UINADS:API-2/2020/KC/1.0

PHARMACOPOEIAL MONOGRAPH

OF

AYUSH KVĀTHA CŪRṆA

THE AYURVEDIC PHARMACOPOEIA OF INDIA

PART-II (FORMULATIONS)

Government of India

Ministry of AYUSH

Pharmacopoeia Commission for Indian Medicine &Homoeopathy

2020

PDH……...

UINADS: API-2/2020/KC/1.0

On behalf of : 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 Ayurvedic 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 Ayurvedic 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 Kvātha Cūrṇa" with Unique Identification Number for AYUSH Drug Standards (UINADS):API-2/2020/KC/1.0. Wherever the UINADS: API-2/2020/KC/1.0 and/or its subsequent version(s) are referred, it stands for "Pharmacopoeial Monograph of Ayush Kvātha Cūrṇa" 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. Ayurvedic Pharmacopoeia of India (API) 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 Kvātha Cūrṇa(KC) 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 Ayurvedic Formulary of India (AFI) 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 Ayurvedic Pharmacopoeia of India (API), Part-I. Where *Water* is used as an ingredient, it should meet the requirements for *Jala* (Potable water) covered by its monograph in the, API, Part-I, Vol.-VI, unless specified otherwise. 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 API, the pharmacopoeial claim is indicated by the letters API; where the ingredient has currently no monograph or it is under process, the letter API 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 as '*prakṣepa dravyas*' 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 (*Ghanasattva*) or liquids/*kvāthas*, particle size is an analytical standard and limits are recommended in the monographs, as follows:

Kvātha cūrṇa: Keeping the traditional practice of '*yavakūṭa'* as the size range for such formulations, 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 Kvāthas (decoctions) 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 *Śodhana*, 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, *p*H 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 *Bhasma/Piṣṭī/Sattva*, 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 $60F_{254}$ 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 API.

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_2H_5OH refer to percentage by volumes at 15.56°.

Temperature: Unless otherwise specified, all temperatures
Solutions: Unless otherwise specified, all solutions are
Reagents and Solutions: Reagents required for the
Filtration: Where it is directed to filter, without further
Therapeutic use(s): Therapeutic uses of the Formulation
Dose(s): The doses mentioned in monograph are in the

The medical practitioner will exercise his own

Storage: Statement under the heading 'Storage'

Specific directions are given in the monograph with

Cold: Any temperature not exceeding 8°and usually

Cool: Any temperature between 8° and 25°. An

Room temperature: The temperature prevailing in a

Warm: Any temperature between 30° and 40°

Excessive heat: Any temperature above 40°

Protection from freezing: Where, in addition to the risk

Storage under non-specific conditions: Where no

Containers: The container is the device that holds the

The container is designed so that the contents may be

It provides the required degree of protection to the

The container should not interact physically or

Prior to its being filled, the container should be clean.

Light-resistant Container: A light resistant container

Well-closed Container: A well-closed container

Tightly-closed Container: A tightly-closed container

Single Unit Container: A single unit container is one

Multiple Unit Container: A multiple unit container is a

*Tamper-evident Container :*A tamper-evident container

Labelling: In general, the labelling of drugs and

INDO-ROMANIC EQUIVALENTS FOR

अ	A	a/*a*	ड	ḌA	ḍa
आ	Ā	ā/*ā*	ढ	ḌHA	ḍha
इ	I	i	ण	ṆA	ṇa
ई	Ī	ī	त	TA	ta
उ	U	u	थ	THA	tha
ऊ	Ū	ū	द	DA	da
ऋ	Ṛ	ṛ	ध	DHA	dha
ए	E	e	न	NA	na
ऐ	AI	ai	प	PA	pa
ओ	O	o	फ	PHA	pha
औ	AU	au	ब	BA	ba
ं	Ṁ	ṁ	भ	BHA	bha
:	Ḥ	ḥ	म	MA	ma
क	KA	ka	य	YA	ya

