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Disclaimer

This Guidance Manual is compiled at the Indian Pharmacopoeia Commission (IPC) after consultations with subject matter experts from industries, regulatory agencies, academia, research institutes, and other stakeholders. The final version of the Guidance Manual is approved by the 'Core Expert Committee' constituted by the IPC. The information contained herein represents the current best practices in the field of pharmacopoeial sciences to comply with the existing regulatory requirements. The guidance provided in this manual is not intended to alter or modify or supplement or in any other way change the contents of the Indian Pharmacopoeia (IP), but is intended to provide general guidance to all users of the IP to ensure proper compliance with the IP standards when standards of drugs are to be determined. The content of this manual should be treated as a guidance material only to ensure compliance of the requirements of the IP and the contained information is subject to review by the IPC. Approaches and methods other than those described in this manual may be adopted if found suitable for applying the IP monographs and if approved by the Competent Authority. Where provisions of the law are mentioned, the law as prevailing at the relevant time shall apply.

Message

I am extremely delighted to note that Indian Pharmacopoeia Commission (IPC) has come up with the publication of revised edition of the 'Guidance Manual for Compliance with Indian Pharmacopoeia (IP)'. Many subject experts from various streams of the pharmaceutical sciences and regulatory organizations have provided their comments and inputs in order to make the document relevant as per current requirements. I am sure that the revised document would be immensely beneficial to the drug manufacturers, testing laboratories and other stakeholders in establishing the compliance with the standards prescribed in the IP. This in turn would help in ensuring the quality of drugs being manufactured and/or marketed in India.

Once again I congratulate IPC and its staff for bringing out this revised edition of the guidance manual and hope that this Institute would continue to serve the nation by publishing more of such informative documents to strengthen the drug regulatory systems in the country.

Best Wishes,

Ms. Preeti Sudan
Chairperson-Governing Body
Indian Pharmacopoeia Commission
Ghaziabad

Message

It gives me immense pleasure to present the revised edition of the 'Guidance Manual for Compliance with Indian Pharmacopoeia (IP)'. The first edition of this guidance manual was published by the Indian Pharmacopoeia Commission (IPC) in the year 2012 and need was felt to revise the same in order to make it relevant as per current regulatory requirements. IPC has an important mandate of setting standards of the drugs being manufactured and marketed in India through publication of IP editions at regular intervals. IP is the book of standards under the provisions of the Drugs and Cosmetics Act 1940 and Rules 1945 there under, and therefore IP standards play key role in ensuring the quality of medicines in the country. Present Guidance Manual is prepared with the objective to help the stakeholders of IP to have better knowledge of the current regulatory pathways and practices so that IP standards can be better understood for their implementation.

I am grateful to the members of Core Expert Committee for their valuable efforts in the form of expert opinions and comments on various important topics which enabled us to bring out this revised edition of the Guidance Manual. Untiring efforts and contributions made by IPC scientists and staff members are also deeply appreciated. Feedback on the content of this manual is always welcomed. I believe that this Guidance Manual will act as a reference book for compliance with the IP standards and found useful by the manufacturers, testing laboratories, and other stakeholders of IP.

Thanking you,

Dr. Jai Prakash
Secretary-cum-Scientific Director (I/c)
Indian Pharmacopoeia Commission
Ghaziabad

Core Expert Committee

Chairperson

Dr. G. N. Singh, Secretary-cum-Scientific Director, Indian Pharmacopoeia Commission, Ghaziabad

Co-Chairperson

Prof. Y. K. Gupta, Former Head, Department of Pharmacology, All India Institute of Medical Sciences, New Delhi

Members

Prof. Saranjit Singh, National Institute of Pharmaceutical Education and Research, Mohali

Prof. Rishendra Verma, ICAR Emeritus Professor, Indian Veterinary Research Institute, Izatnagar

Dr. Ajay Sachan, Assistant Drugs Controller (India), Central Drugs Standard Control Organization, Ghaziabad

~~Dr. D. P. Pathak, Director, Delhi Institute of Pharmaceutical Sciences and Research, New Delhi~~

~~Dr. Madhur Gupta, National Professional Officer, World Health Organization, New Delhi~~

Dr. J. P. Prasad, Scientist Gr. I, National Institute of Biologicals, Noida

Dr. Jai Prakash, Indian Pharmacopoeia Commission, Ghaziabad

Sh. Gaurang Oza, Chief Executive Officer, Vaibhav Analytical Ltd., Ahmedabad

Dr. Vijay Habbu, Senior Vice President, Reliance Industries Ltd., Mumbai

Sh. S. Dey, Former Deputy Drugs Controller (India) and Consultant, Indian Pharmacopoeia Commission, Ghaziabad

~~Dr. Saroj Kr. Ghosh, Biochemist, Central Drugs Laboratory, Kolkata~~

~~Sh. Pradeep Mishra, Professional Secretary, SEARPharm Forum, New Delhi~~

~~Dr. S. S. Venkatakrishnan, Former Drugs Controller, Kerala~~

~~Dr. D. B. A. Narayana, Former Director, Hindustan Lever Research Centre, Bengaluru~~

Sh. Vinod Arora, Former Vice President, Ranbaxy Laboratories Ltd., Gurugram

~~Dr. Prashant Dikshit, Former General Manager, Watson Pharma Pvt. Ltd., Mumbai~~

~~Dr. Vinay Nayak, Director, Surge Chemicals Pvt. Ltd., Mumbai~~

~~Dr. Amit Agarwal, Natural Remedies Pvt. Ltd., Bengaluru~~

Mr. Unikrishnan Panicker (Advocate),

Dr. RN Gupta, Professor, Birla Institute of Technology, Mesra, Ranchi

~~Mr. Ajay Sachan~~

Dr. Gaurav Pratap Singh, Indian Pharmacopoeia Commission, Ghaziabad (Co-ordinator)

List of Contributors

IPC Scientists

Dr. V. Kalaiselvan, Principal Scientific Officer
Dr. Anil Kumar Teotia, Principal Scientific Officer
Dr. Robin Kumar, Principal Scientific Officer
Dr. Anuj Prakash, Senior Scientific Officer
Dr. Meenakshi Dahiya, Senior Scientific Officer
Mr. Alok Sharma, Senior Scientific Officer
Dr. M. Kalaivani, Senior Scientific Officer
Dr. Shashi Bhushan, Senior Scientific Officer
Dr. Manoj Pandey, Scientific Officer
Dr. Pawan K. Saini, Scientific Officer
Ms. Ritu Tiwari, Scientific Assistant
Dr. Manisha Trivedi, Scientific Assistant
Dr. Prasad Thota, Scientific Assistant
Dr. Ajay Kumar, Scientific Assistant
Dr. Shatrunajay Shukla, Scientific Assistant
Mr. Arvind Kumar Sharma, Scientific Assistant
Ms. Shruti Rastogi, Scientific Assistant
Dr. R. S. Ray, Scientific Assistant
Mr. Mukesh Kumar, Scientific Assistant
Ms. Anubhuti Goyal, Scientific Assistant
Mr. Rishi Kumar, Scientific Assistant
Mr. S. K. Sharma, Pharmacopoeia Scientist
Mr. Ramesh Jha, Pharmacopoeia Associate

Abbreviations

API	**:** Active Pharmaceutical Ingredient
CDSCO	**:** Central Drugs Standard Control Organization
DCGI	: Drugs Controller General of India
EU	**:** Endotoxin Unit
GLP	**:** Good Laboratory Practices
IPC	**:** Indian Pharmacopoeia Commission
IP	**:** Indian Pharmacopoeia.
IPRS	**:** Indian Pharmacopoeia Reference Substance(s)
ISO	**:** International Standards Organization
NABL	**:** National Accreditation Board for Testing and Calibration Laboratories
PPM	**:** Parts Per Million
SOP	**:** Standard Operating Procedure
WHO	**:** World Health Organization
STP	**:** Standard Test Procedure
WS	**:** Working Standard
RH	**:** Relative Humidity
RSD	**:** Relative Standard Deviation
USP	**:** United States Pharmacopoeia
BP	**:** British Pharmacopoeia
EP	**:** European Pharmacopoeia
CSIR	**:** Council of Scientific and Industrial Research
NIB	**:** National Institute of Biologicals
IMTECH	**:** Institute of Microbial Technology
NCL	**:** National Chemical Laboratory

Glossary

Active Pharmaceutical Ingredient (API)
A substance that is used as an active constituent of a pharmaceutical dosage form and that, when so used, is responsible for exerting the desired therapeutic pharmacological action. APIs are of chemical or biological origin. API is also known as 'Drug Substance'.

Batch (or Lot)
A defined quantity of starting material, packaging material or product processed in a single process or series of processes so that it is expected to be homogeneous. In continuous manufacture, the batch must correspond to a defined fraction of production, characterized by its intended homogeneity. The batch size can be defined either as a fixed quantity or as the amount produced in a fixed time interval.
The foot notes to clause (v) of Sub-rule (1) of Rule 96 of the Drugs and Cosmetics Rules explain the scope of the word "Batch" and also "batch number for various categories of drugs.

Batch Number (or Lot Number)
A distinctive combination of numbers and/or letters which uniquely identifies a batch ~~on the labels~~, its batch records and corresponding certificate of analysis.

Calibration
The set of operations that establish, under specified conditions, the relationship between values indicated by an instrument or system for measuring (especially weighing), recording and controlling, or the values represented by a material measure, and the corresponding known values of a reference standard. Limits for acceptance of the results of measuring should be established.

Central Drugs Standard Control Organization (CDSCO)
CDSCO is the National Regulatory Authority of India under the Directorate General of Health Services, Ministry of Health & Family Welfare, Government of India. It is headed by the Drugs Controller General of India (DCGI).

Certificate of Analysis (CoA)
The list of test procedures applied to a particular sample with the results obtained and the acceptance criteria applied. It indicates whether or not the sample complies with the specifications.

Certified Reference Material (CRM)
Reference material, characterized by a metrologically valid procedure for one or more specified properties, accompanied by a certificate that provides the value of the specified property, its associated uncertainty and a statement of metrological traceability.

Design Qualification (DQ)
Documented collection of activities that define the functional and operational specifications of the instrument and criteria for selection of the vendor, based on the intended purpose of the instrument.
Note: Selection and purchase of a new instrument should follow a conscious decision process, based on the needs of the technical management. When designing a new laboratory facility, the design specification and the requirements for services should be agreed between the management and the agreed suppliers and the same shall be documented.

~~**Drug Product**~~
~~A finished dosage form (e.g. tablet, capsule or injection) that contains an API, generally, in association with inactive ingredients. Drug product is the finished product of any drug that is ready to use. The drug substance together with the added ingredients (e.g. excipients) is known as drug product.~~

Dosage Forms
The dosage form of a pharmaceutical product denotes its method of entry or delivery into a biological system. Generally, dosage forms are simply classified as solids or liquids. Solid dosage forms include tablets and powders used in propelled inhalants, such as asthma inhalers. Liquid dosage forms can vary greatly in viscosity of their final mixture, and can range from orally-ingested syrups to topical serums to solutions that are administered intravenously.

Expiry Date
The date up to which a drug is expected to remain within specifications, when stored correctly under specified conditions as stated in schedule P of the Drugs and Cosmetics Rules 1945 or under conditions as specified, if not stated in schedule P. Where month only is specified, the date of expiry shall be the last date of the month specified.

Good Laboratory Practices (GLP)
GLP implies the statutory norms prescribed in Schedule L1 of the Drugs and Cosmetics Rules 1945. It is applicable to the laboratories of manufacturing units under rules 74 and 78 of the Drugs & Cosmetics Rules 1945 and approved drugs testing laboratories under rule 150E and is intended to secure effective regulation of the functioning of laboratories for testing of drugs. It prescribes the requirements of infrastructure in terms of building, equipments, personnel and other services and facilities; standard operating procedures to be developed and adopted for equipments, environment, activities of tests and analysis of drugs and associated matters, documentation etc.

Good Manufacturing Practices (GMP)
That part of quality assurance which ensures that pharmaceutical products are consistently produced and controlled to the quality standards appropriate to their intended use and as required by the marketing authorization.

Indian Pharmacopoeia (IP)
IP is published by the Indian Pharmacopoeia Commission in fulfilment of requirement of the Drugs and Cosmetics Act 1940 and Rules 1945 there under. It prescribes the standards of identity, purity and strengths for the drugs manufactured and/or marketed in India. The standards prescribed in IP are authoritative and legally enforceable in the country.

Indian Pharmacopoeia Commission (IPC)
IPC is an autonomous body under the Ministry of Health & Family Welfare, Government of India. The mandate of the commission is to bring out IP, the compendium of the standards for drugs. Additionally, IPC is also vested with the duty of publishing the National Formulary of India (NFI) and providing IP Reference Substances. The IPC has it's headquarter at Raj Nagar, Ghaziabad and the Secretary-cum-Scientific Director is the head of IPC.

Indian Pharmacopoeia Reference Substances (IPRS)
IPRS are highly-characterized physical specimens used in testing by pharmaceutical and related industries to help in ensuring the identity, strength, quality, and purity of medicines. These are primary standards, having appropriate quality within a specified context and are accepted without requiring comparison to another substance.

Installation Qualification (IQ)

The performance of tests to ensure that the analytical equipment used in a laboratory is correctly installed and operates in accordance with established specifications.

Management Review
A formal, documented review of the key performance indicators of a quality management system performed by top management.

Manufacturer
A company that carries out operations such as production, packaging, testing, repackaging, labelling and/or relabelling of pharmaceuticals.

Marketing Authorization
This means the authorization given under the Drugs and Cosmetics Act 1940 and the Rules 1945 there under to import or manufacture a drug and place in the market for its sale, distribution, and use as per rule 2 (ea), 84-E.

Metrological Traceability
Property of a measurement result whereby the result can be related to a reference through a documented, unbroken chain of calibrations, each contributing to the measurement uncertainty.

Operational Qualification (OQ)
Documented verification that the analytical equipment performs as intended over all anticipated operating ranges.

Performance Qualification (PQ)
Documented verification that the analytical equipment operates consistently and gives reproducibility within the defined specifications and parameters for prolonged periods.

Pharmaceutical Aids
Substances which are of little or no therapeutic value, but are necessary in the manufacture, compounding, storage, etc., of dosage forms. They include solvents, diluting agents, suspending agents, emulsifying agents, coloring agents, flavouring agents, preservatives, excipients, ointment bases, vehicles.

Pharmaceutical Excipient
A substance, other than the API, which has been appropriately evaluated for safety and is included in a medicines delivery system to:

- aid in the processing of the medicines delivery system during its manufacture;
- protect, support or enhance stability, bioavailability or patient acceptability;
- assist in pharmaceutical product identification; or
- enhance any other attribute of the overall safety and effectiveness of the medicine during its storage or use.

Primary Reference Substance (or Standard)
A substance that is widely acknowledged to possess the appropriate qualities within a specified context, and whose assigned content is accepted without requiring comparison with another chemical substance. Pharmacopoeial chemical reference substances are considered to be primary reference

substances. In the absence of a pharmacopoeial reference substance, a manufacturer should establish a primary reference substance.

Qualification of Equipment
Action of proving and documenting that any analytical equipment complies with the required specifications and performs suitably for its intended purpose.

Quality Control
All measures taken, including the setting of specifications, sampling, testing and analytical clearance, to ensure that raw materials, intermediates, packaging materials and finished pharmaceutical products conform with established specifications for identity, strength, purity and other characteristics.

Quality Management System
An appropriate infrastructure, encompassing the organizational structure, procedures, processes and resources, and systematic actions necessary to ensure adequate confidence that a product or service will satisfy given requirements for quality (see Part one, section 2).

Quality Manager
A member of staff who has a defined responsibility and authority for ensuring that the management system related to quality is implemented and followed at all times.

Quality Manual
A handbook that describes the various elements of the quality management system for assuring the quality of the test results generated by a laboratory.

Quality Policy
A quality policy is a brief statement that aligns with your organization's purpose and strategic direction, provides a framework for quality objectives, and includes a commitment to meet applicable requirements (such as ISO 17025:2017, customer, statutory or regulatory) as well as to continually improve.

Reference Material
Material sufficiently homogeneous and stable with respect to one or more specified properties, which has been established to be fit for its intended use in a measurement process.

Reference Substance (or Standard)
An authenticated, uniform material that is intended for use in specified chemical and physical tests, in which its properties are compared with those of the product under examination, and which possesses a degree of purity adequate for its intended use (8).

Secondary Reference Substance (or Standard)
A substance whose characteristics are assigned and/or calibrated by comparison with a primary reference substance. The extent of characterization and testing of a secondary reference substance may be less than for a primary reference substance. Often referred to as an in-house working standard.

Specification
A list of detailed requirements (acceptance criteria for the prescribed test procedures) with which the substance or pharmaceutical product has to conform to ensure suitable quality.

Standard Operating Procedure (SOP)
It is an authorized written procedure giving instructions for performing operations usually listed in a chronological order. They are essential for the laboratory quality management system.

System Suitability Test
A test which is performed to ensure that the analytical procedure fulfils the acceptance criteria which had been established during the validation of the procedure. This test is performed before starting the analytical procedure and is to be repeated regularly, as appropriate, throughout the analytical run to ensure that the system's performance is acceptable at the time of the test.

Validation of Analytical Method
The documented process by which an analytical procedure (or method) is demonstrated to be suitable for its intended use.

Verification of Analytical Method
Process by which a pharmacopoeial method or validated analytical procedure is demonstrated to be suitable for the analysis to be performed.

Indian Pharmacopoeia: Legal Status

Indian Pharmacopoeia: Legal Status in the Drugs & Cosmetics Act 1940 and Rules 1945
IP is the official book of standard for drugs in India under the Drugs and Cosmetics Act 1940 and Rules 1945 thereunder. Section 8 relating to import of drugs (into India) and Section 16 of the Act lay down as under:

8. Standards of quality. (1) For the purposes of this Chapter, the expression "standard quality" means-

(a) in relation to a drug, that the drug complies with the standard set out in the Second Schedule…",

16. Standards of quality. (1) For the purposes of this Chapter, the expression "standard quality" means-

(a) in relation to a drug, that the drug complies with the standard set out in the Second Schedule, …..".

Clause 5 of the Second Schedule to the Act states as under:

5. Other drugs:

(a) Drugs included in the Indian Pharmacopoeia	Standards of identity, purity and strength specified in the edition of the Indian Pharmacopoeia for the time being in force and such other standards as may be prescribed. In case the standards of identity, purity and strength for drugs are not specified in the edition of the Indian Pharmacopoeia for the time being in force but are specified in the edition of the Indian Pharmacopoeia immediately preceding, the standards of identity, purity and strength shall be those occurring in such immediately preceding edition of the Indian Pharmacopoeia and such other standards as may be prescribed.
(b) Drugs not included in the Indian Pharmacopoeia but included in the official Pharmacopoeia of any other country.	Standards of identity, purity and strength specified for drugs in the edition of such official Pharmacopoeia of any other country for the time being in force and such other standards as may be prescribed. In case the standards of identity, purity and strength for drugs are not specified in the edition of such official Pharmacopoeia for the time being in force but are specified in the edition immediately preceding, the standards of identity, purity and strength shall be those occurring in such immediately preceding edition of such official Pharmacopoeia and such other standards as may be prescribed.

This status is laid down in the Drugs & Cosmetics Rules 1945 under part XII standards also.

Rule 124 states as under:

R.124- Standards of drugs:

(1) Drugs included in the Indian Pharmacopoeia-

(a) The standards for identity, purity and strength shall be those as may be specified in the edition of the Indian Pharmacopoeia for the time being in force.

(b) In case the standards for identity, purity and strength for drugs are not specified in the edition of the Indian Pharmacopoeia for the time being in force but are specified in the edition of the Indian Pharmacopoeia immediately preceding, the standards for identity,

purity and strength shall be those occurring in such immediately preceding edition of the Indian Pharmacopoeia.

(c) Include rule 124 (c) 124-A, 124-B, 124-C and 124-D

(2) For other drugs-

(a) The standards for identity, purity and strength shall be those as may be specified in the edition of the official pharmacopoeia, for the time being in force, of any country to which the drug claims to comply with.

(b) In case the standards for identity, purity and strength for drugs are not specified in the edition of such official pharmacopoeia for the time being in force, but are specified in the edition immediately preceding, the standards for identity, purity and strength shall be those occurring in such immediately preceding edition of such official pharmacopoeia to which the drug claims to comply with.

Thus all drugs imported into the country except those intended for personal use or in small quantities for tests and analysis, are to comply with the standards set out in the IP (the current or immediate preceding edition as the case may be) and other standards apply only in cases not covered by the IP.

A drug that does not conform to the above standards is one "Not of standard quality" and activities of import, manufacture, sale and distribution of a drug 'not of standard quality, is an offence punishable under the Drugs and Cosmetics Act 1940.

The letters IP in relation to a drug is to be used only for the purpose of informing the user or others as the case may be that the drug is of IP standards. Rule 104 of the Drugs and Cosmetics Rules lays down as:

"**R.104 Use of letters I.P. etc:** The letters 'I.P.' and recognised abbreviations of pharmacopoeias and official compendia of drug standards prescribed under these rules shall be entered on the label of the drug only for the purpose of indicating that the drug is in accordance with standards set out in the Indian Pharmacopoeia or in any such pharmacopoeia or official compendium of drug standards recognised under the Rules."

Labels of drugs are to conform to Rule 96 of the Drugs and Cosmetics Rules and clause (b) of sub-rule (1) of this rule stipulates as under:

R.96. Manner of Labelling: (1).....

(1) Subject to the other provisions of these rules, the following particulars shall be either printed or written in indelible ink and shall appear in a conspicuous manner on the label of the innermost container of any drug and on every other covering in which the container is packed, namely:-

(i) The name of the drug:

For this purpose, the proper name of the drug shall be printed or written in a more conspicuous manner than the trade name, if any, which shall be shown immediately after or under the proper name and shall be –

(a) for drugs included in Schedule F or Schedule F(1), the name given therein;

(b) for drugs included in the Indian Pharmacopoeia or the official pharmacopoeias and official compendia of drug standards prescribed in Rule 124, the name or synonym specified in the respective official pharmacopoeias and official compendia of drug standards followed by the

letters ‘I.P.’ or, as the case may be, by the recognised abbreviations of the respective official pharmacopoeia and official compendia of drug standards;

(c) for drugs included in the National Formulary of India, the name or synonym specified therein followed by the letters ‘N.F.I.’;

(d) for other drugs, the international non-proprietary name, if any, published by the World Health Organization or where an international non-proprietary name is not published, the name descriptive of the true nature or origin of the substance.

Any drug not conforming to the above labelling requirements is a “misbranded drug” as defined and punishable under the law.

Rule 96 as stated above applies to imported drugs also as laid down in Rule 32.

R. 32 Packing and labelling of imported drugs: No drug shall be imported unless it is packed and labelled in conformity with the rules in Parts IX and X and further conforms to the standards laid down in Part XII provided that in the case of drugs intended for veterinary use, the packing and labelling shall conform to the rules in Parts IX and X and Schedule F(1).

These are some of the excerpts from the Drugs & Cosmetics Act and Rules and the users are cautioned not to deviate from the legal provisions in public interest apart from own interests to avoid violations of the law inadvertently or otherwise.

Schedule N of the Drugs & Cosmetics Rules require a pharmacy to keep IP as a book of reference. The requirement is as under:

A Pharmacy shall be provided with the following minimum books necessary for making of official preparations and prescriptions:-

Schedule “V” of the Drugs and Cosmetics Rules, 1945 requires application of IP parameters to Patent and Proprietary Medicines as under:

SCHEDULE V

(See rule 124B)

STANDARDS FOR PATENT OR PROPRIETARY MEDICINES

* * * * *

2. Standards for patent or proprietary medicines, containing vitamins:

Patent or proprietary medicines containing vitamins for prophylactic, therapeutic or paediatric use shall contain the vitamins in quantities not less than and not more than those specified below in single or in two divided daily doses, namely: -

4. General Standards for Different Categories of Patent or Proprietary Medicines:

In the case of pharmaceutical products containing several active ingredients, the selection shall be such that the ingredients do not interact with one another and do not affect the safety and therapeutic efficacy of the product. The combination shall not also lead to analytical difficulties for the purpose of assaying the content of such ingredient separately. The substances added as additives shall be innocuous, shall not affect the safety or therapeutic efficacy of the active ingredients, and shall not affect the assays and identity tests in the amount present.

Subject to the provisions of these rules, patent or proprietary medicines shall comply with the following standards, namely: -

1. Patent or proprietary medicines shall comply with the general requirements of the dosage form under which it falls as given in the **Indian Pharmacopoeia**. If the dosage form is not included in the **Indian Pharmacopoeia**, but is included in any other pharmacopoeia, prescribed for the purpose of the Second Schedule to the Act, it shall comply with the general requirements of the dosage of such pharmacopoeia. Without prejudice to the generality of the foregoing requirements, general requirements shall include compliance with colour consistency, clarity, stability, freedom from contamination with foreign matter or fungal growth, defects like chipping and capping of tablets, cracking of the coating, mottled appearance and other characteristic defects that can be perceived by visual inspection.

2. Without prejudice to the generality of the following paras, dosage forms of patent or proprietary medicines shall comply with the following requirements, namely:-

(a) ***Tablets*****:** Medicines shall comply with requirements for tablets as laid down in the **Indian Pharmacopoeia**. The nature of coating shall be indicated on the label.

Permitted colours may, however, be added and declared on the label. Nature of tablets, such as uncoated, sugar coated or film coated, shall be declared on the label.

(b) ***Capsules***: Medicines shall comply with the requirements for capsules as laid down in the **Indian Pharmacopoeia**. However, the capsules shall be free from distortion or shape, dis-colouration and other physical defects like leakage of powder from joints, pinholes or cracks in the capsules;

(c) ***Liquid oral dosage forms***: Emulsions and suspensions shall disperse uniformly on shaking. Homogeneous solutions shall contain no sediments. The volume of the product (net content) in the container shall be not less than the labelled volume. The limit for ethanol content of pharmaceutical products shall be not less than 90 per cent and not more than 110 per cent of the labelled contents.

(d) ***Injections*****:** Medicines shall comply with the requirements for injections as laid down in the **Indian Pharmacopoeia**.

(e) ***Ointments*****:** Medicines shall comply with the requirements for ointments as laid down in the **Indian Pharmacopoeia**.

3. The content of active ingredients, other than vitamins, enzymes and antibiotics, in patent or proprietary medicines shall be not less than 90 per cent and not more than 110 per cent of the labelled content; however, for enzymes and vitamins, only for lower limit of 90 per cent shall

apply. In all dry formulations containing antibiotics, the limit shall be 90 to 130 per cent of the labelled contents and in case of liquid antibiotic formulations, the limit shall be 90 to 140 per cent of labelled contents.

Fiducial limits for error for microbiological assay of antibiotics may be estimated depending upon the design of assay procedure. Methods, used for assaying active ingredients shall employ the same basic principles and shall use same organisms as given in the latest edition of the **Indian Pharmacopoeia** or shall follow any other methods as approved by the authority competent to grant licence to manufacture.

4. All patent or proprietary medicines containing aspirin shall be subjected to "Free Salicylic Acid Test" and the limit of such acid shall be 0.75 per cent. Except in case of soluble type aspirin in which case the limit of such acid shall be 3 per cent.

5. Patent or proprietary medicine to be tested under the provisions of rule 121-A for pyrogen shall be tested by injecting into rabbits not less than the human dose of the medicine based on body weight of a 60 kg human being. Methodology and limits shall be based on the method recorded in the **Indian Pharmacopoeia**. Dose selected shall be indicated in the protocol but the dose shall be not greater than 5 times the human dose based on body weight of 60 kg for man.

6. In injectable patent or proprietary medicines, the test for freedom from toxicity, shall be performed as described in the **Indian Pharmacopoeia**. Dose selected shall be indicated in the protocol but the dose shall not be less than five times the human dose based on body weight of 60 kg human being.

References

1. The Indian Pharmacopoeia, 2018
2. The Drugs and Cosmetics Act, 1940.
3. The Drugs and Cosmetics Rules, 1945

Process for IP Monograph Development

The inclusion of monographs in IP and their further amendments due to scientific advancements is taken up by IPC from time-to-time. The same is reviewed by specialized Expert Working Groups having members from regulatory authorities, drug control laboratories, pharmaceutical manufacturers, research institutions and other stakeholders. The principle of "openness, justice and fairness" is followed while compiling, verifying, and editing the contents of the IP monographs. Stakeholders contributing in the process for IP monograph development and their amendments are given in Figure 1.

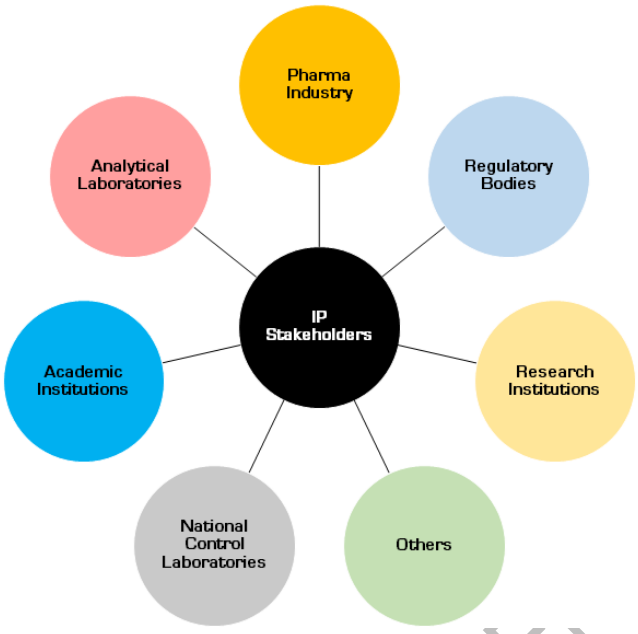


Figure 1. Stakeholders of IP

Process for IP Monograph Development

The process for IP monograph development includes following six steps and is summarized in Figure 2:

Step 1: Preparation of Initial List of Monographs for Inclusion in IP

The scientific staffs of IPC prepare an initial list of APIs and dosage forms for which monographs are to be developed for their inclusion in IP. This list is then thoroughly deliberated in the relevant Expert Working Groups of the IPC. The list of monographs cleared by the Expert Working Groups is then put up to the Scientific Body of the IPC for its review and approval.

Inclusion and Exclusion Criteria for IP

Inclusion Criteria

- Drugs used in National Health Programs of India
- Drugs listed in National List of Essential Medicines (NLEM)
- Drugs approved by CDSCO
- Fixed Dose Combinations approved by CDSCO and recommended by the IPC Experts

Inclusion Criteria contd.

- Drugs considered appropriate by the IPC

Exclusion Criteria

- Drugs banned in India
- Obsolete drugs
- Drugs considered inappropriate by the IPC

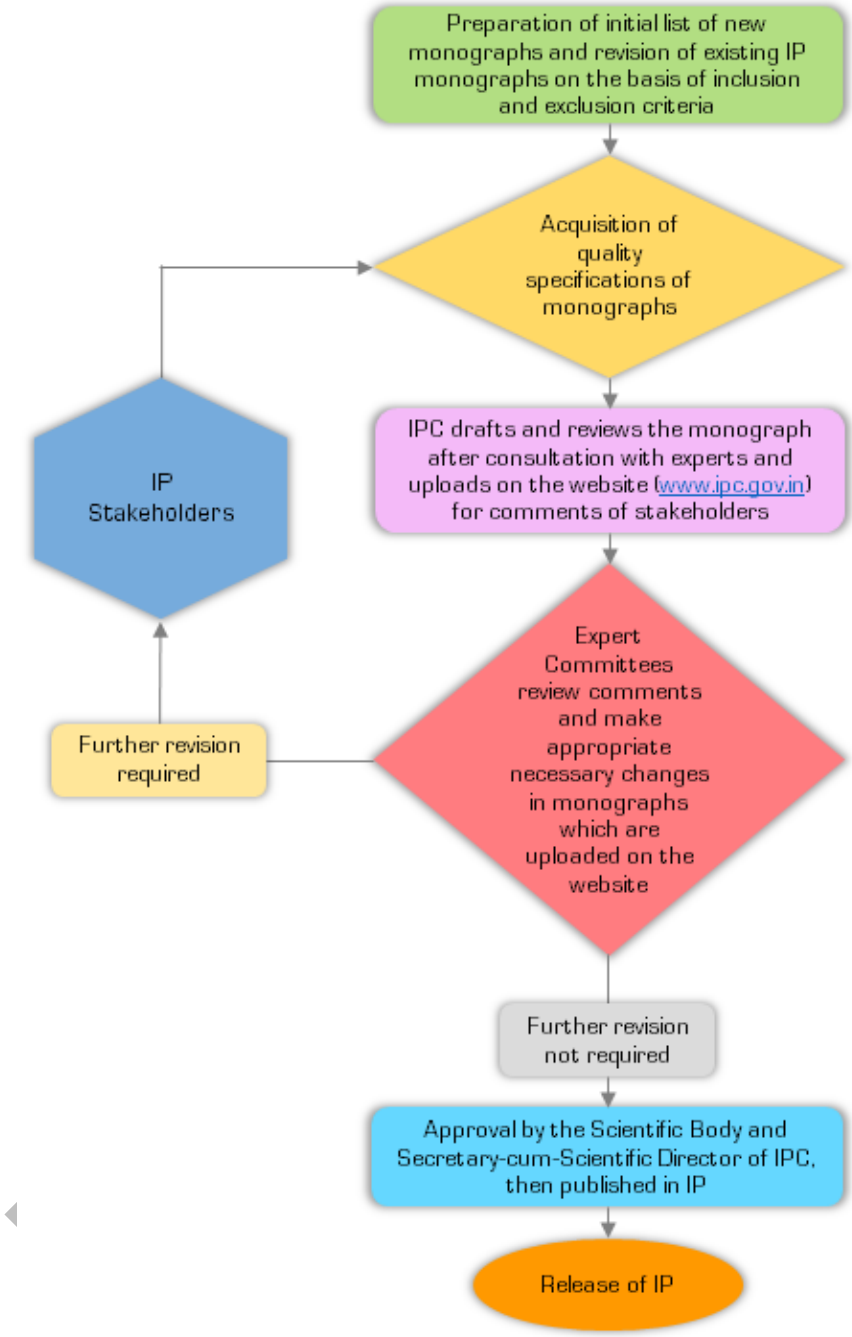


Figure 2. Public Review and Comments Process for IP Monograph Development

Step 2: Acquisition of Monographs from the Stakeholders

The quality specifications of the APIs and/or dosage forms are obtained from the stakeholders along with 'Monographs Inclusion Request Form' which is displayed on IPC website (www.ipc.gov.in) and is re-presented below:

Monograph Inclusion Request Form

1.	Name of the Molecule		
2.	Therapeutic Category		
3.	Name of Monograph		
4.	Is it approved by CDSCO in India? (please tick)		API
			Dosage Form
			Both
			Any Other
5.	In case of Dosage Form, provide details with respect to for how long product is being marketed in India?		
6.	Name of Manufacturers		
7.	Benchmark-International Product/Company		
	Category (Please tick)		National Health Programs of India
			National List of Essential Medicines
			Fixed Dose Combination
			Any Other
8.	Comparative status of proposed monograph in different pharmacopoeias		
9.	Justification for deviation of Innovators specification		
10.	Route of Synthesis (If applicable)		
11.	Analytical Method Validation Data		
12.	Stability Data including data about forced degradation studies		
13.	Rationale of Inclusion of Monograph:		
Reviewers Remarks:			
1. _____ 2. _____			
Authorized by:			
1. _____			

Note: *Duly filled in and approved form is submitted to IPC along with details on test samples for method verification, specifications, STP, WS, reference standard, impurity standard, validation data, stability data, and candidate material for the development of IPRS.*

In addition to the drug monographs, recommendations on IP General Chapters, if any, may also be submitted to IPC. At the time of monograph development, stakeholders are also encouraged to donate candidate material for the development of IPRS.

Step 3: Drafting of Monographs in IP Format

IPC scientific staffs draft the monograph(s) in IP format. Simultaneous verification and/or validation of test methods are performed at the IPC. Different Expert Working Groups examine and review the monographs for their suitability in terms of the technical contents and feasibility of testing.

Step 4: Obtaining Public Comments

The draft monograph(s) are displayed on the IPC website for a period of 45 days and given wider publicity by circulating the list of proposed monographs in IDMA Bulletin/Pharmabiz etc. for inviting public comments.

Step 5: Review of Comments

The comments obtained from stakeholders are examined by the scientific staffs of IPC and/or Expert Working Groups. Further revision, if required, is carried out by IPC staffs and monograph(s) are again displayed on IPC website and circulated to the stakeholders. In case, no further revision is required, the monograph is accepted and processed for its publication. The galley proof of the entire manuscript is reviewed by the IPC scientific staffs and Expert Working Groups. Other requirements such as obtaining ISBN and copyright related provisions are complied with before final publication of IP.

Step 6: Release of IP

The final print of IP, bearing the official seal of IPC and a unique identification number, is released by the competent authority for use by the stakeholders. IP becomes effective from such date as specified and approved by the Secretary-cum-Scientific Director of the IPC.

Queries on IP Monographs and Amendment Lists

IPC receives comments and queries from various stakeholders on IP monographs. These may relate to, but not limited to: inadvertent omissions, composing errors, availability of IPRS and impurity standards, and proposals for technical up-gradations and/or amendments in IP monographs. If any anomaly is pointed out by the stakeholders, the same may be communicated to the IPC by e-mail to lab.ipc@gov.in or by post to the Secretary-cum-Scientific Director, IPC. Based on the feedback received from the stakeholders, amendments are proposed in the IP monographs and General Chapters. Once approved by the Expert Working Group, the same are issued as Amendment List by the Secretary-cum-Scientific Director of IPC.

Composition of IP Standards

IP contains an account of the General Chapters and Individual Monographs on APIs, dosage forms, excipients etc. IP text may be broadly classified into the following four sections with each section having a specific scope. In order to comply with IP standard, the scope and role of each of these sections should be clearly understood.

1. General Notices

General Notices of IP provide the basic guidelines for the interpretation and application of the standards, tests, assays, and other specifications of the IP, as well as to the statements made in the monographs and other texts of the IP. They apply to all monographs and texts contained in IP and provide required information to understand texts and conventional expressions before starting to use monographs. General Notices must be read and understood before proceeding to use monographs.

2. General Chapters

General Chapters provide a comprehensive account of analytical tests and methods that are common to majority of monographs and are cross referred through the citation provided as appendices in the individual monographs. General Chapters are meant to avoid repeated mention of standard methods in each monograph. General Chapters, when referred to in an individual monograph, become part of the standard. General Chapters are mentioned in Volume I of the IP under following categories:

- General Notices
- Test Methods
  - Apparatus
  - Biological Methods
  - Chemical Methods
  - Physical and Physicochemical Methods
  - Pharmaceutical Methods
  - Herbal Products
  - Vaccines
  - Blood and Blood-related Products
- Reference Data
- Reagents and Solutions
- General Tests
- Primary Packages for Pharmaceuticals
- Tables

General Chapters may also be referred for non-pharmacopoeial drugs; however, in such cases, the methods specified in the General Chapters need to be validated, wherever applicable. Some General Chapters are not referred to in any monograph (e.g. Raman Spectrometry, IP Reference Substances, Residual Solvents); however, they provide useful information and guidance for regulatory compliance.

3. General Monographs

General Monographs on dosage forms include requirements of general application and shall apply to all preparations within the scope of the Introduction section of the monographs, except where a preamble limits the application. The requirements are not necessarily comprehensive for a given specific preparation and additional requirements, if any, may be given in the individual monograph. Examples of few General Monographs are as follows:

- Dosage form
- Tablets
- Capsules
- Parenteral Preparations, Oral Liquids etc. (Terms should be as per IP)
- Immunosera for human use
- Immunosera for veterinary use

- Pharmaceutical preparations
- Products of recombinant DNA technology
- Substances for pharmaceutical use
- Vaccines for human use
- Vaccines for veterinary use
- Herbal drugs
- Veterinary drugs

4. Individual Monographs

Individual monographs are the specific monographs either of APIs, Pharmaceutical Aids and Dosage Forms. Individual monographs of IP detail about synonym, molecular structure, molecular formula, molecular weight, definition of article, category, dose, description, potency of the article, identification, tests, impurity profile, assay, specifications of tests, storage and labelling requirements.

