THE AYURVEDIC PHARMACOPOEIA OF INDIA
Foreword
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LEGAL NOTICES

In India, there are laws dealing with drugs that are the subject of monographs which follow. These monographs should be read subject to the restrictions imposed by these laws wherever they are applicable.

It is expedient that enquiry be made in each case in order to ensure that the provisions of the law are being complied with.

In general, the Drugs & Cosmetics Act, 1940 (subsequently amended in 1964 and 1982), the Dangerous Drugs Act, 1930 and the Poisons Act, 1919 and the rules framed there under should be consulted.

Under the Drugs & Cosmetics Act, the Ayurvedic Pharmacopoeia of India (API), Part-I, Vol. VII, is the book of standards for Single Drugs of Minerals, Metals and Marine origin included therein and the standards prescribed in the Ayurvedic Pharmacopoeia of India, Part-I, Vol. VII, would be official. If considered necessary, these standards can be amended and the Chairman of the Ayurvedic Pharmacopoeia Committee authorized to issue such amendments. Whenever such amendments are issued, the Ayurvedic Pharmacopoeia of India, Part-I, Vol. VII, would be deemed to have been amended accordingly.
GENERAL NOTICES

These General Notices provide basic guidelines to help, interpret the provisions and terms used in this volume, in connection with the implementation of the recommended standards and methods of tests prescribed therein. In addition, they also include the features of a monograph that are mandatory standards.

Title: The title of this book is "Ayurvedic Pharmacopoeia of India, Part-I, Vol.VII (Minerals and Metals)". Monographs on twenty-one raw drugs (minerals / metals / ores) are included in this volume.

Monograph: Each Monograph carries prescribed mandatory standards and general information, including those from Ayurvedic Classics. The Monographs are arranged in the English alphabetical order, based on the main Samskṛta official name.

Monograph Title: The title given on top of each monograph indicates the name of the material in Samskṛta, and is the same as mentioned in the single drugs list on the Ayurvedic Formularies for Minerals, Metals and Ores. It is the "Official Name" along with synonyms in Samskṛta. This is followed by a scientific nomenclature in English that is nearest and most appropriate to the article described, and is also "Official".

Synonyms: Taking into account the multilingual nature of the country, other names of the drug have been listed in Indian languages other than Samskṛta as far as these are available. The Urdu and Tamil equivalents, however, are those appearing for the same drug in their respective Formularies.

Broad Classification: The raw materials obtained from the earth's crust and used in the indigenous systems of medicines are of great complexity, being a mixture of various elements, compounds and gangue (waste rock i.e. the non-metalliferous part of an ore, not useful for the intended purpose). They may be chemically classified only in a broad sense, and exact chemical nomenclature cannot be assigned. Therefore, a broad classification, such as Silicate, Sulphide, Oxide and Metal etc., is added for basic information.

Origin and occurrence: The nature and forms in which a raw drug occurs in the earth's crust and its association with other minerals and rocks are included to give an idea about geology of the substance. The occurrence and availability of the article in India are also incorporated. This important information reflects the commercial potential of the article as a source for the drug required by the Ayurvedic, Siddha & Unani practitioners, and to the manufacturers.

Standards: For mandatory purposes, the statements made under the following headings in a monograph shall constitute "Official Standards", ........Title, Definition, Identification (Physical, Optical and Chemical properties), Tests and Assays, Distinction from substitutes and adulterants, Heavy metals, and the "Śodhana" process described and recommended in classics quoted in Appendix -5.
Physical Properties: These include nature, colour, streak, cleavage, fracture, lustre, tenacity, transparency, hardness, specific gravity, taste and magnetism. These are reliable and easy identification features for minerals and metals. Fluorescence and XRD analysis applied for identification of a few articles have also been included. Physical properties serve as the preliminary but powerful parameters for identification of raw drugs. Definitions of geological terminology used in the monographs and methods of determination of various parameters are given in Appendix-1.

Optical Properties: These include the application of optical crystallography in identification of minerals that are crystalline and transparent, irrespective of their chemical composition. This is a time-tested petrographic/mineralogical method of analysis using thin sections of mineral or ore samples, but adapted and applied to powdered materials to suit pharmacopoeial requirement. Several parameters can be computed from only a few fragments using immersion liquids of known Refractive Indices, by a method known as the ‘Immersion Method’. Description of the principle and the method of determination of various constants are given in detail in Appendix-2.

Chemical Properties: Under this, confirmatory identification tests for raw drugs are mentioned. These include charcoal test, flame colouration test, effect of heat, solubility in water and acids and reaction with different acids. The most important, however, are determination of assays for major ingredients and minor constituents, presence of heavy metals and other elements of significance. Details of various tests and methods of determination of elemental assays etc., Details about Chemical & Reagents are given in Appendix-4.

General:

a) Except a few very simple chemical tests which have been given in monographs, all methods of determination of physical, optical and chemical properties are given in detail in the Appendices.

b) Instructions, explanations of technical terms, and the methods, given in different Appendices, are the standard ones employed in the identification of the ores, minerals, metals and inorganic substances. More than one method for determination of a particular parameter in a monograph is given in this volume and the choice of method to be adopted is left to the user, provided the actual method used is on record with laboratory data.

Miscellaneous:

Temperature: Except where specifically mentioned, ambient temperature in Celsius scale is intended.

Pressure: Atmospheric pressure of 760 torr at $0^\circ$, unless stated otherwise.
**Weight and Measures:** 'Constant Weight' implies that under the given circumstances, no two consecutive weights shall differ by more than 0.5 mg per gram of substance taken for analysis. 'Weight taken for analysis' is the amount to be taken for quantitative analysis stated in the procedure within an approximate range, but shall be accurately weighed and shall not differ from the stated weight by more than 10 percent.

**Abbreviations,** used in the monographs, are given in a table on next page.
### Abbreviations for Technical Terms

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<th>Abbreviation</th>
<th>Full Form</th>
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<td>$^0C$</td>
<td>$^0$ Celsius</td>
</tr>
<tr>
<td>$2^d \sin \theta$</td>
<td>$\eta\lambda$</td>
</tr>
<tr>
<td>analytical reagent</td>
<td>AR</td>
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<tr>
<td>atomic absorption spectrophotometry</td>
<td>A.A.S.</td>
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<tr>
<td>gram(s)</td>
<td>g</td>
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<tr>
<td>inductively coupled plasma analyser</td>
<td>ICPA</td>
</tr>
<tr>
<td>kilo newton (pressure unit)</td>
<td>kN</td>
</tr>
<tr>
<td>microgram(s)</td>
<td>$\mu$g</td>
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<tr>
<td>millilitre(s)</td>
<td>ml</td>
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<tr>
<td>milligram(s)</td>
<td>mg</td>
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<tr>
<td>nanogram($10^{-9}$ g)(s)</td>
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<tr>
<td>nanometer(s)</td>
<td>nm</td>
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<tr>
<td>part(s) per million or gram(s) per tonne</td>
<td>ppm</td>
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<tr>
<td>precipitate</td>
<td>ppt.</td>
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<tr>
<td>quantity sufficient</td>
<td>Q.S.</td>
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<tr>
<td>refractive index</td>
<td>R.I.</td>
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<td>refractive index</td>
<td>$\eta$</td>
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<tr>
<td>solution</td>
<td>sol.</td>
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<td>specific gravity</td>
<td>Sp. Gr.</td>
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<td>ultra-violet</td>
<td>UV</td>
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<td>Text</td>
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<tr>
<td>Caraka Sa¹hitā Sūstrasthāna</td>
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<td>National Formulary of Unani Medicines</td>
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<tr>
<td>Rasendracū²āma ’i</td>
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<tr>
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### INDO-ROMANIC EQUIVALENTS OF DEVANĀGARI ALPHABETS

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PREFACE

Ayurveda is very much rich in many aspects and the number of drugs and their sources. The use of a few minerals and metals in Ayurvedic medicines is since Vedic period but scientific and systematic studies were conducted from 8th century onwards forming an integral part of Ayurvedic medicines called Rasaśāstra and the formulations called Rasyogas.

The brief history of Rasaśāstra reveals many aspects of this science when viewed in perspectives of identification, Śodhana (Purification), Māráa (Calcination), indications and dietetic regimen.

Caraka has used ‘Rasa’ in Kustha cikitsā but it is not sure whether it is Pārada or else. Six metals viz. Svaŕa, Rajata, Tāmra, Loha, Vaṣa and Nāga have been coined under drugs of mineral origin. These metals have been indicated for preparation of different devices like Bastinetra, Dhūmanetra, etc. Specialized procedures like Lohādi Rasāyana, Ayaskī has been described so that these metals can be converted into a consumable and efficacious dosage form for therapeutic use. For this, the thin sheets of metals are repeatedly heated and quenched in a series of liquids viz. Triphalā kvātha, Gomūtra, Godugdha, Laváajala, etc. until conversion to fine powder. Apart from metals, various other minerals like Gandhaka, Svaŕamāksika, Haritāla, Manaśilā, Ma²āra, Gairika, etc.; alloys viz. Kāṣya, Pittala and gems viz. Mukta, Pravāla have been mentioned for therapeutic and other uses. Suśruta, in addition to all this, has used Pārada (first non-ambiguous reference) tropically.

A 圻 gasa ṣraha, for the first time, clearly indicates administration of Pārada for its Rasāyana effect. Blowing up metal in a crucible to convert into powder form has also been described by him.

Almost of the marked developments of Rasaśāstra have taken place in this period (from 8th century A. D.). The science attended its peak of advancements and opened new horizons in the field of medicine. Several Rasasiddhas (disciples of Tāntrika doctrine) have contributed to this process. The outcomes of their extensive experimentations have been documented in the form of texts. Nāgārjuna was the most significant contributor to establish the aims, principles and methodologies of Rasaśāstra in an organized and systematic manner. Numerous texts viz. Rasaratnākara, Rasendrama ṣrāla, Rasāŕava have been composed in this period explaining Śodhana, Mārana, properties, therapeutic uses, dose specifications and various formulations of Rasauḍhis. The Śodhana and Māráa methods may either be characteristic for some specific material or applicable to a group/category. Moreover,
specifications for construction of a pharmacy have also been given instructing specific
directions for specific procedures and arrangements required inside and outside the pharmacy
campus.

Mainly three types of Physical treatments are adapted in metal/mineral preparations i.e. Heat,
Beat and Treat. Here the treat means the material is treated with many organic preparations,
the sequence of physical treatments may be varied considering the type of material and
formulation but the sequence may get changed.

In compiling the monographs, the title of each drug had been given in Sanskrit as already
obtained in the Ayurvedic Formulary of India. Then comes the definition physical properties/
Explanations of Geological/ Technical terms – Terminology used in pharmacopoeial
Monographs and methods of determination of the drug giving its identity in scientific
nomenclature and very brief information about its source, occurrence, distribution and
precautions in collection if any, etc.

This is followed by a list of synonyms in Saṁskāra and also the other Indian regional
languages. The monographs then record the Definitions and Methods of determination of
different optical parameters followed by Chemical properties along with qualitative chemical
tests of minerals by Blow pipe method. Apart from this, the distinct photographs of all
included materials are exhibited.

Since the efforts is to compile pharmacopoeial monographs of Ayurvedic drugs, in the accent
of the classical attributes of respective drugs according to the doctrine of Rasa, Gu ā, Vīrya,
Vipāka and Karma has not been lost sight of, though some of them appear to be abstract and
subjective in the absence of established experimental methods to quantify them.

The Legal Notices and General Notices have been given for guidance of the analysts, the
Pharmaceutical suppliers and manufactures and the research workers engaged in this field.
Details about the apparatus, reagents and solutions, tests, methods of preparation of
specimens for microscopic examinations have been given in the Appendices.

The Committee hopes that with the publication of Ayurvedic Pharmacopoeia of India Part I,
Vol. VII comprising of 21 single drugs of mineral, metal origin, as per the format and
procedure laid down, the different research units under Deptt. of AYUSH under the Ministry
of Health and Family Welfare and the other involved laboratories would plan their research
enquiries such that the output of work would be accelerated.
The Committee urges the Government of India to recommend the adoption of these monographs for the purposes of identity, purity and strength of drugs for use in their Government, Semi-Government and Government aided institutions and voluntary public organizations. The Ayurvedic Pharmacopoeia of India, Part-I, Vol. VII may also be notified by Government as a book of reference for implementation of the Drugs and Cosmetics Act, 1940 all over India as Ayurvedic Pharmacopoeia of India Part-I, Vol. I, II, III, IV and V is already included in the First Schedule of Drugs and Cosmetics Act 1940.

This volume is specific in nature consisting of monographs on raw materials of mineral and metals used in the mineral, metallic and herbo-mineral /metallic preparations.

Prof. S.S.Handa                              Dr. S.K.Sharma                              Dr. G.S.Lavekar  
Chairman                                  Vice-Chairman                        Member Secretary  
XIX
ACKNOWLEDGMENT

The Ayurvedic Pharmacopoeia committee records and duly appreciates the contributions made by the project officers and scientific staff of the participating institutions associated with the APC project works for developing quality standards of Minerals and Metals.

The committee expresses gratitude of Ms. Anita Das, Secretary, and Shri Shiv Basant, Joint Secretary, Department of AYUSH for providing constant support for completion of this work and its further continuation and also sincerely thanks to Dr. M.M. Padhi, Deputy Director [Tech.]; Shri. Vasantha Kumar, Asst. Director [Chem.] Dr. Pramila Pant, Research Officer [Chem.], Dr. M.N. Rangne, Dr. Chhote Lal, Dr. AKS Bhadoria and Dr. Bishnupriya Dhar, Research Officer [Phar], Dr. Nikhil Jirankalgikar S.R.F. (Ayu.), Dr. Rajesh Singh S.R.F. [Ayu.], Dr. Sandhya Rani S.R.F. [Ayu.], Mr. Chinmay Rath S.R.F. [Bot.] for their constant efforts in bringing out this volume. Thanks to Mr. Ashish Kumar, Ms. Meenakshi Thakur, Ms. Deepti Anand D.E.O., who took pains in typing and arranging all the technical data into a final shape.
INTRODUCTION

General

The Ayurvedic system of medicine is prevalent in India since the Vedic period and as early as the dawn of human civilization. Though Ayurveda has undergone many changes in the course of its long history, it still remains the mainstay of medical relief to a large section of population of the nation. Due to urbanization and dwindling of forests, the Vaidya by and large is no longer a self contained unit collecting and preparing his own medicines as before. He has now to depend on the newly developed agencies like one collecting and supplying the crude drugs and the other undertaking mass production of medicines in the Ayurvedic Pharmaceutical units run on commercial scale.

2. In view of the new trend in Ayurvedic Pharmaceutical field, Government of India considered it expedient to utilize the existing Drug and Cosmetics Act 1940, to also control to a limited measure the Ayurvedic, Siddha and Unani drugs by amending the Act.

3. The Act was accordingly amended in 1964, to ensure only a limited control over the production and sale of these medicines namely:-

   i. The manufacture should be carried under prescribed hygienic conditions, under supervision of a person having a prescribed qualification;

   ii. The raw materials used in the preparation of drugs should be genuine and properly identified; and

   iii. The formula or the true list of all the ingredients, contained in the drugs, should be displayed on the label of every container.

The present Ayurvedic Pharmacopoeia Committee (APC) was reconstituted or constituted under the Deptt. of AYUSH vide letter No.X-19011/6/94-APC (AYUSH) dated 9th March, 2005 consisting of following members.

1. Ms. Savita Satakopan, M.Sc.  
   (Former Drug Analyst),  
   Government of Gujarat,  
   7/4, Padmam Flats, Seventh Street,  
   Nanganallur, Chennai – 600 061.  
   Chairperson  
   (9th March 2005 to 22nd June 2006)

2. Prof. S.S. Handa, M. Pharma, Ph.D.,  
   (Former Director, RRL, Jammu), 522-A, Block ‘C’,  
   Sushant Lok, Phase-I,  
   Gurgaon, Haryana – 122 001.  
   Chairman  
   (23rd June, 2006 to onwards)
OFFICIAL MEMBERS

1. Dr. S.K. Sharma, M.D. (Ayu.), Ph.D. 
   Advisor (Ayurveda), 
   Department of AYUSH, 
   Red Cross Society Building, 
   New Delhi – 110 001. 
   Vice-Chairman

2. Dr. G.S. Lavekar, AVP; Ph.D. 
   Director, 
   Central Council for Research in Ayurveda & Siddha, 
   61-65, Institutional Area, 
   D-Block, Janakpuri, 
   New Delhi – 110 058. 
   Member-Secretary (Ex-officio)

3. Dr. D.R. Lohar, M.Sc.; Ph.D. 
   Director, 
   Pharmacopoeial Laboratory for Indian Medicine, 
   Central Govt. Offices Complex, Kamla Nehru Nagar, 
   Ghaziabad – 201 002. 
   Member (Ex-officio)

4. Managing Director, 
   Indian Medicines Pharmaceutical Corporation Ltd., 
   Mohan, Via – Ram Nagar, 
   Distt.- Almora, Uttranchal. 
   Member (Ex-officio)

5. Drugs Controller General (India), 
   Ministry of Health & Family Welfare, 
   Nirman Bhawan, New Delhi – 110 011. 
   Member (Ex-officio)

NON-OFFICIAL MEMBERS
Phytochemistry & Chemistry Sub-Committee

1. Prof. V.K. Kapoor, M. Pharm., Ph.D. 
   (Former Dean and Chairman, 
   University Institute of Pharmaceutical Sciences, 
   Panjab University, Chandigarh) 
   1473, Pushpac Complex, 49B, 
   Chandigarh - 160 047. 
   Chairman

2. Prof. S.S. Handa, M. Pharm., Ph.D., 
   (Former Director, RRL), 
   522-A, Block ‘C’, Sushant Lok, Phase-I, 
   Gurgaon, Haryana – 122 001. 
   Member

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3. Dr. P.D. Sethi, M. Pharm., Ph.D.,  
(Former Director,  
Central Indian Pharmacopoeial Laboratory)  
B-140, Shivalik Enclave,  
New Delhi – 110 017.  

Member

Former Chief Manager (Exploration),  
Hindustan Copper Ltd.,  
SF-8, Gayatri Nagar,  
Sector-5, Hiran Magri,  
Udaipur – 313 002. (Rajasthan).  

Member

Pharmacognosy Sub-Committee

1. Ms. S. Satakopan, M.Sc.  
(Former Drug Analyst),  
Government of Gujarat,  
7/4, Padmam Flats, Seventh Street,  
Nanganallur, Chennai – 600 061.  

Chairman

2. Dr. (Mrs.) Shanta Mehrotra, M.Sc., Ph.D.,  
Emeritus Scientist,  
National Botanical Research Institute,  
Rana Pratap Marg, P.B. No.-436,  
Lucknow – 226 001 (U.P.).  

Member

3. Dr. M.A. Iyengar, M. Pharma, Ph.D,  
Prof. of Pharmacognosy (Retd.),  
14, HIG, HUDCO, Manipal – 576 119.  

Member

4. Dr. J. Mohanasundaram, M.D.,  
Former Professor of Pharmacology  
& Deputy Director of Medical Education,  
Chennai.  

Member

Formulary Sub-Committee  
(Rasa Shastra / Bhaishajya Kalpana – Ayurvedic Pharmacy)

1. Prof. S.K. Dixit, A.B.M.S.; D. Ay. M; Ph.D.  
(Former Head, Deptt. of Rasa Shastra, BHU),  
B-3/402, Shivala, Varanasi - 221 005 (UP.).  

Chairman
2. Dr. B.L. Gaur, Ph.D.;
Vice-Chancellor,
Jodhpur Ayurvedic University, Jodhpur, Rajasthan.

Member

3. Prof. Siddhinandan Mishra, G.B.M.S.; Ph.D.
Pharmacy In-charge, SDM Ayurvedic College,
P.O. Kuthpady, Udupi – 574 118
(South Karnataka)

Member

4. Prof. Ved Vrat Sharma, H.P.A.
(Former Principal, DAV Ayurvedic College),
House No. 65, Sector-8, Panchkula, Haryana.

Member

5. Dr. P.K. Prajapati, M.D. (Ay.), Ph. D.,
Reader & Head, Deptt. of Ras Shastra,
IPGT & RA, Gujarat Ayurved University,
Jamnagar, Gujarat – 361 008.

Member

6. Dr. Narendra Bhatt, M.D. (Ay.),
Chief Executive Officer,
Zandu Pharmaceutical Works Ltd.,
70, Ghokhle Road (South), Dadar,
Mumbai – 400 025.

Member

7. Shri Ranjit Puranik,
General Manager,
Shree Dhootapapeshwar Ltd.,
135, Nanubhai Desai Road, Khetwadi,
Mumbai.

Member

Ayurveda Sub-Committee
(Single Drugs of Plants, Minerals, Metals, Animal origin)

1. Prof. V.K. Joshi, M.D. (Ay.), Ph.D.
Deprt. Dravyaguna,
Institute of Medical Sciences,
Banaras Hindu University (BHU),
Varanasi – 221 005 (U.P.).

Chairman

2. Prof. K.C. Chunekar, Ph.D.
(Former Reader, Deptt. of Dravyaguna, BHU),
18/7, Ratan Phatak,
Varanasi, (U.P.).

Member
3. Vaidya Devender Triguna, Ayurvedacharya, “PADAM SHREE”,
143-Sarai Kale Khan,
Nizamuddin East, New Delhi.  

4. Dr. M.R. Uniyal, M.D. (Ay.), Ph.D. 
(Former Director, CRIA, CCRAS), 
Director (Drugs), Maharishi Ayurved Products, 
17/18, NOIDA Export Processing Zone, 
NOIDA – 201 305.  

5. Prof. V.V. Prasad, 
Director, 
Rashtriya Ayurveda Vidyapeeth, 
Dhanvantri Bhawan, 
Road No. 66, Punjabi Bagh (West), 
New Delhi – 110 026.  

1. The term of the Committee shall be for a period of 3 years from the date of its first meeting and the members shall hold office for that period. 

2. The chairman of the APC shall have the powers to form sub-committees whenever required and to co-opt experts from outside for such sub-committees. 

3. The Committee shall have the power to frame procedures of functioning. 

4. The functions of the Committee shall be as follows:  

   i. To prepare a Ayurvedic Pharmacopoeia of India of single and compound drugs. 

   ii. To prescribe the working standards for compound Ayurvedic formulations including tests for identity, purity, strength and quality so as to ensure uniformity of the finished formulations. 

   iii. Keeping in view the time constraint, to identify such methods, procedures and plan of work as would enable to publish the formulary and standards of all commonly used drugs to be brought out in a phased manner.
iv. To prepare remaining parts of the official formulary of compound preparations from the classical texts including standardized composition of reputed institution.

v. To develop and standardize methods of preparations, dosage form toxicity profile etc.

vi. To develop Quality standards, safety, efficacy profile of Intermediates like extracts of Ayurvedic raw drugs.

vii. To develop the Quality standards, safety, efficacy profile of different parts of the plants; as well as to inclusion of new plants as Ayurvedic drugs.

viii. Any other matter relating to the Quality standards, shelf life, identification, new formulations etc.

5. The following are the targets focus of the Committee:

i. To evolve standards of single drugs mentioned in the Ayurvedic Formularies of India.

ii. To evolve standards for compound formulations mentioned in the Ayurvedic Formularies of India & other Ayurvedic formulations of National Priority.

iii. To prepare drafts Standard Operation Procedure of Manufacturing Process (SOP) of Ayurvedic Formularies of India from the classical texts and other authentic sources.
Preamble

The materia medica of the Indian Traditional Systems of Medicine is comprehensive in its resources from nature. Apart from a major share from medicinal plants, both animal products and geochemicals are in trade as well as in use by practitioners. The Formularies till now published by the Ministry of Health and Family Welfare, Govt. of India, lists about 65 substances of Minerals/Metals/Ores as raw materials for use either as single drugs or added to compound formulations. Such a list was prepared from classical medical literature in āyurveda, by eminent vaidyas and practicing āyurvedic pharmacists who were members of the earlier āyurvedic Pharmacopoeia Committees in the seventies of last century. The list was finalized after extensive tours, on-the-spot examinations and consultations with practitioners and user-pharmacies throughout the country. They also, for the first time, equated the recorded names of single drug in Sāvēk’t literature, with its modern scientific nomenclature, with an eye to the future task of evolving quality standards for such raw drugs.

Methodology

The single drug list mentioned above formed the basis for present work. A general format using appropriate geological descriptive titles was drafted by the APC. The format specified the inputs required from the collaborating laboratories under various heads, so that a general description, analytical parameters for identification, their elemental composition, methods of tests and assays and āyurvedic uses etc., would be available for each of the drugs selected. The format was sent along with a preliminary note to the invitees of a Special Committee of Experts, constituted by the Ministry under the APC. The experts were from Wadia Institute of Himalaya Geology, Dehradun, and also from āyurvedic Research Institutes and Universities like Banaras Hindu University (BHU), Varanasi and Gujarat āyurved University (GAU), Jamnagar. An Annexure to this introduction gives the details of the sittings and decisions of this committee.

As a sequel to the recommendation of this committee, further work was initiated by allotting a set of 47 Minerals/Metals/Ores to the National Institute of āyurveda (N.I.A.), Jaipur under the Director as the Principal Investigator and Shri J.K. Dhing, Former Chief Manager (Exploration), Hindustan Copper Ltd., Khetri Nagar and Dr. V.N. Rao, Associate Professor, N.I.A., Jaipur as the Co-Investigators. Using both authentic and marketed samples, analytical work was executed at the laboratories of the Indian Bureau of Mines (IBM), Ajmer and Geological Survey of India (GSI), Jaipur, with Shri Dhing as the Co-ordinator. This volume is based on the results obtained from the above collaborating laboratories and on other inputs from Shri Dhing, Miss Satakopan and the āyurvedic experts of the APC. It recommends specifications and standards for 21 raw drugs and includes other relevant details.
Structure of this Volume

A chapter on General Notices explains the rules and mechanisms that should be followed by analysts while applying the standards and tests in their work. There are various Appendices, detailing the methods of tests and assays, the reagents in use, and the equipments and apparatuses necessary. A special feature is a Glossary, to explain the geological terms used in describing the physical properties of the drugs. This is given to help analysts of pharmaceutical manufacturers and the regulatory bodies who may not be familiar with the terminology used in identification of Minerals/Metals/Ores. On the āyurvedic part of each monograph, the 'āodhana', represents the purity measures as mandatory. Unlike plant or animal products, there is no prior preparation of a mineral drug for the market, by any physical or chemical means. Purity for an āyurvedic Mineral/Metal/Ore does not imply the same as it does for other drugs and does not deal with extraneous or introduced matter. While identity and strength are dealt with by physical and chemical modern tests, the purity requirement of a pharmacopoeial mineral/metal based drug is determined by āyurvedic process described as 'āodhana'. Original Ṛlokas dealing with 'āodhana' are included in Appendix-5. Hopefully, this would curtail the shortcuts now believed to be in use, by side-stepping the tedious 'āodhana' required and also discourages adverse comments that undesired toxic remains are present in such āyurvedic preparations.

A preface (प्रस्तावना) written in Hindi by Dr. B.L. Gaur, currently Vice-Chancellor, Rajasthan āyurved University, Jodhpur (Rajasthan), and a member of the APC, is another feature of departure from routine editorial structure followed for pharmacopoeia. It is necessary to explain the logic behind the use of substances known to be toxic, in medicines for human use, place it in a correct perspective from the Āyurvedic point of view and counter any misinterpretation. The inclusion of geochemicals in the treatment of human diseases by traditional method in Āyurveda should be justifiable. Hence, this special effort.

Scope and Limitations

A pharmacopoeia emerges out of the common interests of stake holders. Its ultimate purpose is to aid the implementation of the requirements under a legislation, which ensures supply of quality drugs to a nation, and also monitors the activities of those responsible for this. The core of objectives for a pharmacopoeia lies in the prescription of quality standards. Such standards should be scientifically sound, technologically attainable (by a small, medium and large scale industry equally), economically viable (for both industry and the common man in relation to the marketed product) and finally, culturally and conceptually compatible to the tenets of the Ayurvedic systems of treatment. These are basic to a well worked out pharmacopoeia for traditional medicines.

Constraints and compromises are inevitable in a pharmacopoeia for traditional medicines, considering that empirical wisdom, accumulated over a few thousand years by a highly evolved society in ancient India, is sought to be shackled in a framework of requirements for the 21st century, in an effort to 'modernize' and keep up a global
participation. In addition, this is attempted in a scientific environment of huge knowledge gaps, owing to absence of research by modern scientists. Although the metal/mineral drugs have been in clinical practice in Ayurveda and in trade since a long time, their role in pharmacology and therapeutics have not been elucidated. The unfortunate effect of this can be seen in the monographs, where assay limits have been set for possible active chemical entities on theoretical grounds and not on clinical data. It has not been possible to decide definitely, which major element or minor element or trace elements present in such a complex material (an ore) would contribute to the recorded curative properties of the drug using that mineral/metal, and, on that evidence, set a range of upper and lower limit. Hopefully, future research may throw light on such aspects and help improve or rationalize the standards.

The sophisticated analytical methods that are invariably carried out using costly imported machinery, is a deterrent to their introduction in the Ayurvedic pharmacopoeia. India still lives in its villages and 75 percent of the rural and remote households still depend on traditional medicines. There is no use in having methods that ensure excellent analytical performance of a drug, but the drug itself is inaccessible to the one who needs it, because of the cost factor. Keeping this aspect in view, the APC has strived for a compromise, using much simpler methods and prescribing standards that are weighted rather heavily on parameters that ensure correct identity, established purity at the expense of an accurate estimation of strength. This is also in keeping with the belief that quality medicines in Ayurveda for public use can well be promoted by using a correctly identified and purified raw material and following GMPs prescribed both by Ayurveda and modern pharmaceutics, even if its mode is unknown.

In the above paragraphs, only a few important impediments to the development of quality standards for Ayurvedic medicines have been outlined so as to keep them at par with accepted norms for modern medicines. There are many more grey areas to be tackled, especially in compound formulations using geochemicals like Bhasmas and Rasayogas, but with these words, we offer this volume for public use and welcome comments and criticisms to enhance its value in future revisions.

Members of APC
MINUTES OF THE 1ST MEETING HELD ON 12TH & 13TH JUNE, 2000 WITH THE
EXPERTS OF ‘RASA SHAstra’, GEOLOGY’ & OTHER SCIENTISTS TO
CLARIFY ISSUES RELATING TO THE MINERALS & METAL SINGLE DRUG
USED IN AYURVEDIC FORMULATIONS.

A meeting was called for by the Adviser (Ayurveda), Ministry of Health & Family
Welfare, on the 12th and 13th of June 2000, to discuss the format, and identity of the mineral
drugs included in the Ayurvedic Formulary of India, Part-I and II. Since the advice of experts
in the subjects Geology and Mineralogy would help the scientist and the physician members
of the Ayurvedic Pharmacopoeia Committee, to work on the monographs for these drugs,
some geologists from well known institutes were invited to participate. Request was also
made for actual samples to be brought to the meeting so that the experts could give their
opinion on them. Several of the Vaidyas had brought whatever samples they had, for
reference at the meeting. The following experts who had been invited were present:

1. Ms. S. Satakopan, Member-APC
   Chairman (In the absence of Adviser)
   Chennai

2. Prof. S.S. Handa, Ex-Director
   Regional Research Laboratory,
   New Delhi

3. Prof. M. Joshi, Professor of Geology,
   Banaras Hindu University
   Varanasi

4. Prof. Ved Vrat Sharma, Retd. Professor of Ras Shastra
   Jalandhar

5. Vd. Nanak Chand Sharma, Director,
   Kaya Maya Pharmacy
   New Delhi

6. Dr. C.B. Jha, Reader,
   Deptt. of Ras Shastra
   Banaras Hindu University
   Varanasi

7. Dr. K.K. Sharma, Wadia Institute of Himalayan Geology,
   Dehradun

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8. Dr. N.S. Virdi  
   Director  
   Wadia Institute of Himalayan Geology  
   Dehradun

9. Dr. Y.B. Tripati  
   Reader,  
   Deptt of Medicinal Chemistry,  
   Institution of Medical Sciences,  
   Banaras Hindu University  
   Varanasi

10. Dr. R.U. Ahmed,  
    Director, PLIM  
    Ghaziabad

11. Dr. Rajeev Kumar,  
    Senior Scientific Officer, PLIM  
    Ghaziabad

12. Dr. P. C. Srivastava  
    Senior Scientific Officer, PLIM  
    Ghaziabad

13. Dr. N.N. Pandey,  
    Chemist  
    Drug Testing Laboratory  
    Haridwar

14. Dr. M.L. Sharma,  
    Deputy Adviser (AY.)  
    Deptt. of ISM & H

15. Dr. Aliya Aman,  
    Deputy Adviser (Unani)  
    Deptt. of ISM & H

16. Dr. J. Pandey,  
    Asstt. Adviser (Ayur)  
    Deptt. of ISM & H

17. Dr. D.C. Katoch,  
    Asstt. Adviser (Ayur)  
    Deptt. of ISM & H

18. Dr. Chhote Lal,  
    S.T. A. (Ayur)  
    Deptt. of ISM & H

19. Dr. A.K.S. Bhadoria,  
    Research Asstt. (Ayur)  
    Deptt. of ISM & H
Following experts could not attend the meeting

1. Dr. I Sanjeev Rao  
2. Dr. (Mrs.) A. S. Paranjape  
3. Prof. R.K. Lal  
4. Dr. Balendu Prakash

Hyderabad  
Varanasi  
Varanasi  
Dehradun

A background note and a list of minerals in use by the practitioners in Ayurveda, Siddha and Unani had earlier been distributed. A draft format for preparing the monographs under suitable parameters was also distributed at the meeting, so that the geologist present at the meeting may suggest additions or alterations, and finalise a format for drafting monographs.

At the beginning, the chairman explained the work to be done, and the help desired from the experts in Geology, on the basis of discussions with the Vaidyas across the table, and their observations on the clinical use of the minerals. After some general discussion, the following business was conducted.

1. The geological experts found the draft format for preparing monographs satisfactory for its purpose and agreed that it gave sufficient characteristics for identity of the minerals and metals.

2. Each item in the list supplied already, with a note on each, giving the available current information was then taken up and decisions taken regarding their identity and scientific names.

3. Since the latest information on the minerals and metals would be available with geologists, they were requested to kindly help the APC by furnishing more details.

   a) Title (this would be the same as that appearing in the list)
   b) Broad classification (Details already given in the format)
प्रस्तावना

आयुर्वेद का उपदेश एवं तदनुसार प्रयोग मनुष्य मात्र के हित के लिये हुआ है। आयुर्वेद के मूलभूत सिद्धांतों में पञ्चमहामूल की अवधारणा एक महत्वपूर्ण सिद्धांत है। शरीर को पात्रवृत्तिक माना गया है तथा इस शरीर का शीर्षन शरीर के स्वरूप के आधार पर होने के साथ देश-काल-बल-वय-प्रकृति-आहार आदि अनेक कारणों से प्रभावित होता है। जिसके कारण एक ही व्यक्ति में भिन-भिन समय में भिन-भिन परिमाण में न्यून या अधिक शीर्षन होता है अथवा एक ही कारण से भिन-भिन परिमाण में न्यून या अधिक शीर्षन होता है। भिन-भिन व्यक्तियों में भिन-भिन परिमाण में यह शीर्षन होता है।

प्राचीन आचार्यों ने गहन आयुर्वेद एवं व्यवस्थित अनुसंधानों के सुनियत-क्रम को प्रयुक्त कर सृष्टेश्वर पुरुष जो तथ्य प्रकाशित किये वे आचर्यजनक हैं। ये तथ्य दो प्रकार से प्रस्तुत हैं - स्पष्टतः नामनिदेशपर्दक एवं संकेतपर्दक। उदाहरण के स्पष्टतः शरीर में जो शीर्षन या क्षण होता है उसका नामन-निदेश है। मांसक्षाय में मांस प्रयोग एवं उदकक्षाय में लघुक्षाय सत्ताश्रय प्रयोग वह नामन-निदेश का उदाहरण है। दूसरा निदेश संकेतपर्दक है, जिसमें रक्तक्षाय में लौह प्रयोग अथवा खिद्र में ताम्र-प्रयोग। इन खिद्र प्रयोग शरीर में करने से क्रमशः रक्तक्षाय का निर्माण एवं शारीरिक प्रभाव के कार्य का व्यवस्थित रूप से सम्पन्न होता है जिससे रक्तक्षाय का अपनयण एवं तवचा के द्वारा अपने स्वास्थ्यक वर्ग को प्राप्त करना है।

यह इस बात का संकेत है कि वनस्पतियों में जो पञ्चमहामूलों के प्रतिनिधि द्रव्य हैं वे शारीरिक तत्त्वों को पुनरक्षत करते हैं। साथ ही शरीर में खिद्र एवं धातुओं के प्रतिनिधि द्रव्य भी होते हैं जो लीह, ताम्र आदि के समस्थाय हैं। इनकी भी पूर्वत वनस्पतियों से हो जाती है, लेकिन इन धातुस्वरूप तत्त्वों की क्षीणता यदि अधिक हो तो तत्त्व वनस्पतियाँ इसकी पूर्वत के लिये पाठ्य हीं होती, अतः उन-उन खिद्रों या धातुओं का अथवा अयस्क का प्रयोग आचर्यक माना जा कर उनका विधिपूर्वक प्रयोग भी आचार्यों ने विनयित किया।

इससे यह स्पष्ट होता है कि आचार्यों के द्वारा यह अनुसंधान किया जा चुका था कि शरीर में कौन से तत्त्व की कमी होने या अधिकता होने से कौन से लक्षण उत्पन्न होते हैं तथा उन्हें दूर करने के लिये कौन से द्रव्य (वनस्पति, खिद्र एवं धातु आदि) उपयुक्त हैं।

वर्तमान काल में भिन्न विषयों के विविधता विषयों के द्वारा एक स्वयं आचर्य उठाई जा रही है कि आयुर्वैदिक रस-भरम शरीर के लिये धातुक एवं आयुर्वैदिक प्रशान हैं। ये बातें ये मोही नहीं कह रहे; अभित नवीनतम अनुसंधानों के आधार पर कह रहे हैं, अतः उन्हें सहसा बूझाना या समझाना नहीं जा सकता।

XXXIII
लेकिन यह भी जान लेना चाहिए कि उनके ये अनुसन्धान-परिषाम एकत्रः सही होते हुये भी
एकान्तः सही नहीं हैं। लेकिन उन्हें यह बता निश्चय मापदंडों एवं विधिविहित प्रक्रियाओं के द्वारा ही
समझायें जा सकती है। जिसके लिये भारत-सरकार के आयुर्व-विभाग ने बीड़ा उठाया है तथा आयुर्वीदीय
औषध-निर्माण की सम्पूर्ण प्रक्रियाओं को आधुनिक मापदंडों पर परिमाणित कर समूर्ण विज्ञान-विशेषज्ञों के
सामने रखने का स्तूप प्रयास किया है।

इस सन्दर्भ में सब से पहले एक बात समझ लेना आवश्यक है कि ये खनिज एवं धातु आदि शरीर
के लिये तभी हितकर हैं जबकि इनका विधिविहित-प्रयोग हो, अन्यथा वे अहितकर ही होते हैं। इनके
विधिविहित-प्रयोग को 4 सत्तरों में विभाजित किया जा सकता है-  

1. खनिज, धातु आदि के उत्तम स्वरूप का चयन  
2. खनिज आदि का समृद्धि शोधन  
3. इनका समृद्धि मारण (भर्तन-निर्माण)  
4. इनका नियत अनुपान एवं समृद्धि पद्ध के साथ प्रयोग

जो काम आयुर्वीदिक फामोकोपिया कमेटी (APC) कर रही है, यह अब नया है, यह कतई नहीं
समझा चाहिए। उपर्युक्त चारों कार्य (या सत्तर) तो सूक्ष्म से सूक्ष्म से होते आये हैं। लेकिन इतना अवश्यक है कि
APC इन्हें नवीनतम मापदंडों के रूप में प्रस्तुत कर रही है। पहले आधार द्रव्य (Raw Drugs) का चयन
शोधन, मारण एवं प्रयोग ये चारों सतर एक ही व्यक्ति, संस्था या समूह के द्वारा समाप्तित किये जाते थे
अतः द्रव्यप्राप्ति में संशय, शोधन में माफिक, निर्माण में समर्पित द्रव्य का अभाव या जुट्टि एवं प्रयोग
में सावित्रियता का अभाव नहीं होता था जो वर्तमान में हो सकता है। द्रव्य का चयन या प्राप्ति अन्य
संस्था या समूह कराताहै, शोधन एवं मारण अवधी के द्वारा समाप्तित होता है तथा प्रयोग किसी अन्य के
द्वारा होता है। अतः नवीन सन्दर्भ में पश्चिम विदेशी मापदंडों का सुनिश्चितकरण एवं प्रचारण प्रस्तावक है।

औषध निर्माण में जिल्ला भी द्रव्य काम में लिये जाते हैं उनमें 15 प्रतिशत द्रव्य खनिज, धातु और
अवयव हैं। एक महत्वपूर्ण उल्लेखनीय तथा यह भी है कि शास्त्रों में उल्लिखित शास्त्रीय योगों में लगभग
53 प्रतिशत ऐसे योग हैं जिनमें खनिज या धातु या अवयव भी हैं। अतः यह - आवश्यक है कि

(क) द्रव्य के परीक्षण की नियत प्रक्रिया हो जो भौतिक और सामाजिक परीक्षण के मापदंड
स्वरूप में हो।  
(ख) इन में पानी जाने वाले घटक तत्त्वों की मात्रा सुनिश्चित हो।  
(ग) प्रमुख कार्यकारी तत्त्व के प्रतिशत की सीमा तय हो।  
(घ) पद्धति के सुनिश्चित शिक्षा होने चाहिए।  
(ङ) ये खनिज आदि उपयोगी स्वरूप में प्राप्त हों।  
(च) इनका शोधन एवं मारण विधिप्राप्त हो।
इनकी एक बार में प्रयुक्त की जाने वाली अधिकतम मात्रा, स्थूलतम मात्रा एवं पूर्णताः प्रयुक्तमान मात्रा सुनिश्चित हो।

इन सब बातों को ध्यान में रखते हुये जो प्रक्रिया अपनाई गई, उसके आधार पर सर्वप्रथम 21 द्रव्यों का परिचय तैयार कर प्रकाशित किया जा रहा है, जिस में प्रमुख विशेषताओं वे हैं- 

(क) आयुद्ध की संहिताओं और नियमों के आधार पर इनके वर्ण, स्वरूप, भारस्थिति एवं गन्धर्व को सम्बन्धित परिभाषा
(ख) इन द्रव्यों के आधार पर रस, गुण, बीमार और योग्यता का उल्लेख
(ग) अभ्यास या अन्य उपाय यदि शास्त्र में उल्लिखित हैं तो उनके आधार पर किये जाने वाले रसायनिक परिष्करणों का उल्लेख।
(घ) आयुतिक अनुसंधानों के आधार पर सुनिश्चित वर्ण-स्वरूप आदि के प्रकाशित तथ्यों का उल्लेख।
(ङ) प्रयोगशास्त्रीय रामाणुण्य प्रतिष्करणों का उल्लेख
(च) मोनोग्राफ निर्माण

इन सब विशेषताओं के आधार पर संकलित, लिखित, उल्लिखित एवं सम्पादित अंशों से पुकार यह प्रथम प्रकाशन अत्यन्त उपयोगी रहेगा तथा इसके निर्माणित तत्त्वों आने सुनिश्चित हैं, यथा-

उपयोगी उचित द्रव्यों की समृद्धिपत्रण एवं सहज उपलब्धि।
उत्तम द्रव्य की उपलब्धि की प्रक्रिया के निर्धारण में शास्त्रीय सहभागिता।
द्रव्य की सन्दर्भता की परिसंतति
आयुद्धिक विज्ञान के सन्दर्भ में मापदण्ड होने से सभी को स्वीकार
अन्य देशों में प्रयोग-प्रक्रिया हेतु समृद्धिपत्रण आदि का निर्माण।
द्रव्य की रासायनिक-प्रक्रिया का आयुद्धिक सन्दर्भ में विशेषणपरिक हृदय ज्ञान होने से इनके लिये शास्त्रों में उल्लिखित शोधन-प्रक्रिया को अपनाने में औषध-निर्माणित अनुसंधानों को प्रेषण मिलेगी।
इस सन्दर्भ में आगे अनुसंधान के रूप खुलने और अनुसंधानकार्यों को प्रेषण मिलेगी।
जो लोग इन से निर्मित औषधियों को घाटक बताते हैं तथा आलोचना करते हैं उनको समृद्धिज्ञान मिलेगा।
सब से बड़ा लाभ यह है कि बाजार में अल्पगुण,अल्पसम्पति, अनुपयोगी द्रव्यों के स्थान पर गुणवान,प्रभावी एवं उपयोगी द्रव्य मिलते लगेंगे।

Drugs and cosmetics act 1940 के अनुसर उपयुक्त मापदण्डों के अनुसर औषध-निर्माण हेतु वाष्प किया जा सकता है तथा इसको समृद्धिज्ञान से लाभ किया जा सकता है।

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XXXV
CONTRIBUTING LABORATORIES & INSTITUTIONS

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3. Regional Ore Dressing Laboratory, Indian Bureau of Mines, Ajmer (Rajasthan), India.
4. Regional Ore Dressing Laboratory, Indian Bureau of Mines, Nagpur (Maharastra), India.
MONOGRAPHS
ABHRAKA
(Biotite Mica)

1. **Definition**: *Abhraka* is Biotite mica, a ferro-magnesium silicate $K (Mg Fe)_3 X(Si_3AlO_11) X (OH)_2$. It is a black variety of mica group of minerals.

2. **Synonyms**:
   2.1 *Saṣkta*: Ghana, Vyoma, Abhra, Niścandra, Vynamaka, Vajra, Vajrābhra, Kṣābhra
   2.2 **Names in other Languages**:
   - Bangali - Abhraka
   - English - Mica
   - Gujarati - Abhraka
   - Hindi - Abhrak, Abarak
   - Kannada - Abhraka
   - Malayalam - Abrakam
   - Marathi - Abhraka
   - Tamil - Abragam, Karuppu Appirakam (S.F.I.)
   - Telugu - Abbarakam
   - Urdu - Abrak Siyah (N.F.U.M.)

3. **Broad Classification**: A Phyllo-Silicate

4. **Origin and occurrence**: *Abhraka* occurs in igneous rocks in pure form as well as in metamorphic rocks as schists and gneisses. It occurs in ore form in pegmatites but pure mineral in sheet-form can be made available through sorting in pegmatite bodies.

   It is found in all igneous terrains of the earth’s crust. In India, it occurs mostly in the mica belts of Rajasthan, Andhra Pradesh and Jharkhand. The main deposits in these states occur at Ajmer, Bhilwara, Tonk and Pali in Rajasthan, Kalichedu, Thalpur and Gudur in Andhra Pradesh, Koderma and Giridih in Jharkhand. Other known occurrences are in the states of Tamil Nadu, Karnataka, Kerala and West Bengal.

