Annex 1

WHO guidelines for selecting marker substances of herbal origin for quality control of herbal medicines

1. Introduction

1.1 Background

With the constant increase in the use of herbal medicines worldwide and the rapid expansion of the global market for them, the safety and quality of herbal materials and finished herbal products has become a major concern for health authorities, pharmaceutical industries and the public. The safety and efficacy of herbal medicines largely depend on their quality. Requirements and methods for quality control of finished herbal products, particularly for mixture herbal products, are far more complex than for chemical medicines. The quality of finished herbal products is also influenced by the quality of the raw materials used.

The World Health Assembly resolution on traditional medicine (WHA56.31), adopted in May 2003, requested WHO to provide technical support to develop methodology to monitor or ensure the quality, efficacy and safety of herbal products.

The International Conference of Drug Regulatory Authorities (ICDRA) in 2002 and 2004, as well as the Meetings of National Centres Participating in the WHO Programme of International Drug Monitoring (in 2000, 2001, 2002 and 2003) requested WHO to develop and continuously update the technical guidelines on quality, safety and efficacy of herbal medicines. One of the challenges in analysing the cause of adverse events reported in connection with use of herbal medicines is the lack of expertise in identifying and testing ingredients and constituents of suspect herbal products at the national pharmacovigilance centres, and/or national quality control laboratories.

To reduce the proportion of adverse events attributable to poor quality of herbal medicines, WHO has committed to developing new guidelines on quality assurance and control of herbal medicines, as well as to updating existing ones.

As a follow-up to the WHO Informal Meeting on Methodologies for Quality Control of Finished Herbal Products, held in Ottawa, Canada in July 2001, WHO decided to develop four new documents to provide technical guidance at the key stages where quality control is required in production of herbal medicines:

(1) *WHO guidelines on good agricultural and collection practices (GACP) for medicinal plants* (published in 2003) (*1*);

(2) *WHO guidelines on assessing quality of herbal medicines with reference to contaminants and residues* (published in 2007) (*2*);

(3) *Good processing practices for herbal materials* (in preparation); and

(4) *Analytical methods for chemical identification of ingredients/ constituents for quality control of herbal medicines* (originally proposed title).

In 2006, WHO revised the *Good manufacturing practices (GMP) supplementary guidelines for manufacture of herbal medicines* (*3*) to take into account the recent update of the WHO core GMP and the fact that many Member States were considering establishing specific GMP for herbal medicines. Subsequently the WHO guidelines on *Good manufacturing practices (GMP) for herbal medicines* were published in 2007 (*4*).

WHO also revised the *Quality control methods for medicinal plant materials* (*5*) updating several chapters relating to determination of major contaminants and residues (e.g. microbial contaminants, toxic heavy metals and pesticide residues) and published it under the title *Quality control methods for herbal materials* (*6*).

1.1.1 Preparation of the document

The original title suggested for these guidelines was *Analytical methods for chemical identification of ingredients/constituents for quality control of herbal medicines*, as mentioned above. In February 2004, WHO convened a working group meeting on quality control of herbal medicines with financial support from Health Canada, in Vancouver, Canada. During this meeting, a brainstorming session was held on how to approach the issue of marker substances in quality assurance and control of herbal medicines. Subsequently, a WHO Consultation on quality control of herbal medicines, held in Abu Dhabi, United Arab Emirates, in July 2005, provided the forum to discuss the working draft of these guidelines. After wide global reviews of subsequent versions of the draft guidelines, the final draft guidelines were reviewed and discussed at the 2nd WHO Consultation on quality control of herbal medicines held in Hong Kong SAR, China, in November 2014. In October 2016, the finalized guidelines were submitted to the fifty-first meeting of the WHO Expert Committee on Specifications for Pharmaceutical Preparations; the Committee endorsed and adopted the guidelines for publication as an annex to the report of the fifty-first meeting and as an independent WHO publication.

1.2 Objectives

The objectives of this document are to:

1) provide selection criteria for marker substances of herbal origin for quality control of herbal medicines;

2) identify methods and techniques for the identification and assay of these substances;
3) provide examples of selected marker substances in selected herbal materials;
4) contribute to the technical guidance on methodologies for quality control of herbal materials, herbal preparations and finished herbal products, in order to meet the quality control requirements;
5) promote the safety and efficacy of herbal medicines by contributing to consistent and reproducible quality.

