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Recent Approaches in Herbal Drug Standardization

Yadav N. P., Dixit V. K.*

Department of Pharmaceutical Sciences, Dr. H. S. Gour University, Sagar, M.P., INDIA

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Abstract
The quality control standards of various medicinal plants used in indigenous system of medicine are becoming more relevant today in view of commercialization of formulations based on medicinal plants. For standardization and quality assurance purposes, following three attributes are desirable i) Authenticity, ii) Purity and iii) Assay. Authenticity relates to proving that the material is true. Authentication in itself involves many parameters including gross morphology, microscopy, chemical analysis and DNA fingerprinting. Purity pertains to evaluating that there are no adulterants present in the plant material. Assay part of standardization is chemical and biological profiling which could assess the chemical effects and curative values get established. The new era of herbal drug standardization includes pharmacognostical, chemical, biological, biopharmaceutical and molecular approaches of drug development and discovery, where biotechnology driven applications play an important role.

Keywords: Herbal Drug Standardization, DNA fingerprinting, Biopharmaceutical characterization, Herbal medicine, Chromatographic Fingerprinting.

INTRODUCTION

Herbal medicines are the synthesis of therapeutic experiences of generations of practicing physicians of indigenous systems of medicine for over hundreds of years and they are known to be oldest health care products that have been used by mankind all over the world in the form of folklore medicines or traditional medicines or ethnic medicines. The therapeutic use of herbal medicines is gaining considerable momentum in the world during the past decade. The World Health Organization (WHO) estimates that herbal medicine is still the mainstay of about 75-80% of the world population, mainly in the developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and lesser side-effects (Kamboj, 2000).

Herbal drug standardization

"Standardization is a system to ensure that every packet of medicine that is being sold has the correct amount and will induce its therapeutic effect (Chaudhry, 1992)."

The overuse of synthetic drugs with impurities, resulting in higher incidence of adverse drug reactions in more advanced communities, has motivated mankind to go back to Nature for safer remedies. Therefore, quality control standards of various medicinal plants used in indigenous system of medicine are becoming more relevant today in view of commercialization of formulations based on medicinal plants, unlike in the past when the traditional doctors would themselves dispense the medicines. Due to varied geographical locations where these plants grow, coupled with the problem of different vernacular names these plants are known by, a great deal of adulteration or substitution is encountered in the commercial markets. Therefore, reproducible standards of each plant are necessary for effective quality control. Although some of these plants are covered in various pharmacopoeias, their standards especially in terms of chemical markers and TLC fingerprinting have not been covered (Indian Herbal Pharmacopoeia, 1998).

An important factor, which can contribute to the consistent quality of Herbal products, is to have

Corresponding author:
V. K. Dixit, Ph.D.
Department of Pharmaceutical Sciences,
Dr. H. S. Gour University,
Sagar, M.P., India,
Email: profvkdixit@rediffmail.com

adequate control on the quality of medicinal plants. Due to the natural heterogenity, the quality of herbal starting materials obtained from wild collections shows more and more fluctuations. Thus cultivation of the most important medicinal plants has been considerably promoted during the last years. It seems to be the only way to meet the increasing demand of consistent qualities of herbal materials taking account of controlled environmental conditions. For this reason WHO recently released guidelines on Good Agricultural and Collection Practices (GACP) for medicinal plants (WHO, 1991). WHO has also issued "Guidelines for quality control methods for medicinal plant materials" in 1992 with a clear objective to provide general test methods for correct botanical evaluation and identification of medicinal plants widely used in traditional and home remedies (WHO, 1998).

The following WHO technical guidelines have been published:

- WHO guidelines on assessing quality and safety of herbal medicines with reference to contaminants and residues.
- WHO GACP monograph for Artemisia Annua L.
- WHO draft guidelines for the selection of substances for quality control of herbal medicines (outline and key technical issues discussed at two WHO working group meetings in 2004 and 2005).
- WHO GMP: Updated supplementary guidelines for manufacture of herbal medicines.
- Support to national capacity building on quality control of herbal medicines.
- WHO Interregional Training Workshop on GACP and GMP for Herbal Medicines, held in China, September 2005.
- Guidelines for the Assessment of Herbal Medicines (WHO, 1991) and Q uality Control Methods for Medicinal Plant Materials (WHO, 1998)

In addition to the test methods, some suggestions regarding general limits for contaminants are also included. Standard monographs on herbs have been published by USA, UK, ESCOP, German-E-Commission, Japanese Pharmacopoeia and perhaps the highest numbers are in Chinese Pharmacopoeia.