5. **Physical properties**: (Definition/Explanation of technical terms and Methods of Determination as given in Appendix –1).

   - **Nature**: Platy (separable in thin layers)
   - **Colour**: Greenish black
   - **Streak**: Greenish black
   - **Cleavage**: Perfect
   - **Fracture**: Uneven
   - **Lustre**: Splendent
   - **Tenacity**: Flexible
6. **Optical Properties:** Anisotropic, Biaxial Negative, small 2V and strong birefringence. Refractive Index: $\eta_\alpha$ 1.565-1.625; $\eta_\beta$ 1.605-1.696; $\eta_\gamma$ 1.605-1.696 (Appendix-2).

7. **Chemical Properties:**
   7.1 **Effect of Heat:** Hold a piece of Abhraka by forceps and heat it over a burner flame in its outer zone (about 1000°). It swells almost double in volume. Colour changes from black to silver moon while, water is released.

   7.2 **Solubility:** Take about 1 g finely powdered (150 mesh) sample of Abhraka in 250 ml beaker. Add 50 ml sulphuric acid. Stir the solution. It decomposes leaving skeleton of silica (distinction from other micas which are not affected by sulphuric acid).

   7.3 **Assay:** Should contain not less than 50% silica (SiO$_2$) when analysed by gravimetric method (Appendix-3.1.3).

   7.4 **Heavy metals and Arsenic:** Should not contain more than the stated limits for the following: - Lead = 45 ppm, Arsenic = 3 ppm, and Cadmium = 2 ppm (Appendix-3.2).

   7.5 **Other Elements:** May contain the following within ± 20% of the stated limits:- Iron = 6%, Aluminium = 5%, Magnesium = 9% and Potassium = 5% (Appendix-3.1 & 3.2).

8. **Śodhana:** Shall not be used in formulation without subjecting it to āyurvedic purification (śodhana).

9. *Abhraka* is always used in the form of *bhasma*, the therapeutic uses, dose and other details of which are given in the monograph of *bhasma*. 
AKĪKA
(Agate)

1. **Definition:** Akīka is a silica mineral (SiO₂). It is the banded form of Chalcedony.

2. **Synonyms:**
   2.1 **Sanskrit:** Akīka
   2.2 **Names in other Languages:**
      - English - Quartz, Chalcedony
      - Hindi - Akīka
      - Punjabi - Mank, Akīka
      - Urdu - Aqeeq (N.F.U.M.)

3. **Broad Classification:** Crypto crystalline Silica Mineral

4. **Origin and occurrence:** Akīka occurs generally in pure form. It occurs all over the earth’s crust in igneous as well as metamorphic rocks. Wherever cavities are developed, silica is deposited and the material becomes Akīka. Banding in Akīka is due to interrupted deposition of silica in cavities. It is a very common semi-precious ornamental stone.

   Akīka occurs mainly as fillings in the voids in the Deccan Trap rocks. The most important occurrence of Akīka in India is Rajpipla area and further west between the mouths of river Tapti and Narmada in Bharuch district, Gujarat. Other occurrences of economic importance are known at Amaravati, Aurangabad, Buldhana, Chandrapur, Jalna, Nasik and Pune in Maharashtra, beds of Krishna and Godavari rivers in Andhra Pradesh, Rajmahal and Sahebganj in Bihar, Dhar and Mandsaur in Madhya Pradesh and Jamnagar, Kutch and Surat in Gujarat.

5. **Physical Properties:** (Definition/Explanation of technical terms and Methods of Determination as given in Appendix–1).

   - **Nature:** Banded form
   - **Colour:** White
   - **Streak:** White
   - **Cleavage:** Not present
   - **Fracture:** Conchoidal
   - **Lustre:** Waxy
   - **Tenacity:** Brittle
   - **Transparency:** Sub- translucent
   - **Hardness:** 6 to 7
   - **Sp. Gr.:** 2.5 to 2.65
   - **Fluorescence:** Remains dark when observed after irradiation with near UV light of 365 to 400 nm
6. **Optical Properties:** *Akīka* is uniaxial, positive, showing birefringence between crossed nicols, with refractive indices between 1.530 and 1.555 with $\eta_w 1.544$ and $\eta_e 1.553$ (Appendix-2).

7. **Chemical Properties:**
   7.1 **Reaction with acids:** Take about 5 g finely powdered (150 mesh) sample of *Akīka* in a test tube. Add 10 ml dilute hydrofluoric acid. It dissolves completely in *hydrofluoric acid*. (Care should be taken in handling *hydrofluoric acid*).

   No other acid has any reaction or effect on *Akīka*.

   7.2 **Assay:** Should contain not less than 95% Silica (SiO$_2$) when analysed by gravimetric method (Appendix-3.1.3).

   7.3 **Heavy metals and Arsenic:** Should not contain more than the stated limits for the following:- Arsenic = 190 ppm, and Cadmium = 1.6 ppm (Appendix-3.2).

8. **Diagnostic property:** Bands of *Akīka* are typically parallel to each other.

9. **Śodhana:** Shall not be used in formulations without subjecting it to śodhana.
   9.1 **Śodhana:** [Ref. Rasatantrasāra Siddha Prayoga Sa-graha, Part-1, page 73]

   i) *Akīka* : 1 part
   ii) Gulāba Jala
      or
      Arka Vedamuśka
      or
      Go-dugdha : Q.S

   **Method:**

   Heat *Akīka* till it becomes red-hot and dip in Gulāba Jala or Arka Vedamuśka or Go-dugdha for 21 times.

10. *Akīka* is used in the form of *pi ¾°ī* or *bhasma*, the details of which are given in the monograph of *pi ¾°ī* or *bhasma*. 
1. **Definition:** *Gairika* is Red Ochre, an oxide of Iron (Fe₂O₃). This mostly earthy variety of hematite mineral is also known as Reddle.

2. **Synonyms:**
   2.1 Ṣaṭkʿa : Kāṣāyakkal, Kāvi, Svarṇagairika
   2.2 Names in other Languages:
   - Bangali - Girimā†
   - English - Ochre, Reddle
   - Gujarati - Geru, Sonāgeru
   - Hindi - Geru, Gerumitti
   - Kannada - Hojātha, Jajoo, Kaavi, Kemmannu
   - Malayalam - Kavimannu
   - Marathi - Gerū, Sonakāva
   - Punjabi - Geri
   - Tamil - Seemaikkaavikkallu, Kāvikkal (S.F.I.)
   - Telugu - Kavirāyī, Kaavi
   - Urdu - Geru (Teen-e-Rumi) (N.F.U.M.)

3. **Broad Classification:** Oxide

4. **Origin and occurrence:** Basically a hematite mineral, *Gairika* is a natural mineral pigment found with other iron-titanium oxide minerals in igneous and metamorphic rocks as accessory mineral. *Gairika* is associated with magnetite. It sometimes contains minor amount of titanium and magnesium. *Gairika* occurs as an alteration product of other iron minerals or by degradation of highly ferruginous rocks in the form of weathered residual concentrations. It is generally found mixed with clay and other impurities.

   Based on their colour, there are two types of ochres found in the country i.e. Red ochre and Yellow ochre. Anhydrous ironoxide is **red ochre** (*Gairika*) and hydrated iron oxide is **yellow ochre**.

   In India, *Gairika* (Red ochre) is widely distributed. *Gairika* deposits are chiefly found in Rajpur, Banaskantha, Jamnagar and Kuchchh in Gujarat, Bellary and Bidar in Karnataka, Gwalior, Jabalpur, Satna, Kailashpur, Madhogarh and Mandla in Madhya Pradesh and Udaipur, Alwar, Bikaner, Chittorgarh and Sawai Madhopur in Rajasthan. Other known occurrences are in Andhra Pradesh and West Bengal.

5. **Physical properties:** (Definition/explanation of technical terms and methods of determination as given in Appendix –1).
   - **Nature** : Massive clayey
 Colour : Reddish brown
 Streak : Reddish brown
 Fracture : Uneven
 Lustre : Earthy
 Tenacity : Brittle
 Transparency : Opaque
 Hardness : 5.5 to 6.5 but much softer in earthy variety
 Sp. Gr. : 2.7

6. Chemical Properties:

6.1 Effect of Heat:
6.1.1 *Gairika* becomes magnetic on heating. Magnetism can be felt using a horse-shoe magnet (Appendix-3.3.3/1.9).

6.1.2 Does not easily fuse in a blow - pipe flame.

6.2 Solubility in Acid: Soluble in hydrochloric acid.

6.3 Assay: *Gairika* should not contain less than 16% Fe or not less than 21% Fe₂O₃ when analysed by gravimetric method (Appendix-3.1.4).

6.4 Heavy metals and Arsenic: *Gairika* should not contain more than the stated limits for the following:- Lead = 6 ppm, Arsenic = 2 ppm and Cadmium = 2 ppm (Appendix-3.2).

6.5 Other elements: May contain the following within ± 20% of the stated limits:- 1% Magnesium and 1% Titanium (Appendix-3.1 & 3.2).

7. Śodhana: Shall not be used in formulations without subjecting it to śodhana.

7.1 Śodhana: [Ref: Rasaratna Samuccaya 3/49]

i) *Gairika* : 1 Part

ii) Go-dugdha : Q.S for Bhāvanā

Method:
Prepare fine powder of *Gairika* and *Bhāvanā* of Go-dugdha should be given three times with sufficient quantity, dry and use for therapeutic purposes.

8. *Gairika* has the following attributes:

8.1 Properties and Actions:

Rasa - Madhura, Kaṭāya
Gu´a - Snigdha, Viśada
Virya - Śīta
Vipāka - Madhura
Karma - Pitta-Nāśaka, Balya, Vra´a Ropa´a, Netrya, Kaphajit

8.2 Therapeutic Uses of śuddha Gairika: Netra roga (diseases of eyes); Raktapitta (bleeding disorder); hikkā (hiccups); vanama (vomiting); viḍāvikāra (disorders due to poison); Rakta pradara (Menorrhagia or Metrorrhagia or both); ka´ḍū (itching); Jvara (fever); dāha (burning sensation); Udara roga (diseases of abdomen)


10. Important Formulations: Ku-kumādi taila, Bh¨garāja taila, Tutthādi lepa, Mahā Jvarā-kuśa rasa, Laghu Sūtaśekhara rasa, Kāmadudhā rasa (Mauktika yukta)
1. **Definition:** Gandhaka is sulphur (S), a non-metallic solid element.

2. **Synonyms:**
   2.1 Sa/vk'ta: Bali, Daityendra, Gandha, Sugandhaka, Valī, Sugandhika

2.2 **Names in other Languages:**
   - Assami: Kibrīt
   - Bangali: Gandhaka
   - English: Sulphur
   - Gujarati: Gandhaka
   - Hindi: Gandhaka
   - Kannada: Gandaga
   - Malayalam: Gandagam
   - Marathi: Gandhaka
   - Punjabi: Gandhaka
   - Tamil: Kandagam, Kantakam (S.F.I.)
   - Telugu: Gandhakam
   - Urdu: Kibreet (Gandhak) (N.F.U.M.)

3. **Broad Classification:** Native element

4. **Origin and occurrence:** Native Gandhaka occurs in the craters and crevices of extinct volcanoes where it has been deposited as a direct sublimation product from volcanic gases. It also occurs around thermal springs, in salt dome cap-rocks and in sedimentary sequences that contain sulphates along with organic materials like bituminous limestone. Gandhaka is frequently associated with gypsum and limestone. Native Gandhaka, which is contaminated with sand or clay, bitumen and such foreign matter, is purified by melting in oven or by distilling in closed vessels.

   In India, the deposits of Gandhaka are very much limited. The only known occurrence is that of Tsokar Lake, Leh district in Jammu and Kashmir. There is no mining for Gandhaka in India. Elemental Gandhaka, however, is recovered commercially as a by-product from fertilizer plants at Panipat in Haryana, Naya Nangal and Bhatinda in Punjab and Oil Refinery at Mathura in Uttar Pradesh. There are huge deposits of elemental Gandhaka buried underground in certain parts of the world especially Poland, Mexico and USA.

5. **Physical properties:** (Definition/explanation of technical terms and methods of determination as given in Appendix–1).

   - Nature: Crystalline lumps
   - Colour: Sulphur yellow
   - Streak: Yellowish white
   - Cleavage: Poor
Fracture : Conchoidal
Lustre : Resinous
Tenacity : Brittle
Transparency : Translucent
Hardness : 1.5 to 2.5
Sp. Gr. : 1.95 to 2.10

6. Chemical Properties:

6.1 Effect of Heat:
6.1.1 On burning a small quantity in a crucible, Gandhaka partially melts with evolution of brownish sulphur fumes.

6.1.2 Gandhaka melts at about 110° where as it burns at 270° in air with a bluish flame yielding sulphur-di-oxide (SO₂).

6.2 Solubility: Insoluble in water as well as any acid, but soluble in carbon-di-sulphide.

6.3 Assay: Should contain not less than 90% Sulphur, when analysed by gravimetric method (Appendix-3.1.8).

6.4 Heavy metals and Arsenic: Should not contain more than the stated limits for the following: Arsenic = 1 ppm and Cadmium = 2 ppm (Appendix-3.2).

7. Śodhana: Shall not be used in formulations without subjecting it to śodhana.

7.1 Śodhana: [Ref: Rasām’īam-2/3]

   i) Gandhaka : 1 part
   ii) Go-dugdha or Bh’garāja rasa (Pl.) : Q.S

Method:
Take fine powder of Gandhaka in a ladle, add little amount of gh īa, melt and pour in to the vessel which contains go-dugdha or bh’garāja svarasa. Collect on cooling to room temperature, wash with plenty of luke warm water, dry, prepare the fine powder and preserve for further purpose.

8. Gandhaka has the following attributes:

8.1 Properties and Actions:
Rasa - Madhura, Ka‘u, Tikta, Ka³āya
Gu´a - U³/₄a, Sara, Snigdha
Vīrya - U³/₄a
Vipāka - Ka‘u
Karma - Rasāyana, Dīpana, Pācana, Viṣahara, Kaphahara, Balya, Medhya, Pittala, Cakṣūṣha, K¨mihara, Sūtajit, Kuṣahara, Āmaśoṣha, Sūtendravīryaprada, Vātahara,

8.2. Therapeutic Uses of śuddha Gandhaka: Ka´ṭū (itching); Kuṣha (diseases of the skin); Visarpa (erysepalis); Dadru (taeniasis); Āmavāta (rheumatism); Kapha roga (disease due to kapha doṣa); Garaviṣa (slow/accumulated poison); Plihā roga (splenic disease); Kṣaya (pthisis); Kāsa (cough); Śvāsa (Asthma); Netra roga (diseases of eyes); Vāta roga (diseases due to Vāta doṣa)

9. Dose: 125 mg - 1 g of śuddha Gandhaka.

10. Important Formulations: - Mahāgandhaka vaṭī, Pañcām ṭa parpaṭī, Candrakalā rasa, Taru´ārka rasa, Rasa parpaṭī, Gandhaka rasāyana.
**GODANTĪ**
(Selenite)

1. **Definition:** *Godantī* is Selenite (CaSO₄, 2H₂O) mineral.

2. **Synonyms:**
   2.1 *Saṇḍakṭa:* Godanta

   2.2 **Names in other Languages:**
   - English - Selenite, Gypsum
   - Gujarati - Godantī, Ghāpa ´a, Chirodī
   - Hindi - Godanti
   - Marathi - Godantī, Śiragola
   - Tamil - Karpura Chilajattu (S.F.I.)
   - Telugu - Hara sothamu
   - Urdu - Godanti (N.F.U.M.)

3. **Broad Classification:** Hydrous calcium sulphate

4. **Origin and occurrence:** *Godantī* occurs as evaporates; extensive sedimentary deposits interbedded with limestone, red shales and clay stones etc. It occurs generally in pure mineral form.

   In India, significant occurrences of *Godantī* are at Nellore, Prakasam and Guntur in Andhra Pradesh and Bikaner, Barmer, Jaisalmer, Nagaur, Ganganagar and Pali in Rajasthan. Major production of *Godantī* comes from these two states only. There are some other occurrences also reported in the states of Gujarat, Jammu and Kashmir, Himachal Pradesh, Uttar Pradesh and Tamil Nadu.

5. **Physical properties:** (Definition / explanation of technical terms and methods of determination as given in Appendix –1).

<table>
<thead>
<tr>
<th>Nature</th>
<th>Crystalline showing elongated tabular crystals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour</td>
<td>Greyish white</td>
</tr>
<tr>
<td>Streak</td>
<td>White</td>
</tr>
<tr>
<td>Cleavage</td>
<td>Perfect</td>
</tr>
<tr>
<td>Fracture</td>
<td>Even</td>
</tr>
<tr>
<td>Lustre</td>
<td>Silky</td>
</tr>
<tr>
<td>Tenacity</td>
<td>Sectile</td>
</tr>
<tr>
<td>Transparency</td>
<td>Translucent</td>
</tr>
<tr>
<td>Hardness</td>
<td>2</td>
</tr>
<tr>
<td>Sp. Gr.</td>
<td>2 to 2.5</td>
</tr>
</tbody>
</table>

6. **Optical properties:** Anisotropic, biaxial positive, large 2V, weak birefringence.

   Refractive index : \( \eta_\alpha, 1.520; \eta_\beta, 1.524; \eta_r, 1.530 \) (Appendix–2).
7. **Chemical Properties:**

7.1 **Test for sulphate radical:** Take about 0.5 g powdered sample in a test tube. Add 10-15 ml dilute hydrochloric acid and boil. Add 10 % barium chloride solution drop wise. The resulting solution gives white precipitate of barium sulphate.

7.2 **Assay:** Should contain not less than 20% Calcium (Ca) or 30% calcium oxide (CaO) when analysed by gravimetric method (Appendix-3.1.5).

7.3 **Heavy metals and Arsenic:** Should not contain more than the stated limits for the following: Lead = 6 ppm, Arsenic = 1 ppm and Cadmium = 4 ppm (Appendix-3.2).

8. **Śodhana:** Shall not be used in formulations without subjecting it to śodhana.

9. **Godantī** is used in the form of *bhasma*, the details of which are given in the monograph of *bhasma*.
GOMEDA
(Garnet)

1. **Definition:** *Gomeda* is Almandite \( \text{Fe}_3\text{Al}_2 (\text{SiO}_4)_3 \), one of the garnet group of minerals.

2. **Synonyms:**
   2.1 **Names in other Languages:**
   - Bangali - Gomeda
   - English - Garnet
   - Gujarati - Gomeda
   - Hindi - Gomeda
   - Marathi - Gomeda
   - Telugu - Gomedikamu

3. **Broad Classification:** Silicate of iron and aluminium

4. **Origin and occurrence:** *Gomeda* occurs in two forms; in-situ deposits and placers. It occurs as in-situ deposits in metamorphic rocks, mostly in schists and gneisses, of argillaceous parentage, crystalline limestone and metamorphosed basic and other igneous rocks. In placer form, it is found as heavy detrital residue in sediments.

   In India, in-situ occurrences of *Gomeda* exist in many parts of the country e.g. Khammam in Andhra Pradesh, Singbhum in Jharkhand, Hassan, Bangalore and Mysore in Karnataka and Ajmer, Bhilwara, Jhujhunu, Sikar, Sirohi and Tonk in Rajasthan. It occurs as primary mineral also in igneous rocks such as mica-pegmatites of Bihar, Andhra Pradesh and Rajasthan. The placer deposits, whereas, occur in beach sands of Kerala, Tamil Nadu and Orissa.

   Varieties of *Gomeda* are valued as semi-precious stones.

5. **Physical properties:** (Definition / explanation of technical terms and methods of determination as given in Appendix –1).

   - **Nature:** Crystal
   - **Colour:** Reddish brown
   - **Streak:** White
   - **Cleavage:** Not present
   - **Fracture:** Sub-conchoidal
   - **Lustre:** Vitreous to resinous
   - **Tenacity:** Brittle
   - **Transparency:** Translucent
   - **Hardness:** 7.0 to 7.5
   - **Sp. Gr.:** 3.5 to 4.0
   - **Fluorescence:** No fluorescence when observed after irradiation with UV rays of 365 to 400 nm
6. **Optical properties:** Isotropic
   Refractive Index: $\eta = 1.83$ (range 1.75 to 1.90) (Appendix–2.4).

7. **Chemical Properties:**

   7.1 **Assay:** Should contain not less than 35% SiO$_2$, 10% Alumina (Al$_2$O$_3$) and 5% Iron (Fe) when analysed by gravimetric method (Appendix-3.1).

   7.2 **Heavy metals and Arsenic:** Should not contain more than the stated limits for the following: - Mercury = 7 ng/g, Arsenic = 2 ppm and Cadmium = traces (Appendix-3.2).

   7.3 **Other Elements:** May contain the following within $\pm 20\%$ of the stated limits:- Calcium = 0.10 %, Magnesium = 0.10 % and Manganese = 11% (Appendix-3.1 & 3.2).

8. **Distinction from Manikyam:**
   Mā́ikya gives a fiery red fluorescence in near UV rays of 365 to 400nm, whereas Gomeda does not fluoresce under the same condition. Mā́ikya is anisotropic, uniaxial and negative.

9. **Śodhana:** Shall not be used in formulations without subjecting it to śodhana.

   9.1 **Śodhana:** [Ref: Rasa Tara- gi¬í-23/123]
      i) Gomeda : 1 Part
      ii) Nimbū svarasa [Fr.] : Q.S for Svedana

   **Method:**
   Prepare small pieces of Gomeda, boil in Nimbū svarasa for 3 hours. On cooling to room temperature, collect Gomeda, dry and use for therapeutic purposes.

10. **Gomeda** is used in the form of pi¾°ī or bhasma, the details of which are given in the monograph of pi¾°ī or bhasma.
1. **Definition:** *Jaharamoharā* is Serpentine, a hydrous silicate of Magnesium $\text{[Mg}_6\text{(Si}_4\text{O}_10)\text{X(OH)}_8\text{]}$. This is also known as Green Marble in trade.

2. **Synonyms:**
   2.1 Saṣkṛta : *Jaharamoharā*
   
   2.2 Names in other Languages:
   - **English** - Serpentine, Green Marble
   - **Hindi** - *Jaharamoharā*
   - **Telugu** - Śālagrāma śilā
   - **Urdu** - Zahar Mohra, Hajr-us-sum, Fad Zahr Madani, Hajr-ul-behr (N.F.U.M.)

3. **Broad Classification:** Hydrous magnesium silicate

4. **Origin and occurrence:** *Jaharamoharā* results from the alteration, either during metamorphism or by late-stage hydrothermal action at temperatures below 400⁰, of rocks rich in magnesium, containing olivine, pyroxene or amphibole. Magnetite usually accompanies this alteration. Some serpentine occurs as large rock masses generally referred to as serpentinites. Nickel in minor amount is generally present in *Jaharamoharā*. A little amount of Calcium, Iron and Aluminium may also be present.

   In India, *Jaharamoharā* occurs mostly in metamorphic terrains of Rajasthan, Karnataka, Jharkhand, Madhya Pradesh and Andhra Pradesh. In Rajasthan, it occurs abundantly in Udaipur and Dungurpur districts.

5. **Physical properties:** (Definition / explanation of technical terms and methods of determination as given in Appendix -1).

   - **Nature** : Crystalline, coarse grained, soapy feel
   - **Colour** : Blackish green
   - **Streak** : Colourless
   - **Cleavage** : Perfect
   - **Fracture** : Conchoidal
   - **Lustre** : Greasy
   - **Tenacity** : Tough
   - **Transparency** : Translucent
   - **Hardness** : 3.5 to 5
   - **Sp. Gr.** : 2.5 to 2.7

6. **Optical properties:** Bi-axial, negative, weakly birefringent with R.I. 1.546 to 1.554 (Appendix- 2).
7. **Chemical Properties:**

7.1 **Effect of Heat:** Take about 1 g powdered sample of *Jaharamoharā* in open test tube. Heat the test tube at around 500\(^\circ\)C. The sample gets decomposed and gives off a little water.

7.2 **Assay:**

7.2.1 Should contain not less than 30% magnesium oxide when analysed by gravimetric method (Appendix-3.1.6).

7.2.2 Should contain not less than 30% Silica (SiO\(_2\)), not less than 5% Ferric oxide (Fe\(_2\)O\(_3\)) and not less than 5% calcium oxide (CaO) when analysed by gravimetric method (Appendix -3.1).

7.2.3 **Heavy metals and Arsenic:** *Jaharamoharā* should not contain more than the stated limits for the following: - Arsenic = 2 ppm and Cadmium = 3 ppm (Appendix-3.2).

7.2.4 **Other elements:** May contain the following within ± 20% of the stated limits:- 0.15 % Nickel when analysed by Atomic Absorption Spectrophotometer (Appendix-3.2.2).

8. **Śodhana:** Shall not be used in formulations without subjecting it to śodhana.

8.1 **Śodhana:** [Ref. Rasatartraśara Siddha Prayoga Sa-graha, Part-1, Page 73 ]

i) *Jaharamoharā* : 1 part

ii) Go-dugdha : Q.S

or

Āmalakī rasa

**Method:**

Heat *Jaharamoharā* till it becomes red hot and dip in Go-dugdha or Āmalakī rasa for 21 times.

9. *Jaharamoharā* is used in the form of pisṭ, the details of which are given in the monograph of piṣṭ.
KĀNTA LAUHA
(Iron Ore)

1. Definition: Kānta Lauha is an Iron ore containing magnetite, a ferric oxide (Fe₃O₄) mineral.

2. Synonyms:
   2.1 Saḥkṣa : Kānta, Kāntaka, Kāntāyasa

2.2 Names in other Languages:
   - English - Magnetite
   - Gujarati - Nāṭīsa
   - Hindi - Cumbaka, Cumbaka patthar
   - Tamil - Kantham (S.F.I.)
   - Telugu - Sudantu rāyi, Ayaskāntamu
   - Urdu - Faulad, Aahan (N.F.U.M.)

3. Broad Classification: Oxide

4. Origin and occurrence: Kānta Lauha occurs in igneous as well as metamorphic rocks as accessory mineral. It is a wide-spread oxide mineral found in earth’s crust in ore form. Kānta Lauha is commonly associated with hematite (Fe₂O₃).

   In India, Kānta Lauha occurs mainly at Guntur in Andhra Pradesh, Salem and Tirucirappalli in Tamil Nadu, Shimoga and Kudramukh in Karnataka, Mandi in Himachal Pradesh, Mayurbhanj in Orissa and Noamundi, Notoburu, Gore Pahar and Biwabathan in Jharkhand. Other known deposits with hematite as predominant mineral occur in the states of Bihar, Goa, Madhya Pradesh and Rajasthan.

5. Physical Properties: (Definition / explanation of technical terms and methods of determination as given in Appendix –1).

   Nature : Lump
   Colour : Greyish black
   Streak : Reddish black
   Cleavage : None
   Fracture : Uneven
   Lustre : Metallic
   Tenacity : Brittle
   Transparency : Opaque
   Magnetism : Magnetic in nature
   Hardness : 5.5 to 6
   Sp.Gr : 5 to 6
6. Chemical Properties:

6.1 Assay:  
*Kānta Lauha* in ore form should contain not less than 60% Iron (Fe) when analysed by gravimetric method (Appendix-3.1.4).

6.2 Heavy metals and Arsenic: *Kānta Lauha* in ore form should not contain more than the stated limits for the following: - Arsenic = 2 ppm and Cadmium = 7 ppm (Appendix-3.2).

6.3 Other Elements: *Kānta Lauha* in ore form may contain the following within ± 20% of the stated limits:- Zinc = 95 ppm, Manganese = 500 ppm and Silver = 5 ppm (Appendix-3.1 & 3.2).

6.4 Acid Insoluble: Take about 1 g of pre-dried finely powdered (150 mesh) sample of *Kānta Lauha* in 250 ml beaker. Add 50 ml dilute hydrochloric acid. Stir the solution and cover the beaker with a watch glass. Heat on the hot plate at 150° and digest for two hours. Cool, filter in buchner funnel, wash with water and ignite at 900°. Weigh the residue. It should not be more than 13% of the initial weight of the sample.

7. Śodhana: Shall not be used in formulations without subjecting it to śodhana.

8. *Kānta Lauha* is used in the form of bhasma, the details of which are given in the monograph of bhasma.
KĀŚĪŚA
(Ferrous Sulphate)

1. **Definition:** Kāśīśa is Ferrous Sulphate (FeSO₄. 7H₂O) also known as Green vitriol, Copper as or melanterite mineral.

2. **Synonyms:**
   2.1 Saḳkṛta: Kāśīśaka, Puṇḍakāśīśa

2.2 **Names in other Languages:**
- **Bangali** - Hirākas
- **English** - Ferrous Sulphate, Green vitriol
- **Gujarati** - Hīrākasī
- **Hindi** - Kasīṣ, HīraKasīṣ
- **Kannada** - Kaasisa, Anabedi
- **Malayalam** - Turusi, Turus
- **Marathi** - Hirākas
- **Tamil** - Annabedhi, Annapeti (S.F.I.)
- **Telugu** - Annabhedi

3. **Broad Classification:** Hydrous ferrous sulphate

4. **Origin and occurrence:** Kāśīśa, a secondary mineral, results from the decomposition of pyrites in the zone of oxidation and is found in small quantities wherever pyrite occurs. It occurs widely as efflorescence on the walls and timbers of mine workings. Kāśīśa is commonly associated with chalcanthite, gypsum and other hydrous basic sulphates. Mostly, commercially manufactured Kāśīśa as a chemical is used for Ayurvedic formulations in the country.

5. **Physical properties:** (Definition / explanation of technical terms and methods of determination as given in Appendix –1).

- **Nature**: Massive lumps
- **Colour**: Greenish white
- **Streak**: White
- **Cleavage**: None
- **Fracture**: Uneven
- **Lustre**: Earthy
- **Tenacity**: Brittle
- **Transparency**: Translucent
- **Hardness**: 1.95 to 2
- **Sp. Gr.**: 1.90 to 1.97
- **Taste**: Sweetish, astringent taste
6. Chemical Properties:

6.1 Effect of Heat:

6.1.1 Heated before blowpipe, Kāśīśa becomes magnetic as felt by a horse-shoe magnet.

6.1.2 On strong heating in a crucible, Kāśīśa looses water with evolution of SO₂ gas giving pungent smell of burning sulphur.

6.1.3 Take about 5 g fine powder each of Kāśīśa and borax. Heat the mixture in a crucible. A brown glassy fused mass results.

6.1.4 Efflorescence: Kāśīśa is efflorescent in dry air. On exposure to moist air, the Kāśīśa crystals rapidly oxidize and become coated with brownish yellow mass.

6.2 Reaction with barium chloride solution: White precipitate of barium sulphate appears on addition of barium chloride solution in Kāśīśa solution, dissolved in purified water.

6.3 Solubility in water: Take about 10 g accurately weighed fine (150 mesh) powder of Kāśīśa in 250 ml beaker. Add 50 ml purified water to it. Stir the solution till Kāśīśa powder stops dissolving. Put the beaker in the sun light till the solution gets completely dried up. Weigh the residue, if any. It should not be more than 5% of the initial weight of the sample. That is, solubility of Kāśīśa in purified water should not be less than 95%.

6.4 Assay: Kāśīśa should contain not less than 25% Iron, not less than 15% Sulphur and not less than 45% SO₄ (Appendix-3.1 & 3.3).

6.5 Heavy metals and Arsenic: Kāśīśa should not contain more than the stated limits for the following: - Arsenic = 2 ppm and Cadmium = 2 ppm (Appendix-3.2).

6.6 Other elements: May contain the following within ± 20% of the stated limits:- Copper (Cu) 188 ppm when analysed by Atomic Absorption Spectrophotometer method (Appendix-3.1.9 & 3.2.2).

7. Śodhana : Shall not be used in formulations without subjecting it to śodhana.

7.1 Śodhana: [Ref: AFI-Part-I; Appendix-II, Śodhana-6]

i) Kāśīśa : 1 Part

ii) Bh¨¬ganira (Bh¨¬garāja) [Pl. ] : Q.S. for Bhāvanā
Method:
Powder kāśīsa, add quantity sufficient bhgarāja svarasa and grind till complete moisture gets evaporated. Repeat the process for three times.

8. **Kūśīśa** has the following attributes:

8.1 **Properties and Actions:**
- **Rasa** - Amla, Tikta, Kaṣaya.
- **Guşā** - Uṣa
- **Vīrya** - Uṣa
- **Vipāka** - Kaṭu
- **Karma** - Vāta-Kaphahara, Keśya, Netrya, Rajah pravartaka, Kṣākara, Sa-kocaka (Astringent), Balya, Keśya, Rañjana, Rakta vardhaka

8.2. **Therapeutic Uses:** kaṭū (itching); viṣa roga (disease due to poison); Mūtrakčchra (dysuria); Aśmarī (calculus); Śvitra (Leucoderma / Vitiligo); Pitta Apasmāra (Epilepsy due to Pitta doṣa); Pāṭu (anaemia); Plīhāvddhi (splenomegaly); Kūmi (Helminthiasis/ worm infestation); Gudabhraṣa (prolapse of rectum); Visarpa (Erysepaltes); Netra roga (disease of eyes); Śleṣma roga (disease due to kapha doṣa)

9. **Dose:** 60 – 250 mg

10. **Important Formulations:** Rajahpravartinī vaṭī, Kūśīśādi taila, Kūśīśādi ghīṭa, Śā-kha drāvaka, Plīhāri vaṭkā
**KHAʾIKĀ**  
(Kaolinite)

1. **Definition:** *Khaʾikā* is Kaolinite \((\text{Al}_2\text{(Si}_2\text{O}_5\text{)(OH)}_4\))\), an aggregate of minerals and colloidal substances commonly known as Clay.

2. **Synonyms:**
   2.1 **Names in other Languages:**
   - Bangali - Khaʾi, Phula Khaʾi
   - English - Clay
   - Gujarati - Khaʾi, Khaʾiūmā†
   - Hindi - Khaʾiya, Khariya, Khaʾi
   - Marathi - Khaʾu
   - Telugu - Sima sunnamu, Sudhā

3. **Broad Classification:** A phyllo-silicate of aluminium

4. **Origin and occurrence:** *Khaʾikā* occurs associated with pre-cambrian granites, gneisses, pegmatites, phyllites and schists; gondwana shales and sand stones as bedded deposits. *Khaʾikā* is formed by the decomposition of other alumino-silicates (feldspars) either by weathering or by hydrothermal activity. It occurs in pure mineral form as lumps consisting of very small particles.

   In India, *Khaʾikā* occurs at several places in different states. Most significant occurrences are Ajmer, Udaipur, Chittorgarh, Bikaner, Barmer and Sawai Madhopur in Rajasthan, Bankura, Birbhum, Purulia and Darjeeling in West Bengal, Chinglapat, Ramanathapuram and South Arcot in Tamil Nadu, Koraput, Cuttak, Keonjhar and Mayurbhanj in Orissa, Cuddapah, Godavari area, Vishakhapatnam and Nellore in Andhra Pradesh, Trivendrum, Ernakulam, Palakkad and Kannur in Kerala and Singhbhum, Bhagalpur, Santhal, Ranchi and Gaya in Jharkhand.

5. **Physical properties:** (Definition / explanation of technical terms and methods of determination as given in Appendix –1).

   - **Nature** : Lumps (whitish to occasionally coloured layers) with greasy feel and earthy odour
   - **Colour** : Whitish, occasionally tinted
   - **Streak** : White
   - **Cleavage** : Not observable
   - **Fracture** : None
   - **Lustre** : Vitreous
   - **Tenacity** : Brittle
   - **Transparency** : Translucent
   - **Hardness** : 2 to 3
   - **Sp.Gr** : 2 to 3
6. Chemical Properties:

6.1 Assay: Kha ḫkā should contain not more than 50% Silica (SiO₂) and not less than 30% Alumina (Al₂O₃) when analysed by gravimetric method (Appendix-3.1).

6.2 Heavy metals and Arsenic: Kha ḫkā should not contain more than the stated limits for the following: - Lead = 15 ppm, Arsenic = 2 ppm and Cadmium = 7 ppm (Appendix-3.2).

6.3 Other Elements: May contain the following within ± 20% of the stated limits:- Calcium = 0.32%, Magnesium = 0.78% with negligible amount of Manganese (20 ppm) and Chromium (250 ppm) (Appendix-3.1 & 3.2).

6.4 Distinctive Properties:

6.4.1 Take a small quantity of Kha ḫkā powder on a micro slide. Mix with one or two drops of alcoholic methylene blue solution (1% w/v in 95% alcohol). Drop a cover slip and examine under medium or low power. Most particles are stained a deep blue (distinction from talc, which is not stained blue).

6.4.2 Take a small quantity of Kha ḫkā powder on a micro slide. Mix with safranin solution (1% w/v in 70% alcohol). Drop a cover slip and examine under medium or low power. None or occasionally very few particles are coloured red (distinction from bentonite, where all particles are stained deep red).

6.4.3 Place a small quantity of Kha ḫkā powder in a watch glass and add a few drops of dilute hydrochloric acid. No gas or bubbles evolved (distinction from chalk).

6.4.4 Place a small quantity of Kha ḫkā powder (150 mesh) on a micro slide and put a cover slip on to make a dry mount. Now add a drop or two of purified water to the edge of the cover slip and allow the water to flow under the cover slip. The water should spread through the powder, which gets easily dispersed without forming a gel (distinction from bentonite and other clays that would swell and form a gel around the edge).

7. Śodhana: Shall not be used in formulations without subjecting it to śodhana.

7.1 Śodhana: [Rasa Tara-गिन्द्र. 21/210]

Method:
Wash in sufficient quantity of purified water, filter through a clean cloth and use for therapeutic purposes.
8. *Kha°ikā* has the following attributes:-

8.1. **Properties and actions:**
- **Rasa** - Tikta, Madhura
- **Gu´a** - Śīta
- **Vīrya** - Śīta
- **Vipāka** - Madhura
- **Karma** - Pitta śāmaka, Vra´a ropa´a, Kapha-dāha-rakta- doʒghnī, Svedādisrāvahara

8.2  **Therapeutic Uses of Śuddha Kha°ikā**: Śotha (inflammation); Netra roga (diseases of eyes); Atīsāra (diarrhoea)

8.3  **External use**: Tvak roga (skin disease); Mukh-danta roga (disease of mouth and tooth) and Dāha (burning sensation)

9. **Dose**: ½ - 1 g of śuddha *Kha°ikā* for both internal & external use

9.1.  **Anupāna**: To be taken along with cold water.

10. **Important Formulations**: Daśana sa¼skāra cū´a (for external)
MA³  ﮇūra
(Iron Slag)

1. **Definition**: Ma˚²ūra is a metallic oxide-cum-silicate of iron, generally having the composition Fe₂SiO₄ and commonly called Slag.

2. **Synonyms**:
   2.1 सा५क्‍ता : Ki 侂, Lohamala, Loha Ki 侂

2.2 **Names in other Languages**:
   - **English** : Slag
   - **Gujarati** : Ma˚²ūra
   - **Hindi** : Ma˚²ūra, Loha ki˚ा, Si¬gha˚ aka
   - **Kannada** : Loha kitta
   - **Malayalam** : Ierumbin Kittam
   - **Marathi** : Ma˚²ūra
   - **Tamil** : Manturam, Irumbukittam (S.F.I.)
   - **Telugu** : Loha kittam
   - **Urdu** : Khabus-ul-Hadeed, Zang-e-Aahan (N.F.U.M.)

3. **Broad Classification**: Metallic oxide-cum-silicate

4. **Origin and occurrence**: Ma˚²ūra is the by-product of the metallurgical process during extraction of Iron (Fe) and Copper (Cu) from their respective ores. It occurs as lumps, boulders or aggregates at the areas where smelting activity is carried out for the extraction of copper and iron. Iron is the main constituent of Ma˚²ūra followed by Silica with minor amounts of Cu, S, Pb, Zn, Ag, Cd and Au.

   Ma˚²ūra is known since ancient times in India and occurs in over 500 years old slag dumps near village Singhana (Khetri), distt. Jhunjhunu (Rajasthan). Ma˚²ūra of similar quality may occur at other places also in the country where smelting of copper ore was carried out in the past.

5. **Physical Properties**: (Definition / explanation of technical terms and methods of determination as given in Appendix –1).

<table>
<thead>
<tr>
<th>Property</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nature</td>
<td>Rough lumpy masses, exhibiting voids</td>
</tr>
<tr>
<td>Colour</td>
<td>Black</td>
</tr>
<tr>
<td>Streak</td>
<td>Black</td>
</tr>
<tr>
<td>Cleavage</td>
<td>None</td>
</tr>
<tr>
<td>Fracture</td>
<td>Conchoidal</td>
</tr>
<tr>
<td>Lustre</td>
<td>Dull</td>
</tr>
<tr>
<td>Tenacity</td>
<td>Brittle but hard</td>
</tr>
<tr>
<td>Transparency</td>
<td>Opaque</td>
</tr>
<tr>
<td>Magnetism</td>
<td>Non-magnetic</td>
</tr>
<tr>
<td>Hardness</td>
<td>6 to 6.5</td>
</tr>
<tr>
<td>Sp. Gr.</td>
<td>3 to 3.8</td>
</tr>
</tbody>
</table>
6. Chemical properties:

6.1 Assay:

6.1.1 Manḍūra should contain not less than 30% Iron (Fe) when analysed by gravimetric method (Appendix-3.1.4).

6.2.1 Manḍūra should contain not less than 30% Silica when analysed by gravimetric method (Appendix-3.1.3).

6.1.3 Manḍūra should show not less than 80% fayalite (Fe₂SiO₄) when studied through XRD method (Appendix-1.13).

6.2 Heavy metals and Arsenic: Manḍūra should not contain more than the stated limits for the following: - Arsenic = 6 ppm and Cadmium = 8 ppm (Appendix-3.2).

6.3 Other Elements: May contain the following within ± 20% of the stated limits: - Copper = 0.45%, Zinc = 50 ppm and Silver = 7 ppm (Appendix-3.2).

7. Śodhana: Manḍūra shall not be used in formulations without subjecting it to śodhana.

8. Manḍūra is used in the form of bhasma, the details of which are given in the monograph of bhasma.
RAJATA
(Silver metal)

1. **Definition:** Rajata (Ag) is a silver-white metal available in the form of ingots.

2. **Synonyms:**
   2.1 Sa\u092f\u093f\u0923\u0940\u0928\u093e\u093e\u094d\u092c\u094d\u093e : Candra, Raupya, Tāra, Rūpyaka, Rūpya, Rūpyamala

2.2 **Names in other Languages:**
   - Bangali - Rūpā
   - English - Silver
   - Gujarati - Rūpum, Cāndī
   - Hindi - Cāndī, Rūpā
   - Latin - Argentum
   - Marathi - Cāndī, Rupe
   - Telugu - Vendi

3. **Broad Classification:** Metal

4. **Origin and occurrence:** Rajata occurs in nature in the free state, occasionally 99% pure, but generally containing copper, gold and other metals. Native Rajata occurs in the earth's crust in two principal ways:
   
   (i) as small amounts in the oxidised zone of ore deposits and
   (ii) as deposits from hydrothermal solutions. It occurs with sulphides, zeolites, calcite, barite, fluorite and quartz. Native Rajata is much rare in occurrence than native Svarła but is widely distributed in small amounts. In recent years, much of the total Rajata production has been obtained as by-product of gold refining. It is obtained from its ores mainly by amalgamation with mercury or by cyanidation if present in a finely divided state. If not finely divided, the ores are concentrated and smelted. But largely Rajata is derived from smelting of Lead, Zinc and Copper ores which are argentiferous. Pure Rajata may contain Svaŕa (Au) up to 10% with trace amounts of Cu and sometimes Pt, Sb, Bi, As and Hg.

   In India, there are no Rajata deposits as such. It is found associated mainly with lead and zinc and to some extent with copper and gold. All galena (lead ore) deposits of Zawar, Rajpura-Dariba, Deri-Basantgarh and Rampura-Aghucha in Rajasthan, Sargipalli in Orissa, Zangamarajupal, Bandalamotu and Nallakonda in Andhra Pradesh, Hesatu-Belhathan in Bihar and Pauri-Garhwal in Uttaranchal invariably contain Rajata. It is available in market in the form of ingots.
5. **Physical properties**: (Definition / explanation of technical terms and methods of determination as given in Appendix –1).

<table>
<thead>
<tr>
<th>Property</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nature</td>
<td>Granular, nuggets and feather like dendrites</td>
</tr>
<tr>
<td>Colour</td>
<td>Silver white, turning greyish black due to tarnishing</td>
</tr>
<tr>
<td>Streak</td>
<td>White</td>
</tr>
<tr>
<td>Cleavage</td>
<td>None</td>
</tr>
<tr>
<td>Fracture</td>
<td>Hackly</td>
</tr>
<tr>
<td>Lustre</td>
<td>Metallic</td>
</tr>
<tr>
<td>Tenacity</td>
<td>Sectile, ductile and malleable</td>
</tr>
<tr>
<td>Transparency</td>
<td>Opaque</td>
</tr>
<tr>
<td>Hardness</td>
<td>2.5 to 3.0</td>
</tr>
<tr>
<td>Sp.Gr</td>
<td>10.1 to 11.1</td>
</tr>
</tbody>
</table>

6. **Chemical Properties**:  

6.1 **Reaction with Acids**: Prepare solution of Rajata in nitric acid and add hydrochloric acid to it. A dense white curdy precipitate of silver chloride is produced. This precipitate is soluble in ammonia.

6.2 **Solubility**: Soluble in nitric acid.

6.3 **Assay**: Rajata should contain not less than 98.5% Silver (Ag) when analysed by A. A. S. (Appendix-3.2.1).

6.4 **Heavy metals and Arsenic**: In Rajata Mercury, Lead, Arsenic and Cadmium should be absent.

6.5 **Other Elements**: May contain the following within ± 20% of the stated limits: Copper = 1.40 %, Sulphur = traces and Gold = 0.001% (Appendix-3.1 & 3.2).

7. **Śodhana**: Shall not be used in formulations without subjecting it to śodhana.

8. **Rajata** is used in the form of bhasma, the details of which are given in the monograph of bhasma.
SĀMUDRA LAVA³ A  
(Sea Salt)

1. **Definition:** Sāmudra Lava ‘a is a halite mineral, described as Sea salt (NaCl).

2. **Synonyms:**
   2.1 Sa³k‘ta : Sāmudra, Sāmudraka

2.2 **Names in other Languages:**
   - Bangali - Karakaca
   - English - Sea salt
   - Gujarati - Mī‘ha
   - Hindi - Pā-ɡā, Pā-ɡānona, Samudri Namak
   - Kannada - Hojathā
   - Marathi - Mī‘ha
   - Tamil - So‘‘uppu, Uppu (S.F.I.)
   - Telugu - Samudrapu Uppu

3. **Broad Classification:** Halite

4. **Origin and occurrence:** Sāmudra Lava ‘a is formed as extensive irregular beds due to evaporation of seawater in saltpans along seashores in India. Sāmudra Lava ‘a occurs in association with gypsum, poly halite, anhydrite, clay, sand stone and calcite etc. in these pans. Some commercial saltpans, known as source of Sāmudra Lava ‘a in India, are Thuthukkudi (Tuticorin) in Tamilnadu, Cambay in Gujarat and Sambar Lake in Rajasthan.