IP Monograph

IP Monograph is the written standard of the quality parameters of a drug (or article) in the IP. It comprises of the name and synonym of the drug (where appropriate) the chemical, empirical and structural formulae of the molecule, its molecular weight, production (in the case of biological products), definition, category, dose, usual strength, description, solubility, tests for identity, tests for impurities, tests for assay or potency in general and in the case of injectables, tests for pyrogen, sterility and in the cases of certain biological products abnormal toxicity, and storage. All the parameters of quality set out in a monograph are designed to determine the quality of a drug. The usual strength, storage conditions as may be mentioned are of known or recommended ones and the requirements as specified in the Drugs and Cosmetics Rules shall prevail in the event of dispute. Where any specific labelling requirement is specified in the IP, the requirements shall be in addition to what is specified in the Rules. A product is not of standard quality unless it complies with all the requirements of the monograph.

IP General Chapters

They provide the information about the common analytical procedures applicable to IP monographs and information pertaining to the quality requirements of pharmaceutical products. The interpretation of a monograph must be in accordance with all the general requirements, testing methods, texts and notices pertaining to it, in the IP.

IPRS

IPRS are mentioned in the Individual Monographs or General Chapters, as the case may be, and therefore are part of the IP standard. IPRS are specifically required for establishing conformance to the IP standard. An IPRS, being an integral and essential component of the IP standard, is an official standard that alone is authoritative in case of doubts or disputes.

Compliance with IP Standard

A drug that is included in IP is considered to comply with the IP standard when it meets all of the requirements stated in the Individual Monograph, applicable General Monographs, General Chapters, General Notices, and any other relevant requirements, if applicable. However, in any

case where requirements differ, the requirements of Individual Monograph shall supersede those of the General Chapters and General Notices. A drug shall comply with the IP standard throughout its shelf-life.

Alternative Methods

The tests and assays described in IP are the official methods upon which the standards of the pharmacopoeia are based. Alternative methods of analysis may be used if they demonstrate advantages over the official IP method e.g. accuracy, sensitivity, precision, selectivity, adaptability to automation or any other suitably justifiedreasons. Wherever applicable, such alternative methods shall be validated based on the intended purpose of use and should be able to give equivalent or better results. Alternative methods should also be submitted to IP for evaluation and inclusion in the monograph, wherever applicable. When a difference appears, or in the event of dispute, the results obtained by using the official methods described in IP shall be authoritative and legally valid.

Indian Pharmacopoeia Reference Substances

Indian Pharmacopoeia Reference Substances (IPRS)

IPRS are highly-characterized physical specimens used in testing to help ensure the identity, strength, quality, and purity of drugs as given in the IP. These are primary standards having appropriate quality within a specified context accepted without requiring comparison to another substance. IPRS are not intended for use as drugs. These are specimens of drug substances, impurities, degradation products, herbal-related and blood related substances, excipients, and test performance calibrators. IPRS are certified, maintained and distributed by the IPC or by laboratories recognised by IPC. Where the letters *RS* appear after the italicized name of the

substance in a test or assay in the individual monograph or appendix, the relevant IPRS must be used. The primary standard is IPRS but may also be used other equivalent Phamacopoeial standard if IPRS not available

Impurity Standards

Impurities are undesirable components of drug substances and drug products other than chemical entity that defines the substance. These are not excipients in drug products. They include degradation products of the drug substances that might have developed on storage and in case of drug products, those that may be formed during manufacturing and storage.

Identification of impurities is done from stability studies, forced degradation studies, and analyses of routine production batches. Impurity standards are needed to analyse the drug substances or drug products in order to ascertain the extent of impurities present therein. Tests for impurities in IP monographs provide information on the extent of known potential or actual impurities, but do not guarantee freedom from all possible impurities. The manufacturer is responsible to limit impurities arising from various sources during manufacturing.

Impurity Categories in Drug Substances

- **Inorganic Impurities:** may result from manufacturing process
- **Organic Impurities:** may be drug-related or process-related
- **Residual Solvents:** inorganic or organic liquids used for preparation of solutions or suspensions during the synthesis of drugs substance

Impurity Categories in Drug Products

- Degradation/reaction products of API
- Products of interaction between various drugs in a combination product

Botanical Reference Substances (BRS)

BRS is a standard whose botanical identity and genuineness has been well established upto both genus and species level. It is used as a reference material for comparison and confirming the identity of the commercial supplies of the respective botanical substances as prescribed under test for identity in the IP monograph. Compliance to identity test using microscopic, chromatographic (TLC/HPTLC fingerprint) and other specified tests will involve use of BRS. It has a shelf-life of 2 years unless otherwise stated. Each BRS should be supplied with documentation describing the above characteristics features. IPC recommends storage of BRS between 2-15° and preferably at 60% RH.

Dissolution Apparatus Calibrator

The IP Prednisone Tablet *RS* is use for the performance verification test for IP Dissolution Apparatus 1 (Paddle Type) and Dissolution Apparatus 2 (Basket Type) as described in the IP General Chapter (2.5.2.).

Storage and Handling of IPRS

In order to serve the intended purpose, it is important that each IPRS is properly stored, handled and used. IPRS should be stored in their original stoppered containers, away from heat and humidity and protected from light, at temperature between 2° to 8°. Special storage conditions, where necessary, are usually provided on the label. Containers should not to be opened until they have attained room temperature, to prevent ingress of moisture by condensation.

Procurement of IPRS from IPC

IPRS may be procured from IPC and their details are given below:

IPRS Available from IPC	
List of IPRS	https://ipc.gov.in
List of Impurities	https://ipc.gov.in
List of BRS	http://www.ipc.gov.in/images/pdf/File788.pdf
Dissolution Apparatus Calibrator (Prednisone Tablet RS)	http://www.ipc.gov.in/mandates/national-formulary-of-india-nfi/ip-prednisone-tablet-dissolution-apparatus-calibrator-is-available-at-ipc,-ghaziabad.html
IPRS Procurement Link	http://www.ipc.gov.in/mandates/national-formulary-of-india-nfi/about-iprs.html
IPRS Price	Rs. 5000/- per vial for private stakeholders and Rs. 2500/- per vial for government stakeholders
Payment Mode	RTGS/NEFT as per details given below
Bank name	Bank of Baroda
Branch	Sanjay Nagar, Ghaziabad
A/c Name	Indian Pharmacopoeia Commission
A/c No.	21860100013540
IFSC Code	BARB0SANGHA (0 = zero)
Type of Account	Saving Account

National Reference Standards on Human Vaccine & Immunosera

National Reference Standards (NRS) on Human Vaccine & Immunosera available from Central Drugs Laboratory, Kasauli are recognised as the IPRS. List of these NRS is given below:

(i) Tetanus Antitoxin
(ii) Diphtheria Antitoxin
(iii) Tetanus Toxoid
(iv) Diphtheria Toxoid
(v) Anti-rabies Serum
(vi) Anti-Rabies Vaccine
(vii) Measles Vaccine
(viii) Mumps Vaccine
(ix) Rubella Vaccine
(x) Pertussis Vaccine
(xi) Pertussis (RWRS) Vaccine
(xii) Bacillus Calmette Guerin Vaccine
(xiii) Cobra Venom
(xiv) Krait Venom
(xv) Russell Viper Venom
(xvi) Saw Scaled Viper Venom
(xvii) Poliomyelitis Vaccine (bivalent OPV 1+3)
(xviii) Poliomyelitis Vaccine (m OPV Type I)
(xix) Poliomyelitis Vaccine (m OPV Type III)
(xx) Polio Antiserum Type I
(xxi) Polio Antiserum Type II

National Reference Standards on Biotech & Blood Reagent Products

NRS on Biotech and Blood Reagent products available from National Institute of Biologicals (NIB), NOIDA are recognised as the IPRS. List of these NRS is given below:

(i) Human insulin
(ii) Insulin lispro
(iii) Anti-A and Anti-B

Reference Microbial Cultures & Their Maintenance

Reference Microbial Cultures are available from the culture collections maintained by CSIR-Institute of Microbial Technology (IMTECH), Chandigarh and CSIR-National Chemical Laboratory (NCL), Pune.

The maintenance of Reference Microbial Cultures is an essential requirement to preserve their viability and characteristics. The pure cultures are transferred periodically onto or into a fresh medium (sub culturing) to allow continuous growth and viability of microorganisms. The transfer is always done under aseptic conditions to avoid contamination.
Since repeated sub culturing is time consuming, it becomes difficult to maintain a large number of pure cultures successfully for a long time. In addition, there is a risk of genetic changes as well as contamination. Therefore, it is now being replaced by some modern methods that do not need frequent sub-culturing. These methods include refrigeration, paraffin coverage method followed by storage at 2° to 8°, cryopreservation, and lyophilization (freeze drying).

Links for Procurement of IPRS from Laboratories Other than IPC
IPRS under following categories may be procured from IPC regonised laboratories:

IPRS Available from Other Laboratories	
NRS on Human Vaccine & Immunosera	**Link:** https://cdlkasauli.gov.in
NRS on Biotech & Blood Reagent Products	**Link:** http://nib.gov.in
Reference Microbial Cultures	**IMTECH Link:** www.imtech.res.in **NCL Link:** http://www.ncl-india.org/ncim

Working Standards
Primary standards are high cost material and supplied by official agencies (e.g. IPRS provided by IPC) usually in low quantities (20-200 mg or less). Therefore, in routine testing, a secondary standard (commonly known as working standard) may be used provided it has been qualified by comparison with a primary standard and its suitability for carrying out the compendial tests has been established. For this purpose, the primary standard is the IPRS but other equivalent pharmacopoeial standards (e.g. BP, USP, EP) may also be used if IPRS is not available. The extent of characterization and testing of a secondary standard may be less than that for a primary standard. Secondary standards are prepared in relatively higher quantities (several grams) and made available for internal use only.

Procedure for Preparation of Working Standards

(i) Source material of satisfactory quality with acceptable purity can be selected from a batch (lot) of the substance originating from the normal production process.

Note: *Working standards that are to be used in assays and other relevant tests (e.g. IR/UV identifications) should possess a high degree of purity. As a guiding principle, a purity of 99.0% or higher is desirable, calculated on the basis of the material in its anhydrous form or free of volatile substances. However, where the selectivity of the analytical procedure for which the chemical reference substance is required is low (identification tests, system suitability tests or chromatographic peak markers) such a degree of purity may not be necessary. In making a decision about the suitability of a chemical reference substance, the most important consideration is the influence of the impurity on the attribute measured in the assay when used in a nonspecific assay procedure. Impurities with physicochemical characteristics similar to those of the main component will not diminish the usefulness of a chemical reference substance, whereas even traces of impurities with significantly different properties may render a substance unsuitable for use as a chemical reference substance.*

If a substance is intended to be used as an impurity standard, the candidate material may be obtained from commercial suppliers, provided that the percentage purity is more than 95% (or 90% if for use in TLC).

(ii) Perform the identification, water content/loss on drying (as applicable), assay/potency/chromatographic purity in triplicate as per the relevant STPs or pharmacopoeial methods by using IPRS or any other available pharmacopoeial reference substance (in case IPRS is not available) of current lot or registration number.

(iii) If the substance is non-pharmacopoeial, analyse as per *in house* methods and specifications.

(iv) Correlation should be checked with two solutions of same dilution of IPRS as per the table below. The acceptance criteria should be between 99.5% to 100.5%.

No. of Standards	No. of Injections
Std-1	5
Std-2	2

$$\frac{\text{Std-1 wt}}{\text{Std-2 wt}} \times \frac{\text{Std-2 Avg. Area}}{\text{Std-1 Avg. Area}} \times 100$$

If not meet the criteria then prepare another Std. and check the correlation with both of the above Std.

If meet than take those Std. in Assay calculation.

Otherwise find the root cause & repeat the whole Analysis

Prepare three set of sample and calculate Assay with both of the Std.

Standard and Test Preparations	No. of Injection
Std-1	5
Std-2	2
Test-1	2
Test-2	2
Test-3	2

Assay Value – P1, P2, P3, P4, P5, P6

Difference between two assays shall not be more than ±0.5%

Average of 6 Value shall be the potency of WS

(v) Consider the average of assay/potency (on *as is* basis). RSD of the analyzed results shall not be more than 0.5%.

Note: *If the obtained assay/potency of working standard is above 100%, it should be reported as 100% on the label.*

(vi) After approval of working standard, subdivide the material and transfer to pre-labelled containers (e.g. 1.0 g or suitable quantity) and numbers (e.g. 12, one for each month).

(vii) Some working standards must be packaged under an inert gas or in conditions of controlled humidity. Therefore, the use of a glovebox or an air-tight cabinet is necessary. Single-use vials or ampoules can be used for hygroscopic materials.

(viii) Seal the containers and store in a refrigerated cabinet (5±3°) and/or desiccators, away from heat, light and moisture. Do not freeze. In specific cases labelled storage condition should be applicable.

Note: *The stability and suitability of the substances should be preserved by keeping them at prescribed temperatures. It should also be remembered that the relative humidity in normal refrigerators or cold rooms may be high; hence ampoules or other tightly closed containers are recommended for improvement of stability and prevent degradation due to absorption of moisture during storage.*

Traceability

Traceability is the property of a result of measurement that can be related to the appropriate standards, generally international or national standards, through an unbroken chain of comparison. In other words, when the result of a measurement is described as traceable, it is essential to specify to what (value of) "appropriate standards" traceability has been established. The assigned value of a secondary standard is traceable to the relevant primary standard.

Labels of Working Standards

Labels of the working standards should mention following minimum details:

Working Standard		
Name	:	
WS No.	:	
Assay/Potency	:	(*as is*)
LOD/Water	:	
Use Before	:	
Traceability	:	
Prepared by	:	
Storage Condition	:	

Validity of Working Standards

Each working standard may be assigned validity up to twelve months subject to verification by intermediate checks. One vial should be used for one month and after one month the remaining quantity in the vial should be destroyed. If the material is very sensitive, validity of working standard should be defined accordingly.

A new working standard should be prepared and standardized at the end of validity of the current working standard. If new material is not available, the existing working standard may be validated and appropriate validity is reassigned (which should be within the expiry date of the material). Whenever a new lot of primary standard becomes official, the working standard shall be re-validated against the new lot of primary standard.

Intermediate Checks of Working Standard

As material can decompose before the expiry date due to several factors like external contamination, environmental change, handling issues etc., therefore intermediate checks as per schedule should be performed to maintain confidence in usage of working standard with respect to critical tests like: water/LOD, chromatographic purity, assay/potency.

Usage and Handling of Working Standard

- Handle the working standards appropriately to avoid cross-contamination.
- Vials/ampoules should not be opened until they have attained room temperature to prevent ingress of moisture by condensation.
- Don't insert spatula or butter paper inside the working standard vial.
- Transfer required quantity approximated on butter paper for weighing purposes.
- Don't transfer the balance quantity in the original vial.

- After use, securely close the vials, seal it and store as recommended.
- If any change in description observed (e.g. lumps, color), donot use the working standard.
- Record the details on consumption of working standard in the log book.

Packaging Components of Working Standards

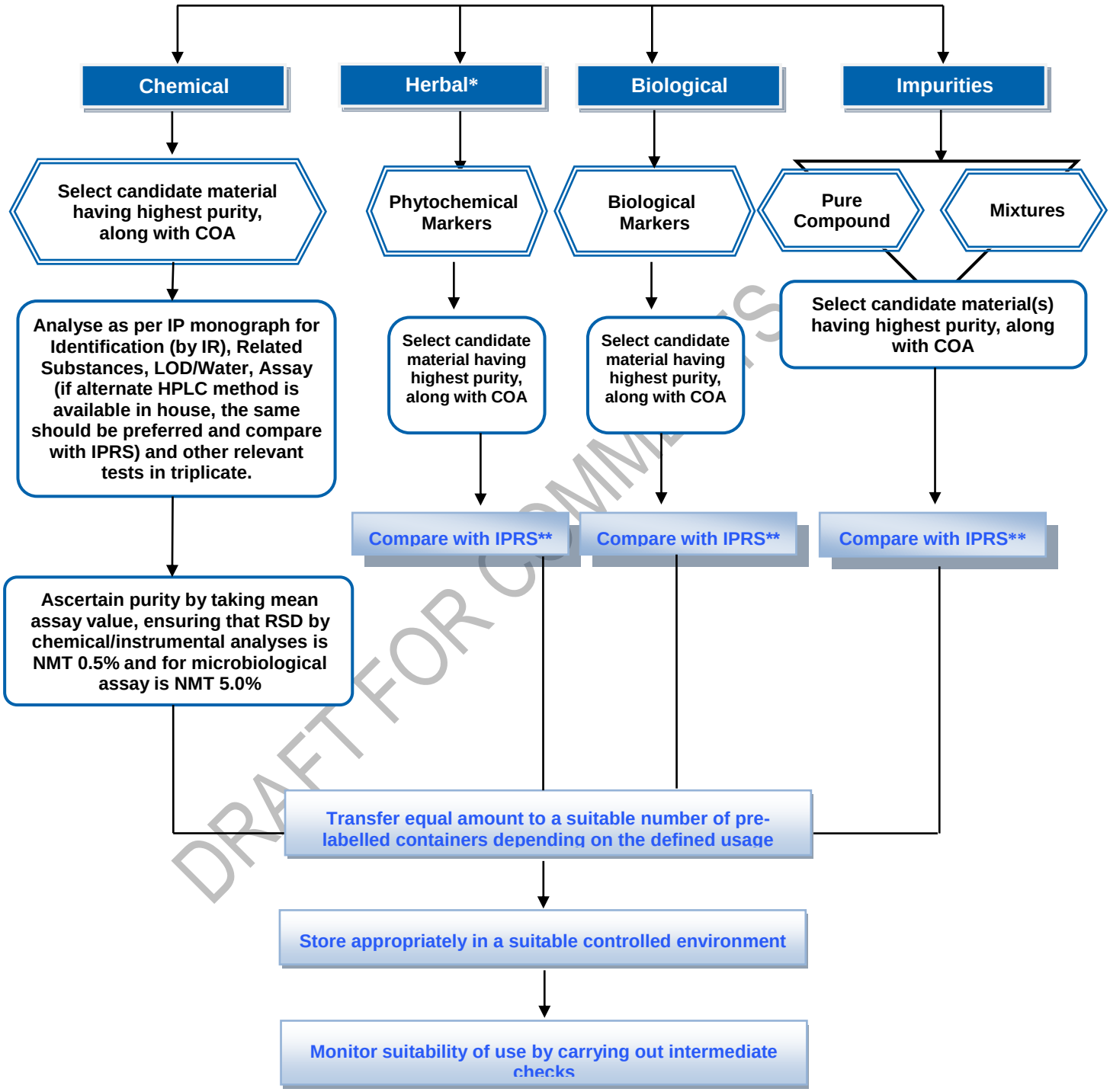
Containers for storage of working standards should be, as far as possible, similar to that of primary standards. However, the suitability of container/closure system should be established by the stakeholders on case-by-case basis. Glass containers for pharmaceutical use should be used. Type I glass (highly resistant borosilicate glass) containers are suitable for most products and are recommended for filling and storage of working standards. Amber coloured glass is recommended for storage of working standards to provide protection from light**.**

Rubber closures can be used. Common rubbers used in pharmaceutical packaging are Butyl rubber, Bromobutyl rubber, Chlorobutyl rubber, Silicone rubber etc.

References

- WHO Technical Report Series No. 943 Annex-3, 2007, General guidelines for the establishment, maintenance and distribution of chemical reference substances, P-59-82.
- Indian Pharmacopoeia 2018, Volume-I, p-1021, 1057-1060.

WORKING STANDARD PREPARATION FLOW DIAGRAM



* *In many of the IP Monographs, there is a requirement of IPRS for herbal extracts.*

** *Till IPRS are made available, the stakeholders can use materials sourced from other pharmacopoeial agencies or taking commercial samples that are standardized for active constituents using RS marker compounds. In case RS markers are not available, the same may be purchased from any authentic source with COA available on record.*

Quantity of Drug Samples Required for Testing

In order to undertake complete testing as per IP, the minimum recommended quantities of drug samples required for testing is given in below table. The quantity suggested is only recommendatory. The actual quantity depends on the strength/potency of the drug present in the individual formulations or the amount of drug that is required for testing all the parameters as outlined in the IP.

S. No.	Name/Type of Drug Samples	Minimum Recommended Quantity*
1.	Tablets	60 Tablets
2.	Capsules	60 Capsules
3.	Syrup/ Suspension/ Emulsion/ Liniment	15 Units
4.	Injection	60 Ampoules/Vials
5.	Large volume parenteral infusion (≥ 100 ml)	30 Bottles
6.	Small volume infusion bottles (<100 ml)	60 Bottles
7.	Powder for injection (sterile)	20 Vials/Ampoules
8.	Dry powder for oral suspension	15 Bottles
9.	Bulk drugs	20 g in sealed glass bottle (5 x 4 bottles)
10.	Ointment/ gel (Non sterile)	15 Tubes
11.	Ointment/ gel (Sterile)	20 Tubes
12.	Eye/Ear drops	35 Vials
13.	Inhaler	15 Nos.
14.	Pessaries	50 Nos.
15.	Suppositories	50 Nos.
16.	Antitoxin/ Anti Serum	10 ml x 10 Vials/Ampoules 1 ml x 50 Vials/Ampoules
17.	Anti Snake Venom Serum/ Antirabies serum (ARS)	10 ml x 5 Vials/Ampoules 5 ml x 10 Vials/Ampoules
18.	Bacterial Vaccines	1 dose x 50 Vials/Ampoules 5 doses x 20 Vials/Ampoules 10 doses x 10 Vials/Ampoules
	BCG	10/20 doses x 40 Vials/Ampoules
19.	Viral Vaccines	1 dose x 50 Vials/Ampoules 5 doses x 20 Vials/Ampoules 10 doses x 10 Vials/Ampoules
	OPV	20 doses x 20 Vials/Ampoules
20.	Surgical Sutures	50 Strands
Immunodiagnostic Kits		
21.	HIV ELISA/CLIA/ELFA	96 Tests x 14 Kits
22.	HBsAg & HCV/Elisa/CLIA/ELFA	96 Tests x 14 Kits
23.	HIV Rapid	1200 Tests
24.	HBsAg & HCV Rapid	1200 Tests
25.	HIV Confirmatory	200 Tests
26.	HBsAg Confirmatory	200 Tests
27.	HCV Confirmatory	200 Tests
28.	Syphilis	1200 Tests
29.	Combo Kits	1200 Tests

Blood Reagents		
30.	Anti-A (Monoclonal), 10ml/5ml	6 Vials
31.	Anti-B (Monoclonal), 10ml/5ml	6 Vials
32.	Anti-AB (Monoclonal), 10ml/5ml	6 Vials
33.	Anti-A (Lectin), 10ml/5ml	6 Vials
34.	Anti-H (Lectin), 10ml/5ml	6 Vials
35.	Anti-D (IgG) Monoclonal, 10ml/5ml	6 Vials
36.	Anti-D (IgM) Monoclonal, 10ml/5ml	6 Vials
37.	Anti-D (IgG + IgM) (Mono-Mono Blend), 10ml/5ml	6 Vials
38.	Blood Grouping Kits (GEL Card for Reverse & Forward Grouping)	5 Boxes
39.	AHG (Anti Human Globulin), 10 ml	6 Vials
40.	BSA (Bovine Serum Albumin), 10 ml	6 Vials
Blood Products		
41.	Human Albumin (5%, 20%, 25%) 50ml/100ml/250ml	10 Bottles
42.	Normal Immunoglobulin I.V. (40 ml/50 ml/100 ml)	6 Bottles
43.	Factor VIII	12 Vials
44.	Factor IX	6 Vials
45.	Fibrin Sealant (1 ml)	20 Kits
	Fibrin Sealant (2 ml)	16 Kits
	Fibrin Sealant (5 ml)	8 Kits
46.	Specific immunoglobulin injection (5 ml)	36 Vials
Recombinant Products		
47.	rh. Insulin & Injection (vials)	28 Vials
	rh. Insulin & Injection [Pre-filled Syringes (PFS)]	56 PFS
48.	rh. Insulin analogues (vials)	28 Vials
	rh. Insulin analogues [Pre-filled Syringes (PFS)]	56 PFS
49.	Exenaxide (analogue), PFS	30 PFS
50.	rh. Erythropoietin injection	12 PFS
51.	Interferon (0.5ml PFS)	20 PFS
Enzymes & Hormones		
52.	Streptokinase (Inj.)	20 Vials
53.	Menotropin	6 Injections
54.	hCG (Human Chorionic Gonadotropin)	6 Injections
55.	Urofollitropin (FSH) - Normal FSH	10 Injections
	- Recombinant FSH	8 Injections
Biochemical Kits/Strips		
56.	Glucose Estimation Kit, 4 x 500 ml	2000 Tests
57.	Glucose Strips	2400
Veterinary Vaccines		
58.	Anthrax Spore Vaccine, Live	9
59.	Avian Infectious Bronchitis Vaccine, Inactivated	6
60.	Avian Infectious Bronchitis Vaccine, Live	9

61.	Avian Infectious Laryngotracheitis Vaccine, Live	12
62.	Avian Spirochaetosis Vaccine	9
63.	Blackquarter Vaccine	9
64.	Bluetongue Vaccine, Inactivated	9
65.	Brucella Abortus (Strain 19) Vaccine, Live	6
66.	Canine Adenovirus Vaccine, Live	12
67.	Canine Coronavirus Vaccine, Inanctivated	9
68.	Canine Distemper Vaccine, Live	12
69.	Canine Leptospirosis Vaccine, Inactivated	9
70.	Canine Parainfluenza Virus vaccine, Live	12
71.	Canine Parvovirus Vaccine, Inactivated	9
72.	Canine Parvovirus Vaccine, Live	12
73.	Ckassical Swine Fever Vaccine, Live	12
74.	Multicomponent Clostridium Vaccine, Inactivated	9
75.	Clostridium Novyi (Type B) Vaccine Inactivated for Veterinary Use	9
76.	Clostridium Septicum Vaccine, Inactivated	6
77.	Duck Pasteurella Vaccine, Inactivated	9
78.	Duck plague Vaccine,Live	9
79.	Egg Drop Syndrome'76 (Adenovirus) Vaccine, Inactivated	9
80.	Enterotoxaemia Vaccine, Inactivated	9
81.	Foot-and-Mouth Disease Vaccine, Inactivated	9
82.	Fowl Cholera Vaccine, Inactivated	9
83.	Fowl Pox Vaccine, Live	12
84.	Goat Pox Vaccine, Live	9
85.	Haemorrhagic Septicaemia Vaccine, Inactivated	9
86.	Haemorrhagic Septicaemia Vaccine-Alum Treated	6
87.	Inclusion Body Hepatitis (IBH) Vaccine, Inactivated	9
88.	Infectious Avian Encephalomyelitis Vaccine, Live	12
89.	Infectious Bursal Disease Vaccine, Inactivated	9
90.	Infectious Bursal Disease Vaccine, Live	12
91.	Infectious Canine Hepatitis Vaccine, Inactivated	9
92.	Infectious Chicken Anemia Vaccine, Inactivated	9
93.	Infectious Chicken Anemia Vaccine, Live	12
94.	Infectious Coryza Vaccine	9
95.	Marek's Disease Vaccine, Live	12
96.	Peste Des Petits Ruminants Vaccine, Live	12
97.	Rabies Veterinary vaccine, Inactivated (cell culture)	9
98.	Ranikhet Disease vaccine, Inactivated	9
99.	Ranikhet Disease vaccine, Live (Lentogenic strain)	12

100.	Ranikhet Disease Vaccine, Live (Mesogenic strain)	12
101.	Reo Virus Vaccine, Inactivated	9
102.	Reo Virus Vaccine, Live	12
103.	Salmonella Abortus Equi Vaccine	9
104.	Salmonella Vaccine, Inactinated	9
105.	Sheep Pox Vaccine, Live Attenuated	9
106.	Tetanus veterinary Vaccine	6

*These quantities are for guidance purpose only. Refer CDSCO for the current list.

Analytical Method Validation

Introduction

Validation is the process by which it is established, by laboratory studies, that the performance characteristics of the method meet the requirements for the intended application. All the analytical methods that are intended for analyzing any sample need to be validated. The suitability of all test methods used should always be verified under the actual conditions of use and should be well documented.

Validation Process

A well planned process should be followed during validation. Possible steps for a complete method validation are listed below:

1. **Develop a Validation Protocol**

   The first step in method validation is to prepare a written and approved protocol with the instructions in a clear step-by-step format. Following is a step-by-step guide for preparing protocols and performing test methods validation with reference to HPLC. One may use similar criteria for all other instrumental test method validation. Typical method validation protocol should include:

   - **Introduction:** Firms validation policy, general description
   - **Organizational structure:** Description of all personal responsibilities for all validation activities
   - **Process and product description:** Makes a brief description of the process and product or reference to adequate documents
   - **Specific process considerations:** describes critical characteristics of the process
   - **Key acceptance criteria:** General statement on acceptance criteria for the process
   - **Documentation format:** The format used for protocol and report is described
   - **Required SOPs:** a list of relevant SOPs should be mentioned
   - **Planning and Scheduling:** describes the resources, equipments and chemicals to be used, including time plan of the project
   - **Change control:** includes description or reference to the critical parameters variations in the process or product

Analytical Method Validation Protocol-Cover Page

Summary Information

Summary Information	
Organization Name	
Site Location	
Department Performing Validation	
Protocol Title	
Validation Number	
Equipment	
Revision Number	

Project Controller

Project Controller	Name	Signature	Date

Document Approval

Document Approval			
Department / Functional Area	**Name**	**Signature**	**Date**
Technical Reviewer			
End Lab Management			
Health & Safety			
Quality Assurance			
Documentation Control (reviewed and archived by)			

2. Validation Parameters

The analytical methods which need to be validated are as following:

- Identification tests: To ensure identity of an analyte
- Quantitative test for impurities: to accurately and quantitatively reflect the purity of a sample
- Limit test for impurities: to reflect purity characteristics of the sample
- Assay of drug substance and drug products: to measure accurately and quantitatively the analyte present in the sample. These methods also include analysis for content uniformity and measurement of analyte from dissolution samples

The characteristics which need to be validated for the different types of method are summarized in following Table.

Validation Characteristics	Assay	Testing for Impurities		Identification
		Quantitative	Limit	
Accuracy	Yes	Yes	No	No
Precision-Repeatability	Yes	Yes	No	No
Precision-Intermediate Precision	Yes	Yes	No	No
Specificity	Yes	Yes	Yes	Yes
Detection Limit	No	No	Yes	No
Quantitation Limit	No	Yes	No	No
Linearity	Yes	Yes	No	No
Range	Yes	Yes	No	No
Robustness	Yes	Yes	No	No

3. Analytical Performance Characteristics

(i) Specificity

Specificity (or selectivity) of the analytical method is defined as the degree to which a method can quantify the analyte in the presence of interferents. Specificity study of the chromatographic method is performed by the separation of the analyte from the other potential components such as impurities, degradants or excipients etc. The selectivity of

chromatographic methods may be assessed by examination of peak homogeneity or peak purity test. Peak purity test shows that there is no co-elution of any sample component. For this, peak purity assessment is done by using PDA or MS detectors. Representative chromatograms with peaks labeled should be included with resolution, plate count and tailing factor reported in the validation report.

- Test procedure
  The specificity of the assay method will be investigated by injecting of the extracted placebo to demonstrate the absence of interference with the elution of analyte.
- Documentation
  Print chromatograms.
- Acceptance criteria
  The excipient compounds must not interfere with the analysis of the targeted analyte.

(ii) Linearity

Linearity of a method is its ability to obtain test results that are directly proportional to the sample concentration over a given range. For HPLC methods, the linear relationship between detector response (peak area and height) and sample concentration is determined. The relationship can be demonstrated directly on drug substance by dilution of standard stock or by separate weighing of the sample components, using the proposed procedures.
Linearity should be evaluated by visual inspection of a plot of signals as a function of analyte concentration or content. If there is linear relationship, test results should be evaluated by appropriate statistical methods, for example, by regression analysis. Data from the regression line is helpful to provide mathematical estimates of the degree of linearity. It is generally expressed in terms of variance around the slope of regression line. In some cases, the analytical responses should be described by the appropriate function of the analyte concentration. The widely used linearity ranges and acceptance criteria for various pharmaceutical methods are listed in following Table.

Test	**Linearity Levels and Ranges**	**Acceptance Criteria**
Assay	Five levels, 50-150% of label claim	Correlation coefficient,R≥0.999
Dissolution	Five to eight levels, 10-150% of label claim	% y intercept NMT 2.0%; R≥0.99
Related Substances	Five levels, LOQ to acceptance criteria	% y intercept NMT 5.0%, R≥0.99

- Test procedure
  Standard solutions will be prepared at six concentrations, typically 25, 50, 75, 100, 150, and 200% of target concentration. Three individually prepared replicates at each concentration will be analyzed. The method of standard preparation and the number of injections will be same as used in the final procedure.
- Documentation
  Record results on a datasheet. Calculate the mean, standard deviation, and RSD for each concentration. Plot concentration (x-axis) versus mean response (y-axis) for eachconcentration. Calculate the regression equation and coefficient of determination (r^2). Record these calculations on the datasheet.

- Acceptance criteria

  The correlation coefficient for six concentration levels will be ≥0.999 for the range of 80% to 120% of the target concentration. The y-intercept must be ≤2% of the target concentration response. A plot of response factor versus concentration must show all values within 2.5% of the target level response factor, for concentrations between 80% and 120% of the target concentration. In general, the coefficient of determination for active ingredients should be ≥0.997, for impurities ≥0.98 and for biologics ≥0.95.

Linearity Data Sheet

Linearity – Data Sheet		Electronic File Name:	
Concentration (mg/ml)	Concentration as % of Analyte Target	Peak Area (mean of three injections)	Peak Area RSD (%)
5 (e.g.)	25		
10	50		
15	75		
20	100		
30	150		
40	200		

(iii) Range

Range of an analytical method is the interval between the upper and lower concentration of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy, and linearity. The range is normally derived from the linearity studies and depends on the intended application of the procedure. The following minimum specified ranges should be considered:

- For the assay method, normally covering from 80% to 120% of the test concentration.
- For content uniformity, covering minimum of 70% to 130% of the test concentration, based on the nature of the dosage form.
- For dissolution testing, ±20% over the specified range.
- For impurity determination, from reporting level of impurity to 120% of the specification.

The range of a method is confirmed when linearity, accuracy and precision criteria are fulfilled.

- Test procedure

  The data obtained during the linearity and accuracy studies will be used to assess the range of the method. The precision data used for this assessment is the precision of the three replicate samples analyzed at each level in the accuracy studies.
- Documentation

  Record the range on the datasheet.

- Acceptance criteria

  The acceptable range will be defined as the concentration interval over which linearity and accuracy are obtained per the above criteria, and in addition, that yields a precision of ≤3% RSD.

Range Data Sheet

Range – Data Sheet	Electronic File Name:
Record Range:	

(iv) Accuracy

The accuracy of an analytical method expresses the closeness of agreement between the value accepted either as a conventional true value or an accepted reference value and the value obtained. Practically no measurement process is ideal, therefore, the true or actual value cannot be exactly known in any particular measurement. The accepted true value for accuracy assessment can be assessed by analyzing a sample with known concentration. The accuracy studies are usually carried out by determining the recovery of the spiked sample of analyte into the matrix of the sample (a placebo) or by comparing the result to the results of a certified reference material of known purity. If the placebo of the sample is not available, the technique of standard addition is used. In case of methods for quantitation of impurities, the sample with known amount of impurities is assessed. Accuracy should be assessed using minimum of nine determinations over a minimum of three concentration levels covering the specified range (for e.g., three concentrations/three replicates each of the total analytical procedure). Accuracy should be reported as percent recovery by the assay of known added amount of analyte in the sample or as the difference between the means and the accepted true value together with the confidence intervals. The concentration should cover the range of concern. The expected recovery depends on the sample matrix, the sample processing procedure, and the analyte concentration. The reported limits for accuracy for drug substances and products are 98.0-102.0% and 97.0-103.0% respectively. For the impurity determination, range from 50-150% of average recovery may be accepted.

- Test procedure
  Spiked samples will be prepared at three concentrations over the range of 50 to 150% of the target concentration. Three individually prepared replicates at each concentration will be analyzed. When it is impossible or difficult to prepare known placebos, use a low concentration of a known standard.
- Documentation
  For each sample, report the theoretical value, assay value, and percent recovery. Calculate the mean, standard deviation, RSD, and percent recovery for all samples. Record results on the datasheet.
- Acceptance criteria
  The mean recovery will be within 90 to 110% of the theoretical value for non-regulated products. For pharmaceutical industry, 100±2% is typical for an assay of an active ingredient in a drug product over the range of 80 to 120% of the target concentration. Lower percent recoveries may be acceptable based on the needs of the methods. The required accuracy is a bias of ≤2% for dosage forms and ≤1% for drug substance.

Accuracy Data Sheet

Accuracy – Data Sheet		Electronic File Name:		
Sample	Percent of Nominal (mean of three injections)	Amount of Standard (mg)		Recovery (%)
		Spiked	Found	
1	75 (e.g.)			
2	100			

3	150			
Mean				
SD				
RSD%				

(v) Precision

Precision of an analytical method expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

Repeatability is the precision under the same operating conditions over a short interval of time. It is also termed as intra-assay precision. It is assessed by making six sample determinations at 100% concentration or by preparing three samples at three concentrations in triplicates covering the specified range for the procedure. It involves repeated determination of same sample.

Intermediate precision expresses within laboratories variation: different days, different analyst, different equipments, etc. It is the term synonymous with the term 'ruggedness'. The extent to which intermediate precision should be established depends on the circumstances under which the procedure is intended to be used. To study intermediate precision, use of an experimental design is encouraged. The intermediate precision is generally studied by multiple preparations of sample and standard solution. Reproducibility is the precision obtained by analysis between laboratories. It is generally assessed during collaborative studies at the time of technology or method transfer. It is assessed by means of an inter-laboratory trial.

The precision data is generally expressed in the form of standard deviation, RSD and confidence interval. To ensure precision of method for major analytes, RSD should be $\leq$2%. For low level impurities, RSD of 5-10% is usually acceptable.

Precision-Repeatability

- Test procedure
  One sample solution containing the target level of analyte will be prepared. Ten replicates will be made from this sample solution according to the final method procedure.
- Documentation
  Record the retention time, peak area, and peak height on the datasheet. Calculate the mean, standard deviation, and RSD.
- Acceptance criteria
  The RSD should be 1% for drug substances and 2% for drug products. For minor components, it should be ±5% but may reach 10% at the limit of quantitation.

Repeatability Data Sheet

Repeatability – Data Sheet		Electronic File Name:	
Injection No.	**Retention Time (min)**	**Peak Area**	**Peak Height**
Replicate 1			
Replicate 2			
Replicate 3			
Replicate 4			

Replicate 5			
Replicate 6			
Replicate 7			
Replicate 8			
Replicate 9			
Replicate 10			
Mean			
SD			
RSD%			

Intermediate Precision

- Test procedure
  Intermediate precision (within-laboratory variation) will be demonstrated by two analysts, using two HPLC systems on different days and evaluating the relative percent purity data across the two HPLC systems at three concentration levels (50%, 100%, 150%) that cover the analyte assay method range 80 to 120%.
- Documentation
  Record the relative % purity (% area) of each concentration on the datasheet. Calculate the mean, standard deviation, and RSD for the operators and instruments.
- Acceptance criteria
  The assay results obtained by two operators using two instruments on different days should have RSD ≤2%.