Standardization serves a number of purposes, including:-

- batch to batch consistency
- confirmation of correct amount of extract per dosage unit
- positive control to indicate possible loss or degradation during manufacturing.

Standardization as defined by American Herbal Products Association:

"Standardization refers to the body of information and controls necessary to produce material of reasonable consistency. This is achieved through minimizing the inherent variation of natural product composition through quality assurance practices applied to agricultural and manufacturing processes (Waldesch *et al*., 2003)."

For pharmaceutical purposes, the quality of medicinal plant material must be as high as that of other medicinal preparations, however, it is impossible to assay for a specific chemical entity when the bioactive ingredient is not known. In practice, assay procedures are not carried out even for those medicinal plant materials where there are known active ingredients. For example much of the ginseng or valerian is bought and sold on the basis of its sensory characters, rather like tea. Further problem is posed by those preparations which contain complex heterogeneous mixtures.

Standardization problem arises from the complex composition of drugs which are used in the form of whole plant, parts of the plant (s) and of plant extracts. Standardization of the presumed active compounds of drug in general does not reflect reality. Only in a few cases does drug activity depend upon single component. Generally, it is the result of concerted activity of several active compounds as well as of inert accompanying substances. Though these inert accompanying components do not directly affect pathological mechanism, it is reasonable to use the complex mixture of components provided by a medicinal plant because these inert components might influence bioavailability and excretions of the active component. Further, by inert plant components the stability of the active component might be increased and the rate of side effects be

Table 1: General Testing Parameters for Characterization and Standardization of Herbal Medicines

	Testing parameters	Guidelines
General data	Geographical Harvesting time Harvesting process Processing	Good Agricultural Practices (GAP)
Description		
Identity	Macroscopic Microscopic Chemical TLC fingerprints	According to Pharmacopoeias
Purity	Foreign matter Ash/Sulfated ash Content of extractable matter Water content	According to Pharmacopoeias
Assay	Constituents with known therapeutic activity (biomarker) Constituents with unknown therapeutic activity (marker substances) Titrimetric Photometric HPLC/GC/TLC	According to Pharmacopoeias
Contaminants	Pesticides Heavy metals Aflatoxins Microbiological purity Radioactivity	Ph. Eur. Recommended limits for herbal drugs(oct. 91) Regulation on aflatoxins (Nov. 90) Ph. Eur. 1997 Suppl. 1999

minimized. If there are different active compounds present in plant drug, they might have additive or potentiating effect.

Directives on the analytical control of a vegetable drug must take account of the fact that the material to be examined has complex and inconsistent composition. Therefore, the analytical limits cannot be so precise as for the pure chemical compound. Vegetable drugs are inevitably "inconsistent" because their composition and, hence their standardization, may be influenced by several factors such as age and origin, harvesting period, method of drying and so on. To eliminate some of inconsistency, one should use cultivated rather than wild plants which are often heterogenous in respect of the above factors and consequently in their content of active principles (Handa, 1995).

The development of parameters for quality control of Herbal drugs is a big task involving biological evaluation for a particular disease area, chemical profiling of the raw material and laying down specifications for the finished product. Therefore, the word "standardization" should encompass the entire field of study from birth of a plant to its clinical application. General testing parameters for characterization and standardization of herbal medicines are given in Table 1.

In 1994, the Dietary Supplement Health and Education Act (DSHEA), enforced by the US Congress, has introduced the term 'dietary supplement', and herbal ingredients are regulated under the act of DSHEA by the terms 'dietary ingredient' and 'new dietary ingredient'. An herbal ingredient may be considered as a 'new dietary ingredient' if it was not marketed in the US in dietary supplements before October 15, 1994. DSHEA requires a manufacturer or distributor to notify the FDA if it intends to market a dietary supplement in which an herbal ingredient may be 'new'. Dietary supplements containing herbal medicine are not subject to the same rigorous standards as are prescriptions and over-the-counter pharmaceutical products. DSHEA places dietary supplements in a special category under the general umbrella of 'foods', not drugs, and requires that every supplement be labeled a dietary supplement. Manufacturers and distributors are responsible for determining if an herbal ingredient is reasonably safe for use in a dietary supplement (Chau *et al.*, 2006).