6. **Physical properties:** (Definition / explanation of technical terms and methods of determination as given in Appendix –1).

<table>
<thead>
<tr>
<th>Nature</th>
<th>Crystalline coarse grained aggregates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour</td>
<td>White / off white</td>
</tr>
<tr>
<td>Streak</td>
<td>None</td>
</tr>
<tr>
<td>Cleavage</td>
<td>Perfect cubic</td>
</tr>
<tr>
<td>Fracture</td>
<td>Conchoidal</td>
</tr>
<tr>
<td>Lustre</td>
<td>Vitreous</td>
</tr>
<tr>
<td>Tenacity</td>
<td>Brittle</td>
</tr>
<tr>
<td>Transparency</td>
<td>Transparent</td>
</tr>
<tr>
<td>Hardness</td>
<td>2.5</td>
</tr>
<tr>
<td>Sp. Gr.</td>
<td>1.98 to 2.2</td>
</tr>
<tr>
<td>Taste</td>
<td>Salty</td>
</tr>
</tbody>
</table>

7. **Optical properties:**

Isotropic with R.I. 1.540 to 1.544 (Appendix-2)

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7. Chemical Properties:

7.1 Effect of Heat:
7.1.1 Take a crystal of Sāmudra Lava´a on a porcelain plate. Blow a burner flame through blowpipe over the Lava´a. The colour of the flame becomes deep yellow indicating the presence of sodium.

7.1.2 When heated through blowpipe, Sāmudra Lava´a crackles and decrepitates and on addition of copper oxide to it, gives the usual blue chlorine flame (Appendix-3.4.3).

7.2 Reaction with silver nitrate: Take 5 g Sāmudra Lava´a and dissolve it in purified water. Add a freshly prepared 5% w/v solution of silver nitrate (AgNO₃) in purified water, drop by drop, to this Lava´a solution. A white precipitate of silver chloride (AgCl) forms.

7.3 Assay:
7.3.1 Sāmudra Lava´a should contain not less than 35% Sodium (Na) when analysed by flame photometry (Appendix-3.2.13).

7.3.2 Sāmudra Lava´a should contain not less than 58% Chlorine (Cl) (Appendix-3.1.10).

7.4 Heavy metals and Arsenic: Sāmudra Lava´a should not contain more than the stated limits for the following: - Lead = 12 ppm, Arsenic = 4 ppm and Cadmium = 4 ppm (Appendix-3.2).

8. Sāmudra Lava´a has the following attributes:

8.1 Properties and Actions:
Rasa - Lava´a
Gu´a - Snigdha, Laghu, Uṣṭa
Vīrya - Nāti Uṣṭa / Nāti Śītala
Vipāka - Madhura
Karma - Vāta-hara, Hṛīdyā, Bhedī, Rucikara, Dīpana, Kaphahara, Śūlaghna, Avidāhī, Pāta Pittala, Snehana, Pācana, Kledana, Balya

8.2 Therapeutic Uses: Ajir´a (dyspepsia), Śoṣha (cachexia), Jir´a carma roga (chronic skin diseases), Galaga´ṣṭi (goiter), Pāṣu (anaemia), Pratiṣyāya (coryza)

9. Dose - According to formulation

10. Important Formulations: Lava´a Bhāskara cūr´a, Sāmudrādya cūr´a, Nārāya´a cūr´a, Mahāśa-kha vaṭi, Kalyānaka gu´a
SAUVĪRĀ¿JANA
(Lead Ore)

1. **Definition:** Sauvīrā¿jana is a lead ore containing galena (PbS) mineral.

2. **Synonyms:**
   2.1 Sa½k’ta : Nīlā¿jana, K ’¾ä¿jana

   2.2 Names in other Languages:
   - Bangali - Suramā
   - English - Galena, Lead ore
   - Gujarati - Suramo
   - Hindi - Suramā, Kālā Suramā
   - Marathi - Suramā
   - Tamil - Vangam (S.F.I.)
   - Telugu - Sauvirã®janamu, Surmã rayi
   - Urdu - Surmah Siyah, Kohal Isphahani, Sang-e-surma (N.F.U.M.)

3. **Broad Classification:** Sulphide

4. **Origin and occurrence:** Sauvīrā¿jana occurs in metamorphic as well as sedimentary rocks in the form of disseminations, veins or lodes. It occurs mostly in pure cubic crystal form and also in ore form associated with rock mass. Sauvīrā¿jana invariably occurs in association with sphalerite (ZnS), their host rock generally being limestone, dolomite and other calc-magnesium rich rocks. Chalcopyrite and pyrite may co-occur in small amount. Silver in minor quantity is always associated. Trace amount of Arsenic, Iron, Cadmium, Gold, Bismuth and Antimony is also reported in Sauvīrā¿jana.

   In India, Sauvīrā¿jana occurs mostly at Zawar, Aghucha, Rajpura-Dariba, Deri - Basanthgarh and Gugra in Rajasthan, Bandalamottu and Zangamarajupal in Andhra Pradesh, Ambaji in Gujarat, Sargipalli in Orissa and Gorubathan in West Bengal. Mining of Sauvīrā¿jana from most of these areas is carried out by Hindustan Zinc Limited (A unit of Vedanta), producing more than 90% lead ore in the country.

5. **Physical Properties:** (Definition / explanation of technical terms and methods of determination as given in Appendix –1).

   - Nature : Usually lumps and heavy cubic crystals
   - Colour : Grey
   - Streak : Grey
   - Cleavage : Cubic
   - Fracture : Even
   - Lustre : Metallic
   - Tenacity : Brittle
   - Transparency : Opaque
6. Chemical properties:

6.1 Charcoal Test: When heated on charcoal through blow pipe, Sauvīrā Janā fuses easily, emits sulphurous fumes and on continued heating yields a globule of metallic lead (Appendix-3.4.4).

6.2 Effect of Acids:
6.2.1 Take about 5 g of finely powdered (150 mesh) Sauvīrā Janā in a test tube. Add 10 ml dilute nitric acid and heat. It evolves brownish fumes.

6.2.2 Take about 5 g finely powdered (150 mesh) Sauvīrā Janā in a test tube. Add 10 ml dilute hydrochloric acid and heat. It evolves hydrogen sulphide (H₂S) gas, as detected by its odour of rotten eggs.

6.3 Assay:
6.3.1 Sauvīrā Janā in ore form should contain not less than 50% Lead (Pb) when analysed by A. A. S. (Appendix-3.2.2).

6.3.2 Sauvīrā Janā in ore form should contain not less than 10% Sulphur when analysed by gravimetric method (Appendix-3.1.8).

6.3.3 Sauvīrā Janā in ore form should contain not less than 500 ppm Silver (Ag) when analysed by A. A. S. (Appendix-3.2.1).

6.4 Heavy metals and Arsenic: Sauvīrā Janā in ore form should not contain more than the stated limits for the following: - Arsenic = 2 ppm and Cadmium = 22 ppm (Appendix-3.2).

6.5 Other Elements: Sauvīrā Janā in ore form may contain the following within ± 20% of the stated limits: - Copper = 70 ppm, Gold = 0.10 ppm and Zinc = 20 ppm (Appendix-3.1 & 3.2).

7. Distinction from Śrotonjana: Cubic cleavage, greater Sp. Gravity and darker colour of Sauvīrā Janā distinguish it from Śroto Janā (Sb₂S₃).

8. Śodhana: Shall not be used in formulations without subjecting it to śodhana.

8.1 Śodhana: [Ref: AFI-Part-1; Appendix-II, Śodhana-3]

i) A Janā : 1 part

ii) Bh̐̃garāja drava (Pl.) : Q.S. for mardana
Method:
Powder the A®jana, add quantity sufficient bh "garāja drava (svarasa) and grind till the contents get dried completely.

9. Sauvīrā®jana has the following attributes:-

9.1 Properties and Actions:

Rasa - Tikta, Ka³āya, Ka¹u
Gu´a - Snigdha
Vīrya - Śīta
Vipāka - Madhura
Karma - Grāhī, Vra´a Śodhana, Ropa´a, Rajorodhaka

9.2 Therapeutic Uses

9.2.1. Śuddha Sauvīrā®jana (External): Netra Roga (diseases of eye)

9.2.2. Bhasma of Sauvīrā®jana: Raktapitta (bleeding disorder); vi³a do¾a (disorders due to poison); Hikkā (hiccup); rajorodha (obstruction of menstrual flow); Raktapradara* (menorrhagia or metrorrhagia or both)

10. Dose: 60-125 mg of the bhasma

*Precaution: It should not be used for more than three days in Raktapradara.

11. Important Formulations: Irimedādi taila (for external use), Nayanām¨tā®jana (for external use).
1. **Definition**: Svaŕa (Au) is a yellow metal available in the form of ingots.

2. **Synonyms**:
   - **Saʌkˈta**: Hā́ka, Hema, Kanaka, Suvaŕa, Hiraˈya, Ka@ana

2.2 **Names in other Languages**:
- **Bangali**: Sonā
- **English**: Gold
- **Gujarati**: Sonu
- **Hindi**: Sonā
- **Latin**: Aurum
- **Marathi**: Sone
- **Telugu**: Bangāram

3. **Broad Classification**: Metal

4. **Origin and occurrence**: Svaŕa is present in the earth's crust and in sea water to the extent of about 4 parts in a hundred million. Most of the Svaŕa that occurs in the earth's crust is in the native state. It occurs in 2 types of deposits; (i) in hydrothermal veins and (ii) in placers. Svaŕa of hydrothermal origin occurs mostly in quartz veins, commonly with pyrite and other sulphides, and gold-silver telluride. The primary deposits of Svaŕa occur in intrusive rocks having composition of diorite, quartz-diorite and granites and their metamorphic equivalents. Placers of Svaŕa occur as rounded or flattened grains and nuggets, in many cases in association with other heavy and resistant minerals. Large quantity of Svaŕa is obtained from sulphides, with which it is probably mechanically mixed.

   Svaŕa occurs very widely diffused in nature, chiefly in the free state, but invariably alloyed with some proportion of Rajata or Tamra, and occasionally with bismuth, mercury and other metals. Native Svaŕa has been known to contain as much as 99.80% gold (Au), but commonly contains 85-95% Au, the balance being usually silver for the most part. The name **electrum** is applied to natural Gold with 20% or more Silver.

   The purity or fineness of Svaŕa is expressed in carats or parts per thousand (1000). Gold containing 999.9 parts per thousand (i.e. 99.99 % Au) is termed as 24 carat Gold. The 22 carat Gold (the standard for Gold coin and jewellery) contains 916.6 parts of gold and 83.4 parts of copper in thousand. That means, 22 carat Gold contains 91.66 % Au and 8.34 % Cu.

   In India, the deposits of primary Svaŕa occur mainly in Hassan and Chitradurga districts in Karnataka, Chigargunta (Chittoor district) in Andhra Pradesh and Kozhikode and Cannore districts in Kerala. Occurrences of Svaŕa have
recently been discovered in the states of Rajasthan and Madhya Pradesh. Main producer of Svar ‘a in country today are Hutti Gold Mines Co. Ltd. (HGML) and Hindustan Copper Ltd. (HCL) as by-product. It is available in market in the form of ingots.

5. **Physical properties:** (Definition / explanation of technical terms and methods of determination as given in Appendix –1).

<table>
<thead>
<tr>
<th>Property</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nature</td>
<td>Granular, dendrites and nuggets</td>
</tr>
<tr>
<td>Colour</td>
<td>Golden yellow</td>
</tr>
<tr>
<td>Streak</td>
<td>Bright yellow</td>
</tr>
<tr>
<td>Cleavage</td>
<td>None</td>
</tr>
<tr>
<td>Fracture</td>
<td>Hackly</td>
</tr>
<tr>
<td>Lustre</td>
<td>Metallic</td>
</tr>
<tr>
<td>Tenacity</td>
<td>Very malleable and ductile</td>
</tr>
<tr>
<td>Transparency</td>
<td>Opaque</td>
</tr>
<tr>
<td>Hardness</td>
<td>2.5 to 3.0 (readily scratched by knife)</td>
</tr>
<tr>
<td>Sp.Gr.</td>
<td>19.30; varies from 15.6 to 18.3 when contains alloying elements like Silver or Copper</td>
</tr>
</tbody>
</table>

6. **Chemical Properties:**

6.1 **Solubility:** Insoluble in any single acid (hydrochloric acid, sulphuric acid or nitric acid) but soluble in aqua-regia (HNO₃: HCl, 1:3)

6.2 **Assay:** Svar ‘a should contain not less than 99.99% Gold (Au) when analysed by Atomic Absorption Spectrorometer (Appendix-3.2.6 & 3.2.10).

6.3 **Heavy metals and Arsenic:** In Svar ‘a Mercury, Lead, Arsenic and Cadmium should be absent.

7. **Distinction from Svar ‘amāk ḫa and Vimala**

7.1 Svar ‘amāk ḫa and Vimala both are brittle and soluble in nitric acid which is not in the case of Svar ‘a.

7.2 Vimala has higher hardness (6-6.5) compared to other two.

7.3 The streak of Svar ‘a is yellow while Svar ‘amāk ḫa gives greenish black streak.

8. **Śodhana:** Shall not be used in formulations without subjecting it to śodhana.

9. Svar ‘a is used in the form of bhasma, the details of which are given in the monograph of bhasma.
SVAR³ AMĀK½IKA
(Copper Ore)

1. Definition: Svaŕamāk¾ika is a copper ore containing Chalcopyrite (CuFeS₂) mineral.

2. Synonyms:
   2.1 Sa½sk¨ta: Dhātumāk¾ika, Māk¾ika, Hemamāk¾ika, Tāpya

   2.2 Names in other Languages:
   - English: Chalcopyrite, Copper ore
   - Gujarati: Māksîka, Sonāmākhī
   - Hindi: Sonāmākhī, Sonāmakkhi,
   - Kannada: Dhātumakśika
   - Malayalam: Makkirakallu
   - Marathi: Daga²ī, Sonāmukhī
   - Tamil: Ponnimilai (S.F.I.)
   - Telugu: Māksîkamu, Svaŕamakhi
   - Urdu: Miss Sokhta (N.F.U.M.)

3. Broad classification: Sulphide

4. Origin and occurrence: Svaŕamāk¾ika, in ore form, occurs mainly in metamorphic rocks of pre-cambrian age. Occurrence of 100% pure Svaŕamāk¾ika in abundance is not common because it is always associated with host rock in the form of disseminations, streaks, stringers, patches, veins or lodes as fracture and cleavage fillings. Svaŕamāk¾ika generally occurs in association with pyrrhotite, pyrite, sphalerite and galena minerals. Gold, Silver, Arsenic, Nickel and Cobalt in trace amounts, are always present in Svaŕamāk¾ika.

   In India, large deposits of Svaŕamāk¾ika occur mainly in Khetri, Distt. Jhunjhunu in Rajasthan, Malanjkhand, Distt. Balagaht in Madhya Pradesh and Ghatshela, Distt. Singhbhum in Jharkhand. Mining of Svaŕamāk¾ika from these 3 areas is carried out by Hindustan Copper Limited (A Govt. of India Enterprise). More than 90% production of this ore in the country comes from these areas only. Small deposits, however, occur in Chitradurga and Hassan in Karnataka, Kurnool and Guntur in Andhra Pradesh and Rangpo in Sikkim.

5. Physical Properties: (Definition / explanation of technical terms and methods of determination as given in Appendix 1).

   Nature: Massive, smooth
   Colour: Golden yellow
   Streak: Greenish black
   Fracture: Uneven
   Lustre: Metallic
   Tenacity: Brittle
6. Chemical Properties:

6.1 **Charcoal Test**: When heated on charcoal, a fragment of *Svar´amākšika* fuses to a black mass without swelling which is strongly magnetic, as felt by a horse-shoe magnet (Appendix-3.4.4).

6.2 **Reaction with Acid**: Take nitric acid in a test tube. Heat to boil for 5 minutes and cool to room temperature. Take 1 g of finely powdered sample in a dry test tube, add 10 ml of the nitric acid to it and heat. The solution becomes bluish black.

6.3 **Assay**:

6.3.1 *Svar´amākšika* in ore form should contain not less than 5% Copper (Cu) (Appendix-3.1.9 & 3.2.2).

6.3.2 *Svar´amākšika* in ore form should contain not less than 20% Iron and 12% Sulphur when analysed by gravimetric method (Appendix-3.1).

6.4 **Heavy metals and Arsenic**: *Svar´amākšika* in ore form should contain not more than the stated limits for the following: - Lead = 70 ppm, Arsenic = 1 ppm and Cadmium = 3 ppm (Appendix-3.2).

6.5 **Other Elements**: *Svar´amākšika* in ore form may contain the following within ± 20% of the stated limits:- Gold = 0.70 ppm, Silver = 48 ppm and Zinc = 800 ppm (Appendix-3.1 & 3.2).

7. **Distinction from Vimala**

7.1 Golden yellow colour and softer than *Vimala* (pyrite), which has hardness 6-6.5.

7.2 *Svar´amākšika*: greenish black streak, *Vimala*: brownish black streak

7.3 Copper content in *Vimala* is negligible.

8. **Śodhana**: Shall not be used in formulations without subjecting it to śodhana.

9. *Svar´amākšika* is used in the form of *bhasma*, the details of which are given in the monograph of *bhasma*.
SVAR³ AMĀK¾IKA -Śj NDRITA  
(Copper Concentrate)

1. **Definition:** Svar´amāk¾ika-Sāndrita is a copper concentrate containing Chalcopyrite (CuFeS₂) mineral with minimum 12 % copper content. (Svar´amāk¾ika-Sāndrita, herein after, is referred as Sāndrita Svar´amāk¾ika in the rest of the monograph).

2. **Broad classification:** Sulphide

3. **Origin and occurrence:** There is no natural occurrence of Sāndrita Svar´amāk¾ika in India or in the world. It is a processed material in the form of fine powder. Beneficiation is done by crushing and grinding of Svar´amāk¾ika and subjecting its slurry to froth flotation. It is important to note that even after processing, it retains its overall mineralogy as well as other properties. The only difference is increase in copper content to more than 12 %. There is a little increase in the content of other major ingredients also. Like Svar´amāk¾ika, it also contains trace but comparatively higher amount of Gold, Silver, Arsenic, Lead, Zinc, Nickel and Cobalt.

In India, large quantity of Sāndrita Svar´amāk¾ika is produced by Hindusthan Copper Limited at Khetri in Rajasthan, Malanjkhand in Madhya pradesh & Ghatsheela in Jharkhand.

3.1 **Preparation of Sandrit Svar´amāk¾ika:**

The Sāndrita Svar´amāk¾ika is produced by crushing and grinding of Svar´amāk¾ika ore lumps to 200 mesh size powder. Substantial amount of water (@ 2.5 M³ per tonne of ore ground) is added during grinding of copper ore to make the material a slurry. This slurry is subjected to froth flotation process, during which pine oil (@ 28 g per tonne of ore ground) and sodium ethyl xanthate (@ 35 g per tonne of ore ground) are added to the flotation cells. The pine oil acts as frother whereas sodium ethyl xanthate as collector. This process enriches the copper content of the slurry just by discarding the gangue material from it. The enriched slurry, which comes out of floatation cells as froth, is dried through hot air dryers. The dried fine powder of 200 mesh size is termed as Concentrate and the process is called beneficiation. The minor amount of pine oil and sodium ethyl xanthate, which was added during floatation process, will totally go away during further processing (i.e. śodhana) by the pharmacies. Hence, the material, which remains after śodhana, will be nothing but Sāndrita Svar´amāk¾ika containing higher content of its ingredients.
4. **Physical Properties**: (Definition / explanation of technical terms and methods of determination as given in Appendix 1).

- **Nature**: Fine powder
- **Colour**: Greenish black with yellow tinge
- **Lustre**: Dull-metallic
- **Sp.Gr**: 3.8 to 4.1

5. **Chemical Properties**:

5.1 **Charcoal Test**: When heated on charcoal, Sāndrita Svar ’āmākḥaka fuses to a black mass which is strongly magnetic, as felt by a horse-shoe magnet (Appendix-3.4.4).

5.2 **Reaction with Acid**: Take nitric acid in a test tube. Heat to boil for 5 minutes and cool to room temperature. Take 1 g powder of Sāndrita Svar ’āmākḥaka in a dry test tube, add 10 ml of the nitric acid to it and heat. The solution becomes bluish black.

5.3 **Assay**:

5.3.1 Sāndrita Svar ’āmākḥaka should contain not less than 12% Copper (Cu) (Appendix-3.1.9 & 3.2.2).

5.3.2 Sāndrita Svar ’āmākḥaka should contain not less than 25% Iron and 28% Sulphur when analysed by gravimetric method (Appendix-3.1).

5.4 **Heavy metals and Arsenic**: Sāndrita Svar ’āmākḥaka should contain not more than the stated limits for the following: Arsenic = 100 ppm and Cadmium = 5 ppm (Appendix – 3.2).

5.5 **Other Elements**: Sāndrita Svar ’āmākḥaka may contain the following within ± 20% of the stated limits:- Gold = 0.40 ppm, Silver = 50 ppm and Zinc = 0.20% (Appendix-3.1 & 3.2).

6. **Śodhana**: Shall not be used in formulations without subjecting it to śodhana.

7. Sāndrita Svar ’āmākḥaka is used in the form of bhasma, the details of which are given in the monograph of bhasma.
TĀMRA
(Copper Metal)

1. Definition: Tāmra is the end product of the metallurgical process of Copper ore. It is available in the form of wires, sheets or rods of Copper which is produced by an electrolytic process.

2. Synonyms:
   2.1 Saṁskāra: Śulvaka, Śulva, Sūrya, Sūryasakhā, Tāmraka, Ravi

2.2 Names in other Languages:
   - Bangali: Tāmā
   - English: Copper
   - Gujarati: Trāmbu, Tāmbu
   - Hindi: Tāmbā
   - Kannada: Tāmbra
   - Latin: Cuprum
   - Malayalam: Chembu
   - Marathi: Tāmbe
   - Tamil: Chempu (S.F.I.)
   - Telugu: Rāgi
   - Urdu: Nuhas, Miss, Tāmba (N.F.U.M.)

3. Broad Classification: Metal

4. Origin and occurrence: Tāmra, in native form, occurs as irregular platy dendrites, crystals and compact mass and is most commonly associated with basic extrusive igneous rocks. Such occurrences are very limited. Electrolytically refined Tāmra is produced mainly in smelter/ refinery plants at Khetri in Rajasthan and Maubhandar-Ghatsheela in Jharkhand, where Copper ore occurs in abundance. Native Tāmra is comparatively less pure than electrolytically refined Copper, which contains 99.50% to 100% Cu.

Native Tāmra as well as electrolytically refined Copper generally contain trace amounts of Gold, Silver, Zinc, Arsenic, Iron, Bismuth and Antimony.

5. Physical properties: (Definition / explanation of technical terms and methods of determination as given in Appendix –1).

   - Nature: Plates, wires or rods; can be bent by hand
   - Colour: Copper red
   - Streak: Copper red
   - Cleavage: None
   - Fracture: Hackly
   - Lustre: Metallic
   - Tenacity: Malleable
   - Transparency: Opaque
Hardness : 2.5 to 3.0
Sp. Gr. : 8 to 9

6. Chemical Properties:

6.1 Reaction with acids: Take about 5 g of finely powdered (150 mesh) Tāmra in a borosil test tube. Add 10 ml nitric acid. It gets completely dissolved in the acid giving a blue solution. Add an excess of ammonia to this blue solution. The colour of solution changes to deep azure blue.

6.2 Assay: Tāmra should contain not less than 99.5% Copper (Cu) (Appendix-3.1.9 & 3.2.2).

6.3 Heavy metals and Arsenic: Tāmra should not contain more than the stated limits for the following: Lead = 5 ppm, Arsenic = 1 ppm and Cadmium = 5 ppm (Appendix-3.2).

6.4 Other Elements: May contain the following within ± 20% of the stated limits:- Zinc = 25 ppm, Silver = 10 ppm and Gold = 135 ppb (Appendix-3.1 & 3.2).

7. Śodhana: Shall not be used in formulations without subjecting it to śodhana.

8. Tāmra is used in the form of bhasma, the details of which are given in the monograph of bhasma.
A≈KA³A
(Borax)

1. Definition: A≈ká is Borax mineral (Na₂B₄O₇, 10 H₂O), also known as Tincal.

2. Synonyms:
   2.1 Sa½k’ta : A≈ká́ka, A≈ga, A≈ka, Saubhāgya

2.2 Names in other Languages:
   Bangali - Sohāgā
   English - Borax, Tincal
   Gujarati - Ta≈kana Khāra, Khadiyo Khāra
   Hindi - Suhāgā
   Kannad - Biligāra, Belgār
   Malayalam - Pongaaram
   Marathi - Ta≈ká’a Khāra
   Punjabi - Sohāgā
   Tamil - Venkaram (S.F.I.)
   Telugu - Veligāram
   Urdu - Tankar, Suhaga (N.F.U.M.)

3. Broad Classification: Borax (Sodium borate)

4. Origin and occurrence: A≈ká occurs as deposits from volcanic emanations (fumaroles), hot springs and in dried up shallow basins (Playa) or saline lakes. It occurs mostly in the waters of various saline lakes in the salt deposits that have been formed through evaporation of such lakes. The origin of A≈ká involves simple concentration and evaporation, which is accompanied by some chemical and mineralogical transformations to give rise to A≈ká (borax). An evaporite mineral, it occurs associated with halites, sulphates, carbonates and other borates like ulexite and colemanite.

   Economically workable deposits of A≈ká are not yet discovered in India, and the domestic need is met by imports of crude borates, which are refined to produce A≈ká and boric acid. Small quantity of A≈ká, since early times, however, was being obtained from salt lakes in Leh district of Jammu and Kashmir and Tibet, where it occurs today also. Non-exploitable occurrences of A≈ká are known in Surendranagar district in Gujarat and Jaipur and Nagaur districts in Rajasthan.

5. Physical properties : (Definition / explanation of technical terms and methods of determination as given in Appendix –1).

   Nature : Crystalline lumps
   Colour : White
   Streak : White
   Cleavage : Poor
Fracture : Conchoidal
Lustre : Vitreous
Tenacity : Brittle
Transparency : Translucent
Hardness : 2 to 2.5
Sp. Gr. : 1.65 to 1.7
Taste : Sweetish alkaline

6. Optical properties.
   Biaxial, Negative, with \( \eta_\alpha, 1.447, \eta_\beta, 1.469 \) and \( \eta_\gamma, 1.472 \) (Appendix-2)

7. Chemical Properties:

7.1 Effect of Heat:
   7.1.1 Heated on a burner flame using blowpipe, \( \bar{a} \ota \ta \acute{a} \) bubbles up and fuses to a clear glassy bead.
   7.1.2 It colours the flame yellow due to sodium and when moistened with sulphuric acid and alcohol, gives a green flame due to boron (Appendix-3.4.3).

7.2 Reaction with acids:
   7.2.1 With hydrochloric acid - gives yellow colour solution in cold condition and on boiling dissolves completely.
   7.2.2 With sulphuric acid - gives colourless solution in cold condition and on boiling dissolves completely.

7.3 Solubility in water: \( \bar{a} \ota \ta \acute{a} \) is completely soluble in purified water producing an alkaline solution as tested by a red litmus paper turning blue.

7.4 Assay:
   7.4.1 \( \bar{a} \ota \ta \acute{a} \) should contain not less than 35% B\(_2\)O\(_3\) (Boron trioxide) (Appendix-3.2.14).
   7.4.2 \( \bar{a} \ota \ta \acute{a} \) should contain not less than 15% Sodium (Na) (Appendix-3.2.13).

7.5 Heavy metals and Arsenic: \( \bar{a} \ota \ta \acute{a} \) should not contain more than the stated limits for the following: - Arsenic = 5 ppm and Cadmium = 4 ppm (Appendix-3.2).

8. Śodhana: Shall not be used in formulations without subjecting it to śodhana.
8.1 Śodhana: [Ref: AFI-Part-I; Appendix-II, Śodhana-19]

Method:
Prepare coarse powder of āka ā and fry over heat source, collect when completely dehydrated.

9. āka ā has the following attributes:

9.1 Properties and Actions:
Rasa - Ka\u
Guṇa - Rūk\a, U\a, Tīk\a, Sāraka
Vīrya - U\a
Vipāka - Ka\u
Karma - H\a, Balya, Sāraka, Kapha nissāraka, Dīpana, Strī pu\a, Mū\a hagarbhapravartaka

9.2 Therapeutic Uses: Kāsa (cough); Śvāsa (Asthma); Vāta roga (diseases due to Vāta do\a); Sthāvara vi\a (poisoning by plant or mineral); Ādhmāna (flatulence with gurgling sound); Vra´a (wound/ulcer)

10. Dose: 125 - 250 mg

11. Important Formulations: Ānandabhīrava rasa, Candrāmēḍa rasa, Icchābhed\a rasa, Saubhāgya va\a, Tribhuvanakīrti rasa.
TUTTHA
(Copper Sulphate)

1. **Definition:** Tuttha is copper sulphate (Cu SO₄, 5H₂O), also known as blue vitriol, copper vitriol, blue stone or chalcanthite mineral.

2. **Synonyms:**
   2.1 Sāśkṛta : Tutthaka

2.2 **Names in other Languages:**
- **Bangali** - Tunte, Tuntiyā
- **English** - Copper Sulphate, Blue vitriol
- **Gujarati** - Morathuthu
- **Hindi** - Niḷā Thothā, Tūtiā
- **Kannada** - Mayuruthutha
- **Malayalam** - Mayilthuththam
- **Marathi** - Moracūda
- **Tamil** - Mayil thuththam, Turken (S.F.I.)
- **Telugu** - Mailu tuttham, Melatutu
- **Urdu** - Tutia, Kabood (N.F.U.M.)

3. **Broad Classification:** Hydrous cupric sulphate

4. **Origin and occurrence:** Tuttha occurs with other hydrated sulphates of copper and iron in the oxidized form near surface zones of copper sulphide ore deposits. It is found generally deposited with mine purified waters, often on the walls of abandoned mine workings. Also, it is found in the zones of weathering of Copper lodes.

   In India, natural occurrence of Tuttha, in abundance, is uncommon. A little amount of Tuttha can be seen near copper mines in Rajasthan, Madhya Pradesh and Jharkhand. Mostly, commercially manufactured Tuttha as a chemical is used for Ayurvedic formulations in the country.

5. **Physical properties:** (Definition / explanation of technical terms and methods of determination as given in Appendix –1).

   - **Nature** : Crystalline lumps
   - **Colour** : Berlin or sky blue
   - **Streak** : Colourless
   - **Cleavage** : Poor
   - **Fracture** : Conchoidal
   - **Lustre** : Vitreous
   - **Tenacity** : Brittle
   - **Transparency** : Translucent
   - **Hardness** : 2 to 2.5
   - **Sp. Gr.** : 2.12 to 2.30
6. Chemical Properties:

6.1 Effect of Heat:

6.1.1 On heating, partially evaporates giving brownish mass.

6.1.2 Heating with sodium carbonate (Na₂CO₃) gives black fused mass.

6.1.3 Heat on charcoal with sodium carbonate (Na₂CO₃) and carbon, it yields metallic copper as observed by its copper red colour.

6.1.4 Heat in closed tube, Tuttha gives off water, observed as droplets on the upper internal wall of the tube (Appendix-3.4.5).

6.2 Solubility in purified water: Take about 10 g accurately weighed fine (150 mesh) powder of Tuttha in 250 ml beaker. Add 50 ml purified water to it. Stir the solution till Tuttha powder stops dissolving. Put the beaker in sun light till the solution gets completely dried up. Weigh the residue, if any. It should not be more than 10% of the initial weight of the sample. That is, solubility of Tuttha in purified water should not be less than 90%.

6.3 Assay: Tuttha should contain not less than 20% Copper (Appendix-3.1.9 & 3.2.2).

6.4 Tuttha should contain not less than 15% Sulphur and not less than 50% SO₄ (Appendix-3.1 & 3.3).

6.5 Heavy metals and Arsenic: Tuttha should not contain more than the stated limits for the following: - Lead = 226 ppm, Arsenic = 4 ppm and Cadmium = 97 ppm (Appendix-3.2).

6.6 Other elements: May contain the following within ± 20% of the stated limits:- Iron 4% when analysed by gravimetric method (Appendix-3.1.4).

7. Śodhana - Shall not be used in formulations without subjecting it to śodhana.

7.1 Śodhana: [Ref: AFI-Part-I; Appendix-II, Śodhana-21]

i) Tuttha : 1 Part
ii) Rakta Candana kvātha [Ht.Wd] : Q.S. for bhāvanā
iii) Maḥā ḫā kvātha [Rt.] : Q.S. for bhāvanā

Method:
Powder Tuttha, add quantity sufficient Rakta Candana Kvātha and grind till complete moisture gets evaporated. Repeat the process for seven times. The process of levigation is to be repeated in all other liquid media i.e. Maḥā Kvātha and Varā Kvātha individually for seven times each.
8. *Tuttha* has the following attributes:

Properties and Actions:

| Rasa     | - Kaºu, Ka¾āya, Madhura |
| Gu’a     | - Laghu, Sara           |
| Vîrya    | - U¼a, Šīta             |
| Vipāka   | - Kaºu                  |
| Karma    | - Kaphapittahara, Lekhana, Bhedana, Balya, Trido¾ghna, Rasāyana, Rucikara, Vāmaka, Var´ya, Garavi¾ghara, Śūlaghna, Cak¾a, Aśmarīhara, Ka´²ūghna, Ka´²īkarmākara, Arśogha, K¨mighna |

8.2. Therapeutic Uses: Kãmi (helminthiasis/worm infestation); Prameha (increased frequency and turbidity of urine); Medoroga (obesity); Śūla (pain/colic); Ku¾ha (diseases of the skin); Śvāsa (Asthma); Amlapitta (hyperacidity); Tvak roga (skin disease); Švitra (Leucoderma /Vitiligo); Arśa (piles); Vra´a (ulcer/wound); Nā² Í Vra´a (sinus); Netra roga (diseases of eyes); Du¾hra´a (non-healing ulcer)

9. Dose: 15 - 30 mg

10. Important Formulations: Jātyādi taila, Nityānanda rasa, Jātyādi gh´īa, Mahā vi¾agarbha taila, Kāśīsādi gh´īa
VAIKRĀNTA
(Tourmaline)

1. **Definition:** Vaikrānta is Tourmaline, sodium aluminium borosilicate of the formula \([(Ca, Na)X(Mg, Al6)X{B3 Al2 Si6X(O,OH)30}]\)

2. **Synonyms:**
   2.1 **Names in other Languages:**
   - Bangali - Cūniviše
   - English - Tourmaline
   - Gujarati - Taramari
   - Hindi - Turamuri
   - Marathi - Turamali, Toramali
   - Telugu - Vaikrantamu

3. **Broad Classification:** Complex ring silicate of boron and aluminium.

4. **Origin and occurrence:** Vaikrānta occurs in granite pegmatites, commonly in radial crystal form. It also occurs in metamorphic rocks, mostly in schists and gneisses. Vaikrānta is a common detrital heavy mineral in sedimentary rocks. Vaikrānta is a wide spread mineral and used also as a Gemstone.

   In India, it occurs in igneous and metamorphic terrains of Rajasthan, Jharkhand, Bihar and Karnataka. In Rajasthan, it mainly occurs in Udaipur, Bhilwara, Ajmer and Rajsamand districts. Vaikrant may be found associated with topaz, spodumene, cassiterite, fluorite and apatite.

5. **Physical properties:** (Definition / explanation of technical terms and methods of determination as given in Appendix –1).

   | Nature         | Crystalline prismatic                  |
   | Colour         | Black                                  |
   | Streak         | Colourless                             |
   | Cleavage       | None, prismatic faces strongly striated vertically and crystals rounded to barrel shaped |
   | Fracture       | Uneven                                 |
   | Lustre         | Vitreous                               |
   | Tenacity       | Brittle and often rather friable       |
   | Transparency   | Translucent                            |
   | Hardness       | 7 to 7.5                               |
   | Sp. Gr.        | 3 to 3.2                               |

6. **Optical properties:**
Uniaxial, negative, \(\eta_o 1.65 \) to 1.69, \(\eta_e 1.63 \) to 1.66, strong birefringence; dichroism present, as seen in parallel polarized light (uncrossed), with change in colour when a fragment is oriented at two positions, right angle to each other, by rotating the stage of microscope (Appendix-2).
7. **Chemical Properties:**

7.1 **Effect of Heat:**

7.1.1 Pyroelectricity- When heated at one end, *Vaikrānta* crystal attracts small bits of papers at the other end.

Heated before blowpipe, *Vaikrānta* swells up and does not fuse easily.

7.2 **Reaction with acids:** *Vaikrānta* does not decompose by any acid.

7.3 **Assay:**

7.3.1 *Vaikrānta* should contain not less than 6% $B_2O_3$ when analysed by ICPA method (Appendix-3.2.14).

7.3.2 *Vaikrānta* should contain not less than 12% ferric oxide ($Fe_2O_3$), not less than 30% Alumina ($Al_2O_3$) and not less than 30% Silica ($SiO_2$) when analysed by gravimetric method (Appendix-3.1).

7.4 **Heavy metals and Arsenic:** *Vaikrānta* should not contain more than the stated limits for the following: Lead = 11 ppm, Arsenic = 4 ppm and Cadmium = 2 ppm (Appendix-3.2).

8. **Śodhana:** Shall not be used in formulations without subjecting it to śodhana.

9. *Vaikrānta* is used in the form of *bhasma*, the details of which are given in the monograph of *bhasma.*
APPENDIX-1

1.1 APPARATUS FOR TESTS AND ASSAYS

1.1.1 - 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. They comply with Indian Standard 4161-1967. They 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.

1.1.2. - 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 –

<table>
<thead>
<tr>
<th>Approximate sieve number*</th>
<th>Nominal mesh aperture size (mm)</th>
<th>Tolerance average aperture size (± mm)</th>
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<tr>
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<td>0.13</td>
</tr>
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<td>250</td>
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</tr>
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<td>180</td>
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</tr>
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<td>53</td>
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</tr>
<tr>
<td>350</td>
<td>45</td>
<td>4.8(3.1)</td>
</tr>
</tbody>
</table>

* Sieve number is the number of meshes in a length of 2.54 cm. in each transverse direction parallel to the wires.

** Figures in brackets refer to close tolerances, those without brackets relate to full tolerances.
1.1.3. -Thermometers


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.

1.1.4 –Ultra-Violet 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%) for lamps of maximum output at 254 nm and 5 μl of a 0.2 per cent w/v solution in ethanol (95%) 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.

1.1.5. -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.

1.1.6. -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.
1.1.7. - Muslin Cloth

Muslin cloth is a cotton fabric where warp is 22 per cm±1 and weft is 18 ±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 coincides 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

2.1 TESTS AND DETERMINATIONS

2.1.1. - Microscopic Identification

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 where they are added ‘in situ’ in powder form as ‘Praksepa Dravyas’. 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, shifting, washing, sedimentation, density separation or by floatation etc., are the preliminary steps. This is followed by clearing the 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 (see later part) 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.

Although monographs prescribe standards only for the ‘Praksepa Dravyas’, characteristics from other ingredients that are processed into extracts or decoctions prior to their addition to a formulation may also be seen in a slide preparation, giving rise to recognisable unique characteristics. In addition, cell or tissue structures common to several ingredients added to a formulation, and therefore not specific to any one of them, would also be present. Caution should therefore be exercised so that such features are not construed as parts from adulterants or substitutes or foreign parts. Proper study of the individual ingredients using authentic material and reference to their monographs in the Ayurvedic Pharmacopoeia for Single Drugs would help avoid errors of this nature. Skill in the recognition of discrete and disoriented tissue components and the knowledge required to ascribe them to their correct source should be acquired by the analyst.

Stains and Reagents for Microchemical Reactions

The Ayurvedic Pharmacopoeia volumes on single drugs already include microchemical reactions for ergastic substances and may be consulted in addition to the following for use on isolated debris:

**Acetic acid:** Dilute 6 ml of glacial acetic acid with 100 ml of distilled water; *used for identification of cystoliths, which dissolve with effervescence.*

**Aniline chloride solution:** Dissolve 2 g in a mixture of 65 ml of 30 per cent ethyl alcohol and 15 ml distilled water and add 2 ml of conc. Hydrochloric acid. *Lignified tissues are stained bright yellow.*

**Bismarck brown:** Dissolve 1 g in 100 ml of 95 per cent of ethyl alcohol; *used as a general stain for macerated material (with Schultze’s).*
**Breamer’s reagent:** Dissolve 1 g of sodium tungstate and 2 g of sodium acetate in sufficient quantity of water to make 10 ml yellowish to brown precipitates; *indicate the presence of tannin.*

**Chlorinated soda solution (Bleaching solution):** Dissolve 75 g of sodium carbonate in 125 ml of distilled water; triturate 50 g of chlorinated lime (bleaching powder) in a mortar with 75 ml of distilled water, adding it little by little. Mix the two liquids and shake occasionally for three or four hours. Filter and store, protected from light. *Used for lighting highly coloured material, by warming in it and washing the tissues thoroughly.*

**Canada balsam (as a Mountant):** Heat Canada balsam on a water bath until volatile matter is removed and the residue sets to a hard mass on cooling. Dissolve residue in xylene to form a thin syrupy liquid. *Used for making permanent mounts of reference slides of selected debris.*

**Chloral hydrate solution:** Dissolve 50 g of chloral hydrate 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.*

**Chloral iodine:** Saturate chloral hydrate solution with iodine, leaving a few crystals undissolved; useful for detecting minute grains of starch otherwise undetectable.

**Chlorziniciodine (Iodinated zinc chloride solution):** Dissolve 20 g of zinc chloride and 6.5 g of potassium iodide in 10 ml of distilled water. Add 0.5 g of iodine and shake for about fifteen minutes before filtering. Dilute if needed prior to use. *Renders cellulosic walls bluish violet and lignified walls yellowish brown to brown.*

**Chromic acid solution:** 10 g of dissolved in 90 ml of dilute sulphuric acid; *macerating agent similar to Schultze’s.*

**Corallin soda:** Dissolve 5 g of corallin in 100 ml of 90 per cent ethyl alcohol. Dissolve 25 g of sodium carbonate in 100 ml distilled water; keep the solutions separate and mix when required, by adding 1 ml of the corallin solution to 20 ml of the aqueous sodium carbonate solution. Prepare fresh each time, as the mixture will not keep for long. *Used for staining sieve plates and callus bright pink and imparts a reddish tinge to starch grains and lignified tissues.*

**Ammoniacal solution of Copper oxide (Cuoxam):** Triturate 0.5 g of copper carbonate in a mortar with 10 ml of distilled water and gradually add 10 ml of strong solution of ammonia (sp. gr. 0.880) with continued stirring; *used for dissolving cellulosic materials.*

**Eosin:** 1 per cent solution in 90 per cent ethyl alcohol; *stains cellulose and aleurone grains red.*

**Ferric chloride solution:** A per cent solution ferric chloride in distilled water. *Taninn containing tissues coloured bluish or greenish black.*

**Glycerin:** Pure or diluted as required with one or two volumes of distilled water. *Used as a general mountant.*

**Haematoxylin, Delafield’s:** Prepare a saturated solution of ammonia alum. To 100 ml of this add a solution of 1 g of Haematoxylin in 6 ml of ethyl alcohol (97 per cent). Leave the mixed solution exposed to air and light in an unstoppered bottle for three or four days. Filter and add to the filtrate 25 ml of glycerin and 25 ml of methyl alcohol. Allow the solution to stand exposed to light, till it acquires a dark colour (about two months). Refilter and store as a stock solution. Dilute it 3 or 4 times volumes with distilled water. *Stains cellulosic fibers blue; used only on water washed material.*
**Iodine water:** Mix 1 volume of decinormal iodine with 4 volumes of distilled water. *Stains starch blue, and reveals crystalloids and globoids when present in aleurone grains.*

**Iodine and Potassium iodide solution:** Dissolve 1 g of potassium iodide in 200 ml of distilled water and 2 g of iodine; *stains lignified walls yellow and cellulosic walls blue.*

**Lactophenol (Amman’s Fluid):** Phenol 20 g, lactic acid 20 g, glycerin 40 g, distilled water 20 ml dissolve; *reveals starch grains in polarised light with a well marked cross at hilum, and also minute crystals of calcium oxalate as brightly polarising points of light.*

**Methylene blue:** A solution in 25 ml of ethyl alcohol (95 per cent). *A general stain for nucleus and bacteria.*

**Millon’s reagent:** Dissolve 1 volume of mercury in 9 volumes of fuming nitric acid (sp. Gr. 1.52), keeping the mixture well cooled during reaction. Add equal volume distilled water when cool. *Stains proteins red.*

**Naphthol solution:** Dissolve 10 g of Naphthol in 100 ml of ethyl alcohol; *a specific stain for detection of inulin; cells containing inulin turn deep reddish violet.*

**Phloroglucinol:** 1 g of phloroglucinol 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 conc. 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.*

**Picric acid solution (Trinitrophenol Solution):** A saturated aqueous solution made by dissolving 1 g of picric acid in 95 ml of distilled water; *stains animal and insect tissues, a light to deep yellow; in a solution with ethyl alcohol, aleurone grains and fungal hyphae are stained yellow.*

**Potash, Caustic:** A 5 per cent aqueous solution; *used to separate tenacious tissues of epidermis and also laticiferous elements and vittae, both of which are stained brown.*

**Ruthenium red:** Dissolve 0.008 g of ruthenium red in 10 ml of a 10 per cent solution of lead acetate; (to be freshly prepared) *used for identification of most kinds of mucilage containing tissues, which turn pink. A 0.0008 g ruthenium red dissolved in 10 ml of distilled water and used immediately stains cuticular tissues in debris to a light pink.*

**Safranin:** A 1 per cent solution in ethyl alcohol 50 per cent; *used to stain lignified cell walls deep red, even after clearing with choral hydrate.*

**Schultze’s Maceration fluid:** Add isolated debris to 50 per cent conc. nitric acid in a test tube and warm over water bath: add a few crystals of potassium chlorate while warming, till tissues soften; cool, wash with water thoroughly and tease out for mounting hard tissues; *isolated cell structures are clearly revealed, but the structures are not useful for measurement of dimensions.*

**Sudan Red III:** Dissolve 0.01 g of sudan red III in 5 ml of ethyl alcohol (90 per cent) and 5 ml of pure glycerin; suberised walls of cork cells, and fatty material in cells are stained bright red.

**Sulphovanadic acid (Mandelin’s reagent):** Triturate 1 g of ammonium vandate with 100 ml conc. sulphuric acid. Allow the deposit to subside and use the clear liquid. *This is to be prepared fresh;
useful for identification of alkaloids, particularly strychnine which turns violet in the cells containing it.

