STANDARDIZATION

OF BOTANICAL PRODUCTS: WHITE PAPER

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Prepared by the Botanical Extracts Committee of the American Herbal Products Association

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INTRODUCTION

In recent years, the United States marketplace has seen an increasing appreciation of the health-promoting benefits of herbal preparations. Both consumers and healthcare practitioners are becoming more receptive to their use. Some people, however, have reservations about the use of herbal products due to the chemical complexity of such products and the perceived difficulty in ensuring batch-to-batch product reproducibility. In order to address this concern, there has been a trend in the marketplace toward the use of "standardized" preparations; and in most cases, the word "standardized" is associated with a quantitative claim for the content of a particular constituent or constituents. The constituent or constituents quantified are commonly known in the U.S. as "marker compounds" or "markers."

The attention focused on marker compounds can imply that the marker content is crucial to guarantee the reproducibility or even the efficacy of the extract. Such assumptions are not always correct. Marker compounds often bear little or no relationship to the efficacy of the preparation. Furthermore, reproducible marker content is indicative of a reproducible product only if used in the context of a complete body of raw material and manufacturing controls. Standardization of a botanical product does not merely mean controlling one or a few constituents; rather, it is a complex process requiring attention to a wide variety of parameters, with the ultimate goal of enhancing the batch-to-batch reproducibility of the entire spectrum of constituents.

This paper describes the many factors that contribute to the proper standardization of a botanical product. The scope of the document covers a variety of preparations, from crude botanicals to extracts to solid oral dosages. Few, if any, products are manufactured using all of the techniques that will be mentioned; rather, each manufacturer must choose those controls most suitable and pertinent to the particular botanical used and the specifications established for the product in question.

Manufacturers are encouraged to share, with interested parties, general information concerning the types of controls used in the standardization of their products. Manufacturers should strive to educate the public about standardization in order for informed decisions to be made with respect to comparisons between products.

This document is intended to foster a heightened awareness of the complexities associated with standardization, and to facilitate informed discussions between raw material suppliers, product manufacturers, practitioners, clinicians, regulators, and consumers.

1. STANDARDIZATION: DEFINED

In broad terms, standardization is the complete body of information and controls that serves to optimize the batch-to-batch consistency of a botanical product. Standardization is achieved by reducing the inherent variation of natural product composition through quality assurance practices applied to agricultural and manufacturing processes.[1, 2]

2. HOW STANDARDIZATION IS ACHIEVED: AN OVERVIEW

In order to produce a consistent product, controls must be implemented over both the raw material supply and the manufacturing process. Controls over raw material sourcing may include specifying the botanical species[3], variety, and plant part; use of consistent growing conditions in terms of location, soil, climate, agricultural practices, and other factors, for both cultivated and wildcrafted herbs; use of consistent and appropriate harvesting practices, including consideration of the stage of development and time of harvesting; consistency in post-harvest processing and storage; and the qualitative and/or quantitative analysis of the chemical profile of the raw material. Each botanical product may require a different combination of controls depending on the nature of the botanical and the goals of the manufacturer. The ultimate purpose of each of these controls is to reduce variability in the composition of the raw material.

A supply of consistent raw materials provide the necessary basis on which manufacturing controls act to yield a reproducible product. Manufacturing controls require well-defined formulas and operating procedures. Important process parameters should be appropriately controlled, such as process times, temperatures, and pressures where relevant, the particle size at various stages of manufacture, the extraction solvent, the extraction process, the proportion of extraction solvent to botanical, and any purification, sanitization, or drying steps, as appropriate for the product or process in question.

As an additional manufacturing control, and to confirm batch-to-batch consistency in chemical composition, many manufacturers perform quantitative analyses of marker compounds. Marker compounds are constituents that occur naturally in the botanical material and are capable of being chemically analyzed. The levels of these constituents are measured in both raw materials and products. This information can then be used for technical purposes during material selection, processing, packaging, and storage. Marker compounds may also relate to the physiological activity of the botanical, although this is not always the case. Marker compounds can serve a variety of useful purposes, but their importance should not be exaggerated. Analysis of one or more marker compounds or

1 Most extract manufacturers, academicians, healthcare professionals, and regulators worldwide believe that marker quantification, along with appropriate raw material and manufacturing controls, is a necessary part of standardization. However, a diverse group of academicians and extract manufacturers do not believe that marker quantification is a requirement for standardization. This subject remains controversial in the U.S. The current document does not attempt to resolve this question.

2 Standardization, in general, seeks to improve the batch-to-batch chemical reproducibility of the product rather than the reproducibility of the biological effect. Some experts believe it more pertinent to control potency or efficacy rather than chemistry. However, due to the difficulty in evaluating in vivo potency or efficacy, this is not an approach commonly used in the commercial marketplace. Trained herbal practitioners often evaluate the "strength" of a preparation by observing its effect in the patient, and adjust the prescribed dose appropriately, thereby achieving a type of efficacy standardization; but this is different than standardizing a product.

