3.2.4.2 Approach

3.2.4.2.1 General chapter

The general testing methods and other specifications which are common in the monographs may be reflected in a General chapter of herbal monographs. It may give an account of tests/procedures or analytical techniques and of general principles. General chapters may contain the following, but are not limited to:

i. general testing methods that include equipments, procedures, methods and limits, etc. for application through individual monographs;
ii. information for the interpretation of the monographs;
iii. sampling procedures;
iv. macroscopic, microscopic, powder microscopy characters;
v. contaminants tests, e.g. foreign organic matter, heavy metals, aflatoxins, microbial contaminants;
vi. physicochemical tests, e.g. extractable values, ash values;
vii. TLC/HPTLC and LC fingerprint for identification;

When a general chapter is referenced in a monograph, acceptance criteria may be presented.

3.2.4.2.2 Individual herbal monographs

The herbal drug monograph in pharmacopoeias may include crude herbs, processed herbs, herbal formulations, etc., that contain as active ingredients parts of plants, or other plant materials, or combinations.

3.2.4.2.2.1 Crude herbs

Crude plant material is, e.g. leaves, flowers, fruit, seed, stems, wood, bark, roots, rhizomes or other plant parts, which may be entire, fragmented or powdered. Herbs are usually in dried form, but sometimes when specified may also be in a fresh form. In specific cases exudates which have not been processed further are also covered under the term herbs.

3.2.4.2.2.2 Processed herbs

Processed herbs means preparations obtained by subjecting herbs to treatment such as extraction, distillation, expression, fractionation, purification, concentration and partial or full fermentation. The basis for finished herbal preparations includes comminuted or powdered herbal materials, or extracts, tinctures and fatty oils of herbal materials. They also include preparations made by steeping or heating herbal materials in alcoholic beverages and/or honey, or in other materials.

3.2.4.2.2.3 Herbal formulations

Herbal preparations made from one or more herbs. If more than one herb is used, the term "mixture herbal product" can also be used. Finished herbal products and mixture herbal products may contain excipients in addition to the active ingredients. However, finished products or mixture products to which chemically-defined active substances have been added, including synthetic compounds and/or isolated constituents from herbal materials, are not considered to be herbal.

3.2.4.3 Monograph development

The following concepts are important in the development and setting of specifications and may be provided for each herbal monograph. The monograph should include: Authorized Title, Definition, Limits of active ingredients, Marker compounds,

Description, Category, Identification, Physicochemical tests and Assay of the marker constituents, Contaminants, Specific testsand Additional requirements.

3.2.4.3.1 Monograph title

For monographs intended for inclusion in pharmacopoeias, the title of the monograph may include the Latin binomial nomenclature or Synonym or Common name or Traditional/Colloquial name, whichever is appropriate, and is followed by the name of plant part(s) or plant product (e.g. resin, gum-resin) and where applicable the processed form.

3.2.4.3.2 Definition

Some or all of the following are usually included in the definition:

- the state of the drug: whole, fragmented, peeled, cut, fresh or dried;
- the complete scientific name of the plant (genus, species, subspecies, variety, author); commonly used synonyms may be mentioned;
- the part or parts of the plant used;
- where appropriate, the stage in the growth-cycle when harvesting takes place, or other necessary information;
- wherever possible, the minimum content of **quantified constituents** (either responsible for the biological activity of the herb (bio-marker) or a chemical compound known to be present in the herb even if not responsible for biological activity (chemical/analytical marker);
- herbal drugs very often contain a mixture of related substances, in which case the total content of **quantified constituents** is determined and expressed as one of the constituents, usually the major constituent; separate limits may be given for different forms of the drug (whole/cut).

3.2.4.3.3 Characters

This section may contain a brief description of the organoleptic characters of the drug such as colour, odour, taste, etc.

3.2.4.3.4 Category

It includes the therapeutic category to which the Title of the monograph belongs.