Intermediate Precision Data Sheet

Intermediate Precision – Datasheet				**Electronic File Name:**		
Sample	**Relative % Purity (% Area)**					
	Instrument 1			**Instrument 2**		
	S1 (50%)	S2 (100%)	S3 (150%)	S1 (50%)	S2 (100%)	S3 (150%)
Operator 1, Day 1						
Operator 1, Day 2						
Operator 2, Day 1						
Operator 2, Day 2						
Mean (Instrument)						
Mean (Operators)						
RSD%	S1 + S1	S2 + S2	S3 + S3			
Instruments						
Operators						

(vi) Limit of Detection (LOD)

The limit of detection of an individual analytical procedure is the lowest amount of analyte in the sample which can be detected but not necessarily quantified as an exact value. The detection limit can be determined in different ways. The simplest approach is based on the signal to noise ratio. The signal to noise ratio is determined by comparing measured signals from samples with known low concentration of analyte with those of blank samples. The concentration showing signal to noise ratio between 3:1 or 2:1 is generally considered as acceptable detection limit.

The other approach is based on the standard deviation of the response and the slope. The detection limit may be expressed as:

$$LOD = 3.3\ \sigma/S$$

where, σ = the standard deviation of the response
S = the slope of the calibration curve

The slope may be estimated from the calibration curve of the analyte. The σ can be estimated as the standard deviation of the blank. The value of σ can also be estimated based on the calibration curve. For this the specific calibration curve should be studied using sample containing analyte in the range of detection limit. The residual standard deviation of a regression line or the standard deviation of the y-intercept of regression lines may be used as standard deviation. Another approach for the estimation of the detection limit is based on visual evaluation. This method is applicable to non-instrumental methods but may be applied to the instrumental methods. The LOD is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected. The relevant chromatograms are sufficient for the justification of the detection limit.

- Test procedure
  The lowest concentration of the standard solution will be determined by sequentially diluting the sample. Six replicates will be made from this sample solution.
- Documentation
  Print the chromatogram and record the lowest detectable concentration and RSD on the datasheet.
- Acceptance criteria
  A signal-to-noise ratio of 3:1.

LOD Data Sheet

Limit of Detection – Data Sheet	Electronic File Name:
Record Sample Data Results: (e.g., concentration, S/N ratio, RSD%)	

(vii) Limit of Quantitation (LOQ)

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in the sample which can be quantitatively determined with suitable precision and accuracy. It is mainly affected by the detector sensitivity and accuracy of sample preparation. The quantitation limit can be determined in the similar way as that of the detection limit. It is the concentration showing signal to noise ratio of 10:1. Based on the standard deviation of the response and the slope it is calculated by the formula:

$$LOQ = 10\ \sigma/S$$

where, σ = the standard deviation of the response
S = the slope of the calibration curve

The value of S and σ are estimated as for the detection limit. The LOQ can also be established from the visual evaluation as the LOD. The analyte concentration should be quantifiable with acceptable accuracy and precision at LOQ level. Typical acceptance criteria for LOQ are mean recovery at this level between 50 - 150 % with % RSD of $\leq$25%.

- Test procedure
  Establish the lowest concentration at which an analyte in the sample matrix can be determined with the accuracy and precision required for the method in question. This value may be the lowest concentration in the standard curve. Make six replicates from this solution.
- Documentation
  Print the chromatogram and record the lowest quantified concentration and RSD on the datasheet. Provide data that demonstrates the accuracy and precision required in the acceptance criteria.
- Acceptance criteria
  The limit of quantitation for chromatographic methods has been described as the concentration that gives a signal-to-noise ratio (a peak with height at least ten times as high as the baseline noise level) of 10:1.2 The quantitation limit is the best estimate of a low concentration that gives an RSD of approximately 10% for a minimum of six replicate determinations.

LOQ Data Sheet

Limit of Quantitation – Data Sheet	Electronic File Name:
Record Sample Data Results: (e.g., concentration, S/N ratio, RSD%)	

(viii) Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. It is partially evaluated during method development stages. The aim of the robustness study is to identify the critical operating parameters for the successful implementation of the method. These parameters should be adequately controlled and a precautionary statement included in the method documentation. In case of an HPLC method, robustness study involves method parameters like pH, flow rate, column temperature and mobile phase composition which are varied within a reasonable range. The system suitability parameters obtained for each condition are studied to check the parameter which significantly affects the method.

Stability of the analytical solution and extraction time are other parameters which are also evaluated as additional parameters during robustness study. Stability of analytical solution is determined by assessing the results obtained by subjecting the analytical solution to the method parameters for longer period of time e.g. 4 hrs, 12 hrs, 24 hrs, 48 hrs etc. The acceptance criteria are based on relative difference between initial value and the value at specified solution stability time. For drug substances and drug products difference should be $\leq$2.0% and for impurity determination, it should be $\leq$10%.

When filtration is done during sample preparation filter paper study can be carried out. It involves analysis by filtering sample solution through different types of filter paper.

The chromatography obtained for a sample containing representative impurities, when using modified parameter(s), will be compared to the chromatography obtained using the target parameters. The effects of the following changes in chromatographic conditions will be determined: methanol content in mobile phase adjusted by ±2%, mobile phase pH adjusted

by ±0.1 pH units, column temperature adjusted by ±5°. If these changes are within the limits that produce acceptable chromatography, they will be incorporated in the method procedure.

Robustness Data Sheet

Robustness – Data Sheet	Electronic File Name:
Explain / Record Sample Data:	

(ix) System Suitability

System suitability testing (SST) is an integral part of many analytical procedures. The tests are based on the concept that the equipment, analytical operations and samples are the integral part of the system that can be evaluated as such. System suitability test provide the added assurance that on a specific occasion the method is giving, accurate and precise results. System suitability test are run every time a method is used either before or during analysis. The results of each system suitability test are compared with defined acceptance criteria and if they pass, the method is deemed satisfactory on that occasion. In case of HPLC methods, system suitability tests ensure the adequacy for performing the intended application on daily basis. The primary SST parameters considered are resolution (Rs), repeatability (% RSD of peak response and retention time), column efficiency (N), and tailing factor (T_f). The other SST parameters include retention factor (k) and separation factor (α). The limits which are considered for the SST parameters are listed following Table.

System Suitability Test	Limits
Resolution (R_S)	>2.0
Repeatability (RSD)	<1.0% for five replicates
Plate count (N)	>2000
Tailing factor (T_f)	≤2.0
Separation factor (α)	>1.0

- Test procedure

  System suitability tests will be performed on both HPLC systems to determine the accuracy and precision of the system by injecting six injections of a solution containing analyte at 100% of test concentration. The following parameters will be determined: plate count, tailing factors, resolution, and reproducibility (percent RSD of retention time, peak area, and height for six injections).

- Documentation

  Print the chromatogram and record the data on the datasheet

- Acceptance criteria

  Retention factor (k): the peak of interest should be well resolved from other peaks and the void volume; generally k should be ≥2.0. Resolution (Rs): Rs should be ≥2 between the peak of interest and the closest eluted peak, which is potentially interfering (impurity, excipient, and degradation product). Reproducibility: RSD for peak area, height, and retention time will be 1% for six injections. Tailing factor (T): T should be 2. Theoretical plates (N): ≥2000.

System Suitability Data Sheet

System Suitability – Data Sheet		Electronic File Name:		
System Suitability Parameter	Acceptance Criteria	Results		Criteria Met/Not Met
		HPLC 1	HPLC 2	
Injection Precision for Retention Time (Min)	RSD ≤ 1%			
Injection Precision for Peak Area (n = 6)	RSD ≤ 1%			
Injection Precision for Peak Height	RSD ≤ 1%			
Resolution (R_s)	Rs = ≥ 2.0			
USP Tailing Factor (T)	T = ≤ 2.0			
Capacity Factor (K)	K = ≥ 2.0			
Theoretical Plates (N)	N = ≥ 2000			

4. Revalidation

Revalidation is necessary whenever a method is changed and the new parameter is outside the operating range. The operating parameters need to be specified with ranges clearly defined. In case of methods for quantitation of impurities, if a new impurity is found that makes the method deficient in its specificity, it needs modification and revalidation. Changes in equipment or chemical quality may also have critical effects on method. So any such change needs revalidation.

Verification of Pharmacopoeial Methods

Method verification is performed for compendial methods to demonstrate their suitability under actual conditions of use for a specific drug substance and/or drug product. As the compendial analytical methods are validated methods, users are not required to validate these methods when first used in their laboratories, but verification of methods shall be performed to establish the objective evidence of suitability of the methods for their intended purpose. Verification consists of assessing selected analytical performance characteristics to generate appropriate data rather than repeating the validation process. If the verification of the compendial method is not successful, it may be concluded that the method may not be suitable for use with the article being tested in that laboratory and an alternate method, as allowed in the General Notices, may be developed and validated.

Verification requirements shall be determined based on an assessment of the complexity of the analytical method as well as the material to which the method is applied. Only those characteristics that are considered to be appropriate for the verification of the particular method need to be evaluated. Although complete revalidation of a compendial method is not required to verify the suitability of a procedure under actual conditions of use, some of the analytical performance characteristics, such as ……, may be used for the verification process. Verification should include an assessment of elements such as the effect of the matrix on the

recovery of impurities and drug substances from the drug product matrix, as well as the suitability of chromatographic conditions and column, the appropriateness of detector signal response, etc.

General Calculations Applied in IP

Pharmacopoeial calculations are indispensable part of almost every monograph procedure. Each monograph procedure uses various techniques, experimental designs, and corresponding formulae to calculate results. Following are examples of general calculations applied in IP:

1. Chemical Analysis

(i) Molarity

In IP, as a practice, all concentrations of solutions are expressed in the terms of molarity. Molarity (M) is defined as the number of moles of solute per litre of solution. The solute is defined as the substance being dissolved, while the solvent is the substance where the solute is dissolved (usually water).

Illustration: *Preparation of 1M NaOH (Sodium hydroxide)*

To prepare 1M NaOH solution, dissolve 40.0 g of sodium hydroxide pellets in 250 mL distilled water and then make up the solution to 1000 mL.

Where, V is volume in ml $M = \frac{1000}{V} X \frac{W}{Mol.Wt.}$

W is weight in gm

M is Molarity

(ii) Normality

Normality (N) is defined as the number of gram equivalent weight of solute per litre of solution. Gram equivalent mass is mass of one mole of element, molecule, ion divided by their valency or number of electrons.

Equivalent weight (E) = $\frac{\text{Molecular weight}}{n}$

Where, n= Number of replaceable H^+ ions (for acids), or Number of replaceable OH^- ions (for bases), or Number of total valence of anions or cations per mole (for salts)

Illustrations

- *H_2SO_4*
  Molecular weight - 98
  Number of replaceable H^+ ions per mole = 2 (as $H_2SO_4=2H^+ + SO_4^{2-}$)
  hence, E = 98/2 =49
  Gram- equivalent of H_2SO_4 is 49 g (26.63 ml)
  Wt per ml = 1.84 g
  1N Sulphuric acid solution is prepared by dissolving 49 g (27 ml) in 250 mL distilled water and then make up the solution to 1.0 litre.
- *NaOH*
  Molecular weight- 40
  Number of replaceable OH^- ions per mole- =1 (as $NaOH= Na^+ + OH^-$)
  hence, E=40/1=40
  Gram- equivalent of NaOH is 40 g
  1N Sodium Hydroxide solution is prepared by dissolving 40 g in 250 mL distilled water and then make up the solution to 1.0 litre.

Where, V is volume in ml $N = \frac{1000}{V} \text{ x } \frac{W}{Equivalent\ Wt.}$

W is weight in gram

N is Normality

(iii) **ppm**

ppm is an abbreviation of parts per million, equivalent to $10^{-6}$and used to describe very low concentrations.
One ppm is equivalent to 1 milligram of substance per litre of solvent (mg/L) or 1 milligram of substance per kilogram of solid or solvent(mg/Kg).

Note:
1 ppm = 1mg/L = 1µg /ml
1000 ppm = 1 g/1000ml
= 1000 mg/1000ml
= 1000000 µg/1000ml
= 1000 µg/ml
= 1000 ppm

ppm shows that the value is multiplied with one million (10^6), and % shows that the value is multiplied with one hundred (10^2).
So in order to convert ppm into %, divide the value by 10000. On the contrary, in order to convert % into ppm, multiply the value by 10000.
Following list is example of conversion.

- 1ppm=0.0001%
- 10ppm=0.001%
- 100ppm=0.01%
- 1000ppm=0.1%
- 10000ppm=1%

Illustration 1: Making up 1000 ppm solutions
1.From the pure metal:
e.g. Make a 1000 ppm standard of sodium (Na) using sodium metal.
weighaccurately 1.0 g of metal, dissolve in 1 : 1 concentrated nitric or hydrochloric acid, and make up to 1000 ml volume with deionised water.
2. From a salt of the metal:
e.g. Make a 1000 ppm standard of Na using the salt NaCl.
Molecular weight of sodium chloride (NaCl) salt = 58.44g.
Atomic weight of sodium(Na) = 23
1g Na in relation to Molecular weight of sodium chloride (NaCl) salt= 58.44 / 23 = 2.542g.
Hence, weigh out 2.542g NaCl and dissolve upto1000ml volume to make a 1000 ppm Na standard solution.

3. From an acidic radical of the salt:
e.g. Make a 1000 ppm phosphate standard using potassium dihydrogen phosphate salt (KH_2PO_4)
Molecular weight of potassium dihydrogen phosphate (KH_2PO_4) = 136.09
Molecular weight of phosphate (PO_4) radical = 95
1g PO4 in relation Molecular weight of potassium dihydrogen phosphate (KH_2PO_4)
= 136.09 / 95 = 1.432g.

Hence, weigh out 1.432g KH2PO4 and dissolve up to 1000 ml volume to make a 1000 ppm PO4 standard.

Illustration 2: Dilution from stock solution

Dilution Formula : C1V1 = C2V2

This equation applies to all dilutions.

C1 = available stock solution concentration

V1 = volume of stock solution required for dilution

C2 = required final concentration

V2 = required final volume

Example:

Prepare 100 ml of 100 ppm sodium solution from 50 ml of 1000 ppm stock solution of sodium (Na) standard using water as diluent.

C1 = 1000 ppm sodium (Na) standard solution

V1 =? ml

C2 = 100 ppm

V2 = 100 ml

V1 = (C2 x V2) / C1

= (100 ppm X 100 ml) / 1000 ppm

= 10 ml

This means pipette out 10.0 ml of the 1000 ppm sodium standard stock solution, dilute up to 100 ml with water to get solution of concentration 100 ppm.

.

2. Titrimetric Analysis

Titration is a method of volumetric analysis. A reagent, called the titrant is prepared as a standard solution. A known concentration and volume of titrant reacts with a solution of analyte to determine concentration. The volume of titrant reacted is called titration volume. There are many types of titrations. The most common types of qualitative titrations are acid-base titrations, redox titrations, precipitation titrations and complexometric titrations.

Illustration: Assay of Albendazole (Acid-Base Titration)

Assay: *Dissolve 0.5 g in 80 ml of anhydrous glacial acetic acid. Titrate with 0.1 M perchloric acid, using crystal violet solution as indicator. Carry out a blank titration. Factor: 1ml of 0.1M perchloric acid is equivalent to 0.02653 g of $C_{12}H_{15}N_3O_2S$ (Albendazole)*

To proceed with assay first 0.1M perchloric acid ($HClO_4$) is to be standardised as per IP.

Standardisation of 0.1M perchloric acid ($HClO_4$)

Weigh accurately about 0.35 g of potassium hydrogen phthalate ($C_8H_5KO_4$), previously powdered lightly and dried at 120° for 2 hours and dissolve it in 50 ml of anhydrous glacial acetic acid. Add 0.1 ml of crystal violet solution and titrate with the perchloric acid solution until the violet colour changes to emerald-green. Perform a blank determination and make any necessary correction.

Acceptance Criteria: The volumetric solutions should not differ from the prescribed strength by more than 10 per cent and the molarity should be determined with a precision of 0.5 per cent.

Factor : 1ml of 0.1M perchloric acid is equivalent to 0.02042 g of $C_8H_5KO_4$

Diluent : anhydrous glacial acetic acid

Indicator : crystal violet solution

Blank : Diluent + Indicator

Note: In potentiometric titrations indicator is not used.

Molarity = Weight of $C_8H_5KO_4$ (g) x Theoretical Molarity x Purity of $C_8H_5KO_4$
(Volume consumed- Blank) ml x Factor (g) x 100

Excel calculation format formolarity determination

	SET 1	***SET 2***	***SET 3***
Purity of potassium hydrogen phthalate	*99.96*		
Theoretical molarity	*0.1*		
Weight of potassium hydrogen phthalate (g)	*0.3505*	*0.3508*	*0.3502*
Blank (ml)	*0.2*	*0.2*	*0.2*
Volume consumed (ml)	*17.2*	*17.2*	*17.1*
Factor	*0.02042*		
Calculated molarity	*0.101*	*0.101*	*0.101*
Relative standard deviation	*0.0*		
Molarity (Mean)	*0.101*		

Calculation Formula:

Assay (% w/w) = (Volume consumed- Blank) X Actual Molarity X Factor X 100
(on as is basis) Weight of sample X Theoretical Molarity

Assay (% w/w) = (Volume consumed- Blank) X Actual Molarity X Factor X 100 X 100
(on dried/anhydrous basis) Weight of sample X Molarity X (100-Loss on drying/Water)

=Assay % w/w (on as is basis) X 100
(100- Loss on drying/ Water)

Excel calculation format of Assay by titration

	SET 1	***SET 2***
Theoretical Molarity	*0.1*	
Molarity calculated	*0.101*	
Weight of sample (g)	*0.5009*	*0.5006*
Blank (ml)	*0.2*	*0.2*
Volume consumed (ml)	*18.8*	*18.8*
Factor	*0.02653*	
Assay (on as is basis) (%w/w)	*99.5*	*99.6*
Loss on drying (LOD) (% w/w)	*0.12*	
Assay (on dried basis) (% w/w)	*99.6*	*99.7*
Mean	*99.6*	

3. Water Determination by Karl Fischer Titration

Karl Fischer titration is a titration method for measuring water content in basically all types of substances.Standardisation of Karl Fischer (KF) Reagentperformed using disodium tartrate. The water equivalence factor F, in mg of water per mL of reagent, is given by the formula:

$$F = \frac{W \times 2(18.02)}{V \times 230.08} \quad or \quad F = \frac{0.1566 \times W}{V}$$

Where, 36.04 is two times the molecular weight of water and 230.08 is the molecular weight of sodium tartrate dihydrate W is the weight in mg of sodium tartrate dihydrate and V is the volume in mL, of the reagent consumed in the titration.

Calculation Formula:

$$\text{Water content \% (w/w)} = \frac{\text{Volume of KF reagent consumed by Test sample} \times \text{Factor} \times 100}{\text{Weight of Test sample} \times 1000}$$

Illustration : Cefaclor (IP2018)

Water (2.3.43).*3.0 to 6.5 per cent, determined on 0.2 g.*

Excel calculation format for water content

KF Reagent Factor (mg/ml)	*5.01*	
	SET 1	***SET 2***
Weight of sample (g)	*0.2016*	*0.2018*
Volume of KF reagent consumed (ml)	*1.65*	*1.65*
Water Content (% w/w)	*4.10*	*4.10*
Mean	*4.10*	

4. Loss on Drying (LOD)

Illustration: Levosalbutamol Sulphate (IP2018)

Loss on Drying (2.4.19). *Not more than 2.0 per cent, determined on 1.0 g at 105°.*

$$\text{LOD (\% w/w)} = \frac{(W2-W3)\ g \times 100}{\text{Sample weight } g}$$

Excel calculation format for LOD

	SET 1	***SET 2***
Wt. of empty bottle in g (W1)	*53.42917*	*48.59443*
Wt. of bottle + sample wt. in g (before drying) (W2)	*54.42919*	*49.59949*
Sample wt. (g)	*1.00002*	*1.00506*
Wt of bottle + sample wt. in g (after drying) (W3)	*54.42010*	*49.58996*
LOD (% w/w)	*0.91*	*0.95*
Mean of LOD (%w/w)	*0.93*	

5. Sulphated Ash

Illustration: Levosalbutamol Sulphate (IP2018)

Sulphated Ash (2.3.18). *Not more than 0.1 per cent.*

$$\text{Sulphated Ash (\% w/w)} = \frac{(W3-W1)\ g \times 100}{\text{Sample weight } g}$$

Excel calculation format for Sulphated Ash

	SET 1	***SET 2***
Wt of empty crucible in g (W1)	*27.17329*	*26.27763*
Wt of crucible + sample wt. in g (before ignition) (W2)	*28.17364*	*27.27839*
Sample wt. (g)	*1.00035*	*1.00076*
Wt of crucible + sample wt. in g (after ignition) (W3)	*27.17374*	*26.27796*
Sulphated Ash (%w/w)	*0.04*	*0.03*
Mean ofSulphated Ash (%w/w)	*0.04*	

6. Specific Optical Rotation (SOR)

Optical rotation, 'α' is the property shown by certain substances of rotating the plane of polarisation of polarised light. Such substances are said to be optically active. This property is characteristic of some crystals and of many pharmaceutical liquids or solutions of solids. Where the property is possessed by a liquid or by a solute in solution, it is generally the result of the presence of one or more asymmetric centres, usually a carbon atom with four different substituents. The measurement of optical rotation, of a pharmaceutical article may be the only convenient means for distinguishing optically active isomers from each other and thus is an important criterion of identity and purity.

For solids

$$[\alpha]^t = \frac{\alpha x\ 100}{{}^{\lambda}c x\ l}$$

For Liquids

$$[\alpha]^{25} = \frac{\alpha}{{}^{\lambda}d^{25} x\ l}$$

where [α] is the specific optical rotation in degrees (°) at wavelength λ, t is the temperature, α is the observed rotation in degrees (°), l is the length of polarimeter tube in decimetres, and c is the concentration of the analyte in g per 100 ml.

In case of liquid samples, d^{25} is specific gravity of liquid or solution at 25° temperature.

Calculation Formula:

SOR (°) = Observed angle of rotation x Dilution of samplex 100
(On anhydrous/ l (dm)x Weight of test samplex (100-water content/Loss onDrying/Solvent content)
dried basis/Solvent content)

Illustration: Levosalbutamol Sulphate (IP2018)

***Specific optical rotation(2.4.22)**: - 30° to - 40°, determined on 1.0 per cent w/v solution.*

Excel calculation format for SOR

	SET 1
Weight of test sample (g)	*1.00032*
Dilution (ml)	*100*
Observed angle of rotation (°)	*-0.35*
Loss on drying (%w/w)	*0.93*
Specific Optical Rotation (°)	*-35.339*

7. Ultra-Violet Spectrophotometer

Illustration: Triamcinolone IP-2018

***Assay:** Dissolve 25 mg in sufficient ethanol (95 percent) to produce 100.0 ml and mix. Dilute 2.0 ml to 50.0 ml with ethanol (95 percent) and measure the absorbance of the resulting solution at the maximum at about 238 nm. (2.4.7)*

Calculations

(i) Using Specific Absorbance

$$\text{Assay (\% w/w)} = \frac{\text{Test absorbance}}{\text{Specific absorbance (E 1\%)}} \times \frac{1}{100} \times \frac{\text{Test dilution}}{\text{Wt. of sample (g)}} \times 100$$

$$\text{Assay (\%w/w) (On dried/anhydrous basis)} = \frac{\text{Assay \% w/w (on as is basis) x100}}{\text{(100- Loss on drying/Water)}}$$

Specific Absorbance	*370*	
	SET 1	***SET 2***
Test Weight(g)	*0.02517*	*0.02519*
Test dilution (ml)	*25.17 mg/100 ml*2ml/50 ml*	*25.19 mg/100 ml*2ml/50 ml*
Test Absorbance	*0.370*	*0.370*
% Assay (on as is basis)	*99.32*	*99.25*
Water content/LOD % (Assumed)	*0.37*	*0.37*
% Assay (on dried /anhydrous basis)	*99.69*	*99.61*
Mean % Assay (on as is basis)	*99.29*	
Mean % Assay (on dried /anhydrous basis)	*99.65*	

(ii) Using Standard Substance

Note: Standard and test solutions are prepared for same concentrations.

$$\text{Assay (\% w/w)} = \frac{\text{Test Absorbance}}{\text{Std Absorbance}} \times \frac{\text{Std weight}}{\text{Std dilution}} \times \frac{\text{Test dilution}}{\text{Test weight}} \times \text{Purity of Standard}$$

	SET 1	***SET 2***
Purity of Standard (% w/w)	*99.8*	
Std Weight(g)	*0.02500*	*0.02500*
Std dilution (ml)	*2500*	*2500*
Test Weight(g)	*0.02517*	*0.02519*
Test dilution (ml)	*25.17 mg/100 ml*2ml/50 ml*	*25.19 mg/100 ml*2ml/50 ml*
Std Absorbance	*0.369*	*0.369*
Test Absorbance	*0.370*	*0.370*
% Assay (on as is basis)	*99.44*	*99.37*
Water content/LOD %	*0.37*	*0.37*
% Assay (on dried /anhydrous basis)	*99.81*	*99.73*
Mean % Assay (on as is basis)	*99.40*	
Mean % Assay (on dried /anhydrous basis)	*99.77*	

8. High Performance Liquid Chromatography (HPLC)

Assay by HPLC involves estimation of the exact amount/quantity of substance present in sample matrix/test solution with respect to standard of known potency.

Preparation of solutions:

- *Blank preparation according to monograph.*
- *Reference solutions/working standard: working standard in diluents/mobile phase according to monograph.*
- *Sample solution/test solution: API or formulation in diluents/mobile phase according to monograph.*

Proposed sequence arrangement in assay is:

- *Blank*
- *Reference solution/Standard preparation (6 injections)*
- *Sample preparation 1-2*
- *Standard preparation(bracketing)*

System suitability parameters such as asymmetry (NMT 2), theoretical plates (NLT 2000) and %RSD (NMT 2%), resolution or any other parameter should be as per monograph.

Illustration: Ethionamide (IP 2018)

Ethionamide contains not less than 98.5 per cent and not more than 101.0 per cent of ethionamide ($C_8H_{10}N_2S$) calculated on dried basis.

Assay. Determine by liquid chromatography (2.4.14)

Reference Solution Preparation (Standard)

Dissolve 50 mg of the ethionamide RS in 100 ml of the mobile phase. Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.

Test Solution Preparation

Dissolve 50 mg of the substance under examination in 100 ml of the mobile phase. Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.

Calculation of assay for API

$$\text{Assay (\%) (On as is basis)} = \frac{\text{Area of Test}}{\text{Area of Std}} \times \frac{\text{Std weight}}{\text{Std Dilution}} \times \frac{\text{Test Dilution}}{\text{Test weight}} \times \text{Purity of Standard}$$

$$\text{Assay (\%) (On dried/anhydrous basis)} = \frac{\text{Assay \% w/w (on as is basis)} \times 100}{\text{(100- Loss on drying/Water)}}$$

Standard Ethionamide	
Purity of Standard	99.5
Weight in mg.	50.55
Dilution	50.55 mg/100 ml→5ml/50ml
No of injection	Area
1	2953606
2	2921057
3	2920293
4	2936718
5	2928947
Mean	2932124
STD DEV	13740
RSD	0.47

Test (Ethionamide API)	
Wt in mg.	50.75
Dilution	50.75 mg/100 ml→5ml/50ml
No of injection	Area
1	2929104
2	2929463
MEAN	2929284
Assay (mg) as is basis	99.01
LOD (% w/w)	0.12
Assay (%w/w) on dried basis	99.13

Calculation of assay for Tablet/Capsule

$$\text{Assay (in mg) (On as is basis)} = \frac{\text{Area of Test} \times \text{Std weight} \times \text{Test Dilution} \times \text{Purity of Standard} \times \text{Average Weight}}{\text{Area of Std} \times \text{Std Dilution} \times \text{Test weight} \times 100}$$

$$\text{Assay (label claim \%) (On as is basis)} = \frac{\text{Assay (in mg)}}{\text{Label claim of formulation (mg)}} \times 100$$

Illustration: Ethionamide Tablets

Ethionamide tablets contain not less than 95.0 per cent and not more than 105.0 per cent of ethionamide.

Assay. Determine by liquid chromatography (2.4.14)

Reference Solution Preparation (Standard)

Dissolve 50 mg of the ethionamide RS in 100 ml of the mobile phase. Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.

Test Solution Preparation

Weigh and powder 20 tablets. Weigh a quantity of the powder containing 50 mg of Ethionamide in 100.0 ml of the mobile phase. Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.

Standard Ethionamide	
Purity of Standard	*99.5*
Weight in mg.	*50.55*
Dilution	*50.55 mg/100 ml→5ml/50ml*
No of injection	*Area*
1	*2953606*
2	*2921057*
3	*2920293*
4	*2936718*
5	*2928947*
Mean	*2932124*
Standard Deviation	*13740*
%RSD	*0.47*

Test (Ethionamide Tablets 250 mg)	
Label Claim in mg	*250*
Weight in mg.	*58.75*
Average weight (mg)	*295*
Dilution	*58.75mg/100ml → 5ml/50ml*
No of injection	*Area*
1	*2901134*
2	*2897463*
MEAN	*2899299*
Assay (mg)	*249.73*
Assay (%)	*99.89*

Calculation of assay for Solution/Syrup/Suspension

$$\text{Assay (in mg/ml)} = \frac{\text{Area of Test} \times \text{Std weight} \times \text{Test Dilution} \times \text{Purity of Standard} \times W}{\text{Area of Std} \times \text{Std Dilution} \times \text{Test weight} \times 100}$$

Where; W = Weight per ml (mg/ml)

$$\text{Assay (label claim \%)} = \frac{\text{Assay (in mg/ml)}}{\text{Label claim of formulation (in mg/ml)}} \times 100$$

Illustration: Trimethoprim and Sulphamethoxazole Oral Suspension (IP2018)

Trimethoprim and Sulphamethoxazole Oral Suspension contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of Trimethoprim, $C_{14}H_{18}N_4O_3$, and Sulphamethoxazole, $C_{10}H_{11}N_3O_3S$.

Assay. Determine by Liquid Chromatography (2.4.14)

Test Solution

Dilute a volume of oral suspension containing 80 mg of sulphamethoxazole with 30 ml of methanol and sonicate for 10 minutes and dilute to 50.0 ml with methanol and filter. Dilute a volume of filtrate to obtain a solution containing 0.016 per cent w/v of sulphamethoxazole with the mobile phase.

Reference Solution

Dilute 5 ml of a solution containing 0.032 percent w/v of trimethoprim RS and 0.16 percent w/v of sulphamethoxazole RS in methanol to 50.0 ml with mobile phase.

Standard		
	Trimethoprim	*Sulphamethoxazole*
Purity of Standard	*99.7*	*99.6*
Weight in mg.	*32.48*	*80.26*
Dilution	*100 ml x 5ml/50 ml*	*50 ml x 5ml/50 ml*
No of injection	*Area (mAU)*	*Area (mAU)*
1	*738407*	*10200012*
2	*738399*	*10195088*
3	*736941*	*10202988*
4	*735672*	*10191089*
5	*740302*	*10191110*
Mean	*737944*	*10196057*
STD DEV	*1743*	*5333*
%RSD	*0.24*	*0.05*

Test (Cotrimoxazole Suspension)		
Co-Trimoxazole 40 mg/200 mg per 5ml Paediatric Suspension		
	Trimethoprim	*Sulphamethoxazole*
Label Claim (mg/5ml)	*40*	*200*
mg/ml	*8*	*40*
weight (mg)	*2405.68*	
weight/ml	*1032.953*	
Dilution	*50 ml→5ml/50 ml*	
No of injection	*Area (mAU)*	*Area (mAU)*
1	*817177*	*11861098*
2	*819000*	*11933215*
MEAN	*818089*	*11897157*
Assay (mg/ml)	*7.71*	*40.05*
Assay (mg/5 ml)	*38.54*	*200.25*
% Assay	*96.35*	*100.13*

9. Dissolution by HPLC

Preparation of solutions:

- *Blank preparation according to monograph.*
- *Reference Solutions/ Working standard: working standard in diluents/mobile phase according to monograph. (Concentration of working standard in ppm should be equivalent with concentration of test in ppm)*
- *Sample solution/ Test solution: 1 tablet/capsule in 250 ml/500 ml/900 ml of dissolution medium according to monograph.*

Calculation for Tablet/Capsule:

Evaluation using UV-Visible Spectrophotometer

$$\text{Content (mg/tab)} = \frac{\text{Abs of test} \times \text{Weight of Std} \times \text{Volume of dissolution medium (dilution)} \times \text{Purity of Standard}}{\text{Abs of Std} \times \text{Dilution of Std} \times \text{1 tablet/capsule} \times 100}$$

$$\text{(\% Content)} = \frac{\text{Content (mg/tab)} \times 100}{\text{Label claim (mg)}}$$

Illustration: Ethionamide Tablets (IP 2018)

Dissolution (2.5.2)

Apparatus No.2,

Medium. 900ml of 0.1M hydrochloric acid

Speed and Time: 100 rpm and 45 minutes

Withdraw a suitable volume of the medium, filter and dilute a suitable volume of the filtrate with the same solvent. Measure the absorbance of the resulting solution at the maximum at about 274 nm (2.7.4). Calculate the content of $C_8H_{10}N_2S$ from the absorbance of a solution of known concentration of ethionamide RS.

D. Not less than 75 per cent of the stated amount of $C_8H_{10}N_2S$

Standard	*Ethionamide*		
Batch No.	*IPRS/235/12*		
Std Wt (mg)	*27.82*		
Purity of Standard	*99.5*		
Dilution preparation	*27.82mg/100ml→2ml/50ml (11.13 ppm)*		
Standard		*Abs at 274nm*	
1	*0.4624*		
2	*0.4624*		
3	*0.4624*		
Mean	*0.4624*		
Test	*Ethionamide Tablets 250 mg*		
Label claim (mg)	*250*		
Dilution preparation	*1Tablet/900mL→2/50 (11.11 ppm)*		
No. of Tab	*Abs at 274 nm*	*Content (mg/tab)*	*Content%*
TEST-1	*0.4579*	*246.70*	*98.68*
TEST-2	*0.4530*	*244.06*	*97.63*
TEST-3	*0.4481*	*241.42*	*96.57*
TEST-4	*0.4604*	*248.05*	*99.22*
TEST-5	*0.4585*	*247.03*	*98.81*
TEST-6	*0.4536*	*244.39*	*97.75*
Max Dissolution (%)	*99.22*		
Min Dissolution (%)	*96.57*		
Mean Dissolution (%)	*98.11*		

Evaluation using HPLC

If evaluation is to be performed using HPLC, proceed as per Assay calculation by HPLC.

$$\text{Content (mg/tab)} = \frac{\text{Area of test} \times \text{Wt of std} \times \text{Volume of dissolution medium (Dilution)} \times \text{Purity of standard}}{\text{Area of std} \times \text{Dilution of std} \times 1 \text{ tablet/capsule} \times 100}$$

$$\text{(\% Content)} = \frac{\text{Dissolution (in mg)} \times 100}{\text{Label claim (mg)}}$$

Proposed sequence arrangement in assay is:

- *Blank*
- *Reference solution/Standard preparation (5 injections)*
- *Sample preparation 1-6*
- *Reference solution/Standard preparation(bracketing)*

System suitability observation such as Asymmetry (NMT 2), Theoretical plates (NLT 2000) and RSD% (NMT 2%) or as per monograph.

10. Uniformity of Content by HPLC

Uniformity of Content is a pharmaceutical analysis parameter for the quality control of capsules/ tablets (formulation). Multiple capsules or tablets are selected at random and a suitable analytical method is applied to assay the individual content of the active ingredient in each capsule or tablet.

Preparation of solutions:

- *Blank preparation, according to monograph.*
- *Reference Solutions/ Working standard: working standard in diluents/mobile phase according to monograph.*
- *Sample solution/ Test solution: 1tablet/capsule in diluents, according to monograph.*

Sequence arrangement in assay is:

- *Blank*
- *Reference solution/Standard preparation (6 injections)*
- *Sample preparation 1-10*
- *Reference solution (bracketing)*

System suitability observation such as Asymmetry (NMT 2), Theoretical plates (NLT 2000) and RSD% (NMT 2%) or as per method/monograph.

Calculation for uniformity of content in tablet/capsule (Formulations):

$$CU\ (in\ mg) = \frac{Area\ of\ test \times Weight\ of\ std \times Dilution\ of\ test \times Potency}{Area\ of\ std \times Dilution\ of\ std \times 1tablet/capsule \times 100}$$

$$CU\ (label\ claim\ \%) = \frac{CU\ (in\ mg)}{Label\ claim\ of\ formulation\ (mg)} \times 100$$

Calculation for tablet/capsule (Formulations) with API used as salt:

$$CU\ (in\ mg) = \frac{Area\ of\ test \times Weight\ of\ std \times Dilution\ of\ test \times Potency \times Factor}{Area\ of\ std \times Dilution\ of\ std \times 1\ tablet/capsule \times 100}$$

$$CU\ (label\ claim\ \%) = \frac{CU\ (in\ mg)}{Label\ claim\ of\ formulation\ (mg)} \times 100$$

$$Factor\ (F) = \frac{Molecular\ weight\ of\ sample\ (Base\ form)}{Molecular\ weight\ of\ sample\ (Salt\ form)}$$

Illustration: Primaquine Tablets

Statement of content: Primaquine Tablets contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of Primaquine $C_{15}H_{21}N_3O$.

Usual strength. 7.5 mg ; 15 mg.

(13 mg of Primaquine Phosphate is approximately equivalent to 7.5 mg of primaquine).

Uniformity of content. (For tablets containing 10 mg or less)

Complies with the test stated under tablets.

Acceptance Limits: Uniformity of Content of Single-Dose Preparations (2.5.4).

Determine by liquid chromatography (2.4.14), as described in the Assay, using the following solutions.

Test solution. Powder one tablet, dissolve in 20 ml of the mobile phase with the aid of ultrasound for 3 minutes and dilute to 50.0 ml with the mobile phase and filter.

Reference solution. Dissolve a weighed quantity of primaquine phosphate RS in the mobile phase and dilute with the mobile phase to obtain a solution having a known concentration similar to the expected concentration of the test solution.

Inject the reference solution and the test solution. Calculate the content of $C_{15}H_{21}N_3O$ in the tablet.

Excel format to show calculation using formula

Standard	*Ethionamide*
Standard	*Primaquine phosphate*
Purity of Standard	*99.5*
Wt in mg	*26.61*
Dilution	*26.61 mg/100ml*
No. of Inlection	*Area (mAU)*
1	*88.643*

2	*87.815*
3	*87.902*
4	*87.103*
5	*85.780*
AVG.	*87.449*
STDEV	*1.08*
%RSD	*1.24*

Primaquine Phosphate eq. to primaquine			
Sample Code	*F3471S5*		
Batch No.	*PE064002AS*		
Label claim(mg)	*7.5*		
Dilution	*1 Tablet/50*		
Tablet No.	*Area (mAU)*	*CONTENT/TAB (mg)*	*Uniformity of Content (%)*
1	*80.362*	*6.93*	*94.30*
2	*84.869*	*7.32*	*99.59*
3	*87.334*	*7.53*	*102.48*
4	*84.333*	*7.27*	*98.96*
5	*92.258*	*7.96*	*108.26*
6	*84.255*	*7.27*	*98.87*
7	*90.691*	*7.82*	*106.42*
8	*81.814*	*7.05*	*96.00*
9	*82.698*	*7.13*	*97.04*
10	*83.597*	*7.21*	*98.09*
AVG.	*85.221*	*7.35*	*100.00*
MIN	*6.93*	*94.30*	
MAX	*7.96*	*108.26*	

Content/Tab (mg) = $\frac{A_{test} \times W_{std} \times \text{Tab Dilution} \times P_{std} \times 259.347}{A_{std} \times \text{STD Dilution} \times 1 \text{ Tab} \times 100 \times 455.340}$

Factor (F) = $\frac{\text{Molecular weight of sample Primaqune (Base form)}}{\text{Molecular weight of sample Primaqune Sulphate (Salt form)}}$

Factor (F) = $\frac{259.347}{455.340}$

Content (%) = $\frac{\text{(Content/Tab)mg} \times 100}{\text{(Avg Content/Tab) mg}}$

11. Bacterial Endotoxin Test (BET)

(i) Maximum Valid Dilution (MVD)

MVD is the maximum allowable dilution of a sample at which the endotoxin limit can be determined. MVD is calculated by following general formula:

$$MVD = \frac{\text{Endotoxin Limit} \times \text{Concentration of the sample solution}^*}{\lambda}$$

Where, λ is the labelled sensitivity of the lysate (EU/ml).

