is around 27°.

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

### 1.5. Nessler Cylinders

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

### 1.6. Weights and Balances

Pharmacopoeial tests and assays require the use of analytical balances that vary in capacity, sensitivity and reproducibility. The accuracy needed for a weighing should dictate the type of balance. Where substances are to

be “accurately weighed”, the weighing is to be performed so as to limit the error to not more than 0.1 per cent.

For example, a quantity of 50 mg is to be weighed to the nearest 0.05 mg; a quantity of 0.1 g is to be weighed to the nearest 0.1 mg; and quantity of 10 g is to be weighed to the nearest 10 mg. A balance should be chosen such that the value of three times the standard deviation of the reproducibility of the balance, divided by the amount to be weighed, does not exceed 0.001.

### 1.7. Muslin Cloth

*Muslin cloth* is a cotton fabric of plain weave where warp is 22 per cm  $\pm 1$  and weft is 18  $\pm 1$  per centimeter.

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

## APPENDIX-2

### MICROSCOPIC IDENTIFICATION OF BOTANICAL SUBSTANCES

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

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

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

#### Stains and Reagents for Microchemical Reactions:

If for some reason the reagents suggested for preparation of material for microscopy in the monographs itself, does not yield expected result/or some reagents suggested here are not available for any sample under test, the relevant reagent given here may be substituted for better results.

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

#### Glycerin, Pure

Glycerin Purified; A clear viscous liquid, not more than 10 Hazen units in colour, miscible with water and with industrial methylated spirit forming clear and colourless solution; Minimum assay by GC 98.0 %; Refractive index 1.471-1.474; weight per ml (at 20°)1.255-1.260; A 20 per cent solution is neutral to litmus

**Glycerin:** Dilute the Glycerin purified as required with one or two volumes of distilled water; Used as a general mountant unless specified otherwise

**Iodine in Potassium iodide solution:** Dissolve 1 g of *potassium iodide* in 200 ml of distilled water, add 2 g of *iodine* to the solution and dissolve it; stains lignified walls yellow and cellulosic walls blue

**Phloroglucinol solution:** 1 g of phloroglucinol AR dissolved in 100 ml of 90 per cent *ethyl alcohol*; mount debris in a few drops, allow to react for a minute, draw off excess of reagent with a filter paper strip, and add a drop of con. *hydrochloric acid* to the slide; lignified tissues acquire a deep purplish red colour; very effective on water washed material but not in chloral hydrate washed debris, for which Alcoholic solution of safranin is more effective. Unless specified otherwise, the term phloroglucinol mentioned in preparation of mounts stands for *Phloroglucinol solution*.

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

**Sudan IV:** Dissolve 0.5 g of Sudan IV AR in 100 ml of 70 per cent *ethyl alcohol*. Wash with 50% *ethyl alcohol* and mount in glycerin; suberized walls of cork cells and fatty material in cells are stained bright red.



**Fig. 1:** Apparatus for volatile oil determination

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 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.

**3.1.7 Determination of pH Value**

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. For the purpose of pharmacopoeia *pH* is defined as the value given by a suitable, properly standardized, *pH* meter capable of reproducing *pH* values to 0.05 *pH* unit using an indicator electrode sensitive to hydrogen ion activity, the glass electrode and a suitable reference electrode. The instrument should be capable of sensing the potential across the electrode pair and for *pH* standardization purposes, applying an adjustable potential to the circuit by manipulation of “standardization,” “zero,” “asymmetry,” or “calibration” control, and should be able to control the change in millivolts per unit change in *pH* reading through a “temperature” and/or “slope” control. Measurements are made at  $25 \pm 2^\circ$ , unless otherwise specified.

To standardize the *pH* meter, select two Buffer Solutions whose difference in *pH* does not exceed 4 units such that the expected *pH* of the material under test falls between them. Commercially available buffer solutions for *pH* meter standardization, having traceability to the National Standards can be used. Take one of the Buffer Solutions in a suitable beaker for Standardization at the temperature at which the test material is to be measured. Set the “temperature” control at the temperature of the solution, and adjust the calibration control to make the observed *pH* value identical with that of the declared *pH*. Rinse the electrodes and beaker with several portions of the second Buffer Solution for Standardization, then fill the beaker with it, at the same temperature as the material to be measured. The *pH* of the second buffer

solution is within  $\pm 0.07$  *pH* unit of the declared value. If a larger deviation is noted, examine the electrodes and if they are faulty, replace them. Repeat the standardization until both Buffer Solutions for Standardization give observed *pH* values within 0.05 *pH* unit of the declared value without further adjustment of the controls. When the system is functioning satisfactorily, rinse the electrodes and the beaker several times with few portions of the test material, take the test material in the beaker, and read the *pH* value. Use *carbon dioxide free water* for solution or dilution of test material in *pH* determinations. In all *pH* measurements, allow sufficient time for stabilization. Unless otherwise specified in the monograph prepare 5 per cent w/v of the sample. Filter if it is not soluble completely and use the filtrate to measure the *pH*.

**3.2 Thin-Layer Chromatography (TLC):**

Thin-layer chromatography is a technique in which a solute undergoes distribution between two phases, stationary phase and a mobile phase. The stationary phase acts as an adsorbent in a relatively thin, uniform layer of dry finely powdered material applied to a glass, plastic or metal sheet. 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 stationary phase, its preparation and its use with different solvents.

Identification can be effected by comparison of spots of identical  $R_f$  value and colour in unknown sample to a reference sample chromatographed on the same plate. A visual comparison of the size and intensity of the spots usually serves for semi-quantitative estimation.

**Apparatus:**

- (a) Flat uniformly thick glass plates of appropriate dimensions coated with a layer of adsorbent that allow the application of the necessary number of the solutions being examined along with reference solutions. The plates are prepared as described below; alternatively, commercially prepared plates may be used.
- (b) An aligning tray or a flat surface on which the plates can be aligned and rested when the coating substance is applied.
- (c) The coating substance consists of finely divided

adsorbent materials, normally between 5 to 40  $\mu\text{m}$  in diameter is suitable for chromatography. It can be applied directly to the plate or can be bonded to the plate by means of plaster of paris (hydrated *calcium sulphate*) or with any other suitable binder. The adsorbent may contain fluorescing material to help in visualising spots that absorb ultra-violet light.

- (d) A spreader which, when moved over the glass plate, will apply a uniform layer of adsorbent of desired thickness over the entire surface of the plate.
- (e) A storage rack to support the plates during drying and transportation.
- (f) A developing chamber that can accommodate one or more plates and can be properly closed and sealed. The chamber is fitted with a plate support rack that supports the plates, back to back, with lid of the chamber in place.
- (g) Graduated micro-pipettes capable of delivering microlitre quantities say 10  $\mu\text{l}$  and less.
- (h) A reagent sprayer that will emit a fine spray and will not itself be attacked by the reagent.
- (i) An ultra-violet light, suitable for observation at short (254 nm) and long (366 nm) ultra-violet wavelengths.

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

**Method:**

Unless unsaturated conditions are prescribed, prepare the tank by lining the walls with sheets of filter paper;

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

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

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

**Visualisation:**

The phrases *ultra-violet light* (254 nm) and *ultra-violet light* (366 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.

## APPENDIX-4

### LIMIT TESTS

#### 4.1. Microbial Limit Tests

**Table-1: Microbial Contamination Limits**

Sl. No.	Parameters	Permissible limits for herbal extracts and powders	Permissible limits for plant materials which will be treated before use
1	<i>Staphylococcus aureus</i> /g	Absent	-
2	<i>Salmonella</i> sp./g	Absent	Absent
3	<i>Pseudomonas aeruginosa</i> /g	Absent	-
4	<i>Escherichia coli</i>	Absent	10
5	Total microbial plate count (TPC)	10 <sup>5</sup> /g*	10 <sup>7</sup>
6	Total Yeast & Mould	10 <sup>3</sup> /g	10 <sup>5</sup>

\*For topical use, the limits shall be 10<sup>7</sup>/g

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

**Preliminary Testing:** The methods given herein are invalid unless it is demonstrated that the test specimens (extracts) to which they are applied do not, of themselves, inhibit the multiplication under the test conditions of microorganisms that can be present. Therefore, prior to doing the tests, inoculate diluted extracts being examined with separate viable cultures of *Escherichia coli*, *Salmonella* species, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. This is done by adding 1 ml of 24 h broth culture containing not less than 1000 microorganisms to the first dilution (in buffer solution pH 7.2, fluid soyabean-casein digest medium or fluid lactose medium) of the test material and following the test procedure. If the organisms fail to grow in the relevant medium the procedure should be modified by (a) increasing the volume of diluents with the quantity of test material remaining the same, or (b) incorporating a sufficient quantity of a suitable inactivating agent in the diluents, or (c) combining the afore mentioned modifications so as to permit growth of the organisms in the media. If inhibitory substances are present in the extracts, 0.5 per cent of soya lecithin

and 4 per cent of polysorbate 20 may be added to the culture medium.

Alternatively, repeat the test as described in the previous paragraph, using fluid casein digest-soya lecithin-polysorbate 20 medium, to demonstrate neutralization of preservatives or other antimicrobial agents in the test material. Where inhibitory substances are contained in the extracts and the latter is soluble, the membrane filtration method described under total aerobic microbial count may be used.