3.2.4.3.5 Identification

The purpose of the Identification category of a monograph is to ensure that the article under examination is in agreement with what is stated in the Definition of the article. All the identifications mentioned below are not necessarily included: some may be absent when they are not feasible or are not significant for the purpose of identification. Macroscopic requirements with appropriate scale (such as grid scale) and microscopic requirements of an herbal monograph should be provided with histological characters and colour photographs.

3.2.4.3.5.1 Macroscopic characters

The important macroscopic botanical characters of the drug are specified to permit a clear identification. A detailed coloured photograph should be provided. When two species/subspecies of the same plant are included in the definition, the individual differences between them are indicated.

3.2.4.3.5.2 Microscopic characters

It involves gross microscopic examination of the drug and it can be used to identify the organized/unorganized drugs by their known histological characters. It is mostly used for qualitative evaluation of organized, crude drugs in entire and powder forms with the help

of a microscope. Using a microscope various cellular tissues and their arrangements such as trichomes, stomata, starch granules and calcium oxalate crystals, etc. can be detected. Crude drug can also be identified microscopically by cutting the thin TS (transverse section)/LS (longitudinal section) and appropriately mounting the slide (example, in case of wood). Quantitative aspects of microscopy include study of stomatal number and index, palisade ratio, vein-islet number, size of starch grains and length of fibres, etc.

3.2.4.3.5.3 Fingerprinting

Chromatographic or spectroscopic patterns, sometimes referred to as "fingerprints", may be used as standards for identification, though they may not be mandatory. These fingerprints can be obtained by HPLC, UHPLC, CE, GC, TLC/HPTLC, IR, mass spectroscopy, etc. Such fingerprints may be suitably referenced within the pharmacopoeia. The fingerprints must be able to distinguish these materials from other materials with potential for species substitution and suspected adulteration. The acceptance criteria for identification tests using chromatographic methods such as HPLC, UHPLC, CE or GC methodology must contain a description of the critical features of the fingerprint chromatograms such as the presence of specified peaks, retention time, their order of elution, and where possible, their relative abundance. For methods of TLC/HPTLC, description must include colour and position of the characteristic bands. A colour image of a typical HPTLC chromatogram should be provided as a guide for the users of the monographs though not as a part of the monograph which may have legal connotations. A critical aspect of the identification of herbal materials by separation techniques is the use of reference standards because they provide assignment at the time of use. In addition to the sample solution, a standard solution containing the reference standard is chromatographed concomitantly. The reference material used in the preparation of a standard solution may be an authenticated Botanical Reference Substance (BRS), a reference standard extract, a single chemical entity or a standardized mixture of substances.

DNA-based identification test may be provided in order to improve authentication of botanical identity, especially in those cases where there is controversial botany or chances of substitution/adulteration.

3.2.4.3.6 Tests

The following tests are indicated to be carried out. Other appropriate tests specific to the material under examination, if required, may be carried out.

3.2.4.3.6.1 Physicochemical evaluation

Physicochemical evaluation is an important parameter in detecting adulteration or improper handling of drugs. It can serve as a valuable source of information and provide an appropriate standard to establish the quality of herbs. These are:

- extractable matter:
  it is considered useful to determine extractable matter only in herbal drugs where no constituent suitable for an assay is known or where the material is used to produce a preparation with a dry residue;
- total ash:
  this test is always included unless otherwise justified. It is to be carried out on the powdered drug;
- acid-insoluble ash:
  this test may be carried out depending on the nature of the particular herbal drug and is used to detect unacceptable quantities of certain minerals.

3.2.4.3.6.2 Loss on drying

Herbal drugs are dried for preservation purposes, if they are insufficiently dried, growth of yeasts or moulds may occur. It is the loss of weight expressed as percentage w/w resulting from water and volatile matter of any kind that can be driven off under

specified conditions. The limit is specified on the basis of the results obtained on a reasonable number of varied samples of acceptable quality.

3.2.4.3.6.3 Swelling index

Applicable to certain hydrocolloid-containing herbal drugs.

3.2.4.3.6.4 Bitterness values

Applicable to herbal drugs containing bitter principles.