*Concentration of the sample solution is expressed as mg/ml in case the endotoxin limit is specified by weight (EU/mg), or as Units/ml in case the endotoxin limit is specified by unit (EU/unit), or as 1.0 ml/ml in case the endotoxin limit is specified by volume (EU/ml).

Illustration: *If an injection has the concentration 40 mg/ml, endotoxin limit is 0.71 EU/mg, and lysate to be used in the test has the sensitivity of 0.03 EU/ml. Then MVD will be calculated as given below:*

$$MVD = \frac{0.71\ EU/mg \times 40\ mg/ml}{0.03\ EU/ml}$$

$$= 946.67\ (1: 946.67)$$

(ii) Endotoxin Limit

Endotoxin limit for a parenteral product defines on the basis of dose (effect of endotoxins are related to the amount of endotoxin in the product dose administrated to a patient). As dose varies from product to product, the endotoxin limit for a product is calculated from the following expression:

$$Endotoxin\ Limit = K/M$$

Where,

K is the threshold pyrogenic dose of endotoxin per kg of body mass. The value of K is the 5.0 EU per kg for parenteral preparations except those administrated intrathecally, and is 0.2 EU per kg for preparations intended to be administrated intrathecally, and

M is the maximum dose administered to an adult (taken as 70 kg for this purpose).

Illustration: *If an injection having maximum dose of 300 mg is administrated intramuscularly to an adult (taken as 70 kg for this purpose), then the endotoxin limit of this injection can be calculated as given below.*

$$Endotoxin\ Limit = \frac{5.0\ EU \times 70\ kg}{300\ mg}$$

$$= 1.16\ EU\ per\ mg$$

Note: *For extract. The value of K is not more than 20.0 EU per medical devices and not more than 2.15 EU per radiopharmaceutical products not administrated intrathically, the endotoxin limit is calculated as 175/V, where V is the maximum recommended dose in ml. For intrathecally administrated radiopharmaceuticals, the endotoxin limit is obtained by the formula 14/V. For formulations (anticancer products) administrated on a per square meter of body surface, the formula is K/M, where K= 2.5 EU per kg and M is the (maximum dose/m^2/hour/×1.80 m^2)/ 70 kg. For medical devices the endotoxin limit is calculated as K×N/V. Where, K is amount of endotoxin per device, N is equal to the number of devices tested and V is equal to the total volume of the medical devices in contact with the cerebrospinal fluid*

12. Antibiotic Assay Calculation (Cup-Plate Method)

Illustration:

Sample Name	: Erythromycin
Test Organism Used	: *Kocuria rhizophila* ATCC-9341
Assumed Potency of Ref. Std.	: 974 mcg/mg (as is basis)
Weight of Ref. Std.	: 10.4 mg
Weight of Sample	: 10.6 mg

Standard Dilution $= \frac{10.4 \times 974}{10} = 1012.96$ mcg/ml or ≈ 1013 mcg/ml

10.4 mg of Ref. Std. + 1.0 ml methanol + 9.0 ml phosphate buffer → 1013 mcg/ml
1.0 ml of 1013 mcg/ml + 9.13 ml phosphate buffer pH 8.0 → 100 mcg/ml
1.0 ml of 100 mcg/ml + 9.0 ml phosphate buffer pH 8.0 → 10 mcg/ml
4.0 ml of 10 mcg/ml + 6.0 ml phosphate buffer pH 8.0 → **4 mcg/ml (S_H)**
1.0 ml of 10 mcg/ml + 9.0 ml phosphate buffer pH 8.0 → **1 mcg/ml (S_L)**

Sample Dilution **=** $\frac{10.6 \times 974}{10}$ = 1032.44 mcg/ml or ≈ 1032 mcg/ml

10.6 mg Sample + 1.0 ml methanol + 9.0 ml phosphate buffer → 1032 mcg/ml
1.0 ml of 1032 mcg/ml + 9.32 ml phosphate buffer pH 8.0 → 100 mcg/ml
1.0 ml of 100 mcg/ml + 9.0 ml phosphate buffer pH 8.0 → 10 mcg/ml
4.0 ml of 10 mcg/ml + 6.0 ml phosphate buffer pH 8.0 → **4 mcg/ml (T_H)**
1.0 ml of 10 mcg/ml + 9.0 ml phosphate buffer pH 8.0 → **1 mcg/ml (T_L)**

Observations

Plate	Sample Zone Diameter (mm)		Standard Zone Diameter (mm)	
	T_H	T_L	S_H	S_L
1	25.3	21.3	25.4	21.3
2	25.0	21.2	25.3	21.2
3	25.1	21.2	25.1	21.4
4	25.2	21.1	25.4	21.5
Average	25.15	21.2	25.3	21.35

Calculation

% potency = antilog (2.0 ± a log I)
Where; I = ratio of dilution

$$a = \frac{(T_H+T_L) - (S_H+S_L)}{(T_H-T_L) + (S_H-S_L)}$$

$$a = \frac{(25.15+21.2) - (25.3+21.35)}{(25.15-21.2) + (25.3-21.35)} = \frac{46.35 - 46.65}{3.95 + 3.95} = -\frac{0.3}{7.9} = -0.0380$$

% of potency = antilog (2.0 − 0.0380×0.6021)
= antilog 1.9771 = 94.86

Potency of sample = $\frac{94.86 \times 974}{100}$ = 923.94 Units/mg (as is basis)

Water content in sample = 3.42%

Potency of sample (on anhydrous basis) = $\frac{923.94 \times 100}{100-3.42}$ = 957.65 Units/mg (on anhydrous basis)

13. Antibiotic Assay Calculation (Turbidimetric Method)

Illustration:

Sample Name : Amikacin sulphate
Test Organism Used : *Staphylococcus aureus* ATCC-29727
Assumed Potency of Ref. Std. : 1000 mcg/mg (as is basis)
Weight of Ref. Std. : 10.2 mg
Weight of Sample : 10.3 mg

Standard Dilution **=** $\frac{10.2 \times 1000}{}$ = 1020 mcg/ml

10

10.2 mg reference standard + 10 ml water → 1020 mcg/ml
1.0 ml of 1020 mcg/ml + 9.20 ml water → 100 mcg/ml
0.64 ml of 100 mcg/ml + 9.36 ml water → **6.4 mcg/ml (Std. 1)**
0.8 ml of 100 mcg/ml + 9.20 ml water → **8 mcg/ml (Std. 2)**
1.0 ml of 100 mcg/ml + 9.0 ml water → **10 mcg/ml (Std. 3)**
1.25 ml of 100 mcg/ml + 8.75 ml water → **12.5 mcg/ml (Std. 4)**
1.56 ml of 100 mcg/ml + 8.44 ml water → **15.6 mcg/ml (Std. 5)**

Sample Dilution **=** $\frac{10.3 \times 1000}{10}$ = 1030 mcg/ml

10.3 mg sample + 10 ml water → 1030 mcg/ml
1.0 ml of 1030 mcg/ml + 9.30 ml water → 100 mcg/ml
1.0 ml of 100 mcg/ml + 9.0 ml water → **10 mcg/ml (equivalent to Std. 3)**

Observations

Concentration of Reference Standard/ Sample		*% Transmittance*			*Average of % Transmittance*	*Log of Concentration*
		I	*II*	*III*		
Std. 1	*a*	*40.474*	*40.431*	*40.428*	*40.444*	*0.8062*
Std. 2	*b*	*42.840*	*42.683*	*42.641*	*42.721*	*0.9031*
Std. 3	*c*	*44.355*	*44.226*	*44.185*	*44.255*	*1*
Std. 4	*d*	*47.304*	*47.295*	*47.299*	*47.299*	*1.0970*
Std. 5	*e*	*53.273*	*50.237*	*50.157*	*51.222*	*1.1931*
Sample		*41.344*	*41.263*	*41.237*	*41.281*	*0.8551*

Calculation

$L = \frac{3a + 2b + c - e}{5}$

$\frac{(3 \times 40.444) + (2 \times 42.721) + 44.255 - 51.222}{5}$

$\frac{121.332 + 85.442 + 44.255 - 51.222}{5} = 39.96$

L = 39.96

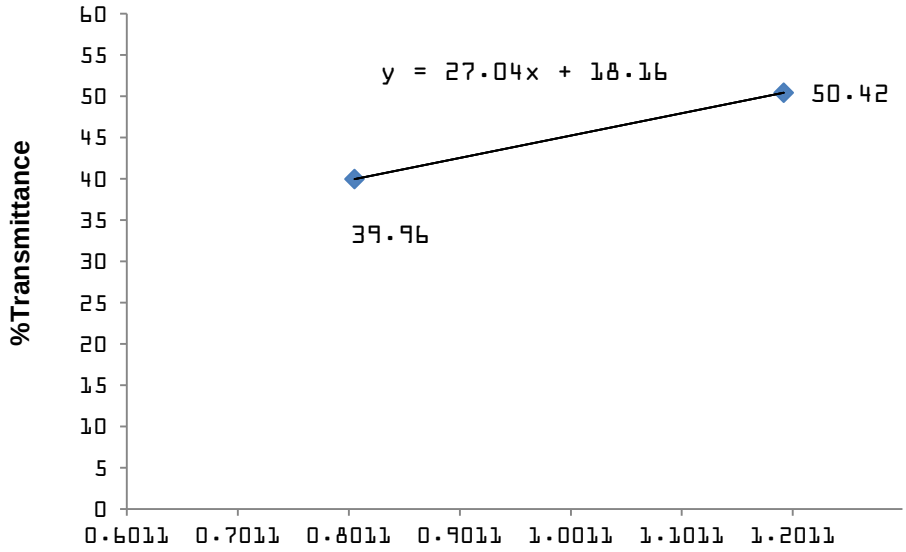
$H = \frac{3e + 2d + c - a}{5}$

$\frac{(3 \times 51.222) + (2 \times 47.299) + 44.255 - 40.444}{5}$

$\frac{153.666 + 94.598 + 44.255 - 40.444}{5} = 50.42$

H = 50.42

Reference Standard Concentration (mcg/ml)	***Log Value of Reference Standard Concentration***	***% Transmittance***
6.4	*0.8062*	*40.444*
8.0	*0.9031*	*42.721*
10	*1.0*	*44.255*
12.5	*1.0970*	*47.299*
15.6	*1.1931*	*51.222*



Concentration of Sample	*% Transmittance*	*Log Value*	*% Potency obtained*
10 mcg/ml	*41.281*	*Y = mx + c* *41.281 = 27.04x + 18.16* *x= 41.281 – 18.16/27.04* *= 23.121/27.04 = 0.8551*	*0.8551 – 1.0 = − 0.1449* *2.0 − 0.1449 = 1.8551* *Antilog of 1.8551 =* ***71.63***

Results

Potency of sample = $\frac{71.63 \times 1000}{100}$ = 716.3 µg/mg (on as is basis)

Environment Monitoring in Analytical Laboratory

Environment monitoring is the process of analyzing an environment sample whether water, soil, air or something else. Environment monitoring is necessary in a number of situations including for regulatory requirements and to examine the impact an activity have on the environment. Laboratory environments often include dust particles, hazardous processes and materials.

The analytical laboratory must be an entity that is legally authorised to function and can be held legally responsible. The environment of the laboratory is the most critical factor that affects the results of drugs during analysis. The most common factor is Heating Ventilation and Air Conditioning System (HVAC) or Air Handling Unit, Ventilation, Temperature & Humidity, and Light Intensity and Disposal of Waste Material for proper working as per the requirements. The laboratory environment should be sufficiently uncrowned, clean and tidy to ensure the quality of the work carried out is not compromised. It is the responsibility of management to maintain the environment of laboratory before functioning it. The premises of the laboratory and cleaning and sanitization of the laboratory is main tool before maintaining the environment of laboratory.

A. Premises of Laboratory

- The laboratory should be situated outside from the crowded area and to avoid the contamination from external environment as open sewage, drain, public lavatory or any factory to produce dust smoke and chemicals.
- Separate lab should be provided each for chemical, biological and microbiological.
- Special area should have for handling highly toxic, hazardous and sensitizing materials.
- Premises of the laboratory should be designed and maintained so as to prevent dust particle, insects and rodent.
- The laboratories shall be provided with adequate lighting and ventilation, air- conditioning to maintain required temperature and relative humidity that will not adversely affect the testing and storage of drugs or the accuracy of the functioning of the laboratory equipments or instruments.
- The air circulation in microbiology laboratory should maintain by airlocks and laminar air flow work station in the area where sterility test is carried out as per Schedule M.
  - Walls, floors and ceiling should be impervious, non-shedding, non-flaking and non-cracking. Flooring should be unbroken and provided with a cove both at the junction between the wall and the floor as well as the wall and the ceiling;
  - Walls shall be flat, and ledges and recesses shall be avoided. Wherever other surfaces join the wall (e,g, sterilizers, electric sockets, gas points etc.) these shall flush the walls. Walls shall be provided with a cove at the joint between the ceiling and the floor;
  - Ceiling shall be solid and joints shall be sealed. Light-fittings and air-grills shall flush with the walls and not hanging from the ceiling, so as to prevent contamination;
  - There shall be no sinks and drains in Grade A and Grade B areas;
  - Doors shall be made of non-shedding material. These may be made preferably of Aluminium or Steel material. Wooden doors shall not be used. Doors shall open towards the higher-pressure area so that they close automatically due to air pressure;
  - Windows shall be made of similar material as the doors, preferably with double panel and shall be flush with the walls. If fire escapes are to be provided, these shall be suitably fastened to the walls without any gaps;

- The furniture used shall be smooth, washable and made of stainless steel or any other appropriate material other than wood.

- Sink and drainage should be only wet lab and washing area. The drainage should be periodically monitored to avoid presence of pathogenic microbes.
- The drainage system facilities shall be such as to facilitate proper maintenance and prevent water logging in the laboratory.

B. Cleaning and Sanitization of laboratory

- The laboratory should be cleaned and maintained in an orderly manner by validated cleaning procedure, so that it is free from accumulated dust and waste.
- Standard Operating Procedure for cleaning and sanitization shall be prepared in accordance with the following requirements, namely:-
  - Preparation of disinfectants solutions, their grade and concentration
  - Specific areas to be cleaned and cleaning intervals
  - Equipment and materials to be used for cleaning purpose
  - Frequencies of use of disinfectant solutions
  - Personal assigned to and responsible to cleaning operation.
- All the disinfectants use in laboratory shall be properly identified with a label.
- Containers of the disinfectants shall bear the following details:-
  - Name of Disinfectant and concentration
  - Date of preparation;
  - Use Before;
  - Name of analytical chemist who prepared the disinfectant solution.
- **Disinfectants-** Disinfectants are antimicrobials agents that are applied to the surface of non living objects to destroy microorganisms that are living on the objects. Disinfectants work by destroying the cell wall of microbes or interfering with their metabolism.

  Sanitizers are substances that simultaneously clean and disinfect.Disinfectants are frequently used in laboratories, production areas and hospitals to kill infectious organisms.

  Generally disinfectants shall be classified into two categories;
  - Black Fluids
  - White Fluids

Black Fluids-These shall be homogeneous dark brown solution of coal tar acid or similar acids derived from petroleum with or without hydrocarbon, and/or other phenolic compounds, and their derivatives and a suitable emulsifier.

White Fluids- These shall be finely dispersed homogeneous white to off- white emulsion consisting of coal tar acids or similar acids derived from petroleum, with or without hydrocarbons, and/or other phenolic compounds, and their derivatives.

Gradation of Disinfectants-Both of the above classes of disinfectants fluids shall be graded on the basis of Rideal Walker Coefficient (RWC) as follows:

Grade	Rideal Walker
1	18
2	10
3	5

Types of Disinfectants-

There are some types of disinfectants which are used in laboratory for cleaning and sanitization.

1. Air Disinfectants- Air disinfectants are typically chemical substances capable of disinfecting microorganisms suspended in the air. An air disinfectant must be dispersed either as an aerosol or vapor at a sufficient concentration in the air to cause the number of viable infectious microorganisms to be significantly reduced.

2. Alcohols- Alcohol and alcohol plus quaternary ammonium cation based compounds comprise a class of proven surface sanitizers and disinfectants approved by the Environment Protection Agency and the Centers for Disease Control for use as a hospital grade disinfectant. Alcohols are most effective when combined with distilled water to facilitate diffusion through the cell membrane; 100% alcohol typically denatures only external membrane proteins. A mixture of 70% ethanol or isopropanol diluted in water is effective against a wide spectrum of bacteria, though higher concentrations are often needed to disinfect wet surfaces. **Tricogel** contains 75% ethanol.

3. Aldehydes-Aldehydeshave a antibacterial, antifungaland are sporicidal activity.Formaldehydeand glutaraldehyde are commonly used aldehydes in disinfectants. 2 % solution inhibits the activity of the enzyme in the cell, denature the cell proteins. Some bacteria have developed resistance to glutaraldehyde, and it has been found that glutaraldehyde can cause asthma and other health hazards; hence ortho-phthalaldehyde is replacing glutaraldehyde. **Glutarex** contains glutaraldehyde is the example of this disinfectant.

4. Oxidizing Agents- Oxidizing agents act by oxidizing the cell membrane of microorganisms, which results in a loss of structure and leads to cell lysis and death. A large number of disinfectants operate in this way. Chlorine and oxygen are strong oxidizers.

- **Hydrogen Peroxide-** Hydrogen peroxide is used in laboratories to disinfect surfaces and it is used in solution alone or in combination with other chemicals as a high level disinfectant. Hydrogen peroxide is sometimes mixed with colloidal silver. It is often preferred because it causes far fewer allergic reactions than alternative disinfectants. A 3% solution is also used as an antiseptic.The antimicrobial action of hydrogen peroxide can be enhanced by surfactants and organic acids. **Gramicide** contains hydrogen peroxide and silver compound.

5. Phenols-Chlorocresol and chloroxylenol are used as disinfectants. These are general disinfectants and also have antifungal and antiviral properties. It denatures the proteins and enzymes of the cell.Dettol contains chloroxylenol and **Lysol** contains p-Chloro-o-benzylphenol is example of this type disinfectant.

6. Quaternary ammonium compounds- Quaternary ammonium compounds (Quats), such as benzalkonium chloride, are a large group of related compounds. A 0.0002% solution is effective against the vegetative cells. It increases the permeability of the cell membrane and causes the coagulation of cell contents.

7. Chlorhexidine Gluconate-It is ageneral antiseptic. A 0.5% solution kills all the bacterial vegetative cells. It destroys the cell content by coagulation. **Savlon** contains chlorhexidine gluconate and cetrimide.

- **Procedure for Cleaning and sanitization**
  - Prepare the disinfectant solution by taking required amount of disinfectant and add purified water to make up desired final volume. Filter the disinfectant under the LAF with the help of filtration assembly.
  - Outer surface of equipment, cabinet and accessories, glass windows and doors with lint free mop soaked with 70% filtered Isopropyl alcohol.

- Mop the floor of Microbial Limit Test lab, Endotoxin lab, Aseptic area, Change room, and Media preparation room with validated disinfectant such as Dettol, Savlon, Benylkonuium Chloride or Lizol with non-fibre shedding mop.
- Clean the doors, walls, ceilings and riser grill with dry non-fibre shedding mop.
- Sanitation of drain point by filling the cup of the drain with disinfectant or flush the drain with disinfectant.
- Inspect and ensure the cleanliness of the area and record.

➢ **Frequencies of cleaning**

- Frequency of the cleaning in laboratory should be twice a day, beginning and at the end of the working hours or whenever required.
- The other ancillary areas shall be cleaned once in a day and whenever required.
- Cleaning of doors, walls, glass windows, riser grill and ceiling shall be weekly or when required.

➢ **Precautions**

- The interior surface of the floor, wall and ceilings should be smooth and cracks free and open joints to avoid accumulation of dust so that permit easily clean and disinfect.
- Perform the area cleaning and sanitation when there is no activity.
- Handle the disinfectant carefully to avoid contact with skin and eyes.
- Use fresh and only validated disinfectants for microbiology laboratory.
- Do not use expired disinfectant solutions.
- Rotate the disinfectant once in a week sequentially to avoid the development of resistance by microorganisms.
- Use separate lint free mop and fresh solution for cleaning of different area to avoid contamination of mop and solution.
- Use only dedicated buckets/containers for preparation and filtration for different disinfectants.

C. Environment Monitoring (in respect of Chemical Laboratory)

➢ Air ventilation system should ensure dust free environment.

➢ The analytical instruments shall be housed in dust-free environment and whenever required, conditions of temperature and humidity shall be maintained and periodic checks on temperature and humidity be made and recorded.

➢ The temperature and humidity within the chemical laboratory are maintained within limits for the proper performance of each test or analysis and maintained according to the manufacturer's specifications for the proper operation of instruments. A comfortable working environment is considered 20 to 25^{o}C with relative humidity of 35 to 50% depending on requirement.

➢ Chemical Laboratory operations often involve use of toxic, hazardous or odoriferous chemicals. The handling of these chemicals particularly digestion activities should be safely performed in a fume hood or fume cupboard. The size and type of fume hood (Bench or Walk-in) shall depend upon lab requirements and volume of work. The non AC labs may use conventional type or automatic by- pass fume hoods. The AC labs shall use auxiliary or variable flow volume fume hoods. A dedicated fume hood is required for extensive usage of perchloric acid greater than 50% of total chemical usage.

➢ The exhaust system of the fume cupboard shall be checked frequently to ensure that it is in order. There should be a water drainage system inside the fume cupboard and shall be checked frequently to ensure that there is no water logging and it is in order.

- No laboratory air is recycled through the building.

D. Environment Monitoring (in respect of Microbiology Laboratory)

Clean rooms have a very special place and purpose in aseptic areas such as sterility lab. The establishment, maintenance, and control of the microbiological quality of the clean room and the controlled environments require engineering and science based approach. Aseptic area maintained by when microorganisms entry are prevented by providing high quality of air in the room, using HEPA (High Efficiency Particulate Air) filters that provide Laminar Air Flow. This chapter will discuss aspects, which represent the regulatory agencies main concern areas.

- **Classification of cleanroom**
  - The design of the laboratory should include consideration for guidelines that are relevant to clean room standards. The design and construction of clean rooms and controlled environments are covered in the ISO 14644 guidelines. There are other standards, which are also referred to by different regions, such as EU-GMPs Annex-1, WHO guidelines, US Federal Standard 209E. All standards generally, define air cleanliness by the absolute concentration of airborne particles. Methods used for the assignment of air classification of controlled environments and monitoring of air borne particles are included.
  - Classification of clean room is based on limits of all non viable particles with sizes equal to or larger than 0.5 µm. The aseptic area in microbiology deals with class 100 and above. Clean rooms are maintained under a state of operational control on the basis of operational data.
  - Separate AHU should be permitted for microbiological lab and different from other labs.Critical areas, such as the sterility testing area, buffer zone area and change room conforming to Grades B, C and D, respectively shall have separate air handling units. The filter configuration in the air handling system shall be suitably designed to achieve the Grade of air as given in Table1.

Table 1- Air Borne Particulate Classification for Aseptic Area in Microbiology Lab

Grade	Maximum number of permitted particles per cubic meter equal to or above			
	At rest		In operation	
	0.5 µm	**5.0 µm**	**0.5 µm**	**5.0 µm**
A	3500	0	3500	0
B	3500	0	350,000	2,000
C	350,000	2,000	35,00,000	20,000
D	35,00,000	20,000	Not defined (c)	Not defined (c)

  - Air borne particles shall be counted by Air borne particulate counter, duly calibrated with national / international traceability.
  - Air conditioning system shall be in continuous operation for at least 24 hours prior to performing these tests. Particulate count for all pre decided location for critical and non-critical area, at the working height, should be taken.
  - **Acceptance Criteria-**The clean room or clean zone shall meet the acceptance criteria for an air borne particulate cleanliness if
    - the average of the particulate count measured at each location falls at or below the class limit, and

- The mean of these averages falls at or below class limit with a 95% confidence limit.

- **Air Velocity Measurement and Air Changes Calculation in clean room**
  - Air Velocity shall be measured by calibrated digital anemometer.
  - Ensure that the blowers are switched "ON" prior to the start of the observations.
  - Measure the air velocity, at 5 different locations for each HEPA filter (Four Corners and center) with the help of calibrated Anemometer at following height
    - At Critical areas where the Filtered Laminar Air Flow is required, air velocity shall be checked at working height (3-4 feet from the floor)
    - At Controlled area, air velocity shall be checked at2-6 inches below the filter face.
  - Calculate the average velocity of the air coming from Supply Grill / Terminal Filter.
  - Calculate the airflow by multiplying the average velocity with the effective filter area.
  - Air flow = Average Velocity × Face Area of the Air Inlet Grill / Filter
    = Ft / Min. × Ft^2
    = Ft^3 / Min. or CFM
  - Calculate the airflow for all the Supply Grill / Terminal Filter in the room and add values to get the total airflow in the room (CFM).
  - Calculate the number of air changes per hour in the room by using the formula :

$$\text{Air Changes / hour} = \frac{\text{Total air flow in the room (CFM)} \times 60}{\text{Room Volume } (Ft^3)}$$

  - **Acceptance Criteria**
    The minimum air changes for Grade B and Grade C areas shall not be less than 20 air changes per hour in a room with good air flow pattern and appropriate HEPA filters. For Grade A laminar air flow work stations, the air flow rate shall be 0.3 meter per second ± 20% (for vertical flows) and 0.45 meter per second ± 20% (for horizontal flows).

- **Integrity Test of HEPA Filters**
  - The measurement is to be done by using a calibrated aerosol photometer having traceability to national/international standards.
  - Feed the DOP (Di Octyl Pthalate) aerosol now replaced by PAO (Poly Alpha Olephin) at the fresh air inlet of AHU.
  - Connect one end of DOP measuring probe to the upstream port of photometer.
  - Photometer will scan the DOP for 100% concentration.
  - Scan the downstream side of the filter with an appropriate photometer probe at a sampling rate of at least 1 ft^3 / min. The probe should scan the entire filter face and frame at a position about 1 to 2 inches from the face of the filter. Scanning shall be done at the rate of maximum 2 feet per minute.
  - **Acceptance Criteria**
    During scanning percentage of the DOP penetration shown by photometer should be less than 0.01% through the filter media and should be ‘zero’ through mounting joints.

- **Differential Pressure of Air**
  - Air Pressure Differential test shall be performed by pre calibrated magnehelic gauge.
  - All HVAC and laminar flow systems shall be in continuous operation before monitoring.

- To avoid unexpected changes in air pressure and to establish a baseline, all doors in the facility must be closed and no man movement to be allowed during the observations. Observe the differential pressure through differential pressure display unit.
- Differential pressure between areas of different environmental standards shall be at least 15 Pascal (0.06 inches or 1.5 mm water gauge).
- Pressure differentials across the two rooms of same class shall be at least 5 Pascals (0.5 mm water gauge).
- The pressure differentials in the change rooms shall be in the descending order from Grade A to Grade D.

➢ **Temperature and Relative Humidity Test**

- AHU shall be in continuous operation for at least 24 hours prior to performing these tests.
- Temperature shall be monitored by calibrated thermometer and humidity by calibrated thermohygrometer.
- A comfortable working environment for microbiology lab is considered at temperature 27 ± 2 ^{0}C with relative humidity of 55 ± 5%respectively.

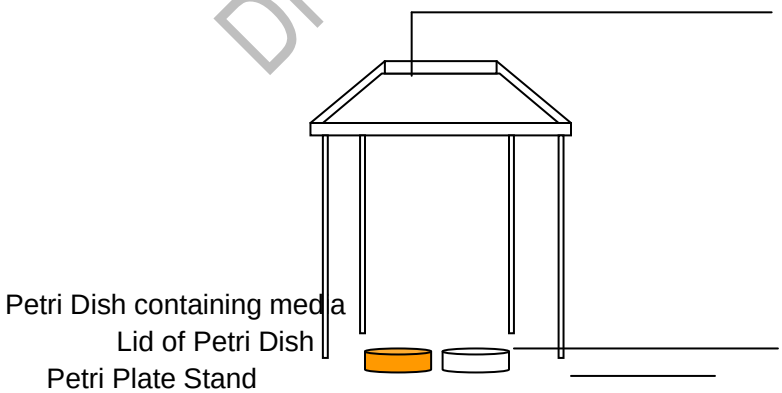
➢ **Air Borne Viable Particle Monitoring**

- Environmental microbiological monitoring should reflect the facility used (Air Lock) and include a combination of air and surface sampling methods appropriate to the facility, such as:

Passive air sampling by Plates Exposure Methods,
Activeair sampling by Air Sampler,
Surface monitoring by RODAC (Replicate Organism Detection and Count) plate or by swabs,
Personnel Monitoring (Gloves etc.) by RODACplate or by swabs.

Passive Air Sampling

– Transfer the Soyabean Casein Digest Agar plates to Aseptic area through pass box.
– Mark the plates indicating location of exposure and date of exposure.
– Take the plates to the respective location, Open the lid of the plate, and keep them aside in such a way that edges of the lid shall be in upward direction as shown in diagram.



- Expose the plates at working height for 4hours at the location mentioned in Table 2.
- At the end of exposure period close the plate with there lids again.
- Bring them for incubation. Incubate the plates first at 30 ^{0}C to 35 ^{0}C for 48hours and then transfer them to 20 ^{0}C to 25 ^{0}C for further 72 hours.
- Keep unexposed media plates of same media as negative control plates along with the sample plates in order to check the sterility of the media.
- Observe the plates first after 48hours and then after 120 hours respectively.

Active Air Sampling

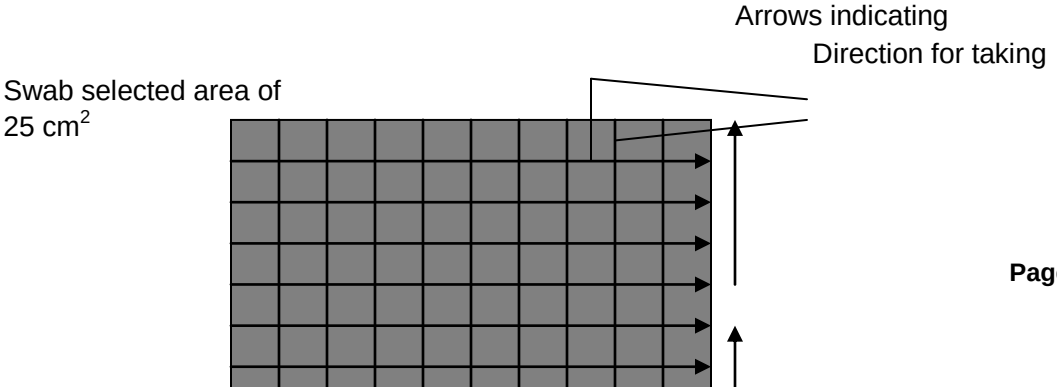
- Transfer the Soyabean Casein Digest Agar plates to Aseptic area through pass box.
- Mark the plates indicating location of air sampling and date of sampling.
- Take the plates and air sampler to the respective location, Open the lid of the plate, and place them in air sampler and operate.
- Time and speed has to set such a way so that it will suck 1000 liter of air.
- After completion of sampling collect all the plates and incubate in inverted position in the incubator first at 30 ^{0}C to 35 ^{0}C for 48 hours and then transfer them to 20 ^{0}C to 25 ^{0}C for further 72 hours.
- Keep unexposed media plates of same media as negative control plates along with the sample plates in order to check the sterility of the media.
- Observe the plates first after 48hours and then after 120 hours respectively.

Surface Monitoring by RODAC or Contact Plate

- Surface monitoring of Walls and floor and equipments lying in class 10000 or Grade C shall be done by this method.
- The size of contact plate shall be 55mm.
- Bring the contact plate in contact with the surface and press it gently there so that surface which is to be monitored shall be brought in contact with the surface.
- Sampling shall be done in duplicate in each area. Each plate shall be marked indicating location of sampling and sampling date.
- Cover the plate with the lid and incubate them first at 30 ^{0}C to 35 ^{0}C temperature for 48 hours and then at 20 ^{0}C to 25 ^{0}C temperature for next 72 hours.
- Observations shall be recorded first after 48hrs and then after 120hrs.

Surface Monitoring by Swab Method

- Surface monitoring of Walls and floor and equipments lying in class 10000 or Grade C shall be done by this method.
- Area of 24 to 30cm^2 shall be selected for taking swabs.
- Swabs shall either be pre- sterilized or non sterile swabs can be sterilized at about 121 ^{0}C for 20 min.
- Sterilized swabs shall be placed in 10mL of sterile 0.9% saline solution
- Rub the swab gently over the surface which is to be monitored as shown in diagram.



5 cm

5 cm

- Keep them back in the tubes containing 0.9% saline solution. Each tube shall be marked with the location and date of sampling.
- Take 1mL of saline suspension containing swab sample in, pre sterilized Petri dish.
- Pour molten Soyabean casein digest agar media, in duplicate. Mark the plates indicating location and date of sampling.
- Let the plates solidify.Incubate them first at 30 ^{0}C to 35 ^{0}C temperature for 48 hours and then at 20 ^{0}C to 25 ^{0}C temperature for next 72 hours.
- Observations shall be recorded first after 48hrs and then after 120hrs.
- Multiply the count observed in each plate with 10, so as to get the actual count of that area.

Personnel Monitoring

- Monitoring of garments and gloves of personnel working in class 10000 areas shall be done by contact plate method.
- Take required number of contact plates for sampling.
- Label them as follows:
  Name of personnel
  Section
  Place of Sampling
  Test
  Date of test
- Press the contact plate gently on the surface of the garments worn by the personnel working in the area and cover it with lid.
- Incubate them first at 30 ^{0}C to 35 ^{0}C temperature for 48 hours and then at 20 ^{0}C to 25 ^{0}C temperature for next 72 hours.
- Monitoring shall be done in duplicate.
- Observations shall be recorded first after 48hrs and then after 120hrs.

Table 2- Locations for Air Viable Count

S. No.	Location
1	Air Lock I
2	Air Lock II
3	Air Lock III
4	Near Autoclave in Buffer room
5	Near Laminar Air flow
6	Near Entry door LAF room
7	Near Return door LAF room
8	Near pass box

9	Return Room

- There shall be a written environmental monitoring program and microbiological results shall be recorded. Recommended limits for microbiological monitoring of clean areas in operation are as given in the Table 3:

Table 3- Recommended Limits of Microbiological Monitoring of Clean Areas "In operation" in Microbiology Lab

Grade	Air sample Cfu/m^3	Settle plates (dia. 90mm) Cfu/4 hrs.	Contact plates (dia. 55mm) cfu per plate	Glove points (five fingers) cfu per glove
A	< 1	< 1	< 1	< 1
B	10	5	5	5
C	100	50	25	---
D	500	100	50	---

NOTE: -These are average values.

Individual settle plates may be exposed for not less than two hours in Grade B, C and D areas and for not less than thirty minutes in Grade A area.

- Appropriate action shall be taken immediately if the result of particulate and microbiological monitoring indicates that the counts exceed the limits. The Standard Operating Procedures shall contain corrective action. After major engineering modification to the HVAC system of any area, all monitoring shall be re-performed before production commences.
- All the environmental parameters shall be verified and established at the time of installation and thereafter monitored at periodic intervals. The recommended frequencies of periodic monitoring shall be as follows
  - Particulate Monitoring in Air – Half Yearly
  - HEPA Filter Integrity Testing (Smoke Test) – Yearly
  - Air Change Rates – Half Yearly
  - Air Pressure Differentials – Daily.
  - Temperature and Humidity – Daily
  - Microbiological Monitoring by Settle Plates and/or Swabs in aseptic areas– Daily, and at decreased frequency in other areas.

NOTE: -The above frequencies of monitoring shall be changed as per the requirements and load in individual cases.

E. Light Intensity

- Sufficient light is necessary at the workplace to get better work output. Less lighting in working area can result in errors in different ways. None should experience eye-strain during his work.
- Poor lighting can affect the health of the people working at workplace causing headache, eyestrain and migraine. It is also linked to Sick Building that causes headache and poor concentration of the people.
- The laboratory maintains illumination sufficient for the procedure being performed.
- Light intensity is measured in Lux. Lux is a standard unit for light intensity measurement. 1 Lux is equal to the light intensity of the surface 1 meter away from a single candle.

- Light intensity is measured by using an instrument called lux meter at the working height about 1 meter from the ground. Light intensity is measured at a minimum of five locations and an average of all readings is calculated to determine the final light intensity of the room.
- The intensity of light in different areas may be varies such as
  - Ideal intensity of light for office is 300-500Lux;
  - For production areas 400 Lux;
  - For sampling and dispense booth above 300 Lux;
  - Generally inspection rooms have light having its intensity above 500 Lux.
- Specialized lighting may be needed in areas where direct sunlight can be deleterious to samples, reagents and media or can interfere with instrumentation or analysis.
- Monitoring of the light intensity in all areas including manufacturing, stores and laboratory should be done yearly.

F. Disposal of Waste Material

- All waste material must be disposed of through the Environment Health and Safety Hazardous Waste Program and bio-medical laboratory waste shall be destroyed as per the provisions of the Bio--Medical waste (Management and Handling) Rules, 1996.
- Staff dealing with hazardous waste disposal should have completed UCR Hazardous Waste Management training.
- Decontamination of waste and the ultimate disposal are closely interrelated. Most glassware, equipment, clothing are decontaminated or autoclaved within the laboratory for reuse. Other contaminated or infectious waste shall be disposed of by using the documented procedures in line with relevant regional or national regulatory standards, as applicable.
- Contaminated material decontaminated by autoclaving and thereafter disposed with incineration as per regulatory norms. Except sharps, all other infectious materials should be transported for disposal after autoclaving in a biohazard identifiable/ colour coded, leak proof pack.
- General hazardous waste disposal guidelines:-
  - Store hazardous waste in closed containers, in secondary containment, and in a designated location. Do not let product enter drains. Discharge into the environment must be avoided.
  - Double-bag dry waste using transparent bags.
  - Waste must be under the control of the person generating and disposing of it.
  - Dispose of routinely generated chemical waste within 90 days.

References:

1. Schedule ‘L-1’ Drugs and Cosmetics Rules 1945.
2. Schedule ‘M’ Drugs and Cosmetics Rules 1945.
3. Specific Criteria for Chemical Testing Laboratories, NABL 102
4. Specific Criteria for Chemical Testing Laboratories, NABL 103

Calibration: Volumetric Glassware

Volumetric apparatus should be calibrated by the analyst before use in the laboratory. Calibration is usually performed by weighing the amount of water delivered by or contained in the volumetric apparatus. This mass is then converted to the desired volume using the density of water:

Volume = Mass/Density

There are following types of glassware which are being used in analytical laboratory and needs calibration.

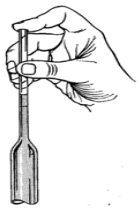
(i) Volumetric flask
(ii) Pipette one mark/bulb pipette
(iii) Pipette graduated
(iv) Burette
(v) Graduated measuring cylinders

Points for Correct Use of Volumetric Glassware

Pipette

The Pipette is used to transfer a volume of solution from one container to another. Most Volumetric Pipettes are calibrated To-Deliver (TD); with a certain amount of the liquid remaining in the tip and as a film along the inner barrel after delivery of the liquid. The liquid in the tip should not be blown-out. Pipettes of the "blow-out" variety will usually have a ground glass ring at the top. And, drainage rates from the pipette must be carefully controlled so as to leave a uniform and reproducible film along the inner glass surface. Measuring Pipettes will be graduated in appropriate units. Once the pipette is cleaned and ready to use, make sure the outside of the tip is dry. Then rinse the pipette with the solution to be transferred. Insert the tip into the liquid to be used and draw enough of the liquid into the pipette to fill a small portion of the bulb. Hold the liquid in the bulb by placing your fore finger over the end of the stem.