3 In the commercial marketplace, botanical materials are typically considered to include plants, algae, and fungi.

even groups of compounds[4] provides only a limited appreciation of the materials' composition, since botanicals commonly contain thousands of individual constituents. Therefore, the presence of a marker compound at a particular level is not, by itself, sufficient to guarantee a standardized product. The analysis of marker compounds is meaningful only when performed in the context of a wide variety of raw material and process controls and in combination with other techniques, such as organoleptic evaluations or chromatographic fingerprinting, which provide a more comprehensive examination of the chemical composition.

3. The Importance and Applicability of Standardization

a. Enhancing Reproducibility

Standardization seeks to enhance the reproducibility of a product's safety and efficacy by providing a more consistent composition. Botanicals contain many compounds that work together after ingestion. Some compounds may enhance or diminish the physiological effects of others. Other compounds, while having no direct physiological effect, may nevertheless influence the stability, solubility, and bioavailability of the physiologically relevant compounds. Therefore, by directly or indirectly enhancing the reproducibility of the complete composition of the product, standardization serves to enhance the batch-to-batch consistency of the product's effect.

b. Material of Reasonably Consistent Composition

Standardization provides material of reasonably consistent composition for the conduct of reliable and reproducible clinical trials. When a clinical trial is conducted, the material used must be standardized and characterized as thoroughly as possible. This allows the results of the trial to be extrapolated to other batches of the same product and to similar products, which have been characterized and standardized in a like manner. On the other hand, the results of the trial should not be extrapolated to products that are significantly different from that used in the trial, since differences in the chemical composition are likely to cause different physiologic effects.

To properly characterize clinical material, researchers should compile and report as much information as possible about the nature of the product used. At a minimum, this should include the species (Latin binomial with authority, e.g. "*Ginkgo biloba* L."), plant part and form of the botanical ingredients. For botanical extracts, it should also include at least one of the following: Either a comprehensive technical description of the extract (i.e., the extraction solvent, extract ratio, native extract content, and excipients) or the compendial standard with which the extract complies. The brand name of the extract is also useful, especially if the brand is well characterized and reproducible. The reporting of the product lot number(s) can facilitate the investigation of anomalous or unexpected results. In addition, any special quality characteristics established for

4 For ease of reading, this document generally uses the term "constituent" or "compound" to refer to that which is quantified, omitting the phrase "or groups of constituents (compounds)." The reader should bear in mind that sometime individual constituents and sometimes groups of constituents are quantified.

the raw material or product should be noted. The content of any marker compounds may be useful information, but is not sufficient by itself to characterize the material. Adequate characterization is essential if researchers are to properly interpret the data obtained, and is particularly important in performing meta-analyses, since only products of similar composition can be meaningfully compared to each other.

c. Standardization with Marker Compounds

Standardization with marker compounds provides an additional tool manufacturers may use for raw material sourcing and process control.[5] With respect to raw materials, markers may help confirm the identity of the material or the absence of known potential adulterants, and provide one piece of evidence that the material is of a composition consistent with previous purchases. With respect to process controls, marker compounds can be used to confirm the integrity of the manufacturing process for each lot, since deviations from the marker levels previously observed may indicate an error or problem during processing. Of particular interest may be the monitoring of volatile or labile marker compounds, which can be used to track the impact of processing on the botanical and ensure that unacceptable loss or degradation is not occurring. Markers can also be used for blend validations, monitoring the stability of preparations, and other purposes. In addition, some markers are relevant to the physiologic activity of the preparation; see section 7(c) below.

4. Raw Material and Process Controls

a. Growth and Harvesting of Botanical Materials

i. **Species and Variety.** In most cases, materials should be sourced from the same species or even variety, in order to minimize inherent botanical variability. However, this is not always necessary; in some genera, such as willow or hawthorn, various species are used interchangeably by herbalists and manufacturers alike. In some rare cases, herbalists consider even plants of different genera interchangeable. Many pharmacopoeial monographs explicitly allow such practices. At the other extreme, for some products, not only the species but also the specific strain is controlled, due to the development of novel cultivars with unique properties or chemical attributes. These include varieties with elevated levels of compounds considered desirable, and varieties with reduced levels of compounds considered toxic.[6] In general, the interchangeability of different botanical materials depends on similarities in their chemical composition, safety, and therapeutic effect. Where significant differences occur (such as the presence of a toxin in one variety or species, or the lack of active constituents in another), the appropriate taxonomic selection is imperative.

5 For a complete, detailed discussion of the appropriate uses of marker compounds, see the publication "Use of marker compounds in manufacturing and labeling botanically derived dietary supplements" published by AHPA in 2000.