### Refractive Indices of Certain Mountants

<table>
<thead>
<tr>
<th>Mountant</th>
<th>Refractive Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>1.333</td>
</tr>
<tr>
<td>Lactophenol</td>
<td>1.444</td>
</tr>
<tr>
<td>Chloral Hydrate solution</td>
<td>1.44 to 1.48</td>
</tr>
<tr>
<td>Olive oil</td>
<td>1.46 to 1.47</td>
</tr>
<tr>
<td>Glycerol</td>
<td>1.473</td>
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<tr>
<td>Castor oil</td>
<td>1.48</td>
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<tr>
<td>Clove oil</td>
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<tr>
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<tr>
<td>Cassia oil</td>
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<tr>
<td>Xylol</td>
<td>1.49</td>
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<tr>
<td>Alcohol</td>
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</tr>
<tr>
<td>Chloroform</td>
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</tbody>
</table>

2.1.2. Microscopical Methods of Examining Crude Vegetable Drugs

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

I. LEAVES, HERBS AND FLOWERS

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

A. Entire and cut materials

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

(ii) **Cut materials** – For examining cut leaves, herbs and flowers, take several pieces in a test tube and employ the same methods as described for entire materials.

Other methods employed for clarification of the material (leaf and stem) are described below :-

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

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

B. Powder

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

II. FRUITS AND SEEDS

A. Entire materials

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

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

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

B. Powder

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

1. Starch – For examining the presence of starch in the seed, take two specimens, one in iodine solution and the other in water. With iodine solution starch turns blue. Shape and the structure of starch grains can be seen in water and their size is measured.

   When examining objects containing starch, prepare specimen by slightly warming in chloral-hydrate solution.

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

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

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

III. BARKS

A. Entire material

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

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

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

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

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

B. Cut materials

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

C. Powder

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

IV. ROOTS AND RHIZOMES

A. Entire materials

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

1. Starch – Starch is detected with iodine solution. For this, prepare specimen with water to measure the granule of starch with an occular micrometer.

2. Inulin – Inulin is detected with Molish’s reagent. For this place a little powder on a slide and apply 1-2 drops of naphthol and a drop of concentrated sulphuric acid, if inulin is present, the powder will appear reddish-violet coloured. Starch also gives this test, so the test for inulin can be done in the absence of starch.

3. Lignified elements – Lignified elements (fibrovascular bundles, mechanical tissue etc.) are detected with phloroglucinol and concentrated hydrochloric acid or safranine solution as mentioned above for barks.

4. Fixed oil – For fixed oil detection use Sudan IV, as mentioned above for fruits and seeds.

   If required for tannin, anthraquinone derivatives test as mentioned above.

B. Cut material

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

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

C. Powder

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

2.1.3. Types of Stomata

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

1. Anomocytic (irregular-celled) – Previously known as ranunculaceous. The stoma is surrounded by a varying number of cells in no way differing form those of the epidermis generally.

2. Anisocytic (unequal-celled) – Previously known as cruciferous or solanaceous. The stoma is usually surrounded by three subsidiary cells, of which one is markedly smaller than the others.

3. Diacytic (cross-celled) – Previously known as caryophyllaceous. The stoma is accompanied by two subsidiary cells whose common wall is at right angles to the guard cells.

4. Paracytic (parallel-celled) – Previously known as rubiaceous. The stoma has one each side one or more subsidiary cells parallel to the long axis of the pore and guard cells.
2.1.4. Determination of Stomatal Index

The stomatal index is the percentage of the number of stomata formed by the total number of epidermal cells, including the stomata, each stoma being counted as one cell.

Place leaf fragments of about $5 \times 5$ mm in size in a test tube containing about 5 ml of chloral hydrate solution and heat in a boiling water-bath for about 15 minutes or until the fragments become transparent. Transfer a fragment to a microscope slide and prepare the mount, the lower epidermis uppermost, in chloral hydrate solution and put a small drop of glycerol-ethanol solution on one side of the cover-glass to prevent the preparation from drying. Examine with a 40x objective and a 6x eye piece, to which a microscopical drawing apparatus is attached. Mark on the drawing paper a cross (x) for each epidermal cell and a circle (o) for each stoma. Calculate the result as follows:

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

Where

S = the number of stomata in a given area of leaf; and
E = the number of epidermal cells (including trichomes) in the same area of leaf.

![Fig. 1 Various types of stomata](image_url)

For each sample of leaf make not fewer than ten determinations and calculate the average index.

2.1.5. Determination of Palisade Ratio

Palisade ratio is the average number of palisade cells under one epidermal cell.

Place leaf fragments of about $5 \times 5$ mm in size in a test-tube containing about 5 ml of chloral hydrate solution and heat in a boiling water-bath for about 15 minutes or until the fragments become transparent. Transfer a fragment to a microscopical slide and prepare the mount of the upper epidermis in chloral hydrate solution and put a small drop of glycerol solution on one side of the cover-glass to prevent the preparation from drying. Examine with a 40x objective and a 6x eye piece, to which a microscopical drawing apparatus is attached. Trace four adjacent epidermal cells on paper; focus gently downward to bring the palisade into view and trace sufficient palisade cells to cover the area of the outlines of the four epidermal cells. Count the palisade cells under the four
epidermal cells. Where a cell is intersected, include it in the count only when more than half of it is within the area of the epidermal cells. Calculate the average number of palisade cells beneath one epidermal cell, dividing the count by 4; this is the “Palisade ratio” (See Fig. 2).

For each sample of leaf make not fewer than ten determinations and calculate the average number.

For Whole or Cut leaves — Take pieces of leaf lamina with an area of not less than 4 square millimeters from the central portion of the lamina and excluding the midrib and the margin of the leaf. Clear the pieces of lamina by heating in a test tube containing chloral hydrate solution on a boiling water-bath for 30 to 60 minutes or until clear and prepare a mount in glycerol-solution or, if desired, stain with safranin solution and prepare the mount in Canada Balsam. Place the stage micrometer on the microscope stage and examine with 4x objective and a 6x eye piece. Draw a line representing 2 mm on a sheet of paper by means of a microscopical drawing apparatus and construct a square on the line representing an area of 4 square millimeters. Move the paper so that the square is seen in the centre of the field of the eyepiece. Place the slide with the cleared leaf piece on the microscope stage and draw in the veins and veinlets included within the square, completing the outlines of those vein-islets which overlap two adjacent sides of the square. Count the number of vein-islets within the square including those overlapping on two adjacent sides and excluding those intersected by the other two sides. The result obtained is the number of vein-islets in 4 square millimeters. For each sample of leaf make not fewer than three determinations and calculate the average number of vein-islets per square millimeter.

For Leaf Fragments having an area less than 4 square millimeters — Take fragments of leaf lamina each with an area of not less than 1 square millimeter, excluding the midrib and the margin of the leaf. Clear and prepare a mount as stated above. Use a 10x objective and a 6x eyepiece and

\[
\text{Fig. 2 Palisade ratio} = \frac{18.4}{4} = 4.5
\]

2.1.6. Determination of Vein-Islet Number

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

For Whole or Cut leaves — Take pieces of leaf lamina with an area of not less than 4 square millimeters from the central portion of the lamina and excluding the midrib and the margin of the leaf. Clear the pieces of lamina by heating in a test tube containing chloral hydrate solution on a boiling water-bath for 30 to 60 minutes or until clear and prepare a mount in glycerol-solution or, if desired, stain with safranin solution and prepare the mount in Canada Balsam. Place the stage micrometer on the microscope stage and examine with 4x objective and a 6x eye piece. Draw a line representing 2 mm on a sheet of paper by means of a microscopical drawing apparatus and construct a square on the line representing an area of 4 square millimeters. Move the paper so that the square is seen in the centre of the field of the eyepiece. Place the slide with the cleared leaf piece on the microscope stage and draw in the veins and veinlets included within the square, completing the outlines of those vein-islets which overlap two adjacent sides of the square. Count the number of vein-islets within the square including those overlapping on two adjacent sides and excluding those intersected by the other two sides. The result obtained is the number of vein-islets in 4 square millimeters. For each sample of leaf make not fewer than three determinations and calculate the average number of vein-islets per square millimeter.

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

2.1.7. Determination of Stomatal Number

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

2.2. -DETERMINATION OF QUANTITATIVE DATA

2.2.1. - Net Content

The content of the final or retail pack shall not be less than 98 percent of the declared net content.

2.2.2. - Foreign Matter

The sample shall be free from visible signs of mold growth, sliminess, stones, rodent excreta, insects or any other noxious foreign matter when examined as given below.

Take a representative portion from a large container, or remove the entire contents of the packing if 100 g or less, and spread in a thin layer in a suitable dish or tray. Examine in daylight with unaided eye. Transfer suspected particles, if any, to a petri dish, and examine with 10x lens in daylight.

2.2.3. - Determination of Total Ash

Incinerate about 2 to 3 g accurately weighed, of the ground drug in a tared platinum or silica dish at a temperature not exceeding 450° until free from carbon, cool and weigh. If a carbon free ash cannot be obtained in this way, exhaust the charred mass with hot water, collect the residue on an ashless filter paper, incinerate the residue and filter paper, add the filtrate, evaporate to dryness, and ignite at a temperature not exceeding 450°. Calculate the percentage of ash with reference to the air-dried drug.

2.2.4. - Determination of Acid Insoluble Ash

To the crucible containing total ash, add 25 ml of dilute hydrochloric acid. Collect the insoluble matter on an ashless filter paper (Whatman 41) and wash with hot water until the filtrate is neutral. Transfer the filter paper containing the insoluble matter to the original crucible, dry on a hot-plate and ignite to constant weight. Allow the residue to cool in a suitable desiccator for 30 minutes and weigh without delay. Calculate the content of acid-insoluble ash with reference to the air-dried drug.
2.2.5. - Determination of Water Soluble Ash

Boil the ash for 5 minutes with 25 ml of water; collect insoluble matter in a Gooch crucible or on an ashless filter paper, wash with hot water, and ignite for 15 minutes at a temperature not exceeding 450°. Subtract the weight of the insoluble matter from the weight of the ash; the difference in weight represents the water-soluble ash. Calculate the percentage of water-soluble ash with reference to the air-dried drug.

2.2.6. - Determination of Sulphated Ash

Heat a silica or platinum crucible to redness for 10 minutes, allow to cool in a desiccator and weigh. Put 1 to 2 g of the substance, accurately weighed, into the crucible, ignite gently at first, until the substance is thoroughly charred. Cool, moisten the residue with 1 ml of sulphuric acid, heat gently until white fumes are no longer evolved and ignite at 800° ± 25° until all black particles have disappeared. Conduct the ignition in a place protected from air currents. Allow the crucible to cool, add a few drops of sulphuric acid and heat. Ignite as before, allow to cool and weigh. Repeat the operation until two successive weighing do not differ by more than 0.5 mg.

2.2.7. - Determination of Alcohol Soluble Extractive

Macerate 5 g of the air dried drug, coarsely powdered, with 100 ml of alcohol the specified strength in a closed flask for twenty-four hours, shaking frequently during six hours and allow to stand for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105°, to constant weight and weigh. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug.

2.2.8. - Determination of Water Soluble Extractive

Proceed as directed for the determination of alcohol-soluble extractive, using chloroform-water instead of ethanol.

2.2.9. - Determination of Ether Soluble Extractive (Fixed Oil Content)

Transfer a suitably weighed quantity (depending on the fixed oil content) of the air-dried, crushed drug to an extraction thimble, extract with solvent ether (or petroleum ether, b.p. 40° to 60°) in a continuous extraction apparatus (Soxhlet extractor) for 6 hours. Filter the extract quantitatively into a tared evaporating dish and evaporate off the solvent on a water bath. Dry the residue at 105° to constant weight. Calculate the percentage of ether-soluble extractive with reference to the air-dried drug.

2.2.10. - Determination of Moisture Content (Loss on Drying)

Procedure set forth here determines the amount of volatile matter (i.e., water drying off from the drug). For substances appearing to contain water as the only volatile constituent, the procedure given below, is appropriately used.

Place about 10 g of drug (without preliminary drying) after accurately weighing (accurately weighed to within 0.01 g) it in a tared evaporating dish. For example, for unground or unpowdered drug, prepare about 10 g of the sample by cutting shredding so that the parts are about 3 mm in thickness.
Seeds and fruits, smaller than 3 mm should be cracked. Avoid the use of high speed mills in preparing the samples, and exercise care that no appreciable amount of moisture is lost during preparation and that the portion taken is representative of the official sample. After placing the above said amount of the drug in the tared evaporating dish, dry at 105° for 5 hours, and weigh. Continue the drying and weighing at one hour interval until difference between two successive weighing corresponds to not more than 0.25 per cent. Constant weight is reached when two consecutive weighing after drying for 30 minutes and cooling for 30 minutes in a desiccator, show not more than 0.01 g difference.

2.2.11. - Determination of Water Insoluble Matter

Take 10 gm of sample, add 200 ml hot distilled H2O and bring to boiling. Allow to cool to room temperature. Filter through a tared gooch crucible having a bed of asbestos or sintered glass filter. Wash the residue with hot water till the filtrate is sugar-free (perform Molisch test). Dry the gooch crucible or sintered glass filter at 135 20 C and weigh. Express as % insoluble matter.
(Ref :- I.S.I Handbook of Food Analysis (Part II) – 1984 page10)

2.2.12. - Determination of Volatile Oil in Drugs

The determination of volatile oil in a drug is made by distilling the drug with a mixture of water and glycerin, collecting the distillate in a graduated tube in which the aqueous portion of the distillate is automatically separated and returned to the distilling flask, and measuring the volume of the oil. The content of the volatile oil is expressed as a percentage v/w.

The apparatus consists of the following parts (see Fig.1). The clevenger’s apparatus described below is recommended but any similar apparatus may be used provided that it permits complete distillation of the volatile oil. All glass parts of the apparatus should be made of good quality resistance glass.

![Fig.1 Apparatus for volatile oil determination](image-url)
The apparatus is cleaned before each distillation by washing successively with acetone and water, then inverting it, filling it with chromic sulphuric acid mixture, after closing the open end at G, and allowing to stand, and finally rinsing with water.

Method of determination

A suitable quantity of the coarsely powdered drug together with 75 ml of glycerin and 175 ml of water in the one litre distilling flask, and a few pieces of porous earthen ware and one filter paper 15 cm cut into small strips, 7 to 12 mm wide, are also put in the distilling flask, which is then connected to the still head. Before attaching the condenser, water is run into the graduated receiver, keeping the tap T open until the water overflows, at P. Any air bubbles in the rubber tubing a—b are carefully removed by pressing the tube. The tap is then closed and the condenser attached. The contents of the flask are now heated and stirred by frequent agitation until ebullition commences. The distillation is continued at a rate, which keeps the lower end of the condenser cool. The flask is rotated occasionally to wash down any material that adheres to its sides.

At the end of the specified time (3 to 4 hours) heating is discontinued, the apparatus is allowed to cool for 10 minutes and the tap T is opened and the tube L, lowered slowly; as soon as the layer of the oil completely enters into the graduated part of the receiver the tap is closed and the volume is read.

The tube L, is then raised till the level of water in it is above the level of B, when the tap T is slowly opened to return the oil to the bulb. The distillation is again continued for another hour and the volume of oil is again read, after cooling the apparatus as before. If necessary, the distillation is again continued until successive readings of the volatile oil do not differ.

The measured yield of volatile oil is taken to be the content of volatile oil in the drug. The dimensions of the apparatus may be suitably modified in case of necessity.

2.2.13. - Special Processes Used in Alkaloidal Assays

2.2.13.a - Continuous extraction of drug

Where continuous extraction of a drug of any other substance is recommended in the monograph, the process consists of percolating it with suitable solvent at a temperature approximately that of the boiling point of the solvent. Any apparatus that permits the uniform percolation of the drug and the continuous flow of the vapour of the solvent around the percolator may be used. The type commonly known as the Soxhlet apparatus (see fig. 2) is suitable for this purpose.

2.2.13.b - Tests for complete extraction of alkaloids

Complete extraction is indicated by the following tests:

When extracting with an aqueous or alcoholic liquid - After extracting at least three times with the liquid, add to a few drops of the next portion, after acidifying with 2 N hydrochloric acid if necessary, 0.05 ml of potassium mercuri-iodide solution or for solanaceous alkaloids 0.05 ml of potassium iodobismuthate solution; no precipitate or turbidity, is produced.

When extracting with an immiscible solvent - After extracting at least three times with the solvent, add to 1 to 2 ml of the next portion 1 to 2 ml of 0.1 N hydrochloric acid, remove the organic solvent by evaporation, transfer the aqueous residue to a test tube, and add 0.05 ml of
potassium mercuri-iodide solution for solanaceous alkaloids 0.05 ml of potassium iodobismuthate solution or for emetine, 0.05 ml of iodine solution; not more than a very faint opalescence is produced.

Fig. 2 - Apparatus for the continuous extraction of Drugs (Soxhlet apparatus)

2.2.14. - Thin-Layer Chromatography (TLC)

Thin-layer chromatography is a technique in which a solute undergoes distribution between two phases, stationary phase acting through adsorption and a mobile phase in the form of a liquid. The adsorbent is a relatively thin, uniform layer of dry finely powdered material applied to a glass, plastic or metal sheet or plate. Precoated plates are most commonly used. Separation may also be achieved on the basis of partition or a combination of partition and adsorption, depending on the particular type of support, its preparation and its use with different solvent.

Identification can be effected by observation of spots of identical Rₜ value and about equal magnitude obtained, respectively, with an unknown and a reference sample chromatographed on the same plate. A visual comparison of the size and intensity of the spots usually serves for semi-quantitative estimation.
Apparatus

(a) Flat glass plates of appropriate dimensions which allow the application at specified points of the necessary quantities of the solution being examined and appropriate reference solutions and which allow accommodation of the specified migration path-length. 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 adsorbent or coating substance consisting of finely divided adsorbent materials, normally 5 µm 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 binders. 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 (365 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 minutes 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. Nowadays 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 x 2 to 6 mm unless otherwise
specified) on a line parallel with, and 20 mm from, one end of the plate, and not nearer than 20 mm
to the sides; the spots should be 15 mm apart. If necessary, the solutions may be applied in
portions, drying between applications. Mark the sides of the plate 15 cm, or the distance specified
in the monograph, from the starting line. Allow the solvent to evaporate and place the plate in the
tank, ensuring that it is as nearly vertical as possible and that the spots or bands are above the level
of the mobile phase. Close the tank and allow to stand at room temperature, until the mobile phase
has ascended to the marked line. Remove the plate and dry and visualise as directed in the
monograph; where a spraying technique is prescribed it is essential that the reagent be evenly
applied as a fine spray.

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 (365 nm) indicate that the plate
should be examined under an ultra-violet light having a maximum output at about 254 or at about
365 nm, as the case may be.

The term secondary spot means any spot other than the principal spot. Similarly, a
secondary band is any band other than the principal band.

Rf Value

Measure and record the distance of each spot from the point of its application and calculate
the Rf value by dividing the distance travelled by the spots by the distance travelled by the front of
the mobile phase.

2.2.15. - Starch Estimation (Mont Gomery, 1957) [Spectrophotometric method]

Prepare 10 per cent homogenate of the plant tissue in 80 per cent ethanol. Centrifuge at
2000 rpm for 15 minutes. To the residue thus obtained, add 4 ml of distilled water, heat on a water
bath for 15 minutes and macerate with the help of glass rod. To each of the samples, add 3 ml of 52
per cent perchloric acid and centrifuge at 2000 rpm for 15 minutes. The supernatant thus obtained
is made up to known volume (generally up to 10 ml or depending on the expected concentration of
starch). Take 0.1 ml aliquot, add 0.1 ml of 80 per cent phenol and 5 ml conc. sulphuric acid, cool
and then read the absorbance at 490 nm.

2.2.16. - Sugar Estimation (Mont Gomery, 1957) [Spectrophotometric Method]

Prepare 10 per cent homogenate of the plant tissue in 80 per cent ethanol. Centrifuge at
2000 rpm for 15 minutes. The supernatant obtained is made up to known volume (generally up to 10
ml or depending on the expected concentration of sugar). Take 0.1 ml aliquot, add 0.1 ml of 80 per
cent phenol and 5 ml conc. sulphuric acid, cool and then read the absorbance at 490 nm.
2.2.17. - Fatty Oil Estimation

To estimate fatty oils, extract accurately weighed air-dried powdered plant material with petroleum ether (40-60°C) in a Soxhlet apparatus. Dry the extract over anhydrous sodium sulphate and remove the solvent under vacuum at 40°C. Weigh the residue and calculate the percentage with reference to the weight of plant material used.

2.2.18. - Test for Argemone Oil (Mustard Oil)

Take 2-3 drops of the oil in a dry test tube and mix successively with one drop of liquid phenol and 2-4 ml of conc. Sulphuric acid and shake. A deep red colour develops within 10-20 seconds if argemone oil is present as adulterant.

2.2.19. - Test for the Presence of Cottonseed oil (Halphen Test)

Take about 5ml of the oil in a test tube and add equal amount of Sulphur solution (1% solution of Sulphur in carbon disulphide and then add an equal volume of amyl alcohol). Mix thoroughly by shaking and heating gently in a water bath (70-80°C) for a few minutes with occasional shaking until the carbon disulphide has boiled off and the sample stops foaming. Place the tube in an oil bath or a saturated brine bath maintained at 110 to 115°C, and hold for 1 to 2 hours. A red colour at the end of this period indicates the presence of cottonseed oil. This test is sensitive to the extent of 0.5 percent of cottonseed oil in other oils.

2.2.20. - Test for Clove Oil -Alkali-Soluble Matter

Place 80 ml of a 5 per cent w/v solution of potassium hydroxide in a 150-ml flask with a long neck, which is graduated in tenths of an ml and is of such diameter that not less than 15cm in length has a capacity of 10ml. The flask before use is cleaned with Sulphuric acid and well rinsed with water. Add 10ml of the oil, cleared by filtration if necessary, and shake thoroughly at five-minute intervals for half an hour, at ambient temperature. Raise the undissolved portion of the oil into the graduated part of the neck of the flask by gradual addition of more of the potassium hydroxide solution; allow standing for not less than twenty-four hours, and read off the volume of the undissolved portion of the oil. The undissolved portion of the oil measures not less than 1.0 ml and not more than 1.5 ml.

2.2.21. - Test for Eucalyptus Oil

Determination of Cineole

Into a stout-walled test tube, about 15 mm in diameter and 80 mm in length place 3 g, accurately weighed, of the oil precisely dried by shaking with anhydrous calcium chloride together with 2.1 g, accurately weighed, of melted o-cresol. Insert a thermometer, graduated in fifths of a degree, and stir the mixture well in order to induce crystallization; note the highest reading of the thermometer. Warm three tube gently, until the contents are completely melted, insert the tube through a bored cork into a wide-mouthed bottle which is to act as an air jacked; allow to cool slowly, until crystallization commences, or until the temperature has fallen to the point previously noted. Stir the contents of the tube vigorously with the thermometer, running the latter on the side of the tube with an up and down motion in order to induce rapid crystallisation. Continue the string and rubbing a long as the temperature rises. Take the highest point as the freezing point.

Remelt the mixture and repeat the determination of the freezing point until two consecutive concordant results are obtained, because the first temperature noted is always lower than the true freezing point.
Find the percentage w/w of cineole corresponding to the freezing-point from the table, obtaining intermediate value, by interpolation (Table 2.1).

<table>
<thead>
<tr>
<th>Freezing-Point</th>
<th>Percent w/w of Cineole</th>
<th>Freezing – point</th>
<th>Percent w/w of Cineole</th>
</tr>
</thead>
<tbody>
<tr>
<td>24°</td>
<td>45.6</td>
<td>30°</td>
<td>53.4</td>
</tr>
<tr>
<td>25°</td>
<td>46.9</td>
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<td>54.7</td>
</tr>
<tr>
<td>26°</td>
<td>48.2</td>
<td>32°</td>
<td>56.0</td>
</tr>
<tr>
<td>27°</td>
<td>49.5</td>
<td>33°</td>
<td>57.3</td>
</tr>
<tr>
<td>28°</td>
<td>50.8</td>
<td>34°</td>
<td>58.6</td>
</tr>
<tr>
<td>29°</td>
<td>53.1</td>
<td>35°</td>
<td>59.9</td>
</tr>
<tr>
<td>30°</td>
<td>61.2</td>
<td>47°</td>
<td>80.0</td>
</tr>
<tr>
<td>31°</td>
<td>62.5</td>
<td>48°</td>
<td>82.1</td>
</tr>
<tr>
<td>32°</td>
<td>63.8</td>
<td>49°</td>
<td>84.2</td>
</tr>
<tr>
<td>33°</td>
<td>65.2</td>
<td>50°</td>
<td>86.3</td>
</tr>
<tr>
<td>34°</td>
<td>66.8</td>
<td>51°</td>
<td>88.8</td>
</tr>
<tr>
<td>35°</td>
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<td>36°</td>
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<td>37°</td>
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<td>96.3</td>
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<tr>
<td>38°</td>
<td>74.2</td>
<td>55°</td>
<td>99.3</td>
</tr>
<tr>
<td>39°</td>
<td>76.1</td>
<td>55.2°</td>
<td>100.0</td>
</tr>
</tbody>
</table>

The o-cresol used must be pure and dry with a freezing-point not below 30°. It is hygroscopic, and should be stored in a small welt-stoppered bottle because the presence of moisture may lower the results, even to the extent of 5 per cent.

2.2.22.- Determination of Acidity

Reagents

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

Procedure

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

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

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

Where V = corrected volume of 0.05 N sod. Hydroxide used

M = weight in gm of the sample taken for test

2.2.23. –Protein Estimation (Lowry et. al 1951)

Homogenise 100 mg plant material with 3 ml of 10% trichloroacetic acid. Centrifuge the homogenate at 10,000 rpm. Discard the supernatant. Treat the pellets obtained after centrifugation with 3 ml IN sodium hydroxide, heat on water bath for 7 minutes and cool. Centrifuge the solution again for five to ten minutes at 5000 rpm. To 0.5 ml of supernatant thus obtained after centrifugation, add 5 ml reagent containing 100 parts of 2% solution of sodium carbonate and one part of 2% solution of sodium potassium tartrate. Allow it so stand for ten to fifteen minutes. Then add 5 ml Folin and Ciocalteu’s Phenol reagent (diluted with distilled water in ratio of 1:1) and allow to stand for half-hour for development of colour and then finally measure the absorbance at 700 nm.

2.2.24. - Method for Alkaloid Estimation

Macerate the plant material with 2 per cent acetic acid in water, filter and concentrate the filtrate under reduced pressure at 45\(^{0}\) to one third of the original volume. Adjust the pH to 2 by 4 M hydrochloric acid. The yellow precipitate will be separated from the solution (A). Dissolve in it 0.1 M to give solution (B). Add Mayer's reagent to the solution A and B to give precipitate of alkaloid-Mayer's reagent complex. Dissolve it again in acetone - methanol - water (6 : 2 : 10) to give solution. Pass this complex finally through Amberlite IRA 400 anion exchange resin (500 g) to give an aqueous solution of alkaloid chlorides.

2.2.25. -Determination of Esters – Boil a convenient quantity of alcohol (90%) thoroughly to expel CO\(_2\) and neutralize it to solution of phenolphthalein. Dissolve about 2 g of the oil or ester, accurately weighed, in 5 ml of the neutralized alcohol contained in a hard glass flask, and neutralize the free acid in the solution with N/10 alcoholic KOH, using 0.2 ml of solution of phenolphthalein as indicator. Add 20 ml of N/2 alcoholic KOH, attach the flask to a reflux condenser, boil on a water bath for 1 hour, and immediately titrate the excess of alkali with N/2 H\(_2\)SO\(_4\), using a further 0.5 ml of solution of phenolphthalein as indicator. Repeat the operation without the oil or ester. The difference between the titrations is equivalent to the alkali required to saponify the esters.

Each ml of N/2 alcoholic KOH is equivalent to-

- 0.0981 g of Bornyl Acetate
- 0.0364 g of Glycerol Triacetate
- 0.0981 g of Linalyl Acetate
- 0.0991 g of Menthyl Acetate
- 0.0761 g of Menthol Salicylate
2.3. - LIMIT TESTS

Table 2.2 - Permissible Limits of Heavy Metals

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Heavy Metal contents</th>
<th>Permissible limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Lead</td>
<td>10 ppm</td>
</tr>
<tr>
<td>2.</td>
<td>Arsenic</td>
<td>3 ppm</td>
</tr>
<tr>
<td>3.</td>
<td>Cadmium</td>
<td>0.3 ppm</td>
</tr>
<tr>
<td>4.</td>
<td>Mercury</td>
<td>1 ppm</td>
</tr>
</tbody>
</table>

2.3.1. - Limit Test for Arsenic

In the limit test for arsenic, the amount of arsenic is expressed as arsenic, As ppm

Apparatus

A wide-mouthed bottle capable of holding about 120 ml is fitted with a rubber bung through which passes a glass tube. The latter, made from ordinary glass tubing, has a total length of 200 mm and an internal diameter of exactly 6.5 mm (external diameter about 8 mm). It is drawn out at one end to a diameter of about 1 mm and a hole not less than 2 mm in diameter is blown in the side of the tube, near the constricted part. When the bung is inserted in the bottle containing 70 ml of liquid, the constricted end of the tube is above the surface of the liquid, and the hole in the side is below the bottom of the bung. The upper end of the tube is cut off square, and is either slightly rounded or ground smooth.

Two rubber bungs (about 25 mm x 25 mm), each with a hole bored centrally and true, exactly 6.5 mm in diameter, are fitted with a rubber band or spring clip for holding them tightly together. Alternatively the two bungs may be replaced by any suitable contrivance satisfying the conditions described under the General Test.

Reagents

Ammonium oxalate AsT: Ammonium oxalate which complies with the following additional test:

Heat 5 g with 15 ml of water, 5 ml of nitric acid AsT, and 10 ml of sulphuric acid AsT in narrow necked, round-bottomed flask until frothing ceases, cool, and apply the General Test; no visible stain is produced.

Arsenic solution, dilute, AsT:

\[\text{Strong Arsenic solution AsT} \quad 1 \text{ ml} \]
\[\text{Water sufficient to produce} \quad 100 \text{ ml} \]

Dilute arsenic solution, AsT must be freshly prepared.

1 ml contains 0.01 mg of arsenic, as.

Arsenic solution, strong, AsT:

\[\text{Arsenic trioxide} \quad 0.132 \text{ g} \]
\[\text{Hydrochloric acid} \quad 50 \text{ ml} \]
\[\text{Water sufficient to produce} \quad 100 \text{ ml} \]
Brominated hydrochloric acid AsT:

- Bromine solution AsT: 1 ml
- Hydrochloric acid AsT: 100 ml

Bromine solution AsT:

- Bromine: 30 g
- Potassium bromide: 30 g
- Water sufficient to produce 100 ml

It complies with the following test:

Evaporate 10 ml on a water-bath nearly to dryness, add 50 ml of purified water, 10 ml of hydrochloric acid AsT and sufficient stannous chloride solution AsT to reduce the remaining bromine and apply the General Test; the stain produced is not deeper than 1 ml standard stain, showing that the proportion of arsenic present does not exceed 1 part per million.

Citric acid AsT: Citric acid which complies with the following additional tests: Dissolve 10 g in 50 ml of water add 10 ml of stannated hydrochloric acid AsT and apply the General Test; no visible stain is produced.

Hydrochloric acid AsT: Hydrochloric acid diluted with water to contain about 32 per cent w/w of hydrochloric acid and complying with the following additional tests:

(i) Dilute 10 ml with sufficient water to produce 50 ml, add 5 ml of ammonium thiocyanate solution and stir immediately; no colour is produced.

(ii) To 50 ml add 0.2 ml of bromine solution AsT, evaporate on a water-bath until reduced to 16 ml adding more bromine solution AsT, if necessary, in order that an excess, as indicated by the colour, may be present throughout the evaporation; add 50 ml of water and 5 drops of stannous chloride solution AsT, and apply the General Test; the stain produced is not deeper than a 0.2 ml standard stain prepared with the same acid, showing that the proportion of arsenic present does not exceed 0.05 part per million.

Hydrochloric acid (constant-boiling composition) AsT: Boil hydrochloric acid AsT to constant boiling Composition in the presence of hydrazine hydrate, using 1 ml of 10 per cent w/v solution in water per litre of the acid.

*Mercuric Chloride Paper: Smooth white filter paper, not less than 25 mm in width, soaked in a saturated solution of mercuric chloride, pressed to remove superfluous solution, and dried at about 60°, in the dark. The grade of the filter paper is such that the weight is between 65 and 120 g per sq. mm; the thickness in mm of 400 papers is approximately equal numerically, to the weight in g per sq. mm.

Nitric acid AsT: Nitric acid which complies with the following additional test:

Heat 20 ml in a porcelain dish with 2 ml of sulphuric acid AsT, until white fumes are given off. Cool, add 2 ml of water, and again heat until white fumes are given off; cool, add 50 ml of water and 10 ml of stannated hydrochloric acid AsT, and apply the General Test; no visible stain is produced.

*NOTE –Murcuric chloride paper should be stored in a stoppered bottle in the dark. Paper which has been exposed to sunlight or to the vapour of ammonia affords a lighter stain or no stain at all when employed in the limit test for arsenic.
**Potassium chlorate AsT:** *Potassium chlorate* which complies with the following additional test:

Mix 5 g in the cold with 20 ml of *water* and 22 ml of *hydrochloric acid AsT*; when the first reaction has subsided, heat gently to expel chlorine, remove the last traces with a few drops of *stannous chloride solution AsT*, add 20 ml of *water*, and apply the General Test; no visible stain is produced.

**Potassium iodide AsT:** *Potassium iodide* which complies with the following additional test:

Dissolve 10 g in 25 ml of *hydrochloric acid AsT* and 35 ml of *water*, add 2 drops of *stannous chloride solution AsT* and apply the General Test; no visible stain is produced.

**Sodium carbonate, anhydrous AsT:** *Anhydrous sodium carbonate* which complies with the following additional test:

Dissolve 5 g in 50 ml of *water*, add 20 ml of *brominated hydrochloric acid AsT*, remove the excess of bromine with a few drops of *stannous chloride solution AsT*, and apply the General Test; no visible stain is produced.

**Sodium Salicylate:** Of the Indian Pharmacopoeia.

**Stannated hydrochloric acid AsT:**

<table>
<thead>
<tr>
<th>Stannous chloride solution AsT</th>
<th>1 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrochloric Acid AsT</td>
<td>100 ml</td>
</tr>
</tbody>
</table>

**Stannous Chloride solution AsT:** Prepared from *stannous chloride solution* by adding an equal volume of *hydrochloric acid*, boiling down to the original volume, and filtering through a fine-grain filter paper.

It complies with the following test:

To 10 ml add 6 ml of *water* and 10 ml of *hydrochloric acid AsT*, distil and collect 16 ml. To the distillate and 50 ml of *water* and 2 drops of *stannous chloride solution AsT* and apply the General Test; the stain produced is not deeper than a 1-ml standard stain, showing that the proportion of arsenic present does not exceed 1 part per million.

**Sulphuric acid AsT:** *Sulphuric acid* which complies with the following additional test:

Dilute 10 g with 50 ml of *water*, add 0.2 ml of *stannous chloride solution AsT*, and apply the General Test; no visible stain is produced.

**Zinc AsT:** *Granulated Zinc* which complies with following additional test:

Add 10 ml of *stannated hydrochloric acid AsT* to 50 ml of *water*, and apply the General Test, using 10 of the zinc and allowing the action to continue for one hour; no visible stain is produced (limit of arsenic). Repeat the test with the addition of 0.1 ml of *dilute arsenic solution AsT*; a faint but distinct yellow stain is produced (test for sensitivity).
**General Method of Testing** - By a variable method of procedure suitable to the particular needs of each substance, a solution is prepared from the substance being examined which may or may not contain that substance, but contains the whole of the arsenic (if any) originally present in that substance. This solution, referred to as the ‘test solution’, is used in the actual test.

**General Test** - The glass tube is lightly packed with cotton wool, previously moistened with *lead acetate solution* and dried, so that the upper surface of the cotton wool is not less than 25 mm below the top of the tube. The upper end of the tube is then inserted into the narrow end of one of the pair of rubber bungs, either to a depth of about 10 mm when the tube has a rounded-off end, or so that the ground end of the tube is flush with the larger end of the bung. A piece of *mercuric chloride paper* is placed flat on the top of the bung and the other bung placed over it and secured by means of the rubber band or spring clip in such a manner that the borings of the two bungs (or the upper bung and the glass tube) meet to form a true tube 6.5 mm in diameter interrupted by a diaphragm of *mercuric chloride paper*.

Instead of this method of attaching the *mercuric chloride paper*, any other method may be used provided (1) that the whole of the evolved gas passes through the paper; (2) that the portion of the paper in contact with the gas is a circle 6.5 mm in diameter; and (3) that the paper is protected from sunlight during the test. The test solution prepared as specified, is placed in the wide-mouthed bottle, 1 g of *potassium iodide* AsT and 10 g of *zinc* AsT added, and the prepared glass tube is placed quickly in position. The action is allowed to proceed for 40 minutes. The yellow stain which is produced on the *mercuric chloride paper* if arsenic is present is compared by day light with the standard stains produced by operating in a similar manner with known quantities of *dilute arsenic solution* AsT. The comparison of the stains is made immediately at the completion of the test. The standard stains used for comparison are freshly prepared; they fade on keeping.

By matching the depth of colour with *standard stains*, the proportion of arsenic in the substance may be determined. A stain equivalent to the 1-ml standard stain, produced by operating on 10 g of substance indicates that the proportion of arsenic is 1 part per million.

**NOTE:** (1) The action may be accelerated by placing the apparatus on a warm surface, care being taken that the *mercuric chloride paper* remains dry throughout the test.

(2) The most suitable temperature for carrying out the test is generally about 40°C but because the rate of the evolution of the gas varies somewhat with different batches zinc AsT, the temperature may be adjusted to obtain a regular, but not violent, evolution of gas.

(3) The tube must be washed with *hydrochloric acid* AsT, rinsed with water and dried between successive tests.

**Standard Stains** - Solutions are prepared by adding to 50 ml of water, 10 ml of *stannated hydrochloric acid* AsT and quantities of *dilute arsenic solutions* AsT varying from 0.2 ml to 1 ml. The resulting solutions, when treated as described in the General Test, yield stains on the *mercuric chloride paper* referred to as the standard stains.

**Preparation of the Test Solution**

In the various methods of preparing the test solution given below, the quantities are so arranged unless otherwise stated, that when the stain produced from the solution to be examined is not deeper than the 1-ml standard stain, the proportion of arsenic present does not exceed the permitted limit.
Ammonium chloride - Dissolve 2.5 g in 50 ml of water, and 10 ml of stannated hydrochloric acid AsT.

Boric acid - Dissolve 10 g with 2 g of citric acid AsT in 50 ml water, and add 12 ml of stannated hydrochloric acid AsT.

Ferrous sulphate - Dissolve 5 g in 10 ml of water and 15 ml of stannated hydrochloric acid AsT and distil 20 ml; to the distillate add a few drops of bromine solution AsT. Add 2 ml of stannated hydrochloric acid AsT, heat under a reflux condenser for one hour, cool, and add 10 ml of water and 10 ml of hydrochloric acid AsT.

Glycerin - Dissolve 5 g in 50 ml of water, and add 10 ml of stannated hydrochloric acid AsT.

Hydrochloric acid - Mix 10 g with 40 ml of water and 1 ml of stannous chloride solution AsT.

Magnesium sulphate - Dissolve 5 g in 50 ml of water and add 10 ml of stannated hydrochloric acid AsT.

Phosphoric acid - Dissolve 5 g in 50 ml of water and add 10 ml of stannated hydrochloric acid AsT.

Potassium iodide - Dissolve 5 g in 50 ml of water and add 2 ml of stannated hydrochloric acid AsT.

Sodium bicarbonate - Dissolve 5 g in 50 ml of water and add 15 ml of brominated hydrochloric acid AsT, and remove the excess of bromine with a few drops of stannous chloride solution AsT.

Sodium hydroxide - Dissolve 2.5 g in 50 ml of water, add 16 ml of brominated hydrochloric acid AsT, and remove the excess of bromine with a few drops of stannous chloride solution AsT.

2.3.2. - 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 minutes. 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 five minutes.

2.3.3. - Limit Test for Heavy Metals

The test for heavy metals is designed to determine the content of metallic impurities that are coloured by sulphide ion, under specified conditions. The limit for heavy metals is indicated in the individual monographs in terms of the parts of lead per million parts of the substance (by weight), as determined by visual comparison of the colour produced by the substance with that of a control prepared from a standard lead solution.

Determine the amount of heavy metals by one of the following methods and as directed in the individual monographs. Method A is used for substances that yield clear colourless solutions
under the specified test conditions. Method B is used for substances that do not yield clear, colourless solutions under the test conditions specified for method A, or for substances which, by virtue of their complex nature, interfere with the precipitation of metals by sulphide ion. Method C is used for substances that yield clear, colourless solutions with sodium hydroxide solution.

**Special Reagents**

**Acetic acid Sp.** *Acetic acid* which complies with the following additional test: Make 25 ml alkaline with *dilute ammonia solution Sp.*, add 1 ml of *potassium cyanide solution Sp.*, dilute to 50 ml with water and add two drops of *sodium sulphide solution*; no darkening is produced.

**Dilute acetic acid Sp.** *Dilute acetic acid*, which complies with the following additional test – Evaporate 20 ml in a porcelain dish, nearly to dryness on a water-bath. Add to the residue 2 ml of the acid and dilute with water to 25 ml, add 10 ml of *hydrogen sulphide solution*. Any dark colour produced is not more than that of a control solution consisting of 2 ml of the acid and 4.0 ml of *standard lead solution* diluted to 25 ml with water.

**Ammonia solution Sp.** *Strong ammonia solution* which complies with the following additional test: Evaporate 10 ml to dryness on a water-bath; to the residue add 1 ml of *dilute hydrochloric acid Sp.* and evaporate to dryness. Dissolve the residue in 2 ml of dilute acetic acid Sp. Add sufficient water to produce 25 ml.

Add 10 ml of *hydrogen sulphide solution*. Any darkening produced is not greater than in a blank solution containing 2 ml of dilute acetic acid Sp. 1.0 ml of *standard lead solution* and sufficient water to produce 25 ml.

**Dilute ammonia solution Sp.** *Dilute ammonia solution* which complies with the following additional test: To 20 ml add 1 ml of *potassium cyanide solution Sp.*, dilute to 50 ml with water, and add two drops of *sodium sulphide solution*; no darkening is produced.

**Hydrochloric acid** *Hydrochloric acid* which complies with the following additional test: Evaporate off the acid in a beaker to dryness on a water-bath. Dissolve the residue in 2 ml of *dilute acetic acid Sp.* dilute to 17 ml with water and add 10 ml of *hydrogen sulphide solution*; any darkening produced is not greater than in a blank solution containing 2.0 ml of *standard lead solution*, 2 ml of *dilute acetic acid Sp.* and dilute to 40 ml with water.

**Dilute hydrochloric acid Sp.** *Dilute hydrochloric acid*, which complies with the following additional test: Treat 10 ml of the acid in the manner described under *Hydrochloric acid Sp.*

**Lead nitrate stock solution** Dissolve 0.1598 g of *lead nitrate* in 100 ml of water to which has been added 1 ml of *nitric acid*, then dilute with water to 1000 ml. This solution must be prepared and stored in polyethylene or glass containers free from soluble lead salts.

**Standard lead solution** On the day of use, dilute 10.0 ml of *lead nitrate* stock solution with water to 100.0 ml. Each ml of *standard lead solution* contains the equivalent of 10 µg of lead. A control comparison solution prepared with 2.0 ml of standard lead solution contains, when compared to a solution representing 1.0 g of the substance being tested, the equivalent of 20 parts per million of lead.

**Nitric acid Sp.** *Nitric acid* which complies with the following additional test: Dilute 10 ml with 10 ml of water, make alkaline with *ammonia solution Sp.*, add 1 ml of *potassium cyanide solution Sp.*, dilute to 50 ml with water, and add two drops of *sodium sulphide solution*; no darkening is produced.
Potassium cyanide solution Sp.: See Appendix 2.3.5.

Sulphuric acid Sp.: Sulphuric acid which complies with following additional test: Add 5 g to 20 ml of water make alkaline with ammonia solution Sp., add 1 ml of potassium cyanide solution Sp., dilute to 50 ml with water and add two drops of sodium sulphide solution; no darkening is produced.

Method A

**Standard solution** - Into a 50 ml Nessler cylinder, pipette 2 ml of standard lead solution and dilute with water to 25 ml. Adjust with dilute acetic acid Sp. or dilute ammonia solution Sp. to a pH between 3.0 and 4.0, dilute with water to about 35 ml, and mix.

**Test solution** - In to a 50 ml Nessler cylinder, place 25 ml of the solution prepared for the test as directed in the individual monograph, or using the stated volume of acid when specified in the individual monograph, dissolve and dilute with water to 25 ml the specified quantity of the substance being tested. Adjust with dilute acetic acid Sp. or dilute ammonia solution Sp. to a pH between 3.0 and 4.0, dilute with water to about 35 ml and mix.

**Procedure** - To each of the cylinders containing the standard solution and test solution, respectively, add 10 ml of freshly prepared hydrogen sulphide solution, mix, dilute with water to 50 ml, allow to stand for five minutes, and view downwards over a white surface; the colour produced in the test solution is not darker than that produced in the standard solution.

Method B

**Standard solution** - Proceed as directed under Method A.

**Test solution** - Weigh in a suitable crucible the quantity of the substance specified in individual monograph, add sufficient sulphuric acid Sp. to wet the sample, and ignite carefully at a low temperature until thoroughly charred. Add to the charred mass 2 ml of nitric acid Sp. and five drops of sulphuric acid Sp. and heat cautiously until white fumes are no longer evolved. Ignite, preferably in a muffle furnace, at 500° to 600° until the carbon is completely burnt off. Cool, add 4 ml of hydrochloric acid Sp., cover, digest on a water bath for 15 minutes, uncover and slowly evaporate to dryness on a water-bath. Moisten the residue with one drop of hydrochloric acid Sp., add 10 ml of hot water and digest for two minutes. Add ammonia solution sp., dropwise, until the solution is just alkaline to litmus paper, dilute with water to 25 ml and adjust with dilute acetic acid Sp. to a pH between 3.0 and 4.0. Filter if necessary, rinse the crucible and the filter with 10 ml of water, combine the filtrate and washings in a 50 ml Nessler cylinder, dilute with water, to about 35 ml, and mix. Procedure: Proceed as directed under Method A.

Method C

**Standard solution** - Into a 50 ml Nessler cylinder, pipette 2 ml of standard lead solution, add 5 ml of dilute sodium hydroxide solution., dilute with water to 50 ml and mix.