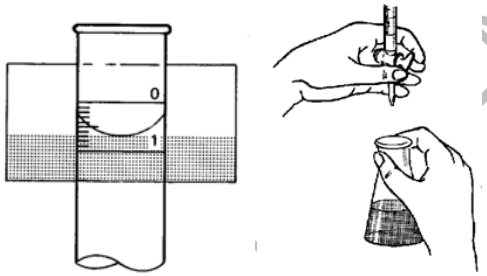
Withdraw the pipette from the liquid and gently rotate it at an angle so as to wet all portions of the bulb. Drain out and discard the rinsing liquid. Repeat this once more. To fill the pipette, insert it vertically in the liquid, with the tip near the bottom of the container. Apply suction to draw the liquid above the graduation mark. Quickly place a fore finger over the end of the stem. Withdraw the pipette from the liquid and use a dry paper to wipe off the stem. Now place the tip of the pipette against the container from which the liquid has been withdrawn and drain the excess liquid such that the meniscus is at the graduation mark. Move the pipette to the receiving container and allow the liquid to flow out (avoiding splashing) of the pipette freely. When most of the liquid has drained from the pipette, touch the tip to the wall of the container until the flow stops and for an additional count of 10.



Burette

The Burette is used to accurately deliver a variable amount of liquid. Fill the burette to above the zero mark and open the stopcock to fill the tip. Work air bubbles out of the tip by rapidly squirting the liquid through the tip or tapping the tip while solution is draining. The initial burette reading is taken a few seconds, ten to twenty, after the drainage of liquid has ceased. The meniscus can be highlighted by holding a white piece of paper with a heavy black mark on it behind the burette.

Place the flask into which the liquid is to be drained on a white piece of paper. (This is done during a titration to help visualize color changes which occur during the titration.) The flask is swirled with the right-hand while the stopcock is manipulated with the left hand. The burette should be opened and allowed to drain freely until near the point where liquid will no longer be added to the flask. Smaller additions are made as the end-point of the addition is neared. Allow a few seconds after closing the stopcock before making any readings. At the end-point, read the burette in a manner similar to that above. As with pipettes, drainage rates must be controlled so as to provide a reproducible liquid film along the inner barrel of the burette.



Volumetric Flasks

The Volumetric Flask is used to prepare Standard Solutions or in diluting a sample. Most of these flasks are calibrated 'To-Contain' (TC) a given volume of liquid. When using a flask, the solution or solid to be diluted is added and solvent is added until the flask is about two-thirds full. It is important to rinse down any solid or liquid which has adhered to the neck. Swirl the solution until it is thoroughly mixed. Now add solvent until the meniscus is at the calibration mark. If any droplets of solvent adhere to the neck, use a piece of tissue to blot these out. Stopper the flask securely and invert the flask at least 10 times.

Volumetric glassware is normally calibrated at 27ºC. However, the temperature generally specified for measurements of volume in analytical operations of the pharmacopoeial, unless otherwise stated, is 25ºC. The discrepancy is inconsequential as long as the room temperature in the laboratory is reasonably constant and is around 27ºC.

Calibration of One Mark/Bulb Pipette

Requirements

- Calibrated balance
- Calibrated thermometer
- Dried beakers which have been previously rinsed with acetone
- Calibrated stop watch

Method:

Determination of volume

a) Clean the pipette thoroughly.
b) Fill up the pipette with distilled water to the mark. Care shall be taken to ensure that there are no air bubbles inside the pipette.
c) Weigh the dried empty beaker (W_1).
d) Deliver the water from the pipette to its maximum in the beaker (W_2). The pipette should be held in vertical direction and its tip should touch the beaker.
e) Weigh the beaker with water (W_2).
f) Note the temperature of the distilled water using a calibrated thermometer.
g) Repeat the whole procedure till difference in two subsequent readings (W_2 & W_1) differ not more than 0.01g.
h) Volume of the pipette is determined by using the following formula

$$V=M/D$$

Where V=Volume of the pipette in cm^3
M=Weight of the water contained in the beaker obtained by the difference of the weights W_2 & W_1 in gms
D=Density of water at 27^oC i.e. 0.9965 g/cc

Determination of Delivery time

a) Fill the pipette with distilled water to the maximum mark
b) Ensure that there are no air bubbles in the pipette
c) Remove the finger from the jet and deliver the water from the pipette in the beaker. Start the stopwatch simultaneously at the start of the delivery of water from the mark on the pipette. Stop the stopwatch as the pipette has emptied at the maximum delivery.
d) Repeat the procedure minimum 3times to determine reproducibility
e) Note the time of delivery in seconds and compare with limits as per nominal capacity and type as per IS-1117-1975.

Precautions

a) The filled pipette should not contain any air bubbles.
b) The temperature of water should be measured after every reading using a calibrated thermometer
c) Whole procedure of Calibration should be undertaken at 27^oC

Reporting

- Report the actual volume of water contained in the pipette.
- Report the delivery time in seconds.

Volumetric Flask

Requirements

- Calibrated balance
- Calibrated thermometer
- Dried beakers which have been previously rinsed with acetone**.**

Procedure

a) Dry the flask under calibration, which has been previously rinsed with acetone.
b) Clean the flask thoroughly.
c) Weigh the dried flask empty (W_1)

d) Fill the flask up to the graduation mark with distilled water.
e) Weigh the water filled flask under the same conditions (W_2).
f) Note the temperature of the water using a calibrated thermometer.
g) Repeat the procedure minimum 3 times to get reproducibility.
h) Volume of the flask is determined by using the following formula

Volume, V=W/ D

i) Where V=Volume of the flask in cm^3
j) W=Weight of the water contained in the flask obtained by difference of the weights in W_2 and W_1.

D=Density of water at 27^oC i.e. 0.9965 g/cc

Precaution

a) The filled flask shall not contain any air bubbles.
b) The temperature of water should be measured after every reading using a calibrated thermometer with trace ability of NABL accredited agency.
c) No droplets of water should adhere to the inner surface of the walls of the flask above the duration mark.
d) Whole procedure of calibration should be undertaken at 27^oC.

Reporting

- Report the actual volume of water contained in the flask at 27^oC.

Graduated Burette

Requirements

- Calibrated balance
- Calibrated thermometer
- Dried beakers which have been previously rinsed by acetone.
- Calibrated stop watch

Determination of Volume

a) Clean the burette thoroughly.
b) Fill up the burette with distilled water to the mark. Care should be taken to ensure that there are no air bubbles inside the burette.
c) Weigh the dried empty beaker (W_1).
d) Deliver the water from the burette into the weighed beaker (W_1) from 0 mark to its maximum capacity.
e) If required the delivery of water may be regulated at 5 different points, for example 50ml burette, maximum points may be 10ml, 20ml, 30ml, 40ml and 50ml.
f) While delivering the water the burette should be held vertically and tip should touch the beaker.
g) Weigh the beaker with water (W_2).
h) Note the temperature of the water by a calibrated thermometer
i) Repeat the procedure till the difference in two subsequent reading (W_2 and W_1) differ not more than 0.01gm.
j) Volume of the burette is determined by using the following formula.

Volume, **V**=**M**/D

Where **V**=Volume of the burette in cm^3
M=Weight of the water contained in the beaker obtained by difference of W_2 and W_1 in grams.
D=Density of water at 27^oC i.e. 0.9965/gm/cc

Determination of Delivery Time

a) Fill the burette with distilled water beyond the 0-ml mark.
b) Ensure that there are no air bubbles in the burette
c) Loose the burette knob to its maximum limit and hold the lower tip of the burette with finger.
d) Remove the finger, the moment lower meniscus reaches 0-ml mark and start the stopwatch.
e) Stop the stopwatch when the lower meniscus reaches its maximum capacity mark.
f) Note the time of delivery.
g) Repeat the procedure 3 to 4 times to determine its reproducibility.

Precautions

a) The filled burette should not contain any air bubbles
b) Temperature of water should be measured after every reading using a calibrated thermometer with traceability to an NABL accredited agency.
c) Whole procedure of calibration should be undertaken at 27^oC.

Reporting

- Report the actual volume of water contained in the burette.
- Report the delivery time in minutes.

Graduated Pipette

Graduated pipette is very similar to graduated burette, both in design and its use. So the calibration can be done using same method described above.

Graduated measuring cylinder

Requirements

- Calibrated balance
- Calibrated thermometer
- Dried beakers which have been previously rinsed with acetone.

Procedure

a) Dry the measuring cylinder under calibration, which has been previously rinsed with acetone.
b) Clean the measuring cylinder thoroughly.
c) Weigh the measuring cylinder empty (W_1).
d) Fill the measuring cylinder with distilled water up to the maximum graduation mark*.
e) Weigh the water filled measuring cylinder under the same condition (W_2).
f) Note the temperature of the water using a calibrated thermometer.
g) Repeat the procedure 3 to 4 times to get reproducibility
h) Volume of the measuring cylinder is determined by using the formula:

Volume, V=W/D
Where V = Volume of the measuring cylinder in cm^3
W = Weight of the water contained in the measuring cylinder obtained by difference of the weights W_2 and W_1.
D = Density of water at 27^oC i.e. 0.9965/g/cc

Precautions

a) The filled cylinder shall not contain any air bubbles.
b) The temperature of water should be measurement after every reading using a calibrated thermometer with trace ability of NABL accredited agency.
c) No droplets of water should be adhering to the inner surface of the walls of the cylinder above the graduation mark.

Reporting

- Report the actual volume of water contained in the measuring cylinder at 27^{o}C.

*Note: More points should be selected to cover full range of the cylinder depending on its use, at least 3-5 points. For example a 50ml may be calibrated at 10ml, 20ml, 30ml, 40ml and 50ml points.

Note: The calibration sticker may be put on the glassware for the identification purpose.

References:

- Published specification in accordance with which the tests are performed : BIS Standards
- IS: 915-1997, one mark volumetric flasks
- IS: 1117-1975, one mark pipettes
- IS: 4162-(part I & II)-1985, Graduated pipettes & graduated burette
- IS: 875-1975, Graduated measuring cylinders
- IS: 9440-1979, Laboratory balances
- IS: 9865-1981, Laboratory standard weights
- IS: 14012-(Part I)-1993 Quality Assurance requirements for measuring equipments.
- IS 8897 –1978 Tables for calibration & method of verification of volumetric glassware

Calibration of HPLC and GC

Calibration of HPLC

Guidance is provided on general procedure applicable for calibration of HPLC taking Agilent Infinity Series (Quaternary Channel) system as an example. Parameters to be considered for calibration of HPLC are:

Parameters to be considered for HPLC Calibration

1. Calibration of Pump

Calibration of pump shall be done on the basis of the following parameters:

a) Flow rate accuracy
b) Flow rate consistency
c) Compositional accuracy (gradient profile)
d) Delay volume of the system

2. Calibration of autosampler

Calibration of autosampler shall be done on the basis of the following parameters:

a) Injection volume accuracy
b) Injection volume precision
c) Injection volume linearity
d) Autosampler temperature accuracy

3. Calibration of column oven

4. Calibration of detector

Calibration of detector shall be done on the basis of the following parameters:

a) Detector linearity
b) Wavelength accuracy

Illustration:

Note*: Following the given HPLC calibration procedure is not mandatory. In-house method of calibration can also be used.

<u>Procedure for the calibration of High Performance Liquid Chromatograph.</u>

Make and Model: Agilent Infinity Series (Quaternary Channel)

Parameters to be considered for HPLC Calibration

General Maintenance

Remove the column from the system and replace with dead volume connector or union. Flush the system using all channels at a flow rate of 2 ml/min with hot HPLC grade water (50-70°) for about half an hour. Following composition can be used: Channel A, 25%; B, 25%; C, 25%; and D, 25%.

1. Calibration of Pump

Calibration of pump should be done on the basis of the following parameters:

a) Flow rate Accuracy

- Remove the column and put all the channels inlets in reservoirs of HPLC grade water. Set the flow rate at 1.0 ml/min. using the following composition: Channel A, 25%; B, 25%; C, 25%; and D, 25%.
- Collect the HPLC grade water from column inlet into a dry 10.0 ml of calibrated volumetric flask and note down the time taken to fill the volumetric flask till the mark using a calibrated stopwatch. Perform the exercise in duplicate.
- Calculate the flow rate as follows:
  Flow rate = Volume in ml /Time in minutes.
- Set the flow rate at 2.0 ml/min. and 3.0 ml/min. and perform the same exercise in duplicate.
- **Acceptance criteria:** The flow rate should be within ±2.0% of the set value.

b) Flow rate Consistency

- Accurately weigh about 100 mg of Caffeine IPRS into a 100.0 ml volumetric flask. Dissolve in about 10 ml of methanol and make up the volume with mobile phase. Further dilute accordingly with mobile phase to get solution having concentration of 10 ppm.
- **Chromatographic conditions:**

Column	: Octadecylsilane (C-18) or Octylsilane (C-8) (250 mm x 4.6 mm x 5 µm)
Mobile phase	: Methanol : Water (50 : 50)
Flow rate	: 1 ml/min.
Injection volume	: 20 µL
Detection	: UV at 272 nm
Run time	: 10 minutes
Retention time of caffeine	: About 5 minutes

- Inject 10 ppm caffeine solution six times and calculate %RSD of the retention time of caffeine as obtained from the chromatograms.
- **Acceptance criteria:** The %RSD of retention time of caffeine should not be more than 1.0%.

c) Compositional Accuracy (Gradient Profile)

- Remove the column from the system and replace with dead volume connector.
- Prepare a 0.25% v/v solution of acetone in water. Flush the channels at a flow rate of 1.0 ml/min. using the composition given below:

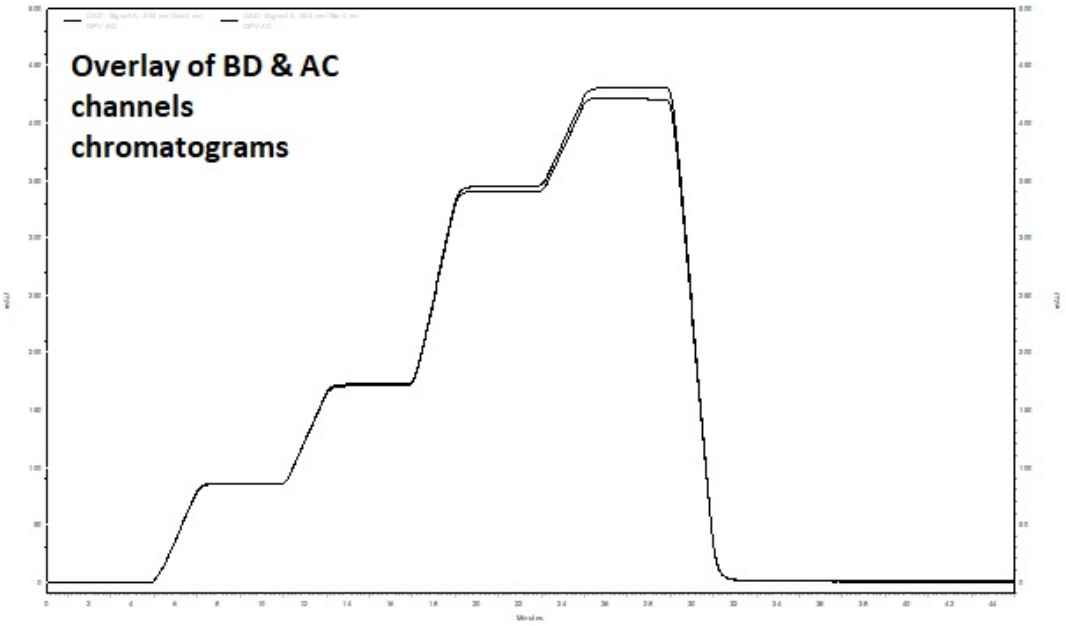
Time (min)	HPLC grade water (Channel A, B) (%)	0.25% v/v Acetone in water (Channel C, D) (%)
0	25 + 25	25 + 25
10	25 + 25	25 + 25
12	50 + 50	0 + 0
20	50 + 50	0 + 0

- Check the compositional accuracy of the HPLC system with the conditions given below:
  Flow rate : 1 ml/min.

Detection : UV at 254 nm
Run time : 30 minutes
Injection delay : 15 minutes

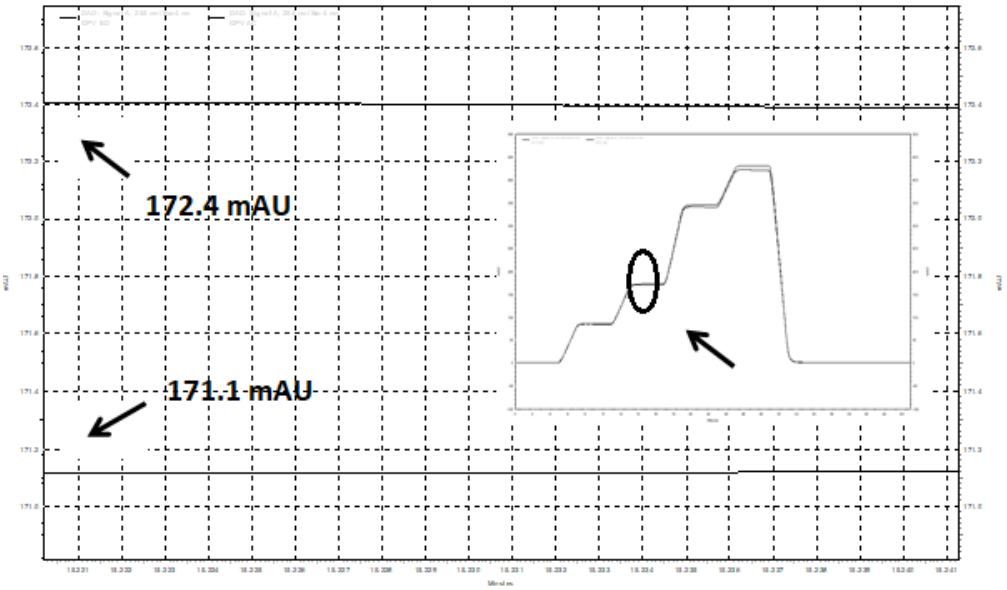
Time (min.)	HPLC grade water (Channel A) (%)	0.25% v/v Acetone in water (Channel C) (%)
0	100	0
4.0	100	0
6.0	80	20
10.0	80	20
12.0	60	40
16.0	60	40
18.0	20	80
22.0	20	80
24.0	0	100
28.0	0	100
30.0	100	0

- Run the gradient using channel combination A and C and repeat the same gradient using channel combination B and D.
- Inject 0 µL or minimum volume of HPLC grade water and record the gradient profile.
- Print the overlay plot of gradient profile of A/C and B/D. Chromatogram is attached for reference.



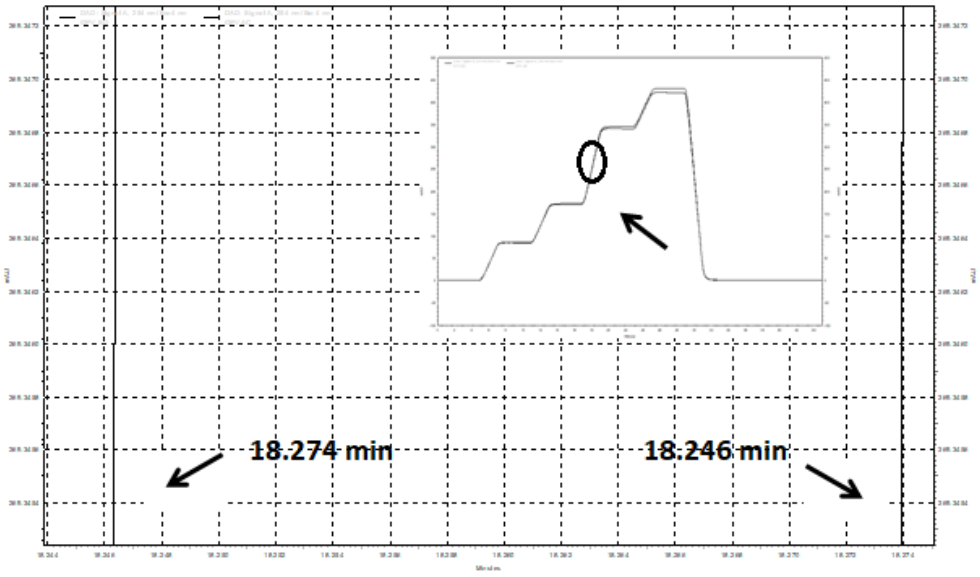
- **Acceptance criteria:** The gradient profile of A/C and B/D should overlay with each other with difference in absorbance should not more than 0.01 AU and the difference in time should not more than 20 seconds. Chromatogram is attached for reference.

Difference in absorbance (LIMIT NMT 0.01 AU)



Difference in Absorbance	172.4-171.1	mAU
	1.3	mAU
	1.3/1000	
	0.0013	AU

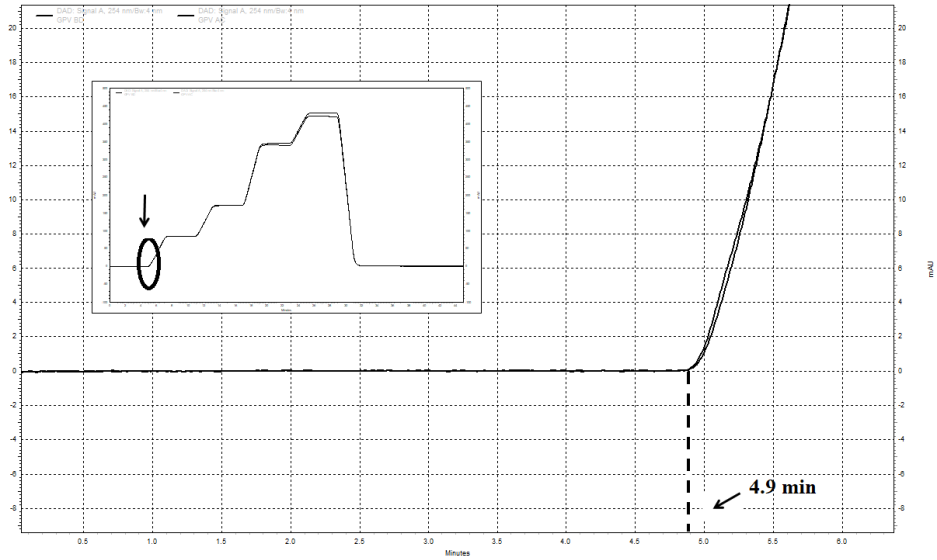
Difference in time (LIMIT NMT 20 SECONDS)



Difference in Time	18.274-18.246	min
	0.028	min
	0.028 x 60	seconds
	1.662	seconds

d) Delay Volume of the System

- Review the gradient profile performed under - Compositional accuracy (Gradient profile). Note the time in minutes taken for the actual first change in absorbance (Lift of the baseline).
- The delay volume of the system can be calculated in terms of ml by subtracting 4 minutes from the actual time in minutes taken for change in absorbance.
- **Acceptance criteria:** The delay volume of the system should be not more than 1.0 ml. Chromatogram is attached for reference.



Delay time	4.9-4.0 min
	0.9 min
Delay volume	Delay time (min) X Flow rate (ml/min)
	0.9 X 1.0
	0.9 ml
Limit	NMT 1.0 ml

2. Calibration of Autosampler

Calibration of autosampler shall be done on the basis of the following parameters:

a) Injection Volume Accuracy

- Purge the instrument with HPLC grade water.
- Fill the HPLC vial with HPLC grade water and close with a cap. Weigh this vial and record weight in gram (W1). Water is used in auto injection volume accuracy test because its density is 0.9982 g/ml at 20°C and 0.9970 g/ml at 25°C. This introduces less than 0.3% error when volume is assumed equal to weight.
- Program HPLC system with a flow rate of 1.0 ml/min. with water and run time of 1 minute.
- Inject 20 µL from HPLC Vial and repeat it for 10 times from the same HPLC vial.
- After completion of 10 injections, remove the vial and weigh again (W2).
- Calculate the average volume (in µL) injected per injection using formula:

  Average Volume = [(W1-W2)/10] x1000= µl/injection
- **Acceptance criteria:** Average volume of injection (µl/injection) should be 20 µl±0.4 µl.

b) Injection Volume Precision

- Flush the HPLC system with HPLC grade water for about half an hour.
- **Chromatographic conditions:**

Column	: Octadecylsilane (C-18) or Octylsilane (C-8) (250 mm x 4.6 mm x 5 µm)
Mobile phase	: Methanol : Water (50 : 50)
Flow rate	: 1 ml/min.
Injection volume	: 20 µL
Detection	: UV at 272 nm
Run time	: 10 minutes
Retention time of caffeine	: About 5 minutes

- Accurately weigh about 100 mg of caffeine in a 100 ml volumetric flask. Dissolve in 10 ml of methanol and make up the volume with mobile phase. Further dilute accordingly with mobile phase to get solution having concentration of 10 ppm.
- Inject this solution six times and calculate %RSD for the area obtained in the chromatograms.
- **Acceptance criteria:** %RSD for the area should not be more than 1.0%.

c) Injection volume linearity

- Inject 10 ppm caffeine solution with duplicate injection of 5 µl, 10 µl, 20 µl, 50 µl and 100 µl.
- From the data obtained, plot a graph of mean area count of duplicate injections on y-axis versus injection volume on the x-axis and calculate the value of R-square.
- **Acceptance criteria:** R-square should be not less than 0.9990.

d) Autosampler Temperature Accuracy

- Set the sample compartment temperature at 40º.
- After about 10 minutes record the observed temperature using a calibrated probe with digital thermometer.
- Repeat the same at 30°, 15°, 10° and 5°.
- **Acceptance criteria:** The observed temperature should be within ±2ºC of the set temperature.

3. Calibration of Column Oven

- Set the column oven temperature at 60º.
- After about 10 minutes, record the temperature using a calibrated probe with a digital thermometer.
- Repeat the same at 50°, 30°, 20° and 10°.
- **Acceptance criteria:** The observed temperature should be within ±2ºC of the set temperature.

4. Calibration of Detector

Set up the HPLC system using chromatographic conditions mention below:

Column	: Octadecylsilane (C-18) or Octylsilane (C-8) (250 mm x 4.6 mm x 5 µm)
Mobile phase	: Methanol : Water (50 : 50)
Flow rate	: 1 ml/min.

Injection volume	: 10 µL
Detection	: UV at 272 nm
Run time	: 10 minutes
Retention time of caffeine	: About 5 minutes

a) Detector Linearity

- Accurately weigh about 100.0 mg of caffeine in a 100 ml volumetric flask. Dissolve in 10 ml of methanol and make up the volume with mobile phase.
- Further dilute accordingly with mobile phase to get solution having concentration of about 0.001, 0.01 and 0.10 mg/ml (1.0, 10.0 and 100.0 µg/ml).
- Inject in duplicate 10 µl of 0.001, 0.01 and 0.10 mg/ml solutions of caffeine prepared above.
- From the data obtained plot a graph of mean area counts of duplicate injection on y-axis versus concentration (µg/ml) on x-axis and calculate the value of R-square.
- **Acceptance criteria:** R-square should not be less than 0.9990.

b) Wavelength Accuracy

For Photo Diode Array Detectors (PDA)

- Set detector wavelength at 200 nm to 400 nm.
- Inject 20 µl of 0.01 mg/ml solution of caffeine.
- Record spectrum and report maxima and minima.
- **Acceptance criteria**
  - Wavelength maxima found should be between 273±2 nm.
  - Wavelength maxima found should be between 205±2 nm.
  - Wavelength minima found should be between 245±2 nm.

For Variable Wavelength Detectors

- Create 32 acquisition program with same parameters except changing wavelength 269 to 278, 239 to 249 and 200 to 210.
- Inject 20 µL of 0.01 mg per mL solution of Caffeine.
- Record spectrum and report maxima and minima.
- **Acceptance criteria**
  - Wavelength maxima found should be between 273 ± 2 nm.
  - Wavelength maxima found should be between 205 ± 2 nm.
  - Wavelength minima found should be between 245 ± 2 nm.

Calibration Frequency

Calibration of HPLC is performed on six month basis or after any major failure or after maintenance. On completion of calibration, the HPLC calibration report shall be filled as per the given format.

	HPLC CALIBRATION REPORT

Instrument No. :
Instrument Make :
Calibration Frequency :
SOP No. :
Calibrated on :
Next Due on :

PUMP				
S. No.	**Parameter**	**Limit**	**Observed Value**	**Complies/Does Not Comply**
1	FLOW RATE ACCURACY	±2.0 % of set value		
	1.0 mL/minute	0.98 – 1.02 mL/minute		
	2.0 mL/minute	1.96 – 2.04 mL/minute		
	3.0 mL/minute	2.94 – 3.06 mL/minute		
2	FLOW RATE CONSISTENCY	%RSD of retention time of Caffeine NMT 1.0%		
3	COMPOSITIONAL ACCURACY	Difference in Absorbance NMT 0.01 AU		
		Difference in Time NMT 20 sec.		
4	DELAY VOLUME	NMT 1.0 mL		
AUTOSAMPLER				
1	INJECTION VOLUME ACCURACY	Average volume for 10 injections 20 ± 0.4 µL		
2	INJECTION VOLUME PRECISION	%RSD NMT 1.0 %		
3	INJECTION VOLUME LINEARITY	R-SQUARE NLT 0.9990		
4	AUTO SAMPLER TEMPERATURE ACCURACY	5 ºC ± 2ºC		
		10 ºC ± 2ºC		
		15ºC ± 2ºC		
		30 ºC ± 2ºC		
		40ºC ± 2ºC		
COLUMN OVEN				
1	TEMPERATURE ACCURACY	10 ºC± 2ºC		
		20 ºC± 2ºC		
		30 ºC± 2ºC		
		50 ºC± 2ºC		
		60ºC± 2ºC		
DETECTOR				
1	LINEARITY	R-Square NLT 0.9990		
2	WAVELENGTH ACCURACY	Maxima 273 ± 2 nm		
		Maxima 205 ± 2 nm		
		Minima 245 ± 2 nm		

Instrument Calibration : **Complies/Does Not Comply**

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Calibration of GC

Guidance is provided on general procedure applicable for calibration of GC taking Agilent 7890A GC system with GC Autosampler-80 system as an example. Parameters to be considered for calibration of GC are:

1. Column Oven Temperature

- Set the column oven temperature for at least 3 points from 40º to 300º.
- After about ten minutes, record the observed temperature using a calibrated temperature probe. Ensure whether the temperature inside the oven is the same as that set. Also, the injector and detector temperatures must equate with the temperature values set for them.
- **Acceptance criteria:** The observed temperature should be within ±2º of the set temperature.

2. Flow rate of gases

Check whether the flow rate of the carrier gas (Nitrogen/Helium) is within the limits of 22.5-27.5 ml/minute, hydrogen gas flow rate is within the limits of 36-44 ml/minute and air flow rate is within the limits of 360-440 ml/minute using a gas flow meter.

3. Detector Performance

a) Detector Precision and Flow rate Consistency

- Chromatographic Parameters:

Oven temperature (1)	: 40º
Time (1)	: 0 min.
Rate (1)	: 25º/min.
Oven temperature (2)	: 90º
Time (2)	: 0 min.
Rate (2)	: 15º/min.
Oven temperature (3)	: 170°
Time (3)	: 15 min
Injector temperature	: 250º
Detector (FID) temperature	: 270º
Carrier gas (N_2/He) flow rate	: 0.5 ml/min
Split ratio	: 10:1
Hydrogen flow	: 40±4 ml/min.
Zero Air flow	: 400±40 ml/min.
Auxiliary flow (N2)	: 25±2.5 ml/min.
Injection volume	: 1.0 µl
Septum purge flow	: 5 ml/min.
Syringe used	: 10 µl
Solvent wash pre injection/	
Pre clean with solvent	: 5
Pre clean with sample	: 3
Sample pumps/ filling strokes	: 5
Post clean with solvent	: 5

- Column: Fused silica column 30 m long, 0.32 mm internal diameter coated with 0.25 µm film of cross linked methyl siloxane (Use HP-1 column or equivalent).
- Standard Mixture: Prepare sufficient quantity of solution containing 0.35 mg/ml of each n-Tetradecane, n-Pentadecane and n-Hexadecane in n-hexane.
- Inject six replicate injections of the standard mixture and calculate the area ratio of Pentadecane and Hexadecane to that of the Tetradecane from the chromatograms.
- Determine the relative standard deviation of the ratio of the area counts of the peaks.
- Retention time of Tetradecane is about 11.6 minutes. The relative retention time of the expected peaks is given below:

Compound name	*Relative retention time*
n-Hexane	~ 0.41
n-Tetradecane	= 1.00
n-Pentadecane	~ 1.16
n-Hexadecane	~ 1.39

- Determine the relative standard deviation of the retention times of n-Tetradecane, n-Pentadecane and n-Hexadecane.
- **Acceptance criteria:**
  - RSD of the ratio of area counts of the peaks corresponding to n-Pentadecane and n-Hexadecane to that of n-Tetradecane in the chromatograms of six replicate injections should not be more than 2.0%.
  - RSD of the retention times of n-Tetradecane, n-Pentadecane and n-Hexadecane in six replicate injections should not be more than 2.0% (Flow rate consistency).

b) Detector Linearity

- Chromatographic Parameters:

Oven temperature (1)	: 50º
Time (1)	: 2.0 min.
Rate (1)	: 10º/min.
Oven temperature (2)	: 200º
Time (2)	: 5.0 min.
Injector temperature	: 250º
Detector (FID) temperature	: 270º
Carrier Gas (N_2 / He) flow rate	: 0.5 ml/min.
Split ratio	: 10:1
Hydrogen flow	: 40±4 ml/min.
Zero Air flow	: 400±40 ml/min.
Auxiliary flow (N2)	: 25±2.5 ml/min.
Injection volume	: 1.0 µl
Septum purge flow	: 5 ml/min.
Syringe used	: 10 µl
Pre clean with solvent 1	: 5
Pre clean with sample	: 3
Sample pumps/ Filling strokes	: 5
Post clean with solvent 1	: 5

- Column: Fused silica column 30 m long, 0.32 mm internal diameter coated with 0.25 µm film of cross linked methyl siloxane (Use HP-1 column or equivalent).

- Prepare five different concentrations of the standard containing 0.25 % v/v, 0.50 % v/v, 0.75% v/v, 1.0% v/v and 1.25% v/v of Methylene Chloride in Toluene.
- Inject 1.0 µl of all the five standards in duplicate.
- From the data obtained, plot regression curve with concentration on x-axis and mean response of five injections on y-axis and calculate the value of R-square.
- Retention time of Toluene is about 7.8 minutes and relative retention time of Methylene Chloride is about 0.64.
- **Acceptance criteria:** R-square should not be less than 0.9990.

Frequency of Calibration

Calibration is to be performed on half yearly basis or after any major failure or after maintenance. On completion of calibration, the GC calibration report shall be filled as per the given format.

	GC CALIBRATION REPORT

Instrument No. :
Instrument Make :
Calibration Frequency :
SOP No. :
Calibrated on :
Next Due on :

S. No.	Parameter	Acceptance Criteria	Observed Value	Complies / Does Not Comply
1	Detector precision	RSD of the ratio of area counts of six replicate injections of pentadecane to tetradecane should NMT 2.0%		
		RSD of the ratio of area counts of six replicate injections of hexadecane to tetradecane should NMT 2.0%		
2	Detector linearity	R-Square NLT 0.9990		
3	Flow rate consistency	RSD of retention time of six replicate injections of hexadecane should NMT 2.0%		
		RSD of retention time of six replicate injections of pentadecane should NMT 2.0%		
		RSD of retention time of six replicate injections of tetradecane should NMT 2.0%		
4	Flow rate of carrier gas (Auxiliary)	25± 2.5 ml/min.		
5	Flow rate of hydrogen gas	40± 4 ml/min.		
6	Flow rate of air gas	400± 40 ml/min.		

Instrument Calibration : **Complies / Does Not Comply**

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Laboratory Waste Material and Residue Sample Storage and Safe Disposal

Disposal of Pharmaceutical Waste

Chapter need to be redrafted for safe disposal of residue sample
Type of material

1) **Chemical for testing**
2) **Reagents for testing**
3) **Microbial Cultures**
4) **Inoculated Microbial Media**
5) **Legal Samples**
6) **Residual Candidate Material**
7) **Residual Biological Products**
8) **Waste Solvents**
9) **Standards**

10) **Test Samples**
    a. **Active Pharmaceutical Ingredients**
    b. **Dosage Forms of various varieties**
    c. **Impurties**
    d. **Radiopharmaceuticals**
    e. **Herbal samples**
    f. **Vaccines and Sera**
    g. **Blood and Blood related product**
11) **Outdated Records**

MSDS- need to be looked into and also storage and disposal and disposal records
Pollution Control Law

Introduction

Pharmaceutical waste is defined as surplus, unneeded, or unwanted material in the laboratory. It is usually the analyst or supervisor who decides whether to declare a given laboratory material a waste. Generally, the expired medicines lose their potency and are capable of producing toxins, causing serious reaction or failure of therapy. Improper disposal of pharmaceuticals may be hazardous if it leads to contamination of water supplies or local sources used by nearby communities or wildlife. Expired drugs may come into the hands of scavengers and children if a landfill is insecure.

Definitions

Chemical Waste: Waste that is or has been contaminated by chemicals and includes expired chemicals.

Hazardous Waste Label: The label that must be affixed to all hazardous waste.

Sharps: Syringes, needles, scalpels, blades and broken glassware; any item having corners, edges or projections capable of cutting or piercing the skin. Sharps can either be clean and free of contamination, or contaminated with chemical materials.

Contaminated Materials: Glass, filter paper, tissues and gloves which are not free of hazardous material.

Disposal Methods

Various disposal methods are briefly described and summarized in Table 1.

Table 1. Summary of disposal methods (*Reference: WHO. Prüss A, Giroult E, Rushbrook P, editors. Management of wastes from health care activities. Geneva: World Health Organization; 1999*)

Disposal Methods	Types of Pharmaceutical	Comments
Return to donor or manufacturer, trans frontier transfer for disposal	All bulk waste pharmaceuticals, particularly antineoplastics.	Usually not practical- Trans frontier procedures may be time consuming.
High temperature incineration with temperatures greatly in excess of 1200°C	Solids, semisolids, powders, antineoplastics, controlled substances.	Expensive.
Medium temperature incineration with two-chamber incinerator with minimum temperature of 850°C.	In the absence of high temperature incinerators, solids, semi-solids, powders. Controlled substances	Antineoplastics best incinerated at high temperature.
Immobilization		
Waste encapsulation	Solids, semi-solids, powders, liquids, antineoplastics, controlled substances.	
Inertization	Solids, semi-solids, powders, antineoplastics, controlled substances	
Landfill		
Highly engineered sanitary landfill	Limited quantities of untreated solids, semi-solids and powders. Disposal of waste pharmaceuticals after immobilization preferable. PVC plastics.	
Engineered landfill	Waste solids, semi-solids and powders, preferably after immobilization. PVC plastics.	
Open uncontrolled non-engineered dump	As last resort untreated solids, semisolids, powders - **must** be covered immediately with municipal waste. **Immobilization** of solids, semi-solids, powders **is preferable**.	Not for untreated controlled substances
Sewer	Diluted liquids, syrups, intravenous fluids, small quantities of diluted disinfectants (supervised).	Antineoplastics, and undiluted disinfectants and antiseptics not recommended.
Fast-flowing watercourse	Diluted liquids, syrups, intravenous fluids; small quantities of diluted disinfectants (supervised).	Antineoplastics, and undiluted disinfectants and antiseptics not recommended.
Burning in open containers	As last resort, packaging, paper, cardboard.	Not acceptable for PVC plastics or pharmaceuticals
Chemical decomposition	Not recommended unless special chemical expertise and materials available.	Not practical for quantities over 50 kg.

Sorting of Materials

The objective of sorting is to separate the pharmaceuticals into categories that require different disposal methods. The appropriate safe disposal method recommended will depend principally on

the pharmaceutical dosage form of the drugs. Segregated temporary storage areas or receptacles must be provided for each sorted category.