6 Such manipulation of an herb's chemical composition must be approached cautiously; refer to section 8(b) below.

ii. **Plant Part.** Standardization usually entails consistent selection of the plant part or parts to be used. While some constituents may be evenly distributed throughout the plant, more often, important components are localized in one part or another. Some of the best-known examples come from the food supply; tomato fruits and potato tubers are edible, but the leaves of these plants are toxic when consumed. The difference between various plant parts is not always so dramatic, but is still often crucial. For example, ephedra aerial parts contain hypertensive alkaloids such as ephedrine, while ephedra roots contain hypotensive alkaloids such as ephedradines. Since the composition of each plant part is different, traditional use and modern science usually dictate use of a specific part or a specific combination of parts of the plant.

iii. **Growing Conditions.** Growing conditions have a significant impact on the chemical composition of the plant. Soil type, rainfall, temperature, humidity, sun intensity and exposure, and seasonal or year-to-year variations therein, as well as climate and other features of the local physical environment, all influence the vigor of the plant; suboptimal conditions may prevent the plant from reaching its full biochemical potential. Agricultural practices, including irrigation and the application of fertilizers or pesticides, can also have significant effects. Finally, a plant's physical and chemical state can be altered by biotic influences in its environment; for example, competition with other plants for space and grazing by animals can influence the growth and development of the plant. Fungal and bacterial infections, as well as insect predations, can stimulate the plant's production of protective chemicals. Factors such as these should be considered in the development of a standardized product, and controls implemented as appropriate.

Good agricultural and wildcrafting practices designed to minimize undesirable impurities in the botanical material should be strictly followed. These include the avoidance of growing sites contaminated by heavy metals, radioactivity, pesticide residues, and other toxins at unacceptable levels. Local air quality with respect to airborne contaminants should be considered. Any manure applied should be thoroughly composted to avoid contamination with pathogens. Irrigation water should meet appropriate water quality standards and be as free as possible of toxic contaminants and pathogens. If precautions such as these are not taken, the resulting botanical material may be too contaminated to be suitable for use.[7]

A variety of federal and state governments have established criteria for the certification of "organic" botanical materials, which are grown and processed using natural techniques to maximize plant health and minimize chemical contaminants.[8] The use of certified organic materials may offer significant quality and marketing advantages to the manufacturer.

iv. **Harvest and Post-Harvest Practices.** Harvesting and post-harvesting practices are particularly critical. It is important that the plant be collected at

7 *The manufacturer must determine the acceptable levels of individual contaminants on a product-by-product basis; see section 5(f)(v) below.*

8 *In Europe these practices are known as "biological cultivation."*

the appropriate stage of development and under the proper conditions, which may include the proper time of year and sometimes even the optimal time of day. Harvesting may also need to occur under dry conditions, since wet soil, dew, rain, or high humidity may promote the growth of microorganisms; if harvest is performed under wet conditions, extra care should be taken to minimize this problem.

After harvest, plants should be properly cleaned to remove physical contaminants and extraneous matter, and in most cases should be dried promptly. Drying is often a critical step; delayed or incomplete drying may allow significant microbial or enzymatic degradation. On the other hand, drying at too high a temperature may also cause degradation. Drying methods should be described (e.g., sun drying vs. oven drying) and, if appropriate, drying conditions should be optimized and/or monitored with respect to temperature, humidity, light intensity, air flow, time, and final moisture content. Material to be used fresh must usually progress immediately to the next stage of processing.

In some instances, special preliminary processing steps, such as aging or steaming, are traditionally used for the plant, in order to remove toxins, enhance or reduce the plant's potency, or otherwise alter its biochemical effect. Examples of these processes include the aging of cascara bark to moderate its cathartic effect, the prolonged boiling of aconite to reduce toxicity, and the prolonged soaking of cassava to remove cyanogenic glycosides. Other traditional practices include steaming, curing, washing, pickling, and stir-frying with water, wine, honey, or other ingredients.

v. **Storage Conditions.** Proper storage is essential to preserve the quality of the herb; the material should be protected from heat, moisture, dirt, insect or rodent infestations, and sometimes light. In general, the quality of the material should be maintained through proper storage conditions rather than the application of chemical fumigants or irradiation.[9] Where chemical fumigation or irradiation is used, such practices must comply with national usage and application requirements and should be disclosed to any purchasers of the bulk herbal material.

vi. **Comparison of Sourcing Strategies.** Different manufacturers adopt different strategies with respect to sourcing their botanical materials. These decisions are influenced by a variety of factors, including the sourcing options available in the marketplace (e.g., whether cultivated or wildcrafted supplies are available) and the particular characteristics desired in the raw material and/or finished product (e.g., content of particular constituents, absence of particular toxins, organoleptic qualities such as flavor or color, various physical characteristics, etc.).

A wide variety of botanicals have been brought under cultivation, but many botanicals are still available only in wildcrafted form. The lack of cultivated supplies may be due to limited demand for a particular botanical; the

9 See section 5(f)(ii) below.

abundance of the botanical in the wild making cultivation unnecessary; or to practical difficulties experienced in growing the plant outside its natural ecological niche. Conversely, the development of cultivated supplies may be stimulated by real or perceived diminishment of the plant's abundance in the wild; by the desire to ensure a reliable and consistent supply at low or at least predictable prices; or by the desire to develop or maintain unique cultivars.

The manufacturer must decide, often on a product-by-product basis, the best way to obtain the needed materials. Sometimes companies opt for a high degree of vertical integration, controlling the raw material supply from growth of the plants through harvest to final processing. This is often motivated by the desire to control particular aspects of the cultivation and/or harvesting that the company considers critical to the consistency or quality of their product. The critical factors may be the location or conditions in which plants are grown or cultivated; the particular subspecies, variety, or cultivar used; details related to the time or manner of harvest; or the necessity of limiting the time between harvest and further processing, among others.