**Test solution** - Into a 50 ml Nessler cylinder, place 25 ml of the solution prepared for the test as directed in the individual monograph; or, if not specified otherwise in the individual monograph, dissolve the specified quantity in a mixture of 20 ml of water and 5 ml of dilute sodium hydroxide solution. Dilute 50 ml with water and mix.
Procedure - To each of the cylinders containing the standard solution and the test solution, respectively add 5 drops of sodium sulphide solution, mix, allow to stand for five minutes and view downwards over a white surface; the colour produced in the test solution is not darker than that produced in the standard solution.

2.3.4. - 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 1000.0 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 five minutes. 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 five minutes.

2.3.5. - Limit Test for Lead

The following method is based on the extraction of lead by solutions of dithizone. All reagents used for the test should have as low a content of lead as practicable. All reagent solutions should be stored in containers of borosilicate glass. Glassware should be rinsed thoroughly with warm dilute nitric acid, followed by water.

Special Reagents

(1) Ammonia-cyanide solution Sp.: Dissolve 2 g of potassium cyanide in 15 ml of strong ammonia solution and dilute with water to 100 ml.

(2) Ammonium citrate solution Sp.: Dissolve 40 g of citric acid in 90 ml water. Add two drops of phenol red solution then add slowly strong ammonia solution until the solution acquires a reddish colour. Remove any lead present by extracting the solution with 20 ml quantities of dithizone extraction solution until the dithizone solution retains its orange-green colour.

(3) Dilute standard lead solution: Dilute 10.0 ml of standard lead solution with sufficient 1 per cent v/v solution of nitric acid to produce 100 ml. Each ml of this solution contains 1 µg of lead per ml.

(4) Dithizone extraction solution: Dissolve 30 mg of diphenylthiocarbazone in 1000 ml of chloroform and add 5 ml of alcohol. Store the solution in a refrigerator. Before use, shake a suitable volume of the solution with about half its volume of 1 per cent v/v solution of nitric acid and discard the acid.

(5) Hydroxylamine hydrochloride solution Sp.: Dissolve 20 g of hydroxylamine hydrochloride in sufficient water to produce about 65 ml. Transfer to separator, add five drops of thymol blue
solution, add strong ammonia solution until the solution becomes yellow. Add 10 ml of a 4 per cent w/v solution of sodium diethyldithiocarbamate and allow to stand for five minutes. Extract with successive quantities, each of 10 ml, of chloroform until a 5 ml portion of the extract does not assume a yellow colour when shaken with dilute copper sulphate solution. Add dilute hydrochloric acid until the solution is pink and then dilute with sufficient water to produce 100 ml.

(6) Potassium cyanide solution Sp.: Dissolve 50 g of potassium cyanide in sufficient water to produce 100 ml. Remove the lead from this solution by extraction with successive quantities, each of 20 ml of dithizone extraction solution until the dithizone solution retains its orange-green colour. Extract any dithizone remaining in the cyanide solution by shaking with chloroform. Dilute this cyanide solution with sufficient water to produce a solution containing 10 g of potassium cyanide in each 100 ml.

(7) Standard dithizone solution: Dissolve 10 ml of diphenylthiocarbazone in 1000 ml of chloroform. Store the solution in a glass-stoppered, lead-free bottle, protected from light and in a refrigerator.

(8) Citrate-cyanide wash solution: To 50 ml of water add 50 ml of ammonium citrate solution Sp. and 4 ml of potassium cyanide solution Sp., mix, and adjust the pH, if necessary, with strong ammonia solution to 9.0.

(9) Buffer solution pH 2.5: To 25.0 ml of 0.2 M potassium hydrogen phthalate add 37.0 ml of 0.1 N hydrochloric acid, and dilute with sufficient water to produce 100.0 ml.

(10) Dithizone-carbon tetrachloride solution: Dissolve 10 mg of diphenylthiocarbazone in 1000 ml of carbon tetrachloride. Prepare this solution fresh for each determination.

(11) pH 2.5 wash solution: To 500 ml of a 1 per cent v/v nitric acid add strong ammonia solution until the pH of the mixture is 2.5, then add 10 ml of buffer solution pH 2.5 and mix.

(12) Ammonia-cyanide wash solution: To 35 ml of pH 2.5 wash solution add 4 ml of ammonia-cyanide solution Sp., and mix.

Method

Transfer the volume of the prepared sample directed in the monograph to a separator and unless otherwise directed in monograph, add 6 ml of ammonium citrate solution Sp., and 2 ml hydroxyamine hydrochloride solution Sp., (For the determination of lead in iron salts use 10 ml of ammonium citrate solution Sp.). Add two drops of phenol red solution and make the solution just alkaline (red in colour) by the addition of strong ammonia solution. Cool the solution if necessary, and add 2 ml of potassium cyanide solution Sp. Immediately extract the solution with several quantities each of 5 ml, of dithizone extraction solution, draining off each extract into another separating funnel, until the dithizone extraction solution retains its green colour. Shake the combine dithizone solutions for 30 seconds with 30 ml of a 1 per cent w/v solution of nitric acid and discard the chloroform layer. Add to the solution exactly 5 ml of standard dithizone solution and 4 ml of ammonia-cyanide solution Sp. and shake for 30 seconds; the colour of the chloroform layer is of no deeper shade of violet than that of a control made with a volume of dilute standard lead solution equivalent to the amount of lead permitted in the sample under examination.
2.3.6. - Limit Test for Sulphates

Reagents

Barium sulphate reagent: Mix 15 ml of 0.5 M barium chloride, 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, dilute to 100 ml with water, and mix. Barium sulphate reagent must be freshly prepared.

0.5 M Barium chloride: Barium chloride dissolved in water to contain in 1000 ml 122.1 g of BaCl₂, 2H₂O.

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 five minutes. 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 five minutes.

2.3.7. - Heavy Metals 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.

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

2. 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 vapor 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.

3. **Monochromator:** Its function is to separate the specified wavelength radiation from the electromagnetic radiations erradiated 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.

4. **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.

5. **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 preparation 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).

2.3.8. - Determination of Lead, Cadmium, Arsenic, Mercury and Copper

(1) Determination of Lead(Pb) (Graphite Oven Method):

**Determination conditions:** Reference condition: dry temperature: 100-120°C, maintain 20 seconds; ash temperature: 400-750°C, maintain 20-25 seconds; atomic temperature: 1700-2100°C, 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°C.

**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 µl to determine their absorbance according to the above method of “Preparation of calibration curve”. Calculate the content of lead (Pd) 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 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 µg per ml As, stored at 0-5°.

**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 minutes. 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°C.

**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°C 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 solutions 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(Cu) (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°C.

**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.

### 2.3.9 Determination of Calcium Oxide

**Apparatus**

(1) Calibrated Brix spindle  
(2) Brix Cylinder  
(3) Conical flasks - 250 ml capacity  
(4) Beakers – 100 and 200 ml capacity  
(5) Funnels  
(6) Pipettes- calibrated at 10 ml

**Reagents**

(1) EDTA solution – Weigh accurately 6.6473 gm EDTA into a beaker, dissolve in distilled water and make upto 1000 ml to obtain exactly M / 56 solution  
(2) Ammonia Liquor  
(3) Lead Subacetate  
(4) Potassium Ferrocyanide powder  
(5) Potassium iodide  
(6) Eriochrome Black – T – weigh 0.1 eriochrome black T in a 100 ml volumetric flask and dissolve the same in rectified spirit or absolute alcohol. Make upto volume and use as indicator

**Procedure**

Make a 15 0 Brix solution of the sample. Transfer about 150 ml of the solution to a conical flask. Clarify the solution with Lead subacetate. Transfer about 60 ml of the clarified solution to a dry conical flask or flask previously rinsed with the clarified solution. Add Potassium Ferrocyanide powder little by little till no further precipitate forms. Shake thoroughly and filter. Test the filterate with Pot. Iodide.. Collect the lead free filterate in a conical flask Pipette out 10 ml of lead free filterate in a clean conical flask previously rinsed with distilled water and dried. Add 5 – 6 drops of liquor ammonia and 4-5 drops of indicator when a pink colour appears. Titrate against EDTA solution shaking the flask after each addition of EDTA solution. The end point is indicated by a sharp change of colour from red to blue. Note down the volume of the titrant

**Calculation**

Calcium oxide mg / 100 gm = V X 100 mg per litre of diluted solution

(Ref :- I.S.I. Handbook of Food Analysis (Part II) – 1984 page 9)

### 2.4. - MICROBIAL LIMIT TESTS

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 pharmaceutical
substances. The term ‘growth’ is used to designate the presence and presumed proliferation of viable micro-organisms.

**Preliminary Testing**

The methods given herein are invalid unless it is demonstrated that the test specimens to which they are applied do not, of themselves, inhibit the multiplication under the test conditions of micro-organisms that can be present. Therefore, prior to doing the tests, inoculate diluted specimens of the substance being examined with separate viable cultures of *Escherichia coli*, *Salmonella* species, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. This is done by adding 1 ml of not less than 10⁻³ dilutions of a 24 h broth culture of the micro-organisms 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 diluent 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 aforementioned modifications so as to permit growth of the organisms in the media. If inhibitory substances are present in the sample, 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 product 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 micro-organisms. 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 at 115°C for 30 minutes.

In preparing media by the formulas given below, dissolve the soluble solids in the water, using heat if necessary, to effect complete solution and add solutions of hydrochloric acid or sodium hydroxide in quantities sufficient to yield the required pH in the medium when it is ready for use. Determine the pH at 25°C ± 2°C.

**Baird-Parker Agar Medium**

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreatic digest of casein</td>
<td>10.0 g</td>
</tr>
<tr>
<td>Beef extract</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>1.0 g</td>
</tr>
<tr>
<td>Lithium chloride</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Agar</td>
<td>20.0 g</td>
</tr>
</tbody>
</table>
Glycine \hspace{1cm} 12.0 \hspace{1cm} g  
Sodium pyruvate \hspace{1cm} 10.0 \hspace{1cm} g  
Water to \hspace{1cm} 1000 \hspace{1cm} ml

Heat with frequent agitation and boil for 1 minute. Sterilise, cool to between 45\(^{0}\) and 50\(^{0}\), and add 10 ml of a one per cent w/v solution of sterile potassium tellurite and 50 ml of egg-yolk emulsion. Mix intimately 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 seconds). Adjust the pH after sterilization to 6.8 ± 0.2.

**Bismuth Sulphite Agar Medium**

Solution (1)

\[
\begin{align*}
\text{Beef extract} & \quad 6 \quad g \\
\text{Peptone} & \quad 10 \quad g \\
\text{Agar} & \quad 24 \quad g \\
\text{Ferric citrate} & \quad 0.4 \quad g \\
\text{Brilliant green} & \quad 10 \quad mg \\
\text{Water to} & \quad 1000 \quad ml
\end{align*}
\]

Dissolve with the aid of heat and sterilise by maintaining at 115\(^{0}\) for 30 minutes.

Solution (2)

\[
\begin{align*}
\text{Ammonium bismuth citrate} & \quad 3 \quad g \\
\text{Sodium sulphite} & \quad 10 \quad g \\
\text{Anhydrous disodium hydrogen Phosphate} & \quad 5 \quad g \\
\text{Dextrose monohydrate} & \quad 5 \quad g \\
\text{Water to} & \quad 100 \quad ml
\end{align*}
\]

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\(^{0}\) and pour.

Bismuth Sulphite Agar Medium should be stored at 2\(^{0}\) to 8\(^{0}\) for 5 days before use.

**Brilliant Green Agar Medium**

\[
\begin{align*}
\text{Peptone} & \quad 10.0 \quad g \\
\text{Yeast extract} & \quad 3.0 \quad g \\
\text{Lactose} & \quad 10.0 \quad g \\
\text{Sucrose} & \quad 10.0 \quad g \\
\text{Sodium chloride} & \quad 5.0 \quad g \\
\text{Phenol red} & \quad 80.0 \quad g \\
\text{Brilliant green} & \quad 12.5 \quad mg \\
\text{Agar} & \quad 12.0 \quad g \\
\text{Water to} & \quad 1000 \quad ml
\end{align*}
\]
Mix, allow to stand for 15 minutes, sterilise by maintaining at 115° for 30 minutes 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. Sterilise by heating in an autoclave at 121° for 15 minutes.

**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
- Cetrimide: 0.3 g
- 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. Sterilise at 121° for 15 minutes.

**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 minutes. With continuous stirring, bring gently to the boil and maintain at boiling point 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.

**Fluid Casein Digest-Soya Lecithin-Polysorbate 20 Medium**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreatic digest of casein</td>
<td>20 g</td>
</tr>
<tr>
<td>Soya lecithin</td>
<td>5 g</td>
</tr>
<tr>
<td>Polysorbate 20</td>
<td>40 ml</td>
</tr>
<tr>
<td>Water to</td>
<td>1000 ml</td>
</tr>
</tbody>
</table>

Dissolve the pancreatic digest of casein and soya lecithin in water, heating in a water-bath at 48° to 50° for about 30 minutes to effect solution. Add polysorbate 20, mix and dispense as desired.

**Fluid Lactose Medium**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef extract</td>
<td>3.0 g</td>
</tr>
<tr>
<td>Pancreatic digest of gelatin</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Lactose</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Water to</td>
<td>1000 ml</td>
</tr>
</tbody>
</table>

Cool as quickly as possible after sterilization. Adjust the pH after sterilization to 6.9 ± 0.2.

**Lactose Broth Medium**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef extract</td>
<td>3.0 g</td>
</tr>
<tr>
<td>Pancreatic digest of gelatin</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Lactose</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Water to</td>
<td>1000 ml</td>
</tr>
</tbody>
</table>

Adjust the pH after sterilisation to 6.9±0.2.

**Levine Eosin-Methylene Blue Agar Medium**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreatic digest of gelatin</td>
<td>10.0 g</td>
</tr>
<tr>
<td>Dibasic potassium phosphate</td>
<td>2.0 g</td>
</tr>
<tr>
<td>Agar</td>
<td>15.0 g</td>
</tr>
<tr>
<td>Lactose</td>
<td>10.0 g</td>
</tr>
<tr>
<td>Eosin Y</td>
<td>400 mg</td>
</tr>
<tr>
<td>Methylene blue</td>
<td>65 mg</td>
</tr>
<tr>
<td>Water to</td>
<td>1000 ml</td>
</tr>
</tbody>
</table>

Dissolve the pancreatic digest of gelatin, dibasic potassium phosphate and agar in water with warming and allow to cool. Just prior to use, liquefy the gelled agar solution and the remaining ingredients, as solutions, in the following amounts and mix. For each 100 ml of the liquefied agar solution use 5 ml of a 20 per cent w/v solution of lactose, and 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 sterilisation to 7.1±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 mg
- Crystal violet: 1 mg
- Water to: 1000 ml

Boil the mixture of solids and water for 1 minute to effect solution. Adjust the pH after sterilisation to 7.1 ± 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 mg
- Water to: 1000 ml

Adjust the pH after sterilisation to 7.3±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

Mix, heat with frequent agitation and boil for 1 minute to effect solution. Adjust the pH after sterilisation to 7.4 ± 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 mg
- Water to: 1000 ml

Dissolve with the aid of heat. Adjust the pH to 8.0 to 8.4 with 5M sodium hydroxide and boil for 10 minutes. Filter, and sterilise by maintaining at 115° for 30 minutes and adjust the pH to 7.3±0.1.
Pseudomonas Agar Medium for Detection of Flourescein

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreatic digest of casein</td>
<td>10.0</td>
<td>g</td>
</tr>
<tr>
<td>Peptic digest of animal tissue</td>
<td>10.0</td>
<td>g</td>
</tr>
<tr>
<td>Anhydrous dibasic potassium phosphate</td>
<td>1.5</td>
<td>g</td>
</tr>
<tr>
<td>Magnesium sulphate hepta hydrate</td>
<td>1.5</td>
<td>g</td>
</tr>
<tr>
<td>Glycerin</td>
<td>10.0</td>
<td>ml</td>
</tr>
<tr>
<td>Agar</td>
<td>15.0</td>
<td>g</td>
</tr>
<tr>
<td>Water to</td>
<td>1000</td>
<td>ml</td>
</tr>
</tbody>
</table>

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 sterilisation to 7.2 ± 0.2.

Pseudomonas Agar Medium for Detection of Pyocyanin

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreatic digest of gelatin</td>
<td>20.0</td>
<td>g</td>
</tr>
<tr>
<td>Anhydrous magnesium chloride</td>
<td>1.4</td>
<td>g</td>
</tr>
<tr>
<td>Anhydrous potassium sulphate</td>
<td>10.0</td>
<td>g</td>
</tr>
<tr>
<td>Agar</td>
<td>15.0</td>
<td>g</td>
</tr>
<tr>
<td>Glycerin</td>
<td>10.0</td>
<td>ml</td>
</tr>
<tr>
<td>Water to</td>
<td>1000</td>
<td>ml</td>
</tr>
</tbody>
</table>

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 sterilisation to 7.2 ± 0.2.

Sabouraud Dextrose Agar Medium

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dextrose</td>
<td>40</td>
<td>g</td>
</tr>
<tr>
<td>Mixture of equal parts of peptic digest of animal tissue and Pancreatic digest of casein</td>
<td>10</td>
<td>g</td>
</tr>
<tr>
<td>Agar</td>
<td>15</td>
<td>g</td>
</tr>
<tr>
<td>Water to</td>
<td>1000</td>
<td>ml</td>
</tr>
</tbody>
</table>

Mix, and boil to effect solution. Adjust the pH after sterilisation to 5.6 ± 0.2.

Sabouraud Dextrose Agar Medium with Antibiotics

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

Selenite F Broth

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>5</td>
<td>g</td>
</tr>
<tr>
<td>Lactose</td>
<td>4</td>
<td>g</td>
</tr>
<tr>
<td>Disodium hydrogen phosphate</td>
<td>10</td>
<td>g</td>
</tr>
<tr>
<td>Sodium hydrogen selenite</td>
<td>4</td>
<td>g</td>
</tr>
<tr>
<td>Water to</td>
<td>1000</td>
<td>ml</td>
</tr>
</tbody>
</table>

Dissolve, distribute in sterile containers and sterilise by maintaining at 100° for 30 minutes.
**Fluid Selenite-Cystine Medium**

Pancreatic digest of casein   5.0  g  
Lactose     4.0  g  
Sodium phosphate    10.0  g  
Sodium hydrogen selenite   4.0  g  
L-Cystine      10.0  mg  
Water to      1000  ml  

Mix and heat to effect solution. Heat in flowing steam for 15 minutes. Adjust the final pH to 7.0 ± 0.2. Do not sterilise.

**Tetrathionate Broth Medium**

Beef extract     0.9  g  
Peptone     4.5  g  
Yeast extract     1.8  g  
Sodium chloride    4.5  g  
Calcium carbonate    25.0  g  
Sodium thiosulphate    40.7  g  
Water to      1000  ml  

Dissolve the solids in water and heat the solution to boil. On the day of use, add a solution prepared by dissolving 5 g of potassium iodide and 6 g of iodine in 20 ml of water.

**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  mg  
Water to      1000  ml  

Heat just to boiling; do not reheat. Adjust the pH so that after heating it is 7.0 ± 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  mg  
Agar     12.0  g  
Water to      1000  ml
Mix, allow standing for 15 minutes, bringing to boil and maintain at boiling point until solution is complete, mix, distributing in tubes and sterilising by maintaining at 115° for 30 minutes. Allow to stand in a sloped form with a butt about 2.5 cm long.

**Urea Broth Medium**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium dihydrogen</td>
<td>9.1 g</td>
</tr>
<tr>
<td>orthophosphate</td>
<td></td>
</tr>
<tr>
<td>Anhydrous disodium hydrogen</td>
<td>9.5 g</td>
</tr>
<tr>
<td>phosphate</td>
<td></td>
</tr>
<tr>
<td>Urea</td>
<td>20.0 g</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>0.1 g</td>
</tr>
<tr>
<td>Phenol red</td>
<td>10 mg</td>
</tr>
<tr>
<td>Water to</td>
<td>1000 ml</td>
</tr>
</tbody>
</table>

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

**Vogel-Johnson Agar Medium**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreatic digest of casein</td>
<td>10.0 g</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Mannitol</td>
<td>10.0 g</td>
</tr>
<tr>
<td>Mannitol</td>
<td>10.0 g</td>
</tr>
<tr>
<td>Dibasic potassium phosphate</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Lithium chloride</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Glycerin</td>
<td>10.0 g</td>
</tr>
<tr>
<td>Agar</td>
<td>16.0 g</td>
</tr>
<tr>
<td>Phenol red</td>
<td>25.0 mg</td>
</tr>
<tr>
<td>Water to</td>
<td>1000 ml</td>
</tr>
</tbody>
</table>

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

**Xylose-Lysine-Desoxycholate Agar Medium**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xylose</td>
<td>3.5 g</td>
</tr>
<tr>
<td>L-Lysine</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Lactose</td>
<td>7.5 g</td>
</tr>
<tr>
<td>Sucrose</td>
<td>7.5 g</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>3.0 g</td>
</tr>
<tr>
<td>Phenol red</td>
<td>80 mg</td>
</tr>
<tr>
<td>Agar</td>
<td>13.5 g</td>
</tr>
<tr>
<td>Sodium desoxycholate</td>
<td>2.5 g</td>
</tr>
<tr>
<td>Sodium thiosulphate</td>
<td>6.8 g</td>
</tr>
<tr>
<td>Ferric ammonium citrate</td>
<td>800 mg</td>
</tr>
<tr>
<td>Water to</td>
<td>1000 ml</td>
</tr>
</tbody>
</table>

Heat the mixture of solids and water, with swirling, just to the boiling point. Do not overheat or sterilise. 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 micro-organisms that should be revealed in the test.

2.4.1. - Total Aerobic Microbial Count

Pretreat the sample of the product being examined as described below.

**Water-soluble products:** Dissolve 10 g or dilute 10 ml of the 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 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 preparation being examined, unless otherwise specified, with 5 g of polysorbate 20 or polysorbate 80. If necessary, heat to not more than 40\(^\circ\). 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\(^\circ\) if necessary. Maintain this temperature for the shortest time necessary for formation of an emulsion and in any case for not more than 30 minutes. If necessary, adjust the pH to about 7.

**Examination of the sample:** Determine the total aerobic microbial count in the substance 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 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\(^\circ\) to 35\(^\circ\) in the test for bacteria and 20\(^\circ\) to 25\(^\circ\) in the test for fungi. Count the number of colonies that are formed. Calculate the number of micro-organisms per g or per ml of the preparation being examined, if necessary counting bacteria and fungi separately.
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 preparation and about 15 ml of liquefied casein soyabean digest agar at not more than 45\(^0\). Alternatively, spread the pretreated preparation on the surface of the solidified medium in a Petri dish of the same diameter. If necessary, dilute the pretreated 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\(^0\) to 35\(^0\) 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\(^0\) to 25\(^0\) 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 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 2.3, indicate the most probable number of micro-organisms per g or per ml of the test specimen.

Table 2.3 – Most Probable Total Count by Multiple-Tube Or Serial Dilution Method

<table>
<thead>
<tr>
<th>Observed combination of numbers of tubes showing growth in each set</th>
<th>No.of mg (or ml) of specimen per tube</th>
<th>Most probable number of micro-organisms per g or per ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100 (100 µl)</td>
<td>10 (10 µl)</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>
2.4.2. - Tests for Specified Micro-Organisms

Pretreatment of the 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 hours for gelatin) and shake again. Loosen the cap and incubate at $37^0$ for 18 to 24 hours.

**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 $36^0$ to $38^0$ for 48 hours. 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 peptone water. Incubate in a water-bath at $43.5^0$ to $44.5^0$ for 24 hours 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*.

**Alternative test:** By means of an inoculating loop, streak a portion from the enrichment culture (obtained in the previous test) on the surface of MacConkey agar medium. Cover and invert the dishes and incubate. Upon examination, if none of the colonies are brick-red in colour and have a surrounding zone of precipitated bile the sample meets the requirements of the test for the absence of *Escherichia coli*.

If the colonies described above are found, transfer the suspect colonies individually to the surface of Levine eosin-methylene blue agar medium, plated on Petri dishes. Cover and invert the plates and incubate. Upon examination, if none of the colonies exhibits both a characteristic metallic sheen under reflected light and a blue-black appearance under transmitted light, the sample meets the requirements of the test for the absence of *Escherichia coli*. The presence of *Escherichia coli* may be confirmed by further suitable cultural and biochemical tests.
**Salmonella**: Transfer a quantity of the pretreated 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 hours and shake again. Loosen the cap and incubate at 35° to 37° for 24 hours.

**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° to 38° for 48 hours. 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° to 38° for 18 to 24 hours. Upon examination, if none of the colonies conforms to the description given in Table 2.4, the sample meets the requirements of the test for the absence of the genus *Salmonella*.

If any colonies conforming to the description in Table 2.4 are produced, carry out the secondary test.

**Secondary test**: Subculture any colonies showing the characteristics given in Table 2.4 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 hours. 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 the urea broth, indicate the presence of *Salmonella*. If acid but no gas is produced in the stab culture, 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*.

**Table 2.4 – Test for Salmonella**

<table>
<thead>
<tr>
<th>Medium</th>
<th>Description of colony</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bismuth sulphite agar</td>
<td>Black or green</td>
</tr>
<tr>
<td>Brilliant green agar</td>
<td>Small, transparent and colourless, or opaque, pinkish or white (frequently surrounded by a pink or red zone)</td>
</tr>
<tr>
<td>Deoxycholate-citrate agar</td>
<td>Colourless and opaque, with or without black centres</td>
</tr>
<tr>
<td>Xylose-lysine-deoxy-cholate agar</td>
<td>Red with or without black centres</td>
</tr>
</tbody>
</table>

**Pseudomonas aeruginosa**: Pretreat the 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° to 37° for 24 to 48 hours. 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° to 37° for 18 to 24 hours.

If, upon examination, none of the plates contains colonies having the characteristics listed in Table 3 for the media used, the sample meets the requirement for freedom from *Pseudomonas*.
If any colonies conforming to the description in Table 2.5 are produced, carry out the oxidase and pigent 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 2.5 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 dihydrochloride 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 2.4 – Tests for *Pseudomonas aeruginosa***

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

*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 4 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 2.5 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 hours and subsequently at suitable intervals up to 24 hours. If no coagulation in any degree is observed, the sample meets the requirements of the test for the absence of *Staphylococcus aureus*.

**Table 2.5 – Tests for *Staphylococcus aureus***

<table>
<thead>
<tr>
<th>Selective medium</th>
<th>Characteristic colonial morphology</th>
<th>Gram stain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vogel-Johnson agar</td>
<td>Black surrounded by yellow zones</td>
<td>Positive cocci (in clusters)</td>
</tr>
<tr>
<td>Mannitol-salt agar</td>
<td>Yellow colonies with yellow zones</td>
<td>Positive cocci (in clusters)</td>
</tr>
<tr>
<td>Baird-Parker agar</td>
<td>Black, shiny, surrounded by clear zones of 2 to 5 mm</td>
<td>Positive cocci (in clusters)</td>
</tr>
</tbody>
</table>
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° to 35° for 18 to 24 hours or, for Candida albicans, at 20° for 48 hours.

*Staphylococcus aureus* (ATCC 6538; NCTC 10788)
*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 micro-organisms per ml. Use the suspension of each of the micro-organisms 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 micro-organisms.

Validity of the tests for specified micro-organisms: 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° to 35° for 18 to 24 hours. Dilute portions of each of the cultures using buffered sodium chloride-peptone solution pH 7.0 to make test suspensions containing about 10³ viable micro-organisms per ml. Mix equal volumes of each suspension and use 0.4 ml (approximately 10² micro-organisms of each strain) as an inoculum in the test for *E. coli, Salmonella, P. aeruginosa* and *S. aureus*, in the presence and absence of the preparation being examined, if necessary. A positive result for the respective strain of micro-organism should be obtained.

### Microbial Contamination Limits

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Parameters</th>
<th>Permissible limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Staphylococcus aureus</em>/g.</td>
<td>Absent</td>
</tr>
<tr>
<td>2.</td>
<td><em>Salmonella sp./g.</em></td>
<td>Absent</td>
</tr>
<tr>
<td>3.</td>
<td><em>Pseudomonas aeruginosa</em>/g</td>
<td>Absent</td>
</tr>
<tr>
<td>4.</td>
<td><em>Escherichia coli</em></td>
<td>Absent</td>
</tr>
<tr>
<td>5.</td>
<td>Total microbial plate count (TPC)</td>
<td>10⁵/g*</td>
</tr>
<tr>
<td>6.</td>
<td>Total Yeast &amp; Mould</td>
<td>10³/g</td>
</tr>
</tbody>
</table>

*For topical use, the limit shall be 10⁷/g.

### 2.5. - 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. The item includes substances intended for use as growth-regulators, defoliants or desiccants and any substance applied to crops either before or after harvest to protect the commodity from deterioration during storage and transport.
**Limits:** Unless otherwise indicated in the monograph, the drug to be examined at least complies with the limits indicated in Table -1. The limits applying to pesticides that are not listed in the table and whose presence is suspected for any reason comply with the limits set by European Community directives 76/895 and 90/642, including their annexes and successive updates. Limits for pesticides that are not listed in Table.-1 nor in EC directives are calculated using the following expression:

\[
\frac{ADI \times M}{MDD \times 100}
\]

*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 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.

The competent authority may grant total or partial exemption from the test when the complete history (nature and quantity of the pesticides used, date of each treatment during cultivation and after the harvest) of the treatment of the batch is known and can be checked precisely.

**Sampling**

**Method:** For containers up to 1 kg, take one sample from the total content, thoroughly mixed, sufficient for the tests. For containers between 1 kg and 5 kg, take three samples, equal in volume, from the upper, middle and lower parts of the container, each being sufficient to carry out the tests. Thoroughly mix the samples and take from the mixture an amount sufficient to carry out the tests. For containers of more than 5 kg, take three samples, each of at least 250 g from the upper, middle and lower parts of the container. Thoroughly mix the samples and take from the mixture an amount sufficient to carry out the tests.

**Size of sampling:** If the number (n) of containers is three or fewer, take samples from each container as indicated above under Method. If the number of containers is more than three, take n+1 samples for containers as indicated under Method, rounding up to the nearest unit if necessary.
The samples are to be analysed immediately to avoid possible degradation of the residues. If this is not possible, the samples are stored in air-tight containers suitable for food contact, at a temperature below $0^\circ$, protected from light.

**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.

2.5.1. - Qualitative and Quantitative Analysis of Pesticide Residues

The analytical procedures used are validated according to the regulations in force. In particular, they satisfy the following criteria:

- the chosen method, especially the purification steps, are suitable for the combination pesticide residue/substance to be analysed and not susceptible to interference from co-extractives; the limits of detection and quantification are measured for each pesticide-matrix combination to be analysed.

- between 70 per cent to 110 per cent of each pesticide is recovered.

- the repeatability of the method is not less than the values indicated in Table 2.6.

- the reproducibility of the method is not less than the values indicated in Table 2.7.

- the concentration of test and reference solutions and the setting of the apparatus are such that a linear response is obtained from the analytical detector.

### Table 2.6

<table>
<thead>
<tr>
<th>Substance</th>
<th>Limit (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alachlor</td>
<td>0.02</td>
</tr>
<tr>
<td>Aldrin and Dieldrin (sum of)</td>
<td>0.05</td>
</tr>
<tr>
<td>Azinphos-methyl</td>
<td>1.0</td>
</tr>
<tr>
<td>Bromopropylate</td>
<td>3.0</td>
</tr>
<tr>
<td>Chlordane (sum of cis-, trans – and Oxytordane)</td>
<td>0.05</td>
</tr>
<tr>
<td>Chlorfenvinphos</td>
<td>0.5</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>0.2</td>
</tr>
<tr>
<td>Chlorpyrifos-methyl</td>
<td>0.1</td>
</tr>
<tr>
<td>Cypermethrin (and isomers)</td>
<td>1.0</td>
</tr>
<tr>
<td>DDT (sum of p,p-'DDT, o,p-'DDT, p,p-'DDE and p,p-'TDE)</td>
<td>1.0</td>
</tr>
<tr>
<td>Deltamethrin</td>
<td>0.5</td>
</tr>
<tr>
<td>Diazinon</td>
<td>0.5</td>
</tr>
<tr>
<td>Dichlorvos</td>
<td>1.0</td>
</tr>
<tr>
<td>Dithiocarbamates (as CS2)</td>
<td>2.0</td>
</tr>
<tr>
<td>Endosulfan (sum of isomers and Endosulfan sulphate)</td>
<td>3.0</td>
</tr>
<tr>
<td>Endrin</td>
<td>0.05</td>
</tr>
<tr>
<td>Ethion</td>
<td>2.0</td>
</tr>
<tr>
<td>Fenitrothion</td>
<td>0.5</td>
</tr>
<tr>
<td>Fenvalerate</td>
<td>1.5</td>
</tr>
<tr>
<td>Fonofos</td>
<td>0.05</td>
</tr>
</tbody>
</table>
Table -2.7

<table>
<thead>
<tr>
<th>Concentration of the pesticide (mg/kg)</th>
<th>Repeatability (difference, $\pm$ mg/kg)</th>
<th>Reproducibility (difference, $\pm$ mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.010</td>
<td>0.005</td>
<td>0.01</td>
</tr>
<tr>
<td>0.100</td>
<td>0.025</td>
<td>0.05</td>
</tr>
<tr>
<td>1.000</td>
<td>0.125</td>
<td>0.25</td>
</tr>
</tbody>
</table>

2.5.2. - Test for Pesticides

Organochlorine, Organophosphorus and Pyrethroid Insecticides

The following methods may be used, in connection with the general method above, 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.

1. Extraction

To 10 g of the substance being examined, coarsely powdered, add 100 ml of acetone and allow to stand for 20 min. Add 1 ml of a solution containing 1.8 µg/ml of carbophenothion in 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°C until the solvent has almost completely evaporated. To the residue add a few milliliters 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).
2. Purification

2.1 Organochlorine, organophosphorus and pyrethroid insecticides:

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 µm).

- as mobile phase toluene at a flow rate of 1 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, in 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.

2.2 Organochlorine and pyrethroid insecticides: 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 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).

2.5.3. - 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 \( \mu \text{m} \) 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 a atomic emission spectrometry detector. Maintaining the temperature of the column at \( 80^0 \) for 1 min, then raising it at a rate of \( 30^0/\text{min} \) to \( 150^0 \), maintaining at \( 150^0 \) for 3 min, then raising the temperature at a rate of \( 4^0/\text{min} \) to \( 280^0 \) and maintaining at this temperature for 1 min and maintaining the temperature of the injector port at \( 250^0 \) and that of the detector at \( 275^0 \). 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 2.8 Calculate the content of each insecticide from the peak areas and the concentrations of the solutions.

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 \( \mu \text{l} \) 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.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Relative retention times</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dichlorvos</td>
<td>0.20</td>
</tr>
<tr>
<td>Fonofos</td>
<td>0.50</td>
</tr>
<tr>
<td>Diazinon</td>
<td>0.52</td>
</tr>
<tr>
<td>Parathion-methyl</td>
<td>0.59</td>
</tr>
<tr>
<td>Chlorpyrifos-methyl</td>
<td>0.60</td>
</tr>
<tr>
<td>Pirimiphos-methyl</td>
<td>0.66</td>
</tr>
<tr>
<td>Malathion</td>
<td>0.67</td>
</tr>
<tr>
<td>Parathion</td>
<td>0.69</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>0.70</td>
</tr>
<tr>
<td>Methidathion</td>
<td>0.78</td>
</tr>
<tr>
<td>Ethon</td>
<td>0.96</td>
</tr>
<tr>
<td>Carbophenothion</td>
<td>1.00</td>
</tr>
<tr>
<td>Azinphos-methyl</td>
<td>1.17</td>
</tr>
<tr>
<td>Phosalon</td>
<td>1.18</td>
</tr>
</tbody>
</table>

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 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 2.9. Calculate the content of each insecticide from the peak areas and the concentrations of the solutions.

**Table 2.9- Relative Retention Times of Insecticides**

<table>
<thead>
<tr>
<th>Substance</th>
<th>Relative retention times</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Hexachlorocyclohexane</td>
<td>0.44</td>
</tr>
<tr>
<td>Hexachlorobenzene</td>
<td>0.45</td>
</tr>
<tr>
<td>β-Hexachlorocyclohexane</td>
<td>0.49</td>
</tr>
<tr>
<td>Lindane</td>
<td>0.49</td>
</tr>
<tr>
<td>δ-Hexachlorocyclohexane</td>
<td>0.54</td>
</tr>
<tr>
<td>ε-Hexachlorocyclohexane</td>
<td>0.56</td>
</tr>
<tr>
<td>Heptachlor</td>
<td>0.61</td>
</tr>
<tr>
<td>Aldrin</td>
<td>0.68</td>
</tr>
<tr>
<td>cis-Heptachlor-epoxide</td>
<td>0.76</td>
</tr>
<tr>
<td>o,p-′DDE</td>
<td>0.81</td>
</tr>
<tr>
<td>α-Endosulfan</td>
<td>0.82</td>
</tr>
<tr>
<td>Dieldrin</td>
<td>0.87</td>
</tr>
<tr>
<td>p,p-′DDE</td>
<td>0.87</td>
</tr>
<tr>
<td>o,p-′DDD</td>
<td>0.89</td>
</tr>
<tr>
<td>Endrin</td>
<td>0.91</td>
</tr>
<tr>
<td>β-Endosulfan</td>
<td>0.92</td>
</tr>
<tr>
<td>o,p-′DDT</td>
<td>0.95</td>
</tr>
<tr>
<td>Carbophenothion</td>
<td>1.00</td>
</tr>
<tr>
<td>p,p-′DDT</td>
<td>1.02</td>
</tr>
<tr>
<td>cis-Permethrin</td>
<td>1.29</td>
</tr>
<tr>
<td>trans-Permethrin</td>
<td>1.31</td>
</tr>
<tr>
<td>Cypermethrin*</td>
<td>1.40</td>
</tr>
<tr>
<td>Fenvalerate*</td>
<td>1.47 and 1.49</td>
</tr>
<tr>
<td>Deltamethrin</td>
<td>1.54</td>
</tr>
</tbody>
</table>

*The substance shows several peaks.

**2.6. - GAS CHROMATOGRAPHY**

Gas chromatography (GC) is a chromatographic separation technique based on the difference in the distribution of species between two non-miscible phases in which the mobile phase is a carrier gas moving through or passing the stationary phase contained in a column. It is applicable to substances or their derivatives, which are volatilized under the temperatures employed.

GC is based on mechanisms of adsorption, mass distribution or size exclusion.

**Apparatus**

The apparatus consists of an injector, a chromatographic column contained in an oven, a detector and a data acquisition system (or an integrator or a chart recorder). The carrier gas flows through the column at a controlled rate or pressure and then through the detector.
The chromatography is carried out either at a constant temperature or according to a given temperature programme.

Injectors

Direct injections of solutions are the usual mode of injection, unless otherwise prescribed in the monograph. Injection may be carried out either directly at the head of the column using a syringe or an injection valve, or into a vaporization chamber which may be equipped with a stream splitter.

Injections of vapour phase may be effected by static or dynamic head-space injection systems.

Dynamic head-space (purge and trap) injection systems include a sparging device by which volatile substances in solution are swept into an absorbent column maintained at a low temperature. Retained substances are then desorbed into the mobile phase by rapid heating of the absorbent column.

Static head-space injection systems include a thermostatically controlled sample heating chamber in which closed vials containing solid or liquid samples are placed for a fixed period of time to allow the volatile components of the sample to reach equilibrium between the non-gaseous phase and the vapour phase. After equilibrium has been established, a predetermined amount of the head-space of the vial is flushed into the gas chromatograph.

Stationary Phases

Stationary phases are contained in columns, which may be:

- a capillary column of fused-silica close wall is coated with the stationary phase.
- a column packed with inert particles impregnated with the stationary phase.
- a column packed with solid stationary phase.

Capillary columns are 0.1 mm to 0.53 mm in internal diameter (Φ) and 5 to 6 m in length. The liquid or stationary phase, which may be chemically bonded to the inner surface, is a film 0.1 µm to 5.0 µm thick.

Packed columns, made of glass or metal, are usually 1 m to 3 m in length with an internal diameter (Φ) of 2 mm to 4 mm. Stationary phases usually consist of porous polymers or solid supports impregnated with liquid phase.

Supports for analysis of polar compounds on columns packed with low-capacity, low-polarity stationary phase must be inert to avoid peak tailing. The reactivity of support materials can be reduced by silanising prior to coating with liquid phase. Acid-washed, flux-calcinated diatomaceous earth is often used. Materials are available in various particle sizes, the most commonly used particles are in the ranges of 150 µm to 180 µm and 125 µm to 150 µm.

Mobile Phases

Retention time and peak efficiency depend on the carrier gas flow rate; retention time is directly proportional to column length and resolution is proportional to the square root of the column length. For packed columns, the carrier gas flow rate is usually expressed in milliliters per minute at atmospheric pressure and room temperature, flow rate is measured at the detector outlet, either with a calibrated mechanical device or with a bubble tube, while the column is at operating
temperature. The linear velocity of the carrier gas through a packed column is inversely proportional to the square root of the internal diameter of the column for a given flow volume. Flow rates of 60 ml/min in a 4 mm internal diameter column and 15 ml/min in a 2 mm internal diameter column, give identical linear velocities and thus similar retention times.

Helium or nitrogen is usually employed as the carrier gas for packed columns, whereas commonly used carrier gases for capillary columns are nitrogen, helium and hydrogen.

Detectors

Flame-ionisation detectors are usually employed but additional detectors which may be used include: electron-capture, nitrogen-phosphorus, mass spectrometric, thermal conductivity, Fourier transform infrared spectrophotometric and others, depending on the purpose of the analysis.

Method

Equilibrate the column, the injector and the detector at the temperatures and the gas flow rates specified in the monograph until a stable baseline is achieved. Prepare the test solution(s) and the reference solutions(s) as prescribed. The solutions must be free from solid particles.

Criteria for assessing the suitability of the system are described in the chapter on Chromatographic separation techniques. The extent to which adjustments of parameters of the chromatographic system can be made to satisfy the criteria of system suitability are also given in this chapter.

2.7. - TEST FOR AFLATOXINS

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 B₁, B₂, G₁ and G₂ 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: Grind about 200 g of plant material to a fine powder. Transfer about 50 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 minutes 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 separatory funnel. Add 40 ml of sodium chloride solution and 25 ml of hexane and shake for 1 minute. Allow the layers to separate and transfer the lower aqueous layer to a second separatory funnel. Extract the aqueous layer in the separatory funnel twice, each time with 25 ml of methylene chloride, by shaking for 1 minute. Allow the layers to separate each time, separate the lower organic layer and collect the combined organic layers in a 125 ml conical flask. Evaporate the organic solvent to dryness on a water bath. Cool the residue. If interferences exist in the residue, proceed as directed for Cleanup 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 minutes. 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 separatory funnel.”

**Cleanup Procedure**: Place a medium-porosity sintered-glass disk or a glass wool plug at the bottom of a 10 mm x 300 mm chromatographic tube. Prepare slurry of 2 g of silica gel with a mixture of **ethyl 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 sulfate**. 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 minute. 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 minute; 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 0.2 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 aflatoxin B1, aflatoxin B2, aflatoxin G1 and aflatoxin G2 in a mixture of **chloroform** and **acetonitrile** (9.8:0.2) to obtain a solution having concentrations of 0.5 µg /per ml each for aflatoxin B1 and G1 and 0.1µg per ml each for aflatoxins for B2 and G2.

**Procedure**: Separately apply 2.5 µl, 5 µl, 7.5 µl 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 mixture. 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 15 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 365 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**, 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 aflatoxin 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**.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Aflatoxins</th>
<th>Permissible Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>B1</td>
<td>0.5 ppm</td>
</tr>
<tr>
<td>2.</td>
<td>G1</td>
<td>0.5 ppm</td>
</tr>
<tr>
<td>3.</td>
<td>B2</td>
<td>0.1 ppm</td>
</tr>
<tr>
<td>4.</td>
<td>G2</td>
<td>0.1 ppm</td>
</tr>
</tbody>
</table>

*For Domestic use only
APPENDIX - 3

3.1. PHYSICAL TESTS AND DETERMINATIONS

3.1.1. - REFRACTIVE INDEX

The refractive index ($\eta$) of a substance with reference to air is the ratio of the sine of the angle of incidence to the sine of the angle of refraction of a beam of light passing from air into the substance. It varies with the wavelength of the light used in its measurement.

Unless otherwise prescribed, the refractive index is measured at 25°C (±0.5) with reference to the wavelength of the D line of sodium ($\lambda = 589.3$ nm). The temperature should be carefully adjusted and maintained since the refractive index varies significantly with temperature.

The Abbe’s refractometer is convenient for most measurements of refractive index but other refractometer of equal or greater accuracy may be used. Commercial refractometers are normally constructed for use with white light but are calibrated to give the refractive index in terms of the D line of sodium light.

To achieve accuracy, the apparatus should be calibrated against distilled water which has a refractive index of 1.3325 at 25°C or against the reference liquids given in the Table 3.1.

Table 3.1

<table>
<thead>
<tr>
<th>Reference Liquid</th>
<th>$\eta_{D^{20^0}}$</th>
<th>Temperature Co-efficient $\Delta n/\Delta t$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon tetrachloride</td>
<td>1.4603</td>
<td>-0.00057</td>
</tr>
<tr>
<td>Toluene</td>
<td>1.4969</td>
<td>-0.00056</td>
</tr>
<tr>
<td>$\alpha$-Methylnaphthalene</td>
<td>1.6176</td>
<td>-0.00048</td>
</tr>
</tbody>
</table>

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

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

3.1.2. - WEIGHT PER MILLILITRE AND SPECIFIC GRAVITY

A. Weight per millilitre: The weight per millilitre of a liquid is the weight in g of 1 ml of a liquid when weighed in air at 25°C, unless otherwise specified.

Method

Select a thoroughly clean and dry pycnometer. Calibrate the pycnometer by filling it with recently boiled and cooled water at 25°C and weighing the contents. Assuming that the weight of 1 ml of water at 25°C when weighed in air of density 0.0012 g per ml, is 0.99602 g. Calculate the capacity of the pycnometer. (Ordinary deviations in the density of air from the value given do not affect the result of a determination significantly). Adjust the temperature of the substance to be examined, to about 20°C and fill the pycnometer with it. Adjust the temperature of the filled pycnometer to 25°C, remove any excess of the substance and weigh. Subtract the tare weight of the pycnometer from the filled weight of the pycnometer. Determine the weight per milliliter dividing
the weight in air, expressed in g, of the quantity of liquid which fills the pycnometer at the specified 
temperature, by the capacity expressed in ml, of the pycnometer at the same temperature.

**B. Specific gravity:** The specific gravity of a liquid is the weight of a given volume of the liquid at 
25\(^0\) (unless otherwise specified) compared with the weight of an equal volume of water at the same 
temperature, all weighing being taken in air.

**Method**

Proceed as described under wt. per ml. Obtain the specific gravity of the liquid by dividing 
the weight of the liquid contained in the pycnometer by the weight of water contained, both 
determined at 25\(^0\) unless otherwise directed in the individual monograph.