The sorting process includes:

- identifying each item;
- making a decision on whether it is usable;
- if usable, leaving packaging intact;
- if not usable, making a judgement on the optimal method of disposal and sorting accordingly;
- leaving packages and boxes intact until reaching their location, prior to definitive disposal or transport to an institution for use.

Materials to be disposed off should be segregated. Different methods are employed depending on:

(i) Type of Dosage Forms- Tablets, Capsules, Powders, Injectables, Creams, Ointments, Liquids, Ampoules, Vials, Intra-venous Infusions etc.

(ii) Chemical Nature of Drugs e.g. Antineoplastics/Anticancer, β-Lactams, Hormones, Steroids, Anti-infective, Narcotics, Antiseptics and Psychotropic substances etc. Tertiary (Printed/Labelled Corrugated Boxes) and Secondary (Printed Cartons/Paper box) packaging materials are removed and destroyed with the help of heavy duty paper shredder.

Recommended disposal methods of Pharmaceutical Dosage Forms by sorting categories

(i) Tablets/Capsules

Up to 50 tablets or capsules soak in about 100 ml of water and collect the same in a polyethylene bag containing used Tea/Coffee grind. Seal the bag and put in trash. Big quantity Pulverize using heavy duty crusher. Collect in a poly bag and seal. Dispose it in high temperature incinerator (Temp. 850^{o} C to 1200^{o} C)/approved site for solid waste disposal by the Pollution Control Board of the State.

(ii) Injectables-ampoules/vials

Up to 50 Ampules/Vials (up to 10 ml)-break ampoules/open vials and collect liquid in a polyethylene bag containing used Tea/Coffee grind. Seal the bag and put in trash. For bigger quantity, use heavy duty crusher to separate liquid and dilute it with water and transfer it to Effluent Treatment Plant (ETP) of the manufacturing unit. Broken glass/vials (after removal of label), rubber stoppers and seals should be disposed off as scrap. Powder Injectables (in vials/ampoules) to be disposed off in an incinerator as indicated above.

(iii) Oral liquids and Intravenous fluids

Small quantity – Dilute the liquid with water and drain it. For bigger quantity, dilute collected liquid with water and transfer it to ETP of the manufacturing unit. Liquids with high solid contents to be disposed off in an incinerator as indicated above.

(iv) Semi solids

Small quantity, mix it with used Tea/Coffee grind in a polyethylene bag. Seal the bag and put in a trash. Deshape the containers/remove the label and discard the containers. Semisolids in bigger quantity to be disposed off in an incinerator mentioned earlier.

Containers-Tubes to be deshaped and remove the label from glass/plastic container before disposal as a scrap.

Specific categories

(i) Anti-infectives-β-lactams:

Small quantity of all β-lactam antibiotics to be destroyed by soaking in 1N Sodium Hydroxide for 30 minutes or 1% Hydroxylamine in Water for 10 minutes and trash. Bigger quantity to be disposed off in an incinerator (Temp. 850^{o} C to 1200^{o} C) indicated above.

(ii) Anti-infectives-others

Tetracyclines- Small quantity to be soaked in 10% of Calcium Hydroxide/any other Calcium salt in Water for 30 minutes and trash. Macrolides- (Erythromycin, Clarithromycin etc.) Small quantity, soak in 1N Hydrochloride Acid and trash. Aminoglycosides (Gentamicin, Amikacin etc.) Small quantity dilute with large volume of water and drain it. Bigger quantity of all the above anti-infective should be disposed of in an incinerator as mentioned above.

(iii) Steroids

Small quantity-Soak in 1N Sodium Hydroxide for 30 minutes and trash. Bigger quantity-all dosage forms (taken out from the primary packing materials) to be incinerated at the temperature range indicated above.

(iv) Hormones

Small quantity – Aqueous solution to be exposed to UV for 20 minutes and trash. Estrogens- Small quantity in aqueous solution should be exposed to ultrasound at 0.6 and 2 kw in a sonicator for 60 mins. And trash. Bigger quantity-all solid dosage forms (taken out from primary packaging materials) to be incinerated as indicated above.

(v) Disinfectants

Small quantity-use it. Bigger quantity-Not more than 50L. Dilute with enough quantity of water to ensure dilution with loss of activity and drain it in ETP.

(vi) Controlled substances

Small quantity-Flush down the toilet to avoid misuse. Bigger quantity-All dosage forms (take out from primary packaging material) to be incinerated as mentioned above.

Disposal by incineration is preferred over chemical in activation for all dosage forms/APIs.

Table 2. Summary of pharmaceutical categories and disposal methods (*Reference: WHO. Prüss A, Giroult E, Rushbrook P, editors. Management of wastes from health care activities. Geneva: World Health Organization; 1999*)

Disposal methods	Types of pharmaceutical	Comments
Solids	Landfill	No more than 1% of the daily municipal waste should be disposed of daily in an untreated form (non-immobilized) to a landfill
Semi-solids	Waste encapsulation	
Powders	Waste inertization	
	Medium and high temperature incineration	
Liquids	Sewer	Antineoplastics not to sewer
	High temperature incineration	
Ampoules	Crush ampoules and flush diluted fluid to Sewer	Antineoplastics not to sewer
Anti-infective drugs	Waste encapsulation	Liquid antibiotics may be diluted with water, left to stand for several weeks and discharged to a sewer.
	Waste inertization	
	Medium and high temperature incineration	
Antineoplastics	Return to donor or manufacturer	Not to landfill unless encapsulated
	Waste encapsulation	Not to sewer.
	Waste inertization	No medium temperature incineration.
	Medium and high temperature incineration	

Controlled drugs	Waste encapsulation	Not to landfill unless encapsulated
	Waste inertization	
	Medium and high temperature incineration	
Aerosol canisters	Landfill Waste encapsulation	Not to be burnt: may explode.
Disinfectants	To sewer or fast-flowing watercourse: small quantities of diluted disinfectants (max. 50 litres per day under supervision)	No undiluted disinfectants to sewers or water courses. Maximum 50 litres per day diluted to sewer or fast-flowing watercourse. No disinfectants at all to slow moving or stagnant watercourses.
PVC plastic, glass	Landfill	Not for burning in open containers.
Paper, cardboard	Recycle, burn, landfill	

Laboratory Procedures: General Considerations

- Hazardous waste should be collected in a suitable container and labeled for its content, waste accumulation start date and date of disposal.
- All hazardous wastes must undergo segregation, safe treatment, accurate and complete labelling and storage. Mixing should be avoided wherever possible.
- Waste chemicals and solvents are stored in suitable areas whilst awaiting collection and must not be accumulated.
- Untrained staff is not to handle hazardous wastes and must not be given responsibility for them.

Chemical Waste

- Solvent wastes are to be collected in waste containers, labeled as “Solvent waste for Disposal”.
- Polar and non-polar wastes must be kept separate.
- Acid wastes must be placed in appropriate waste bottles and the solution kept neutral before disposal.
- Dry chemicals should be placed in a drum, labeled "Waste Dry Chemicals for Disposal".

Glass Bottles

- Remove caps of empty chemical and solvent bottles. Remove or deface the labels from bottles.
- Rinse the empty solvent bottles with water before discarding.
- Bottles and its stoppers shall be discarded separately.
- All the bottles shall be collected and displaced from laboratory at designated places before disposal.

Sharps

- Small bins are used for broken glassware in laboratories having a lid that is firmly attached.
- The waste bins are for sharps disposal such as syringe, needles, scalpel, blades, etc.
- Sharps must be placed into the “Sharps Disposal Container”.

Contaminated waste material

Used gloves, tissues and wipes go into the laboratory contaminated material bin.

Packing Materials

- Plastic containers, polythene bags, cartons, etc. are packed in a big polythene cover.
- Glass and plastic containers should be sorted separately.
- Clean paper and cardboard goes into the paper recycling box.

Packing Hazardous Waste

- Do not mix waste types. Always use a separate container for each type of waste.
- All material must be stored in an appropriate container that will not be degraded by the waste contents.
- Ensure the container is not leaking and there is no spillage on the exterior of the container.
- Liquid material should only be stored 80% full.
- Containers must be labelled with its content of waste. Unknown materials should not be picked up.

Labeling Hazardous Waste

- Containers for laboratory waste must be appropriately labeled.
- Each individual bin must be clearly labeled with a waste label.
- All required information must appear on the label to ensure safe handling and to enable proper disposal.
- Full chemical names must be used, as short forms or acronyms do not provide accurate identification.

Record and Disposal

- Samples are disposed off under the supervision of a designated person.
- Disposal of waste should be carried out in compliance with all the relevant environmental and health and safety regulations.
- Solvent wastes are disposed off in the ETP.

Safety and Precautions

- Always wear safety glasses, a mask, a laboratory coat and an appropriate pair of gloves for personal protection when handling waste.
- Work in the fume hood, if necessary.
- Laboratory waste should be minimized wherever and whenever an opportunity arises.
- Hazardous materials should be substituted with less hazardous or non-hazardous materials.
- Use the minimum volume of chemicals possible to achieve research objectives**.**
- Waste segregation of non-hazardous and hazardous materials should be done.

Disposal of Microbiological Media

- Wear personal protective garments like nose mask, rubber hand gloves and apron.
- Collect all the glass wares like petri plates, flasks, and test tubes etc. containing media contaminated with microorganism from the incubators.
- Add disinfectant like 70% IPA, 2.5% Savlon or 2.5% Dettol to the media plates, flasks and tubes to be discarded having growth or without any growth.
- After adding disinfectant leave the apparatus for half an hour.
- Collect the media in disposable poly bag, and apparatus like petri plates, flasks and tubes in separate disposable poly bags.
- Autoclave them for 30 minutes at 15 lbs pressure and 121^0C.
- All details pertaining to in the format given in Annexure-1.

Residual Sample Handling

In general as per Sch-L1, the residual sample shall be retained in proper storage condition for a period of one year after the final report.
But any type of samples (IPRS, NDS, and Miscellaneous) after testing at Indian Pharmacopoeial Laboratory and release of results of analysis by concerned division with the approval of Quality Assurance division at IPC, the remaining samples called as “Residual samples” and to be “stored in well closed containers in proper condition in defined place for five years” in case of NDS, “stored in well closed containers in proper condition in defined place for limited time duration and after that time, they are destroyed” in case of Miscellaneous samples for testing, but generally remaining quantity of sample is stored for three months or may be longer depending upon the nature of the sample. If raw material/sample available as residual sample at IPRS division is sufficient then testing is done only at IPC upto 5 years and “stored in well closed containers in proper condition in defined place for five years” in case of IPRS.

Storage of residual samples

Residual Samples for IPRS: Residual raw materials/samples for IPRS to be stored in controlled temperature at 2 to 8^{o} C in walk in type cooling chamber.

Residual Samples for NDS: Residual raw materials/samples for NDS to be stored in well closed containers in proper condition as mentioned on the product label and as follows:

Temperature	**Storage Area**
Upto 25 ^{o} C	Keep at room temperature
2 to 8^{o} C	Refrigerator
Upto -20 ^{o} C	Deepfreezer
Upto -80 ^{o} C	Ultra deep freezer

Residual Samples (Miscellaneous) for Testing: Residual raw materials/standards/samples (Miscellaneous) for testing at Indian Pharmacopoeial laboratory to be stored in well closed containers in proper condition a mentioned on the product label and as follows:

Temperature	**Storage Area**
Upto 25 ^{o} C	Keep at room temperature
2 to 8^{o} C	Refrigerator
Upto -20 ^{o} C	Deep freezer
Upto -80 ^{o} C	Ultra deep freezer

Recording & Archival of files related to samples/residual samples

- The laboratory must establish and maintain procedures for the identification collection, indexing, retrieval, storage, maintenance, and disposal of all quality documents.
- All the raw data, documentation, Standard Operative Procedures, protocols, and final reports are to be retained and there shall be archives for orderly storage and expeditious retrieval of all raw data, documentation, protocols, interim and final report. The archive shall provide a suitable environment that will prevent modification, damage, or deterioration and/or loss.
- The condition under which the original documents are stored must ensure their security and confidentiality.
- Paper documents shall not be kept for long periods under high humidity and raw data in the form of tape and discs are to be preserved with care.
- In case of storage of only optical disc, the life of disc shall be longer than the storage time.

- Raw data on thermal paper might fade away with time; therefore, a photocopy of the thermal paper shall also be retained in the archive.
- Time for which records are retained shall be prescribed in the documents

References

1. 5th ed. India: Government of India, Ministry of Health and Family Welfare. Indian Pharmacopoeia Commission; 2016. National Formulary of India.
2. Biomedical Waste Management Rules. G.S.R. 343 (E) dated 28th March 2016.

Drugs Testing Laboratories: General Requirements

Introduction

Following are general requirements for drug testing laboratories:

- Laboratory should have adequate space for performing its activities with proper layout of different sections of the laboratory for ensuring smooth and efficient functioning.
- Regulation and control of entry of persons not associated with the laboratory.
- Proper installation of the equipments enabling their efficient operation and maintenance.
- Supply of stabilized electrical power through recommended fixtures.

- Adequate lighting and ventilation; air conditioning in specific laboratories with temperature and humidity control.
- Supply of different types of water suitable for the purpose of washing, preparing solutions and media, etc.
- Supply of fuel gas, vacuum line, compressed air, as required.
- All gadgets required for observing safety measures, hygiene, and sanitation.
- Facility for inactivation of the hazardous chemical, biological and other wastes before disposal.
- Facilities for disposal of organic, inorganic, biological, chemical and other general wastes as per Bio-Medical Waste (Management and Handling) Rules.
- Aseptic area for microbiological testing.
- Proper space for keeping samples and reference materials with controlled temperature and humidity facilities.
- Proper storage space for chemicals and solvents.
- Adequately furnished store section.
- Environment controlled with fire-proof arrangement as may be needed.
- Spare glassware, accessories of equipments and separate area for keeping obsolete ware before disposal.
- Well equipped area for stability studies.
- Animal house, where the sample testing requires the use of animals. Layout enables orderly location of the sections and the ancillary areas associated to ensure efficient functioning.
- Library and Information Resource Centre.

List of Areas in a Testing Laboratory

- Reception
- Administrative office and meeting room
- Sample receipt and storage area
- Report dispatch room
- Kinds of laboratories: Balance room, Wet lab, Instrumentation lab (Spectrophotometry, Chromatography, Dissolution, NMR, MS etc.)
- Stability testing area
- Chemical and solvent storage area
- Control sample storage area
- Quality assurance room
- Record archive room
- Waste collection, storage and disposal area
- Utilities: Generator room, Water, Compressed air, Gases (Nitrogen), Water treatment plant
- Air Handling Unit
  ~~(Grey, White and Black Area)~~

Requirements of Equipment

Equipment shall be installed at locations designed, constructed, adapted and maintained to suit the operation to be carried out. The instruments shall be installed in dust-free and safe environment and conditions of temperature and humidity shall be maintained and periodic check on temperature and humidity shall be made and recorded. Instruments requiring the calibration shall be calibrated at regular intervals. There shall be written instructions in the form of SOP for operation, maintenance and calibration of instruments. Most instruments need vibration proof flat

level and hard surface for installation. Uninterrupted power supply is also needed to perform the operations properly. The Equipment Manual provided by the manufacturer shall be referred to for the various qualification criteria, operative and maintenance procedures to generate good quality data.

The equipment listed here are the minimum requirements for performing the tests specified under the various monographs and for various dosage forms given in IP. The actual requirements of instruments and their specifications will vary depending upon the activities to be carried out in a laboratory. The analyst performing the tests shall suggest instrument specifications as per requirements.

- Proper infrastructure is required for the laboratory to perform tests specified in the various monographs of the IP. Users are cautioned that the requirements specified are not exhaustive ones and the needs of the individual laboratory are to be decided based on the nature and extent of activities.
- The infrastructure facilities required include proper environmental controls also for performance of microbiological tests, instrumentation tests etc. Certain tests are required to be performed at controlled temperatures and in diminished light or darkness. Improper environmental conditions could affect the results of the tests performed.
- Proper installation and uninterrupted / steady power supply are required for the proper performance of most equipment. The user manual of the equipment shall be checked for proper installation, operation, service and maintenance.
- Necessary accessories essential for performing the tests specified shall be available.
- Maintenance contracts are essential for the proper performance of equipment and accuracy of the results.
- Operation of equipment, cleaning and maintenance etc., are all to be done by competent persons.
- Calibration of equipment should be carried out as per the defined schedule and in accordance with the SOP(s).
- Qualification of equipment, systems and validation of methods, wherever applicable, are also important to generate accurate results.

The following is the list of equipment expected to be available in an Analytical Laboratory performing IP tests

Representative list of equipment for a first-stage and medium-sized pharmaceutical quality control laboratory

First-stage laboratory

Equipment and major instruments	***Quantity***	
Top-loading balance	1	
Analytical balance (5 digits)		1 or 2
Melting-point apparatus		1
pH meter (with assorted electrodes)	1	
Microscope		1
Polarimeter	1	
High-performance liquid chromatograph with ultraviolet detector		2
Ultraviolet/visible spectrophotometer		1

Infrared spectrophotometer with pellet press		1
Karl Fischer titrator (semi-micro determination of water)		1
Agate mortar with pestle		1
Equipment for thin-layer chromatography		1
Thin-layer chromatography spotter	1	
Developing chambers		6 + 1a
Atomizers		6
Ultraviolet viewing lamp		1
Disintegration test equipment (1 basket for 6 tablets)		1
Dissolution apparatus		1
Soxhlet extraction apparatus (60 ml)		3 + 1a
Micrometer callipers	1	
Pycnometers		2
Burettes/pipettes (10 ml and 25 ml/1, 2, 5, 10, 20, 25, 50 ml)		3 of each
Desiccator		1 + 1a
Centrifuge (table-top model, 4-place swing rotor)		1
Water-bath (20 litres)		1
Hot plates with magnetic stirrers		3
Vacuum pump (rotary, oil)		1
Drying oven (60 litres)		1
Vacuum oven (17 litres)		1
Muffle furnace		1
Refrigerator (explosion-proof)		1
Water distilling apparatus (8 litres/hour)		1
Water deionizer (10 litres/hour)		1
Dehumidifier (where needed)		1
Fume hood		1
Optional items		
Analytical microbalance		1
Flame photometer (including air compressor)		1
Refractometer		1
Viscometer		1
Vortex mixer		1
Shaker (wrist-action)		1
Pipette rinser		1
Constant temperature water-bath		1
Ultrasonic cleaner (5 litres)	1	

Medium-sized laboratory

Equipment and major instruments	***Quantity***
Top-loading balance	1 or 2
Analytical balance (5 digits)	2
Analytical microbalance	1
Microscope	1 or 2
Equipment for thin-layer chromatography	1
Thin-layer chromatography multispotter	1

Developing chambers		6
Atomizers		6
Ultraviolet viewing lamp		1
Potentiometric titrimeter		1
Micro-Kjeldahl equipment (including fume flasks)		1
Soxhlet extraction apparatus (60 ml)		3
Pycnometers		2
Burettes/pipettes (10 ml and 25 ml/1, 2, 5, 10, 20, 25, 50 ml)		6 of each
Micrometer callipers	1	
Heating mantles for flasks (assorted sizes: 50, 200 and 2000 ml)	6	
Sieves (assorted sizes)		1 set
Centrifuge (floor model)		1
Shaker (wrist-action)		1
Vortex mixers		2
Water-bath (electrical, 20 litres)		2 or 3
Hot plates with magnetic stirrers		3 or 4
Vacuum pump (rotary, oil)		2
Vacuum rotary evaporator		1
Drying oven (60 litres)		2 or 3
Muffle furnace (23 litres)		1
Vacuum oven (17 litres)		1
Desiccators	2	
Refrigerator (explosion-proof)		2
Freezer		1
Ultrasonic cleaners (5 litres)		2
Laboratory glassware washing machine		1
Water distilling apparatus (8 litres/hour)		1
Water deionizing equipment (10 litres/hour)		1
Fume hoods		2
Melting-point apparatus		1
Polarimeter	1	
pH meters (with assorted electrodes)		2
High-performance liquid chromatograph with variable wavelength		1
Ultraviolet/visible detector		3 or 4
Ultraviolet/visible spectrophotometer, double-beam		1
Infrared spectrophotometer with pellet press		1
Agate mortar with pestle		1
Gas chromatograph (flame ionization, direct and static head space injection)		1
Refractometer		1
Karl Fischer titrators (1 semi-micro and 1 coulometric for microdetermination of water)		2
Oxygen flask combustion apparatus		1
Disintegration test equipment (1 basket for 6 tablets)		1
Dissolution test equipment (for 6 tablets/capsules)	1	

Optional items		
Atomic absorption spectrophotometer		1
Spectrofluorometer	1	
High-performance liquid chromatograph detectors:		
— fluorescence		1
— diode-array		1
— refractive index		1
— evaporative light scattering (ELSD)		1
— charged aerosol (CAD)		1
— mass spectrometric (MS)	1	
Gas chromatograph detectors:		
— conductivity		1
— nitrogen/phosphorous (NPD)		1
— mass spectrometric (MS)		1
Capillary electrophoresis equipment		1
Thin-layer chromatography scanner	1	
Crushing strength tester		1
Friability tester		1
Viscometer		1
Ice machine		1
Solvent-recovery apparatus		1
Equipment for microbiology unit		
pH meter		1
Ultraviolet/visible spectrophotometer, single-beam	1	
Microscopes (for bacteriology)		2
Membrane filter assembly for sterility tests	1	
Colony counter with magnifier		1
Laminar air flow unit		1
Hot-air sterilizer		1
Incubators, 60 litres	2 or 3	
Anaerobic jar		1
Zone reader		1
Centrifuge		1
Water-bath (thermostatically controlled)		2
Autoclaves (100 litres, top-loading)		2
Refrigerators (340 litres)		2
Deep freeze		1
Laboratory glassware washing machine		1
Equipment for pharmacognosy/phytochemistry unit		
Grinder/mill (for preparation of sample of herbal materials)	1	
Top loading balance		1
Sieves		1 set
Microscope		1
Soxhlet extraction apparatus		2 or 3
Water-bath	1	

Heating mantles for flasks		1 or 2
Hot plates with magnetic stirrers		2
Equipment for thin-layer chromatography		1 or 2
Developing chambers		3 or 4
Desiccators	2	
Rotary vacuum apparatus		1
Distillation equipment		1
Conical percolators	2 or 3	
Apparatus for determination of water content by azeotropic method[b]		1
Apparatus for determination of volatile oils[b]	1	
Apparatus for determination of arsenic limit test[c]		1

a Needed in the case that herbal medicines are also tested.
b *Quality control methods for medicinal plant materials.* Geneva, World Health Organization, 1998.
c *WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues.* Geneva, World Health Organization, 2006.

Glassware

- Different glassware is required for day-to-day use. Adequate supply of glassware should be made available. The glassware shall be of appropriate quality (preferably type A and B). Damaged glassware shall not be used.
- Calibrate the glassware in accordance with the procedure established wherever applicable. Maintain calibration schedule and record of calibration.
- Establish appropriate cleaning procedures for the glassware and removal of grease, etc. Glassware shall not remain unclean after use. Drying of calibrated glassware in ovens shall be at low temperature to preserve their volumetric accuracy.
- Details of cleaning procedure of glassware can be found in IP General Chapter 5.1.

Consumables

- These include reference substances, chemicals, solvents, reagents, media for sterility tests etc. Several consumables are required in a laboratory for day-to-day use for performing tests and analysis. It is important and essential that all consumables that are regularly or frequently used and required are stored in sufficient quantities to perform tests without interruption. It is also important that consumables of the right quality and quantity are procured from identified sources. In the case of consumable that are rarely used and not readily stocked, sources of repute and reliable suppliers shall be identified and arrangements made for supplies as and when needed.
- Representative list of critical consumables-

S. No.	Name of the Chemicals
1.	Methanol
2.	Acetonitrile
3.	Ethyl Alcohol
4.	N,N Dimethyl Formamide
5.	Acetone
6.	N-Hexane
7.	2-Propanol

8. Ethyl Acetate
9. Glacial Acetic Acid
10. Isobutyl Methyl Ketone
11. n-Butanol
12. Sodium Tartrate Dihydrate
13. Sodium Hydroxide
14. Dichloromethane
15. Cyclohexane
16. Hydrochloric Acid
17. Ammonia Solution
18. Sulphuric Acid
19. Potassium Chloride
20. Potassium Hydroxide
21. Potassium Bromide (IR Grade)
22. Sucrose

- Note: This list is not exhaustive, It may vary as per the user requirement.

Representative list of critical consumables used in Microbiology Laboratory

1. Soyabean Casein Digest Medium
2. Soyabean Casein Digest Agar
3. Sabouraud's Dextrose Agar
4. Sabouraud Dextrose Broth
5. Enterobacteria Enrichment Broth-Mossel
6. Violet Red Bile Glucose Agar
7. MacConkey Broth
8. MacConkey Agar
9. Rappaport Vassiliadis Salmonella Enrichment Broth
10. Wilson and Blair's BBS Agar
11. GN Broth
12. Xylose-Lysine-Deoxycholate Agar
13. Mannitol Salt Agar
14. Cetrimide Agar
15. Reinforced Medium for Clostridia
16. Columbia Agar
17. Antibiotic Assay Medium A
18. Antibiotic Assay Medium G
19. Antibiotic Assay Medium B
20. Antibiotic Assay Medium No. 11
21. Limulus Amebocyte Lysate
22. Purified Water IP

- There should be proper arrangements to store the consumables. Inflammable ones shall be preserved in cool places well protected and with proper safety measures. Corrosive items shall also be preserved in proper containers. Due care shall be exercised in the handling of

inflammable and corrosive consumables. Care should be taken to avoid damages to the labels of the containers of materials, damages to workstations and equipments etc.

- Reference substances, chemicals and reagents shall be handled by authorized persons only.
- Standard solutions prepared shall be properly labelled with 'use before date'.

Reference Books and Journals and Other Resource Materials

Laboratory shall procure authentic and latest version of IP and its Addendum in sufficient numbers for use in different divisions of the laboratory. IP is now available in a DVD also. Authentic copies of other reference books including other leading pharmacopoeias may also be kept.

Personnel

- Technical and supportive staffs are the important personnel of the laboratory. Entry to the laboratory shall be restricted to the personnel working there only. Visitors and others shall not be allowed to handle equipments or materials.
- The personnel shall be given periodical training to update their knowledge and skill.
- Ensure proper supervision of trainees and new recruits.

Supportive facilities such as power, water, gas supply etc.

- Standby electrical power source shall be available to provide uninterrupted power supplies to the laboratory in general and to work-stations and equipments that are to function continuously without interruption in particular.
- Where gas cylinder is used as the source of gas supply for the burner/ stove, the cylinder shall be located outside the work area, safely secured. The supply pipeline shall be easily identifiable and insulated, if needed.
- Water supply shall be available for washing areas and to the water shower provided for use in emergencies.
- Water purifier/ water distillation unit required for preparation of standard solutions and other preparations shall also be made available to provide adequate supply of the water of desired quality.

Records

- Records are to be maintained by persons responsible for the activity concerned.
- Records shall be authentic with the entries/details there in properly authenticated by the competent person responsible for the activity. Raw data records are the basis for deciding the quality of the material tested. Corrections/over-writing/erasing of entries etc. in records in general and in raw data records in particular are irrelevant and shall be avoided. Inadvertent actions of such nature shall be properly explained and authenticated. Un-authenticated records have no merits.

Procedures

- **General**: Well laid down procedures are essential to ensure performance of an activity properly, efficiently and in a precise manner every time. SOPs are to be prepared and adopted for every activity that could influence its result directly or indirectly.

- This manual lists some of the SOPs but these are not the whole of the procedures. As stated elsewhere also in this manual, SOPs are to be prepared and adopted by the laboratories to have proper systems in place to control all activities.

Stability Testing of Drug Substances and Drug Products

1. Introduction

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1.2 Scope of these guidelines
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2. Guidelines

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2.2 Drug Product (Finished Pharmaceuticals Product)

2.2.1 General
2.2.2 Photostability testing
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2.2.11 In-use stability
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3. Glossary

4. References

1.1 Objective of these guidelines

These guidelines seek to exemplify the core stability data package required for registration of new drug substances / active pharmaceutical ingredients (APIs) and new drug products / finished pharmaceutical products (FPPs), However, alternative approaches can be found in when they are scientifically justified. Further guidance can be used International Conference on Harmonisation (ICH) guidelines and in the WHO *guidelineson the active pharmaceuticals ingredients master file procedure.*

It is recommended that these guidelines should also be applied to products that are already being marketed, with allowance for an appropriate transition period, e.g. upon re-registration or upon re-evaluation.

1.2 Scope of these guidelines

These guidelines apply to new and existing APIs and address information to be submitted in original and subsequent applications for marketing authorization of their related FPP for human use. These guidelines are not applicable to stability testing for biologicals.

1.3 General Principles

The purpose of stability testing is to provide evidence of how the quality of an API or FPP varies with time under the influence of variety of environmental factors such as temperature, humidity and light. The stability programme also includes the study of product-related factors that influence its quality, for example, interaction of API with excipients container closure systems and packaging materials. In fixed-dose combination FPPs (FDCs) the interaction between two or more APIs also has to be considered.

The choice of test conditions defined in this guidance is based on an analysis of the effects of climatic conditions in the three regions of the EU, Japan, and the United States. The mean kinetic temperature in any part of the world can be derived from climatic data, and the world can be divided into four climatic zones, I-IV. This guidance addresses climatic zones I and II. The principle has been established that stability information generated in any one of three regions of the,EC (In ICH) Japan, and United States would be mutually acceptable to the other two regions, provided the information is consistent with this guidance and the labelling is in accord with national / regional requirements.

The design of the stability testing programme should take into account the intended market and the climatic conditions in the area in which the drug products will be used.

Four climatic zones can be distinguished for the purpose of worldwide stability testing, as follows:

- Zone I: Temperate; 21°C and 45% RH
- Zone II: Subtropical and Mediterranean climate; 25°C and 60% RH
- Zone III: Hot and dry; 30°C and 35% RH
- Zone IVA: Hot and humid climate; 30°C and 65% RH
- Zone IVB: Hot and very humid climate; 30°C and 75% RH

Since there are only a few countries in zone I, the manufacturer would be well advised to base stability testing on the conditions in climatic zone II when it is intended to market products in temperate climates. For countries where certain regions are situated in zones III or IVA or IVB, and also with a view to the global market, it is recommended that stability testing programmes should be based on the conditions corresponding to climatic zone IVA or IVB.

As a result of stability testing a re-test period for the API (in exceptional cases, e.g. for unstable APIs, a shelf-life is given) or a shelf-life for the FPP can be established and storage conditions can be recommended. The classification of India in the climatic zone as specified in the WHO Technical Report Series No. 953 is 30°C and 70% RH.

2.1 DRUG SUBSTANCE (Active Pharmaceutical Ingredient)

2.1.1 General

Information on the stability of the drug substance is an integral part of the systematic approach to stability evaluation.Potential attributes to be tested on an API during stability testing are listed in the examples of testing parameters reproduced from WHO Technical Report Series No. 953 (Appendix 2 of the WHO Technical Report Series 953 is reproduced as Appendix 1 to this article).

The re-test period or shelf-life assigned to the API by the API manufacturer should be derived from stability testing data.

2.1.2 Stress Testing

Stress testing of the drug substance can help identify the likely degradation products, which can in turn help establish the degradation pathways and the intrinsic stability of the molecule and validate the stability indicating power of the analytical procedures used. The nature of the stress testing will depend on the individual drug substance and the type of drug product involved.

Stress testing is to be carried out on a single batch of the drug substance. The testing should include the effect of temperatures (in 10°C increments (e.g., 50°C, 60°C) above that for accelerated testing), humidity (e.g., 75 percent relative humidity or greater) and, where appropriate, oxidation, and photolysis on the drug substance. The testing should also evaluate the susceptibility of the drug substance to hydrolysis across a wide range of pH values when in solution or suspension. Photostability testing should be an integral part of stress testing. The standard conditions for photostability testing are described in ICH *Q1B Photostability Testing of New Drug Substances and Products*.

Examining degradation products under stress conditions is useful in establishing degradation pathways and developing and validating suitable analytical procedures. However, such examination may not be necessary for certain degradation products if it has been demonstrated that they are not formed under accelerated or long-term storage conditions. Results from these studies will form an integral part of the information provided to regulatory authorities.

2.1.3 Selection of Batches

Data from formal stability studies should be provided on at least three primary batches of the drug substance. The batches should be manufactured to a minimum of pilot scale by the same synthetic route as production batches and using a method of manufacture and procedure that simulates the final process to be used for production batches. The overall quality of the batches of drug substance placed on formal stability studies should be representative of the quality of the material to be made on a production scale. Other supporting data can be provided.
For existing active substances that are known to be stable, data from at least two primary batches should be provided.

2.1.4 Container Closure System

The stability studies should be conducted on the drug substance packaged in a container closure system that is the same as or simulates the packaging proposed for storage and distribution.

2.1.5 Specification

Specification, which is a list of tests, references to analytical procedures, and proposed acceptance criteria, is addressed in *Specifications: Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products.*

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

2.1.6Analytical methods *(Not in ICH Q1A or WHO 953)*

A systematic approach should be adapted to the presentation and evaluation of stability information, which should include, as necessary, physical, chemical, biological and microbiological test characteristics.

Test methods to demonstrate the efficacy of additives, such as antimicrobial agents, should be used to determine whether such additives remain effective and unchanged throughout the projected shelf-life.

Analytical methods should be validated or verified, and the accuracy as well as the precision (standard deviations) should be recorded. The assay methods chosen should be those indicative of stability. The test of related compounds or products of decompositions should be validated to demonstrate that they are specific to the products being examined and are of adequate sensitivity.

2.1.7 Testing Frequency

For long-term studies, frequency of testing should be sufficient to establish the stability profile of the drug substance. For drug substances with a proposed re-test period of at least 12 months, the frequency of testing at the long-term storage condition should normally be every 3 months over the first year, every 6 months over the second year, and annually thereafter through the proposed re-test period or Shelf Life (WHO).

At the accelerated storage condition, a minimum of three time points, including the initial and final time points (e.g., 0, 3, and 6 months), from a 6-month study is recommended. Where an expectation (based on development experience) exists that the results from accelerated studies are likely to approach significant change criteria, increased testing should be conducted either by adding samples at the final time point or including a fourth time point in the study design.

When testing at the intermediate storage condition is called for as a result of significant change at the accelerated storage condition, a minimum of four time points, including the initial and final time points (e.g., 0, 6, 9, 12 months), from a 12-month study is recommended.

2.1.8 Storage Conditions

In general, a drug substance should be evaluated under storage conditions (with appropriate tolerances) that test its thermal stability and, if applicable, its sensitivity to moisture. The storage

conditions and the lengths of studies chosen should be sufficient to cover storage, shipment, and subsequent use.

Storage conditions tolerances are defined as the acceptable variations in temperature and relative humidity of storage facilities for stabilities studies. The equipment used should be capable of controlling the storage conditions within the ranges defined in these guidelines. The storage conditions should be monitored and recorded. Short term environmental changes due to opening the door of the storage facility are accepted as unavoidable. The effect of the excursion due to equipment failure should be assessed, addressed and reported if judged to affect stability results. Excursions that exceed the defined tolerances for more than 24 hours should be described in the study report and their effects assessed.

The long-term testing should cover a minimum of 12 months' duration on at least three primary batches at the time of submission and should be continued for a period of time sufficient to cover the proposed re-test period. Additional data accumulated during the assessment period of the registration application should be submitted to the authorities if requested. Data from the accelerated storage condition and, if appropriate, from the intermediate storage condition can be used to evaluate the effect of short-term excursions outside the label storage conditions (such as might occur during shipping).

Long-term, accelerated, and, where appropriate, intermediate storage conditions for drug substances are detailed in the sections below. The general case should apply if the drug substance is not specifically covered by a subsequent section. Alternative storage conditions can be used if justified.

a. General case (2.1.8.1)

Study	**Storage condition**	**Minimum time period covered by data at submission**
Long-term*	25°C ± 2°C/60% RH ± 5% RH or 30°C ± 2°C/65% RH ± 5% RH or 30°C ± 2°C/75% RH ± 5% RH	12 months or 6 months as described in point 2.1.7
Intermediate**	30°C ± 2°C/65% RH ± 5% RH	6 months
Accelerated	40°C ± 2°C/75% RH ± 5% RH	6 months

* It is up to the applicant to decide whether long-term stability studies are performed at 25°C ± 2°C/60% RH ± 5% RH or 30°C ± 2°C/65% RH ± 5% RH.

** If 30°C ± 2°C/65% RH ± 5% RH is the long-term condition, there is no intermediate condition.

If long-term studies are conducted at 25°C ± 2°C/60% RH ± 5% RH and *significant change* occurs at any time during 6 months' testing at the accelerated storage condition, additional testing at the intermediate storage condition should be conducted and evaluated against significant change criteria. Testing at the intermediate storage condition should include all tests, unless otherwise justified. The initial application should include a minimum of 6 months' data from a 12-month study at the intermediate storage condition.

Significant change for a drug substance is defined as failure to meet its specification.

b. Drug substances intended for storage in a refrigerator (2.1.8.2)

Study	Storage condition	Minimum time period covered by data at submission
Long-term*	5°C ± 3°C	12 months
Accelerated	25°C ± 2°C/60% RH ± 5% RH or 30°C ± 2°C/65% RH ± 5% RH or 30°C ± 2°C/75% RH ± 5% RH	6 months

Data from refrigerated storage should be assessed according to the evaluation section of this guidance, except where explicitly noted below.

If significant change occurs between 3 and 6 months' testing at the accelerated storage condition, the proposed re-test period should be based on the real time data available at the long-term storage condition.

If significant change occurs within the first 3 months' testing at the accelerated storage condition, a discussion should be provided to address the effect of short-term excursions outside the label storage condition (e.g., during shipping or handling). This discussion can be supported, if appropriate, by further testing on a single batch of the drug substance for a period shorter than 3 months but with more frequent testing than usual. It is considered unnecessary to continue to test a drug substance through 6 months when a significant change has occurred within the first 3 months.

c. Drug substances intended for storage in a freezer (2.1.8.3)

Study	Storage condition	Minimum time period covered by data at submission
Long-term	-20°C ± 5°C	12 months

For drug substances intended for storage in a freezer, the retest period should be based on the real time data obtained at the long-term storage condition. In the absence of an accelerated storage condition for drug substances intended to be stored in a freezer, testing on a single batch at an elevated temperature (e.g., 5°C ± 3°C or 25°C ± 2°C) for an appropriate time period should be conducted to address the effect of short-term excursions outside the proposed label storage condition (e.g., during shipping or handling).

d. Drug substances intended for storage below -20°C (2.1.8.4)

Drug substances intended for storage below -20°C should be treated on a case-by-case basis.

2.1.9 Stability Commitment

When available long-term stability data on primary batches do not cover the proposed retest period granted at the time of approval, a commitment should be made to continue the stability studies postapproval to firmly establish the retest period.

Where the submission includes long-term stability data on three production batches covering the proposed retest period, a postapproval commitment is considered unnecessary. Otherwise, one of the following commitments should be made:

- If the submission includes data from stability studies on at least three production batches, a commitment should be made to continue these studies through the proposed retest period.

- If the submission includes data from stability studies on fewer than three production batches, a commitment should be made to continue these studies through the proposed retest period and to place additional production batches, to a total of at least three, on long-term stability studies through the proposed retest period.

- If the submission does not include stability data on production batches, a commitment should be made to place the first three production batches on long term stability studies through the proposed retest period.

The stability protocol used for long-term studies for the stability commitment should be the same as that for the primary batches, unless otherwise scientifically justified.

2.1.10 Evaluation

The purpose of the stability study is to establish, based on testing a minimum of three batches of the drug substance and evaluating the stability information (including, as appropriate, results of the physical, chemical, biological, and microbiological tests), a retest period applicable to all future batches of the drug substance manufactured under similar circumstances. The degree of variability of individual batches affects the confidence that a future production batch will remain within specification throughout the assigned retest period.

The data may show so little degradation and so little variability that it is apparent from looking at the data that the requested retest period will be granted. Under these circumstances, it is normally unnecessary to go through the formal statistical analysis; providing a justification for the omission should be sufficient.