In other circumstances, companies opt to source their materials on the world market, purchasing the botanical material in whole,[10] dried form from a variety of brokers. This system is used where the manufacturer has determined that material of an acceptable quality and consistency is readily available, obviating the need for tighter control of the supply chain. This system also allows the manufacturer to moderate variability by mixing lots from various sources, and to compensate for local supply difficulties caused by extremes of weather or other factors. However, purchase on the world market often necessitates the use of extensive and discerning examinations and/or tests of all purchased material, to ensure the identity, purity, quality, and consistency of the materials.

b. Process Control

i. **Formula.** The materials to be used in the manufacture of a standardized product must be carefully defined. This includes the botanical material itself, as discussed above. It also includes any extraction solvents to be used, since these will affect the range of native constituents obtained, the total amount of extractives, and the activity of enzymes in the extract. Solvents may also reduce the microbial load in the material or product. Finally, any excipients must be appropriately specified; these include carriers, fillers, flow agents, stabilizers, preservatives, and other ingredients that serve a technical purpose in the product.

The formula must specify not only the nature but also the amount of each ingredient. It may be necessary to specify a range of quantities rather than a unique quantity, to allow the manufacturer to compensate for natural

10 Materials sourced on the world market are often purchased in whole form to allow proper identification of the botanical material, to minimize the chance of economic adulteration, and to ensure the freshness and quality of the material. Once the botanical is reduced to a powder it can become difficult or impossible to identify the species, and the degradation of constituents may accelerate. Furthermore, the grinding of materials may be used to hide the presence of extraneous materials or plant parts, fillers, fungal contamination, etc. On the other hand, within some markets it is common for materials to be traded in powdered form; such commerce requires proper controls over the supply chain to ensure the integrity of the materials.

variability in the botanical material. However, where ingredient quantities are allowed to vary, the range must be defined in advance and should be kept as narrow as possible. This is particularly true for excipient quantities in extracts where the active constituents are not definitively known.[11] In such cases, the entire native extract[12] is considered responsible for the physiologic effect of the preparation, so the dosage of native extract must be controlled carefully in a standardized product.

ii. **Processes.** The manufacturing process must be defined with an appropriate level of detail. Each step in the process must be identified, and critical parameters for each step must be established. The following are some typical steps:

1. **Milling.** Crude botanical materials, extracts in powdered form, and other materials may need to be milled. Milling reduces the material to the desired particle size, which should be clearly specified. Particle size can impact characteristics such as extraction efficiency, blend uniformity, and raw material and product shelf life, among others. Finely milled materials may be especially unstable due to their high surface area, which increases exposure of the native constituents to air.

In addition to the particle size, the type of mill, speed, screen size, use of cryogenic or other cooling systems, etc. should be carefully defined. The most suitable milling method depends upon the hardness of the material, the particle size desired, and the stability of the material's constituents. Botanicals should usually be milled gently, especially those containing important volatile or labile components, since elevated temperatures can significantly degrade the material.

2. **Extraction.** To produce a consistent extract, the type of extraction process must be specified and important process variables controlled. Some common extraction processes include infusion, decoction, maceration, and percolation. The type of extraction process affects the chemical mixture that results, as do process variables such as the particle size of the botanical material, the extraction solvent(s), the quantities of botanical material and solvent(s) or the ratio between them, and parameters such as times, temperatures, and pressures.

Subsequent processing of the extract begins with the physical separation of the herb residue (marc) from the extract. It may also include removal of the extraction solvent, removal of other undesirable constituents or contaminants,[13] or treatments such as pasteurization to reduce the microbiological load. Excipients may be added in accordance with the formula, and in some cases the extract is dried into a powder. In each case, the appropriate combination of process specifications must be customized by the manufacturer, depending on the plant and plant part, type of extraction, and the manufacturer's goals for the product.

11 See section 7(c) for further information about physiologically active vs. inactive constituents.

12 In this context the term "native extract" refers to material consisting only of components native to the original plant or naturally formed during extraction, excluding any excipients or other added substances. See the glossary for further information.

13 See sections 5(f)(v), 7, and 8 for further information.

3. **Blending.** Blending is necessary whenever a product is made using more than one ingredient or lot of ingredients. Such circumstances can include the addition of excipients to an extract or other product; the blending of raw material or extract lots; and the blending of compound formulas.[14] Mixing steps, especially where dry powders are blended, should be controlled to ensure uniformity of the resulting mixture. Attention must be paid to the blending time, equipment speed, container geometry, powder density, and particle size, as applicable for the product. In some cases, quantitation of marker compounds can be used to verify blend uniformity.

   Whenever materials are blended together, consideration must be given to possible interactions between the materials, which may either enhance or diminish the preparation's desired effect. For example, lecithin can increase the bioavailability of ingredients such as grape seed extract. On the other hand, exposure of garlic to water can cause the premature conversion of alliin to allicin. Such potential interactions must be considered when developing the product.