1.3 Glossary

The terms used in this document are defined below. Additional terms that may be used in pharmaceutical texts referring to herbal medicines are also included. These terms and their definitions have been selected and adapted from other WHO documents and guidelines that are widely used by the WHO Member States. The citation numbers in parentheses following a term refer to the publication, as listed in the reference list, from which the term has been derived.

These definitions may differ from those included in national regulations, and are therefore provided for reference only.

1.3.1 Terms related to herbal medicines

Medicinal plants are plants (wild or cultivated) used for medicinal purposes (*1*, *3*, *4*).

Medicinal plant materials: see *Herbal materials*

Herbal medicines include ***herbs*** and/or ***herbal materials*** and/or ***herbal preparations*** and/or ***finished herbal products*** in a form suitable for administration to patients (Box A1.1).

Note: In some countries herbal medicines may contain, by tradition, natural organic or inorganic active ingredients that are not of plant origin (e.g. animal and mineral materials).

Box A1.1
Definition of herbs, herbal materials, herbal preparations and finished herbal products

Herbs are crude plant material which may be entire, fragmented or powdered. Herbs include, e.g. the entire aerial part, leaves, flowers, fruits, seeds, roots, bark (stems) of trees, tubers, rhizomes or other plant parts.

Box A1.1 *continued*

Herbal materials include, in addition to herbs, other crude plant materials. Examples of these other plant materials include gums, resins, balsams and exudates.

Herbal preparations are produced from herbal materials by physical or biological processes.

These processes may be extraction (with water, alcohol, supercritical carbon dioxide (CO_2)), fractionation, purification, concentration, fermentation and other processes. They also include processing herbal materials with a natural vehicle or steeping or heating them in alcoholic beverages and/or honey, or in other materials.

The resulting herbal preparations include, among others, simply comminuted (fragmented) or powdered herbal materials as well as extracts, tinctures, fatty (fixed) or essential oils, expressed plant juices, decoctions, cold and hot infusions.

Finished herbal products consist of one or more herbal preparations made from one or more herbs (i.e. from different herbal preparations made of the same plant as well as herbal preparations from different plants. Products containing different plant materials are called "mixture herbal products").

Finished herbal products and mixture herbal products may contain excipients in addition to the active ingredients. However, finished products or mixture herbal 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".

Substitute is a herbal material or herbal preparation that is replaced by another, appropriately labelled herbal material, or herbal preparation consistent with the national pharmacopoeia, or traditional (or complementary and alternative) medicine practice.

Adulterant is herbal material, a herbal constituent or other substance that is either deliberately or non-intentionally (through cross-contamination or contamination) added to a herbal material, herbal preparation, or finished herbal product.

1.3.2 Terms related to constituents of herbal medicines

Constituents are chemically defined substances or group(s) of substances found in a herbal material or herbal preparations.

Therapeutic activity refers to the successful prevention, diagnosis and treatment of physical and mental illnesses. Treatment includes beneficial alteration or regulation of the physical and mental status of the body and development of a sense of general well-being as well as improvement of symptoms.

Active ingredients refer to constituents with known therapeutic activity, when they have been identified. Where it is not possible to identify the active ingredients, the whole herbal medicine may be considered as one active ingredient.

Constituents with known therapeutic activity are substances or group(s) of substances which are chemically defined and known to contribute to the therapeutic activity of the herbal material or of a preparation (*3*, *4*).

Constituents with recognized pharmacological (biological) activities are characteristic constituents (substances or group(s) of substances) which are chemically defined and where the relevance of the pharmacological (biological) activities for the therapeutic or toxicological effects of the herbal material or herbal preparation has not yet been fully established.

Characteristic constituents are chemically defined substances or group(s) of substances that are specific for one medicinal plant or for certain plant species, families or genera.

Toxic constituents are substances or group(s) of substances that are chemically defined and their toxic property is predominant, although they may contribute to the therapeutic activities of the herbal material or herbal preparation.

1.3.3 Terms related to standardization and quality control of herbal materials and herbal preparations

Reference substances are chemically defined molecular entities (appropriate for intended uses in standardization or quality control of herbs and herbal materials).

Markers (marker substances) are reference substances that are chemically defined constituents of a herbal material. They may or may not contribute to the therapeutic activity. However, even when they contribute to the therapeutic activity, evidence that they are solely responsible for the clinical efficacy may not be available.

Primary chemical reference substances are substances that are widely acknowledged to have the appropriate qualities within a specified context, and whose assigned content when used as a (mostly as an assay) standard is accepted without requiring comparison to another chemical substance (*7*).