An approach for analyzing the data on a quantitative attribute that is expected to change with time is to determine the time at which the 95 percent one-sided confidence limit for the mean curve intersects the acceptance criterion. If analysis shows that the batch-to-batch variability is small, it is advantageous to combine the data into one overall estimate. This can be done by first applying appropriate statistical tests (e.g., p values for level of significance of rejection of more than 0.25) to the slopes of the regression lines and zero time intercepts for the individual batches. If it is inappropriate to combine data from several batches, the overall retest period should be based on the minimum time a batch can be expected to remain within acceptance criteria.

The nature of any degradation relationship will determine whether the data should be transformed for linear regression analysis. Usually the relationship can be represented by a linear, quadratic, or cubic function on an arithmetic or logarithmic scale. Statistical methods should be employed to test the goodness of fit of the data on all batches and combined batches (where appropriate) to the assumed degradation line or curve.

Limited extrapolation of the real time data from the long-term storage condition beyond the observed range to extend the retest period can be undertaken at approval time if justified. This justification should be based, for example, on what is known about the mechanism of degradation, the results of testing under accelerated conditions, the goodness of fit of any mathematical model, batch size, and/or existence of supporting stability data. However, this extrapolation assumes that the same degradation relationship will continue to apply beyond the observed data.

Any evaluation should cover not only the assay, but also the levels of degradation products and other appropriate attributes.

2.1.11 Statements/Labelling

A storage statement should be established for the labelling in accordance with relevant national/regional requirements. The statement should be based on the stability evaluation of the drug substance. Where applicable, specific instructions should be provided, particularly for drug substances that cannot tolerate freezing. Terms such as *ambient conditions* or *room temperature* should be avoided.

A retest period should be derived from the stability information, and a retest date should be displayed on the container label if appropriate.

2.1.12 Ongoing stability studies (Not in ICH)

The stability of API should be monitored according to a continuous and appropriate programme that will permit the detection of any stability issue (e.g. changes in levels of degradation products). The purpose of ongoing stability programme is to monitor the API and to determine that the API (drug substances) remains, and can be expected to remain, within specifications under the storage conditions indicated on the label, within the re-test period in all future batches. The ongoing stability programme should be described in a written protocol and the results presented in a formal report. The protocol for an ongoing stability programme should extend to the end of the re-test period and shelf-life and should include, but not be limited to, the following parameters:

- Number of batch (es) and different batch sizes, if applicable;
- Relevant physical, chemical, microbiological and biological test methods;
- Acceptance criteria;
- reference to test methods;(WHO)
- Description of the container closer system(s);
- Testing frequency;
- Description of the conditions of storage (standardized condition for long term testing as described in these guidelines, and consistent with the API labelling, should be used); and

- Other applicable parameters specific to the API.

At least one production batch per year of API (unless none is produced during that year) should be added to the stability monitoring programme and tested at least annually to confirm the stability. In certain situation additional batches should be included in the ongoing stability programme. For example, an ongoing stability study should be conducted after any significant change or significant deviation to the synthetic route, process or container closer system which may have an impact upon the stability of the API.

Out of specification results or significant atypical trends should be investigated. Any confirmed significance change, out of specification results, or significant atypical trends should be reported immediately to the relevant product manufacturer. The possible impact on batches on the market should be considered in consultation with the relevant product manufacturers and the competent authorities. The summery of all the data generated, including any interim conclusion on the programme, should be maintained. This summary should be subjected to periodic review.

2.2 DRUG PRODUCT (Finished Pharamaceutical Product)

2.2.1 General

The design of the formal stability studies for the drug product should be based on knowledge of the behaviour and properties of the drug substance (API), results from stability studies on the drug substance, and experience gained from preformulation or (clinical formulation (WHO)) studies. The likely changes on storage and the rationale for the selection of attributes to be tested in the formal stability studies should be stated.

2.2.2 Photostability Testing (Not in WHO TRS 953)

Photostability testing should be conducted on at least one primary batch of the drug product if appropriate. The standard conditions for photostability testing are described in ICH Q1B guidelines.

2.2.3 Selection of Batches

Data from stability studies should be provided on at least three primary batches of the drug product. The primary batches should be of the same formulation and packaged in the same container closure system as proposed for marketing. The manufacturing process used for primary batches should simulate that to be applied to production batches and should provide product of the same quality and meeting the same specification as that intended for marketing. In the case of conventional dosage forms with APIs that are known to be stable, data from at least two primary batches should be provided.

Two of the three batches should be at least pilot scale batches, and the third one can be smaller if justified. Where possible, batches of the drug product should be manufactured by using different batches of the drug substance.

Stability studies should be performed on each individual strength and container type and size of the drug product unless bracketing or matrixing is applied. Other supporting data can be provided.

2.2.4 Container Closure System

Stability testing should be conducted on the dosage form packaged in the container closure system proposed for marketing (including, as appropriate, any secondary packaging and container label)(In ICH not in WHO). Any available studies carried out on the drug product outside its immediate container or in other packaging materials can form a useful part of the stress testing of the dosage form or can be considered as supporting information, respectively.

2.2.5 Specification

Stability studies should include testing of those attributes of the drug product that are susceptible to change during storage and are likely to influence quality, safety, and/or efficacy. The testing should cover, as appropriate, the physical, chemical, biological, and microbiological attributes, preservative content (e.g., antioxidant, antimicrobial preservative), and functionality tests (e.g., for a dose delivery system). Analytical procedures should be fully validated and stability indicating. Whether and to what extent replication should be performed will depend on the results of validation studies.

Shelf life acceptance criteria should be derived from consideration of all available stability information. It may be appropriate to have justifiable differences between the shelf life and release acceptance criteria based on the stability evaluation and the changes observed on storage.

Any differences between the release and shelf life acceptance criteria for antimicrobial preservative content should be supported by a validated correlation of chemical content and preservative effectiveness demonstrated during drug development on the product in its final formulation (except for preservative concentration) intended for marketing. A single primary stability batch of the drug product should be tested for antimicrobial preservative effectiveness (in addition to preservative content) at the proposed shelf life for verification purposes, regardless of whether there is a difference between the release and shelf life acceptance criteria for preservative content.

Specification, which is a list of tests, references to analytical procedures, and proposed acceptance criteria, including the concept of different acceptance criteria for release and shelf life specifications, is addressed in ICH Q6A and Q6B. In addition, specification for degradation products in a drug product is addressed in ICH *Q3B Impurities in New Drug Products*.(In ICH only)

2.2.6 Analytical methods (Not in ICH Q1A or WHO 95)

A systematic approach should be adopted to the presentation and evaluation of stability information, which should include, as necessary, physical, chemical, biological and microbiological test characteristics.

Analytical methods should be validated or verified, and the accuracy as well as the precision (standard deviations) should be recorded. The assay methods chosen should be those indicative of stability. The test of related compounds or products of decompositions should be validated to demonstrate that they are specific to the products being examined and are of adequate sensitivity.

2.2.7 Testing Frequency

For long-term studies, frequency of testing should be sufficient to establish the stability profile of the drug product. For products with a proposed shelf life of at least 12 months, the frequency of testing at the long-term storage condition should normally be every 3 months over the first year, every 6 months over the second year, and annually thereafter through the proposed shelf life.

At the accelerated storage condition, a minimum of three time points, including the initial and final time points (e.g., 0, 3, and 6 months), from a 6-month study is recommended. Where an expectation (based on development experience) exists that results from accelerated testing are likely to approach significant change criteria, increased testing should be conducted either by adding samples at the final time point or by including a fourth time point in the study design.

When testing at the intermediate storage condition is called for as a result of significant change at the accelerated storage condition, a minimum of four time points, including the initial and final time points (e.g., 0, 6, 9, 12 months), from a 12-month study is recommended.

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

2.2.8 Storage Conditions

In general, a FPP should be evaluated under storage conditions (with appropriate tolerances) that test its thermal stability and, if applicable, its sensitivity to moisture or potential for solvent loss. The storage conditions and the lengths of studies chosen should be sufficient to cover storage, shipment, and subsequent use with due regard to the climatic conditions in which the product is indented to be marketed.

Photostability testing, which is an integral part of stress testing, should be conducted on at least one primary batch of the drug product if appropriate. More details can be found in other guidelines.

Stability testing of the drug product after constitution or dilution, if applicable, should be conducted to provide information for the labelling on the preparation, storage condition, and in use period of the constituted or diluted product. This testing should be performed on the constituted or diluted product through the proposed in-use period on primary batches as part of the formal stability studies at initial and final time points, and if full shelf life, long-term data will not be available before submission, at 12 months or the last time point for which data will be available. In general, this testing need not be repeated on commitment batches.

The long-term testing should cover a minimum of six or12 months' duration on at least three primary batches at the time of submission and should be continued for a period of time sufficient to cover the proposed shelf life. Additional data accumulated during the assessment period of the registration application should be submitted to the authorities if requested. Data from the accelerated storage condition and, if appropriate, from the intermediate storage condition can be used to evaluate the effect of short-term excursions outside the label storage conditions (such as might occur during shipping).

Long-term, accelerated, and, where appropriate, intermediate storage conditions for drug products are detailed in the sections below. The general case should apply if the drug product is not specifically covered by a subsequent section. Alternative storage conditions can be used if justified.

a. **General case** (2.2.8.1)

Study	**Storage condition**	**Minimum time period covered by data at submission**
Long-term*	25°C ± 2°C/60% RH ± 5% RH or 30°C ± 2°C/65% RH ± 5% RH 30 °C ± 2 °C/75% RH ± 5% RH 30 °C ± 2 °C/70 % RH ± 5% RH(India Specific as per WHO TRS 953)	12 months or 6 months as referred to in section 2.2.8
Intermediate**	30°C ± 2°C/65% RH ± 5% RH	6 months
Accelerated	40°C ± 2°C/75% RH ± 5% RH	6 months

* It is up to the applicant to decide whether long-term stability studies are performed at 25°C ± 2°C/60% RH ± 5% RH or 30°C ± 2°C/65% RH ± 5% RHor 30 °C ± 2 °C/75% RH ± 5% RH is determined by the climatic zone in which the FPP is intended to be marketed . Testing at a more severe long-term condition can be an alternative to storage at 25 °C/60% RH or 30 °C/65% RH.(WHO)

** If 30°C ± 2°C/65% RH ± 5% RHor 30 °C ± 2 °C/75% RH ± 5% RH(WHO) is the long-term condition, there is no intermediate condition.

If long-term studies are conducted at 25°C ± 2°C/60% RH ± 5% RH and *significant change* occurs at any time during 6 months' testing at the accelerated storage condition, additional testing at the intermediate storage condition should be conducted and evaluated against significant change criteria. The initial application should include a minimum of 6 months' data from a 12-month study at the intermediate storage condition.

In general, *significant change* for a drug product is defined as one or more of the following (as appropriate for the dosage form):

- A 5 percent change in assay from its initial value, or failure to meet the acceptance criteria for potency when using biological or immunological procedures (Note: Other values may be applied, if justified, to certain products, such as multivitamins and herbal preparations)

- Any degradation product's exceeding its acceptance criterion

- Failure to meet the acceptance criteria for appearance, physical attributes, and functionality test (e.g., color, phase separation, resuspendibility, caking, hardness, dose delivery per actuation). However, some changes in physical attributes (e.g., softening of suppositories, melting of creams) may be expected under accelerated conditions.

- Failure to meet the acceptance criterion for pH (liquid formulation)

- Failure to meet the acceptance criteria for dissolution for 12 dosage units

b. FPPs (WHO) packaged in impermeable containers (2.2.8.2)

Parameters required to classify the packaging materials as permeable or impermeable depend on the characteristics of the packaging material, such as thickness and permeability coefficient. The suitability of the packaging material used for a particular product is determined by its product characteristics. Containers generally considered to be moisture impermeable include glass ampoules.

Sensitivity to moisture or potential for solvent loss is not a concern for **FPPs (WHO)** packaged in impermeable containers that provide a permanent barrier to passage of moisture or solvent. Thus, stability studies for products stored in impermeable containers can be conducted under any controlled or ambient humidity condition.

c. FPPs (WHO) packaged in semipermeable containers (2.2.8.3)

Aqueous-based products packaged in semipermeable containers should be evaluated for potential water loss in addition to physical, chemical, biological, and microbiological stability. This evaluation can be carried out under conditions of low relative humidity, as discussed below. Ultimately, it should be demonstrated that aqueous-based **FPPs (WHO)** stored in semipermeable containers can withstand low relative humidity environments. Other comparable approaches can be developed and reported for non aqueous, solvent-based products.

Study	**Storage condition**	**Minimum time period covered by data at submission**
Long-term*	25°C ± 2°C/40% RH ± 5% RH or 30°C ± 2°C/35% RH ± 5% RH	12 months
Intermediate**	30°C ± 2°C/65% RH ± 5% RH	6 months
Accelerated	40°C ± 2°C/not more than (NMT) 25% RH	6 months

* It is up to the applicant to decide whether long-term stability studies are performed at 25°C ± 2°C/40% RH ± 5% RH or 30°C ± 2°C/35% RH ± 5% RH.
** If 30°C ± 2°C/35% RH ± 5% RH is the long-term condition, there is no intermediate condition.

When long-term studies are conducted at 25°C ± 2°C/40% RH ± 5% RH and significant change other than water loss occurs during the 6 months' testing at the accelerated storage condition, additional testing at the intermediate storage condition should be performed, as described under the

general case, to evaluate the temperature effect at 30°C. A significant change in water loss alone at the accelerated storage condition does not necessitate testing at the intermediate storage condition. However, data should be provided to demonstrate that the drug product will not have significant water loss throughout the proposed shelf life if stored at 25°C and the reference relative humidity of 40 percent RH.

A 5 percent loss in water from its initial value is considered a significant change for a product packaged in a semi-permeable container after an equivalent of 3 months' storage at 40°C/NMT 25 percent RH. However, for small containers (1 ml or less) or unit-dose products, a water loss of 5 percent or more after an equivalent of 3 months' storage at 40°C/NMT 25 percent RH may be appropriate if justified.

An alternative approach to studying at the reference relative humidity as recommended in the table above (for either long-term or accelerated testing) is performing the stability studies under higher relative humidity and deriving the water loss at the reference relative humidity through calculation. This can be achieved by experimentally determining the permeation coefficient for the container closure system or, as shown in the example below, using the calculated ratio of water loss rates between the two humidity conditions at the same temperature. The permeation coefficient for a container closure system can be experimentally determined by using the worst case scenario (e.g., the most diluted of a series of concentrations) for the proposed drug product.

Example of an approach for determining water loss:(WHO have different value in the table)

For a product in a given container closure system, container size, and fill, an appropriate approach for deriving the water loss rate at the reference relative humidity is to multiply the water loss rate measured at an alternative relative humidity at the same temperature by a water loss rate ratio shown in the table below. A linear water loss rate at the alternative relative humidity over the storage period should be demonstrated. For example, at a given temperature (e.g., 40**°**C), the calculated water loss rate during storage at NMT 25 percent RH is the water loss rate measured at 75 percent RH multiplied by 3.0, the corresponding water loss rate ratio.

Alternative relative humidity	**Reference relative humidity**	**Ratio of water loss rates at a given temperature**
60% RH	25% RH	1.9
60% RH	40% RH	1.5
65% RH	35% RH	1.9
75% RH	25% RH	3.0

Valid water loss rate ratios at relative humidity conditions other than those shown in the table above can also be used.

d. FPPs(WHO) intended for storage in a refrigerator (2.2.8.4)

Study	Storage condition	Minimum time period covered by data at submission
Long-term	5°C ± 3°C	12 months
Accelerated	25°C ± 2°C/60% RH ± 5% RH or 30 °C ± 2 °C/65% RH ± 5% RH or 30 °C ± 2 °C/75% RH ± 5% RH (WHO)	6 months

Whether accelerated stability studies are performed at 25 ± 2 °C/60% RH ± 5% RH or 30 °C ± 2 °C/65% RH
± 5% RH or 30 °C ± 2 °C/75% RH ± 5% RH is based on a risk-based evaluation. Testing at a more severe
accelerated condition can be an alternative to the storage condition at 25 °C/60% RH or 30 °C/65% RH.(WHO)
If the FPP is packaged in a semi-permeable container, appropriate information should be provided to assess the extent of water loss.

Data from refrigerated storage should be assessed according to the evaluation section of this guidance, except where explicitly noted below.

If significant change occurs between 3 and 6 months' testing at the accelerated storage condition, the proposed shelf life should be based on the real time data available from the long-term storage condition.

If significant change occurs within the first 3 months' testing at the accelerated storage condition, a discussion should be provided to address the effect of short-term excursions outside the label storage condition (e.g., during shipment and handling). This discussion can be supported, if appropriate, by further testing on a single batch of the drug product for a period shorter than 3 months but with more frequent testing than usual. It is considered unnecessary to continue to test a product through 6 months when a significant change has occurred within the first 3 monthsof accelerated studies at the specificcondition chosen in accordance with the risk analysis.(WHO)

e. FPPs intended for storage in a freezer (2.2.8.5)

Study	Storage condition	Minimum time period covered by data at submission
Long-term	-20°C ± 5°C	12 months

For FPPs intended for storage in a freezer, the shelf life should be based on the real time data obtained at the long-term storage condition. In the absence of an accelerated storage condition for drug products intended to be stored in a freezer, testing on a single batch at an elevated

temperature (e.g., 5°C ± 3°C or 25°C ± 2°Cor 30 °C ± 2 °C) (WHO) for an appropriate time period should be conducted to address the effect of short-term excursions outside the proposed label storage condition.

f. FPPs intended for storage below -20°C (2.2.8.6)

Drug products intended for storage below -20°C should be treated on a case-by-case basis.

Stability commitment

2.2.9 Evaluation

A systematic approach should be adopted in the presentation and evaluation of the stability information, which should include, as appropriate, results from the physical, chemical, biological, and microbiological tests, including particular attributes of the dosage form (e.g., dissolution rate for solid oral dosage forms).

The purpose of the stability study is to establish, based on testing a minimum of three batches of the FPP a shelf life and label storage instructions applicable to all future batches of the drug product manufactured and packaged under similar circumstances. The degree of variability of individual batches affects the confidence that a future production batch will remain within specification throughout its shelf life.

Where the data show so little degradation and so little variability that it is apparent from looking at the data that the requested shelf life will be granted, it is normally unnecessary to go through the formal statistical analysis; providing a justification for the omission should be sufficient.However, a provisional shelf-life of 24 months may be established providedthe following conditions are satisfied:

• The API is known to be stable (not easily degradable).
• Stability studies, as outlined above in section 2.1.12, have been performed and no signify cant changes have been observed.
• Supporting data indicate that similar formulations have been assigned ashelf-life of 24 months or more.
• The manufacturer will continue to conduct long-term studies until theproposed shelf-life has been covered, and the results obtained will besubmitted to the national medicines regulatory authority.

An approach for analyzing data of a quantitative attribute that is expected to change with time is to determine the time at which the 95 percent one-sided confidence limit for the mean curve intersects the acceptance criterion. If analysis shows that the batch-to-batch variability is small, it is advantageous to combine the data into one overall estimate. This can be done by first applying appropriate statistical tests (e.g., p values for level of significance of rejection of more than 0.25) to the slopes of the regression lines and zero time intercepts for the individual batches. If it is inappropriate to combine data from several batches, the overall shelf life should be based on the minimum time a batch can be expected to remain within acceptance criteria.The nature of the degradation relationship will determine whether the data should be transformed for linear regression analysis. Usually the relationship can be represented by a linear, quadratic, or cubic function on an arithmetic or logarithmic scale. Statistical methods should be employed to test the goodness of fit on all batches and combined batches (where appropriate) to the assumed degradation line or curve.

Limited extrapolation of the real time data from the long-term storage condition beyond the observed range to extend the shelf life can be undertaken at approval time if justified. This justification should be based, for example, on what is known about the mechanisms of degradation, the results of testing under accelerated conditions, the goodness of fit of any mathematical model, batch size, and/or existence of supporting stability data. However, this extrapolation assumes that the same degradation relationship will continue to apply beyond the observed data.

Any evaluation should consider not only the assay but also the degradation products and other appropriate attributes. Where appropriate, attention should be paid to reviewing the adequacy of the mass balance and different stability and degradation performance.

2.2.10 Statements/Labelling

A storage statement should be established for the labelling in accordance with relevant national requirements. The statement should be based on the stability evaluation of the drug product. Where applicable, specific instruction should be provided, particularly for drug products that cannot tolerate freezing. Terms such as *ambient conditions* or *room temperature* should be avoided.

There should be a direct link between the label storage statement and the demonstrated stability of the drug product. An expiration date should be displayed on the container label.

2.2.11In- use stability

The purpose of in-use stability testing is to provide information for the labelling on the preparation, storage conditions and utilization period of multidose products after opening, reconstitution or dilution of a solution, e.g. an antibiotic injection supplied as powder for reconstitution.

As far as possible the test should be designed to simulate the use of the (Drug product) in practice, taking into consideration the filling volume of the container and any dilution or reconstitution before use. At intervals comparable to those which occur in practice appropriate quantities should be removed by the withdrawal methods normally used and described in the product literature.

The physical, chemical and microbial properties of the (Drug product) susceptible to change during storage should be determined over the period of the proposed in-use shelf-life. If possible, testing should be performed at intermediate time points and at the end of the proposed in-use shelf-life on the final amount of the (Drug products) remaining in the container. Specific parameters, e.g. for liquids and semi-solids, preservations, per content and effectiveness, need to be studied.

A minimum of two batches, at least pilot-scale batches, should be subjected to the test. At least one of these batches should be chosen towards the end of its shelf-life. If such results are not available, one batch should be tested at the final point of the submitted stability studied.

This testing should be performed on the reconstituted or diluted (Drug product) throughout the proposed in-use period on primary batches as part of the stability studied at the initial and final time points and, if full shelf-life, long-term data are not available before submission, at 12 months or the last time point at which data will be available.

In general this testing need not be repeated on commitment batches (see 2.2.10)

In-use stability

2.2.12 Variations

Once the FPP has been registered, additional stability studies are required whenever variations that may affect the stability of the API or FPP are made, such as major variations.

The following are examples of such changes:

- change in the manufacturing process;
- change in the composition of the FPP;
- change of the immediate packaging;
- change in the manufacturing process of an API.

In all cases of variations, the applicant should investigate whether the intended change ill or will not have an impact on the quality characteristics of API and/or FPP and consequently on their stability.

The scope and design of the stability studies for variations and changes are based on the knowledge and experience acquired on API and FPP.

The results of these stability studies should be communicated to the regulatory authorities concerned.

2.2.13Ongoing Stability Studies

After marketing authorization has been granted, the stability of the FPP should be monitored according to a continuous appropriate programme that will permit the detection of any stability issue (e.g. changes in levels of impurities or dissolution profile) associated with the formulation in the container closure system in which it is marked. The purpose of the ongoing stability programme is to monitor the product over its shelf-life and to determine that the product remains, and can be expected to remain, within specifications under the storage conditions on the label.

This mainly applies to the FPP in the container system in which it is supplied, but consideration should also be given to inclusion in the programme of bulk products. For example, when the bulk product is stored for a long period before being packaged and/or shipped from a manufacturing site to a packaging site, the impact on the stability of the packaged product should be evaluated and studied. Generally this would form part of development studies, but where this need has not been foreseen, inclusion of a one-off study in the ongoing stability programme could provide the necessary data. Similar considerations could apply to intermediates that are over prolonged periods.

The ongoing stability programme should be described in a written protocol and results formalized as a report.

The protocol for an ongoing stability programme should extend to the end of the shelf-life period and should include, but not be limited to, the following parameters:

- number of batch (es) per strength and different batch size, if applicable. The batch size should be recorded, if different batch sizes are employed;
- relevant physical, chemical, microbiological and biological test methods;
- acceptance criteria;
- reference to test methods;(WHO)
- description of the container closure system (s);
- testing frequency;
- description of the conditions of storage (standardized conditions for long-term testing as described in these guidelines, and consistent with the product labelling, should be used); and
- other applicable parameters specific to the FPP.

The protocol for the ongoing stability programme can be different from that of the initial long-term stability study as submitted in the marketing authorization dossier provided that this is justified and documented in the protocol (for example, the frequency of testing, or when updating to meet revised recommendations).

The number of batches and frequency of testing should provide sufficient data to allow for trend analysis. Unless otherwise justified, at least one batch per year product manufactured in every strength and every primary packaging type, if relevant, should be included in the stability programme (unless none is produced during that year). The principle of bracketing and matrixing designs may be applied if scientifically justified in the protocol.

In certain situations additional batches should be included in the ongoing stability programme. For example, an ongoing stability study should be conducted after any significant change or significant deviation to the process or container closure system. Any reworking, reprocessing or recovery operation should also be considered for inclusion.

Out-of-specification results or significant atypical trends should be investigated. Any confirmed significant change, out-of-specification result, or significant atypical trend should be reported immediately to the relevant competent authorities. The possible impact on batches on the market should be considered in consultation with the relevant competent authorities.

A summary of all the data generated, including any interim conclusions on the programme, should be written and maintained. This summary should be subjected to periodic review.

2.2.14 Stability Commitment

When available long-term stability data on primary batches do not cover the proposed shelf life granted at the time of approval, a commitment should be made to continue the stability studies post approval to firmly establish the shelf life.

Where the submission includes long-term stability data from three production batches covering the proposed shelf life, a post approval commitment is considered unnecessary. Otherwise, one of the following commitments should be made:

- If the submission includes data from stability studies on at least three production batches, a commitment should be made to continue the long-term studies through the proposed shelf life and the accelerated studies for 6 months.

- If the submission includes data from stability studies on fewer than three production batches, a commitment should be made to continue the long-term studies through the proposed shelf life and the accelerated studies for 6 months, and to place additional production batches, to a total of at least three, on long-term stability studies through the proposed shelf life and on accelerated studies for 6 months.

- If the submission does not include stability data on production batches, a commitment should be made to place the first three production batches on long term stability studies through the proposed shelf life and on accelerated studies for 6 months.

The stability protocol used for studies on commitment batches should be the same as that for the primary batches, unless otherwise scientifically justified.

Where intermediate testing is called for by a significant change at the accelerated storage condition for the primary batches, testing on the commitment batches can be conducted at either the intermediate or the accelerated storage condition. However, if significant change occurs at the accelerated storage condition on the commitment batches, testing at the intermediate storage condition should also be conducted.(Only In ICH)

2.3 **STABILITY TESTING OF BIOTECHNOLOGICAL/BIOLOGICAL PRODUCTS (**ICH **Q5C)-It may be included**

3. GLOSSARY / DEFINITIONS

The following definitions are provided to facilitate interpretation of the guidance.

Accelerated testing: Studies designed to increase the rate of chemical degradation or physical change of a drug substance or drug product by using exaggerated storage conditions as part of the formal stability studies. Data from these studies, in addition to long-term stability studies, can be used to assess longer term chemical effects at no accelerated conditions and to evaluate the effect of short-term excursions outside the label storage conditions such as might occur during shipping. Results from accelerated testing studies are not always predictive of physical changes.

Active pharmaceuticals ingredient (API): Any substance or mixture of substances intended to be used in the manufacture of pharmaceutical dosage form and that, when so used, becomes an active ingredient of that pharmaceutical dosage form. Such substances are intended to furnish

pharmacological activity or other direct effect in the diagnosis, cure, mitigation, treatment, or prevention of disease or to affect the structure and function of the body.

Batch: The design quantity of starting material, packaging material or finished pharmaceutical product (FPP) processed in a single process or series of processes so that it is expected to be homogeneous. It may sometimes be necessary to divide a batch into a number of sub-batches, which are later brought together to form a final homogeneous batch. In the case of terminal sterilization, the batch size is determined by the capacity of the autoclave. In continuous manufacture, the batch must correspond to a defined fraction of the production, characterized by its intended homogeneity. The batch size can be defined either as a fixed quantity or as the amount produced in a fixed time interval.

Bracketing: The design of a stability schedule such that only samples on the extremes of certain design factors (e.g., strength, package size) are tested at all time points as in a full design. The design assumes that the stability of any intermediate levels is represented by the stability of the extremes tested. Where a range of strengths is to be tested, bracketing is applicable if the strengths are identical or very closely related in composition (e.g., for a tablet range made with different compression weights of a similar basic granulation, or a capsule range made by filling different plug fill weights of the same basic composition into different size capsule shells). Bracketing can be applied to different container sizes or different fills in the same container closure system.

Climatic zones: The four zones in the world that are distinguished by their characteristic, prevalent annual climatic conditions. This is based on the concept described by W. Grimm (*Drugs Made in Germany*, 28:196-202, 1985 and 29:39-47, 1986).

Commitment batches: Production batches of a drug substance or drug product for which the stability studies are initiated or completed post approval through a commitment made in the registration application.

Container closure system: The sum of packaging components that together contain and protect the dosage form. This includes primary packaging components and secondary packaging components if the latter are intended to provide additional protection to the drug product. A packaging system is equivalent to a container closure system.

Dosage form: A pharmaceutical product type (e.g., tablet, capsule, solution, cream) that contains a drug substance generally, but not necessarily, in association with excipients.

Drug product: The dosage form in the final immediate packaging intended for marketing.

Drug substance: The unformulated drug substance that may subsequently be formulated with excipients to produce the dosage form.

Excipient: Anything other than the drug substance in the dosage form.

Expiration date: The date placed on the container label of a drug product designating the time prior to which a batch of the product is expected to remain within the approved shelf life specification, if stored under defined conditions, and after which it must not be used.

Formal stability studies: Long-term and accelerated (and intermediate) studies undertaken on primary and/or commitment batches according to a prescribed stability protocol to establish or confirm the re-test period of a drug substance or the shelf life of a drug product.

Impermeable containers: Containers that provide a permanent barrier to the passage of gases or solvents (e.g., sealed aluminium tubes for semi-solids, sealed glass ampoules for solutionsand aluminium/aluminium blisters for solid dosage forms.)(WHO).

Intermediate testing: Studies conducted at 30°C/65% RH and designed to moderately increase the rate of chemical degradation or physical changes for a drug substance or drug product intended to be stored long-term at 25°C.

Long-term testing: Stability studies under the recommended storage condition for the retest period or shelf life proposed (or approved) for labelling.

Mass balance: The process of adding together the assay value and levels of degradation products to see how closely these add up to 100 percent of the initial value, with due consideration of the margin of analytical error.

Matrixing: The design of a stability schedule such that a selected subset of the total number of possible samples for all factor combinations is tested at a specified time point. At a subsequent time point, another subset of samples for all factor combinations is tested. The design assumes that the stability of each subset of samples tested represents the stability of all samples at a given time point. The differences in the samples for the same drug product should be identified as, for example, covering different batches, different strengths, different sizes of the same container closure system, and, possibly in some cases, different container closure systems.

Ongoing stability study: The study carried out by the manufacturer on production batches according to a predetermined schedule in order to monitor, confirm and extend the projected re-test period (or shelf-life) of the API, or confirm or extend the shelf-life of the FPP.

Mean kinetic temperature: A single derived temperature that, if maintained over a defined period of time, affords the same thermal challenge to a drug substance or drug product as would be experienced over a range of both higher and lower temperatures for an equivalent defined period. The mean kinetic temperature is higher than the arithmetic mean temperature and takes into account the Arrhenius equation.

When establishing the mean kinetic temperature for a defined period, the formula of J. D.

Haynes *(J. Pharm. Sci.,* 60:927-929, 1971) can be used.

New molecular entity: An active pharmaceutical substance not previously contained in any drug product registered with the national or regional authority concerned. A new salt, ester, or noncovalent bond derivative of an approved drug substance is considered a new molecular entity for the purpose of stability testing under this guidance.

Pilot scale batch: A batch of a drug substance or drug product manufactured by a procedure fully representative of and simulating that to be applied to a full production scale batch. For solid oral dosage forms, a pilot scale is generally, at a minimum, one-tenth that of a full production scale or 100,000 tablets or capsules, whichever is larger.

Primary batch: A batch of a drug substance or drug product used in a formal stability study, from which stability data are submitted in a registration application for the purpose of establishing a retest period or shelf life, respectively. A primary batch of a drug substance should be at least a pilot scale batch. For a drug product, two of the three batches should be at least pilot scale batch, and the third batch can be smaller if it is representative with regard to the critical manufacturing steps. However, a primary batch may be a production batch.

Production batch: A batch of a drug substance or drug product manufactured at production scale by using production equipment in a production facility as specified in the application.

Provisional shelf-life: A provisional expiry date which is based on acceptable accelerated and available long-term data for the FPP to be marketed in the proposed container closure system.

Release specification: The combination of physical, chemical, biological, and microbiological tests and acceptance criteria that determine the suitability of an API or FPP at the time of its release.

Re-test date: The date after which samples of the drug substance should be re-examined to ensure that the material is still in compliance with the specification and thus suitable for use in the manufacture of a given drug product.

Re-test period: The period of time during which the drug substance is expected to remain within its specification and, therefore, can be used in the manufacture of a given drug product, provided that the drug substance has been stored under the defined conditions. After this period, a batch of drug substance destined for use in the manufacture of a drug product should be re-tested for compliance with the specification and then used immediately. A batch of drug substance can be re-tested multiple times and a different portion of the batch used after each re-test, as long as it continues to comply with the specification. For most biotechnological/biological substances known to be labile, it is more appropriate to establish a shelf- life than a re-test period. The same may be true for certain antibiotics.

Semi-permeable containers: Containers that allow the passage of solvent, usually water, while preventing solute loss. The mechanism for solvent transport occurs by absorption into one container surface, diffusion through the bulk of the container material, and desorption from the other surface. Transport is driven by a partial pressure gradient. Examples of semi-permeable containers include plastic bags and semi-rigid, low-density polyethylene (LDPE) pouches for large volume parenterals (LVPs), and LDPE ampoules, bottles, and vials.

Shelf life (also referred to as expiration dating period): The time period during which a drug product is expected to remain within the approved shelf life specification, provided that it is stored under the conditions defined on the container label.

Specification: A list of tests, references to analytical procedures, andappropriate acceptance criteria, which are numerical limits, ranges or other criteria forthe tests described. It establishes the set of criteria to which an API or FPPshould conform to be considered acceptable for its intended use. (WHO)

Specification, Release: The combination of physical, chemical, biological, and microbiological tests and acceptance criteria that determine the suitability of a drug product at the time of its release.

Specification, Shelf life: The combination of physical, chemical, biological, and microbiological tests and acceptance criteria that determine the suitability of a drug substance throughout its re-test period, or that a drug product should meet throughout its shelf life.

Stability indicating methods: Validated analytical procedures that can detect the changes with time in the chemical, physical or microbiological properties of the API or FPP, and that are specific so that the content of the API, degradation products, and other components of interest can be accurately measured without interference.

Stability studies (stability testing): Long-term and accelerated (and intermediate) studies undertaken on primary and/or commitment batches according to a prescribed stability protocol to establish or confirm the re-test period (or shelf-life) of an API or shelf-life of an FPP.

Storage condition tolerances: The acceptable variations in temperature and relative humidity of storage facilities for formal stability studies. The equipment should be capable of controlling the storage condition within the ranges defined in this guidance. The actual temperature and humidity (when controlled) should be monitored during stability storage. Short-term spikes due to opening of doors of the storage facility are accepted as unavoidable. The effect of excursions due to equipment failure should be addressed and reported if judged to affect stability results. Excursions that exceed the defined tolerances for more than 24 hours should be described in the study report and their effect assessed.

Stress testing (drug substance): Studies undertaken to elucidate the intrinsic stability of the drug substance. Such testing is part of the development strategy and is normally carried out under more severe conditions than those used for accelerated testing.

Stress testing (drug product): Studies undertaken to assess the effect of severe conditions on the drug product. Such studies include photostability testing (see ICH Q1B) and specific testing of certain products (e.g., metered dose inhalers, creams, emulsions, refrigerated aqueous liquid products).

Supporting stability data: Data, other than those from formal stability studies that support the analytical procedures, the proposed retest period or shelf life, and the label storage statements.

Such data include (1) stability data on early synthetic route batches of drug substance, small scale batches of materials, investigational formulations not proposed for marketing, related formulations, and product presented in containers and closures other than those proposed for marketing; (2) information regarding test results on containers; and (3) other scientific rationales.

Utilization period: A period of time during which a reconstituted preparation of the finished dosage form in amultidose container can be used.

4. References

1. Guidelines for stability testing of pharmaceutical products containing well established drug substances in conventional dosage forms. In: WHO Expert Committee on Specifications for Pharmaceuticals Preparations. Thirty-fourth report. Geneva, World Health Organization, 1996, Annex 5 (WHO Technical Report, No. 863)

   These guidelines were revised at the thirty-seventh and fortieth meetings of the WHO Expert Committee on specifications for Pharmaceutical Preparations. In: WHO Expert Committee on specifications for Organizations, 2003 (WHO Technical Report Series, No. 908), p.13 and WHO Expert Committee on Specifications for Pharmaceutical Preparations. Fortieth report. Geneva, World Health Organizations, 2006 (WHO Technical Report Series, No. 937), p. 12.

2. The following ICH Guidelines may be consulted in the context of stability testing:

   International Conference on Harmonisation. ICH Q1A (R2): Stability testing of new drug substances and products (http://www.ich.org/LOB/media/MEDIA419.pdf).

   International Conference on Harmonisation. ICH Q1B: Photostability testing of new drug substances and products (http://www.ich.org/LOB/media/MEDIA412.pdf).

   International Conference on Harmonisation. ICH Q1C: Stability testing of new drug dosage forms (http://www.ich.org/LOB/media/MEDIA413.pdf).

   International Conference on Harmonisation. ICH Q1D: Bracketing and matrixing designs for stability testing of new drug substances and products (http://www.ich.org/LOB/media/MEDIA414.pdf).

   International Conference on Harmonisation. ICH Q1E: Evaluation for stability data (http://www.ich.org/ LOB/ media/MEDIA415.pdf).

   International Conference on Harmonisation. ICH Q2R1: Validation of analytical procedures: text and methodology (http://www.ich.org/LOB/media/MEDIA417.pdf).

   International Conference on Harmonisation. ICH Q6A: Specification: Test procedures and acceptance criteria for new drug substances and new drug products: Chemical substances (http://www.ich.org/LOB/ media/MEDIA430.pdf).

Further information can be found on the ICH homepage:
http://www.ich.org/ cache/compo/276-254-1.html.

3. WHO Expert Committee on Specifications for Pharmaceutical Preparations. Geneva, World Health Organizations, (WHO Technical Report Series, No. 953, 2009), p. 87

4. ASEAN Guidelines on stability study of drug product, 9th ACCSQ-PPWG Meeting, Philippines, 21-24 February 2005, version 22 February 2005.

Appendix 1

Examples of testing parameters

Section I for active pharmaceutical ingredients

In general, appearance, assay and degradation products should be evaluated for all active pharmaceutical ingredients (APIs). Other API parameters that may be susceptible to change should also be studied where applicable.

Section II for finished pharmaceutical products

The following list of parameters for each dosage form is presented as a guide to the types of tests to be included in a stability study. In general, appearance, assay and degradation products should be evaluated for all dosage forms, as well as the preservative and antioxidant content if applicable.

The microbial quality of multiple-dose sterile and non-sterile dosage forms should be controlled. Challenge tests should be carried out at least at the beginning and at the end of the shelf-life. Such tests would normally be performed as part of the development programme, for example, within primary stability studies. They need not be repeated for subsequent stability studies unless a change has been made which has a potential impact on microbiological status.

It is not expected that every test listed be performed at each time point. This applies in particular to sterility testing, which may be conducted for most sterile products at the beginning and at the end of the stability test period. Tests for pyrogens and bacterial endotoxins may be limited to the time of release. Sterile dosage forms containing dry materials (powder fi lled or lyophilized products) and solutions packaged in sealed glass ampoules may need no additional microbiological testing beyond the initial time point. The level of microbiological contamination in liquids packed in glass containers with flexible seals or in plastic containers should be tested no less than at the beginning and at the end of the stability test period; if the long-term data provided to the regulatory authorities for marketing authorization registration do not cover the full shelf-life period, the level of microbial contamination at the last time point should also be provided.