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

#### **Media**

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

Where agar is specified in a formula, use agar that has a moisture content of not more than 15 per cent. Where *water* is called for in a formula, use purified *water*. Unless otherwise indicated, the media should be sterilized by heating in an autoclave (15 psi) at 121° for 15 min. In preparing media by the formulas given below, dissolve the soluble solids in the *water*, using heat if necessary, to effect complete solution, add solutions of 0.1 N *hydrochloric acid* or 0.1 N *sodium hydroxide* in quantities sufficient to yield the required pH in the medium when it is ready for use. Determine the pH at 25° ± 2°.

**Baird-Parker Agar Medium**

Pancreatic digest of casein	10.0 g
Beef extract	5.0 g
Yeast extract	1.0 g
Lithium chloride	5.0 g
Agar	20.0 g
Glycerin	12.0 g
Sodium pyruvate	10.0 g
Water to	1000 ml

Heat with frequent agitation and boil for 1 minute. Sterilize, cool in between 45°-50°, add 10 ml of a one per cent w/v solution of sterile *potassium tellurite* and 50 ml of egg yolk emulsion. Mix thoroughly, but gently and pour into plates. (Prepare the egg-yolk emulsion by disinfecting the surface of whole shell eggs, aseptically cracking the eggs, and separating out intact yolks into a sterile graduated cylinder.

Add sterile saline solution, get a 3 to 7 ratio of egg-yolk to saline. Add to a sterile blender cup and mix at high speed for 5 sec). Adjust the pH after sterilization to 6.8 ± 0.2.

**Bismuth Sulphite Agar Medium**

Solution (1)

Beef extract	6.0 g
Peptone	10.0 g
Agar	24.0 g
Ferric citrate	0.4 g
Brilliant green	10.0 mg
Water to	1000 ml

Dissolve with the aid of heat and sterilize by maintaining at 115° for 30 min.

Solution (2)

Ammonium bismuth citrate	3.0 g
Sodium sulphite	10.0 g
Anhydrous disodium hydrogen phosphate	5.0 g
Dextrose monohydrate	5.0 g
Water to	100 ml

Mix, heat to boiling, cool to room temperature, add 1 volume of solution (2) to 10 volumes of solution (1)

previously melted and cooled to a temperature of 55° and pour.

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

**Brilliant Green Agar Medium**

Peptone	10.0 g
Yeast extract	3.0 g
Lactose	10.0 g
Sucrose	10.0 g
Sodium chloride	5.0 g
Phenol red	80.0 g
Brilliant green	12.5 mg
Agar	12.0 g
Sodium chloride	5.0 g
Water to	1000 ml

Mix, allow to stand for 15 min, sterilize by maintaining at 115° for 30 min and mix before pouring.

**Buffered Sodium Chloride-Peptone Solution pH 7.0**

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

0.1 to 1.0 per cent w/v polysorbate 20 or polysorbate 80 may be added\*.

**Casein Soyabean Digest Agar Medium**

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

Adjust the pH after sterilization to 7.3 ± 0.2.

**Cetrimide Agar Medium**

Pancreatic digest of gelatin	20.0 g
Magnesium chloride	1.4 g
Potassium sulphate	10.0 g

\*Note: Sterilize at 121° for 15 minutes

Agar	13.6 g
Glycerin	10.0 g
Water to	1000 ml

Heat to boiling for 1 minute with shaking. Adjust the pH so that after sterilization\* it is 7.0 to 7.4.

**Desoxycholate-Citrate Agar Medium**

Beef extract	5.0 g
Peptone	5.0 g
Lactose	10.0 g
Trisodium citrate	8.5 g
Sodium thiosulphate	5.4 g
Ferric citrate	1.0 g
Sodium desoxycholate	5.0 g
Neutral red	0.02 g
Agar	12.0 g
Water to	1000 ml

Mix and allow to stand for 15 min. Gently boil with continuous stirring and continue boiling until solution is complete. Cool to 80°, mix, pour and cool rapidly.

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

**Lactose Broth Medium**

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

Adjust the pH after sterilization to  $6.9 \pm 0.2^*$ .

**Levine Eosin-Methylene Blue Agar Medium**

Pancreatic digest of gelatin	10.0 g
Dibasic potassium phosphate	2.0 g
Agar	15.0 g
Lactose	10.0 g
Eosin Y	400 mg
Methylene blue	65.0 mg
Water to	1000 ml

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

**MacConkey Agar Medium**

Pancreatic digest of gelatin	17.0 g
Peptone (meat and casein, equal parts)	3.0 g
Lactose	10.0 g
Sodium chloride	5.0 g
Bile salts	1.5 g
Agar	13.5 g
Neutral red	30.0 mg
Crystal violet	1.0 mg
Water to	1000 ml

Boil the mixture of solids and *water* for 1 minute to effect solution. Adjust the pH after sterilization to  $7.1 \pm 0.2$ .

**MacConkey Broth Medium**

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

Adjust the pH after sterilization to  $7.3 \pm 0.2^*$ .

**Mannitol-Salt Agar Medium**

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

\*Note: Sterilize at 121° for 15 minutes

Mix, heat with frequent agitation and boil for 1 minute to effect solution. Adjust the *pH* after sterilization to  $7.4 \pm 0.2^*$ .

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

**Nutrient Broth Medium**

Beef extract	10.0 g
Peptone	10.0 g
Sodium chloride	5.0 mg
Water to	1000 ml

Dissolve with the aid of heat. Adjust the *pH* to 8.0 to 8.4 with 5 M *sodium hydroxide* and boil for 10 min. Filter and sterilize by maintaining at 115° for 30 min and adjust the *pH* to  $7.3 \pm 0.1$ .

**Pseudomonas Agar Medium for Detection of Flourescein**

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

Dissolve the solid components in *water* before adding *glycerin*. Heat with frequent agitation and boil for 1 min to effect solution. Adjust the *pH* after sterilization to  $7.2 \pm 0.2^*$ .

**Pseudomonas Agar Medium for Detection of Pyocyanin**

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

Dissolve the solid components in *water* before adding *glycerin*. Heat with frequent agitation and boil for 1 minute to effect solution. Adjust the *pH* after sterilization to  $7.2 \pm 0.2^*$ .

**Sabouraud Dextrose Agar Medium**

Dextrose	40.0 g
Peptic digest of animal tissue and pancreatic digest of casein (1:1)	10.0 g
Agar	15.0 g
Water to	1000 ml

Mix, and boil to effect solution. Adjust the *pH* after sterilization to  $5.6 \pm 0.2^*$ .

**Sabouraud Dextrose Agar Medium with Antibiotics**

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

**Selenite F Broth**

Peptone	5.0 g
Lactose	4.0 g
Disodium hydrogen phosphate	10.0 g
Sodium hydrogen selenite	4.0 g
Water to	1000 ml

Dissolve, distribute in sterile containers and sterilize by maintaining at 100° for 30 min.

**Tetrathionate-Bile-Brilliant Green Broth Medium**

Peptone	8.6 g
Dehydrated ox bile	8.0 g
Sodium chloride	6.4 g
Calcium carbonate	20.0 g
Potassium tetrathionate	20.0 g
Brilliant green	70.0 mg
Water to	1000 ml

Heat just to boiling; do not reheat. Adjust the *pH* so that after heating it is  $7.0 \pm 0.2$ .

**Triple Sugar-Iron Agar Medium**

Beef extract	3.0 g
Yeast extract	3.0 g
Peptone	20.0 g
Lactose	10.0 g
Sucrose	10.0 g
Dextrose monohydrate	1.0 g

Ferrous sulphate	0.2 g
Sodium chloride	5.0 g
Sodium thiosulphate	0.3 g
Phenol red	24.0 mg
Water to	1000 ml

Mix, allow standing for 15 min, bringing to boil and maintain at boiling point until solution is complete, mix, distributing in tubes and sterilising by maintaining at 121° for 15 min. Allow to stand in a sloped form with a butt about 2.5 cm long.

**Urea Broth Medium**

Potassium dihydrogen orthophosphate	9.1 g
Anhydrous disodium hydrogen phosphate	9.5 g
Urea	20.0 g
Yeast extract	0.1 g
Phenol red	10.0 mg
Water to	1000 ml

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

**Vogel-Johnson Agar Medium**

Pancreatic digest of casein	10.0 g
Yeast extract	5.0 g
Mannitol	10.0 g
Dibasic potassium phosphate	5.0 g
Lithium chloride	5.0 g
Glycerin	10.0 g
Agar	16.0 g
Phenol red	25.0 mg
Water to	1000 ml

Boil the solution of solids for 1 minute. Sterilize, cool to between 45-50° and add 20 ml of 1 per cent w/v sterile solution of *potassium tellurite*. Adjust the pH after sterilization to 7.0 ± 0.2.\*

**Xylose-Lysine-Desoxycholate Agar Medium**

Xylose	3.5 g
l-Lysine	5.0 g
Lactose	7.5 g
Sucrose	7.5 g

\*Note: Sterilize at 121° for 15 minutes

Yeast extract	3.0 g
Phenol red	80.0 mg
Agar	13.5 g
Sodium desoxycholate	2.5 g
Sodium thiosulphate	6.8 g
Ferric ammonium citrate	800 mg
Water to	1000 ml

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

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

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

**4.1.1 Total Aerobic Microbial Count:**

Pretreat the extracts and raw materials being examined as described below.