Recently, many international authorities and agencies including the WHO, European Agency

for the Evaluation of Medicinal Products and European Scientific Cooperation of Phytomedicine, US Agency for Heath Care Policy and Research, European Pharmacopoeia Commission, Department of Indian System of Medicine and such have started creating new mechanism to induce quality control and standardization of botanical medicine.

RECENT APPROACHES IN HERBAL DRUG STANDARDIZATION

DNA fingerprinting

Correct identification and quality assurance of the starting material is an essential prerequisite in herbal medicine to ensure reproducible quality of herbal medicine, which contributes to its safety and efficacy (Joshi *et al*., 2004; Straus, 2002; De Smet, 2002).

Methods of identification and emerging techniques

Most of the regulatory guidelines and pharmacopoeias suggest macroscopic and microscopic evaluation and chemical profiling of the botanical materials for quality control and standardization (WHO, 1998; Indian Herbal Pharmacopoeia, 2002; British Herbal Pharmacopoeia, 1996). Chemical profiling establishes a characteristic chemical pattern for a plant material, its fractions or extracts. Thin layer chromatography (TLC) and high performance thin layer chromatography (HPTLC) are routinely used as valuable tools for qualitative determination of small amounts of impurities.

In order to ensure efficacy, selection of the correct chemotype of the plant is necessary. Even when there are many known chemotypes of a plant species, selection of the right chemotype to which clinical effects are attributed is difficult. Another difficulty encountered in the selection of the correct plant material is to establish the identity of certain species that may be known by different binomial botanical names in different regions.

In view of these limitations there is need for a new approach that can complement or, in certain situations, serve as an alternative. Molecular markers generally refer to biochemical constituents, including primary and secondary metabolites and other macromolecules such as nucleic acids. Secondary metabolites as markers have been extensively used in quality control and standardization of botanical drugs. DNA markers are reliable for informative polymorphisms as the genetic composition is unique for each species and is not affected by age, physiological conditions as well as environmental factors (Chan, 2003). DNA can be extracted from fresh or dried (Warude *et al*., 2000) organic tissue of the botanical material; hence the physical form of the sample for assessment does not restrict detection.

Various types of DNA-based molecular techniques (Joshi *et al*., 1999) are utilized to evaluate DNA polymorphism. These are hybridization-based methods, polymerase chain reaction (PCR)-based methods and sequencing-based methods.

Hybridization-based methods include restriction fragment length polymorphism (RFLP) (Botstein *et al.*, 1980**)** and variable number tandem repeats (Nakamura *et al*., 1987). Labelled probes such as random genomic clones, cDNA clones, probes for microsatellite (Litt *et al*., 1989) and minisatellite (Jeffrey *et al*., 1985) sequences are hybridized to filters containing DNA, which has been digested with restriction enzymes. Polymorphisms are detected by presence or absence of bands upon hybridization.

PCR-based markers involve *in vitro* amplification of particular DNA sequences or loci, with the help of specific or arbitrary oligonucleotide primers and the thermostable DNA polymerase enzyme. PCR-based techniques where random primers are used, include random amplified polymorphic DNA (RAPD) (Williams *et al*., 1990; Welsh *et al*., 1990**)**, arbitrarily primed PCR (AP–PCR) (Welsh *et al*., 1991) and DNA amplification fingerprinting (DAF) (Caetano-Anolles *et al*., 1993; Caetano-Anolles *et al*., 1991). A recent approach known as amplified fragment length polymorphism (AFLP) (Zabeau, 1993) is a technique that is based on the detection of genomic restriction fragments by PCR amplification. Adaptors are ligated to the ends of restriction fragments followed by amplification with adaptor-homologous primers. AFLP has the capacity to detect thousands of independent loci and can be used for DNAs of any origin or complexity (Kumar, 1999).

DNA sequencing can also be used as a definitive means for identifying species. Variations due to transversion, insertion or deletion can be assessed directly and information on a defined locus can be obtained.