ख	KHA	kha	र	RA	ra
ग	GA	ga	ल	LA	la
घ	GHA	gha	व	VA	va
ङ	ṄA	ṅa	श	ŚA	śa
च	CA	ca	ष	ṢA	ṣa
छ	CHA	cha	स	SA	sa
ज	JA	ja	ह	HA	ha
झ	JHA	jha	क्ष	KṢA	kṣa
ञ	ÑA	ña	त्र	TRA	tra
ट	ṬA	ṭa	ज्ञ	JÑA	jña
ठ	ṬHA	ṭha			

ABBREVIATIONS FOR TECHNICAL TERMS

°C	-	-	°
Analytical	-	-	AR
concentrated	-	-	con.
gram(s)	-	-	g
hour(s)	-	-	h
kilogram(s)	-	-	kg
Kvātha Cūrṇa			Kv.
litre(s)	-	-	l
micron	-	-	µ
milligram(s)	-	-	mg
millilitre(s)	-	-	ml
Minute(s)	-	-	min
ortho	-	-	*o*
para	-	-	*p*
parts per	-	-	ppb
parts per	-	-	ppm
quantity	-	-	Q.S.
Reference	-	-	RS
Second(s)	-	-	sec
volume	-	-	vol.

volume in	-	-	v/v
weight	-	-	wt.
weight in	-	-	w/v
weight in	-	-	w/w

ABBREVIATIONS FOR PLANT PARTS

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

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Name of Laboratory	Personnel involved
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Pharmacopoeia Commission for Indian Medicine &
Gratitude is expressed for the expert advice and critical
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Efforts of scientific staff of the Commission Dr. G. V.
Involvement of Dr. Anupam Maurya, Scientific Officer
In last, thanks are due to all those who have directly

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

KVĀTHA CŪRṆA

Definition

Certain drugs or combination of drugs are made into

Method of preparation

Drugs are cleaned and dried. They are coarsely

Characteristics

The characteristic features are dried and coarsely

Directions for Preparation of Ayush Kvātha as an

Add 3 g of Ayush Kvātha Cūrṇa to 150 ml (approx.1

Note: *The specific directions for preparation of Ayush*

Mode of administration:

Consume while luke warm. Guḍa (jaggery) / Drākṣā

Storage

It should be stored in suitable air-tight container

APPENDIX - 1

APPARATUS FOR TESTS AND ASSAYS

Definition:

Ayush Kvātha Cūrṇa is a

Formulation composition:

1.	Tulsi	*Ocimum*	Lf.	Kv.Cū.	4
2.	Dalchini	*Cinnamomum*	St.	Kv.Cū.	2
3.	Sunthi	*Zingiber*	Rz.	Kv.Cū.	2
4.	Krishna	*Piper*	Fr.	Kv.Cū.	1

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

Identification:

Microscopy (Appendix 2)*:*
Take about 2 g of coarse
Fragments of upper and lower
Thin-layer Chromatography:
Carry out *Thin-layer*

Physico-chemical parameters:

Loss on drying at 105°: Not

Other requirements:

Microbial limits: Complies with

Storage:

Store in a cool place in

Important Therapeutic uses:

Pācana, Kāsa, Śvāsa,

Dose:

3 g once or twice a day in

Directions for Preparation:

Add 3 g of Ayush Kvātha

Note: The specific

Mode of administration:

Consume while luke warm.

Precaution:

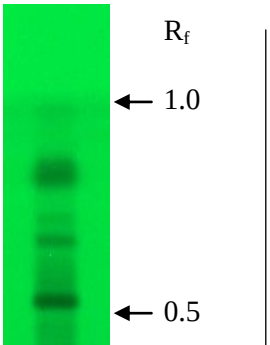
Pregnant women should take

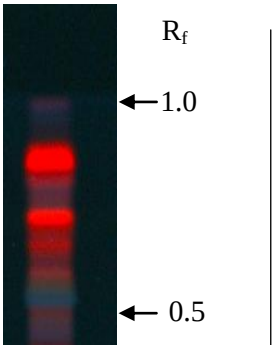
1.1. Sieves

Sieves for pharmacopoeial

Sieves conform to the

IS 460 (Pt I) (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





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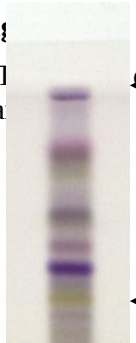
Exa