Secondary chemical reference substances (also called *working standards*) are substances whose characteristics are assigned and/or calibrated by comparison with a primary chemical reference substance.

The extent of characterization and testing of a secondary chemical reference substance may be less than for a primary chemical reference substance (*7*).

International Chemical Reference Substances (ICRS) are primary chemical reference substances established on the advice of the WHO Expert Committee on Specifications for Pharmaceutical Preparations.

They are supplied primarily for use in physical and chemical tests and assays described in the specifications for quality control of medicines published in *The International Pharmacopoeia* or proposed in draft monographs. The ICRS may be used to calibrate secondary standards (*7*).

Certified reference substances are primary reference substances certified by regulatory bodies.

Pharmacopoeial reference substances (standards) are primary reference substances established and distributed by pharmacopoeial authorities following the general principles of the *ISO Guide 34* (*8*).

Note: a different approach is used by the pharmacopoeial authorities to give the user the information provided by certificate of analysis and expiration dates (*7*).

Reference materials refer to materials other than substances appropriate for intended uses in standardization or quality control of herbs and herbal materials. Reference materials include, among others, herbarium samples, authentic specimens of herbal materials (such as extracts and their fractions), herbal reference preparations and authentic spectra or fingerprints.

2. Selection criteria for substances of herbal origin relevant for standardization and quality control of herbal medicines

2.1 General considerations in the standardization and quality control of herbal materials, herbal preparations and herbal medicines

Herbal materials, herbal preparations and finished herbal products are very complex. This can make the identification and quantification of herbal medicines very difficult and the detection of adulteration is very challenging.

It should be emphasized that the identification of herbal medicines using markers, and quantification of marker substances in herbal medicines are not in themselves sufficient to guarantee the quality of herbal medicines. Quality control must cover all steps of their production and must be complemented by good agricultural and collection practices (GACP) and good manufacturing practices (GMP) (such as those described in references *1* and *4*), as appropriate.

Criteria for the selection of reference substances and quality control of herbal medicines should take into account that various ingredients may have different levels of influence on the final quality, safety and efficacy. For this reason, the order of selection of the substances for identification and quantification should follow the rules presented below.

1) If **constituents with known therapeutic activity (activities)** have been identified, they should be used as markers.
2) If 1. is not the case, but **constituent(s) with recognized pharmacological activity (activities) is (are) known**, they should be used as markers.
3) If the above cases are not applicable, the identity and quantity of herbal materials, preparations and medicines may be established by the production process and by analysing marker substance(s) containing other characteristic constituent(s).

Note that identification of herbal materials, and also to some extent herbal preparations and finished herbal products, may be be done or may be complemented by microscopic, macroscopic or DNA analytical methods using appropriate reference materials and descriptions.

2.2 Purpose and expected functions of relevant marker substances

Markers used as chemical reference substances should be international chemical or pharmacopoeial reference substances. If others are used, markers for quantitative determination should be of high purity as required by national regulations, determined by validated analytical methods, including physical and chemical ones. These analyatical methods may be different from those employed for quantifying herbal materials. For markers used for identification, lower purity may be suitable.

The general requirements for markers are:

- identity, specificity and selectivity using the specified analytical method(s);
- should be present in traceable quantity for identification or sufficient quantity for assay;
- should be easily obtained,[1] stable under specified storage conditions;
- should be easily detected and quantified analytically.[1]

[1] This does not apply to substances described under 2.2.4.

2.2.1 Marker substances of constituents with known therapeutic activity[2]

PURPOSE AND FUNCTION

Markers of constituents with known therapeutic activity should serve their appropriate purpose (identification or quantification).

SELECTION CRITERIA

The criteria for selection of a marker substance of constituents with known therapeutic activity are as follows:

- The marker must be readily available (for example, as an international or pharmacopoeial reference substance). New markers may only be selected if no such reference substance is available. In that case, detailed documentation should be provided on the identity and properties of the selected markers.
- It should be relatively easy to separate or distinguish the marker analytically from other structurally similar herbal constituents.
- Markers should be detectable and quantifiable with available analytical instrumental methods (such as thin-layer chromatography (TLC), high-performance thin-layer chromatography (HPTLC), gas chromatography (GC) or high-performance liquid chromatography (HPLC).
- Different marker substances may be selected for the same herbal medicines depending on the analytical instrumental methods available.