Chromatographic fingerprinting

Chromatographic fingerprinting has been in use for a long time for single chemical entity drug substances. Recently it has become one of the most powerful approaches to quality control of herbal medicines. The use of chromatographic fingerprinting for herbal drugs tends to focus on identification and assessment of the stability of the chemical constituents observed by chromatography. Chemical and chromatographic techniques may also be used to aid in identification of a herbal material or extract. Chromatographic techniques such as HPLC, thin layer chromatography (TLC), gas chromatography (GC), and capillary electrophoresis have been used for identity tests. Examples have been found in the literature where marker compounds and chromatographic profiles ("fingerprints") are used to help in identification of herbals, and in assessment of their potency and stability (Lazarowych *et al*., 1998). The British Herbal Pharmacopoeia, 1996 has had an emphasis on using TLC profiles to characterize herbal materials, relying on the use of different spray reagents and TLC profiles to identify characteristic and active principles of herbal materials. Chromatographic (HPTLC) fingerprinting of poly herbal formulations has also been reported by B. L. Chauhan *et al.* (Chauhan *et al*., 1994).

HPLC profiles have also been used to distinguish different types and sources of Ginseng (Chuang *et al*., 1995). The authors report that 37 commercial samples of ginseng were analyzed for ginsengosides and malonyl ginsengosides. When embarking on development of assays, it must first be decided which compounds to quantitate. If a principle active component is known, it is most logical to quantitate this compound. Where active ingredients contributing to therapeutic efficacy are known, botanical preparations should be standardized to these compounds. Where the active ingredients are not yet known, a marker substance which should be specific for the botanical could be chosen for analytical purposes, although it should only serve for internal batch control. Single or multiple markers can be used to ensure that the concentration and ratio of components in an herbal mixture are present in reproducible levels in raw materials, manufacturing intermediates, and in the final dosage forms. In this way, multiple markers or chromatographic fingerprints give information assisting manufacturing control and assuring batch-to-batch consistency (Lazarowych *et al*., 1998).

Biopharmaceutical characterisation of herbal medicinal products

In contrast to chemically defined drug products, the biopharmaceutical quality and behaviour of *herbal medicinal products* (**HMPs**) often are not well documented. In most cases *in vitro/in vivo* biopharmaceutical characterisation is complicated by the complex composition of herbal drug preparations, extensive metabolism of constituents and the resulting analytical difficulties.

The *active pharmaceutical ingredient* (**API**) of HMPs is generally defined to be the whole herbal preparation, e.g. the extract in its entirety. Individual or groups of constituents have only in selected cases been identified to be responsible for the therapeutic activity.

As the whole herbal drug preparation, e.g. extract, is regarded as the active pharmaceutical ingredient (API), several extract types, depending on the pharmaceutical, analytical, pharmacological-toxicological and clinical findings, can be identified as:

- Extracts (Type A) containing constituents (single or groups) that are solely responsible for the known and acknowledged/well documented therapeutic activity. Adjustment (standardisation) to a defined content is acceptable.
- Extracts (Type B1) containing chemically defined constituents (single or groups) possessing relevant pharmacological properties (active markers). These substances are likely to contribute to the clinical efficacy; however, evidence that they are solely responsible for the clinical efficacy is not yet available. The characterisation of these extracts should take into consideration as far as possible the particular state of knowledge concerning the documented efficacy, quality and safety of an extract. Standardisation by blending different lots of a herbal drug before extraction, or by mixing different lots of

herbal drug preparations is appropriate. Adjustment using excipients is not acceptable.

- Extracts (Type B2) containing no constituents documented as being determinant or relevant for efficacy, or as having pharmacological or clinical relevance. In these cases, chemically defined constituents (markers) without known therapeutic activity may be used for control purposes. These markers may be used to monitor good manufacturing practice or as an indication for the assay/content of the drug product.

This classification implies that an extract may be progressed from type B2 to B1 and even to type A as further knowledge is acquired about the extract (Lang, 2001).

Relevance of the biopharmaceutical classification system for HMPs

The Biopharmaceutical Classification System (**BCS**), which was originally developed for chemically defined synthetic drug substances may be helpful for HMPs as well (Blume *et al*., 2000).