*Note:* The raw material needs to be ground as a coarse powder before analysis.

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

**Products insoluble in water (non-fatty):** Suspend 10 g or 10 ml of the extract preparation being examined, unless otherwise specified, in buffered *sodium chloride-peptone* solution pH 7.0 or any other suitable medium shown not to have antimicrobial activity under the conditions of the test and dilute to 100 ml with the same medium. If necessary, divide the preparation being examined and homogenize the suspension mechanically. A suitable surface-active agent such as 0.1 per cent w/v of *polysorbate 80* may

be added to assist the suspension of poorly wettable substances. If necessary, adjust the pH of the suspension to about 7.

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

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

**Membrane filtration:** Use membrane filters 50 mm in diameter and having a nominal pore size not greater than 0.45 µm the effectiveness of which in retaining bacteria has been established for the type of preparation being examined. Transfer 10 ml or a quantity of each dilution containing 1 g of the preparation being examined to each of two membrane filters and filter immediately. If necessary, dilute the pretreated extract preparation so that a colony count of 10 to 100 may be expected. Wash each membrane by filtering through it three or more successive quantities, each of about 100 ml, of a suitable liquid such as buffered *sodium chloride-peptone* solution pH 7.0. For fatty substances add to the liquid *polysorbate 20* or *polysorbate 80*. Transfer one of the membrane filters, intended for the enumeration of bacteria, to the surface of a plate of *casein soyabean digest agar* and the other, intended for the enumeration of fungi, to the surface of a plate of *Sabouraud dextrose agar* with antibiotics.

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

**Table-2: Most Probable Total Count by Multiple-Tube or Serial Dilution Method**

Observed combination of numbers of tubes showing growth in each set			Most probable number of microorganisms per g or per ml
Number of mg (or ml) of specimen per tube			
100 (100 µl)	10 (10 µl)	1 (1 µl)	
3	3	3	>1100
3	3	3	1100
3	3	2	500
3	3	1	200
3	3	0	290
3	2	3	210
3	2	2	150
3	2	1	90
3	2	0	160
3	1	3	120
3	1	2	70
3	1	1	40
3	1	0	95
3	0	2	60
3	0	1	40
3	0	0	23

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

**Plate count for fungi:** Proceed as described in the test for bacteria but use *sabouraud dextrose agar with antibiotics* in place of *casein soyabean digest agar* and incubate the plates at 20-25° for 5 days, unless a more reliable count is obtained in a shorter time. Calculate

the results using plates with not more than 100 colonies.

**Multiple-tube or serial dilution method:** In each of fourteen test-tubes of similar size place 9.0 ml of sterile *fluid soyabean casein digest medium*. Arrange twelve of the tubes in four sets of three tubes each. Put aside one set of three tubes to serve as controls. Into each of three tubes of one set (“100”) and into fourth tube (A) pipette 1 ml of the solution of suspension of the test specimen (extract) and mix. From tube A pipette 1 ml of its contents into the one remaining tube (B) not included in the set and mix. These two tubes contain 100 mg (or 100 µl) and 10 mg (or 10 µl) of the specimen respectively. Into each of the second set (“10”) of three tubes pipette 1 ml from tube A, and into each tube of the third set (“1”) pipette 1 ml from tube B. Discard the unused contents of tube A and B. Close well and incubate all of the tubes. Following the incubation period, examine the tubes for growth. The three control tubes remain clear. Observations in the tubes containing the test specimen, when interpreted by reference to Table-2, indicate the most probable number of microorganisms per g or per ml of the test specimen.

#### 4.1.2 Tests for Specified Microorganisms:

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

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

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

**Secondary test:** Add 0.1 ml of the contents of the tubes containing (a) 5 ml of MacConkey broth and (b) 5 ml of *peptonewater*. Incubate in a water-bath at 43.5 - 44.5° for 24 h and examine tube (a) for acid and gas and tube (b) for *indole*. To test for indole, add 0.5 ml of Kovac’s reagent, shake well and allow to stand for 1

minute; if a red colour is produced in the reagent layer *indole* is present. The presence of acid and gas and of *indole* in the secondary test indicates the presence of *Escherichia coli*.

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

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

**Table-3: Test for *Salmonella***

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

**Primary test:** Add 1.0 ml of the enrichment culture to each of the two tubes containing (a) 10 ml of selenite F broth and (b) tetrathionate-bile-brilliant green broth and incubate at 36-38° for 48 h. From each of these two cultures subculture on at least two of the following four agar media: *bismuth sulphate agar*, *brilliant green agar*, *deoxycholate citrate agar* and *xylose-lysine deoxycholate agar*. Incubate the plates at 36-38° for 18 to 24 h. Upon examination, if none of the colonies conforms to the description given in Table-3, the sample meets the requirements of the test for the absence of the genus *Salmonella*. If any colonies conforming to the description in Table-3 are produced, carry out the secondary test.

**Secondary test:** Subculture any colonies showing the characteristics given in Table-3 in triple sugar-iron agar by first inoculating the surface of the slope and then making a stab culture with the same inoculating

needle, and at the same time inoculate a tube of urea broth. Incubate at 36° to 38° for 18 to 24 h. The formation of acid and gas in the stab culture (with or without concomitant blackening) and the absence of acidity from the surface growth in the triple sugar iron agar, together with the absence of a red colour in urea broth indicates the presence of *Salmonella*. If acid but no gas is produced in the cultures, the identity of the organisms should be confirmed by agglutination tests.

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

***Pseudomonas aeruginosa*:** Pre-treat the extract preparation being examined as described above and inoculate 100 ml of *fluid soyabean-casein digest medium* with a quantity of the solution, suspension or emulsion thus obtained containing 1 g or 1 ml of the preparation being examined. Mix and incubate at 35-37° for 24 to 48 h. Examine the medium for growth and if growth is present, streak a portion of the medium on the surface of *cetrimide agar medium*, each plated on petri dishes. Cover and incubate at 35-37° for 18 to 24 h. If, upon examination, none of the plates contains colonies having the characteristics listed in Table-4 for the media used, the sample meets the requirement for freedom from *Pseudomonas aeruginosa*. If any colonies conforming to the description in Table-4 are produced, carry out the oxidase and pigment tests.

Streak representative suspect colonies from the agar surface of cetrimide agar on the surfaces of *Pseudomonas agar medium* for detection of fluorescein and *Pseudomonas agar medium* for detection of pyocyanin contained in petri dishes. Cover and invert the inoculated media and incubate at 33° to 37° for not less than 3 days. Examine the streaked surfaces under ultra-violet light. Examine the plates to determine whether colonies conforming to the description in Table-4 are present. If growth of suspect colonies occurs, place 2 or 3 drops of a freshly prepared 1 per cent w/v solution of *N,N,N',N'-tetramethyl-4-phenylenediamine di-hydrochloride* on filter paper and smear with the colony; if there is no development of a pink colour, changing to purple, the

sample meets the requirements of the test for the absence of *Pseudomonas aeruginosa*.

**Table-4: Tests for *Pseudomonas aeruginosa***

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

***Staphylococcus aureus*:** Proceed as described under *Pseudomonas aeruginosa*, if upon examination of the incubated plates, none of them contains colonies having the characteristics listed in Table-5 for the media used, the sample meets the requirements for the absence of *Staphylococcus aureus*. If growth occurs, carry out the coagulase test. Transfer representative suspect colonies from the agar surface of any of the media listed in Table-5 to individual tubes, each containing 0.5 ml of mammalian, preferably rabbit or horse, plasma with or without additives.

Incubate in water-bath at 37° examining the tubes at 3 h and subsequently at suitable intervals up to 24 h. If no coagulation in any degree is observed, the sample meets the requirements of the test for the absence of *Staphylococcus aureus*.

**Table-5: Tests for *Staphylococcus aureus***

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

**Validity of the tests for total aerobic microbial count:**

Grow the following test strains separately in tubes containing fluid soyabean-casein digest medium at 30-

35° for 18 to 24 h or, for *Candida albicans*, at 20° for 48 h.

*Staphylococcus aureus* (ATCC 6538; NCTC 0788)

*Bacillus subtilis* (ATCC 6633; NCIB 8054)

*Escherichia coli* (ATCC 8739; NCIB 8545)

*Candida albicans* (ATCC 2091; ATCC 10231)

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

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

#### Validity of the tests for specified microorganisms:

Grow separately the test strains of *Staphylococcus aureus* and *Pseudomonas aeruginosa* in fluid soyabean-casein digest medium and *Escherichia coli* and *Salmonella typhimurium* at 30-35° for 18 to 24 h. Dilute portions of each of the cultures using buffered sodium chloride-peptone solution pH 7.0 to make test suspensions containing about 10<sup>3</sup> viable microorganisms per ml. Mix equal volume of each suspension and use 0.4 ml (approximately 10<sup>2</sup> microorganisms of each strain) as an inoculum in the test for *E. coli*, *S. typhimurium*, *P. aeruginosa* and *S. aureus*, in the presence and absence of the extract preparation being examined, if necessary. A positive result for the respective strain of microorganism should be obtained.