The list of tests presented for each dosage form is not intended to be exhaustive, nor is it expected that every test listed be included in the design of a stability protocol for a particular fi nished pharmaceutical product (FPP) (for example, a test for odour should be performed only when necessary and with consideration for the analyst's safety).

The storage orientation of the product, i.e. upright versus inverted, may need to be included in a protocol when contact of the product with the closure system may be expected to affect the stability of the products contained, or where there has been a change in the container closure system.

Tablets

Dissolution (or disintegration, if justified), water content and hardness/ friability.

Capsules

• Hard gelatin capsules: brittleness, dissolution (or disintegration, if justified), water content and level of microbial contamination.

• Soft gelatin capsules: dissolution (or disintegration, if justified), level of microbial contamination, pH, leakage, and pellicle formation.

Oral solutions, suspensions and emulsions

Formation of precipitate, clarity (for solutions), pH, viscosity, extractables, level of microbial contamination.

Additionally for suspensions, dispersibility, rheological properties, mean size and distribution of particles should be considered. Also polymorphic conversion may be examined, if applicable.

Additionally for emulsions, phase separation, mean size and distribution of dispersed globules should be evaluated.

Powders and granules for oral solution or suspension

Water content and reconstitution time.

Reconstituted products (solutions and suspensions) should be evaluated as described above under “Oral solutions suspensions and emulsions”, after preparation according to the recommended labelling, through the maximum intended use period.

Metered-dose inhalers and nasal aerosols

Dose content uniformity, labelled number of medication actuations per container meeting dose content uniformity, aerodynamic particle size distribution, microscopic evaluation, water content, leak rate, level of microbial contamination, valve delivery (shot weight), extractables/
leachables from plastic and elastomeric components, weight loss, pump delivery, foreign particulate matter and extractables/leachables from plastic and elastomeric components of the container, closure and pump. Samples should be stored in upright and inverted/on-the-side orientations.

For suspension-type aerosols, microscopic examination of appearance of the valve components and container's contents for large particles, changes in morphology of the API particles, extent of agglomerates, crystal growth, foreign particulate matter, corrosion of the inside of the container or deterioration of the gaskets.

Nasal sprays: solutions and suspensions

Clarity (for solution), level of microbial contamination, pH, particulate matter, unit spray medication content uniformity, number of actuations meeting unit spray content uniformity per container, droplet and/ or particle size distribution, weight loss, pump delivery, microscopic evaluation (for suspensions), foreign particulate matter and extractables/leachables from plastic and elastomeric components of the container, closure and pump.

Topical, ophthalmic and otic preparations

Included in this broad category are ointments, creams, lotions, paste, gel,solutions, eye drops and cutaneous sprays.

• Topical preparations should be evaluated for clarity, homogeneity, pH,suspendability (for lotions), consistency, viscosity, particle size distribution (for suspensions, when feasible), level of microbial contamination/sterility and weight loss (when appropriate).
• Evaluation of ophthalmic or otic products (e.g. creams, ointments,solutions and suspensions) should include the following additional attributes: sterility, particulate matter and extractable volume.
• Evaluation of cutaneous sprays should include: pressure, weight loss, net weight dispensed, delivery rate, level of microbial contamination, spray pattern, water content and particle size distribution (for suspensions).

Suppositories

Softening range, disintegration and dissolution (at 37 °C).

Small volume parenterals (SVPs)

Colour, clarity (for solutions), particulate matter, pH, sterility, endotoxins. Stability studies for powders for injection solution should include monitoring for colour, reconstitution time and water content. Specifi c parameters to be examined at appropriate intervals throughout the maximum intended use period of the reconstituted drug product, stored under condition(s) recommended on the label, should include clarity, colour, pH, sterility, pyrogen/endotoxin and particulate matter. It may be appropriate to consider monitoring of sterility after reconstitution into a product, e.g. dual-chamber syringe, where it is claimed that reconstitution can be performed without compromising sterility.

• The stability studies for Suspension for injection should include, in addition, particle size distribution, dispersibility and rheological properties.
• The stability studies for Emulsion for injection should include, in addition,phase separation, viscosity, mean size and distribution of dispersed phase globules.

Large volume parenterals (LVPs)

Colour, clarity, particulate matter, pH, sterility, pyrogen/endotoxin and volume.

Transdermal patches
In vitro release rates, leakage, level of microbial contamination/sterility, peel and adhesive forces.

Model SOP

	INDIAN PHARMACOPOEIA COMMISSION			
	Standard Operating Procedure		**Page No.**	… of …
			SOP No.	IPC/SOP/001
	Section	Quality Assurance	**Revision No.**	00
	Effective Date	DD.MM.YYYY	**Review Date**	DD.MM.YYYY
Title: Preparation and Control of Standard Operating Procedure				

1.0 OBJECTIVE

To lay down the procedure for preparation, review, approval, distribution, withdrawal, archiving and disposition of Standard Operating Procedure (SOP)**.**

2.0 SCOPE

This SOP shall be applicable to all SOPs of IndianPharmacopoeia Commission (IPC).

3.0 RESPONSIBILITY

3.1 All the Officers and Section In-charge of IPC shall ensure that this parent SOP has been reflected in the Section/Departmental SOPs.

3.2 Quality Manager/Quality Assurance person designated by the Director shall ensure overall compliance.

4.0 PROCEDURE

4.1 Construction of SOP (Refer Annexure I)

SOP is constructed mainly in three parts, viz. header, front page and body. The contents of each parts of SOP shall be as follows:

4.1.1 Header

The header on each page shall provide following details:

(a) OrganizationLogo

The logo of the organization shall be printed.

(b) Name of the Laboratory

Name of the laboratory shall be written.

(c) Section

'Standard Operating Procedure' shall be written in this cell.

(d) Page No.

Page No. indicates serial no. of pages in "X of Y" pattern. Formats attached as annexure shall not be given page number in continuation of SOP pages. However, any annexure of more than one page shall be given the page number in the similar pattern of "X of Y" (Independent for each format).

	Name	Designation	Signature	Date
Prepared by	**Dept. Personnel**			DD.MM.YYYY
Reviewed by	**HOD/ QM/TM**			DD.MM.YYYY
Approved by	**Director**			DD.MM.YYYY

(e) SOP No.

SOP No shall be an alphanumeric unique number.

(f) Section

Particular section of the department or operation (if any) where the SOP shall be applicable. If any specific section does not exist in any department then word "General" shall be written under this column.

(g) Revision No.

Revision no. shall be numeric, consisting of maximum two digits.

(h) Effective Date

This date shall specify the date on which the SOP becomes effective. The difference between approval date and effective date shall not be more than 30 days.

(i) Review Date

This column shall specify the month, in which the individual SOP is to be reviewed. Review date should be two years from the month of the effective date. However, any SOP can be reviewed earlier than this date as per the requirement.

(j) Title

Indicates the title of the SOP, mentioning heading of the document.

4.1.2 Footer on Each Page (K)

Footer on each page of the SOP shall have Prepared by, Reviewed by and Approved by following with Name, Designation, Signature and Date.

4.1.3 Body

The SOP shall be prepared as per the below given sequence under the following headings:

(a) Objective

Shall write in a nutshell the purpose and use of the SOP, starting with "To lay down a procedure or prescribed for ……".

(b) Scope

Shall specify the boundary limits for the application of the SOP.

(c) Responsibility

Shall specify the personnel responsible for the development, implementation and monitoring of the SOPs.

(d) Procedure

Procedure shall provide the step wise actions to be taken for implementation of the SOP. It shall also provide the applicable references to other quality documents as and when required.

(e) Safety &Precautions (If any)

Shall mention the salient features of operational and behavioral precautions and safety measures.

(f) Revision History

Shall trace the chronological changes, reasons thereof at least last three revisions from the current version in short form or in single line. Revision history shall contain the change control no.

(g) References

Mention the document(s), if reference has been made into the preparation of the particular SOP.

(h) Abbreviation

Full form of an abbreviation shall appear in the text at the place where it appeared for the first time before start of use of an abbreviation. However, a list of full form of all the abbreviations used in the document shall also to be detailed under this column.

(i) Annexure

The list of annexure related to the document shall be mentioned under this heading along with individual specific format number of listed annexure but without mention of revision number e.g. Annexure-III of this document shall be written as "Record of Distribution, Withdrawal and Disposition of Document XYZ/03" where X is the code of department and YZ is the number of this document and "03" is for Annexure-III.

(j) Distribution

The list of departments to which the SOP and documents shall be distributed as a controlled copy shall be provided in a tabular form in Annexure-III, XYZ/04.

4.2 Formatting of the SOP

4.2.1 The SOP shall be prepared in accordance with the guideline given in this SOP.

4.2.2 The title and text shall be clearly understood and written in clear, plain English so that everyone who carries out a particular activity /operation /function shall perform in the same way and safely.

4.2.3 The language used shall be in directive form that gives step-wise instructions, the words like "shall", "must" are to be used rather than "should", "may" or "might" to the possible extent.

4.2.4 The page setting and text shall conform to the following specifications.

Paper Size	A4 Size

Language	English
Font Type/Size (Header)	Times New Roman, Bold/ 12 Point
Font Type/Size (Text)	Times New Roman, plain / 12 Point
Font size (Sections)	Times New Roman, bold / 12 Point
Printing	One side
Space Between Paragraph	Single

4.2.5 Text shall be divided into logical sections numbered 1.0, 2.0, and 3.0 etc. whereas 1.0 relates to Purpose; 2.0 relate to Scope; 3.0 relates to Responsibility etc. Type the section head in Uppercase (Capital) letters.

4.2.6 These sections shall be sub-divided into logical sections. 1.1; 1.2; 2.1; 2.2; 2.3 etc. Where required, these sub-divisions may further be sub-divided, as 1.1.1; 1.1.2; 1.1.3 etc. Type the subsections in sentence case. In case, in any sub section does not have any guideline or in complete sentence is intended to be written, bullets can be used instead of use of numeric system.

4.2.7 Each SOP shall be allocated a unique SOP No. by the concerned department and same shall be verified by QA personnel. The number shall be alphanumeric with maximum 15 characters. The SOP Number shall be written in the following sequence:

O/XXX/YYY/ZZ

Where,

"IPC" : The First characters in alphabetic digits shall be used for mention of organization (location) code e.g. IPC/XXXX/001/00

"/" : The second character shall be used as a "Slash"

"XXXX" : The third characters in alphabetic digits shall be used for mention of SOP's category code e.g. (QSP, INST, and GEN), (IPC/QSP/001/00, IPC/INST/001/00, IPC/GEN/001/00).

"/" : The fifth character shall be used as a "Slash"

"YYY" : The next three characters in numeric digits shall be used for allotting the serial number to SOP in ascending order starting from 001.

"/" : The next character shall be used as a "slash"

"ZZ" : The last two characters in two numeric digits shall be used for mention of version/revision number of SOP starting from 00.

e.g. the document number of First version of first SOP shall be allotted by QA manager.

4.2.8 All annexure should be numbered as Annexure I, II, and III as follows. All annexures/formats be a part of respective SOP and shall be attached to the SOP. Each annexure/ format of the SOP shall be numbered as follows:

O/XX/NNN/YY/nn

Where,

O : is Organization / location code

XXXX : is SOP category code,

YYY : is serial no of the SOP,

ZZ : is the version no.

e.g. IPC/QSP/001/02 is the second revision of the first format of this SOP.

4.2.9 The Name, Designation, Signature and Date shall be entered in blue pen only. Effective date and Review due on must be handwritten. Date shall be written as alphanumeric, starting with the day as two numeric characters, followed by the month in alphabetic characters and the year in two numeric characters showing the last two numbers of the year.

4.2.10 The gap between date mentioned in the control copy stamp and effective date shall be enough, to take care of time taken in distribution of SOP to other departments. SOP shall be made effective only after training

4.3 Writing of SOP

4.3.1 Person designated by the Director / section Incharge of concerned department shall write SOP.

4.3.2 After completion of draft of SOP, the concerned person shall circulate it to the persons/departments being involved in the operation of the SOP, for inviting their opinion /comments.

4.3.3 Person responsible for preparation of SOP in consultation of section In-charge shall incorporate the relevant portion of received comments / opinion in the draft SOP.

4.4 Review of the SOP

4.4.1 Section Incharge of the concerned department shall designate the person for review of the draft SOP.

4.4.2 In case, other department(s) is getting affected by the procedure of SOP under question, draft SOP shall also be sent to the related department(s) for review purpose.

4.4.3 Reviewer's further comments, if any, shall be incorporated in the final version of SOP in consultation with section Incharge of concerned department(s).

4.4.4 The reviewer(s) shall sign the final version in the provided columns. Number of reviewers may be more than two, depending upon the Cross functional activities.

4.4.5 Person responsible for preparation of SOP shall arrange to send the final version of SOP duly signed by him/her and by the reviewer(s), to the QA or to the person designated by Quality Manager / Director for final approval.

4.5 Approval of the SOP

4.5.1 Director / Quality Manager or to the person designated by Director shall approve the SOP.

4.5.2 Quality Manager or the person designated by Director shall arrange the mention of effective date and review due on in respective columns and affixing "**MASTER COPY**" (Black in color) on the bottom of the right corner of each page in the original signed copy.

4.5.3 QA, as a custodian shall retain this **"MASTER COPY"**.

4.5.4 Functional department shall organize for training of the concerned personnel responsible before executing the SOP.

4.6 Distribution of SOP

4.6.1 Copies of the SOP shall be distributed to all the concerned sectional / departmental Incharge. QA personnel shall take photocopies from Master Copy for the desired number of copies.

4.6.2 QA personnel shall stamp the Photocopy of SOP as "**CONTROLLED COPY**" (Blue in colour) on the bottom near to Master copy of each page the "Copy No." shall given by QA personnel as per codification of departments.

4.6.3 Incase, SOP is required to be submitted to an external party, QA personnel responsible of maintaining the master copy, shall take photocopy of master copy and shall stamp **"UNCONTROLLED COPY"** [Red in color.] on the bottom near to Master of each page.

4.6.4 The impression of all the stamps is attached as Annexure VI.

4.7 Revision of SOP

4.7.1 All SOPs shall be reviewed on or before date mentioned as review due on.

4.7.2 Any revision in the SOP or annexure(s) shall be through Change control system. Refer SOP on change control.

4.7.3 In case of any revision in the existing SOP, same procedure shall be adopted for preparation of revised SOP as mentioned in the procedure of preparation of SOP starting from clause 4.3.

4.7.4 In case ,during or before periodic review of SOP , any change / revision in the SOP or in Annexure(s) or in both is required ,then SOP or Annexure(s) or both as per the case shall be reprinted after incorporating suggested and approved changes as per change control system.

4.7.5 In the case of revision in the main SOP {with or without annexure(s)}, a next revision number shall be given to the SOP and the summary of revisions made shall be documented under the column of revision history.

4.7.6 In case of revision only in the annexure(s) no next revision number to the SOP shall be given. In this case next revision number shall be given to a particular revised annexure(s). The revision history and effective date of annexure(s) shall be maintained in the Master list of Records .In this case only revised annexure(s) shall be printed for master copy and distribution.

4.7.7 Concerned department shall arrange to send the print of revised annexure(s) to QM or person designated by Director for further action of attachment with master copy and for distribution.

4.7.8 QM or person designated by Director shall affix the stamp of master copy on the original copy of revised annexure(s) and shall attach with the already issued master copy of SOP.

4.7.9 QM or person designated by Director shall take photocopy (ies) of issued annexure(s) and distribute to the concerned department(s) and retrieving the old version.

4.7.10 In case, during periodic review of SOP and Any master documents, no revision is required in the existing version of SOP, annexure(s) and any master documents, the same form of SOP along with annexure(s) and any master documents shall be extended for use by any department with the approval of QM or the person designated by Director.

4.7.11 In case of 4.7.10, concerned department shall get the Review Report and Extension Authorization (Annexure-IV,) issued from QA, QM or the person designated by Director for allowing its use before or up to next review due on.

4.7.12 QM or the person designated by Director shall stamp master Copy on the original copy of Review Report and Extension Authorization (Annexure-IV,) and attached with the master document and shall issue the controlled copy of this form to the concerned department(s) to which earlier copy of SOP was distributed.

4.7.13 Personal responsible for maintaining the SOPs in the concerned department(s), shall ensure that received copy of Review Report and Extension Authorization (Annexure-V) is attached with the controlled copy of said SOP, already available in the department.

4.8 Withdrawal and Archiving of SOP

4.8.1 Withdrawal and archiving of SOP and or related annexure(s) shall be done by QA department. The controlled copy of the previous version of the SOP and or related annexure(s) shall be withdrawn by QA Department before effective date of issues document. All the retrieved Controlled Copies shall be destroyed by QA or the person designated by Director. The activity is recorded on annexure II.

4.8.2 The master copy of archived SOP and or related annexure(s) shall be stamped **''OBSOLETE''** (Green in color), on the middle of each page of the SOP. Obsolete copy of master SOP shall be preserved for any future reference by QM or person designated by Director. The record of Obsolete SOP shall be maintained as per Annexure - III.

5.0 SAFETY AND PRECAUTION

Do not use any SOP if it is not signed and issued by QA Personnel's or the authorized signatories. Do not use pencil for recording on SOP. Do not use adhesive tape or whitener on SOP.

6.0 REVISION HISTORY

7.0 REFERENCES

In-House

8.0 ABBREVIATIONS

SOP	:	Standard Operating Procedure
QM	:	Quality Manager
QA	:	Quality Assurance
Dep.	:	Department

9.0 ANNEXURE(s)

Annexure 1	:	Format for SOP preparation IPC/QSP/001/00/F-01
Annexure 2	:	Record of Distribution, Withdrawal and Disposition of Document(s) IPC/QSP/001/00/F-02
Annexure 3	:	Record of Obsolete Document(s) IPC/QSP/001/00/F-03
Annexure 4	:	Review Report and Extension Authorization IPC/QSP/001/00/F-04
Annexure 5	:	Impression of StampsIPC/QSP/001/00/F-05

Annexure 6 : Organization / Departmental Code IPC/QSP/001/00/F-06

IPC/QSP/001/00/F-01

	INDIAN PHARMACOPOEIA LABORATORY
	ANNEXURE 1: FORMAT FOR STANDARD OPERATING PROCEDURE

Header on Each Page

<table>
<tr><td rowspan="5">A</td><td colspan="4">B</td></tr>
<tr><td colspan="2" rowspan="2">C</td><td>D</td><td></td></tr>
<tr><td>E</td><td></td></tr>
<tr><td>F</td><td></td><td>G</td><td></td></tr>
<tr><td>H</td><td></td><td>I</td><td></td></tr>
<tr><td colspan="5">J</td></tr>
</table>

Body

1.0 Objective
2.0 Scope
3.0 Responsibility
4.0 Procedure
5.0 Safety & precautions (If any)
6.0 Revision History
7.0 References
8.0 Abbreviation
9.0 Annexure

Footer Each Page (K)

	Name	**Designation**	**Signature**	**Date**
Prepared by				
Reviewed by				
Approved by				

IPC/QSP/001/00/F-02

सत्यमेव जयते IPC	**INDIAN PHARMACOPOEIA LABORATORY**
	ANNEXURE 2 : RECORD OF DISTRIBUTION OF DOCUMENT(s)

S. No.	**Name of Document**	**Document No.** (With revision number)	**Date of Issue**	**Department**	**Record Holder** (Signature/Date)

IPC/QSP/001/00/F-03

	INDIAN PHARMACOPOEIA LABORATORY
	ANNEXURE 3 : RECORD OF OBSOLETE DOCUMENT(s)

S. No.	Name of Document	Document No.	Date of Discontinuation	Record Holder (Signature/Date)

सत्यमेव जयते IPC	**INDIAN PHARMACOPOEIA LABORATORY**
	ANNEXURE 4 : REVIEW REPORT AND EXTENSION AUTHORIZATION

Date:

Title:

Document No.:

Effective Date:

Current Review due on:

Review Remarks / Comments:

Review done by
(Signature and Date)

Proposed Extension Period:

Next Review due on:

Extension Requested by
(Signature and Date)
(Head of the Department)

Extension Approved By
(Signature and Date)
(QM or Designated authorized person from Director)

सत्यमेव जयते IPC	**INDIAN PHARMACOPOEIA LABORATORY**
	ANNEXURE 5 : IMPRESSION OF STAMPS

MASTER COPY (Black Color)

CONTROLLED COPY (Blue Color)

UNCONTROLLED COPY (Red Color)

OBSOLETE COPY (Green Color)

	INDIAN PHARMACOPOEIA LABORATORY
	ANNEXURE 6 :ORGANIZATION / DEPARTMENTAL CODE

S. No.	AUTHORIZED HOLDERS	COPY NO.
1.	Master copy (Quality Manager)	00
2.	Secretary-cum-Scientific Director / Incharge of Institute	01
3.	Quality Manager	02
4.	Technical Manager	03
5.	Library & Information Officer (Publication/Documentation)	04
6.	Auditors Copy	05
7.	Section- In charge , Standard Development	06
8.	Section-In charge, Analytical Research Development	07
9.	Quality Assurance	08
10.	Section-In charge / HOD's (Technical Services)	09

Note:*Any other organizations/departments may include.*

List of SOPs

General SOPs

1. SOP for preparation, issue & control of new documents/amendments of documents
2. SOP for change control procedure
3. SOP for maintaining confidentiality
4. SOP for review of requests, tenders & contracts
5. SOP for sub-contracting of testing items
6. SOP for purchase, receipt, storage and disposal of consumable items
7. SOP for dealing with complaints
8. SOP for control of non-conformance, corrective & preventive action
9. SOP for improve effectiveness of management system
10. SOP for dealing with deviations
11. SOP for retention & custody and retrieval of records
12. SOP for planning and conducting an internal audit
13. SOP for management review meeting
14. SOP for planning, and conducting training program
15. SOP for controlling the access to the lab
16. SOP for house keeping
17. SOP for validation of analytical method
18. SOP for estimation of uncertainty of measurement
19. SOP for preparation of specifications, Installation and maintenance of Instruments
20. SOP for in house maintenance and calibration of equipments
21. SOP for awarding AMC to outside agencies
22. SOP for purchase of capital equipments
23. SOP for maintenance and use of reference material
24. SOP for reference material handling and storage
25. SOP for repeat analysis of sample
26. SOP for organizing & participation in inter laboratory proficiency testing
27. SOP for issue, dispatch & retention of test reports
28. Procedure for electronic storage, amendment and transmission of results/data
29. SOP for internal quality check
30. SOP for reporting of results
31. SOP for laboratory waste disposal
32. SOP for handling of Out of Specification (OOS) results
33. SOP for handling of incident during operation
34. SOP for preparation and control of master list of documents
35. SOP for procedure of feed back
36. SOP for intermediate check of instruments
37. SOP for quality risk management.
38. SOP for handling of Out of Trend (OOT) Analysis

Technical SOPs

39. Operation, maintenance, and calibration of equipments (separate SOP for each equipment)

Important Links

Central Drugs Standard Control Organization, New Delhi	
National Institute of Biologicals, Noida	www.nib.gov.in
Indian Veterinary Research Institute, Izzatnagar	
Central Drugs Laboratory, Kasauli	
National Accreditation Board of Testing and Calibration Laboratories, Gurugram	

Frequently Asked Questions

(The Questions and Answers given below are based on the queries received by IPC from the stakeholders. The users of IPC products and services are encouraged to help the commission by communicating their comments and suggestions)

B. General Questions on IPC and IP

1. What is the legal status of Indian Pharmacopoeia?

Indian Pharmacopoeia (abbreviated as IP) is the official book of standards for drugs in India under the Second Schedule of the Drugs and Cosmetics Act 1940 and Rules 1945 there under.

2. When is IP published and what are the nature of supplements to the IP?

IP is published periodically and regularly by IPC, presently after every four years, In between the two editions, the amendment lists are released from time to time containing corrections, upgradations etc. and uploaded on the IPC website. Also IPC publishes addenda intermittently between the two editions of IP.

3. Where is the IP available from?

IP can be procured from the office of the Secretary-cum-Scientific Director, Indian Pharmacopoeia Commission, Sector 23, Raj Nagar, Ghaziabad201 002 (UP), India or from authorized distribution network (for details see the website: www.ipc.gov.in). The Pharmacopoeia is available in hard and/or soft copy (DVD).

4. What is effective date for new edition of IP, amendment list and addendum?

The effective date for the main edition of IP is mentioned inside in the initial pages of volume I. The effective date is usually six months after the publication. The amendment list becomes effective on the date of release. The effective date of addendum is presently three months after the publication.

5. What is IP guidance manual?

IP Guidance Manual is a book published to enable the users of IP to perform the activities related to performance of the tests or associated activities prescribed in the IP and also to understand or interpret the requirements of IP for proper compliance of the requirements. Guidance manual provide specific features of IP, Validation of analytical procedures, procurement, availability and use of IP reference substances, calibration of equipment and glassware, SOP's and infrastructure requirement of drug testing laboratory.

6. What are the roles of IPC?

IPC is a multifunctional body with primary objective of publishing the Indian Pharmacopoeia on a regular basis. Along with this role, it also prepares and supplies IPRS for drug substances, impurities and herbal marker substances. In parallel it is involved in publication of NFI and also is responsible for the PvPI and MvPI. In addition, IPC regularly conducts training programmes for stakeholders. Indian Pharmacopoeia Laboratory is the only accredited by WHO-prequalified government laboratory. R&D, FDC, NDS, Monograph development. IPC is WHO Collaboration centre for Public Health Programme and Regulatory Services. The patent and proprietary medicine should comply with the general requirements of the dosage forms under which it falls as given in the Indian Pharmacopoeia.

7. **What is the status of accreditations of IPC and IP Lab?**

IPC have accreditations for:
NABL Accreditation (ISO 17025:2005) for Chemical and Biological
NABL Accreditation for (ISO 17034:2016) for Reference Material Producer (RMP)
NABL Accreditation for (ISO 17043:2010) for Proficiency Test (PT) Provider
WHO Pre-Qualification for Quality Control Laboratory

8. **How the quality of monographs in IP differ from other National/International Pharmacopoeial monographs?**

Indian Pharmacopoeia has been taking lead in many aspects over other leading compendia. Also, IP has been the first in the world to develop monographs on antiretroviral drugs and many rational fixed dose combinations. It is for the first time the monograph on veggie capsules has been introduced in the latest edition of IP. IPC has formal arrangement with USPC for sharing monographs. IPC has observer status with EDQM. Moreover IPC is working towards being a partner with world Pharmacopoeias in the harmonization process. Largely the standards of Indian Pharmacopoeia are at par with global compendia, except the rationalization in some situations, to comply with Indian Laws and Standards, yet keeping the public health above all.

9. **How one can participate in the work of IPC?**

Interested parties can participate in the following ways:

(i) Monograph Development: IPC encourages to submit new draft monographs (online through IPC website or offline) along with required documents as per the check list for monograph development. Draft monograph may be the starting point for an official public standard.

(ii) Revision/Upgradation of Existing Monograph: Revision/upgradation to the existing General Chapters and Monographs in the current official edition of the IP may be proposed.

(iii) IPRS Development: A candidate material for the development of IPRS may also be donated.

10. **What are draft amendments proposed to be included in IP?**

Draft amendments to IP are the ones that are drafted on the recommendation of expert committees and made available on the IPC website (www.ipc.gov.in) for comments of stakeholders. The same are finalized after the technical discussion/deliberation of the received comments by the technical experts. After finalization, the same is/are displayed/published on the website of IPC and compiled later into the amendment list and addendum.

11. **What are the draft monographs?**

Any new monograph to be introduced into IP is done in accordance with the laid down criteria and vetted by the expert committee. Such monographs are drafted in IP format, and displayed on the website (www.ipc.gov.in) for stakeholder's comments. They may be considered for next addendum/edition of IP.

12. **What if a drug is banned in India by CDSCO, but monograph is/are present in IP, can such a drug/product still be manufactured and marketed in India?**

In case a ban order is issued by CDSCO for any drug and its products, automatically the existing monographs in current or previous editions of IP become inapplicable. The Monographs of the IP should be applied subject to the restriction imposed by Indian drug regulatory law(s).

13. **What is the status of previous edition of IP?**

For this, as also mentioned above, the Second Schedule of the Drugs and Cosmetics Act, 1940 states as follows: "In case the standards of identity, purity and strength for drugs are not specified

in the edition of the Indian Pharmacopoeia for the time being in force but are specified in the edition of the Indian Pharmacopoeia immediately preceding, the standards of identity, purity and strength shall be those occurring in such immediately preceding edition of the Indian Pharmacopoeia and such other standards as may be prescribed".

14. **If an error in a monograph has been noticed, whom to contact in IPC?**
In case of any error or mistake found in current edition of IP, including supplements, the same needs to be reported to IPC by communicating to the following address by post/email.

By post: Secretary-cum-Scientific Director
Indian Pharmacopoeia Commission
Ministry of Health & Family Welfare
Government of India
Sector-23, Raj Nagar,
Ghaziabad (U.P.)-201002, India
Phone: +91-120-2783400, 2783401, 2783392
Fax**:** +91-120-2783311

By e-mail: lab.ipc@gov.in

15. **Once an error is reported and data has been supplied by the stakeholder, what happens next?**
The data is examined by the relevant technical expert committees of IPC.The revised method is verified by Indian Pharmacopoeia Laboratory and the amendment is published accordingly.

C. Notifications and General Chapters

16. **Are test protocols of new drugs available with IPC?**
Yes, IPC may be verifying test protocols of certain drugs that have been introduced recently in the Indian market on approval of CDSCO. It is not considered as public information till a draft monograph is called for comments.

17. **Is Usual Strengths in IP considered mandatory?**
The usual strengths prescribed in IP are suggestive but of a drug does not restrict other strengths, if approved by the Regulatory Authority, to comply with the standards specified in the Indian Pharmacopoeia and labeled as 'IP'.

18. **Is solubility mandatory to comply with?**
No, please refer Appendix 2.4.26 for further detail and are intended as information on the approximate solubility at a temperature between 15^{o} and 30^{o}, unless otherwise stated, and are not to be considered as official requirements. However, a test for solubility stated in a monograph constitutes part of the standards for the substance that is the subject of that monograph.

19. **How much deviation is allowed from a relative retention time prescribed in a monograph?**
There are no acceptance criteria suggested to variation in relative retention times, as IP at this stage is not specifying the brand of the column.

20. **How to use Correction Factor and Relative Response Factor given in certain Monographs?**
In case where Correction Factors are mentioned in the monograph, the same shall be applied by direct multiplication with area of the respective peak. For Relative Response Factor, the respective peak area should be divided by it**.**

21. **Can my company label its product as IP to indicate compliance to a proposed draft monograph?**
A company is not permitted to label their article as “IP” on the basis of adherence to a proposed draft monograph. “IP” labeling is permitted only when the monographs becomes official.

22. **Does the requirement of individual monograph supercede the general chapter requirement?**
Yes, as a general rule the monographs are to be read in tandem with the relevant general chapters and any other text in the IP. But there may be cases where acceptance criteria and/or methodology may be different in the monograph for specific test, owing to which the monograph would supercede the general chapter requirement(s). In situations, where a specific monograph is silent on specific requirement outlined in the general chapter, the requirement of the latter would prevail.

23. **Is complying to description part in the monograph mandatory?**
The statements under the heading description are for preliminary evaluation, unless otherwise specified.For example, a note in general chapter on solubility specifies as: “A test for solubility becomes the test for purity only where a special quantitative test given in the individual monograph and is an official requirement.”The failure in description may lead to declaration of not of standard quality. Hence the stakeholder should carefully assess the status of the description with respect to quality of the pharmaceutical.

24. **Can we use alternative method for analysis as a substitute for official method published in IP monograph?**
Yes, alternative methods of analysis may be used forin- house control purposes, provided thoseareproven to give results of equivalent accuracy and enable an unequivocal decision to be made as to whether compliance with the standards of the monograph would be achieved if the official methods were used for testing. Such alternative methods must be validated and are subject to approval by the competentauthority. In the event of doubt or dispute, the methods of analysis of the Pharmacopoeia are alone authoritative and only the result obtained by the method given in the Pharmacopoeia is conclusive.

25. **What does the word “solution” imply where the name of solvent is not stated in the test?**
Where the name of solvent is not stated, “solution” implies a solution in water. The water used at the minimum complies with the requirements of the monograph on Purified Water.

26. **Can IPC consider to include superior method developed by stakeholder to replace existing IP Method?**
There are several considerations that are to be considered by a pharmacopoeial agency for inclusion of a method in a monograph, including ruggedness and complete validation. The technical expert committee of IPC responsible for the monograph developmentusuallycritically evaluates any submitted proposal and data, followed by thorough testing at Indian Pharmacopoeial Laboratory or collaborative laboratory of IPC.If accepted, the monograph may be amended to incorporate superior method. It may be pertinent to mention that the stakeholders may be asked to provide all the candidate materials employed in the proposed ‘superior’ method.

27. **My company only manufactures a few products. Can I buy a single monograph of the IP?**

Not really. As a practice, it is desired that the stakeholders, in particular the manufacturer possess anoriginal copy of IP in force at that time.

28. **I am having difficulty in meeting the system suitability criteria in a monograph, what changes can I make to the chromatographic conditions?**
The changes,which may be made to the chromatographic conditions in order to achieve compliance with the system suitability criteria mentioned in the individual monograph,are outlined under "Adjustment to chromatographic conditions" in General Chapter 2.4.13. Gas Chromatography, and 2.4.14. Liquid Chromatography. As long as you stay within the permissible changes, further validation of the method is not required. Any change outside the defined limits requires that the modified method is revalidated to meet system suitability criteria.

29. **To what extent revalidation is needed for the Pharmacopoeial Methods at the users end?**
Pharmacopoeial methods are considered validated and, therefore, their complete revalidation is not required when first used in the laboratory. However, the user may need to verify applicability of the method with respect to use in qualitative and quantitative testing.Verification of the method shall be performed to establish the objective of suitability of the methods for their intended purpose. Verification consists of assessing selected analytical performance characteristics (like specificity in qualitative tests and accuracy, precision and bias in quantitative tests) to generate appropriate data rather than repeating the validation process.

D. Indian Pharmacopoeia Reference Substances (IPRS)

30. **What is IPRS?**
These are highly-characterized physical specimens used in testing by pharmaceutical and related industries to help ensure the identity, strength, quality, and purity of medicines. These are Primary standards, having appropriate quality within a specified context and are accepted without requiring comparison to another substance. Their applications are limit tests for impurities or related compounds, assays for drug substances & formulations, system suitability tests and chromatographic & spectrophotometric procedures.

31. **What is the status of availability of reference substances and impurity standards at IPC?**
The lists of IP Reference Substances and Impurity Standards available with IPC are given on IPC website
(https://ipc.gov.in/images/List_of_IP_Reference_Substances_available_at_IPC_Ghaziabad-converted.pdf).

32. **From where to procure IPRS (National Reference Standard) of human vaccine and immunosera?**
These may be procured from
CDL, Kasauli-173204, Himachal Pradesh.
Website www.cdlkasauli.gov.in

33. **From where to procure reference microbial cultures?**
Reference Microbial Cultures may be procured from IMTECH, Chandigarh (www.imtech.res.in) at following address:
MTCC (Microbial Type Culture Collection Bank CSIR-Institute of Microbial Technology (IMTECH) Sector-39A, Chandigarh-160036)
NCIM (National Collection of Industrial Microorganisms, NCL, Pune)

(http://www.ncl-india.org/ncim)
ATCC (American Type Culture Collection Centre Maryland USA)
NCTC (National Collection of Type Culture, London)
National Centre for Veterinary Type Cultures, Sirsa Road, Hisar-125001

E. Microbiological Testing

34. Why microbial reference cultures should not be used in microbiological analysis if they are more than 5 passages from the original culture?

In order to prevent any phenotypic change in the microbial reference culture used, the microorganism used in the test should not be more than 5 passages from the original culture. One passage is defined as inoculation of the organisms from existing culture to a fresh medium followed by incubation and growth.

35. What is the purpose of the negative control?

The purpose of negative control is to show that there is no contamination during the microbiological analysis of the product. If a positive result is obtained with a negative control, the test can be regarded as invalid and may be repeated. Negative controls should be included every time the product is tested.

36. In case of total fungal count at which incubation period (5 or 7 days) the test can be considered complete?

Fungal cells can be stressed under the environmental factors. These cells have multiple mechanisms to recover from enforced stress. Hence it is advisable to provide an extra period of recovery by extending till 7 days, if there is no growth in 5 days.

For in House testing at the stakeholders end, the test can be considered complete in 5 days when the validated data generated prove that there is no growth observed at the end of 7 days and even 5 days.

37. What does the factor of 2 mean?

It means that the result can be twice that of the inoculum. For example with an inoculum of 100 CFU, acceptable counts are: 100/2 = 50 CFU to 100 x 2 = 200 CFU.

38. What is the maximum valid dilution (MVD) and how it is calculated?

MVD is the maximum allowable dilution of a sample at which the endotoxin limit can be determined. MVD is calculated by following general formula.

$$MVD = \frac{\text{Endotoxin Limit} \times \text{Concentration of the test solution}}{\text{Lysate sensitivity } (\lambda)}$$

39. How glassware is depyrogenated?

Glassware can be depyrogenated by heating in a hot air oven at 250° for 30 minutes.

F. Water

40. What is the testing criterion of specific pathogen in purified water?

The requirement of the test for absence of organisms (specific pathogens) in purified water depends on the route of administration of the product in which the purified water is used. For example, if the purified water is used only for Oral Dosage from (either solid or liquid) then it should be tested for absence of *E. coli, Salmonella, Bile tolerant Gram Negative Bacteria* and *Shigella*, but if purified water is used in the preparation of Vaginal Tablets (Pessaries) then it

should be tested for *Candida albicans, Staphylococcus* as well as *Pseudomonas* as the first two organisms can cause vaginal infection and the last one can cause urinary tract infection. Similarly, if purified water is used in preparation of a Cream which is meant to apply on burn or wound, it should be tested for the absence of *Staphylococcus* and *Pseudomonas* because they can cause infection of burns or wound. This is the reason that the specified pathogens for purified water should be tested.

G. Monographs

41. We performed the TLC procedure as specified in the IP monograph and the spots were not visible, can you revise the method?

The reasons for non-visibility of spot should be considered before revising the method. A few important factors are improper storage of TLC plates, adequate pre-treatment of TLC plate where necessary. Was the chamber saturated, sealed and lined? Were the spots dry before the plate was placed in the chamber? After the drying stage of the test the TLC plate may be re-sprayed and re-dried to ensure a reaction is complete; ensure the spray system is adequate i.e. produces a fine mist as opposed to droplets.

42. The monograph for "active substance x" has been revised to include an HPLC method for related substances. Why has the same method not been included in the corresponding formulation monographs?

The policy of the IPC is that wherever possible the same methods should be included in formulation monographs as the corresponding active substance monographs. However, it is not always possible to include the same methods due to interference from excipients and/or extraction problems.

43. The monograph indicates that the IPRS should be used in the standard preparation; can I use a different grade of material?

In routine testing, a secondary standard commonly known as a working standard may be used provided it has been characterized and calibrated by comparison with a primary reference substance and its suitability for carrying out the compendial tests has been established. The secondary standard should be assigned shelf life.

Where a pharmacopoeial reference standard is referred to in a monograph or general chapter, it represents the official standard that is alone authoritative in case of doubt or dispute.

44. The monograph for "active substance x" has been revised to include an HPLC method for related substances. Why has the same method not been included in the corresponding formulation monographs?

The policy of the IPC is that wherever possible, the same methods should be included in formulation monographs as that of the corresponding active substance monographs. However, it is not always possible to include the same methods in formulation due to interference from excipients and/or extraction related issues problems.

45. How to contact IPC?

By email: lab.ipc@gov.in
By post: Indian Pharmacopoeia Commission
Ministry of Health & Family Welfare
Government of India
Sector 23, Raj Nagar,

Ghaziabad (U.P.) 201 002, India
Phone: +91-120-2783400, 2783401, 2783392
Fax**:** +91-120-2783311