of metal wire

a. 5.60 mm IS Sieve

b. 425 μm IS Sieve

Nominal aperture sizes of 1 mm

Glycerin, Pure
Glycerin Purified; A clear
Glycerin: Dilute the Glycerin
Iodine in Potassium iodide

APPENDIX-3

QUANTITATIVE PHYSICO-CHEMICAL TESTS, ASSAYS AND INSTRUMENTATION TECHNIQUES

1.2. Thermometers

Unless otherwise specified,
The thermometers are of the

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

An instrument consisting of

1.4. Volumetric Glassware

Volumetric apparatus is
Pharmacopoeial assays involving

1.5. Nessler Cylinders

Nessler cylinders which are

1.6. Weights and Balances

Pharmacopoeial tests and assays

1.7. Muslin Cloth

Muslin cloth is a cotton fabric
Method: Take a cardboard or

Phloroglucinol solution: 1 g of
Safranin: A one per cent
Sudan IV: Dissolve 0.5 g of

3.1 Determination of Quantitative data

3.1.1 Determination of Loss on Drying:

Dry the evaporating dish for

3.1.2 Determination of Total Ash:

Incinerate about 2 to 3 g,

3.1.3 Determination of Acid-insoluble Ash:

To the crucible containing

3.1.4 Determination of Alcohol-soluble Extractive:

Macerate 5 g of the air-dried

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
Monographs where the test is
Stains and Reagents for
If for some reason the
Chloral Hydrate Solution:

For example, a quantity of

3.1.5 Determination of Water-soluble Extractive:

Proceed as directed for the

3.1.6 Determination of Volatile Oil in Drugs:

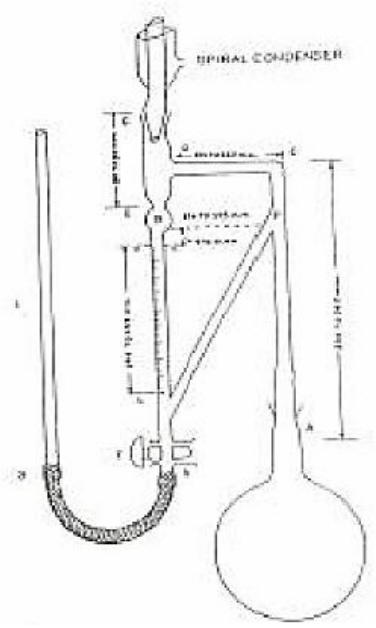


Fig. 1: Apparatus for volatile

The determination of volatile

3.1.7 Determination of pH Value

The *p*H value of an aqueous

To standardize the *p*H

3.2 Thin-Layer Chromatography (TLC):

Thin-layer chromatography is

Identification can be effected by

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 µm in diameter is suitable for chromatography. It can be applied directly to the plate or can be bonded to the plate by means of plaster of paris (hydrated *calcium sulphate*) or with any other suitable 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 µl and less.

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

(i) An ultra-violet light, suitable for observation at short (254 nm) and long (366 nm) ultra-violet wavelengths.