3.1.3. - DETERMINATION OF pH VALUES

The pH value of an aqueous liquid may be defined as the common logarithm of the 
reciprocal of the hydrogen ion concentration expressed in g per litre. Although this definition 
provides a useful practical means for the quantitative indication of the acidity or alkalinity of a 
solution, it is less satisfactory from a strictly theoretical point of view. No definition of pH as a 
measurable quantity can have a simple meaning, which is also fundamental and exact.

The pH value of a liquid can be determined potentiometrically by means of the glass 
electrode, a reference electrode and a pH meter either of the digital or analogue type.

3.2. - DETERMINATION OF MELTING RANGE AND CONGEALING RANGE

3.2.1. Determination of Melting Range

The melting-range of a substance is the range between the corrected temperature at which 
the substance begins to form droplets and the corrected temperature at which it completely melts, as 
shown by formation of a meniscus.

**Apparatus**

(a) A capillary tube of soft glass, closed at one end, and having the following dimensions:

(i) thickness of the wall, about 0.10 to 0.15 mm.
(ii) length about 10 cm or any length suitable for apparatus used.
(iii) internal diameter 0.9 to 1.1 mm for substances melting below 100\(^0\) or 0.8 to 1.2 
mm for substances melting above 100\(^0\).

**Thermometers**

Accurately standardized thermometers covering the range 10\(^0\) to 300\(^0\) the length of two 
degrees on the scale being not less than 0.8 mm. These thermometers are of the mercury-in-glass, 
solid-stem type; the bulb is cylindrical in shape, and made of approved thermometric glass suitable 
for the range of temperature covered; each thermometer is fitted with a safety chamber. The 
smallest division on the thermometer scale should vary between 0.1\(^0\) to 1.5\(^0\) according to the 
melting point of the substance under test.

The following form of heating apparatus is recommended.
A glass heating vessel of suitable, construction and capacity fitted with suitable stirring
device, capable of rapidly mixing the liquids.

Suitable liquids for use in the heating vessel:

**Glycerin**  
Upto 150°

Sulphuric acid to which a small crystal of potassium nitrate or 4
Drops of nitric acid per 100 ml has been added  
Upto 200°

A liquid paraffin of sufficiently high boiling range  
Upto 250°

Seasame oil  
Upto 300°

30 parts of potassium sulphate, dissolved by heating in 70
parts of sulphuric acid  
Upto 300°

Any other apparatus or method, preferably, the electric method may be used subject to a
check by means of pure substances having melting temperature covering the ranges from 0° to 300°
and with suitable intervals.

The following substances are suitable for this purpose.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Melting range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vanillin</td>
<td>81° to 83°</td>
</tr>
<tr>
<td>Acetanilide</td>
<td>114° to 116°</td>
</tr>
<tr>
<td>Phenacetin</td>
<td>134° to 136°</td>
</tr>
<tr>
<td>Sulphanilamide</td>
<td>164° to 166.5°</td>
</tr>
<tr>
<td>Sulphapyridine</td>
<td>191° to 193°</td>
</tr>
<tr>
<td>Caffeine (Dried at 100°)</td>
<td>234° to 237°</td>
</tr>
</tbody>
</table>

**Procedure**

**Method I:** Transfer a suitable quantity of the powdered and thoroughly dried substance to a dry
capillary tube and pack the powder by tapping the tube on a hard surface so as to form a tightly
packed column of 2 to 4 mm in height. Attach the capillary tube and its contents to a standardized
thermometer so that the closed end is at the level of the middle of the bulb; heat in a suitable
apparatus (preferably a round-bottom flask) fitted with an auxiliary thermometer regulating the rise
of temperature in the beginning to 3° per minute. When the temperature reached is below the lowest
figure of the range for the substance under examination, the heating of the apparatus is adjusted as
desired; if no other directions are given, the rate of rise of temperature should be kept at 1° to 2° per
minute. The statement ‘determined by rapid heating’ means that the rate of rise of temperature is 5°
per minute during the entire period of heating.

Unless otherwise directed, the temperature at which the substance forms droplets against the
side of the tube and the one at which it is completely melted as indicated by the formation of a
definite meniscus, are read.

The following emergent stem corrections should be applied to the temperature readings.

Before starting the determination of the melting temperature the auxiliary thermometer is
attached so that the bulb touches the standard thermometer at a point midway between the
gradation for the expected melting temperature and the surface of the heating material. When the
substance has melted, the temperature is read on the auxiliary thermometer. The correction figure to
be added to the temperature reading of the standardized thermometer is calculated from the following formula

\[ 0.00015 \times N (T-t) \]

Where ‘T’ is the temperature reading of the standardized thermometer.

‘t’ is the temperature reading of the auxiliary thermometer.

‘N’ is the number of degrees of the scale of the standardized thermometer between the surface of the heating material and level of mercury.

The statement “melting range, a\(^0\) to b\(^0\)” means that the corrected temperature at which the material forms droplets must be at least a\(^0\), and that the material must be completely melted at the corrected temperature, b\(^0\).

**Method II:** The apparatus employed for this test is the same as described for method I except for such details as are mentioned in the procedure given below

**Procedure:** A capillary tube open at both ends is used for this test. Melt the material under test at as low a temperature as possible. Draw into the capillary a column of the material about 10 mm high. Cool the charged tube in contact with ice for at least 2 hours. Attach the tube to the thermometer by means of rubber band and adjust it in the heating vessel containing water so that the upper edge of the material is 10 mm below the water level. Heat in the manner as prescribed in Method I until the temperature is about 5\(^0\) below the expected melting point and then regulate the rate of rise of temperature to between 0.5\(^0\) to 1\(^0\) per minute. The temperature at which the material is observed to rise in the capillary tube is the melting temperature of the substance.

3.2.2. - Determination of Congealing Range

The congealing temperature is that point at which there exists a mixture of the liquid (fused) phase of a substance and a small but increasing proportion of the solid phase. It is distinct from the freezing point which is the temperature at which the liquid and solid phase of a substance are in equilibrium. In certain cases, this may happen over a range of temperatures.

The temperature at which a substance solidifies upon cooling is a useful index of its purity if heat is liberated when solidification takes place.

The following method is applicable to substances that melt between –20\(^0\) and 150\(^0\).

**Apparatus**

A test-tube (About 150 mm × 25 mm) placed inside another test-tube (about 160 mm × 40 mm) the inner tube is closed by a stopper that carries a stirrer and a thermometer (About 175 mm long and with 0.2\(^0\) graduations) fixed so that the bulb is about 15 mm above the bottom of the tube. The stirrer is made from a glass rod or other suitable material formed at one end into a loop of about 18 mm overall diameter at right angles to the rod. The inner tube with its jacket is supported centrally in a 1-litre baker containing a suitable cooling liquid to within 20 mm of the top. The thermometer is supported in the cooling bath.
Method

Melt the substance, if a solid, at a temperature not more than $20^\circ$ above its expected congealing point, and pour it into the inner test-tube to a height of 50 to 57 mm. Assemble the apparatus with the bulb of the thermometer immersed half-way between the top and bottom of the sample in the test-tube. Fill the bath to almost 20 mm from the top of the tube with a suitable fluid at a temperature 4$^\circ$ to 5$^\circ$ below the expected congealing point. If the substance is a liquid at room temperature, carry out the determination using a bath temperature about 15$^\circ$ below the expected congealing point. When the sample has cooled to about 5$^\circ$ above its expected congealing point stir it continuously by moving the loop up and down between the top and bottom of the sample at a regular rate of 20 complete cycles per minute. If necessary, congelation may be induced by scratching the inner walls of the test-tube with the thermometer or by introducing a small amount of the previously congealed substance under examination. Pronounced supercooling may result in deviation from the normal pattern of temperature changes. If it happens, repeat the test introducing small fragments of the solid substance under examination at 1$^\circ$ intervals when the temperature approaches the expected congealing point.

Record the reading of the thermometer every 30 seconds and continue stirring only so long as the temperature is falling. Stop the stirring when the temperature is constant to start to rise slightly. Continue recording the temperature for at least 3 minutes after the temperature again begins to fall after remaining constant.

The congealing point will be mean of not less than four consecutive readings that lie within a range of 0.2$^\circ$.

3.2.3. - DETERMINATION OF BOILING RANGE

The boiling-range of a substance is the range of temperature within which the whole or a specified portion of the substance distils.

Apparatus

The boiling-range is determined in a suitable apparatus, the salient features of which are described below:

(a) Distillation flask: The flask shall be made of colourless transparent heat-resistant glass and well annealed. It should have a spherical bulb having a capacity of about 130 ml. The side tube slopes downwards in the same plane as the axis of the neck at angle of between 72$^\circ$ to 78$^\circ$. Other important dimensional details are as under:

<table>
<thead>
<tr>
<th>Dimension</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Internal diameter of neck</td>
<td>15 to 17 mm</td>
</tr>
<tr>
<td>Distance from top of neck to center of side tube</td>
<td>72 to 78 mm</td>
</tr>
<tr>
<td>Distance from the center of the side tube to surface of the Liquid when the flask contains 100 ml liquid</td>
<td>87 to 93 mm</td>
</tr>
<tr>
<td>Internal diameter of side tube</td>
<td>3.5 to 4.5 mm</td>
</tr>
<tr>
<td>Length of side tube</td>
<td>97 to 103 mm</td>
</tr>
</tbody>
</table>

(b) Thermometer: Standardised thermometers calibrated for 100 mm immersion and suitable for the purpose and covering the boiling range of the substance under examination shall be employed;
the smallest division on the thermometer scale may vary between $0.2^0$ to $1^0$ according to requirement.

(c) **Draught Screen**: suitable draught screen, rectangular in cross section with a hard asbestos board about 6 mm thick closely fitting horizontally to the sides of the screen, should be used. The asbestos board shall have a centrally cut circular hole, 110 mm in diameter. The asbestos board is meant for ensuring that hot gases from the heat source do not come in contact with the sides or neck of the flask.

(d) **Asbestos Board**: A 150 mm square asbestos board 6 mm thick provided with a circular hole located centrally to hold the bottom of the flask, shall be used. For distillation of liquids boiling below $60^0$ the hole shall be 30 mm in diameter; for other liquid it should be 50 mm in diameter. This board is to be placed on the hard asbestos board of the draught screen covering its 110 mm hole.

(e) **Condenser**: A straight water-cooled glass condenser about 50 cm long shall be used.

**Procedure**: 100 ml of the liquid to be examined is placed in the distillation flask, and a few glass beads or other suitable substance is added. The bulb of the flask is placed centrally over a circular hole varying from 3 to 5 cm in diameter (according to the boiling range of the substance under examination), in a suitable asbestos board. The thermometer is held concentrically in the neck of the flask by means of a well fitting cork in such a manner that the bulb of the thermometer remains just below the level of the opening of the side-tube. Heat the flask slowly in the beginning and when distillation starts, adjust heating in such a manner that the liquid distils at a constant rate of 4 to 5 ml per minute. The temperature is read when the first drop runs from the condenser, and again when the last quantity of liquid in the flask is evaporated.

The boiling ranges indicated, apply at a barometric pressure of 760 mm of mercury. If the determination is made at some other barometric pressure, the following correction is added to the temperatures read:

$$K - (760 - p)$$

Where $p$ is the barometric pressure (in mm) read on a mercury barometer, without taking into account the temperature of the air;

$K$ is the boiling temperature constant for different liquids having different boiling ranges as indicated below:

<table>
<thead>
<tr>
<th>Observed Boiling range</th>
<th>‘K’</th>
</tr>
</thead>
<tbody>
<tr>
<td>Below $100^0$</td>
<td>0.04</td>
</tr>
<tr>
<td>$100^0$ to $140^0$</td>
<td>0.045</td>
</tr>
<tr>
<td>$141^0$ to $190^0$</td>
<td>0.05</td>
</tr>
<tr>
<td>$191^0$ to $240^0$</td>
<td>0.055</td>
</tr>
<tr>
<td>above $240^0$</td>
<td>0.06</td>
</tr>
</tbody>
</table>

If the barometric pressure is below 760 mm of mercury the correction is added to the observed boiling-range; if above, the correction is subtracted.

The statement ‘distils between $a^0$ and $b^0$’, means that temperature at which the first drop runs from the condenser is not less than $a^0$ and that the temperature at which the liquid is completely evaporated is not greater than $b^0$. 

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3.3. - DETERMINATION OF OPTICAL ROTATION AND SPECIFIC OPTICAL ROTATION

A. Optical Rotation: Certain substances, in a pure state, in solution and in tinctures possess the property of rotating the plane of polarized light, i.e., the incident light emerges in a plane forming an angle with the plane of the incident light. These substances are said to be optically active and the property of rotating the plane of polarized light is known as optical rotation. The optical rotation is defined as the angle through which the plane of polarized light is rotated when polarized light obtained from sodium or mercury vapour lamp passes through one decimeter thick layer of a liquid or a solution of a substance at a temperature of 25\(^0\) unless as otherwise stated in the monograph. Substances are described as dextrorotatory or laevoretatory according to the clockwise or anticlockwise rotation respectively of the plane of polarized light. Dextrorotation is designated by a plus (+) sign and laevorotation by a minus (-) sign before the number indicating the degrees of rotation.

**Apparatus:** A polarimeter on which angular rotation accurate 0.05\(^0\) can be read may be used.

**Calibration:** The apparatus may be checked by using a solution of previously dried sucrose and measuring the optical rotation in a 2-din tube at 25\(^0\) and using the concentrations indicated in Table.

<table>
<thead>
<tr>
<th>Concentration (g/100 ml)</th>
<th>Angle of Rotation (+) at 25(^0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.0</td>
<td>13.33</td>
</tr>
<tr>
<td>20.0</td>
<td>26.61</td>
</tr>
<tr>
<td>30.0</td>
<td>39.86</td>
</tr>
<tr>
<td>40.0</td>
<td>53.06</td>
</tr>
<tr>
<td>50.0</td>
<td>66.23</td>
</tr>
</tbody>
</table>

**Procedure:** For liquid substances, take a minimum of five readings of the rotation of the liquid and also for an empty tube at the specified temperature. For a solid dissolve in a suitable solvent and take five readings of the rotation of the solution and the solvent used. Calculate the average of each set of five readings and find out the corrected optical rotation from the observed rotation and the reading with the blank (average).

B. Specific Rotation: The apparatus and the procedure for this determination are the same as those specified for optical rotation.

Specific rotation is denoted by the expression

\[ [\alpha]_x^t \]

‘\(t\)’ denotes the temperature of rotation; ‘\(\alpha\)’ denotes the wave length of light used or the characteristic spectral line. Specific rotations are expressed in terms of sodium light of wave length 589.3 mw (D line) and at a temperature of 25\(^0\), unless otherwise specified.

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

For liquid substances
For solutions of substances

\[ [\alpha]^t = \frac{a \times 100}{l \cdot c} \]

Where \( a \) is the corrected observed rotation in degrees
\( l \) is the length of the polarimeter tube in decimeters.

\( D \) is the specific gravity of the liquid
\( C \) is the concentration of solution expressed as the number of g of the substance in 100 ml of solution.

### 3.4. - DETERMINATION OF VISCOSITY

Viscosity is a property of a liquid, which is closely related to the resistance to flow.

In C.G.S. system, the dynamic viscosity (\( \eta \)) of a liquid is the tangential force in dryness per square centimeter exerted in either of the two parallel planes placed, 1 cm apart when the space between them is filled with the fluid and one of the plane is moving in its own plane with a velocity of 1 cm per second relatively to the other. The unit of dynamic viscosity is the poise (abbreviated \( P \)). The centi poise (abbreviated \( cp \)) is 1/100th of one poise.

While on the absolute scale, viscosity is measured in poise or centi poise, it is not convenient to use the kinematic scale in which the units are stokes (abbreviated \( S \)) and centi-stokes (abbreviated \( CS \)). The centistokes is 1/100th of one stoke. The kinematic viscosity of a liquid is equal to the quotient of the dynamic viscosity and the density of the liquid at the same temperature, thus:

\[ \text{Dynamic Viscosity} = \frac{\text{Kinematic Viscosity}}{\text{Density}} \]

Viscosity of liquid may be determined by any method that will measure the resistance to shear offered by the liquid.

Absolute viscosity can be measured directly if accurate dimensions of the measuring instruments are known but it is more common practice to calibrate the instrument with a liquid of known viscosity and to determine the viscosity of the unknown fluid by comparison with that of the known.

**Procedure:** The liquid under test is filled in a U tube viscometer in accordance with the expected viscosity of the liquid so that the fluid level stands within 0.2 mm of the filling mark of the viscometer when the capillary is vertical and the specified temperature is attained by the test liquid. The liquid is sucked or blown to the specified weight of the viscometer and the time taken for the meniscus to pass the two specified marks is measured. The kinematic viscosity in centistokes is calculated from the following equation:

\[ \text{Kinematic viscosity} = kt \]
Where \( k \) = the constant of the viscometer tube determined by observation on liquids of known kinematic viscosity; \( t \) = time in seconds for meniscus to pass through the two specified marks.

3.5. - DETERMINATION OF TOTAL SOLIDS

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

**Method 1**: Transfer accurately 50 ml of the clear Asava/ Aristha an evaporable dish and evaporate to a thick extract on a water bath. Unless specified otherwise, extract the residue with 4 quantities, each of 10 ml, of dehydrated ethanol with stirring and filter. Combine the filtrates to another evaporating dish which have been dried to a constant weight and evaporate nearly to dryness on a water bath, add accurately 1 g of diatomite (dry at 105\(^0\) for 3 hours and cooled in a desiccator for 30 min), stir thoroughly, dry at 105\(^0\) for 3 hours, cool the dish in a desiccator for 30 min, and weigh immediately. Deduct the weight of diatomite added, the weight of residue should comply with the requirements stated under the individual monograph.

**Method 2**: Transfer accurately 50 ml of the clear Asava/ Aristha to an evaporable dish, which has been dried to a constant weight and evaporate to dryness on a water bath, then dry at 105\(^0\) for 3 hours. After cooling the dish containing the residue in a desiccator for 30 min, weigh it immediately. The weight of residue should comply with the requirements stated under the individual monograph.

3.6. - SOLUBILITY IN WATER

Take 100 ml of distil water in a Nessler cylinder and add air-dried and coarsely powdered drug up to saturation. Then stir the sample continuously by twirling the spatula (rounded end of a microspatula) rapidly. After 1 minute, filter the solution using Hirsch funnel, evaporate the filtrate to dryness in a tared flat bottomed shallow dish and dry at 105\(^0\) to constant weight and calculate the solubility of the drug in water (wt. in mg/100ml).

3.7. - DETERMINATION OF SAPONIFICATION VALUE

The saponification value is the number of mg of potassium hydroxide required to neutralize the fatty acids, resulting from the complete hydrolysis of 1 g of the oil or fat, when determined by the following method:

Dissolve 35 to 40 g of potassium hydroxide in 20 ml water, and add sufficient alcohol to make 1,000 ml. Allow it to stand overnight, and pour off the clear liquor.

Weigh accurately about 2 g of the substance in a tared 250 ml flask, add 25 ml of the alcoholic solution of potassium hydroxide, attach a reflux condenser and boil on a water-bath for one hour, frequently rotating the contents of the flask cool and add 1 ml of solution of phenolphthalein and titrate the excess of alkali with 0.5 N hydrochloric acid. Note the number of ml required (a). Repeat the experiment with the same quantities of the same reagents in the manner omitting the substance. Note the number of ml required (b) Calculate the saponification value from the following formula:—

\[
\text{Saponification Value} = \frac{(b-a) \times 0.02805 \times 1.000}{W}
\]

Where ‘W’ is the weight in g of the substance taken.
3.8. - DETERMINATION OF IODINE VALUE

The Iodine value of a substance is the weight of iodine absorbed by 100 part by weight of the substance, when determined by one of the following methods:-

Iodine Flasks - The Iodine flasks have a nominal capacity of 250 ml.

A. Iodine Monochloride Method - Place the substance accurately weighed, in dry iodine flask, add 10 ml of carbon tetrachloride, and dissolve. Add 20 ml of iodine monochloride solution, insert the stopper, previously moistened with solution of potassium iodide and allow to stand in a dark place at a temperature of about 17° or thirty minutes. Add 15 ml of solution of potassium iodide and 100 ml water; shake, and titrate with 0.1 N sodium thiosulphate, using solution of starch as indicator. Note the number of ml required (a). At the same time carry out the operation in exactly the same manner, but without the substance being tested, and note the number of ml of 0.1 N sodium thiosulphate required (b).

Calculate the iodine value from the formula:-

\[
\frac{(b-a) \times 0.01269 \times 100}{W}
\]

Iodine value = -------------------------------

Where 'W' is the weight in g of the substance taken.

The approximate weight, in g, of the substance to be taken may be calculated by dividing 20 by the highest expected iodine value. If more than half the available halogen is absorbed, the test must be repeated, a smaller quantity of the substance being used.

Iodine Monochloride Solution: The solution may be prepared by either of the two following methods:

1. Dissolve 13 g of iodine in a mixture of 300 ml of carbon tetrachloride and 700 ml of glacial acetic acid. To 20 ml of this solution, add 15 ml of solution of potassium iodide and 100 ml of water, and titrate the solution with 0.1 N sodium thiosulphate. Pass chlorine, washed and dried, through the remainder of the iodine solution until the amount of 0.1 N sodium thiosulphate required for the titration is approximately, but more than, doubled.

2. Iodine trichloride 8 g
   Iodine 9 g
   Carbon tetrachloride 300 ml
   Glacial acetic acid, sufficient to produce 1000 ml

Dissolve the iodine trichloride in about 200 ml of glacial acetic acid, dissolve the iodine in the carbon tetrachloride, mix the two solutions, and add sufficient glacial acetic acid to produce 1000 ml. Iodine Monochloride Solution should be kept in a stoppered bottle, protected from light and stored in a cool place.

B. Pyridine Bromide Method - Place the substance, accurately weighed, in a dry iodine flask, add 10 ml of carbon tetrachloride and dissolve. Add 25 ml of pyridine bromide solution, allow to stand for ten minutes in a dark place and complete the determination described under iodine monochloride method, beginning with the words. Add 15 ml.
The approximate weight in gram, of the substance to be taken may be calculated by dividing 12.5 by the highest expected iodine value. If more than half the available halogen is absorbed the test must be repeated, a small quantity of the substance being used.

Pyridine bromide Solution: Dissolve 8 g pyridine and 10 g of sulphuric acid in 20 ml of glacial acetic acid, keeping the mixture cool. Add 8 g of bromine dissolved in 20 ml of glacial acetic acid and dilute to 100 ml with glacial acetic acid.

Pyridine bromide Solution should be freshly prepared.

3.9. - DETERMINATION OF ACID VALUE

The acid value is the number of mg of potassium hydroxide required to neutralize the free acids in 1 g of the substance, when determined by the following method:

Weigh accurately about 10 g of the substance (1 to 5) in the case of a resin into a 250 ml flask and add 50 ml of a mixture of equal volumes of alcohol and solvent ether, which has been neutralized after the addition of 1 ml of solution of phenolphthalein. Heat gently on a water-bath, if necessary until the substance has completely melted, titrate with 0.1 N potassium hydroxide, shaking constantly until a pink colour which persists for fifteen seconds is obtained. Note the number of ml required. Calculate the acid value from the following formula:

\[
\text{Acid Value} = \frac{a \times 0.00561 \times 1000}{W}
\]

Where ‘a’ is the number of ml of 0.1 N potassium hydroxide required and ‘W’ is the weight in g of the substance taken.

3.10. - DETERMINATION OF PEROXIDE VALUE

The peroxide value is the number of milliequivalents of active oxygen that expresses the amount of peroxide contained in 1000 g of the substance.

Method

Unless otherwise specified in the individual monograph, weigh 5 g of the substance being examined, accurately weighed, into a 250-ml glass-stoppered conical flask, add 30 ml of a mixture of 3 volumes of glacial acetic acid and 2 volumes of chloroform, swirl until dissolved and add 0.5ml volumes of saturated potassium iodide solution. Allow to stand for exactly 1 minute, with occasional shaking, add 30 ml of water and titrate gradually, with continuous and vigorous shaking, with 0.01M sodium thiosulphate until the yellow colour almost disappears. Add 0.5 ml of starch solution and continue the titration, shaking vigorously until the blue colour just disappears (a ml). Repeat the operation omitting the substance being examined (b ml). The volume of 0.01M sodium thiosulphate in the blank determination must not exceed 0.1 ml.

Calculate the peroxide value from the expression

\[
\text{Peroxide value} = 10 \frac{(a - b)}{W}
\]

Where \(W\) = weight, in g, of the substance.
3.11. - DETERMINATION OF UNSAPONIFIABLE MATTER

The unsaponifiable matter consists of substances present in oils and fats, which are not saponifiable by alkali hydroxides and are determined by extraction with an organic solvent of a solution of the saponified substance being examined.

Method

Unless otherwise specified in the individual monograph, introduce about 5 g of the substance being examined, accurately weighed, into a 250-ml flask fitted with a reflux condenser. Add a solution of 2 g of potassium hydroxide in 40 ml of ethanol (95 per cent) and heat on a water-bath for 1 hour, shaking frequently. Transfer the contents of the flask to a separating funnel with the aid of 100 ml of hot water and, while the liquid is still warm, shake very carefully with three quantities, each of 100 ml, of peroxide-free ether. Combine the ether extracts in a second separating funnel containing 40 ml of water, swirl gently for a few minute, allow to separate and reject the lower layer. Wash the ether extract with two quantities, each of 40 ml, of water and with three quantities, each of 40 ml, of a 3 per cent w/v solution of potassium hydroxide, each treatment being followed by a washing with 40 ml of water. Finally, wash the ether layer with successive quantities, each of 40 ml, of water until the aqueous layer is not alkaline to phenolphthalein solution. Transfer the ether layer to a weighed flask, washing out the separating funnel with peroxide-free ether. Distil off the ether and add to the residue 6 ml of acetone. Remove the solvent completely from the flask with the aid of a gentle current of air. Dry at 100° to 105° for 30 minutes. Cool in a desiccator and weigh the residue. Calculate the unsaponifiable matter as per cent w/w.

Dissolve the residue in 20 ml of ethanol (95 per cent), previously neutralised to phenolphthalein solution and titrate with 0.1M ethanolic potassium hydroxide. If the volume of 0.1M ethanolic potassium hydroxide exceeds 0.2 ml, the amount weighed cannot be taken as the unsaponifiable matter and the test must be repeated.

3.12. - DETECTION OF MINERAL OIL (HOLDE’S TEST)

Take 22 ml of the alcoholic potassium hydroxide solution in a conical flask and add 1ml of the sample of the oil to be tested. Boil in a water bath using an air or water cooled condenser till the solution becomes clear and no oily drops are found on the sides of the flask. Take out the flask from the water bath, transfer the contents to a wide mouthed warm test tube and carefully add 25ml of boiling distilled water along the side of the test tube. Continue shaking the tube lightly from side to side during the addition. The turbidity indicates presence of mineral oil, the depth of turbidity depends on the percentage of mineral oil present.

3.13. - RANCIDITY TEST (KREIS TEST)

The test depends upon the formation of a red colour when oxidized fat is treated with conc. hydrochloric acid and a solution of phloroglucinol in ether. The compound in rancid fats responsible for the colour reaction is ephydrin aldehyde. All oxidized fats respond to the Kreis test and the intensity of the colour produced is roughly proportional to the degree of oxidative rancidity.

Procedure

Mix 1 ml of melted fat and 1 ml of conc. hydrochloric acid in a test tube. Add 1 ml of a 1 per cent solution of phloroglucinol in diethyl ether and mix thoroughly with the fat-acid mixture. A pink colour formation indicates that the fat is slightly oxidized while a red colour indicates that the fat is definitely oxidized.

The Reichert-Meissl value is the number of millitres of 0.1N aqueous sodium hydroxide solution required to neutralize steam volatile water soluble fatty acids distilled from 5g of an oil/fat under the prescribed conditions. It is a measure of water soluble steam volatile fatty acids chiefly butric and caprole acids present in oil or fat.

The Polenske value is the number of millitres of 0.1N aqueous alkali solution required to neutralize steam volatile water insoluble fatty acids distilled from 5 g of the oil/fat under the prescribed conditions. It is a measure of the steam volatile and water insoluble fatty acids, chiefly caprylic, capric and lauric acids present in oil and fat.

**Principle:**

The material is saponified by heating with glycerol sodium hydroxide solution and then split by treatment with dilute sulfuric acid. The volatile acids are immediately steam distilled. The soluble volatile acids in the distillate are filtered out and estimated by titration with standard sodium hydroxide solution.

**Reagents**

a. Glycerine: Analytical reagent grade
d. Dilute sulfuric acid solution: Approximately 1.0 N
e. Sodium hydroxide solution: 0.1N solution in water, accurately standardized
f. Phenolphthalein indicator: Dissolve 0.1g of phenolphthalein in 100 ml of ethyl alcohol
g. Ethyl alcohol: 90% by volume and neutral to phenolphthalein

**Procedure**

Weigh accurately 5 ± 0.1 g of filtered oil or fat sample into a clean, dry, 300 ml distilling flask. Add 20 g of glycerine and 2 ml of concentrated sodium hydroxide solution, and heat with swirling over a flame until completely saponified, as shown by the mixture becoming perfectly clear. Cool the content slightly and add 90 ml of boiling distilled water, which has been vigorously boiled for about 15 min. After thorough mixing the solution should remain clear. If the solution is not clear (indicating incomplete saponification) or is darker than light yellow (indicating overheating), repeat the saponification with a fresh sample of the oil or fat. If the sample is old, the solution may some times be dark and not clear.

Add about 0.1 g of pumic stone grains, and 50 ml of dilute sulfuric acid solution. Immediately connect the flask to the distillation apparatus. Heat very gently until the liberated fatty acids melt and separate. Then set the flame so that 110 ml of distillate shall be collected within 19 to 21 min. The beginning of the distillation is to be taken as the moment when the first drop forms in the still head. Collect the distillate in a graduated flask. The temperature of the issuing distillate should be between 180 to 210.

When the distillate exactly reaches the 110 ml mark on the flask, remove the flame and quickly replace the flask by a 25 ml measuring cylinder. Stopper the graduated flask and without mixing place it in a water bath maintain at 15° for 10 min so that the 110 ml graduation mark is 1 cm below the water level in the bath. Remove the graduated flask from the cold water bath, dry the outside and mix the content gently by inverting the flask 4 or 5 times without shaking. Avoid wetting the stopper with the insoluble acids. Filter the liquid through a dry, 9 cm Whatman No.4 filter paper. The filtrate should be clear. Pipette 100 ml of the filtrate and add 5 drops of the
phenolphthalein solution, and titrate against standard 0.1 N sodium hydroxide solution. Run a Blank Test without the fat, but using the same quantities of the reagents.

**Calculation**

Reichert-Meissl Value = (A-B) x N x 11

where,

A = Volume in ml of standard sodium hydroxide solution required for the test;
B = Volume in ml of standard sodium hydroxide solution required for the blank;
N = Normality of standard sodium hydroxide solution.

After titrating the soluble volatile acids, detach the still head and rinse the condenser with three successive 15 ml portions of cold distilled water passing each washing separately through the measuring cylinder, 110 ml graduated flask and the filter paper and allow all of it to pass through. Discard all the washings. Place the funnel on a clean conical flask. Dissolve the insoluble fatty acids by three similar washings of the condenser, the measuring cylinder, the 110 ml flask with stopper, and the filter paper with 15 ml portions of ethyl alcohol. Combine the alcoholic washings in a clean flask, add 5 drops of phenolphthalein indicator solution, and titrate with standard (0.1N) sodium hydroxide solution.

Polenske Value = 10 x V x N

where,

V = Volume in ml of standard sodium hydroxide solution required for the test;
N = Normality of the standard sodium hydroxide solution.

**3.15. - DETERMINATION OF ALCOHOL CONTENT**

The ethanol content of a liquid is expressed as the number of volumes of ethanol contained in 100 volumes of the liquid, the volumes being measured at 24.9° to 25.1°. This is known as the “percentage of ethanol by volume”. The content may also be expressed in g of ethanol per 100 g of the liquid. This is known as the ‘percentage of ethanol by weight’.

Use Method I or Method II, as appropriate, unless otherwise specified in the individual monograph.

**Method I**

Carry out the method for gas chromatography, using the following solutions. Solution (1) contains 5.0 per cent v/v of ethanol and 5.0 per cent v/v of 1-propanol (internal standard). For solution (2) dilute a volume of the preparation being examined with water to contain between 4.0 and 6.0 per cent v/v of ethanol. Prepare solution (3) in the same manner as solution (2) but adding sufficient of the internal standard to produce a final concentration of 5.0 per cent v/v.

The chromatographic procedure may be carried out using a column (1.5 m x 4 mm) packed with porous polymer beads (100 to 120 mesh) and maintained at 150°, with both the inlet port and the detector at 170°, and nitrogen as the carrier gas.

Calculate the percentage content of ethanol from the areas of the peaks due to ethanol in the chromatogram obtained with solutions (1) and (3).
Method II

For preparations where the use of Industrial Methylated Spirit is permitted in the monograph, determine the content of ethanol as described in Method I but using as solution (2) a volume of the preparation being examined diluted with water to contain between 4.0 and 6.0 per cent v/v of total ethanol and methanol.

Determine the concentration of methanol in the following manner. Carry out the chromatographic procedure described under Method I but using the following solutions. Solution (1) contains 0.25 per cent v/v of methanol and 0.25 per cent v/v of 1-propanol (internal standard). For solution (2) dilute a volume of the preparation being examined with water to contain between 0.2 per cent and 0.3 per cent v/v of methanol. Prepare solution (3) in the same manner as solution (2) but adding sufficient of the internal standard to produce a final concentration of 0.25 per cent v/v.

The sum of the contents of ethanol and methanol is within the range specified in the individual monograph and the ration of the content of methanol to that of ethanol is commensurate with Industrial Methylated Spirit having been used.

Method III

This method is intended only for certain liquid preparations containing ethanol. Where the preparation contains dissolved substances that may distil along with ethanol Method III B or III C must be followed.

Apparatus

The apparatus (see Fig. 3) consists of a round-bottomed flask (A) fitted with a distillation head (B) with a steam trap and attached to a vertical condenser (C). A tube is fitted to the lower part of the condenser and carries the distillate into the lower part of a 100-ml or 250-ml volumetric flask (D). The volumetric flask is immersed in a beaker (E) containing a mixture of ice and water during the distillation. A disc with a circular aperture, 6 cm in diameter, is placed under the distillation flask (A) to reduce the risk of charring of any dissolved substances.

Method III A

Transfer 25 ml of the preparation being examined, accurately measured at 24.90 to 25.10, to the distillation flask. Dilute with 150 ml of water and add a little pumice powder. Attach the distillation head and condenser. Distil and collect not less than 90 ml of the distillate into a 100-ml volumetric flask. Adjust the temperature to 24.90 to 25.10 and dilute to volume with distilled water at 24.90 to 25.10. Determine the relative density at 24.90 to 25.10. The values indicated in column 2 of Table 3.2 are multiplied by 4 in order to obtain the percentage of ethanol by volume contained in the preparation. If the specific gravity is found to be between two values, the percentage of ethanol should be obtained by interpolation. After calculation of the ethanol content, report the result to one decimal place.

NOTE – (1) If excessive frothing is encountered during distillation, render the solution strongly acid with phosphoric acid or treat with a small amount of liquid paraffin or silicone oil.

(2) The distillate should be clear or not more than slightly cloudy. If it is turbid or contains oily drops, follow Method IIIC. When steam-volatile acids are present, make the solution just alkaline with 1M sodium hydroxide using solid phenolphthalein as indicator before distillation.
Method III B

Follow this method or the following one if the preparation being examined contains appreciable proportions of volatile materials other than ethanol and water.

Mix 25 ml of the preparation, accurately measured at 24°C to 25.1°C, with about 100 ml of water in a separating funnel. Saturate this mixture with sodium chloride, add about 100 ml of hexane and shake vigorously for 2 to 3 minutes. Allow the mixture to stand for 15 to 20 minutes. Run the lower layer into the distillation flask, wash the hexane layer in the separating funnel by shaking vigorously with about 25 ml of sodium chloride solution, allow to separate and run the wash liquor into the first saline solution. Make the mixed solutions just alkaline with 1M sodium hydroxide using solid phenolphthalein as indicator, add a little pumice powder and 100 ml of water, distil 90 ml and determine the percentage v/v of ethanol by Method IIIA beginning at the words “Adjust the temperature…”.

Fig. 3. Apparatus for Determination of Ethanol by Distillation Method.
Table 3.2

<table>
<thead>
<tr>
<th>Specific gravity at 25°</th>
<th>Ethanol content*</th>
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<tr>
<td>1.0000</td>
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<tr>
<td>0.9985</td>
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<td>0.9685</td>
<td>25</td>
</tr>
</tbody>
</table>

* per cent v/v at 15.56°.

Method III C

Transfer 25 ml of the preparation, accurately measured at 24.9° to 25.1°, to the distillation flask. Dilute with 150 ml of water and add a little pumice powder. Attach the distillation head and condenser. Distil and collect about 100 ml. Transfer to a separating funnel and determine the percentage v/v of ethanol by Method III B beginning at the words “Saturate this mixture…”.
4.1. REAGENTS AND SOLUTIONS

**Acetate buffer 5.5 pH** – Dissolve 21.5 g of *sodium acetate* (AR) in 300 ml *purified water* containing 2 ml *glacial acetic acid* and dilute to 1000 ml.

**Acetic Acid** – Contains approximately 33 per cent w/v of C₂H₄O₂. Dilute 315 ml of glacial acetic acid to 1000 ml with *water*.

**Acetic Acid, Glacial** – CH₃COOH = 60.05.

Contains not less than 99.0 per cent w/w of C₂H₄O₂. About 17.5 N in strength.

**Description** – At temperature above its freezing point a clear colourless liquid, odour, pungent and characteristic; crystallises when cooled to about 10° and does not completely re-melt until warmed to about 15°.

**Solubility** – Miscible with *water*, with *glycerin* and most fixed and volatile oils.

**Boiling range** – Between 117° and 119°.

**Congealing temperature** – Not lower than 14.8°.

**Wt. per ml** – At 25° about 1.047 g.

**Heavy metals** – Evaporate 5 ml to dryness in a porcelain dish on water-bath, warm the residue with 2 ml of 0.1 N *hydrochloric acid* and water to make 25 ml; the limit of heavy metals is 10 parts per million, Appendix 2.3.3.

**Chloride** – 5 ml complies with the limit test for chlorides, Appendix 2.3.2.

**Sulphate** – 5 ml complies with the limit test for sulphates,

**Certain aldehydic substances** – To 5 ml add 10 ml of *mercuric chloride solution* and make alkaline with *sodium hydroxide solution*, allow to stand for five minutes and acidify with dilute *sulphuric acid*; the solution does not show more than a faint turbidity.

**Formic acid and oxidisable impurities** – Dilute 5 ml with 10 ml of water, to 5 ml of this solution add 2.0 ml of 0.1 N potassium dichromate and 6 ml of sulphuric acid, and allow to stand for one minute, add 25 ml of water, cool to 15°, and add 1 ml of freshly prepared potassium iodide solution and titrate the liberated iodine with 0.1 N sodium thiosulphate, using starch solution as indicator. Not less than 1 ml of 0.1N sodium thiosulphate is required.

**Odorous impurities** – Neutralise 1.5 ml with *sodium hydroxide solution*; the solution has no odour other than a faint acetous odour.

**Readily oxidisable impurities** – To 5 ml of the solution prepared for the test for Formic Acid and Oxidisable Impurities, add 20 ml of water and 0.5 ml of 0.1 N *potassium permanganate*; the pink colour does not entirely disappear within half a minute.

**Non-volatile matter** – Leaves not more than 0.01 per cent w/w of residue when evaporated to dryness and dried to constant weight at 105°.
**Assay** – Weigh accurately about 1 g into a stoppered flask containing 50 ml of water and titrate with N sodium hydroxide, using phenolphthalein solution as indicator. Each ml of sodium hydroxide is equivalent to 0.06005 g of C₂H₄O₂.

**Acetic Acid, Lead-Free** – Acetic acid which complies with following additional test, boil 25 ml until the volume is reduced to about 15 ml, cool make alkaline with lead-free ammonia solution, add 1 ml of lead free potassium cyanide solution, dilute to 50 ml with water, add 2 drops of sodium sulphide solution; no darkening is produced.

**Acetone** – Propan-2-one; (CH₃)₂CO = 58.08

**Description** – Clear, colourless, mobile and volatile liquid; taste, pungent and sweetish; odour characteristic; flammable.

**Solubility** – Miscible with water, with alcohol, with solvent ether, and with chloroform, forming clear solutions.

**Distillation range** – Not less than 96.0 per cent distils between 55.5° and 57°.

**Acidity** – 10 ml diluted with 10 ml of freshly boiled and cooled water; does not require for neutralisation more than 0.2 ml of 0.1 N sodium hydroxide, using phenolphthalein solution as indicator.

**Alkalinity** – 10 ml diluted with 10 ml of freshly boiled and cooled water, is not alkaline to litmus solution.

**Methyl alcohol** – Dilute 10 ml with water to 100 ml. To 1 ml of the solution add 1 ml of water and 2 ml of potassium permanganate and phosphoric acid solution. Allow to stand for ten minutes and add 2 ml of oxalic acid and sulphuric acid solution; to the colourless solution add 5 ml of decolorised magenta solution and set aside for thirty minutes between 15° and 30°; no colour is produced.

**Oxidisable substances** – To 20 ml add 0.1 ml of 0.1 N potassium permanganate, and allow to stand for fifteen minutes; the solution is not completely decolorised.

**Water** – Shake 10 ml with 40 ml of carbon disulphide; a clear solution is produced.

**Non-volatile matter** – When evaporated on a water-bath and dried to constant weight at 105°, leaves not more than 0.01 per cent w/v residue.

**Acetone Solution, Standard** – A 0.05 per cent v/v solution of acetone in water.

**Alcohol** –

**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 C₂H₅OH 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 0.1N sodium hydroxide to produce a pink colour.

**Specific gravity** – Between 0.8084 and 0.8104 at 25º.

**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 10º for thirty minutes; the solution remains clear.

**Methanol** – To one drop add one 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 60º for ten minutes; 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 15º and then add from a carefully cleaned pipette 0.1 ml 0.1 N potassium permanganate. Mix at once by inverting the stoppered cylinder and allow to stand at 15º for five minutes; 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 three minutes.

**Aldehydes and ketones** – Heat 100 ml of hydroxylamine hydrochloride solution in a loosely stoppered flask on a water-bath for thirty minutes, 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 ten minutes 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 105º for one hour; 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:

**Aldehyde** – 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 twenty four hours, remove the alcohol by distillation, dilute to 200 ml with a 2 per cent v/v solution of sulphuric acid, and set aside for twenty four hours; no crystals are produced.

**Alcohol, Sulphate-free.** – Shake alcohol with an excess of anion exchange resin for thirty minutes and filter.
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.

Ammonia Solution, Iron-free – Dilute ammonia solution which complies with the following additional test:

Evaporate 5 ml nearly to dryness on a water-bath add 40 ml of water, 2 ml of 20 per cent w/v solution of iron free citric acid and 2 drops of thioglycollic acid, mix, make alkaline with iron-free ammonia solution and dilute to 50 ml with water, no pink colour is produced.

Ammonia buffer solutions 9.5 pH – Dissolve 67.5 g ammonium chloride in 300 ml purified water, add 570 ml ammonia solution and dilute to 1000 ml.

Ammonium Chloride Solution – A 10.0 per cent w/v solution of ammonium chloride in water.

Ammonium molybdate -NH₄Mo₇O₂₄.4H₂O=1235.86
Analytical reagent grade of commerce.
White crystal or crystalline masses, sometimes with a yellowish or green tint.

Ammonium Thiocyanate – NH₄SCN = 76.12.

Description – Colourless crystals.

Solubility – Very soluble in water, forming a clear solution, readily soluble in alcohol.

Chloride – Dissolve 1 g in 30 ml of solution of hydrogen peroxide, add 1 g of sodium hydroxide, warm gently, rotate the flask until a vigorous reaction commences and allow to stand until the reaction is complete; add a further 30 ml of hydrogen peroxide solution boil for two minutes, cool, and add 10 ml of dilute nitric acid and 1 ml of silver nitrate solution; any opalescence produced is not greater than that obtained by treating 0.2 ml of 0.01 N hydrochloric acid in the same manner.

Sulphated ash – Moisten 1 g with sulphuric acid and ignite gently, again moisten with sulphuric acid and ignite; the residue weighs not more than 2.0 mg.

Ammonium Thiocyanate, 0.1N – NH₄SCN = 76.12; 7.612 in 1000 ml. Dissolve about 8 g of ammonium thiocyanate in 1000 ml of water and standardise the solution as follows:

Pipette 30 ml of standardised 0.1 N silver nitrate into a glass stoppered flask, dilute with 50 ml of water then add 2 ml of nitric acid and 2 ml of ferric ammonium sulphate solution and titrate with the ammonium thiocyanate solution to the first appearance of a red brown colour. Each ml of 0.1N silver nitrate is equivalent to 0.007612 g of NH₄SCN.

Ammonium Thiocyanate Solution – A 10.0 per cent w/v solution of ammonium thiocyanate solution.

Aniline chloride solution – To 100 ml of aniline, add 30 ml of hydrochloric acid. (10:3).

Anisaldehyde-Sulphuric Acid Reagent – 0.5 ml anisaldehyde is mixed with 10 ml glacial acetic acid, followed by 85 ml methanol and 5 ml concentrated sulphuric acid in that order.

The reagent has only limited stability and is no longer usable when the colour has turned to redviolet.
Arsenomolybdic Acid Reagent - 250 mg of ammonium molybdate was dissolved in 45 ml of distilled water. To this, 2.1 ml of concentrated H$_2$SO$_4$ was added and mixed well. To this solution, 3 mg of Na$_2$ASO$_4$.7H$_2$O dissolved in 25 ml of distilled water, mixed well and placed in incubator maintained at $37^\circ$ C for 24 h.

Borax - Sodium Tetraborate, Na$_2$B$_4$O$_7$. 10H$_2$O = 381.37. Contains not less than 99.0 per cent and not more than the equivalent of 103.0 per cent of Na$_2$B$_4$O$_7$. 10H$_2$O.

**Description** – Transparent, colourless crystals, or a white, crystalline powder; odourless, taste, saline and alkaline. Effloresces in dry air, and on ignition, loses all its water of crystallisation.

**Solubility** – Soluble in water, practically insoluble in alcohol.

**Alkalinity** – A solution is alkaline to litmus solution.

**Heavy metals** – Dissolve 1 g in 16 ml of water and 6 ml of $N$ hydrochloric acid and add water to make 25 ml; the limit of heavy metals is 20 parts per million, Appendix 2.3.3.