3.2.4.3.6.5 Contaminants – general

3.2.4.3.6.5.1 Foreign organic matter

It is the material consisting of any or all of the following:

- parts or organs of plant from which the drug is derived other than the parts/organs named in the definition and description or for the limit is prescribed in the individual monograph;
- any part or organs of plant other than those named in the definition and description;
- matter not coming from the source ;
- moulds, insects or other animal contaminant.

Generally a limit of 2% of foreign matter is imposed, unless otherwise prescribed in a specific monograph. Where a limit for foreign matter greater than 2% is to be prescribed, it is stated in the specific monograph with an indication of the type of foreign matter. Where necessary, the monograph should indicate how the foreign matter is identified.

3.2.4.3.6.5.2 Heavy metals

The test is prescribed where there is the potential for contamination by heavy metals. The limit of heavy metals is indicated in the individual monograph in terms of ppm, i.e. the parts of lead per million parts of the substance under examination.

3.2.4.3.6.5.3 Microbial contamination

The pharmacopoeial monographs should specify the total count of aerobic microorganisms, the total count of yeasts and moulds and the absence of specific pathogenic bacteria (e.g. *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella* and *Salmonella* species)

3.2.4.3.6.5.4 Aflatoxins

Aflatoxins are one of the most toxic classes of mycotoxins; they arise from the growth of many species of Aspergillus, a fungus. Wherever fungal contamination of the herbal article is likely, it is advisable to include a test for aflatoxins.

3.2.4.3.6.6 Contaminants – specific

An individual herbal monograph may require certain specifications that are peculiar to that monograph, especially when safety is an issue. Limits may be set in certain specific monographs for the characters that are undesirable or have negative botanic characteristics. When one desires a limit for harmful substances that are present either naturally in the substance or formed as a result of post-harvest processing practices, such submissions must be accompanied by toxicity data.

3.2.4.3.7 Assay

Wherever possible, an assay is included. Assay is carried out using suitable instruments such as UV spectrophotometer, LC, GC or by HPTLC, etc., to check and quantify one or more markers, specifying either the percentage or relative proportions of them.

3.2.4.3.8 Additional information

3.2.4.3.8.1 Storage

Storage conditions are applicable unless otherwise specified: Store protected from light. Where applicable, additional specific conditions are given in the individual monograph.

3.2.4.3.8.2 Labelling

Labelling of herbal products includes the label both upon the immediate container and other associated labelling and written, printed or graphic materials. The label states the Latin binomial followed by the authorized name; the plant part(s), plant product or processed form contained in the container or from which the article was derived. Content, in percentage, of active principles or marker compounds should be stated. Labelling should be in accordance with the applicable drug laws.

3.2.4.4 Botanical Reference Substances/Phytochemical Reference Substances

This section includes list of all authorized BRS/Phytochemical Reference Substances (PRS) or biomarkers that are required for comparison to conduct the monograph tests, method development and evaluation. These substances are plant parts/isolated compounds certified to have come from a plant that have been highly characterized and approved by the competent authority.

These substances are used as a reference material for comparison and confirming the identity and quantity of herbal samples under examination. Thus, a standard operating procedure giving detailed steps for preparation, qualification, validation and revalidation may be incorporated. Proper documentation including the certificate of analysis and their issuance to the end-users may be done.

An adequate sampling plan of the source material for these substances from different locations/seasons may also be suggested to map the "natural window of the chemistry" for developing the monographs.

A collaborative ring testing from at least three accredited/certified laboratories should be done and the results obtained should be reviewed before assigning or fixing the limits.

[*3.2.5 Monographs on other products*] *Action:* on hold, to be discussed later]

4. REFERENCE STANDARDS

[Argentinian Pharmacopoeia, BP, IPC, USP, WHO]

4.1 Reference substances are referred to in pharmacopoeias. They are an integral part of the procedures in a pharmacopoeia, and their use demonstrates compliance with the corresponding documentary standard. They constitute authenticated benchmarks for those analytical tests and assays that are based on comparison of the certified attribute(s) of a sample with those of the reference standard. They enable the analyst to achieve accurate and traceable results. In particular, reference substances are used to identify, to determine the purity or to assay pharmaceutical substances and preparations. They are further used to verify the performance of test methods or to calibrate analytical instruments.