4. **Unit Dosing.** Unit dosing is important whenever portions of material are filled into individual units such as bottles, tablets, or capsules. In such cases, care must be taken to ensure each dose delivers the expected quantity of the botanical. The content uniformity of the dosages may be measured directly using markers or, for single-ingredient products, by testing the unit weight of the dosages. For multi-ingredient products, the dosage weight variation provides an indication of content uniformity only if the material has a uniform composition before unit dosing.

   The quantity of the dose should be sufficient to produce the desired physiological effect without engendering safety concerns. Furthermore, the form of the dose (liquid, capsule, tablet, etc.) should be chosen appropriately with respect to delivery of the material to the desired portion of the gastrointestinal tract and/or with respect to the necessary solubilization of components. The type of dosage form can significantly influence the preparation's safety or efficacy.

5. **Packaging and Storage.** Packaging and storage conditions must be chosen to maintain the product's integrity and quality throughout its intended shelf life. Many botanicals require protection from light, oxygen, moisture, and/or heat. Protection against the first three is often achieved through the use of impervious packaging containers, such as tinted glass bottles or foil bags. Protection against heat is usually provided through the use of a temperature-controlled storage environment. Desiccants, oxygen-absorbing packets, or insulated packaging are other options. Ideally, manufacturers should verify the adequacy of their packaging and storage conditions through stability tests examining the product's physical, chemical, and/or microbiological characteristics throughout its shelf life.

14 A compound formula is one containing more than one botanical species or plant part.

5. EXAMINATIONS, TESTS AND OTHER DATA

a. Organoleptic Examinations

Organoleptic examinations of raw materials, in process materials and finished products, are an excellent analytical tool when used by knowledgeable personnel. Whereas chromatography and other, more technological analytical methods are limited to examining a few constituents or groups of constituents, organoleptic examinations of the material's color, odor, and taste provide a way to evaluate a material's overall quality and chemical composition, and can often detect nuances that would be missed in quantitative analyses. For this reason, the acknowledged standard for evaluation of products such as fine wines, olive oil, and flavor extracts remains the organoleptic test. Other botanical preparations can benefit from this procedure as well.

Organoleptic testing requires that analysts have personal experience in the evaluation of each botanical, because the complexities of sensory data are difficult to convey in written or verbal form. An informed evaluation can be based on knowledge gained via expert tutelage, or on comparison of the sample to an authenticated specimen of the desired quality. For record keeping purposes, evaluators should record descriptive data (e.g., bitter, minty, astringent, aromatic, strong) about each sample. It may also be useful to control the environment in which the evaluation is performed (with respect to odors, lighting, noise, etc.), to have samples evaluated by more than one analyst or on repeated occasions, and to instruct evaluators to excuse themselves from testing if they are sick or distracted.

b. Macroscopic and Microscopic Examinations

Visual examinations of a material's macroscopic and microscopic characteristics can provide confirmation of the plant part, and often the genus or species. Materials should also be checked for general quality characteristics such as the color and the presence of physical contaminants or adulterants.

Macroscopic examinations of gross plant morphology are the primary tool by which botanists establish the identity of a plant species, and therefore form a crucial step in proper raw material sourcing. However, macroscopic examinations are possible only while the material remains in whole form or relatively large pieces. Furthermore, positive identification may require observation of the plant *in situ* at one or more particular stages of development, which may or may not be the optimal stage for harvest of the material; therefore, post-harvest material may or may not display the characteristics necessary for positive identification. Where possible, processors should maintain a system of authenticated voucher specimens combined with detailed record keeping to demonstrate the proper identification of materials.

Microscopic examinations, unlike macroscopic examinations, can be used not only for whole materials but also for cut or ground materials. Microscopy alone usually cannot provide positive identification, but it can provide supporting evidence since many microscopic features are diagnostic of plant identity.

Microscopy using polarized light or specialized stains can be especially powerful in confirming identity and detecting adulteration. Microscopic examinations can sometimes also be applied to powdered extracts to discover the presence of undisclosed fillers, added purified chemicals, dirt, metal, mold, or other undesirable components.

Macroscopic and microscopic examinations of materials in trade require the analyst to recognize numerous botanical structures and attributes. Samples can be evaluated based on a variety of information, including personal experience and training; drawings or photographs published in authoritative references; and comparisons to authenticated specimens.

c. Fingerprinting

A chromatographic[15] "fingerprint" is a visual pattern of peaks or bands that results from examining a material using techniques such as HPLC, GC, or TLC.[16] Chromatography separates compounds in the sample from one another so that, ideally, each can be detected individually. Chromatographic fingerprints typically include up to several dozen bands or peaks.[17] While this may represent only a small fraction of the compounds in the botanical, it is nevertheless a useful diagnostic tool for characterizing the material.

Fingerprints are commonly used to confirm identity; each botanical material gives a characteristic fingerprint under defined test conditions, so conformance to the expected fingerprint provides evidence of proper identification. The expected fingerprint can be based either on the simultaneous analysis of an authenticated reference material, or on descriptions of the characteristic fingerprint published in pharmacopoeia or other authoritative sources. Fingerprinting alone is not a foolproof means of identification, because sometimes a group of botanical materials display very similar fingerprints. Furthermore, a single botanical material can give somewhat different fingerprints depending on growing conditions or other factors.[18] Nevertheless, a properly optimized and well-documented method can often distinguish not only one species from another, but also different plant parts within the same species, different drying or processing conditions, and other variations.