The BCS takes into account the physicochemical characteristics of a compound, in particular their solubility (in aqueous buffer systems of physiological pH) and permeability through gastrointestinal membranes. According to this BCS, APIs are classified into four groups (group I high solubility, high permeability; group II low solubility, high permeability; group III high solubility, low permeability; group IV low solubility, low permeability). In this context high solubility is defined such that the highest dosage strength is soluble (> 90 %) in 250 ml buffer and high permeability if more than 80 % of the dose is absorbed after oral administration.

The Note for Guidance reflects that the investigation of the solubility and the rapid dissolution of the API is of superior importance than the permeability of the active pharmaceutical ingredient.

It seems scientifically plausible that bioavailability of an active ingredient depends on solubility and permeability as well. However, only solubility/dissolution may be pharmaceutically controlled and influenced (e.g. by the pharmaceutical formulation).

Therefore for “immediate release” of herbal medicinal products the solubility of the total extract and known pharmacological active constituents are crucial parameters for a waiver of bioequivalence or clinical studies.

Determination of the solubility of extracts and active markers in BCS-buffers

It is examined if the quantity of extract contained in the highest strength of the product dissolves in 250 ml of solvents (buffers) I-III (I: pH 1.0; II: pH 4.6; III : pH 6.8) at 37°C.

After stirring for 60 minutes the residues are filtered and gravimetrically determined after drying at 100 ^{0}C for 2 h. As plant extracts may often contain rather insoluble “matrix” components such as tannins, proteins and other polymeric compounds assumed not to be linked to efficacy, extracts with a solubility of > 90% are classified as very soluble. Extracts with a lower solubility than 90% are classified to be problematical. Active markers are also classified as problematical if their solubility from extracts is <90%.

Plant extracts can be classified according to the knowledge on their composition and efficacy into 3 categories (A, B1 and B2). Herbal medicinal products containing extracts of categories A or B1 should comply with the Note for Guidance on the Investigation of Bioavailability and Bioequivalence (Lang, 2001).

EMEA (European Agency for the Evaluation of Medicinal Products) GUIDELINES FOR EVALUATION OF HERBAL MEDICINES (EMEA, 2820/00)

Herbal medicinal products have been defined as the medicinal products containing as active substances exclusively herbal drug or herbal drug preparations.

The following tests and acceptance criteria considered generally applicable to all herbal medicinal products:

Assay

In the case of products containing herbal drugs and/or herbal drug preparations with constituents of known therapeutic activity, validated assays of

the content of these constituents are required along with details of the analytical procedure(s).

In the case of products containing herbal drug(s) and /or herbal drug preparations where the constituents responsible for the therapeutic activity are unknown, assays of marker substances or other justified determinations are required.

Impurities

- Impurities arising from the herbal drug(s) and/or herbal drug preparations e.g. contaminants such as pesticide/fumigant residues, heavy metals, are normally controlled during the testing of the herbal drug preparation (herbal drug) and it is not necessary to test for these in the herbal medicinal product.
- Similarly, residual solvent arising from the manufacture of the herbal drug preparation (e.g. an extract) need not be controlled in the finished herbal medicinal product provided it is appropriately controlled in the extract specification. However, solvents used for example in tablet coating will need to be controlled in the dosage form.
- Major impurities arising from degradation of the herbal drug preparation (herbal drug) should be monitored in the herbal medicinal product. Acceptance limits should be stated for individual specified degradation products, which may include both identified products as appropriate and total degradation products.

Microbial limits

There is a need to specify the total count of aerobic microorganisms, the total count of yeasts and moulds, and the absence of specific objectionable bacteria. These limits should comply with the European Pharmacopoeia. The frequency of testing should be justified.

Specific tests/criteria

In addition to the universal tests listed above, the following tests may be considered applicable to herbal medicinal products on a case by case basis. Individual tests/ criteria should be included in the specification when the tests have an impact on the quality of the herbal medicinal product for batch control. Tests other than those listed below may be needed in particular situations or as new information becomes available.

Tablets (coated and uncoated) and hard capsules

A) Dissolution/Disintegration:

In the case of immediate release herbal medicinal products and without constituents with known therapeutic activity, the test for in-vitro active ingredient release can be omitted.