**Iron** – 0.5 g complies with the *limit test for iron*, Appendix 2.3.4.

**Chlorides** – 1 g complies with the *limit test for chlorides*, Appendix 2.3.2.

** Sulphates** – 1 g complies with the *limit test for sulphates*, Appendix 2.3.6.

**Assay** – Weigh accurately about 3 g and dissolve in 75 ml of water and titrate with 0.5 $N$ hydrochloric acid, using methyl red solution as indicator. Each ml of 0.5 $N$ hydrochloric acid is equivalent to 0.09534 g of Na$_2$B$_4$O$_7$.10H$_2$O.

**Storage** – Preserve Borax in well-closed container.

Bromine – Br$_2$ = 159.80.

**Description** – Reddish-brown, fuming, corrosive liquid.

**Solubility** – Slightly soluble in water, soluble in most organic solvents.

**Iodine** – Boil 0.2 ml with 20 ml of water, 0.2 ml of $N$ sulphuric acid and a small piece of marble until the liquid is almost colourless. Cool, add one drop of liquefied phenol, allow to stand for two minutes, and then add 0.2 g of potassium iodide and 1 ml of starch solution; no blue colour is produced.

**Sulphate** – Shake 3 ml with 30 ml of dilute ammonia solution and evaporate to dryness on a water bath, the residue complies with the *limit test for sulphates*, Appendix 2.3.6.

**Bromine Solution** – Dissolve 9.6 ml of bromine and 30 g of potassium bromide in sufficient water to produce 100 ml.

**Bromophenol Blue Indicator** – Dissolve 0.1 g of bromophenol blue in 3.0 ml of 0.05 N sodium hydroxide solution and 5 ml of ethyl alcohol (90 percent by volume) by gently warming. Make up the volume of the solution with ethyl alcohol (20 percent v/v) to 250 ml in a volumetric flask.

**Canada Balsam Reagent** – General reagent grade of commerce.
Carbon Tetrachloride – CCl₄ = 153.82

Description – Clear, colourless, volatile, liquid; odour, characteristic.

Solubility – Practically insoluble in water; miscible with ethyl alcohol, and with solvent ether.

Distillation range – Not less than 95 per cent distils between 76⁰ and 77⁰.

Wt. per ml – At 20⁰, 1.592 to 1.595 g.

Chloride, free acid – Shake 20 ml with 20 ml of freshly boiled and cooled water for three minutes and allow separation to take place; the aqueous layer complies with the following test:

Chloride – To 10 ml add one drop of nitric acid and 0.2 ml of silver nitrate solution; no opalescence is produced.

Free acid – To 10 ml add a few drops of bromocresol purple solution; the colour produced does not indicate more acidity than that indicated by the addition of the same quantity of the indicator to 10 ml of freshly boiled and cooled water.

Free chlorine – Shake 10 ml with 5 ml of cadmium iodide solution and 1 ml of starch solution, no blue colour is produced.

Oxidisable impurities – Shake 20 ml for five minutes with a cold mixture of 10 ml of sulphuric acid and 10 ml of 0.1 N potassium dichromate, dilute with 100 ml of water and add 3 g of potassium iodide: the liberated iodine requires for decolourisation not less than 9 ml of 0.1 N sodium thiosulphate.

Non-volatile matter – Leaves on evaporation on a water-bath and drying to constant weight at 105⁰ not more than 0.002 per cent w/v of residue.

Caustic Alkali Solution, 5 per cent – Dissolve 5 g of potassium or sodium hydroxide in water and dilute to 100 ml.

Charcoal, Decolourising – General purpose grade complying with the following test.

Decolourising powder – Add 0.10 g to 50 ml of 0.006 per cent w/v solution of bromophenol blue in ethanol (20 per cent) contained in a 250 ml flask, and mix. Allow to stand for five minutes, and filter; the colour of the filtrate is not deeper than that of a solution prepared by diluting 1 ml of the bromophenol blue solution with ethanol (20 per cent) to 50 ml.

Chloral Hydrate – CCl₃.CH(OH)₂ = 165.40.

Description – Colourless, transparent crystals, odour, pungent but not acrid; taste, pungent and slightly bitter, volatilises slowly on exposure to air.

Solubility – Very soluble in water, freely soluble in alcohol, in chloroform and in solvent ether.

Chloral alcoholate – Warm 1 g with 6 ml of water and 0.5 ml of sodium hydroxide solution: filter, add sufficient 0.1 N iodine to impart a deep brown colour, and set aside for one hour; no yellow crystalline precipitate is produced and no smell of iodoform is perceptible.
Chloride – 3 g complies with the limit test for chlorides, Appendix 2.3.2.

Assay – Weigh accurately about 4 g and dissolve in 10 ml of water and add 30 ml of N sodium hydroxide. Allow the mixture to stand for two minutes, and then titrate with N sulphuric acid using phenolphthalein solution as indicator. Titrate the neutralised liquid with 0.1 N silver nitrate using solution of potassium chromate as indicator. Add two-fifteenth of the amount of 0.1 N silver nitrate used to the amount of N sulphuric acid used in the first titration and deduct the figure so obtained from the amount of N sodium hydroxide added. Each ml of N sodium hydroxide, obtained as difference; is equivalent to 0.1654 g of C₂H₃Cl₃O₂.

Storage – Store in tightly closed, light resistant containers in a cool place.

Chloral Hydrate Solution – Dissolve 20 g of chloral hydrate in 5 ml of water with warming and add 5 ml of glycerin.

Chloral Iodine Solution – Add an excess of crystalline iodine with shaking to the chloral hydrate solution, so that crystals of undissolved iodine remain on the bottom of bottle. Shake before use as the iodine dissolves, and crystals of the iodine to the solution. Store in a bottle of amber glass in a place protected from light.

Chloroform – CHCl₃ = 119.38

Description – Colourless, volatile liquid; odour, characteristic. Taste, sweet and burning.

Solubility – Slightly soluble in water; freely miscible with ethyl alcohol and with solvent ether.

Wt. per ml. : Between 1.474 and 1.478 g.

Boiling range – A variable fraction, not exceeding 5 per cent v/v, distils below 60° and the remainder distils between 50° to 62°.

Acidity – Shake 10 ml with 20 ml of freshly boiled and cooled water for three minutes, and allow to separate. To a 5 ml portion of the aqueous layer add 0.1 ml of litmus solution; the colour produced is not different from that produced on adding 0.1 ml of litmus solution to 5 ml of freshly boiled and cooled water.

Chloride – To another 5 ml portion of the aqueous layer obtained in the test for Acidity, add 5 ml of water and 0.2 ml of silver nitrate solution; no opalescence is produced.

Free chlorine – To another 10 ml portion of the aqueous layer, obtained in the test for Acidity, add 1 ml of cadmium iodide solution and two drops of starch solution; no blue colour is produced.

Aldehyde – Shake 5 ml with 5 ml of water and 0.2 ml of alkaline potassium mercuri-iodide solution in a stoppered bottle and set aside in the dark for fifteen minutes; not more than a pale yellow colour is produced.

Decomposition products – Place 20 ml of the chloroform in a glass-stoppered flask, previously rinsed with sulphuric acid, add 15 ml of sulphuric acid and four drops of formaldehyde solution, and shake the mixture frequently during half an hour and set aside for further half an hour, the flask being protected from light during the test; the acid layer is not more than slightly coloured.

Foreign organic matter – Shake 20 ml with 10 ml of sulphuric acid in a stoppered vessel previously rinsed with sulphuric acid for five minutes and set aside in the dark for thirty minutes,
both the acid and chloroform layers remain colourless. To 2 ml of the acid layer add 5 ml of water; the liquid remains colourless and clear, and has no unpleasant odour. Add a further 10 ml of water and 0.2 ml of silver nitrate solution; no opalescence is produced.

**Foreign odour** – Allow 10 ml to evaporate from a large piece of filter paper placed on a warm plate; no foreign odour is detectable at any stage of the evaporation.

**Non volatile matter** – Not more than 0.004 per cent w/v determined on 25 ml by evaporation and drying at 105°.

**Storage** – Store in tightly-closed, glass-stoppered, light-resistant bottles.

**Copper Sulphate** – CuSO\(_4\).5H\(_2\)O = 249.68

Contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of CuSO\(_4\).5H\(_2\)O.

**Description** – Blue triclinic prisms or a blue, crystalline powder.

**Solubility** – Soluble in water, very soluble in boiling water, almost insoluble in alcohol; very slowly soluble in glycerin.

**Acidity and clarity of solution** – 1 g, dissolved in 20 ml of water, forms a clear blue solution, which becomes green on the addition of 0.1 ml of methyl orange solution.

**Iron** – To 5 g, add 25 ml of water, and 2 ml of nitric acid, boil and cool. Add excess of strong ammonia solution, filter, and wash the residue with dilute ammonia solution mixed with four times its volumes of water. Dissolve the residue, if any, on the filter with 2 ml of hydrochloric acid, diluted with 10 ml of water; to the acid solutions add dilute ammonia solution till the precipitation is complete; filter and wash; the residue after ignition weighs not more than 7 mg.

**Copper Sulphate, Anhydrous** – CuSO\(_4\) =159.6

Prepared by heating copper sulphate to constant weight at about 230°.

**Copper Sulphate Solution** – A10.0 per cent w/v solution of copper sulphate in water.

**Cresol Red** – 4,4’-(3H-2, 1-Benzoxathiol-3 ylidene) di-O-cresol SS-dioxide; C\(_{12}\)H\(_8\)O\(_5\)S = 382.4.

Gives a red colour in very strongly acid solutions, a yellow colour in less strongly acid and neutral solutions, and a red colour in moderately alkaline solutions (pH ranges, 0.2 to 1.8, and 7.2 to 8.8).

**Cresol Red Solution** – Warm 50 ml of cresol red with 2.65 ml of 0.05 M sodium hydroxide and 5 ml of ethanol (90 per cent); after solution is effected, add sufficient ethanol (20 per cent) to produce 250 ml.

**Sensitivity** – A mixture of 0.1 ml of the solution and 100 ml of carbon dioxide-free water to which 0.15 ml of 0.02 M sodium hydroxide has been added is purplish-red. Not more than 0.15 ml of 0.02 M hydrochloric acid is required to change the colour to yellow.

**Diphenylamine barium sulphonate** – Dissolve 0.25 g in 100 ml water.
**Disodium Ethylenediamine tetraacetate** – (Disodium Acetate) \(C_{10}H_{14}N_{2}Na_{2}O_{8}.2H_{2}O = 372.2\), Analytical reagent grade.

**Dragendorff’s Reagent** –

**Solution 1** – Dissolve 0.85 g of *bismuth oxy nitrate* in 40 ml of water and 10 ml of acetic acid.

**Solution 2** – Dissolve 8 g of *potassium iodide* in 20 ml of water.

Mix equal volumes of solution 1 and 2, and to 10 ml of the resultant mixture add 100 ml of water and 20 ml of acetic acid.

**Dithizone**-1,5-Diphenylthiocarbazone; Diphenylthiocarbazone; \(C_{6}H_{5}N:NCSNHNHC_{6}H_{5}\= 56.32\)  
Analytical Reagent grade of commerce.  
Almost black powder; mp, about 168\(^0\), with decomposition.  
Store in light-resistant containers.

**Eosin** – Acid Red 87; Tetrabromoflurescein disodium salt; \(C_{20}H_{6}O_{5}Br_{4}Na_{2}\ =691.86\).

**Description** – Red powder, dissolves in water to yield a yellow to *purplish-red* solution with a greenish-yellow fluorescence.

**Solubility** – Soluble in water and in alcohol.

**Chloride** – Dissolve 50 mg in 25 ml of water, add 1 ml of nitric acid, and filter; the filtrate complies with the *limit test for chlorides*, Appendix 2.3.2.

**Sulphated ash** – Not more than 24.0 per cent, calculated with reference to the substance dried at 110\(^0\) for two hours, Appendix 2.2.6.

**EDTA solution 0.05 M** – Dissolve 18.6120 g of sodium salt of EDTA in purified water and make up to 1000 ml.

**Eosin Solution** – A 0.5 per cent w/v solution of eosin in water.

**Eriochrome Black T** – Mordant Black 11; Sodium 2(1-hydroxy-2-naphthylazo) 5-nitro-2-naphtol-4-sulphonate; \(C_{20}H_{12}N_{3}NaO_{7}S\ = 461.38\).

Brownish black powder having a faint, metallic sheen, soluble in alcohol, in *methyl alcohol* and in hot water.

**Eriochrome Black T indicator 0.1 per cent solution** – Dissolve 0.10 g indicator in 100 ml of Methanol.

**Ethyl Acetate** – \(CH_{3}. CO_{2}C_{2}H_{5} = 88.11\).  
Analytical reagent grade.

A colourless liquid with a fruity odour; boiling point, about 77\(^0\); weight per ml about 0.90g.

**Ethyl Alcohol** – \(C_{2}H_{5}OH = 46.07\).

Absolute Alcohol; Dehydrated Alcohol.
**Description** – Clear, colourless, mobile, volatile liquid; odour, characteristic and spirituous; taste, burning; hygroscopic. Readily volatilisable even at low temperature and boils at 78° and is flammable.

**Solubility** – Miscible with water, with solvent ether and with chloroform.

Contains not less than 99.5 per cent w/w or 99.7 per cent v/v of C\textsubscript{2}H\textsubscript{5}OH.

**Identification** – Acidity or Alkalinity; Clarity of Solution; Methanol; Foreign organic substances; Isopropyl alcohol and butyl alcohol; Aldehydes and ketones; fusel oil constituents; Non-volatile matter; complies with the requirements described under Alcohol.

**Specific gravity** – Between 0.7871 and 0.7902, at 25°.

**Storage** – Store in tightly closed containers in a cool place away from fire and protected from moisture.

**Labelling** – The label on the container states “Flammable”.

**Fehling’s Solution** –

A. Dissolve 69.278 g of CuSO\textsubscript{4} 5H\textsubscript{2}O in water and make the volume up to 1 litre  
B. Dissolve 100 g of sodium hydroxide and 340 g of Sodium potassium tartrate in water and make the volume to 1 litre.

Mix equal volumes of A and B before the experiment.

**Formaldehyde Solution** – Formalin; HCHO =30.03

Formaldehyde Solution is a solution of formaldehyde in water with methyl alcohol added to prevent polymerisation. It contains not less than 34.0 per cent w/w and not more than 38.0 per cent w/w of CH\textsubscript{2}O.

**Description** – Colourless liquid; odour, characteristic, pungent and irritating; taste, burning. A slight white cloudy deposit is formed on long standing, especially in the cold, due to the separation of paraformaldehyde. This white deposit disappears on warming the solution.

**Solubility** – Miscible with water, and with alcohol.

**Acidity** – To 10 ml add 10 ml of carbon dioxide free water and titrate with 0.1 N sodium hydroxide using bromothymol blue solutions as indicator; not more than 5 ml of 0.1 N sodium hydroxide is required.

**Wt. per ml** – At 20°, 1.079 to 1.094 g.

**Assay** – Weigh accurately about 3 g and add to a mixture of 50 ml of hydrogen peroxide solution and 50 ml of N sodium hydroxide, warm on a water-bath until effervescence ceases and titrate the excess of alkali with N sulphuric acid using phenolphthalein solution as indicator. Repeat the experiment with the same quantities of the same reagents in the same manner omitting the formaldehyde solution. The difference between the titrations represents the sodium hydroxide required to neutralise the formic acid produced by the oxidation of the formaldehyde. Each ml of N sodium hydroxide is equivalent to 0.03003 g of CH\textsubscript{2}O.
Storage – Preserve Formaldehyde Solution in well-closed container preferably at a temperature not below 15\(^\circ\)C.

**Formaldehyde Solution, Dilute** – Dilute 34 ml of formaldehyde solution with sufficient water to produce 100 ml.

**Folin Ciocalteu Reagent** – Dilute commercially available Folin-Ciocalteu reagent (2N) with an equal volume of distilled water. Transfer it in a brown bottle and store in a refrigerator (\(4^\circ\)). It should be golden in colour. Do not use it if it turns olive green.

**Formic acid** - 
\[ \text{HCOOH} = 46.03 \]

**Description** - Colourless liquid, odour, very pungent, highly corrosive; wt per ml. about 1.20 g, contains about 90.0 per cent of HCOOH and is about 23.6 M in strength.

**Assay** - Weigh accurately, a conical flask containing 10 ml of water, quickly add about 1 ml of the reagent being examined and weigh again. Add 50 ml of water and titrate with 1 M sodium hydroxide using 0.5 ml of phenolphthalein solution as indicator. Each ml of 1 M sodium hydroxide is equivalent to 0.04603 g of HCOOH.

**Glycerine** – 
\[ \text{C}_3\text{H}_8\text{O}_3 = 82.09 \]

**Description** – Clear, colorless, liquid of syrupy consistency; odourless, taste sweet followed by a sensation of warmth. It is hygroscopic.

**Solubility** – Miscible with water and with alcohol; practically insoluble in chloroform, in solvent ether and in fixed oils.

**Acidity** – To 50 ml of a 50 per cent w/v solution add 0.2 ml of dilute phenolphthalein solution; not more than 0.2 ml of 0.1 N sodium hydroxide is required to produce a pink colour.

**Wt. per ml** – Between 1.252 g and 1.257 g, corresponding to between 98.0 per cent and 100.0 per cent w/w of C\(_3\)H\(_8\)O\(_3\).

**Refractive index** – Between 1.470 and 1.475 determined at 20\(^\circ\).

**Arsenic** – Not more than 2 parts per million, Appendix 2.3.1.

**Copper** – To 10 ml add 30 ml of water, and 1 ml of dilute hydrochloric acid, and 10 ml of hydrogen sulphide solution; no colour is produced.

**Iron** – 10 g complies with the limit test for iron, Appendix 2.3.4.

**Heavy metals** – Not more than 5 parts per million, determined by Method A on a solution of 4 g in 2 ml of 0.1 N hydrochloric acid and sufficient water to produce 25 ml, Appendix 2.3.3.

**Sulphate** – 1 ml complies with the limit test for sulphates, Appendix 2.3.6.

**Chloride** – 1 ml complies with the limit test for chloride, Appendix 2.3.2.

**Acraldehyde and glucose** – Heat strongly; it assumes not more than a faint yellow, and not a pink colour. Heat further; it burns with little or no charring and with no odour of burnt sugar.
**Aldehydes and related substances** – To 12.5 ml of a 50 per cent w/v solution in a glass-stoppered flask add 2.5 ml of water and 1 ml of decolorised magenta solution. Close the flask and allow to stand for one hour. Any violet colour produced is not more intense than that produced by mixing 1.6 ml of 0.1 N potassium permanganate and 250 ml of water.

**Sugar** – Heat 5 g with 1 ml of dilute sulphuric acid for five minutes on a water-bath. Add 2 ml of dilute sodium hydroxide solution and 1 ml of copper sulphate solution. A clear, blue coloured solution is produced. Continue heating on the water-bath for five minutes. The solution remains blue and no precipitate is formed.

**Fatty acids and esters** – Mix 50 ml with 50 ml of freshly boiled water and 50.0 ml of 0.5N sodium hydroxide, boil the mixture for five minutes. Cool, add a few drops of phenolphthalein solution and titrate the excess alkali with 0.5 N hydrochloric acid. Perform a blank determination, not more than 1 ml of 0.5 N sodium hydroxide is consumed.

**Sulphated ash** – Not more than 0.01 per cent, Appendix 2.2.6.

**Storage** – Store in tightly-closed containers.

**Glycerin Solution** – Dilute 33 ml of glycerin to 100 ml with water and add a small piece of camphor or liquid phenol.

**n-Hexane** - \( \text{C}_6\text{H}_{14} \), = 86.18

Analytical reagent grade of commerce containing not less than 90.05 of \( n \)-Hexane.

Colourless, mobile, highly flammable liquid, bp 68\(^{\circ}\); wt per ml, about 0.674 g.

**Hydrochloric Acid** – HCl = 36.46

Concentrated Hydrochloric Acid

**Description** – Clear, colourless, fuming liquid; odour, pungent.

**Arsenic** – Not more than 1 part per million, Appendix 2.3.1.

**Heavy metals** – Not more than 5 parts per million, determined by Method A on a solution prepared in the following manner: Evaporate 3.5 ml to dryness on a water-bath, add 2 ml of dilute acetic acid to the residue, and add water to make 25 ml, Appendix 2.3.3.

**Bromide and iodide** – Dilute 5 ml with 10 ml of water, add 1 ml of chloroform, and add drop by drop, with constant shaking, chlorinated lime solution; the chloroform layer does not become brown or violet.

**Sulphite** – Dilute 1 ml with 10 ml of water, and add 5 drops of barium chloride solution and 0.5 ml of 0.001 N iodine; the colour of the iodine is not completely discharged.

**Sulphate** – To 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, Appendix. 2.3.7.

**Free chlorine** – Dilute 5 ml with 10 ml of freshly boiled and cooled water, add 1 ml of cadmium iodide solution, and shake with 1 ml of chloroform; the chloroform layer does not become violet within one minute.
**Sulphated ash** – Not more than 0.01 per cent, Appendix 2.2.6.

**Assay** – Weigh accurately about 4 g into a stoppered flask containing 40 ml of water, and titrate with \( N \) sodium hydroxide, using methyl orange solution as indicator. Each ml of \( N \) sodium hydroxide is equivalent to 0.03646 g of HCl.

**Storage** – Store in glass-stoppered containers at a temperature not exceeding 30\(^0\).

**Hydrochloric Acid, \( x \) N** – Solution of any normality \( x \) N may be prepared by diluting 84 x ml of hydrochloric acid to 1000 ml with water.

**Hydrochloric Acid** – (1 per cent w/v) Dilute 1 g of hydrochloric acid to 100 ml with water.

**Dilute Hydrochloric Acid** –

**Description** – Colourless liquid.

**Arsenic, Heavy metals bromoide and iodide, Sulphate, free chlorine** – Complies with the tests described under Hydrochloric Acid, when three times the quantity is taken for each test.

**Assay** – Weigh accurately about 10 g and carry out the Assay described under Hydrochloric Acid.

**Storage** – Store in stoppered containers of glass or other inert material, at temperature below 30\(^0\).

**Hydrochloric Acid, \( N \)** – HCl = 36.460

36.46 g in 1000 ml

Dilute 85 ml of hydrochloric acid with water to 1000 ml and standardise the solution as follows:

Weigh accurately about 1.5 g of anhydrous sodium carbonate, previously heated at about 270\(^0\) for one hour. Dissolve it in 100 ml of water and add two drops of methyl red solution. Add the acid slowly from a burette with constant stirring, until the solution becomes faintly pink. Heat again to boiling and titrate further as necessary until the faint pink colour no longer affected by continued boiling. Each 0.5299 g of anhydrous sodium carbonate is equivalent to 1 ml of \( N \) hydrochloric acid.

**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 water, 2 ml of a 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 water; no pink colour is produced.

**Hydrogen Peroxide Solution** – (20 Vol.) \( \text{H}_2\text{O}_2 = 34.02 \)

Analytical reagent grade of commerce or hydrogen peroxide solution (100 Vol.) diluted with 4 volumes of water.

A colourless liquid containing about 6 per cent w/v of \( \text{H}_2\text{O}_2 \); weight per ml, about 1.02 g.

**Hydroxylamine Hydrochloride; Hydroxylammonium Chloride** – \( \text{NH}_2\text{OH}.\text{HCl} = 69.49 \).

Contains not less than 97.0 per cent w/w of \( \text{NH}_2\text{OH} \). 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, Appendix 2.2.6.

Assay – Weigh accurately about 0.1 g and dissolve in 20 ml of water, add 5 g of ferric ammonium sulphate dissolved 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 \( \text{NH}_2\text{OH} \cdot \text{HCl} \).

**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.

**Mercuric Chloride** – \( \text{HgCl}_2 = 271.50 \).

Contains not less than 99.5 per cent of \( \text{HgCl}_2 \);

**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 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 minutes. Add 20 ml of acetic acid and 35 ml of 0.1 \( N \) iodine. Shake continuously for about ten minutes or until the precipitated mercury is completely redissolved, and titrate the excess of iodine with 0.1 \( N \) sodium thiosulphate. Each ml of 0.1 \( N \) iodine is equivalent to 0.01357 g of \( \text{HgCl}_2 \).

**Mercuric Chloride, 0.2 M** – Dissolve 54.30 g of mercuric chloride in sufficient water to produce 1000 ml.

**Mercuric Chloride Solution** – A 5.0 per cent w/v solution of mercuric chloride in water.

**Mercuric Potassium Iodide Solution** – See Potassium - Mercuric Iodide solution.

**Methyl Alcohol** : Methanol : \( \text{CH}_3\text{OH} = 32.04 \).

**Description** – Clear, Colourless liquid with a characteristic odour.

**Solubility** – Miscible with water, forming a clear colourless liquid.

**Specific Gravity** – At \( 25^\circ \), not more than 0.791.

**Distillation range** – Not less than 95 per cent distils between 64.5\( ^\circ \) and 65.5\( ^\circ \).

**Refractive Index** – At \( 20^\circ \), 1.328 to 1.329.
Acetone – Place 1 ml in a Nessler cylinder, add 19 ml of water, 2 ml of a 1 per cent w/v solution of 2-nitrobenzaldehyde in alcohol (50 per cent), 1 ml of 30 per cent w/v solution of sodium hydroxide and allow to stand in the dark for fifteen minutes. The colour developed does not exceed that produced by mixing 1 ml of standard acetone solution, 19 ml of water, 2 ml of the solution of 2-nitrobenzaldehyde and 1 ml of the solution of sodium hydroxide and allowing to stand in the dark for fifteen minutes.

Acidity – To 5 ml add 5 ml of carbon dioxide-free water, and titrate with 0.1 N sodium hydroxide, using bromothymol blue solution as indicator; not more than 0.1 ml is required.

Non-volatile matter – When evaporated on a water-bath and dried to constant weight at 105°, leaves not more than 0.005 per cent w/v of residue.

Methyl Alcohol, Dehydrated – Methyl alcohol, which complies with the following additional requirement.

Water – Not more than 0.1 per cent w/w.

Methyl Orange – Sodium-p-di methylamineazobenzene sulphate, C₁₄H₁₄O₃N₃SNa.

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 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 0.1 N 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, C₁₅H₁₅O₂N₃.

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

Methyl red solution – Dissolve 100 mg in 1.86 ml of 0.1 N sodium hydroxide and 50 ml of alcohol and dilute to 100 ml with water.

Test for sensitivity – A mixture of 0.1 ml of the methyl red solution and 100 ml of freshly boiled and cooled water to which 0.05 ml of 0.02 N hydrochloric acid has been added is red. Not more than 0.01 ml of 0.02 N sodium hydroxide is required to change the colour to yellow.

Colour change – pH 4.4 (red) to pH 6.0 (yellow).

Molish’s Reagent – Prepare two solutions in separate bottles, with ground glass stoppers:

(a) Dissolve 2 g of α-naphthol in 95 per cent alcohol and make up to 10 ml with alcohol (α-naphthol can be replaced by thymol or resorcinol). Store in a place protected from light. The solution can be used for only a short period.

(b) Concentrated sulphuric acid.
Nitric Acid – Contains 70.0 per cent w/w of HNO₃ (limits, 69.0 to 71.0). About 16 N in strength.

Description – Clear, colourless, fuming liquid.

Wt. per ml. – At 20⁰, 1.41 to 1.42 g.

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

Iron – 0.5 ml of complies with the limit test for iron, Appendix 2.3.4.

Lead – Not more than 2 parts per million, Appendix 2.3.5.

Chloride – 5 ml neutralised with dilute ammonia solution, complies with the limit test for chlorides, Appendix 2.3.2.

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, Appendix 2.3.7.

Sulphated ash – Not more than 0.01 per cent w/w, Appendix 2.2.6.

Assay – Weigh accurately about 4 g into a stoppered flask containing 40 ml of 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 HNO₃.

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

Nitric Acid, Dilute – Contains approximately 10 per cent w/w of HNO₃. Dilute 106 ml of nitric acid to 1000 ml with water.

Petroleum Light – Petroleum Spirit.

Description – Colourless, very volatile, highly flammable liquid obtained from petroleum, consisting of a mixture of the lower members of the paraffin series of hydrocarbons and complying with one or other of the following definitions:

Light Petroleum – (Boiling range, 30⁰ to 40⁰).

Wt. per ml. – At 20⁰, 0.620 to 0.630 g.

Light Petroleum – (Boiling range, 40⁰ to 60⁰).

Wt. per ml – At 20⁰, 0.630 to 0.650 g.

Light Petroleum – (Boiling range, 60⁰ to 80⁰).

Wt. per ml. – At 20⁰, 0.670 to 0.690.

Light Petroleum – (Boiling range, 80⁰ to 100⁰).

Wt. per ml. – At 20⁰, 0.700 to 0.720
**Light Petroleum** – (Boiling range, $100^0$ to $120^0$).

**Wt. per ml** – At $20^0$, 0.720 to 0.740 g.

**Light Petroleum** – (Boiling range, $120^0$ to $160^0$).

**Wt. per ml** – At $20^0$, about 0.75 g.

**Non-volatile matter** – When evaporated on a water-bath and dried at $105^0$, leaves not more than 0.002 per cent w/v of residue.

**Patterns & Reeders indicators 0.1 per cent solution** – Dissolve 0.01g indicator in 100 ml of Methanol.

**Phenolphthalein** – $C_{20}H_{14}O_4$.

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

**Phenolphthalein indicator** – Dissolve 0.5 gm Phenolphthalein in 100 ml of 50% ethyl alcohol (v/v).

**Phenolphthalein Solution** – Dissolve 0.10 g in 80 ml of alcohol and dilute to 100 ml with 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)

**Phloroglucinol** – 1, 3, 5 – Trihydroxybenzene, $C_6H_3(OH)_3 \cdot 2H_2O$.

**Description** – White or yellowish crystals or a crystalline powder.

**Solubility** – Slightly soluble in water; soluble in alcohol, and in solvent ether.

**Melting range** – After drying at $110^0$ for one hour, $215^0$ to $219^0$.

**Sulphated ash** – Not more than 0.1 per cent, Appendix 2.2.6.

Phloroglucinol should be kept protected from light.

**Phosphoric Acid** – $H_3PO_4 = 98.00$.

(Orthophosphoric Acid; Concentrated Phosphoric Acid).

**Description** – Clear and colourless syrupy liquid, corrosive.

**Solubility** – Miscible with water and with alcohol.

**Phosphoric Acid, x N** – Solutions of any normality, x N may be prepared by diluting 49 x g of phosphoric acid with water to 1000 ml.
Phosphoric Acid, Dilute –  
Contains approximately 10 per cent w/v of H₃PO₄. 
Dilute 69 ml of phosphoric acid to 1000 ml with water.

Potassium Chloride – KCl = 74.55  
Analytical reagent grade

Potassium Chromate – K₂CrO₄ = 194.2  
Analytical reagent grade

Potassium Chromate Solution – A 5.0 per cent w/v solution of potassium chromate.  
Gives a red precipitate with silver nitrate in neutral solutions.

Potassium Cupric-Tartrate Solution – Cupric Tatrate Alkaline Solution: Fehling’s Solution.

(1) Copper Solution – Dissolve 34.66 g of carefully selected small crystals of copper sulphate, showing no trace of efflorescence or of adhering moisture, in sufficient water to make 500 ml. Keep this solution in small, well-stoppered bottles.

(2) Alkaline Tartrate Solution – Dissolve 176 g of sodium potassium tartrate and 77 g of sodium hydroxide in sufficient water to produce 500 ml. 
Mix equal volumes of the solutions No. 1 and No. 2 at the time of using.

Potassium Dichromate – K₂Cr₂O₇ = 294.18. 
Contains not less than 99.8 per cent of K₂Cr₂O₇.

Description – Orange-red crystals or a crystalline powder.

Solubility – Soluble in water

Chloride – To 20 ml of a 5 per cent w/v solution in water and 10 ml nitric acid, warm to about 50° and add a few drops of silver nitrate solution; not more than a faint opalescence is produced.

Assay – Carry out the assay described under Potassium Chromate, using 2 g. Each ml of 0.1 N sodium thiosulphate is equivalent to 0.004904 g of K₂Cr₂O₇.

Potassium Dichromate Solution – A 7.0 per cent w/v solution of potassium dichromate in water.

Potassium Dichromate, Solution 0.1N – K₂Cr₂O₇ = 294.18, 4.903 g in 1000 ml.  
Weigh accurately 4.903 g of potassium dichromate and dissolve in sufficient water to produce 1000 ml.
Potassium Dihydrogen Phosphate - $\text{KH}_2\text{PO}_4 = 136.1$

Analytical reagent grade of commerce.

Potassium Ferrocyanide – $\text{K}_4\text{Fe(CN)}_6.3\text{H}_2\text{O} = 422.39$.

Contains not less than 99.0 per cent of $\text{K}_4\text{Fe(CN)}_6.3\text{H}_2\text{O}$.

**Description** – Yellow, crystalline powder.

**Solubility** – Soluble in water.

**Acidity or Alkalinity** – A 10 per cent w/v solution in water is neutral to litmus paper.

**Assay** – Weigh accurately about 1g and dissolve in 200 ml of water, add 10 ml of sulphuric acid and titrate with 0.1 $N$ potassium permanganate. Each ml of 0.1 $N$ potassium permanganate is equivalent to 0.04224 g of $\text{K}_4\text{Fe(CN)}_6.3\text{H}_2\text{O}$.

**Potassium Ferrocyanide Solution** – A 5.0 per cent w/v solution of potassium ferrocyanide in water.

Potassium Hydrogen Phthalate – $\text{CO}_2\text{H} \cdot \text{C}_6\text{H}_4 \cdot \text{CO}_2\text{K} = 204.22$.

Contains not less than 99.9 per cent and not more than the equivalent of 100.1 per cent of $\text{C}_8\text{H}_5\text{O}_4\text{K}$ calculated with reference to the substance dried at 110°C for one hour.

**Description** – White, crystalline powder.

**Solubility** – Slowly soluble in water, forming clear, colourless solution.

**Acidity** – A 2.0 per cent w/v solution in carbon dioxide free water gives with bromophenol blue solution the grey colour indicative of $pH$ 4.0.

**Assay** – Weigh accurately about 9 g, dissolve in 100 ml of water and titrate with $N$ sodium hydroxide using phenolphthalein solution as indicator. Each ml of $N$ Sodium hydroxide is equivalent to 0.2042 g of $\text{C}_8\text{H}_5\text{O}_4\text{K}$.

**Potassium Hydrogen Phthalate, 0.02 M** – Dissolve 4.084 g of Potassium hydrogen phthalate in sufficient water to produce 1000 ml.

**Potassium Hydrogen Phthalate, 0.2 M** – Dissolve 40.84 g of potassium hydrogen phthalate in sufficient water to produce 1000 ml.

**Potassium Hydroxide** – Caustic Potash: $\text{KOH} = 56.11$

Contains not less than 85.0 per cent of total alkali, calculated as KOH and not more than 4.0 per cent of $\text{K}_2\text{CO}_3$.

**Description** – Dry white sticks, pellets or fused mass; hard, brittle and showing a crystalline fracture; very deliquescent; strongly alkaline and corrosive.

**Solubility** – Freely soluble in water, in alcohol and in glycerin; very soluble in boiling ethyl alcohol.
Aluminium, iron and matter insoluble in hydrochloric acid – Boil 5 g with 40 ml of dilute hydrochloric acid, cool, make alkaline with dilute ammonia solution, boil, filter and wash the residue 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.

Chloride – 0.5 g dissolved in water with the addition of 1.6 ml of nitric acid, complies with the limit test for chlorides, Appendix 2.3.2.

Heavy metals – Dissolve 1 g in a mixture of 5 ml of water and 7 ml of dilute hydrochloric acid. Heat to boiling, add 1 drop of phenolphthalein solution and dilute ammonia solution dropwise to produce a faint pink colour. Add 2 ml of acetic acid and water to make 25 ml; the limit of heavy metals is 30 parts per million, Appendix 2.3.3.

Sulphate – Dissolve 1 g in water with the addition of 4.5 ml of hydrochloric acid; the solution complies with the limit test for sulphates, Appendix 2.3.6.

Sodium – To 3 ml of a 10 per cent w/v solution add 1 ml of water, 1.5 ml of alcohol, and 3 ml of potassium antimonate solution and allow to stand; no white crystalline precipitate or sediment is visible to the naked eye within fifteen minutes.

Assay – Weigh accurately about 2 g, and dissolve in 25 ml of water, add 5 ml of barium chloride solution, and titrate with N hydrochloric acid, using phenolphthalein solution as indicator. To the solution in the flask add bromophenol blue solution, and continue the titration with N hydrochloric acid. Each ml of N hydrochloric acid, used in the second titration is equivalent to 0.05611 g of total alkali, calculated as KOH.

Storage – Potassium Hydroxide should be kept in a well-closed container.

Potassium Hydroxide, x N – Solution of any normality, x N, may be prepared by dissolving 56.11x g of potassium hydroxide in water and diluting to 1000 ml.

Potassium Hydroxide Solution – Solution of Potash.

An aqueous solution of potassium hydroxide containing 5.0 per cent w/v of total alkali, calculated as KOH (limits, 4.75 to 5.25).

Assay – Titrate 20 ml with N sulphuric acid, using solution of methyl orange as indicator. Each ml of N sulphuric acid is equivalent to 0.05611 g of total alkali, calculated as KOH.

Storage – Potassium hydroxide solution should be kept in a well-closed container of lead-free glass or of a suitable plastic.

Potassium Iodide – KI = 166.00

Description – Colourless crystals or white powder; odourless, taste, saline and slightly bitter.

Solubility – Very soluble in water and in glycerin; soluble in alcohol.

Arsenic – Not more than 2 parts per million, Appendix 2.3.1.

Heavy metals – Not more than 10 parts per million, determined on 2.0 g by Method A, Appendix 2.3.3.
**Barium** – Dissolve 0.5 g in 10 ml of water and add 1 ml of dilute sulphuric acid; no turbidity develops within one minute.

**Cyanides** – Dissolve 0.5 g in 5 ml of warm water, add one drop of ferrous sulphate solution and 0.5 ml of sodium hydroxide solution and acidify with hydrochloric acid; no blue colour is produced.

**Iodates** – Dissolve 0.5 g in 10 ml of freshly boiled and cooled water, and add 2 drops of dilute sulphuric acid and a drop of starch solution; no blue colour is produced within two minutes.

**Assay** – Weigh accurately about 0.5 g, dissolve in about 10 ml of water and add 35 ml of hydrochloric acid and 5 ml of chloroform. Titrate with 0.05 M potassium iodate until the purple colour of iodine disappears from the chloroform. Add the last portion of the iodate solution drop-wise and agitate vigorously and continuously. Allow to stand for five minutes. If any colour develops in the chloroform layer continue the titration. Each ml of 0.05 M potassium iodate is equivalent to 0.0166 mg of KI.

**Storage** – Store in well-closed containers.

**Potassium Iodide, M** – Dissolve 166.00 g of potassium iodide in sufficient water to produce 1000 ml.

**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 Iodide and Starch solution must be recently prepared.

**Potassium Iodide Solution** – A 10 per cent w/v solution of potassium iodide in water.

**Potassium Iodobismuthate Solution** – Dissolve 100 g of tartaric acid in 400 ml of water and 8.5 g of bismuth oxynitrate. Shake during one hour, add 200 ml of a 40 per cent w/v

**Potassium Iodobismuthate Solution, Dilute** – Dissolve 100 g of tartaric acid in 500 ml of water and add 50 ml of potassium iodobismuthate solution.

**Potassium Mercuric-Iodide Solution** – Mayer’s Reagent.

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.

**Potassium Mercuric-Iodide Solution, Alkaline (Nessler’s Reagent)**

To 3.5 g of potassium iodide add 1.25 g of mercuric chloride dissolved in 80 ml of water, add a cold saturated solution of mercuric chloride in water, with constant stirring until a slight red precipitate remains. Dissolve 12 g of sodium hydroxide in the solution, add a little more of the cold saturated solution of mercuric chloride and sufficient water to produce 100 ml. Allow to stand and decant the clear liquid.

**Potassium Permanganate** – KMnO₄ = 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.

**Chloride and Sulphate** – 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 colour-less; filter. A 20 ml portion of the filtrate complies with the limit test for chloride, Appendix 2.3.2., and another 20 ml portion of the filtrate complies with the limit test for sulphates, Appendix 2.3.7.

**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 KMnO4.

**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 substance, 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** – 158.03. 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 0.003161 g of KMnO4.

**Potassium Tellurite**: K₂ TeO₃ (approx)

General reagent grade of commerce.

**Purified Water** – H₂O = 18.02.

**Description** – Clear, colourless liquid, odourless, tasteless.

Purified water is prepared from potable water by distillation, ion-exchange treatment, reverse osmosis or any other suitable process. It contains no added substances.

**pH** – Between 4.5 and 7.0 determined in a solution prepared by adding 0.3 ml of a saturated solution of potassium chloride to 100 ml of the liquid being examined.

**Carbon dioxide** – To 25 ml add 25 ml of calcium hydroxide solution, no turbidity is produced.

**Chloride** – To 10 ml add 1 ml of dilute nitric acid and 0.2 ml of silver nitrate solution; no opalescence is produced, Appendix 2.3.2.

**Sulphate** – To 10 ml add 0.1 ml of dilute hydrochloric acid and 0.1 ml of barium chloride, the solution remains clear for an hour, Appendix 2.3.6.
Nitrates and Nitrites – To 50 ml add 18 ml of acetic acid and 2 ml of naphthylamine-sulphanilic acid reagent. Add 0.12 g of zinc reducing mixture and shake several times. No pink colour develops within fifteen minutes.

Ammonium – To 20 ml add 1 ml of alkaline potassium mercuric-iodide solution and after five minutes view in a Nessler cylinder placed on a white tile; the colour is not more intense than that given on adding 1 ml of alkaline potassium mercuric-iodide solution to a solution containing 2.5 ml of dilute ammonium chloride solution (Nessler’s) 7.5 ml of the liquid being examined.

Calcium – To 10 ml add 0.2 ml of dilute ammonia solution and 0.2 ml of ammonium oxalate solution; the solution remains clear for an hour.

Heavy metals – Adjust the pH of 40 ml to between 3.0 and 4.0 with dilute acetic acid, add 10 ml of freshly prepared hydrogen sulphide solution and allow to stand for ten minutes; the colour of the solution is not more than that of a mixture of 50 ml of the liquid being examined and the same amount of dilute acetic acid added to the sample, Appendix 2.3.3.

Oxidisable matter – To 100 ml add 10 ml of dilute sulphuric acid and 0.1 ml of 0.1 N potassium permanganate and boil for five minutes. The solution remains faintly pink.

Total Solids – Not more than 0.001 per cent w/v determined on 100 ml by evaporating on a water bath and drying in an oven at 105°C for one hour.

Storage – Store in tightly closed containers.

Resorcinol solution – Dissolve 1 g resublimed resorcinol in 100 ml hydrochloric acid (sp gr 1.18 to 1.19).

Silver Nitrate Solution – A freshly prepared 5.0 per cent w/v solution of silver nitrate in water.

Silver Nitrate, 0.1 N – AgNO₃ = 169.87; 16.99 g in 1000 ml. Dissolve about 17 g in sufficient water to produce 1000 ml and standardise the solution as follows:

Weigh accurately about 0.1 g of sodium chloride previously dried at 110°C for two hours and dissolve in 5 ml of water. Add 5 ml of acetic acid, 50 ml of methyl alcohol and three drops of eosin solution is equivalent to 1 ml of 0.1 N silver nitrate.

Sodium Bicarbonate – NaHCO₃ = 84.01

Description – White, crystalline powder or small, opaque, monoclinic crystals; odourless; taste, saline.

Solubility – Freely soluble in water; practically insoluble in alcohol.
Carbonate – 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, Appendix 2.3.1.

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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, Appendix 2.3.4.

Heavy metals –Not more than 5 parts per million, determined by Method A 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 drop wise to give the solution a faint pink colour. Cool and dilute to 25 ml with water, Appendix 2.3.3.

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, Appendix 2.3.2.

Sulphates –Dissolve 2 g in water with the addition of 2 ml of hydrochloric acid; the solution complies with the limit test for sulphates, Appendix 2.3.6.

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 solutions as indicator. Each ml of 0.5 N sulphuric acid is equivalent to 0.042 g of NaHCO₃.

Storage –Store in well-closed containers.

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

Sodium Carbonate – Na₂CO₃. 10H₂O =286.2.

Analytical reagent grade.

Sodium Chloride – NaCl = 58.44

Analytical reagent grade.

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 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, Appendix 2.3.1.
**Heavy metals** – Not more than 30 parts per million, determined by Method A, Appendix 2.3.3. 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 cobaltinitrite solution; no precipitate is formed.

**Chloride** – 0.5 g dissolved in water with the addition of 1.8 ml of nitric acid, complies with the limit test for chlorides, Appendix 2.3.2.

**Sulphates** – 1 g dissolved in water with the addition of 3.5 ml of hydrochloric acid complies with the limit test for sulphates, Appendix 2.3.6.

**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 Potassium Tartrate** – Rochelle Salt COONa.CH(OH). CH(OH), COOK. 4H\(_2\)O = 282.17

Contains not less than 99.0 per cent and not more than the equivalent of 104.0 per cent of \( \text{C}_4\text{H}_2\text{O}_6\text{KNa}. 4\text{H}_2\text{O} \).

**Description** – Colourless crystals or a white, crystalline powder; odourless; taste saline and cooling. It effloresces slightly in warm, dry air, the crystals are often coated with a white powder.

**Solubility** – Soluble in water; practically insoluble in alcohol.

**Acidity or Alkalinity** – Dissolve 1 g in 10 ml of recently boiled and cooled water, the solution requires for neutralisation not more than 0.1 ml of 0.1 \( N \) sodium hydroxide or of 0.1 \( N \) hydrochloric acid, using phenolphthalein solution as indicator.

**Iron** – 0.5 g complies with the limit test for iron, Appendix 2.3.4.

**Chloride** – 0.5 g complies with the limit test for chlorides, Appendix 2.3.2.

**Sulphate** – 0.5 g complies with the limit test for sulphate, Appendix 2.3.6.

**Assay** – Weigh accurately about 2 g and heat until carbonised, cool, and boil the residue with 50 ml of water and 50 ml of 0.5 \( \text{N sulphuric acid} \); filter, and wash the filter with water; titrate the excess
of acid in the filtrate and washings with 0.5 N sodium hydroxide, using methyl orange solution as indicator. Each ml of 0.5 N sulphuric acid is equivalent to 0.07056 g of C₄H₄O₆KNa. 4H₂O.

**Sodium Sulphate (anhydrous)** – Na₂SO₄ = 142.04

Analytical reagent grade of commerce.
White, crystalline powder of granules; hygroscopic.