It can be useful to examine the intensity of various bands or peaks in the fingerprint. The relative levels of key constituents can then be determined. Information of this type can sometimes distinguish subtle differences between botanical materials. For example, the ratio of R_g1 to R_b1 ginsenosides in ginseng

15 Chromatography involves first the separation and then the detection of the compounds present in a mixture. Common detection methods include a variety of spectroscopic techniques; for the purpose of fingerprinting, detection methods that provide as much information as possible, such as photo diode array detectors, are usually preferable. Spectroscopic techniques used by themselves, such as near infrared spectroscopy, Fourier-transform infrared spectroscopy, and ultraviolet-visible spectroscopy, can also produce visual patterns that are to some extent characteristic of the material. However, such patterns are typically less specific than chromatographic fingerprints because no separation of compounds has taken place.

16 HPLC: High performance liquid chromatography; GC: gas chromatography; TLC: thin layer chromatography.

17 The number and resolution of the peaks or bands depends on the particular techniques used for sample preparation and chromatography. Full separation of the compounds in a fingerprint can require extensive optimization of the analytical method and may not be necessary or desirable. Test methods often achieve only partial separation, so that each peak or band actually represents several compounds. The compounds observed may be known markers or may remain unidentified.

18 Therefore, to ensure proper identification multiple types of information should be used, including gross morphology, microscopic morphology, agricultural or wildcrafting records, etc., as available.

roots may be used to distinguish between preparations made from different species of ginseng, different parts of the plant, and different ages of roots.

Fingerprinting can be used for a variety of purposes in addition to confirming the identity of raw materials and finished products. For example, it can be used in process development to quickly evaluate the effect of various experimental process variations. It can also be used as a process control, with the fingerprint verified at key junctures in the manufacturing process. Fingerprints can help monitor the stability of various materials and products, by watching for the disappearance of expected bands, appearance of unexpected bands, or changes in the color or intensity of bands. In the latter fashion, fingerprinting can also be used to determine the adequacy of various packaging regimes or to evaluate the sensitivity of the material to various types of stress (light, moisture, heat, oxygen).

d. Quantitative Analysis of Compounds

Quantitative data about particular constituents in raw materials, in-process materials, and finished products is often used as a process control.[19] In cases where a preparation's active compound has been positively identified, this may also provide the basis for batch adjustments.[20] Quantitative data is often used as a shorthand notation to communicate to external parties the standardized nature of a preparation; however, it must be remembered that such data represent only a fraction of the effort invested in producing a properly standardized product.

To communicate accurately with external parties, it is important that quantitative test methods be specific as to the compound(s) measured and well-validated as to performance. In general, chromatographic methods give more accurate results than less-selective methods, such as titrations or UV-visible spectroscopy. Test methods also must be suitable for the matrix in which the marker occurs. For example, different sample preparation methods may be necessary for the analysis of a crude botanical vs. an extract. Analyses of mixtures of botanicals or vitamins in combination with botanicals can be particularly difficult. The need to optimize methods for different matrices may mean that manufacturers cannot rely solely on public methods from authoritative sources such as AHP, AOAC, INA, or USP.[21] Nevertheless, it is preferable to use such authoritative methods as the starting point for method optimization rather than proprietary methods.

For internal process control purposes, the requirements for test methods are less stringent. An extremely high degree of accuracy may not be required for in-process or batch-to-batch monitoring. Methods which are relatively non-specific or which suffer from poor recovery may be acceptable for these purposes. However, the precision of the method remains critical, since it is impossible to judge the reproducibility of a manufacturing process using an analytical method which is not itself reproducible.

Whenever quantitative representations about a product are made to external parties, the test method on which those representations are based should be

19 See section 3(c) above and section 8(a) below.
20 See section 8(b) below.
21 AHP: American Herbal Pharmacopoeia; AOAC: AOAC International; INA: Institute for Nutraceutical Advancement; USP: United States Pharmacopeia.

disclosed. Otherwise, it is difficult to interpret the results, since some methods will consistently yield higher or lower results than others. The details of any proprietary method should be made available, and ideally the manufacturer should be able to characterize the extent to which results obtained with the proprietary method will agree or differ from results obtained with publicly validated or compendial methods.

e. Bioassays

In some cases, it is possible to devise a relatively simple test to gauge the physiological activity of a product *in vivo* or *in vitro.* A classic *in vivo* test evaluates the strength of digitalis preparations by observing its effect in pigeons.[22] More commonly, botanicals are tested *in vitro* by examining the product's effect on cellular metabolism, gene expression, the uptake or release of particular chemicals, binding to or activation of particular receptors, or the behavior or survival of cells or tissues under defined conditions.