#### 4.2 Test for Aflatoxins (HPTLC Method):

**Table-6: Permissible Limit of Aflatoxins**

Sl. No.	Aflatoxin	Permissible Limit
1	B <sub>1</sub>	< 2 ppb
2	B <sub>1</sub> +B <sub>2</sub> +G <sub>1</sub> +G <sub>2</sub>	< 5 ppb

**Caution:** Aflatoxins are highly dangerous and extreme care should be exercised in handling aflatoxin materials. This test is provided to detect the possible

presence of aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> in any material of plant origin. Unless otherwise specified in the individual monograph use the following method.

#### Zinc Acetate - Aluminum Chloride Reagent:

Dissolve 20 g of zinc acetate and 5 g of aluminum chloride in sufficient water to make 100 ml.

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

**Test Solution 1:** Transfer about 5 g of the powdered material, accurately weighed, to a glass-stoppered flask. Add 200 ml of a mixture of methanol and water (17: 3). Shake vigorously by mechanical means for not less than 30 min and filter. [Note - If the solution has interfering plant pigments, proceed as directed for Test Solution 2]. Discard the first 50 ml of the filtrate and collect the next 40 ml portion. Transfer the filtrate to a separating funnel. Add 40 ml of sodium chloride solution and 25 ml of n-hexane and shake for 1 min. Allow the layers to separate and transfer the lower aqueous layer to a second separating funnel. Extract the aqueous layer in the separating funnel twice, each time with 25 ml of methylene chloride, by shaking for 1 min. Allow the layers to separate each time, separate the lower organic layer and remove the solvent from the combined and evaporate layers on a water bath. Cool the residue. If interferences exist in the residue, proceed as directed for Clean-up Procedure; otherwise, dissolve the residue obtained above in 0.2 ml of a mixture of chloroform and acetonitrile (9.8:0.2) and shake by mechanical means, if necessary.

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

**Clean-up Procedure:** Place a medium-porosity sintered-glass disk or a glass wool plug at the bottom of a 10 mm × 300 mm chromatographic tube. Prepare slurry of 2 g of silica gel with a mixture of diethyl ether and hexane (3:1), pour the slurry into the column and wash with 5 ml of the same solvent mixture. Allow the absorbent to settle and add to the top of the column a layer of 1.5 g of anhydrous sodium sulphate.

Dissolve the residue obtained above in 3 ml of *methylene chloride* and transfer it to the column. Rinse the flask twice with 1 ml portions of *methylene chloride*, transfer the rinses to the column and elute at a rate not greater than 1 ml per min. Add successively to the column 3 ml of *hexane*, 3 ml of *diethyl ether* and 3 ml of *methylene chloride* elute at a rate not greater than 3 ml per min; and discard the eluates. Add to the column 6 ml of a mixture of *methylene chloride* and *acetone* (9:1) and elute at a rate not greater than 1 ml per minute, preferably without the aid of vacuum. Collect this eluate in a small vial, add a boiling chip if necessary and evaporate to dryness on a water bath. Dissolve the residue in 2 ml of a mixture of *chloroform* and *acetonitrile* (9.8:0.2) and shake by mechanical means if necessary.

**Aflatoxin Solution:** Dissolve accurately weighed quantities of aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> in a mixture of *chloroform* and *acetonitrile* (9.8:0.2) to obtain a solution having concentration of 1.0 µg/ml each for aflatoxins B<sub>1</sub> and G<sub>1</sub>, 0.2 µg/ml each for aflatoxins B<sub>2</sub> and G<sub>2</sub>.

**Procedure:** Separately apply 2.5, 5, 7.5 and 10 µl of the *Aflatoxin Solution* and three 10 µl applications of either *Test Solution 1* or *Test Solution 2* to a suitable thin-layer chromatographic plate coated with a 0.25-mm layer of chromatographic silica gel. Superimpose 5 µl of the *Aflatoxin Solution* on one of the three 10 µl applications of the *Test Solution*. Allow the spots to dry and develop the chromatogram in an unsaturated chamber containing a solvent system consisting of a mixture of *chloroform*, *acetone* and *isopropyl alcohol* (85:10:5) until the solvent front has moved not less than 8 cm from the origin. Remove the plate from the developing chamber, mark the solvent front and allow the plate to air-dry. Locate the spots on the plate by examination under UV light at 366 nm: the four applications of the *Aflatoxin Solution* appear as four clearly separated blue fluorescent spots; the spot obtained from the *Test Solution* that was superimposed on the *Aflatoxin Solution* is no more intense than that of the corresponding *Aflatoxin Solution*; and no spot from any of the other *Test Solutions* corresponds to any of the spots obtained from the applications of the *Aflatoxin Solution*. If any spot of aflatoxins is obtained in the *Test Solution*, the colour match the position of each fluorescent spot of the *Test Solution* with those of the *Aflatoxin Solution* to identify the type of aflatoxin present. The intensity of the aflatoxins spot, if present

in the *Test Solution*, when compared with that of the corresponding aflatoxin in the *Aflatoxin Solution* will give an approximate concentration of aflatoxin in the *Test Solution*.

### 4.3 Pesticide Residue:

**Definition:** For the purposes of the Pharmacopoeia, a pesticide is any substance or mixture of substances intended for preventing, destroying or controlling any pest, unwanted species of plants or animals causing harm during or otherwise interfering with the production, processing, storage, transport or marketing of vegetable drugs.

**Table-7: 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 <i>p,p'</i> -DDT, <i>o</i> , <i>p'</i> -DDT, <i>p</i> , <i>p'</i> -DDE and <i>p</i> , <i>p'</i> -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

Substance	Limit (mg/kg)
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

**Note:** Apart from the above, if any pesticides applied to the herb before or after harvesting should also be tested. The limit should be calculated using the following formula.

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

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

M= body mass in kilograms (60 kg),

MDD= daily dose of the drug, in kilograms

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

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

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

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

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

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

**Test for Pesticides:**

The following methods may be used depending on the substance being examined, it may be necessary to modify, sometimes extensively, the procedure described hereafter. In any case, it may be necessary to use, in addition, another column with a different polarity or another detection method (mass spectrometry) or a different method (immunochemical methods) to confirm the results obtained. This procedure is valid only for the analysis of samples of vegetable drugs containing less than 15 per cent of water. Samples with a higher content of water may be dried, provided it has been shown that the drying procedure does not affect significantly the pesticide content.

**Extraction (Method-I):** To 10 g of the substance being examined, add 100 ml of acetone and allow to stand for 20 min. Add 1 ml of a solution containing 1.8  $\mu\text{g/ml}$  of carbophenothionin toluene. Homogenise using a high-speed blender for 3 min. Filter and wash the filter cake with two quantities, each of 25 ml of acetone. Combine the filtrate and the washings and heat using a rotary evaporator at a temperature not exceeding 40° until the solvent has almost completely evaporated. To the residue add a few millilitres of toluene and heat again until the acetone is completely removed. Dissolve the residue in 8 ml of toluene. Filter through a membrane filter (45  $\mu\text{m}$ ), rinse the flask and the filter with toluene and dilute to 10.0 ml with the same solvent (solution A).

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

- A stainless-steel column 0.30 m long and 7.8 mm in internal diameter packed with styrene-

divinylbenzene copolymer (5  $\mu\text{m}$ )

- As mobile phase *toluene* at a flow rate of 1 ml/min

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

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

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

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

**Extraction (Method-II):** To 25 g of the substance being examined, add 300 ml of *acetonitrile: water* (3:1) and homogenise using a high-speed blender for 5 min. Filter and wash the filter cake with two quantities, each of 25 ml of *acetonitrile water* mixture. Transfer filtrate and rinse to a separating funnel. Add 50 ml of saturated *sodium chloride* and mix vigorously

for 30 seconds. Add 50 ml *hexane* to the separating funnel and extract. Repeat extraction with *hexane* for another two times. Collect the *hexane* layer and pass the combined *hexane* layer through *anhydrous sodium sulphate*. Collect the *hexane* and evaporate to dryness. Dissolve the residue in 25 ml *hexane*.

**Florisil column clean up:** Use florisil solid phase extraction cartridges. Using bulb pipet transfer 2 ml of the *hexane* solution containing the pesticide residue in to the florisil cartridge. Elute with 12 ml of 15 per cent *diethyl ether* in *hexane*. Further elute with 12 ml of 50 per cent *diethyl ether* in *hexane*. Collect the elutes separately and evaporate and dry using rotary evaporator. Dissolve in 0.2 ml of *n-hexane* containing 10 ng/ml of *carbophenothion* and sonicate.

#### Quantitative Analysis:

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

**Test solution:** Concentrate solution B in a current of *helium for chromatography* almost to dryness and dilute to 100  $\mu\text{l}$  with *toluene*.

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

The chromatographic procedure may be carried out using:

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

Maintaining the temperature of the column at 80° for 1 min, then raising it at a rate of 30°/min to 150°, maintaining at 150° for 3 min, then raising the temperature at a rate of 4°/min to 280° and maintaining at this temperature for 1 min and

maintaining the temperature of the injector port at 250° and that of the detector at 275°. Inject the chosen volume of each solution. When the chromatograms are recorded in the prescribed conditions, the relative retention times are approximately those listed in Table-8. Calculate the content of each insecticide from the peak areas and the concentrations of the solutions.