**Sodium Thiosulphate** – Na₂S₂O₃. 5H₂O =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 33⁰.

**Solubility** – Very soluble in water; insoluble in alcohol.

**pH** – Between 6.0 and 8.4, determined in a 10 per cent w/v solution.

**Arsenic** – Not more than 2 parts per million, Appendix 2.3.1.

**Heavy metals** – Not more than 20 parts per million, determined by Method A, Appendix 2.3.3. in a solution prepared in the following manner: Dissolve 1 g in 10 ml of water, slowly add 5 ml of dilute hydrochloric acid and evaporate the mixture to dryness on a water-bath. Gently boil the residue with 15 ml of water for two minutes, and filter. Heat the filtrate to boiling, and add sufficient bromine solution to the hot filtrate to produce a clear solution and add a slight excess of bromine solution. Boil the solution to expel the bromine completely, cool to room temperature, then add a drop of phenolphthalein solution and sodium hydroxide solution until a slight pink colour is produced. Add 2 ml of dilute acetic acid and dilute with water to 25 ml.

**Calcium** – Dissolve 1 g in 20 ml of water, and add a few ml of ammonium oxalate solution; no turbidity is produced.

**Chloride** – Dissolve 0.25 g in 15 ml of 2N nitric acid and boil gently for three to four minutes, cool and filter; the filtrate complies with the limit test for chlorides, Appendix 2.3.2.

**Sulphate and Sulphite** – Dissolve 0.25 g in 10 ml of water, to 3 ml of this solution add 2 ml of iodine solution, and gradually add more iodine solution, dropwise until a very faint-persistant yellow colour is produced; the resulting solution complies with the limit test for sulphates, Appendix 2.3.7.

**Sulphide** – Dissolve 1 g in 10 ml of water and 10.00 ml of a freshly prepared 5 per cent w/v solution of sodium nitroprusside; the solution does not become violet.

**Assay** – Weigh accurately about 0.8 g and dissolve in 30 ml of water. Titrate with 0.1 N iodine, using 3 ml of starch solution as indicator as the end-point is approached. Each ml of 0.1 iodine is equivalent to 0.02482 g of Na₂S₂O₃.5H₂O.

**Storage** – Store in tightly-closed containers.

**Sodium Thiosulphate, 0.1 N** – Na₂S₂O₃.5H₂O. = 248.17, 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 in sufficient water to produce 250 ml. To 50 ml of this solution, add 2 g of potassium iodide and 3 ml of 2 N 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 0.1 N sodium thiosulphate frequently.

**Soxhlet Modification of Fehling’s solution** – Prepare by mixing equal volumes of Solution A and Solution B immediately before using.

**Copper Sulphate Solution (Solution A)** – Dissolve 34.639 g of copper sulphate crystals (CuSO₄·5H₂O) in water, dilute to 500 ml and filter through glass wool or filter paper.

**Potassium Sodium Tartrate (Rochelle Salt) Solution (Solution B)** – Dissolve 173 g of potassium sodium tartrate and 50 g of sodium hydroxide in water, dilute to 500 ml. Let the solution stand for a day and filter.

**Standard Invert Sugar Solution** – Weigh accurately 0.95 g sucrose and dissolve it in 500 ml water. Add 2 ml of concentrated hydrochloric acid, boil gently for 30 minutes and keep aside for 24 hours. Neutralize with sodium carbonate and make the final volume to 1000 ml; 50 ml of this solution contains 0.05 g invert sugar.

**Stannous Chloride – SnCl₂·2H₂O = 225.63.**

Contains not less than 97.0 per cent of SnCl₂·2H₂O.

**Description** – Colourless crystals.

**Solubility** – Soluble in dilute hydrochloric acid.

**Arsenic** – Dissolve 5.0 g in 10 ml of hydrochloric acid, heat to boiling and allow to stand for one hour; the solution shows no darkening when compared with a freshly prepared solution of 5.0 g in 10 ml of hydrochloric acid.

**Sulphate** – 5.0 g with the addition of 2 ml of dilute hydrochloric acid, complies with the limit test for sulphates, Appendix 2.3.7.

**Assay** – Weigh accurately about 1.0 g and dissolve in 30 ml of hydrochloric acid in a stoppered flask. Add 20 ml of water and 5 ml of chloroform and titrate rapidly with 0.05 M potassium iodate until the chloroform layer is colourless. Each ml of 0.05 M potassium iodate is equivalent to 0.02256 g of SnCl₂·2H₂O.

**Stannous Chloride Solution** – May be prepared by either of the two methods given below: Dissolve 330 g of stannous chloride in 100 ml of hydrochloric acid and add sufficient water to produce 1000 ml.

Dilute 60 ml of hydrochloric acid with 20 ml of water, add 20 g of tin and heat gently until gas ceases to be evolved; add sufficient water to produce 100 ml, allowing the undissolved tin to remain in the solution.

**Starch Soluble** – Starch, which has been treated with hydrochloric acid until after being washed, it forms an almost clear liquid solution in hot water.

**Description** – Fine, white powder.
Solubility – Soluble in hot water, usually forming a slightly turbid solution.

Acidity or Alkalinity – Shake 2 g with 20 ml of water for three minutes and filter; the filtrate is not alkaline or more than faintly acid to litmus paper.

Sensitivity – Mix 1 g with a little cold water and add 200 ml boiling water. Add 5 ml of this solution to 100 ml of water and add 0.05 ml of 0.1 N iodine. The deep blue colour is discharged by 0.05 ml of 0.1 N sodium thiosulphate.

Ash – Not more than 0.3 per cent, Appendix 2.3.

Starch Solution – Triturate 0.5 g of soluble starch, 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.

Sulphamic Acid – NH$_2$SO$_3$H = 97.09.

Contains not less than 98.0 per cent of H$_3$NO$_3$S.

Description – White crystals or a white crystalline powder.

Solubility – Readily soluble in water. Melting Range –203$^\circ$ to 205$^\circ$, with decomposition.

Sulphuric Acid – H$_2$SO$_4$ = 98.08.

When no molarity is indicated use analytical reagent grade of commerce containing about 98 per cent w/w of sulphuric acid. An oily, corrosive liquid weighing about 1.84 g per ml and about 18 M in strength.

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

Solutions of sulphuric acid contain about 10 per cent w/v of H$_2$SO$_4$ per g mol.

Sulphuric Acid, Dilute – Contains approximately 10 per cent w/w of H$_2$SO$_4$.

Dilute 57 ml of sulphuric acid to 1000 ml with water.

Sulphuric Acid, Chlorine-free – Sulphuric acid which complies with the following additional test:

Chloride – Mix 2 ml with 50 ml of water and add 1 ml of solution of silver nitrate, no opalescence is produced.

Sulphuric Acid, Nitrogen-free – Sulphuric acid which contains not less than 98.0 per cent w/w of H$_2$SO$_4$ and complies with the following additional test:

Nitrate – Mix 45 ml with 5 ml of water, cool and add 8 mg of diphenyl benzidine; the solution is colourless or not more than very pale blue.

Sulphuric acid + orthophosphoric acid mixture – take 60 ml water, add 15 ml conc. sulphuric acid and 15 ml H$_3$PO$_4$ cool and dilute to 1000 ml.
Tartaric Acid – (CHOH. COOH)₂ =150.1

Analytical reagent grade.

Thioglycollic Acid – Mercapto acetic acid, – HS. CH₂COOH =92.11.

Contains not less than 89.0 per cent w/w of C₂H₄O₂S, as determined by both parts of the Assay described below:

**Description** – Colourless or nearly colourless liquid; odour strong and unpleasant.

**Iron** – Mix 0.1 ml with 50 ml of water and render alkaline with *strong ammonia solution*; no pink colour is produced.

**Assay** – Weigh accurately about 0.4 g and dissolve in 20 ml of water and titrate with 0.1 N sodium hydroxide using cresol red solution as indicator. Each ml of 0.1 N sodium hydroxide is equivalent to 0.009212 g of C₂H₄O₂S.

To the above neutralised solution and 2 g of sodium bicarbonate and titrate with 0.1 N iodine. Each ml of 0.1 N iodine is equivalent to 0.009212 g of C₂H₄O₂S.

**Triethanolamine 20per cent Solution** – 200 ml of triethanolamine, adds 800 ml water and make up to 1000 ml.

**Toluene** – Methyl benzene, C₆H₅.CH₃ =102.14.

Analytical grade reagent of commerce.

Clear, colourless liquid, odour, characteristic; bp about 110⁰, wt per ml, about 0.870 g.

**Water** – See purified water.

**Water, Ammonia-free** – Water, which has been boiled vigorously for a few minutes and protected from the atmosphere during cooling and storage.

**Xylenol Orange** – [3H-2,1-Benzoxathiol–3-ylidene bis – (6-hydroxy-5-methyl-m-phenylene) methylidenenitrilo] tetra acetic acid SS-dioxide or its tetra sodium salt.

Gives a reddish-purple colour with mercury, lead, zinc and contain other metal ions in acid solution. When metal ions are absent, for example, in the presence of an excess of disodium ethylenediamine tetraacetate, this solution is yellow.

**Xylenol Orange Solution** – Dissolve 0.1 g of *xylenol orange* with 100 ml of water and filter, if necessary.

**Zinc Acetate** – analytical grade reagent of commerce.

**Zinc Acetate – Aluminum Chloride Reagent:** Dissolve 20 g of *zinc acetate* and 5 g of *aluminum chloride* in sufficient water to make 100 ml.

**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- 5

5.1. CHEMICAL TESTS AND ASSAYS

5.1.1. - ESTIMATION OF TOTAL PHENOLICS

Prepare a stock solution (1 mg/ml) of the extract in methanol. From the stock solution, take suitable quantity of the extract into 25-ml volumetric flask and add 10 ml of water and 1.5 ml of Folín Ciocalteau reagent. Keep the mixture for 5 min, and then add 4 ml of 20 per cent sodium carbonate solution and make up to 25 ml with double distilled water.

Keep the mixture for 30 min and record absorbance at 765 nm. Calculate percentage of total phenolics from calibration curve of gallic acid prepared by using the above procedure and express total phenolics as percentage of gallic acid.

5.1.2. - ESTIMATION OF TOTAL TANNINS

Defat 2 g of sample with 25 ml petroleum ether for 12 h. Boil the marc for 2 h with 300 ml of double distilled water. Cool, dilute up to 500 ml and filter. Measure 25 ml of this infusion into 2-litre porcelain dish; add 20 ml Indigo solution and 750 ml double distilled water. Titrate it with 0.1N potassium permanganate solution, 1 ml at a time, until blue solution changes to green. Thereafter add drops wise until solution becomes golden yellow in colour.

Similarly, titrate mixture of 20-ml Indigo solution and 750 ml of double distilled water. Calculate the difference between two titrations in ml.

Each ml of 0.1N potassium permanganate solution is equivalent to 0.004157 g of total tannins.

5.1.3. - ESTIMATION OF SUGARS

Method A:

Estimate total soluble and reducing sugars according to Nelson – Somogyi photometric method for the determination of glucose.

Preparation of calibration curve for d-glucose (Dextrose)

Dissolve accurately weighed 500 mg of dextrose in a 100-ml volumetric flask (5 mg / ml). From the above stock solution pipette out aliquots of 0.05 ml to 0.3 ml in to 10- ml volumetric flask and makeup the volume with double distilled water. Add 1 ml of alkaline reagent to each tube (25 parts of Reagent I + 1 part of Reagent II).

Reagent I: Dissolve 25 g of anhydrous sodium carbonate 25 g of Rochelle salt or sodium potassium tartrate, 20 g of sodium bicarbonate and 200 g of anhydrous sodium sulphate in about 800 ml of water and dilute to 1 L.

Reagent II : Add 15 per cent copper sulphate containing concentrated sulphuric acid per 100 ml to the tube. Mix the contents and heat for 20 min in a boiling water-bath. Then cool the tubes and add the solution 1 ml of arsenomolybdic acid reagent (dissolve 250 mg of ammonium molybdate in 45 ml of purified water. To this, add 2.1 ml of concentrated sulphuric acid and mix well. To this solution, dissolve 3 g of sodium arsenate in 25 ml of purified water, mix well and place in incubator maintained at 37 °C for 24 hr). Dilute the contents of the test tube to 10 ml by adding purified water mix well and then read color intensity at 520 nm using a ultra violet visible
spectrophotometer. Record the absorbance and plot a standard curve of absorbance vs. concentration.

5.1.3.1. - Reducing sugars

For reducing sugars, weigh accurately 500 mg of the sample, dissolve in 100 ml of double distilled water and make up the volume to 100 ml in a volumetric flask. Then follow method as mentioned for the preparation of calibration curve.

5.1.3.2. - Total sugars

Place 25 ml of the solution from the 100 ml stock solution prepared for the reducing sugars in a 100 ml beaker. To this, add 5 ml of hydrochloric acid: purified water (1:1 v/v), mix well and allow to stand at room temperature for 24 hr for inversion. Neutralize the sample with 5 N sodium hydroxide and make up to 50 ml with purified water. From this diluted sample, use 1 ml of aliquot for the estimation of total soluble sugars using the method described in preparation of calibration curve for dextrose.

5.1.3.3. - Non-reducing sugars

Non-reducing sugars are determined by subtracting the content of reducing sugars from the amount of total sugars.

Preparation of reagent:

Fehling’s solution:

A) Dissolve 69.278 g of copper sulphate in water and make the volume up to 1 liter.

B) Dissolve 100 g of sodium hydroxide and 340 g sodium potassium tartarate in purified water and make the volume to 1 liter.

Mix equal volumes of A and B before the experiment.

Clarifying reagent:

Solution 1: Dissolve 21.9 g of zinc acetate and 3 ml of glacial acetic acid in purified water and make the volume to 100 ml.

Solution II: Dissolve 10.6 g of potassium ferrocyanide in water and make up to 100 ml.

Reducing sugars: Take suitable amount of the sample and neutralize with sodium hydroxide solution (10per cent in water). Evaporate the neutralized solution to half the volume on a water bath at 50°C to remove the alcohol. Cool the solution add 10 ml of the clarifying solution I followed by 10 ml of the clarifying solution II. Mix, filter through a dry filter paper and make up the volume to 100 ml. Take 10 ml of the Fehling’s solution and from a burette and add sugar solution (above prepared sample) in a drop wise manner and heat to boiling over the hot plate (maintained at 80°C) until the mixture of Copper (Fehling’s solution) appears to be nearly reduced. Add 3-5 drops of 1per cent methylene blue and continue the titration till the blue colour is discharged. Note down the readings and calculate the percentage of glucose.

Non-reducing sugars: Take suitable amount of the sample and neutralize with sodium hydroxide solution (10per cent in water). Evaporate the neutralized solution to half the volume on a water bath
at 50°C to remove the alcohol. Cool the solution add 10 ml of the clarifying solution I followed by 10 ml of the clarifying solution II. Mix, filter through a dry filter paper. To the Filter add 15 ml of 0.1 N hydrochloric acid. Cover with stopper and heat to boiling for two minutes. Add phenolphthalein and neutralize with sodium hydroxide solution (10 per cent). Transfer to 100 ml volumetric flask and make the volume to 100 ml and perform the titration as done for the reducing sugars. Calculate the percentage of the total sugars. Subtract the percentage of the reducing sugars from the sugars to obtain non reducing sugars.

5.1.4. FIEHE’S TEST

**Reagents**
Resorcinol solution – Dissolve 1 g resublimed resorcinol in 100 ml hydrochloric acid (sp gr 1.18 to 1.19).
Ether – sulphuric ether.

**Procedure** – Transfer about 5 g of the honey sample into a mortar, using a pestle, mix the honey with 10 ml of ether. Decant the ether extract into a porcelain dish. Repeat the extraction twice in the same manner and collect the extract in the same dish. Allow the extracts to evaporate to dryness at room temperature and add a large drop of freshly prepared resorcinol solution. The production of cherry red colour appearing instantly indicates a positive reaction. Faint pink colour disappearing after a short time or yellow to salmon pink colours indicate a negative reaction.

5.1.5. ANILINE CHLORIDE TEST

**Reagent**
aniline chloride solution – To 100 ml of aniline, add 30 ml of hydrochloric acid (10:3).

**Procedure** – Place 5 g of the sample in a porcelain dish and add, while stirring, 2.5 ml of recently prepared aniline chloride solution. In the presence of commercial invert sugar within one minute, the reagent assumes orange red colour turning dark red. Yellow to salmon shades have no significance.

5.1.6. DETERMINATION OF SULPHUR DIOXIDE

Sulphur dioxide is determined by the modified Monier-William’s Method –
The apparatus as assembled is shown below

---

**Assembly for determination of Sulphur dioxide**
Reagents

(a) **Sodium Carbonate Solution** - 10 percent (m/v). aqueous

(b) **Bromophenol Blue Indicator** – Dissolve 0.1 g of bromophenol blue in 3.0 ml of 0.05 N sodium hydroxide solution and 5 ml of ethyl alcohol (90 percent by volume) by gently warming. Make up the volume of the solution with ethyl alcohol (20 percent v/v) to 250 ml in a volumetric flask.

(c) **Hydrogen peroxide solution** - Dilute a 30 percent (m/v) hydrogen peroxide solution with about twice its volume of water and neutralize the free sulphuric acid that may be present in the hydrogen peroxide solution with barium hydroxide solution, using bromophenol blue indicator solution. Allow the precipitate of barium sulphate to settle, and filter. Determine the concentration of hydrogen peroxide in the filtrate by titrating with standard potassium permanganate solution. Dilute the filtrate with cold water so as to obtain a 3 percent (m/v) solution of hydrogen peroxide.

(d) **Concentrated Hydrochloric acid** - sp.gr. 1.16

(e) **Carbon dioxide gas** - from a cylinder.

(f) **Standard sodium hydroxide solution** -0.1 N, standardized at the time of the experiment using bromophenol blue indicator solution.

Procedure
Assemble the apparatus as shown above. Introduce into the flask C, 300 ml of water and 20 ml of concentrated hydrochloric acid through the dropping funnel E. Run a steady current of cold water through the condenser F. Boil the mixture contained in the flask G for a short time to expel the air from the system in current of carbon dioxide gas previously passed through the wash bottle A. Weigh accurately about 100 g of the material and mix with the minimum quantity of water so as to make the diluted material easily flow down to the dropping funnel. Introduce the diluted material into the flask C through the dropping funnel E. Wash the dropping funnel with a small quantity of water and run the washing into the flask C. Again boil the mixture contained in the flask C in a slow current of carbon dioxide gas (passed previously through the wash bottle A) for one hour. Just before the end of the distillation, stop the flow of water in the condenser. (This causes the condenser to become hot and drives over residual traces of sulphur dioxide retained in the condenser.) When the delivery tube H, just above the Erlenmeyer flask j, becomes hot to touch, remove the stopper J immediately. Wash the delivery tube H and the contents of the Peligot tube L with water into Erlenmeyer flask. Cool the contents of the Erlenmeyer flask to room temperature, add a few drops of bromophenol blue indicator and titrate with standard sodium hydroxide solution. (Bromophenol blue is unaffected by carbon dioxide and gives a distinct change of color in cold hydrogen peroxide solution).

Carry out a blank determination using 20 ml of Conc., Hydrochloric acid diluted with 300 ml of water.

Calculation

\[
\text{Sulphur dioxide, mg/kg} = \frac{0.032000 \times (V-v) \times 1000 \times 1000 \times N}{W}
\]

Where

\( V \) = volume in ml of standard sodium hydroxide solution required for the test with the material

\( v \) = volume in ml of standard sodium hydroxide solution required for the blank determination;

\( N \) = normality of standard sodium hydroxide solution; and

\( W \) = weight in g of the material taken for the test
5.1.7. DETERMINATION OF TOTAL REDUCING SUGARS, SUCROSE AND FRUCTOSE – GLUCOSE RATIO

TOTAL REDUCING SUGARS

Reagents

Soxhlet Modification of Fehling’s solution – Prepare by mixing equal volumes of Solution A and Solution B immediately before using.

Copper sulphate solution (Solution A) – Dissolve 34.639 g of copper sulphate crystals (CuSO₄·5H₂O) in water, dilute to 500 ml and filter through glass wool or filter paper.

Standardization of copper sulphate solution – Using separate pipettes, pipette accurately 5 ml of Solution A and 5 ml of Solution B into a conical flask of 250 ml capacity. Heat this mixture to boiling on an asbestos gauze and add standard invert sugar solution from a burette, about one millilitre less than the expected volume which will reduce the Fehling’s solution completely 48 ml. Add one ml of methylene blue indicator while keeping the solution boiling. Complete the titration within three minutes, the end point being indicated by change of colour from blue to red. From the volume of invert sugar solution used, calculate the strength(s) of the copper sulphate solution by multiplying the titre value by 0.001 (mg/ml of the standard invert sugar solution). This would give the quantity of invert sugar required to reduce the copper in 5 ml copper sulphate solution.

Potassium Sodium Tartrate (Rochelle Salt) Solution (Solution B) – Dissolve 173 g of potassium sodium tartrate and 50 g of sodium hydroxide in water, dilute to 500 ml. Let the solution stand for a day and filter.

Hydrochloric acid – Sp gr 1.18 at 20 °C (approximately 12 N).

Standard Invert Sugar Solution – Weigh accurately 0.95 g sucrose and dissolve it in 500 ml water. Add 2 ml of concentrated hydrochloric acid, boil gently for 30 minutes and keep aside for 24 hours. Neutralize with sodium carbonate and make the final volume to 1000 ml; 50 ml of this solution contains 0.05 g invert sugar.

Methylene Blue Indicator – 0.02 per cent in water.

Procedure – Place accurately weighed about one gram (W) of the prepared sample of honey into a 250 ml volumetric flask and dilute with about 150 ml of water. Mix thoroughly the contents of the flask and make the volume to 250 ml with water. Using separate pipettes, take accurately 5 ml of each of Solution A and Solution B, in a porcelain dish. Add about 12 ml of honey solution from a burette and heat to boiling over an asbestos gauze. Add 1 ml of methylene blue indicator and while keeping the solution boiling complete the titration, within three minutes, the end point being indicated by change of colour from blue to red. Note the volume (H) in ml of honey solution required for the titration.

Calculation

Total reducing sugars, per cent by mass = \( \frac{250 \times 100 \times S}{H \times M} \)

Where

\( S \) = strength of copper sulphate solution,
\( H \) = volume in ml of honey solution required for titration, and
\( M \) = mass in g of honey.
**SUCROSE**

**Procedure** – To 100 ml of the stock honey solution, add one ml of concentrated hydrochloric acid and heat the solution to near boiling. Keep aside overnight. Neutralize this inverted honey solution with sodium carbonate and determine the total reducing sugars as described as above.

**Calculation**

Sucrose, per cent by mass = [(reducing sugars after inversion, per cent by mass) – (reducing sugars before inversion, per cent by mass)] x 0.95

**FRUCTOSE – GLUCOSE RATIO**

**Reagents**

*Iodine Solution* – 0.05 N.

*Sodium hydroxide solution* – 0.1 N.

*Sulphuric Acid* – concentrated.

*Standard Sodium Thiosulphate Solution* – 0.05 N.

**Procedure** – Pipette 50 ml of honey solution in a 250 ml stoppered flask. Add 40 ml of iodine solution and 25 ml of sodium hydroxide solution. Stopper the flask and keep in dark for 20 minutes. Acidify with 5 ml of sulphuric acid and titrate quickly the excess of iodine against standard sodium thiosuphate solution. Conduct a blank using 50 ml of water instead of honey solution.

**Calculations**

Approximately glucose,  
Per cent by mass \((w)\) = \(\frac{(B – S) x 0.004502 x 100}{a}\)

where

\(B\) = volume of sodium thiosulphate solution required for the blank,  
\(S\) = volume of sodium thiosulphate solution required for the sample, and  
\(a\) = mass of honey taken for test.

Approximate  
Fructose, per cent  
By mass \((x)\) = Approximate total reducing sugars, per cent – \(w\)  
\(0.925\)

True glucose, per cent by mass \((y)\) = \(w – 0.012 \times \)

True fructose,  
Per cent by mass \((z)\) = Approximate reducing sugars, per cent – \(y\)  
\(0.925\)

True reducing sugars, per cent by mass = \(y+z\)
Fructose – glucose ratio = True fructose, per cent by mass (z)  
True glucose, per cent by mass (y)

5.2.- ESTIMATION OF CURCUMIN BY TLC DENSITOMETER

Sample solution - Extract 5 g of availeha with methanol (25 ml x 4), filter, pool, concentrate and make up the volume to 25 ml with methanol.

Standard solution - Prepare a stock solution of curcumin (160 µg/ml) by dissolving 4 mg of accurately weighed curcumin in methanol and making up the volume to 25 ml with methanol. Transfer the aliquots (0.4 – 1.4 ml) of stock solution to 10 ml volumetric flasks and make up the volume with methanol to obtain standard solutions containing 6.4 to 22.4 µg/ml curcumin, respectively.

Calibration curve - Apply 10 µl of the standard solutions (64 to 224 ng) on a precoated TLC plate of uniform thickness. Develop the plate in the solvent system toluene: ethyl acetate: methanol (5 : 0.5 : 1) to a distance of 10 cm. Scan the plate densitometrically at 429 nm. Record the peak area and prepare the calibration curve by plotting peak area vs concentration of curcumin applied.

Estimation of curcumin in the drug - Apply 5 µl of the test solution on a precoated silica gel 60 F254 TLC plate. Develop the plate in the solvent system toluene: ethyl acetate: methanol (5: 0.5: 1) and record the chromatogram as described above for the calibration curve. Calculate the amount of curcumin present in the sample from the calibration curve of curcumin.

5.2.1. - Determination of Aluminium

Solutions

10 per cent sodium hydroxide solution – Dissolve 10 g sodium hydroxide in 100 ml purified water.

EDTA solution 0.05 M – Dissolve 18.6120 g of sodium salt of EDTA in purified water and make up to 1000 ml.

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.

Acetate buffer 5.5 pH – Dissolve 21.5 g of sodium acetate (AR) in 300 ml purified water containing 2 ml glacial acetic acid and dilute to 1000 ml

Xylenol orange indicator – Dissolve 0.2 g of xylenol orange indicator in 100 ml purified water with 2 ml acetic acid.

Procedure

Take suitable aliquot from the stock solution in 250 ml beaker. Take 50 ml of 10 per cent sodium hydroxide solution in another beaker. Neutralize the aliquot with sodium hydroxide solution. Transfer the 10 per cent sodium hydroxide solution to aliquot with constant stirring. Add a pinch of sodium carbonate into the solution. Boil the content on burner. Cool and filter through Whatman 40 No. filter paper with pulp in 600 ml beaker. Wash the precipitate with hot water 6-8 times. Acidify the filtrate with dil. hydrochloric acid and adjust pH 5.5. Add, in excess normally 25 ml 0.05M EDTA solution. Add 25 ml acetate buffer solution. Boil the solution; cool and again adjust pH 5 – 5.5. Add 5-6 drops of xylenol orange indicator. The colour changes from golden...
yellow to orange red at the end point. Take 25 ml 0.05 M EDTA solution and run a blank. Each of 1M EDTA is equivalent to 0.05098 g of Al₂O₃.

5.2.2. - Determination of Borax

Powder 5-6 g of drug and incinerated at 450° for 3 hours to get it ash. Dissolve the ash in 20 ml of purified water and left for 15 minutes, filter, wash the residue with 80 ml of purified water for 4-5 washings. If necessary, shake the contents and titrate with 0.5N hydrochloric acid using solution of methyl orange as an indicator. Each ml of 0.5N hydrochloric acid is equivalent to 0.09536 g of Na₂B₄O₇.10H₂O.

5.2.3. - Determination of Calcium

Solutions

20 per cent Potassium hydroxide solution – Dissolve 200 g potassium hydroxide in purified water and make up to 1000 ml.
Ammonia buffer solutions 9.5 pH – Dissolve 67.5 g ammonium chloride in 300 ml purified water, add 570 ml ammonia solution and dilute to 1000 ml.
EDTA (Ethylene Diethyl Tetra Acetic acid) solution 0.05 M – Dissolve 18.6120 g of solution salt of EDTA and in water and make up to 1000 ml.

Procedure

Take one part of filtrate reserved from Iron (Fe) estimation. Add 5 ml Triethanolamine 20 per cent solution. Add a pinch of Hydroxylamine hydrochloride. Add 25-30 ml potassium hydroxide 20 per cent solution. Add 4-5 drops of Patterns and Reeders indicator, which imparts rose red colour. Titrate the solution against standard EDTA solution. The colour changes from rose red to Prussian blue mark end point.

Each ml of 1M EDTA solution is equivalent to 0.04008 g Calcium.

5.2.4. - Determination of Copper(Cu)

Solutions

Standard 0.1 N sodium thiosulphate solutions
Potassium iodide.
Starch 1 per cent solution – Dissolve 1 g in water, boil and make up 100 ml.
Procedure

Take suitable aliquot from the stock solution in a beaker. Add approx. 1.0 g sodium fluoride. Add ammonium solution and precipitate solution. Add acetic acid to dissolve the precipitate. Boil and cool in water bath. Add approx 1.0 g potassium iodide. Titrate the liberated iodine against 0.1 N sodium thiosulphate (hypo) solutions by adding starch solution as indicator. The liberated iodine colour blackish brown changes to white at the end point. Calculate copper value against 1 ml of hypo solution titrating against standard 1000 ppm copper solution.

Each ml of 1N Na₂S₂O₃ solution is equivalent to 0.06357 g of Copper

5.2.5. - Determination of Iron (Fe)

Preparation of sample solution

Ignite a suitable quantity of the sample (in the presence of organic matter) in a crucible in a muffle furnace at 500-550⁰ until the residue is free from organic matter. Moisten with 5-10 ml of hydrochloric acid, boil for two min, add 30 ml of water, heat on the water bath for few min, filter and wash thoroughly the residue with water and make up to volume in a volumetric flask.

Solutions

Stannous chloride solution – Dissolve 5 g stannous chloride (A.R) in 25 ml Conc. hydrochloric acid and dilute to 100 ml (5 per cent solution).

Mercuric chloride – saturated solution in water.

Sulphuric acid + orthophosphoric acid mixture – take 60 ml water, add 15 ml conc. sulphuric acid and 15 ml H₃PO₄ cool and dilute to 1000ml.

Diphenylamine barium sulphonate – Dissolve 0.25 g in 100 ml water.

0.1 N Standard potassium dichromate solution - Dissolve 4.9035 g AR grade in water and dilute to 1000 ml.

Procedure

Take /withdraw a suitable aliquot from the stock solution in 250 ml in duplicate. Dilute to about 100 ml with distilled water. Add 1-2 drops of methyl red indicator. Add 1-2 g ammonium chloride. Add dil. Ammonium solution till brown precipitate appears. Boil the solution with ppt. for 4-5 minutes. Cool the content and filter through Whatman 41 no. filter paper. Wash the residue with hot water 4-6 times. Dissolve the residue in dil. HCl in 250 ml beaker. Wash with hot water and make the volume to 100 ml approx. Boil the solution on burner. Reduce the Fe³⁺ to Fe²⁺ by adding stannous chloride solution drop wise till solution becomes colourless.


Each ml of 1N K₂Cr₂O₇ solution is equivalent to 0.05585 g Iron
Each ml of 1N K₂Cr₂O₇ solution is equivalent to 0.7985 g Fe₂O₃
5.2.6. - Determination of Magnesium

Take another part of filtrate reserved from Fe estimation. Add 5 ml triethanolamine 20 per cent solution. Add a pinch of hydroxylamine hydrochloride. Add 25-30 ml ammonia buffer 9.5 pH. Add 4-5 drops of eriochrome black T indicator. The colour changes from rose red to blue marks the end point.

Each ml of 1M EDTA solution is equivalent to 0.0409 g of MgO.

5.2.7. - Determination of Mercury

Powder 0.5 g drug and treat with 7 ml of conc. nitric acid and 15 ml of conc. sulphuric acid in a kjeldahl flask; heat under reflux gently at first then strongly for 30 minutes. Cool and add 50 ml conc. nitric acid boil so as to remove the brown fumes. Continue the addition of nitric acid and boiling until the liquid is colourless; cool, wash the condenser with 100 ml of water, remove the flask and add 1.0 per cent potassium permangnate solution drop wise until pink colour persists. Decolourize it by adding 6.0 per cent hydrogen peroxide drop wise to remove excess of potassium permangnate followed by 3.0 ml of conc. nitric acid and titrate with 0.1N ammonium thiocyanate solutions using ferric alum as indicator.

Each ml. of 0.1N NH₄SCN solution is equivalent to 0.01003 g Mercury.

5.2.8. - Determination of Silica (SiO₂)

Weigh 0.5 g (in case of high silica) or 1.0 g (low silica) finely powdered and dried sample in a platinum crucible (W₁). Add 4-5 g anhydrous sodium carbonate into the crucible. Mix thoroughly and cover the crucible with lid, if necessary. Place the crucible in muffle furnace. Allow the temperature to rise gradually to reach 900-950⁰ and keep on this temp. for about ½ hour to complete the fusion. Take out the crucible and allow cool at room temperature. Extract the cooled mass in 25-30 ml dil hydrochloric acid in 250 ml beaker. Heat on hot plate/burner to dissolve the contents. Wash the crucible with distilled water. Keep the beaker on water bath and allow dry the mass. Dehydrate back and powder the mass. Take out the beaker and allow cooling at room temperature. Add 25-30 ml hydrochloric acid dilute to 100 ml distilled water. Boil the content and allow cool. Filter through Whatman no 40. filter paper. Wash the residue with hot water 6-8 times. Place the residue along with filter paper in platinum crucible. Ignite at 900-950⁰ for 2-3 min. Allow to cool and weigh as SiO₂.

5.2.9. - Estimation of Sodium and Potassium by Flame Photometer

Preparation of Standard Solutions

Weigh 2.542 g of AR sodium chloride and dissolve in purified water and make upto 1000 ml in a volumetric flask. Dilute 1 ml of the stock solution to 100 ml. This gives standard of 1mg of sodium per 100 ml (10 ppm). Prepare 20, 30, 40 and 50 ppm standard solution.

Weigh 1.9115g of AR potassium chloride and dissolve in purified water and make up to 1000 ml in a volumetric flask. Dilute 1ml of the stock solution to 100ml. This gives standard of 1mg of sodium per 100 ml (10 ppm). Prepare 20, 30, 40 and 50 ppm standard solution.

Preparation of Sample Solution

Weigh 10 g of sample in a preweighed silica dish and heat in a muffle furnace for 1hr at 600⁰. Cool and dissolve the ash in purified water and make up to 100 ml in a volumetric flask.
Switch on the instrument first and then the pump. Keep distilled water for aspiration and allow it to stand for 15 min (warming time). Open the glass cylinder and ignite the flame. Adjust the instrument to zero.

Introduce the maximum concentration solution and adjust it to 100. Again introduce the purified water so that instrument shows zero. Then introduce the standard solution in ascending concentration. Note down the reading each time. Introduce the purified water for aspiration in between the standard solutions. Introduce the sample solution and if it is within the range take the reading. If it exceeds limit 100 then dilute the solution till the reading is within the range. Plot the curve with concentration in ppm against reading obtained. Find out the concentration of the sample solution. Take two or three readings and find out the average. Find out the concentration of sodium and potassium.

5.2.10. - Determination of Sodium Chloride

Dissolve about 2-3g accurately weighed drug in 25 ml of purified water and left for 30 minutes, filter. Wash the filter paper completely with purified water and the filtrate is made 100 ml in volumetric flask, make the solution homogeneous, titrate 25 ml of this solution with 0.1 N silver nitrate solution using potassium chromate as indicator. The end point shows the light brick red colour.

Each ml. of 0.1 N Ag NO₃ solution is equivalent to 0.005845 g of NaCl.

5.2.11. - Determination of Sulphur

Solution

Carbon tetrachloride saturated with Bromine

Barium chloride – 10 per cent solution in water.

Procedure

Take 0.5 – 1 g powdered sample in 250 ml beaker. Add 10 ml carbon tetrachloride saturated with bromine. Keep in cold condition in fume chamber over night. Add 10 – 15 ml conc. nitric acid. Digest on water bath. Add 10 ml conc. hydrochloric acid, digest it to expel nitrate fumes till syrupy mass. Cool and extract with hydrochloric acid, make volume to 100 ml. Boil and filter through Whatman No 40. filter paper. Wash the residue with hot water. Filter through Whatman 41 No. paper in 600 ml beaker. Acidify the filtrate with hydrochloric acid. Add 20 ml of 10 per cent Barium chloride solution. Stir the solution and digest on burner. Allow to settle BaSO₄ precipitate over night. Filter the precipitate through Whatman No. 42 filter paper. Wash the precipitate with water. Ignite the precipitate in muffle furnace in pre weighed platinum crucible up to 850º. Allow to cool and weigh.

Each g of weight of precipitate is equivalent to 0.13734 g of Sulphur.

5.2.12. - Qualitative Reactions of Some Radicals

Sodium

Sodium compounds, moistened with hydrochloric acid and introduced on a platinum wire into the flame of a Bunsen burner, give a yellow colour to the flame.
Solutions of sodium salts yield, with solution of uranyl zinc acetate, a yellow crystalline precipitate.

**Potassium**

Potassium compounds moistened with hydrochloric acid and introduced on platinum wire into the flame of a Bunsen burner, give a violde colour to the flame.

Moderately strong solutions of potassium salts, which have been previously ignited to remove ammonium salts, give a white, crystalline precipitate with perchloric acid.

Solutions of potassium salts, which have been previously ignited to free them from ammonium salts and from which iodine has been removed, give a yellow precipitate with solution of sodium cobaltinitritre and acetic acid.

**Magnesium**

Solution of magnesium salts yield a white precipitate with solution of ammonium carbonate, especially on boiling, but yield no precipitate in the presence of solution of ammonium chloride.

Solution of magnesium salts yield a white crystalline precipitate with solution of sodium phosphate in the presence of ammonium salts and dilute ammonia solution.

Solution of magnesium salts yield with solution of sodium hydroxide a white precipitate insoluble in excess of the reagent, but soluble in solution of ammonium chloride.

**Carbonates and Bicarbonates**

Carbonates and bicarbonates effervesce with dilute acids, liberating carbon dioxide; the gas is colourless and produces a white precipitate in solution of calcium hydroxide.

Solutions of carbonates produce a brownish-red precipitate with solution of mercuric chloride; Solutions of bicarbonates produce a white precipitate.

Solutions of carbonates yield, with solution of silver nitrate, a white precipitate which becomes yellow on the addition of an excess of the reagent and brown on boiling the mixture. The precipitate is soluble in dilute ammonia solution and in dilute nitric acid.

Solutions of carbonates produce, at room temperature, a white precipitate with solution of magnesium sulphate. Solutions of bicarbonates yield no precipitate with the reagent at room temperature, but on boiling the mixture a white precipitate is formed.

Solutions of bicarbonates, on boiling, liberate carbon dioxide which produces a white precipitate in solution of calcium hydroxide.

**Sulphates**

Solutions of sulphates yield, with solution of barium chloride, a white precipitate insoluble in hydrochloric acid.

Solutions of sulphates yield, with solution of lead acetate, a white precipitate soluble in solution of ammonium acetate and in solution of sodium hydroxide.
Chlorides

Chlorides, heated with manganese dioxide and sulphuric acid, yield chlorine, recognisable by its odour and by giving a blue colour with potassium iodide and solution of starch.

Calcium

Solutions of calcium salts yield, with solution of ammonium carbonate, a white precipitate which after boiling and cooling the mixture, is insoluble in solution of ammonium chloride.
APPENDIX-6

6.1. WEIGHTS AND MEASURES

6.1.1. - METRIC EQUIVALENTS OF CLASSICAL WEIGHTS AND MEASURES

Weights and measures described in Ayurvedic classics and their metric equivalents adopted by the Ayurvedic Pharmacopoeia Committee

The following table of metric equivalents of weights and measures, linear measures and measurement of time used in the Ayurvedic classics have been approved by the Ayurvedic Pharmacopoeia committee in consultation with Indian Standards Institution.

I. WEIGHTS AND MEASURES

<table>
<thead>
<tr>
<th>Classical Unit</th>
<th>Metric Equivalent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Ratti or Guj</td>
<td>= 125 mg</td>
</tr>
<tr>
<td>8 Ratti or Guj</td>
<td>= 1 g</td>
</tr>
<tr>
<td>12 Mças</td>
<td>= 1 Kar²a</td>
</tr>
<tr>
<td>2 Kar²as (Tolas)</td>
<td>= 1 ¹uktis</td>
</tr>
<tr>
<td>2 ¹uktis</td>
<td>= 1 Palam</td>
</tr>
<tr>
<td>(4 Kar²as or Tolas)</td>
<td>= 1 Prasṭis</td>
</tr>
<tr>
<td>2 Palams</td>
<td>= 1 Ku²ava</td>
</tr>
<tr>
<td>2 Ku²avas</td>
<td>= 1 Mṇika</td>
</tr>
<tr>
<td>2 Mṇikas</td>
<td>= 1 Prastha</td>
</tr>
<tr>
<td>4 Prasthas</td>
<td>= 1 ²haka</td>
</tr>
<tr>
<td>4 ²hakas</td>
<td>= 1 D’o’a</td>
</tr>
<tr>
<td>2 D’o’as</td>
<td>= 1 ½rpa</td>
</tr>
<tr>
<td>2 ½rpas</td>
<td>= 1 D’o’i</td>
</tr>
<tr>
<td>4 D’o’is</td>
<td>= 1 Khṛi</td>
</tr>
<tr>
<td>1 Palam</td>
<td>= 1 Tula</td>
</tr>
<tr>
<td>100 Palams</td>
<td>= 1 Bhṛa</td>
</tr>
</tbody>
</table>

In case of liquids, the metric equivalents would be the corresponding litre and milliliter.

II. LINEAR MEASURES

<table>
<thead>
<tr>
<th>Classical Unit</th>
<th>Inches</th>
<th>Metric Equivalents</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Yavodara</td>
<td>1/8 of ¾&quot;</td>
<td>0.24 cm</td>
</tr>
<tr>
<td>2. Aṅgula</td>
<td>¾&quot;</td>
<td>1.95 cm</td>
</tr>
<tr>
<td>3. Bitahasti</td>
<td>9&quot;</td>
<td>22.86 cm</td>
</tr>
<tr>
<td>4. Aratni</td>
<td>10 ½&quot;</td>
<td>41.91 cm</td>
</tr>
<tr>
<td>5. Hasta</td>
<td>18&quot;</td>
<td>45.72 cm</td>
</tr>
<tr>
<td>6. N’pahasta</td>
<td>22&quot;</td>
<td>55.88 cm</td>
</tr>
<tr>
<td>(Rṣahasta)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. Vyama</td>
<td>72&quot;</td>
<td>182.88 cm</td>
</tr>
</tbody>
</table>

III. MEASUREMENT OF TIME

<table>
<thead>
<tr>
<th>Unit</th>
<th>Equivalent (in hours, minutes &amp; seconds)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 Kṛha</td>
<td>= 1 Lava</td>
</tr>
<tr>
<td>2 Lavas</td>
<td>= 1 Nime³a</td>
</tr>
<tr>
<td>3 Nime³as</td>
<td>= 1 Ka³ha</td>
</tr>
<tr>
<td>1 Ghati</td>
<td>= 24 Minutes</td>
</tr>
<tr>
<td>30 Ka³has</td>
<td>= 2 Minutes</td>
</tr>
<tr>
<td>20 Kal³ + 3</td>
<td>= 20 seconds</td>
</tr>
<tr>
<td>Ka³has</td>
<td>= 48 Minutes</td>
</tr>
<tr>
<td>30 Muh³rta</td>
<td>= 24 Hrs.</td>
</tr>
<tr>
<td>15 Ahor³ra</td>
<td>= 15 Days</td>
</tr>
<tr>
<td>2 Pak³ras</td>
<td>= 30 Days/One Month</td>
</tr>
<tr>
<td>2 Mṛṣa</td>
<td>= 60 Days/Two Months</td>
</tr>
<tr>
<td>3 Štus</td>
<td>= 6 Months</td>
</tr>
<tr>
<td>2 Samvaṣas</td>
<td>= 12 Months/One Year</td>
</tr>
<tr>
<td>5 Samvaṣara</td>
<td>= 5 Years</td>
</tr>
<tr>
<td>1 Ahorṛa of Devas</td>
<td>= 1 Year</td>
</tr>
<tr>
<td>1 Ahorṛa of Pitaras</td>
<td>= 1 Month</td>
</tr>
</tbody>
</table>
6.2. - METRIC SYSTEM

**Measure of Mass (Weights)**

1 Kilogram (Kg) – is the mass of the International Prototype Kilogram.
1 Gramme (g) – the 1000\(^{th}\) part of 1 Kilogram.
1 Milligram (mg) – the 1000\(^{th}\) part of 1 gramme.
1 Microgram (µg) – the 1000\(^{th}\) part of 1 milligram.

**Measures of capacity (Volumes)**

1 Litre (1) is the volume occupied at its temperature of maximum density by a quantity of water having a mass of 1 Kilogram.
1 Millilitre (ml) the 1000\(^{th}\) part of 1 litre.

The accepted relation between the litre and the cubic centimetre is 1 litre ≈ 1000.027 cubic centimeters.

**Relation of capacity of Weight (Metric)**

One litre of water at 20\(^{0}\) weighs 997.18 grammes when weighed in air of density 0.0012 gramme per millilitre against brass weights of density 84 grammes per millilitre.

**Measures of Length**

1 Metre (m) is the length of the International Prototype Metre at 0.
1 Centimetre (cm) – the 100\(^{th}\) part of 1 metre.
1 Millimetre (mm) – the 1000\(^{th}\) part of 1 metre.
1 Micron (µ) – the 1000\(^{th}\) part of 1 millimetre
1 Millimicron (mµ) – the 1000\(^{th}\) part of micron
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Definitions

Rasa:

The term ‘Rasa’ refers to the direct and immediate action of a drug when it comes in contact with the sense organ of taste i.e. tongue. The existence of different types of rasas (tastes) in different substances is attributed to their varying pancabhautika composition. The ‘Rasa’ of different substances have definite relationship to the increase or decrease of Dosha and they have certain actions in the body. The drugs are selected keeping in view their rasas (taste) and the predominate doshas in the body of the patient. There are six types of rasas (tastes). In other contexts the word rasa also applied to nutrition to the end product of digestion of food, to the first dhatu (tissue) and to the principal metal drug Mercury etc.


The term ‘guna’ refers to the physico-chemical and also the pharmacodynamic properties of drugs and dietary articles which are responsible for the action of the respective drugs/diets in the body. A total of 41 gunas are described in Ayurveda but out of these twenty are more important.

3. Sheet – Cold   4. Ushna – Hot
5. Snigdha – Unctuousness   6. Ruksha – Non – unctuousness or dryness
17. Shkshama – Fineness   18. Sthla – Bulkiness

Vipaka:

Vipaka is the action of the drug after it has undergone digestive and assimilative transformations. The Vipaka of a drug overcomes the action of ‘rasa’ (taste) but is itself overcome by virya; vipaka refers to drug metabolism i.e. action of a drug through drug metabolism. The texts describe three kinds of drug metabolism viz. Katu (pungent) amla (sour) madhura (sweet) responsible in turn for increase in vata, pitta and kapha respectively.

Virya:

Virya refers to the potency of a drug/drug action such an action is not accounted for the rasa, guna or vipaka of a drug. According to the most commonly held view virya is of two kinds: usna (literal meaning; hot) and sita (literal meaning; cold).