Bioassays are an increasingly popular quality control tool because they provide a measure of the botanical's activity without requiring any of the plant's "active compounds" to be identified. However, bioassay data cannot be relied upon without careful scrutiny of the method used and consideration of possible confounding factors. Bioassays are commonly devised based on what is assumed – but usually not proven – to be a botanical material's mechanism of action. Furthermore, many herbs exert their effect through more than one mechanism involving more than one physiologic pathway. Factors such as gastrointestinal solubility, bioavailability, transformations in the gut caused by enzymes or microflora, and other physiologic parameters can also be critical to the proper activity. Consequently, there may be no direct correlation between *in vitro* activity and *in vivo* potency. It is preferable for bioassays to be validated against clinical endpoints, but the expense for this process can be prohibitive, and even a validated bioassay cannot fully replace clinical trials.

f. Purity

The purity of botanical material can affect the safety or efficacy of the preparation, and impurities present at excessive or toxic levels can render the material adulterated. There are several types of impurities to be considered during standardization.

i. **Physical.** Contaminants such as insects, filth, dirt, debris, foreign species, and unintended plant parts will, at a minimum, dilute the material and thereby reduce its effect. Contaminants such as foreign plants may also exert their own physiologic action; they may act with or against the primary botanical material, thereby altering its effect in significant ways. Some foreign species may be toxic. Foreign matter of all types should be reduced to an appropriate or safe level before proceeding, which may be accomplished by sifting or by handpicking through shipments of raw materials.

22 This is not intended to endorse the use of such tests. Many AHPA members eschew the use of animal testing.

ii. **Microbiological.** High levels of microbes may be unacceptable in the material, since actively growing bacteria or fungi will degrade the plant's constituents. Pathogens must be absent, especially those that produce toxins such as aflatoxins or enterotoxins.

However, it is normal and not necessarily undesirable for a botanical material to contain significant levels of viable microorganisms.[23] Microbes are a natural part of the environment and the food supply; they perform beneficial functions in the soil, in the plant, and in the intestinal tract. Once a botanical material is dried, the low water content discourages microbial growth and consequent deterioration in quality, at least so long as warm, moist storage conditions are avoided. Even in fresh materials, where microbial growth may proceed rapidly, the presence of microbes is not necessarily problematic so long as the material is moved to the next process step (e.g., pressing or extraction) in a timely manner.[24]

Extremely low levels of microorganisms may indicate the material was sterilized with irradiation or chemicals such as ethylene oxide. These practices are, at best, of questionable value in the production of botanical products. They are often used to compensate for poor handling, drying, and storage practices and can therefore conceal significant quality concerns. For example, there may be microbially-produced toxins remaining in the material after the microbes are killed. Sterilants can also destroy labile constituents, degrade organoleptic qualities such as flavor or color, and leave behind toxic or reactive residues. Therefore, the indiscriminate use of these sterilants should be avoided.

Rather than irradiation or chemical sterilization, levels of microorganisms should be controlled through use of good agricultural practices, such as thorough composting of manure or the spraying of crops with solutions of innocuous bacterial strains, both of which promote the competitive growth of harmless bacteria at the expense of pathogens. Proper harvesting and cleaning followed by prompt and thorough dehydration of the material will also limit microbial levels. Common subsequent steps such as extraction using organic solvents (e.g., ethanol) or boiling in hot water will reduce the microbial loadfurther.

Should additional sanitization be desirable, techniques such as steam sterilization (for crude botanical materials) or ultra-high temperature treatment (for extracts) are generally preferable to irradiation or chemical sterilization processes. However, these must also be used cautiously, otherwise significant degradation of plant constituents can occur.

iii. **Exogenous Chemicals.** Many types of pesticides and heavy metals are toxins or carcinogens whose ingestion should always be minimized. Other possible contaminants include aflatoxins, radioactive substances, solvent residues, and residues from fumigants such as ethylene oxide.

23 Fresh and dried agricultural products commonly contain anywhere from hundreds of thousands to many millions of colony forming units (cfu) per gram without presenting a health risk.

24 "A timely manner" may be on the order of days or even hours, depending on the botanical and the desired product.

iv. **Endogenous Chemicals.** Naturally occurring plant toxins must also be considered; the levels of these can vary between or even within species. Variations can be due to differences in the variety or genotype, or to external factors such as growing location, weather, and agricultural practices. Biotic interactions can also cause increased production of natural defensive chemicals, as the plant strives to protect itself from competitors, microbes, insects, and herbivores. In some cases, it is the active constituents themselves which have the potential for toxicity if consumed in too high or too frequent a dose. In other cases the toxins are unrelated to the desirable effects of the botanical, and can be removed if technology permits. In either case, attention to the levels of such compounds can be important.[25]

v. **Acceptable Levels.** The acceptable level of any particular contaminant or impurity must be determined on a case-by-case basis by the manufacturer. Specific upper limits often depend on the national, compendial, or regulatory standards with which the manufacturer wishes to comply. They also depend on what, if any, processing will occur before the material is ingested by the consumer. Many common processing steps provide significant decontamination, such as boiling to kill bacteria. Similarly, the levels of pesticides, heavy metals, and other undesirable chemicals can be reduced or eliminated during a well-designed extraction process.