Notes

- Derivatives of the naturally occurring markers may be used where the latter are not easy to detect, are not stable or are not easily obtained.
- Different marker substances may be selected for the same herbal materials depending on the different forms of herbal preparations or finished herbal products.
- A group of markers may be selected if a single marker is not sufficient to identify and evaluate the herbal materials or finished herbal products.

[2] They could also be named "reference substances". However, taking the complex nature of all kinds of herbal medicines into account, it is unlikely that one single compound would be solely responsible for the therapeutic action. Thus, substances of constituents with known therapeutic activity are generally also called "markers".

2.2.2 Marker substances of constituents with recognized pharmacological activities

PURPOSE and FUNCTION
Markers of constituents with recognized pharmacological activities should serve as qualitative and quantitative measures in herbal medicines.

SELECTION CRITERIA
The criteria for selection of a marker substance of constituents with recognized pharmacological activities are as follows:

- They occur naturally in sufficient quantities in herbal materials.
- *Markers for quantification*: should be representative of the main therapeutic or pharmacological profiles of the herbal materials and finished products.
- *Markers for identification*: should be specific for one plant or for certain plant species and genera. If not, other marker(s) should be selected for specific identification.
- They should be detectable and quantifiable by available instrumental analytical methods (such as TLC, HPTLC, HPLC, GC) or by another relevant analytical method.
- Different substances may be selected for the same herbal materials depending on the different forms of the herbal preparations (including different, e.g. aqueous and alcoholic extracts) or different therapeutic indications.
- A group of substances may be selected if a single one is not sufficient to evaluate the herbal material or finished herbal product.

2.2.3 Marker substances of characteristic constituents

PURPOSE and FUNCTION
The main purpose of markers of characteristic constituents is identification and quantification of herbal materials in herbal preparations and finished herbal products.

SPECIFIC REQUIREMENTS
Markers for identification:

- The marker should be specific for one plant. If not, the marker should be specific for a certain plant species, genus and family.

  Note: a plant family may contain many classes of biologically diverse species and chemically diverse ingredients, but the plants in the

same genus are normally genetically close and contain structurally similar secondary metabolite constituents.

- If not specific for one plant, the marker should be specific at least for one herbal material or preparation in a mixture herbal preparation or herbal medicines.
- The marker should consist of one substance or group of substances, or characteristic pattern of substances.

  Note: A pattern of substances characteristic for a specific herb may replace a single substance.

Markers for quantification:

- The marker for quantification should be available in sufficient quantity for assay.

SELECTION CRITERIA
The criteria for selection marker substances of characteristic constituents are as follows:

- They should occur naturally in sufficient quantities in herbal materials.
- An authentic reference should be available.
- Spectral data on the substance should be recorded in an available library or database.
- TLC chromatogram pattern or other analytical identification should be illustrated in an available source.
- A simple identification and quantification test should be described for the substance or its chemical class.
- There should be adequate experimental evidence that the substance or group of substances is characteristic of the given herbal medicine.

2.2.4 Marker substances for toxic constituents

PURPOSES AND FUNCTION
Marker substances for toxic constituents are used to define maximum acceptable concentrations of toxic constituents in herbal materials, herbal preparations or finished herbal products.

REQUIREMENTS

- As a consequence of the composition of the herbal material or product, such a limit test is needed.

- There should be a defined upper tolerable limit for the mode of application and posology intended (e.g. oral, topical, inhalation, short-term, subchronic or chronic application).
- A toxicological evaluation is required, but experience with traditional use should be taken into account.
- Genotoxicity, mutagenicity and carcinogenicity should also be considered when establishing toxicity criteria.
- An analytical detection procedure for the established tolerable limits should be available.
- These requirements should always be met by the finished herbal product destined for human use, since processing and conservation may alter toxicity.

SELECTION CRITERIA

- An appropriate reference substance should be available.
- For identity, specificity and selectivity are important characteristics.
- Limit of detection and limit of quantitation values for the target herbal medicines should be specified.
- Highly sensitive instrumental analytical methods (such as TLC, HPTLC, GC, HPLC, GC/mass spectrometry (MS), liquid chromatography (LC)/MS) should be available for detection of toxic substances.
- Simple identification tests for groups of toxic substances, such as alkaloids or terpenoids should be available.

Note: The criteria used for selecting marker substances for toxic constituents apply to detection of a toxic substance specific to a particular herbal material. To ensure its safety for human consumption, the toxic constituents of a herbal material, its herbal preparation or its finished herbal product should be identified accurately.