Preparation of plates: Unless

Method:

Unless unsaturated conditions

For two-dimensional

When the method prescribed

Visualisation:

The phrases *ultra-violet light*

APPENDIX-4

LIMIT TESTS

R_f Value:

Measure and record the

4.1. Microbial Limit Tests

Table-1: Microbial

Sl.	Parameters	Permissible	Permissible
1	*Staphylococcus*	Absent	-
2	*Salmonella*	Absent	Absent
3	*Pseudomonas*	Absent	-
4	*Escherichia coli*	Absent	10
5	Total	1 0^5/g*	10^7
6	Total Yeast	10^3/g	10^5

*For topical use, the limits

The following tests are
Preliminary Testing: The
Alternatively, repeat the test as
If in spite of incorporation

Media

Culture media may be
Where agar is specified in a

Baird-Parker Agar

Pancreatic digest of	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
Add sterile saline solution,

Bismuth Sulphite Agar

Solution (1)

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

Dissolve with the aid of heat

Solution (2)

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

Mix, heat to boiling, cool to
Bismuth Sulphite Agar

Brilliant Green Agar

Peptone	10.0 g
Yeast extract	3.0 g
Lactose	10.0 g
Sucrose	10.0 g
Sodium chloride	5.0 g
Brilliant green	12.5
Agar	12.0 g
Sodium chloride	5.0 g
Water to	1000

Mix, allow to stand for 15

Buffered Sodium Chloride-

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

0.1 to 1.0 per cent w/v

Casein Soyabean Digest

Pancreatic digest of	15.0 g
Papaic digest of	5.0 g
Sodium chloride	5.0 g
Agar	15.0 g
Water to	1000

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

Cetrimide Agar

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

Heat to boiling for 1 minute

Desoxycholate-Citrate Agar

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	5.0 g
Neutral red	0.02 g
Agar	12.0 g
Water to	1000 ml

**Note:* Sterilize at 121° for 15 minutes in an

Mix and allow to stand for 15
Care should be taken not to

Lactose Broth Medium

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

Adjust the *p*H after

Levine Eosin-Methylene

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

Dissolve the pancreatic digest

MacConkey Agar

Pancreatic digest of	17.0 g
Peptone (meat and	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
Crystal violet	1.0
Water to	1000

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

MacConkey Broth

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

Adjust the pH after

Mannitol-Salt Agar

Pancreatic digest of	5.0 g
Peptic digest of animal	5.0 g
D-Mannitol	10.0 g
Sodium chloride	75.0 g
Agar	15.0 g
Phenol red	25
Water to	1000

Mix, heat with frequent

Nutrient Agar Medium:

Nutrient Broth Medium

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

Dissolve with the aid of heat.

Pseudomonas Agar Medium for

Pancreatic digest of	10.0 g
Peptic digest of animal	10.0 g
Anhydrous dibasic	1.5 g
Magnesium sulphate	1.5 g
Glycerin	10.0 ml
Agar	15.0 g
Water to	1000

Dissolve the solid components

Pseudomonas Agar Medium for

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

Dissolve the solid components

Sabouraud Dextrose Agar

Dextrose	40.0 g
Peptic digest of animal	10.0 g
Agar	15.0 g
Water to	1000 ml

Mix, and boil to effect

Sabouraud Dextrose Agar

To 1 litre of Sabouraud

Selenite F Broth

Peptone	5.0 g

**Note:* Sterilize at 121° for 15 minutes in an

Disodium hydrogen	10.0 g
Sodium hydrogen	4.0 g
Water to	1000

Dissolve, distribute in sterile

Tetrathionate-Bile-Brilliant

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

Heat just to boiling; do not

Triple Sugar-Iron

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
Water to	1000

Mix, allow standing for 15

Urea Broth Medium

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

Mix, sterilize by filtration

Vogel-Johnson Agar

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

Boil the solution of solids for

Xylose-Lysine-Desoxycholate

Xylose	3.5 g
l-Lysine	5.0 g
Lactose	7.5 g
Sucrose	7.5 g
Sodium chloride	5.0 g
Yeast extract	3.0 g
Phenol red	80.0 mg
Agar	13.5 g
Sodium desoxycholate	2.5 g
Sodium thiosulphate	6.8 g
Ferric ammonium	800 mg
Water to	1000 ml

Heat the mixture of solids

Sampling: Use 10 ml or 10 g

Precautions: The microbial limit

4.1.1 Total Aerobic Microbial Count:

Pretreat the extracts and raw

Note: The raw material needs

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 *p*H 7.0 or any other suitable medium shown to have no antimicrobial activity under the conditions of test and adjust the volume to 100 ml with the same medium. If necessary, adjust the *p*H to about 7.

