Guidance Document

Indian Pharmacopoeia Reference Substances

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Disclaimer

This Guidance Document is compiled by the Indian Pharmacopoeia Commission (IPC) after consultations with the 'Core Expert Committee' constituted by the IPC for this purpose. The information contained herein represents the current best practices in the field of pharmacopoeial sciences to demonstrate compliance with the existing regulatory requirements. The guidance provided in this document 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 help in ensuring proper compliance with the IP requirements when standards of drugs are to be determined. The content of this document shall be treated as non-mandatory guidance and the information contained herein is subject to review by the IPC. Approaches and methods other than those described in this Guidance Document may be adopted if found suitable and justified. Where provisions of the law exist, the law as prevailing at the relevant time shall apply.

Introduction

Indian Pharmacopoeia Reference Substances (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 products, phytopharmaceuticals, 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 shall be used. In cases where IPRS is not available, other equivalent phamacopoeial standard may be used.

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 analysis 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 or total 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 authenticity has been well established up to 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. IPC recommends storage of BRS between 2-8°.

Dissolution Apparatus Calibrator

The IP Prednisone Tablet *RS* is used for the performance verification test for IP Dissolution Apparatus 1 (Basket Type) and Dissolution Apparatus 2 (Paddle 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	
IPC Website	https://ipc.gov.in
IPRS Price	Rs. 5000/- per vial for private stakeholders and Rs. 2500/- per vial for government stakeholders + GST
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 usually in low quantities (e.g. IPRS provided by IPC). 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 (in grams) and made available for internal use only. The traceability between the secondary and the primary standard must be documented. If the substance is non-pharmacopoeial, analyse as per *in house* methods and specifications.

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 1: *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.*

Note 2: *Working standard should be traceable to certified reference standards (CRM). Traceability refers to the value of a standard where it can be related to stated references (national or international standards) through an unbroken chain of comparisons. Therefore, for developing working standards, a method should be adopted where traceability/comparison shall be shown with CRM.*

(ii) Perform the identification test preferably by IR or as given in the specification or IP monograph. IR spectrum should be compared against official reference standard or spectra given in the IP.

(iii) Perform water content/loss on drying and assay 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.

(iv) Working standards, where pharmacopoeial reference substance is not available or if the substance is non-pharmacopoeial, should be qualified as per *in house* methods and specifications or by the absolute analytical procedure (i.e. by defined potency). Absolute method can be titrimetric provided titrimetric methods are available. In any case, a chromatographic method is preferable.

(v) The working standards for pharmacopoeial substances should be standardized as per official specifications. Working standard should be re-standardized in case of major change in test for related substance and/or assay.

(vi) Evaluate system suitability, correlation and assay with the standard solution. A typical injection sequence should be as follows:

S. No.	Injection	No. of Injection(s)
1	Blank	1
2	System suitability (if required)	1
3	Standard-1	5
4	Standard-2	2
5	Sample-1	2
6	Sample-2	2
7	Sample-3	2
8	Bracketing standard	1

(vii) Correlation should be checked between standard preparations 1 and 2 using following formula.

$$\text{Correlation} = \frac{\text{Average area of Standard-1}}{\text{Average area of Standard-2}} \times \frac{\text{Wt. of Standard-2}}{\text{Wt. of Standard-1}} \times 100$$

Acceptance criteria: Correlation between the response of two successive reference standard 1 & 2 preparations shall be between 99.0-101.0%. In case of standards where multiple peaks are present- correlation for principal peak (main peak with highest area)should be considered.

(viii) If the correlation and/or system suitability falls outside the limits, do not proceed for the test samples. Retest analysis should be carried out after root cause investigations.

(ix) Calculate the Assay (on *as is* basis) of the working standard from the average area responses of the standard preparation-1 and sample preparations 1, 2 and 3 by using the following formula:

$$\text{Assay (\% w/w) (on as is basis)} = \frac{\text{Test area x Wt. of Standard x Test dilution x Purity of Standard}}{\text{Standard area x Standard dilution x Wt. of test sample}}$$

$$\text{Assay (\% w/w) (on dried/anhydrous basis)} = \frac{\text{Assay (\% w/w) (on as is basis) x 100}}{\text{(100 – Water content/LOD)}}$$

(x) When the material is standardized by an absolute analytical technique (such as Titrimetric), the assay should also be evaluated in triplicate and the mean value should be used as the potency of the material standardized as working standard.