The toxicity may assessed for control by the absence of a constituent or by establishing and testing allowable tolerable limit(s) for the toxic constituents using selected marker(s) and analytical methods. For example, the absence of thiaminase enzyme activity in horsetail (*Equisetum arvense*) as well as a method for the detection of kavalactone (a hepatotoxic agent) in kava kava (*Piper methysticum*) should be required.

If it is not possible to exclude the toxic effect, e.g. because there is no appropriate marker constituent or because of the lack of an analytical method or specific method of preparation, the herbal material or its herbal preparation should not be used in finished herbal products.

2.3 Use of reference materials

Various types of herbal reference materials are used as complements to analytical methods that were performed using markers and especially when no reference substances for the above-mentioned markers are available. They may also be used when markers are available, but are not adequate for identification of the herbal materials, preparations or finished products.

For example, herbal medicines may contain a group of specified constituents or constituents with recognized pharmacological activities, such as flavonoids, alkaloids and saponins. There are also cases (e.g. well-identified extracts) when the reference material might be more stable than single ingredients with a high degree of purity (primary or secondary reference standards).

PURPOSE AND FUNCTION

Identification and quantification of herbs, herbal materials and herbal medicines.

REQUIREMENTS

- Botanical reference materials and/or herbal preparations should be described by national pharmacopoeias or materia medica (e.g. those of China, Indonesia and Japan).
- Herbarium samples and authentic herbal material for microscopic and macroscopic comparison should be developed in cooperation with botanists for systematic authentication.
- If herbal preparations are used as the reference standard, full documentation on the preparation needs to be submitted to allow full traceability.
- Reference materials should be prepared following methods described in guidelines on validation.

SELECTION CRITERIA

- Herbal reference extracts should be prepared in accordance with standard operating procedures and the characteristic and/or active constituents should be well demonstrated on chromatograms (obtained by instrumental analytical methods such as TLC, high-performance thin-layer chromatography (HPTLC), HPLC, GC) and spectra (such as nuclear magnetic resonance (NMR) or MS) under specified conditions.
- The herbal reference extracts or herbal reference preparations and their main constituents should be stable and identifiable using available analytical instruments and analytical methods.

- There should be predetermined in-house criteria on how to use herbal reference preparations for identification of specified finished herbal products produced by manufacturers.

3. Analytical methods for substances of herbal origin in herbal medicines

This section describes testing methods employed for quality control.

3.1 General considerations regarding the test methods to be employed

Analytical methods used for quality control of herbal materials, herbal preparations and finished herbal products are generally based on:

a) chemical reactions;
b) chromatographic procedures (such as TLC, HPTLC, GC and HPLC), including fingerprinting;
c) spectroscopic and spectrometric methods;
d) a combination of b) and c); and
e) others.

Test methods should be specific and selective for the selected substances in the herbal materials, herbal preparations or finished herbal products. Test methods must be validated. It might be necessary to revalidate the method if the substance is tested at different stages of the production process (e.g. herbal preparations such as extracts and finished herbal products) because other substances, e.g. excipients may influence the analytical procedures.

Test methods, if applicable, should be able to detect substitutes or adulterants that are likely to be present in the sample.

- *Herbal materials, herbal preparations or finished herbal products with constituents with known therapeutic activity*

  The analytical methods used for quality control should be capable of detecting and quantifying the constituents with known therapeutic activity. The use of reference substances for the therapeutically important constituents in the analysis is recommended.

- *Herbal materials, herbal preparations or finished herbal products with constituents with recognized pharmacological activities*

  The analytical methods used for quality control should be capable of detecting and quantifying the constituents with recognized

pharmacological activities. The use of reference substances for the therapeutically important constituents in the analysis is recommended.

- *Herbal materials, herbal preparations or finished herbal products with characteristic constituents (whose pharmacologically or therapeutically active constituents are unknown)*

  When the pharmacological and the therapeutically active constituents are unknown, the identification and assay procedures should be based on characteristic constituents (markers) and fingerprint chromatograms, or on characteristic microscopic or macroscopic features of the herbal materials, herbal preparations or finished herbal products. Reference samples should be used in the analysis, where available.

Where the local laboratory has limited capacity, the use of dependable but simple basic technical and testing methods is recommended. However, the producer should not be discouraged from developing and applying more sophisticated methods for testing products intended for export.

3.2 Monographs

It is recommended that pharmacopoeial monographs prepared by national or regional authorities should incorporate substances and constituents for quality control of herbal materials, herbal preparations or finished herbal products.

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