Products insoluble in water

Fatty products: Homogenise

Examination of the sample:

Membrane filtration: Use

Incubate the plates for 5 days,

Table-2: Most Probable

Observed Number of mg (or			Most microorganisms
100 (100	10 (10	1 (1 µl)	
3	3	3	>1100
3	3	3	1100

**Note:* Sterilize at 121° for 15 minutes in an

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:

Plate count for fungi:

Multiple-tube or serial

4.1.2 Tests for Specified Microorganisms:

Pre-treatment of the extract

Escherichia coli: Place the

Primary test: Add 1.0 ml of

Secondary test: Add 0.1 ml of

Carry out a control test by

***Salmonella*:**Transfer a quantity of the

Table-3: Test for *Salmonella*

Medium	Descriptionof colony
Bismuth	Black or green
Brilliant green	Small, transparent
Deoxycholate-	Colourless and
Xylose-lysine-	Red with or

Primary test: Add 1.0 ml of

Secondary test: Subculture any

Carry out the control test by

Pseudomonas aeruginosa: Pre-

Streak representative suspect

Table-4: Tests for

Medium	Characteristic colonial morphology	Fluorescence in UV	Oxidase test	Gram
Cetrimide	Generally	Greenish	Positive	Negative
Pseudomonas medium of	Generally colourless to yellowish	Yellowish	Positive	Negative
Pseudomonas medium of	Generally greenish	Blue	Positive	Negative

Staphylococcus aureus: Proceed

Incubate in water-bath at 37°

Table-5: Tests for

Selective	Characteristic	Gram stain
Vogel-	Black	Positive (in
Mannitol-	Yellow colonies	Positive (in
Baird-	Black, shiny, of 2 to 5 mm	Positive (in

Validity of the tests for total

Grow the following test

Staphylococcus (ATCC 6538;

Bacillus (ATCC 6633;

Escherichia coli (ATCC 8739;

Candida (ATCC 2091;

Dilute portions of each of the

A count for any of the test

Validity of the tests for

4.2 Test for Aflatoxins (HPTLC Method):

Table-6: Permissible Limit

Sl.	Aflatoxin	Permissible Limit
1	B_1	< 2 ppb
2	$B_1+B_2+G_1+G_2$	< 5 ppb

Caution: Aflatoxins are highly

Zinc Acetate - Aluminum

Sodium Chloride Solution:

Test Solution 1: Transfer

Test Solution 2: Collect 100

Clean-up Procedure: Place a

Aflatoxin Solution: Dissolve

Procedure: Separately apply

4.3 Pesticide Residue:

Definition: For the purposes of

Table-7: Permissible Limits

Substance	Limit
Alachlor	0.02
Aldrin and Dieldrin	0.05
Azinphos-methyl	1.0
Bromopropylate	3.0
Chlordane (sum of *cis*-,	0.05
Chlorfenvinphos	0.5
Chlorpyrifos	0.2
Chlorpyrifos-methyl	0.1
Cypermethrin (and	1.0

Substance	Limit
DDT (sum of *p,p'*-	1.0
Deltamethrin	0.5
Diazinon	0.5
Dichlorvos	1.0
Dithiocarbamates (as CS_2)	2.0
Endosulfan (sum of	3.0
Endrin	0.05
Ethion	2.0
Fenitrothion	0.5
Fenvalerate	1.5
Fonofos	0.05
Heptachlor (sum of	0.05
Hexachlorobenzene	0.1
Hexachlorocyclohexane	0.3
Lindane (γ-	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	1.0

Note: Apart from the above,

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

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 µg/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 μm), 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 and7.8 mm in internal diameter packed with styrene-divinylbenzene copolymer (5 μm)
- As mobile phase *toluene* at a flow rate of1 ml/min

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

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

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 to1.5 per cent of the mass of silica gel used; shake vigorously until agglomerates have disappeared and continue shaking for 2 h using a mechanical shaker. Condition the column using 1.5 ml of *hexane.* Prepacked columns containing about 0.50 g of a suitable silica gel may also be used, provided they are previously validated.