(xi) Overall percent RSD of replicate and bracketing standard injection should not be more than 2%. The acceptance criteria for percent RSD of assay may be taken as under:

S. No.	Test	Mean Results	RSD
1	Assay	Assay	NMT 1.0 %
		Assay by Microbiological Method	NMT 5.0%

(xii) If Assay of working standard is found more than 100.0%w/w, it shall be considered and reported as 100.0%w/w (other than biological standard).

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

Traceability

Traceability is the property of a result of measurement that can be related to the international or national standards, through an unbroken chain of calibrations. In other words, when the result of a measurement is described as traceable, it is essential to specify value of appropriate primary standard. The assigned value of a secondary/working standard should be traceable to the relevant primary standard.

Recording and Documentation of Working Standard

Analytical raw data of the working standards qualification, COA or the batch/lot no. and validity statement shall be compiled along with the required chromatograms.

Labels of Working Standards

Labels of the working standards should mention following minimum details:

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

Validity of Working Standards

Each working standard should be assigned validity up to twelve months or can be extended further which depend upon number of factors, including chemical and physical properties of the substance, stability of substance, storage condition employed, extent of use (how often the container is opened and closed). It is recommended that one vial should be used as per user requirement but in any case, it should not be used beyond one month.

The stock of working standard shall be packed in bottle/container of suitable capacity and

preserved under appropriate storage conditions. If the working standard is hygroscopic in nature or molecule/material is very sensitive/unstable, validity of working standard should be defined accordingly such as the vial should be used for one time only and water content required to be performed by using minimum quantity of material as available.
A new working standard should be prepared and standardized before the end of validity of the current working standard. If new material is not available, the existing working standard may be re-validated and appropriate validity is reassigned (which should be within the expiry date of the material). Whenever a new lot of primary standard (i.e. IPRS) becomes official, the working standard should be prepared or re-validated only against the new lot of primary standard.

Intermediate Checks of Working Standard

Working Standard 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 and assay. However retested working standard should never be used in case of the following test results:
If after the validity date or during intermediate checks or use, any change in description is observed, the same shall be discarded. A thorough investigation should be done to find out the reason for such change and documented for further corrective action.
In case where result of retesting of In-house qualified working standards is noncompliant, a retrospective check of tests performed using subjected In-house qualified working standards since its previous examination should be carried out. For evaluation of outcomes of retrospective checks and consideration of possible corrective actions, risk analysis shall be applied as per applicable SOP on Risk management.

Usage and Handling of Working Standard

- Handle the working standards carefully to avoid cross-contamination in order to ensure that integrity is safeguard and maintained throughout their period of use.
- Refrigerated Vials/ampoules/bottles should not be opened until they have attained room temperature and should be kept in desiccator having self-indicating silica to prevent ingress of moisture by condensation.
- Clean and dry dispensing aids shall be used for withdrawal of standard.
- Don’t insert spatula or butter paper inside the working standard vial and always dispense the material on the weighing paper by gentle tapping.
- Transfer required quantity approximated on butter paper for weighing purposes and extra material (if any) taken out from bottle/vial of working standard during weighing shall not be returned back to the original bottle/vial and shall be disposed off.
- After use, securely close the vials, seal it and store as recommended.
- If any change in description observed (e.g. lumps, colour), don’t use the working standard.
- Record the details on consumption of working standard in the assigned log book.
- The outdated vials of working standard shall be disposed off as per the current version of applicable SOP on Laboratory Waste Management.

Packaging Components and storage of Working Standards

Containers for storage of working standards should be, as far as possible, similar to that of primary standards. 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 of Butyl rubber, Bromobutyl rubber, Chlorobutyl rubber, Silicone rubber recommended in pharmaceutical packaging should be used.

Seal the working standard containers and store in a refrigerated cabinet (2-8˚) and/or in desiccators, away from heat, light and moisture, do not freeze. In specific cases labelled storage condition should be applicable. Working standards, packaged under an inert gas or in conditions of controlled humidity, the use of a glove box or an air-tight cabinet is necessary. Single-use vials or ampoules should be used for hygroscopic materials.

Containers for storage of working standards should be air tight and light protected depending on the physical and chemical properties as under but not limited to;

- sensitive to oxygen, storage under inert gas
- hygroscopic, storage in desiccators having self-indicating silica
- sensitive to temperature, refrigerated or deep frozen

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.*

References

1. WHO Technical Report Series No. 943 Annex-3, General guidelines for the establishment, maintenance and distribution of chemical reference substances, P-59-82.
2. ISO 17034:2016. General requirements for the competence of reference material producers. Geneva: International Organization for Standardization. 2016.