For immediate release products containing herbal drug preparations, which are highly soluble throughout the physiological pH range, disintegration testing may sometimes be sufficient. Disintegration testing is most appropriate when a relationship to dissolution has been established or when disintegration is shown to be more discriminating than dissolution. In such cases dissolution testing may not always be necessary, or may be proposed as a periodic test. Single-point measurements are normally considered to be suitable for immediate-release dosage forms. For modified-release dosage forms, appropriate test conditions and sampling procedures should be established. For example, multiple-time-point sampling should be performed for extended-release dosage forms, and two-stage testing may be appropriate for delayed-release dosage forms. In these cases it is important to consider the populations of individuals or target animal species who will be taking the herbal medicinal product (e.g. achlorhydric elderly) when designing the tests and acceptance criteria.

B) Hardness/Friability:

It is normally appropriate to perform hardness and / or friability testing as an in-process control. Under these circumstances, it is normally not necessary to include these attributes in the specification. If the characteristic of hardness and friability have a critical impact on herbal medicinal product quality (e.g., chewable tablets), acceptance criteria should be included in the specification.

C) Uniformity of dosage units:

This term includes both uniformity of content and uniformity of mass; a pharmacopoeial procedure should be used. If appropriate, these tests may be performed as in-process controls; the acceptance criteria should be included in the specification.

D) Water content:

A test for water content should be included when appropriate. The acceptance criteria may be

justified with data on the effects of or water absorption on the herbal medicinal product. In some cases, a Loss on Drying procedure may be adequate; however, a detection procedure which is specific for water (e.g., Karl Fischer titration) is required.

E) Microbial limits:

Microbial limit testing is seen as an attribute of Good Manufacturing practice, as well as of quality assurance. It is advisable to test the herbal medicinal product unless its components are tested before manufacture and the manufacturing process is known, through validation studies, not to carry a significant risk of microbial contamination. Reference should be made to the European Pharmacopoeia general text on the Microbiological Quality of Pharmaceutical Preparations for guidance on acceptable limits. Periodic testing may be appropriate.

Chemistry-Manufacturing-Control (CMC) considerations for herbal products

Unlike standard chemically-defined drugs, herbal products have often had substantial human use prior to clinical trial evaluation. To capitalize on the use of this information in protocols to evaluate these products, it is important that the chemistry, manufacturing, and control of the product to be used mimics that for the traditionally-used formulation.

Evaluation of herbal products does not require attempts to purify the medicines down to known or otherwise single chemical constituents. For herbal products, “analysis of the active pharmaceutical ingredient(s)” may be best approached by analysis of one or more hypothesized active ingredient(s), analysis of a chemical constituent that constitutes a sizable percentage of the total ingredients, and a chemical fingerprint of the total ingredients. The latter two analyses are surrogates for analysis of the unknown constituents that contribute to efficacy. Variation of content from batch to batch is an important issue for herbal products, therefore several analytical procedures are needed to adequately quantify their constituents (WHO, 2005).

CONCLUSION

For standardization and quality assurance purposes, following three attributes are desirable i) Authenticity, ii) Purity and iii) Assay. Authenticity as the name suggests relates to proving that the material is true, i.e. it corresponds to the right identity. Authentication in itself involves many parameters including gross morphology, microscopy, chemical analysis and DNA fingerprinting.

Purity pertains to evaluating that there are no adulterants present in the plant material. Assay part of standardization is chemical and biological profiling which could assess the chemical effects and curative values get established. Safety for use could also be assessed through this parameter. In biological assays, the drug activity is evaluated through a pharmacological model. Chemoprofiling is a versatile technique and can be made to good use in standardization. Fingerprinting in essence is chemoprofiling, which means establishing a characteristic chemical pattern for the plant material or its cut or fraction or extract (Bhutani, 2000).

Herbal drug technology is used for converting botanical materials into medicines, where standardization and quality control with proper integration of modern scientific techniques and traditional knowledge is important. The routine methods of herbal drug standardization address quality related issue using botanical and organoleptic parameters of crude drugs, and chemoprofiling assisted characterization with spectroscopic techniques but the new era of herbal drug standardization includes pharmacognostical, chemical, biological, biopharmaceutical and molecular approaches.

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