**Table-8: Relative Retention Times of Pesticides**

Substance	Relative retention times
Dichlorvos	0.20
Fonofos	0.50
Diazinon	0.52
Parathion-methyl	0.59
Chlorpyrifos-methyl	0.60
Pirimiphos-methyl	0.66
Malathion	0.67
Parathion	0.69
Chlorpyrifos	0.70
Methidathion	0.78
Ethion	0.96
Carbophenothion	1.00
Azinphos-methyl	1.17
Phosalon	1.18

**B. Organochlorine and Pyrethroid Insecticides:**

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

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

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

**Table-9: Relative Retention Times of Insecticides**

Substance	Relative retention times
α-Hexachlorocyclohexane	0.44

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

\*The substance shows several peaks.

The chromatographic procedure may be carried out using:

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

- a device allowing direct cold on-column injection maintaining the temperature of the column at 80° for 1 min, then raising it at a rate of 30°/min to 150°, maintaining at 150° for 3 min, then raising the temperature at a rate of 4°/min to 280° and maintaining at this temperature for 1 min and maintaining the temperature of the injector port at 250° and that of the detector at 275°. Inject the chosen volume of each solution. When the chromatograms are recorded in the prescribed conditions, the relative retention times are approximately those listed in Table-9. Calculate the content of each insecticide from the peak areas and the concentrations of the solutions.

#### 4.4 Heavy Metals and Arsenic

**Table-10: Permissible Limits of Heavy Metals and Arsenic**

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

#### Determination of Arsenic, Cadmium, Mercury, and Lead by Atomic absorption spectrophotometry

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

#### Apparatus

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

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

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

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

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

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

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

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

The commonly used wavelength region is 190.0 - 900.0 nm.

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

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

## Procedure

### Method (Direct Calibration Method)

Prepare not less than 3 reference solutions of the element being examined of different concentrations, covering the range recommended by the instrument manufacturer and add separately the corresponding reagents as that for the test solution and prepare the blank reference solution with the corresponding reagents. Measure the absorbances of the blank reference solution and each reference solution of different concentrations separately, record the readings and prepare a calibration curve with the average value of 3 readings of each concentration on the ordinate and the corresponding concentration on the abscissa. Prepare a test solution of the substance being examined as specified in the monograph, adjust the concentration to fall within the concentration range of the reference solution. Measure the absorbance 3 times, record the readings and calculate the average value. Interpolate the mean value of the readings on

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

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

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

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

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

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

#### Preparation of test solution:

*Method:* Weigh accurately 0.5 g of the coarse powder of the substance being examined, transfer into a casparian flask, add 5-10 ml of the mixture of *nitric acid* and *perchloric acid*(4 : 1), add a small hopper on the flask-top, macerate overnight, heat to slake on the electric hot plate, keep somewhat-boiling, if brownish-black, add again a quantity of the above mixture,

continuously heat till the solution becomes clean and transparent, then raise temperature, heat continuously to thick smoke, till white smoke disperse, the slaked solution becomes colourless and transparent or a little yellow, cool, transfer it into a 50-ml volumetric flask, wash the container with 2 per cent *nitric acid* solution add the washing solution into the same volumetric flask and dilute with the same solvent to the volume, shake well. Prepare synchronously the reagent blank solution according to the above procedure.

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

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

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

**Preparation of Cd standard stock solution:** Measure accurately a quantity of Cd single element standard solution to prepare standard stock solution Cd with 2 per cent *nitric acid*, which containing 0.4  $\mu\text{g}$  per ml Cd, stored at 0-5°.

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

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

**Determination:** Pipette accurately 10-20  $\mu\text{l}$  of the test solution and its corresponding reagent blank solution respectively, determine their absorbance according to

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

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

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

**Preparation of As standard stock solution:** Measure accurately a quantity of As single element standard solution to prepare standard stock solution with 2 per cent *nitric acid* solution, which contains 1.0  $\mu\text{g}$  per ml As, stored at 0-50.

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

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

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

the content of As in the test solution from the calibration curve.

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

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

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

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

#### Preparation of test solution:

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

**Determination:** Pipette accurately a quantity of the test solution and its corresponding reagent blank

solution, respectively, proceed as described under "Preparation of calibration curve" beginning at the words "add 1 ml of 25 per cent *potassium iodide* solution". Calculate the content of mercury (Hg) in the test solution from the calibration curve.

#### (5) Determination of Copper (flame method):

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

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

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

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

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

### 4.5 Limit Test for Sulphates:

#### Reagents

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

**0.5 M Barium Chloride:** Barium chloride AR dissolved in *water* to contain in 1000 ml 122.1 g of barium chloride.

**Method:** Dissolve the specified quantity of the substance in *water*, or prepare a solution as directed in the text, transfer to a *Nessler cylinder*, and add 2 ml of *dilute hydrochloric acid*, except where *hydrochloric*

*acid* is used in the preparation of the solution. Dilute to 45 ml with *water*, add 5 ml of *barium sulphate reagent*. Stir immediately with a glass rod, and allow to stand for 5 min. The turbidity produced is not greater than the *standard turbidity*, when viewed transversely. **Standard turbidity:** Place 1.0 ml of 0.1089 per cent w/v solution of potassium sulphate and 2 ml of *dilute hydrochloric acid* in a *Nessler cylinder*, dilute to 45 ml with *water*, add 5 ml of *barium sulphate reagent*, stir immediately with a glass rod and allow to stand for 5 min.

#### 4.6 Limit Test for Chlorides:

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

#### Standard Opalescence:

Place 1.0 ml of a 0.05845 per cent w/v solution of *sodium chloride* and 10 ml of *dilute nitric acid* in a *Nessler cylinder*. Dilute to 50 ml with *water* and add 1 ml of *silver nitrate solution*. Stir immediately with a glass rod and allow to stand for 5 min.

#### 4.7 Limit Test for Iron

**Standard Iron solution:** Weigh accurately 0.1726 g of *ferric ammonium sulphate* and dissolve in 10 ml of 0.1 N *sulphuric acid* and sufficient *water* to produce 1000 ml. Each ml of this solution contains 0.02 mg of Fe.

Method: Dissolve the specified quantity of the substance being examined in 40 ml of *water*, or use 10 ml of the solution prescribed in the monograph, and

transfer to a *Nessler cylinder*. Add 2 ml of a 20 per cent w/v solution of *iron-free citric acid* and 0.1 ml of *thioglycollic acid*, mix, make alkaline with *iron-free ammonia solution*, dilute to 50 ml with *water* and allow to stand for 5 min. Any colour produced is not more intense than the standard colour.

**Standard colour:** Dilute 2.0 ml of *standard iron solution* with 40 ml of *water* in a *Nessler cylinder*. Add 2 ml of a 20 per cent w/v solution of *iron-free citric acid* and 0.1 ml of *thioglycollic acid*, mix, make alkaline with *iron-free ammonia solution*, dilute to 50 ml with *water* and allow to stand for 5 min.

**Note :** Appendix 4.5 to 4.7 are required in order to ascertain quality of certain chemicals mentioned in Appendix 5.

## APPENDIX-5

## SPECIFICATIONS OF REAGENTS/CHEMICALS AND SOLUTIONS

**Acetone - Propan-2-one;  $(\text{CH}_3)_2\text{CO} = 58.08$  (67-64-1)**

Analytical reagent grade of commerce; A volatile, flammable liquid; boiling point about  $56^\circ$ ; weight per ml about 0.79 g; Complies with the following test: *Water* Not more than 0.3 per cent w/w, using anhydrous *pyridine* as the solvent

**Acetonitrile- Methyl Cyanide;  $\text{CH}_3\text{CN} = 41.05$** 

General laboratory reagent grade of commerce; Colourless liquid; boiling point about  $81^\circ$ ; weight per ml about 0.78 g; *Acetonitrile intended for use in spectrophotometry complies with the following test:* Transmittance: not less than 98 per cent in the range 255 to 420 nm using *water* as the blank

**Alcohol -  $\text{C}_2\text{H}_5\text{OH}$** 

*Description:* Clear, colourless, mobile, volatile liquid; odour characteristic and spirituous; taste burning; readily volatilised even at low temperature, and boils at about  $78^\circ$ , flammable; *Alcohol* containing not less than 94.85 per cent v/v and not more than 95.2 per cent v/v of  $\text{C}_2\text{H}_5\text{OH}$  at  $15.56^\circ$

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

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

*Specific gravity:* Between 0.8084 and 0.8104 at  $25^\circ$

*Clarity of solution:* Dilute 5 ml to 100 ml with *water* in glass cylinder; the solution remains clear when examined against a black background. Cool to  $10^\circ$  for 30 min; the solution remains clear.

*Methanol:* To one drop, add one drop of *water*, one drop of *dilute phosphoric acid*, and one drop of *potassium permanganate solution*. Mix, allow to stand for one minute and add *sodium bisulphite solution* dropwise, until the *permanganate* colour is discharged. If a brown colour remains, add one drop of *dilute phosphoric acid*. To the colourless solution, add 5 ml of freshly prepared *chromotropic acid* solution and heat on a water-bath at  $60^\circ$  for 10 min; no violet colour is produced.