Maintenance of a collection of pharmacopoeial reference materials requires ongoing effort. This effort includes continued suitability-for-use studies, stability

studies, addition of new reference materials for new monographs and monographs that are being updated, and studying candidate materials for replacement lots.

4.2 Reference substances may be fully characterized by physical or chemical means alone, or may require the use of biological- or microbiological tests. The characterization of reference substance by biological and microbiological methods may follow special rules, which are not yet fully covered in this chapter.

4.3 The pharmacopoeial authority should apply appropriate principles during the establishment, storage and distribution of reference substances to guarantee that they are suitable for their intended use. All operations should be carried out under a defined quality management system. It is desirable that the quality management system be assessed as satisfactory by an independent body.

4.4 Source material for the establishment of reference substances may be synthesized and purified for this purpose or may be selected from the pharmaceutical production of the substance referred to in the monograph provided that the purity and homogeneity are suitable. In some cases, for example, in order to improve the stability of the reference substance, it may be useful to select an alternative salt (or salt vs base), solvate or hydrate.

4.5 The source material should be tested with suitable analytical techniques aiming to characterize all relevant quality attributes. The identity is confirmed and the purity is determined usually based on results obtained with the validated methods of the respective monographs. However, the use of further analytical techniques may be appropriate in order to fully characterize the candidate material. Absolute methods (for example, volumetric titrations, differential scanning calorimetry) may be employed to complement and verify the results of relative methods, where appropriate.

4.6 The extent of testing and the number of laboratories involved in characterizing the material depend on the intended use of the reference substance to be established. If required, assay standards are characterized in interlaboratory trials to better estimate the trueness of the assigned value or to determine the associated uncertainty.

4.7 A thorough purity investigation of the candidate material is usually performed with the aim to identify and quantify all relevant components (i.e. main component, organic and inorganic impurities, water and residual solvents).The cumulative percentage of all quantified components should yield 100% (mass balance approach).

4.8 The content assigned to a quantitative reference standard depends on the purity of the candidate material and is specific to the method for which the standard will serve as a reference.

4.9 Reference standards are dispensed into suitable containers under appropriate filling and closure conditions, to ensure the integrity of the reference material. The containers employed should minimize the risk of decomposition, contamination and moisture uptake.

4.10 The labelling should provide all information necessary to use the reference substance as intended, i.e. the name of the reference substance, the batch number, storage conditions, etc. If intended for quantification the assigned content is also given. An accompanying leaflet may be considered to be part of the labelling.

4.11 Reference substances should be stored in their packaging and distributed under conditions suitable to ensure their stability.

4.12 The stability of reference standards stored for distribution is monitored regularly. The frequency and extent of the reexaminations are based on the:

- liability of the reference standard to degradation;
- container and closure system;
- storage conditions;
- hygroscopicity;
- physical form;
- intended use.

4.13 The analytical methods employed to verify the stability are often chosen among those performed during the establishment of the reference standard. The maximum permitted deviation from the assigned value should be predefined and, if exceeded, the batch should be reestablished or replaced.

4.14 When reference substances are used for purposes other than those for which they were established, the responsibility for assessing the suitability rests with the user or the authority that prescribes or authorizes this use.

4.15 Infrared reference spectra may be provided for use in identification test as described in monographs of pharmacopoeias.

4.16 Pharmacopoeial reference materials should be made available to the public without restriction, other than as required to comply with laws specifically regulating the shipment or export of certain materials.

5. ANALYTICAL TEST PROCEDURES AND METHODOLOGIES (ANALYTICAL METHOD) [IPC]

Analytical test procedures and methodologies are employed to establish the identity, purity, impurity, strength, quality and potency of drug substances and drug products. An analytical method mentioned in a pharmacopoeia should be simple, reliable, accurate, sensitive and specific.