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

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 of50 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 μl with *toluene.*

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

The chromatographic procedure may be carried out using:

- a fused-silica column 30 m long and 0.32 mm in internal diameter the internal wall of which is covered with a layer 0.25 μm thick of poly (dimethyl) siloxane

- *hydrogen for chromatography* as the carrier gas. Other gases such as helium for chromatography or *nitrogen for chromatography* may also be used provided the chromatography is suitably validated.
- a phosphorus-nitrogen flame-ionisation detector or 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
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 eradiated 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 1per cent *ammonium dihydrogen phosphate* and 0.2 per cent *magnesium nitrate*, shake well, pipette accurately10-20 μl 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 μg 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 μl the above solutions respectively, inject them into the graphite oven, determine their absorbance, and then draw the calibration curve with absorbance as vertical axis and concentration as horizontal ordinate.

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

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

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

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

Preparation of As standard stock solution: Measure accurately a quantity of As single element standard solution to prepare standard stock solution with 2 per cent *nitric acid* solution, which contains 1.0 μg 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 with2 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;$(CH_3)_2CO$ = 58.08 (67-64-1)

Analytical reagent grade of commerce; A volatile, flammable liquid; boiling point about 56°; 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; CH_3CN = 41.05

General laboratory reagent grade of commerce; Colourless liquid; boiling point about 81°;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 - C_2H_5OH

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

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°

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° 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° 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° 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° 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 *Nesseler cylinder*, and titrate with 0.05 N *sodium hydroxide* until the colour matches that of the remainder of the *hydroxylamine hydrochloride solution* contained in a similar cylinder, both solutions being viewed down the axis of the cylinder. Not more than 0.9 ml of 0.05 N *sodium hydroxide* is required.

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

Non-volatile matter: Evaporate 40 ml in a tared dish on a water-bath and dry the residue at 105° 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 a 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: H_3N = 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 - NH_4Cl

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 - $NH_4H_2PO_4$ = 115.03

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

Ammonium Nitrate - NH_4NO_3 =80.04

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

Ascorbic acid - $C_6H_8O_6$ =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 $C_6H_8O_6$.

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 - $CaCl_2.2H_2O$=147.01

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

Carbophenothion: $C_{11}H_{16}ClO_2PS_3$ = 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; $CHCl_3$= 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°; d_{20}^{20} 1.475 to 1.481

Chromotropic Acid - $C_{10}H_8O_8S_2$

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 - $C_4H_{10}O$ = 74.12 (60-29-7)

Analytical reagent grade of commerce; A volatile, highly flammable, colourless liquid; boiling point, 34° to 35°; d_{20}^{20}, 0.7 13 to 0.7 15; 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-$Fe(NH_4)(SO_4)_2,12H_2O$ 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-$NH_2OH.HCl$ = 69.49

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

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. 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_3H_8O = 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 $(NO_3)_2.6H_2O$ = 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 iodidein 20 ml of water, mix and add sufficient water to produce 100 ml.

Mercuric Chloride- $HgCl_2$ = 271.50

Contains not less than 99.5 per cent of $HgCl_2$

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

Solubility: Soluble in water; freely soluble in alcohol

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

Assay: Weigh accurately about 0.3 g and dissolve in 85 ml of *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 $HgCl_2$.