*Foreign organic substances:* Clean a glass-stoppered cylinder thoroughly with *hydrochloric acid*, rinse with *water* and finally rinse with the *alcohol* under examination. Put 20 ml in the cylinder, cool to about  $15^\circ$  and then add from a carefully cleaned pipette, 0.1 ml of 0.1 N *potassium permanganate*. Mix at once by inverting the stoppered cylinder and allow to stand at  $15^\circ$  for 5 min; the pink colour does not entirely disappear.

*Isopropyl alcohol and t-butyl alcohol:* To 1 ml, add 2 ml of *water* and 10 ml of *mercuric sulphate* solution and heat in a boiling water-bath; no precipitate is formed within 3 min.

*Aldehydes and ketones:* Heat 100 ml of *hydroxylamine hydrochloride* solution in a loosely stoppered flask on a water-bath for 30 min, cool, and if necessary, add sufficient 0.05 N *sodium hydroxide* to restore the green colour. To 50 ml of this solution, add 25 ml of the *alcohol* and heat on a water bath for 10 min in a loosely stoppered flask. Cool, transfer to a *Nessler cylinder*, and titrate with 0.05 N *sodium hydroxide* until the colour matches that of the remainder of the *hydroxylamine hydrochloride solution* contained in a similar cylinder, both solutions being viewed down the axis of the cylinder. Not more than 0.9 ml of 0.05 N *sodium hydroxide* is required.

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

*Non-volatile matter:* Evaporate 40 ml in a tared dish on a water-bath and dry the residue at  $105^\circ$  for 1 h; the weight of the residue does not exceed 1 mg.

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

*Labelling:* The label on the container states "Flammable".

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

*Aldehydes:* To 25 ml, contained in 300 ml flask, add 75 ml of *dinitrophenyl hydrazine solution*, heat

on a water bath under a reflux condenser for 24 h, remove the *alcohol* by distillation, dilute to 200 ml with

2 per cent v/v solution of *sulphuric acid*, and set aside for 24 h; no crystals are produced.

*Alcohol, Sulphate-free:* Shake *alcohol* with an excess of anion exchange resin for 30 min and filter.

**Ammonia:**  $\text{H}_3\text{N} = 17.03$

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

**Ammonium Chloride -  $\text{NH}_4\text{Cl}$**

*Description:* A white, crystalline, granular powder; odourless; taste saline and cooling; somewhat hygroscopic

*Solubility:* Soluble in 2.6 parts of water, in 1.4 parts of boiling water and in about 100 parts of alcohol

*Reaction:* pH of a 5 percent w/v solution between 4.5 and 6.0

*Sulphates:* 2 g complies with the *limit test for sulphates*

*Sulphated ash:* Not more than 0.1 percent

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

**Ammonium dihydrogen phosphate -  $\text{NH}_4\text{H}_2\text{PO}_4 = 115.03$**

Ammonium dihydrogen orthophosphate: Ammonium Phosphate; Monobasic; Analytical reagent grade of commerce; Odourless crystals or crystalline powder

**Ammonium Nitrate -  $\text{NH}_4\text{NO}_3 = 80.04$**

Analytical reagent grade of commerce, White crystalline solid, highly soluble in water

**Ascorbic acid -  $\text{C}_6\text{H}_8\text{O}_6 = 176.13$**

*Description:* Colourless crystals or white to very pale yellow crystalline powder; odourless; on exposure to light it gradually darkens.

*Solubility:* Freely soluble in water, sparingly soluble in ethanol (95%); insoluble in chloroform, in ether and in benzene

*Storage:* Store in tightly-closed, light-resistant containers and avoid contact with metals. It

undergoes rapid decomposition in solutions in contact with air.

*Standards:* Ascorbic Acid contains not less than 99.0 per cent and not more than 100.5 per cent of  $\text{C}_6\text{H}_8\text{O}_6$ .

**Bromophenol Blue Solution:** Strong bromophenol blue solution; Ethanolic bromophenol blue solution

Dissolve 0.1 g of bromophenol blue AR with gentle heating in 1.5 ml of 0.1M *sodium hydroxide* and 20ml of *ethanol* (95 percent), and add sufficient *water* to produce 100 ml.

Complies with the following test:

*Sensitivity:* A mixture of 0.05 ml of the solution and 20 ml of *carbon dioxide free water* to which 0.05 ml of 0.1M *hydrochloric acid* has been added is yellow. Not more than 0.1 ml of 0.1M *sodium hydroxide* is required to change the colour to bluish violet.

**Calcium Chloride -  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O} = 147.01$**

*Calcium Chloride Solution:* 10% w/v solution of calcium chloride AR in *water*

**Carbophenothion:**  $\text{C}_{11}\text{H}_{16}\text{ClO}_2\text{PS}_3 = 342.9$

Carbophenothion appears as an off-white to amber liquid with a mild odour of rotten eggs; Used as an insecticide and acaricide, primarily for citrus crops and deciduous fruits and nuts

**Chloroform - Trichloromethane;  $\text{CHCl}_3 = 119.4$  (67-66-3)**

Analytical reagent grade of commerce containing 0.4 to 1.0 per cent w/w of *ethanol*

A colourless liquid with a sweet, penetrating odour; boiling point about  $60^\circ$ ;  $d_{20}^{20}$  1.475 to 1.481

**Chromotropic Acid -  $\text{C}_{10}\text{H}_8\text{O}_8\text{S}_2$**

Molecular weight: 320.29

*Chromotropic Acid Solution:* Dissolve 50 mg of chromotropic acid AR in 100 ml of mixture of *sulphuric acid* and *water* in 9: 4 proportion.

**Diethyl ether -  $\text{C}_4\text{H}_{10}\text{O} = 74.12$  (60-29-7)**

Analytical reagent grade of commerce; A volatile, highly flammable, colourless liquid; boiling point,  $34^\circ$  to  $35^\circ$ ;  $d_{20}^{20}$ , 0.713 to 0.715; Do not distil unless the ether complies with the following test for peroxides:

*Peroxides:* Place 8 ml of *potassium iodide* and starch solution in a 12-ml ground-glass-stoppered cylinder of about 1.5 cm in diameter. Fill completely with the reagent being examined, shake vigorously and allow to stand in the dark for 30 min. No colour is produced.

Store protected from light at a temperature not exceeding 15°. The name and concentration of any added stabiliser are stated on the label.

**Dimethyl Yellow Solution:** A 0.2 per cent w/v solution of dimethyl yellow AR in *ethanol* (90 %)

Complies with the following test: A solution containing 2 g of *ammonium chloride* in 25 ml of *carbon dioxide free water*, to which is added 0.1 ml of the *dimethyl yellow solution*, is yellow; Not more than 0.1 ml of 0.1M *hydrochloric acid* is required to change the colour to red

**Dinitrophenyl hydrazine:**  $C_6H_6N_4O_4 = 198.14$

Analytical reagent grade of commerce

**Ferric Ammonium Sulphate-** $Fe(NH_4)(SO_4)_2 \cdot 12H_2O$   
Ferric alum; Ammonium Iron (III) Sulphate = 482.18

Analytical reagent grade of commerce; Pale violet crystals or nearly colourless crystalline powder

*Ferric Ammonium Sulphate Solution:* A 8.0 per cent w/v solution of *ferric ammonium sulphate*

**Formaldehyde-**  $HCHO = 30.03$

Analytical grade reagent of commerce; colourless, aqueous solution with a lachrymatory vapour; weight per ml about 1.08 g; contains not less than 34.0 per cent w/v of  $HCHO$ ; to be stored protected from moisture preferably at temperature not below 15°

*Assay:* Dilute 5 ml to 1000 ml with *water*. To 10 ml of the solution, add 25 ml of 0.05M iodine AR and 10 ml of 1M *sodium hydroxide* solution. Allow to stand for 5 min, add 12 ml of 1M *hydrochloric acid* and titrate the excess of iodine with 0.1M *sodium thiosulphate* using 1 ml of starch solution, added towards the end of the titration, as indicator. Perform a blank determination and make any necessary correction. 1 ml of 0.05M iodine is equivalent to 0.0015 g of  $HCHO$

**Heptane; n-Heptane**  $C_7H_{16} = 100.20$

General laboratory reagent grade of commerce; Clear, colourless, volatile, flammable, liquid; boiling point about 98°; weight per ml, about 0.69 g

**Hydrochloric acid - HCl = 36.46 (7647-01-0)**

Where no molarity is indicated use analytical reagent grade of commerce with a relative density of about 1.18, containing not less than 35 per cent w/w and not more than 38 per cent w/w of  $HCl$  and about 11.5 M in strength

This may also be referred wherever con. *Hydrochloric Acid* is mentioned

A colourless, fuming liquid

Solutions of molarity xM should be prepared by diluting 85x ml of *hydrochloric acid* to 1000 ml with *water*. Store in a container of *polyethylene* or other non-reacting material at a temperature not exceeding 30°.

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

**Hydroxylamine hydrochloride-** $NH_2OH \cdot HCl = 69.49$

Contains not less than 97.0 per cent w/w of  $NH_2OH \cdot HCl$

*Description:* Colourless crystals, or a white, crystalline powder

*Solubility:* Very soluble in water; soluble in alcohol

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

*Sulphated ash:* Not more than 0.2 per cent

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

*Hydroxylamine Hydrochloride Solution:* Dissolve 1 g of *hydroxylamine hydrochloride* in 50 ml of *water* and add 50 ml of *alcohol*, 1 ml of *bromophenol blue*

*solution* and 0.1 N *sodium hydroxide* until the solution becomes green.