A pharmacopoeia provides physical, physicochemical, chemical, pharmaceutical and biological methods for analysis of quality of drug substances (APIs) and drug products (finished dosage forms). The type of method applied for analysis depends on the nature of the drug.

The principles of method validation apply to all types of analytical procedures. It is established by demonstrating documentary evidence with respect to any particular drug substance or drug product. In certain products like biological, biotechnological, botanical or radiopharmaceutical drugs some specifications may not be applicable. For example, many bioassays are based on animal challenge models, immunogenicity assessments or other immunoassays that have unique features that should be considered.

System suitability testing should be an integral part of analytical method

5.1 Types of analytical procedure

5.1.1 Regulatory analytical procedure

A regulatory analytical procedure is the analytical procedure used to evaluate a defined characteristic of the drug substance or drug product. The analytical procedures that are legally recognized are the regulatory analytical procedures for compendial items.

5.1.2 Alternative analytical procedure

An alternative analytical procedure is an analytical procedure that is not a regulatory analytical procedure. A proper validation of the alternative analytical procedure should be done to show its performance equal to or better than the regulatory analytical procedure. If an alternative analytical procedure is used, it is necessary to provide a rationale for its inclusion and identify its use (e.g. release, stability testing), validation data and comparative data to that of regulatory analytical procedure.

5.1.3 Stability-indicating assay

A stability-indicating assay is a validated quantitative analytical procedure that can detect the changes with time in the pertinent properties of the drug substance and drug product. A stability- indicating assay accurately measures the active ingredients, without interference from degradation products, process impurities, excipients or other potential impurities.

5.2 Validation of analytical methods

It is the process of demonstrating that analytical procedures are suitable for their intended use. The methods validation process for analytical procedures begins with the planned and systematic collection of the validation data to support the analytical procedures. A full validation report should be generated and any deviation/change during the process should be justified and approved. All analytical procedures are of equal importance from a validation perspective. In general, validated analytical procedures should be used, irrespective of whether they are for in-process, release, acceptance or stability testing. Each quantitative analytical procedure should be designed to minimize assay variation.

5.2.1 Validation characteristics

The validation of analytical procedures is mentioned in detail in ICH guideline (Q2R1) "Validation of Analytical Procedures: Text and Methodology". Although not all of the validation characteristics are needed for all types of tests.

A brief account of the same is as under:

A. **Specificity:** Specificity is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present. Typically these should include impurities, degradants, matrix, etc.

B. **Linearity:** Linearity of an analytical procedure is its ability (within a given range) to obtain test results that are directly proportional to the concentration (amount) of analyte in the sample.

C. **Range:** The range of an analytical method is the interval between the upper and lower concentration (amounts) of analyte (including these concentrations) for which it has been demonstrated that the analytical procedure should has suitable level of precision, accuracy and linearity.

D. **Accuracy:** Accuracy of an analytical procedure expresses the closeness of an agreement between the value which is accepted either as a conventional true value as accepted reference value and the value found. This is sometimes termed trueness.

E. **Precision:** The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision should be considered at three levels: repeatability, intermediate precision and reproducibility.

- **Repeatability (intra-assay precision):** repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed as intra-assay precision.
- **Intermediate precision:** intermediate precision expresses within-laboratory variations: different days, different analysts or equipment, etc.
- **Reproducibility:** reproducibility expresses the precision between laboratories (collaborative studies, usually applied to standardization of methodology).

F. **Detection limit:** the detection limit of an analytical procedure is the lowest concentration of analyte in a sample that should be detected but not necessarily quantitated as an exact value.

G. **Quantitation limit:** the quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which should be quantitatively determined with suitable precision and accuracy.

H. **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. Such testing should be performed during development of the analytical procedure.

5.2.2 Other method validation information

Methods validation information should also include:

- data to demonstrate the stability of all analytical sample preparations through the time required to complete the analysis;
- legible reproductions of representative instrument output or recordings (e.g. chromatograms) and raw data output (e.g. integrated areas), as appropriate;
- instrument output for placebo, standard and sample should also be provided;
- representative calculations using submitted raw data, to show how the impurities in drug substance are calculated;
- information from stress studies;
- impurities labeled with their names and location identifiers (e.g. RRT for chromatographic data) for the impurity analytical procedure.