Mercuric Sulphate- $HgSO_4$

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; CH4O = 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

Methyl Orange: Sodium-p-

An orange-yellow powder

*Methyl Orange Solution***:**

Test for sensitivity: A mixture

Colour change: pH 3.0 (red)

Methyl red-p-

A dark red powder or

Methylene chloride -

Analytical reagent grade of

n-Hexane - Hexane = 86.18

Analytical reagent grade of

Nitric acid*:* HNO_3 = 63.013

Contains 70.0 per cent w/w of

Description: Clear, colourless,

Weight per ml: At 20°, 1.41

Copper and Zinc: Dilute 1 ml

Iron: 0.5 ml of complies

Lead: Not more than 2 ppm

Chlorides: 5 ml neutralised

Sulphates: To 2.5 ml, add 10

Sulphated ash: Not more than

Assay: Weigh accurately

Nitric Acid, xN: Solutions of

Nitric Acid, Dilute: Contains

Oracet blue, Solvent blue

Analytical reagent grade of

Oxalic Acid - $(COOH)_2.2H_2O$

Analytical grade reagent of

Perchloric Acid- $HClO_4$ =

A solution in *purified water*

Clear, colourless liquid; very

Phenolphthalein - $C_{20}H_{14}O_4$

A white to yellowish-white

Phenolphthalein Solution:

Test for sensitivity: To 0.1

Colour change: pH 8.2

Phosphoric Acid - H_3PO_4=

Description: Clear, colourless

Solubility: Miscible with water

Phosphoric Acid, xN: Solutions

Phosphoric Acid, Dilute:

Potassium iodide -KI = 166.0

Analytical reagent grade of

Potassium Iodide and Starch

Potassium permanganate–

Description: Dark purple,

Solubility: Soluble in *water*;

Chlorides and Sulphates:
Assay: Weigh accurately
Storage: Store in well-closed
Caution: Great care should be
Potassium Permanganate
Potassium Permanganate, 0.1
3.161 g in 1000 ml
Dissolve about 3.3. g of
Sodium bicarbonate
Description: White, crystalline
Solubility: Freely soluble in
*Carbonate*s: *p*H of a freshly
Aluminium, calcium and
Arsenic: Not more than 2 parts
Iron: Dissolve 2.5 g in 20 ml
Heavy metals: Not more than
Chlorides: Dissolve 1.0 g in
Sulphates: Dissolve 2 g in
Ammonium compounds: 1 g
Assay: Weigh accurately
Storage: Store in well-closed
Sodium Bicarbonate Solution:
Sodium Bisulphite - $NaHSO_3$
Sodium Bisulphite Solution:
Sodium borohydride$NaBH_4$
Analytical reagent grade of
Caution: The heat of this

Sodium carbonate-$Na_2CO_3.10H_2O$ = 286.2 (6132-02-1)

Analytical reagent grade of commerce; Melting point greater than 300°

Sodium chloride - 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–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 a2.5 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 cobalt nitrite 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 Na_2CO_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 - Na_2SO_4= 142.0 (7757-82- 6)

Analytical reagent grade of commerce complying with the following test:

Loss on drying: When dried at 130°, loses not more than 0.5 per cent of its weight

Sodium Thiosulphate - $Na_2S_2O_3.5H_2O$ = 248.17

Description: Large colourless crystals or coarse, crystalline powder; odourless; taste saline;

deliquescent in moist air and effloresces in dry air at temperature above 33°

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 starchAR, 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 starchmust be recently prepared.

Sulphuric acid- H_2SO_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; C_7H_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°

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 z*inc acetate* in 50 ml *purified water* and fewdrops 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

Marica	*Piper nigrum* L.
Tulasī	*Ocimum tenuiflorum* L. syn. *Ocimum sanctum* L.
Tvak	*Cinnamomum verum* J. Presl syn. *Cinnamomum zeylanicum* Blume.
Śuṇṭhī	*Zingiber officinale* Rosc.

APPENDIX-7

LIST OF DISEASES/TECHNICAL TERMS AND THEIR ENGLISH EQUIVALENTS

Disease/Technical Terms	English Equivalent
Kāsa	cough
Pācana	enhancing digestion
Pratiśyāya	cold/catarrah
Śvāsa	dyspnoea
Ūrjaskara	health promoting

APPENDIX-8

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