**Isopropyl alcohol - Propane-2-ol; C<sub>3</sub>H<sub>8</sub>O = 60.1**

An isomer of 1-propanol; A colourless liquid having disinfectant properties; Volatile, colourless liquid with a sharp musty odour like rubbing alcohol; Flash point of 11.6°; Vapours heavier than air and mildly irritating to the eyes, nose, and throat

**Magnesium Nitrate: Mg (NO<sub>3</sub>)<sub>2</sub>.6H<sub>2</sub>O = 256.41**

Analytical reagent grade of commerce; Colourless crystals; deliquescent

**Mayer's reagent: Potassium Mercuric-Iodide Solution**

Add 1.36 g of mercuric chloride dissolved in 60 ml of *water* to a solution of 5 g of potassium iodide in 20 ml of *water*, mix and add sufficient water to produce 100 ml.

**Mercuric Chloride- HgCl<sub>2</sub> = 271.50**

Contains not less than 99.5 per cent of HgCl<sub>2</sub>

*Description:* Heavy, colourless or white, crystalline masses, or a white crystalline powder

*Solubility:* Soluble in water; freely soluble in alcohol

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

*Assay:* Weigh accurately about 0.3 g and dissolve in 85 ml of *purified water* in a stoppered flask, add 10 ml of *calcium chloride solution*, 10 ml of *potassium iodide solution*, 3 ml of *formaldehyde solution* and 15 ml of *sodium hydroxide solution*, and shake continuously for two min. Add 20 ml of *acetic acid* and 35 ml of 0.1N iodine. Shake continuously for about ten min, or until the precipitated mercury is completely re-dissolved, and titrate the excess of iodine with 0.1N *sodium thiosulphate*. Each ml of 0.1N iodine is equivalent to 0.01357 g of HgCl<sub>2</sub>.

**Mercuric Sulphate- HgSO<sub>4</sub>**

Molecular Weight: 296.65

Mercuric Sulphate Solution: Mix 5 g of yellow mercuric oxide AR with 40 ml of *water*, add while stirring, 20 ml of *sulphuric acid* and 40 ml of *water* and continue stirring until complete dissolution.

**Methanol - Methyl alcohol; CH<sub>4</sub>O = 32.04 (67-56-1)**

Analytical reagent grade of commerce; A colourless liquid; boiling point 64° to 65°,  $d_{20}^{20}$  0.791 to 0.793

When 'methanol' is followed by a percentage figure, an instruction to use *methanol* diluted with *water* to produce the specified percentage v/v of *methanol* is implied.

**Methyl Orange: Sodium-p-dimethylamineazobenzene sulphate - C<sub>14</sub>H<sub>14</sub>O<sub>3</sub>N<sub>3</sub>SNa**

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

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

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

*Colour change:* pH 3.0 (red) to pH 4.4 (yellow)

**Methyl red-p-Dimethylaminoazobenzene-o-carboxylic acid, C<sub>15</sub>H<sub>15</sub>O<sub>2</sub>N<sub>3</sub>.**

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

**Methylene chloride - Dichloromethane; CH<sub>2</sub>Cl<sub>2</sub> = 84.93**

Analytical reagent grade of commerce; Volatile, sweet smelling liquid; boiling point about 40°; weight per ml about 1.32 g

**n-Hexane - Hexane = 86.18**

Analytical reagent grade of commerce containing not less than 90 per cent of n-hexane; Colourless, mobile, highly inflammable liquid; boiling point about 68°, weight per ml, about 0.674 g

**Nitric acid: HNO<sub>3</sub> = 63.013**

Contains 70.0 per cent w/w of HNO<sub>3</sub> (limits, 69.0 to 71.0); About 16N in strength

*Description:* Clear, colourless, fuming liquid

*Weight per ml:* At 20°, 1.41 to 1.42 g

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

*Iron*: 0.5 ml of complies with the limit test for iron

*Lead*: Not more than 2 ppm

*Chlorides*: 5 ml neutralised with *dilute ammonia solution*, complies with the limit test for chlorides

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

*Sulphated ash*: Not more than 0.01 per cent w/w

*Assay*: Weigh accurately about 4 g into a stoppered flask containing 40 ml of *purified water* and titrate with N *sodium hydroxide* using *methyl orange solution* as indicator. Each ml of N sodium hydroxide is equivalent to 0.06301 g of  $\text{HNO}_3$ .

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

*Nitric Acid, Dilute*: Contains approximately 10 per cent w/w of *nitric acid*; Dilute 106 ml of *nitric acid* to 1000 ml with purified water

**Oracet blue**, Solvent blue 19,  $\text{C}_{21}\text{H}_{16}\text{N}_2\text{O}_2 = 328.4$

Analytical reagent grade of commerce

**Oxalic Acid** -  $(\text{COOH})_2 \cdot 2\text{H}_2\text{O} = 126.07$

Analytical grade reagent of commerce; colourless crystals

**Perchloric Acid**-  $\text{HClO}_4 = 100.46$

A solution in *purified water* containing between 70 percent and 72 per cent w/v of *perchloric acid* and about 12M in strength

Clear, colourless liquid; very corrosive and may deflagrate on contact with oxidisable substances; weight per ml about 1.7 g

**Phenolphthalein** -  $\text{C}_{20}\text{H}_{14}\text{O}_4$

A white to yellowish-white powder, practically insoluble in water, soluble in alcohol

*Phenolphthalein Solution*: Dissolve 0.10 g of *phenolphthalein* in 80 ml of *alcohol* and dilute to 100 ml with purified water.

*Test for sensitivity*: To 0.1 ml of the *phenolphthalein solution*, add 100 ml of freshly boiled and cooled *water*, the solution is colourless. Not more than 0.2

ml of 0.02 N *sodium hydroxide* is required to change the colour to pink.

*Colour change*: pH 8.2 (colourless) to pH 10.0 (red)

**Phosphoric Acid** -  $\text{H}_3\text{PO}_4 = 98.00$  (Orthophosphoric Acid; Concentrated Phosphoric Acid)

*Description*: Clear, colourless syrupy liquid; corrosive

*Solubility*: Miscible with water and with alcohol

*Phosphoric Acid, xN*: Solutions of any normality, xN may be prepared by diluting 49x g of *phosphoric acid* with *purified water* to 1000 ml.

*Phosphoric Acid, Dilute*: Contains approximately 10 per cent w/v of *phosphoric acid*; Dilute 69 ml of *phosphoric acid* to 1000 ml with *purified water*

**Potassium iodide** -KI = 166.0 (7681-11-0)

Analytical reagent grade of commerce; A white, crystalline powder

*Potassium Iodide and Starch Solution*: Dissolve 10 g of *potassium iodide* in sufficient *water* to produce 95 ml and add 5 ml of starch solution.

**Potassium permanganate**–  $\text{KMnO}_4 = 158.03$

*Description*: Dark purple, slender, prismatic crystals, having a metallic lustre, odourless; taste, sweet and astringent

*Solubility*: Soluble in *water*; freely soluble in *boiling water*

*Chlorides and Sulphates*: Dissolve 1 g in 50 ml of *boiling water*, heat on a water-bath, and add gradually 4 ml or a sufficient quantity of *alcohol* until the meniscus is colourless and filter. A 20 ml portion of the filtrate complies with the limit test for *chlorides* and another 20 ml portion of the filtrate complies with the limit test for *sulphates*.

*Assay*: Weigh accurately about 0.8 g, dissolve in *water* and dilute to 250 ml. Titrate with this solution, 25.0 ml of 0.1 N *oxalic acid* mixed with 25 ml of *water* and 5 ml of *sulphuric acid*. Keep the temperature at about 70° throughout the entire titration. Each ml of 0.1 N *oxalic acid* is equivalent to 0.00316 g of  $\text{KMnO}_4$ .

*Storage*: Store in well-closed containers.

*Caution*: Great care should be observed in handling *potassium permanganate*, as dangerous explosions are liable to occur if it is brought into contact with

organic or other readily oxidisable substances, either in solution or in the dry condition.

*Potassium Permanganate Solution:* A 1.0 per cent w/v solution of *potassium permanganate* in *water*.

*Potassium Permanganate, 0.1 N Solution:*

3.161 g in 1000 ml

Dissolve about 3.3 g of *potassium permanganate* in 1000 ml of *water*, heat on a water-bath for one hour and allow to stand for two days. Filter through glass wool and standardise the solution as follows: To an accurately measured volume of about 25 ml of the solution in a glass-stoppered flask, add 2 g of *potassium iodide* followed by 10 ml of *N sulphuric acid*. Titrate the liberated *iodine* with standardised 0.1 N *sodium thiosulphate*, adding 3 ml of *starch solution* as the end point is approached. Correct for a blank run on the same quantities of the same reagents. Each ml of 0.1 N *sodium thiosulphate* is equivalent to 0.003161g of  $\text{KMnO}_4$ .