5.2.2.1 Identification

Identification of drugs may be carried out by IR, differential scanning colorimetry (DSC), X-ray diffraction (XRD), UV, LC, TLC/HPTLC, etc., or by applying other suitable techniques. A specific identification test should be included for the active ingredient whenever possible. In cases where a non-specific identification analytical procedure is proposed for the active ingredient, two independent analytical procedures are generally sufficient.

5.2.1.1 Impurities

The validation characteristics under quantitative testing for impurities apply, regardless of which methodology is used to quantitate impurities. If the same analytical procedure is proposed as a limit test, validation characteristics under limit testing for impurities will apply.

5.2.1.2 Assay

Assay includes the content of the active ingredient and measurement of content in dissolution and content uniformity of samples.

5.2.1.3 Specific tests

Specific tests to control the quality of drug substance, excipient or drug product can include tests such as particle size analysis, droplet distribution, spray pattern, dissolution (excludes measurement), optical rotation and methodologies such as Raman spectroscopy, DSC, etc. The validation characteristics may differ for various analytical procedures.

5.3 Analytical methodology/analytical procedures

The quality data can only be generated if all standards, samples, reagents, instruments used for the analyses are suitable and each analytical step is performed in a systematic manner.

- ***Sample preparation***

For the analysis sufficient amount of sample should be available. It should be randomly collected. Analytical method designed/selected should be capable of measuring the analyte of interest even in presence of interferants (sample matrix, excipients, etc.).

- ***Standard preparation***

Procedures for the preparation of all standard solutions (e.g. stock solution, working standard solutions, internal standards) should be well documented.

- ***Reagent preparation***

There should be a system in place to ensure the quality of reagents. The reagents should be obtained only from reputed/accredited suppliers. The supplier should provide documentary evidence of any accreditation status. Reagents and solutions should be labelled to indicate identity (with concentration if appropriate), expiry date and specific storage instructions. Information concerning source, preparation date and stability should be available.

Many analytical methods are used in pharmacopoeial monographs. A few widely used methods are described below.

5.3.1 Dissolution test

In vitro dissolution testing as applied to solid-dosage forms measures the amount of drug dissolved in a known volume of liquid medium at a predetermined time, using a specified apparatus designed to carefully control the parameters of dissolution testing. It can help pinpoint formulations that may present potential bioequivalence problem. Once a formulation has been shown to be bioavailable, dissolution testing is of great value in assuring lot-to-lot bioequivalence.

Dissolution test is incorporated in pharmacopoeias to give a direction to stakeholders to formulate drug dosage forms and to develop quality control specifications for its manufacturing process. It applies to tablets and capsules.

The dissolution procedure description and validation should include the following.

5.3.1.1 Dissolution medium

Solvent specified in the individual monograph should be used.

5.3.1.2 Procedure

A dissolution test consists of a dissolution procedure and method of analysis (automated on-line analysis or manual sampling followed by HPLC analysis). The written procedure should cover the following items:

- Apparatus;
- preparation of standard;
- preparation of sample;
- method of analysis (e.g., UV, HPLC, etc.);
- sampling procedure (e.g. intervals, filtration, handling of samples, dilutions);
- calculations;
- acceptance criteria.

Regardless of the method of analysis, system suitability criteria should be described.

Blank and standard solution spectra or chromatograms should be included.

5.3.1.3 Acceptance criteria

In case of solid dosage forms, the pharmacopoeias give a limit:

- immediate release typically means that 75% of the API is dissolved within 45 minutes:
  - rapidly dissolving: ≥ 85% in ≤ 30 minutes
  - very rapidly dissolving: ≥ 85% in ≤ 15 minutes.

In case of immediate-release dosage forms as per pharmacopoeias, the requirements are met if the quantities of active ingredient(s) dissolved from the dosage forms tested conform to the following table, unless otherwise specified in the individual monograph.