### Sodium Bicarbonate

*Description:* White, crystalline powder or small, opaque, monoclinic crystals; odourless; taste, saline

*Solubility:* Freely soluble in *water*; practically insoluble in *alcohol*

*Carbonates:* pH of a freshly prepared 5.0 per cent w/v solution in *carbon dioxide free water*, not more than 8.6

*Aluminium, calcium and insoluble matter:* Boil 10 g with 50 ml of *water* and 20 ml of *dilute ammonia solution*, filter, and wash the residue with *water*; the residue, after ignition to constant weight, not more than 1 mg

*Arsenic:* Not more than 2 parts per million

*Iron:* Dissolve 2.5 g in 20 ml of *water* and 4 ml of *iron-free hydrochloric acid*, and dilute to 40 ml with *water*; the solution complies with the *limit test for iron*.

*Heavy metals:* Not more than 5 parts per million, on a solution prepared in the following manner: Mix 4.0 g with 5 ml of *water* and 10 ml of *dilute hydrochloric acid*, heat to boiling, and maintain the temperature for one minute. Add one drop of *phenolphthalein solution* and sufficient *ammonia solution* dropwise to give the solution a faint pink colour. Cool and dilute to 25 ml with *water*.

*Chlorides:* Dissolve 1.0 g in *water* with the addition of 2 ml of *nitric acid*; the solution complies with the *limit test for chlorides*.

*Sulphates:* Dissolve 2 g in *water* with the addition of 2 ml of *hydrochloric acid*; the solution complies with the *limit test for sulphates*.

*Ammonium compounds:* 1 g warmed with 10 ml of *sodium hydroxide solution* does not evolve ammonia.

*Assay:* Weigh accurately about 1 g, dissolve in 20 ml of *water*, and titrate with 0.5 N *sulphuric acid* using *methyl orange solution* as indicator. Each ml of 0.5 N *sulphuric acid* is equivalent to 0.042 g of  $\text{NaHCO}_3$ .

*Storage:* Store in well-closed containers.

*Sodium Bicarbonate Solution:* A 5 per cent w/v solution of *sodium bicarbonate* in *water*

### Sodium Bisulphite - $\text{NaHSO}_3$

*Sodium Bisulphite Solution:* Dissolve 10 g of sodium bisulphite AR in sufficient *water* to produce 30 ml. The solution should be freshly prepared.

### Sodium borohydride $\text{NaBH}_4$ or $\text{BH}_4\text{Na} = 37.84$

Analytical reagent grade of commerce; white to grayish crystalline powder; decomposes by *water* to form sodium hydroxide, a corrosive material, and hydrogen, a flammable gas

*Caution:* The heat of this reaction may be sufficient to ignite the hydrogen. The material itself is easily ignited and burns vigorously once ignited.

### Sodium carbonate $\text{Na}_2\text{CO}_3 \cdot 10\text{H}_2\text{O} = 286.2$ (6132-02-1)

Analytical reagent grade of commerce; Melting point greater than  $300^\circ$

### Sodium chloride - $\text{NaCl} = 58.44$

Analytical reagent grade of commerce

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

### Sodium hydroxide $\text{NaOH} = 40.00$

*Description:* White sticks, pellets, fused masses, or scales; dry, hard brittle and showing a crystalline fracture; very deliquescent; strongly alkaline and corrosive

*Solubility:* Freely soluble in *water* and in *alcohol*

*Aluminium, iron and matter insoluble in hydrochloric acid:* Boil 5 g with 50 ml of dilute hydrochloric acid, cool, make alkaline with *dilute ammonia solution*, boil, filter, and wash with a 2.5 per cent w/v solution of *ammonium nitrate*; the insoluble residue after ignition to constant weight weighs not more than 5 mg.

*Arsenic:* Not more than 4 parts per million

*Heavy metals:* Not more than 30 parts per million, in a solution prepared by dissolving 0.67 g in 5 ml of *water* and 7 ml of 3 N *hydrochloric acid*. Heat to boiling, cool and dilute to 25 ml with *water*.

*Potassium:* Acidify 5 ml of a 5 per cent w/v solution with *acetic acid* and add 3 drops of *sodium cobaltnitrite solution*; no precipitate is formed.

*Chlorides:* 0.5 g dissolved in *water* with the addition of 1.8 ml of *nitric acid*, complies with the limit test for *chlorides*.

*Sulphates:* 1 g dissolved in *water* with the addition of 3.5 ml of *hydrochloric acid* complies with the limit test for *sulphates*.

*Assay:* Weigh accurately about 1.5 g and dissolve in about 40 ml of *carbon dioxide free water*. Cool and titrate with N *sulphuric acid* using *phenolphthalein solution* as indicator. When the pink colour of the solution is discharged, record the volume of acid solution required, add *methyl orange solution* and continue the titration until a persistent pink colour is produced. Each ml of N *sulphuric acid* is equivalent to 0.040 g of total alkali calculated as NaOH and each ml of acid consumed in the titration with *methyl orange* is equivalent to 0.106 g of  $\text{Na}_2\text{CO}_3$ .

*Storage:* Store in tightly closed containers.

*Sodium Hydroxide, x N:* Solutions of any normality, xN may be prepared by dissolving 40 x g of *sodium hydroxide* in *water* and diluting to 1000 ml.

*Sodium Hydroxide Solution:* A 20.0 per cent w/v solution of *sodium hydroxide* in *water*

*Sodium Hydroxide Solution, Dilute:* A 5.0 per cent w/v solution of *sodium hydroxide* in *water*

**Sodium sulphate Anhydrous** -  $\text{Na}_2\text{SO}_4 = 142.0$  (7757-82-6)

Analytical reagent grade of commerce complying with the following test:

Loss on drying: When dried at  $130^\circ$ , loses not more than 0.5 per cent of its weight

**Sodium Thiosulphate** -  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O} = 248.17$

*Description:* Large colourless crystals or coarse, crystalline powder; odourless; taste saline; deliquescent in moist air and effloresces in dry air at temperature above  $33^\circ$

*Sodium Thiosulphate 0.1N Solution:* 24.82 g in 1000 ml

Dissolve about 26 g of *sodium thiosulphate* and 0.2 g of *sodium carbonate* in *carbon dioxide-free water* and dilute to 1000 ml with the same solvent. Standardise the solution as follows:

Dissolve 0.300 g of potassium bromate AR in sufficient *purified water* to produce 250 ml. To 50 ml of this solution, add 2 g of *potassium iodide* and 3 ml of 2N *hydrochloric acid* and titrate with the *sodium-thiosulphate solution* using *starch solution*, added towards the end of the titration, as indicator until the blue colour is discharged. Each 0.002784 g of *potassium bromate* is equivalent to 1 ml of 0.1N *sodium thiosulphate*.

Note: Re-standardise 0.1N *sodium thiosulphate* frequently.

**Starch Solution:** Triturate 0.5 g of soluble starch AR, with 5 ml of *water*, and add this, with constant stirring, to sufficient *water* to produce about 100 ml. Boil for a few minutes, cool, and filter. Solution of starch must be recently prepared.

**Sulphuric acid-**  $\text{H}_2\text{SO}_4 = 98.08$  (7664-93-9)

When no molarity is indicated, use analytical reagent grade of commerce containing about 96 per cent w/w of sulphuric acid and about 18 M in strength; an oily, corrosive liquid; weight per ml about 1.84 g

When solutions of molarity xM are required, they should be prepared by carefully adding 54x ml of *sulphuric acid* to an equal volume of *water* and diluting to 1000 ml with *water*.

When '*sulphuric acid*' is followed by a percentage figure, an instruction to add, carefully, *sulphuric acid* to *water* to produce the specified percentage v/v (or, if required, w/w) proportion of *sulphuric acid* is implied.

**Toluene-** Methylbenzene;  $\text{C}_7\text{H}_8 = 92.14$  (108-88-3)

Analytical reagent grade of commerce; A colourless liquid with a characteristic odour; weight per ml 0.865 to 0.870 g; boiling point about  $110^\circ$

**Water**

HPLC Grade, Ultra-pure water

*Water, carbon dioxide-free:* Water that has been boiled vigorously for a few min and protected from the atmosphere during cooling and storage

**Zinc acetate**

Analytical grade reagent of commerce

*Zinc acetate solution 0.05M* - Dissolve 10.9690 g of *zinc acetate* in 50 ml *purified water* and few drops of *glacial acetic acid* and dilute to 1000 ml.

**APPENDIX-6**

**LIST OF SINGLE DRUGS USED IN FORMULATION**

**List of Single Drugs of Plant origin used in Formulations, with Botanical Nomenclature**

Darchini	<i>Cinnamomum verum</i> J. Presl syn. <i>Cinnamomum zeylanicum</i> Blume.
Filfil Siyah	<i>Piper nigrum</i> L.
Rehan	<i>Ocimum tenuiflorum</i> L. syn. <i>Ocimum sanctum</i> L.
Zanjabeel	<i>Zingiber officinale</i> Rosc.

**APPENDIX-7**

**List of Diseases/Technical terms and their English equivalents**

<b>Disease/Technical Terms</b>	<b>English Equivalent</b>
Butlan al-Dhawq / Fasad al-Dhawq	ageusia/dysgeusia
Nazla-o-Zukām	coryza and catarrh
Şihhat Afzā	health promotion
Su‘āl	cough
Su‘al-Haḍm	dyspepsia
‘Ushr al-Tanaffus	difficulty in breathing

**APPENDIX-8**  
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