However, the reverse is also true: Chemical contaminants can be greatly concentrated during extraction, especially if the initial liquid extract is subsequently dried into a powder. As a result, it is important for extract manufacturers to give careful consideration to the effect of their process on any contaminants that may be present. Some extract manufacturers validate their process to determine the degree of decontamination or concentration it produces, and use the resulting information to establish limits for impurities in the raw materials. This must usually be done on a case-by-case basis for each botanical and each extraction process. Each manufacturer must establish purity specifications for their raw materials based on their particular manufacturing process.

The acceptable level of any particular toxin, impurity, or contaminant in a finished product can also depend on the dose, the target consumer, and the intended distribution channels for the product. Ingredients or preparations used in large or frequent doses may require more stringent standards than those whose use is minor. Products intended for use in sensitive populations such as children, the elderly, or seriously ill patients may also require extra caution. And finally, some preparations can safely be distributed only through the hands of knowledgeable practitioners, who will ensure safety either by limiting the dose, combining the botanical with other materials to mitigate its effect, and/or by monitoring the patient's tolerance for the preparation.

25 One situation where endogenous toxins can cause problems is in the standardization of botanical drugs intended for clinical trials. Variations in the type and quantity of toxin may correlate to variations in serum liver enzymes that will be detected during monitoring for these enzymes.

g. Other Data

Several other types of information can also play a useful role in standardization.

1. **Ash.** The ash in crude botanical material is determined by heating the material at a temperature sufficiently high to vaporize organic compounds. The ash left behind is a mixture of minerals, which should represent the native mineral content of the botanical. The ash can be further analyzed to determine the levels of acid-insoluble ash and water-soluble ash. Ash levels higher than expected may indicate an excessive amount of dirt or other debris in the material.

ii. **Extractives.** The amount of material that can be extracted from the botanical using various solvents is an important piece of information for process development and routine raw material sourcing. During product development, a variety of extraction solvents may be tested to determine which solvents solubilize which components. It can also be determined which solvent extracts the largest amount of extractives from the botanical. The latter can be determined by drying and weighing a series of experimental extracts and calculating the yields. This process also provides the manufacturer with information concerning the expected concentration ratio of finished powdered extracts. For example, if a particular solvent system yields extractives representing approximately 20% of the original material, the manufacturer knows the finished native extract will have a ratio of approximately 5:1.[26] Thereafter, to maintain a similar final ratio, the amount of extractives in each raw material lot needs to be controlled within appropriate ranges.[27]

iii. **Genetic Analyses.** Genetic analyses such as those using PCR are finding increasing application in the analysis of botanical materials.[28] So long as sufficient DNA is present in the material, PCR can identify the species and even the variety, cultivar, or genotype. Other genetic techniques can examine gene expression or the resulting protein production, thus providing another useful bioassay for the evaluation of materials.

6. Documentation and Records

Careful documentation and record-keeping form an integral part of the standardization process. Records routinely kept may include agricultural or wildcrafting records, manufacturing and packaging records, sampling and testing records, product specifications, and certificates of analysis. Other significant work, such as product development studies, process validation studies, stability studies, method validation studies, toxicological studies, and clinical trials or other evaluations of efficacy, should also be thoroughly documented.

26 See the glossary for a definition of extract ratios.
27 Ratios can also be adjusted within defined limits by the addition of diluents; refer to section 8(b) below.
28 PCR: Polymerase chain reaction.

The extent to which any company's records can be relied upon depends on the quality of its record-keeping practices. Records should be thorough, and data should be recorded concomitantly with the process being documented. Critical data and calculations should be checked and countersigned. Records should be completed in a timely fashion, and reviewed promptly by a responsible individual who will sign for their veracity.

A system of lot or batch numbers should be employed to facilitate cross referencing of finished product and raw material records. Records pertaining to the acquisition, sampling, and testing of raw materials should be completed and approved prior to release of the raw materials into production. And records of the manufacture, sampling, and testing of each production batch should be completed and approved prior to release of the product for sale. Batch-specific record-keeping also allows the company to monitor trends and provides the basis for the establishment of control limits.[29]

Proper documentation preserves an important body of information, experience, and knowledge for future reference. No matter how thoroughly a product or process is investigated, unforeseen questions may arise later. The answers to such questions are more easily researched if detailed records have been kept. Manufacturers report that details such as the grade or even brand of solvent used for analytical testing or the pack size in which particular raw materials are purchased have, in some instances, been the source of one difficulty or another. One cannot predict precisely which information will prove important; therefore, it behooves the manufacturer to maintain records which are as complete as possible concerning all aspects of its operations.

Thorough documentation also provides evidence to external parties of the manufacturer's adherence to good manufacturing practices[30] and commitment to quality. While much of this information will never be made widely available to the public, appropriate records must be available for review by regulatory agencies, auditors, and sometimes customers or business partners.

Although many manufacturing details are considered proprietary and cannot be revealed to the outside world, customers of all types have a right to understand the nature of the products they buy, at least in general terms. Therefore, manufacturers should be ready and willing to provide information for this purpose. This includes, for example, such government-mandated information as the species, plant part, and excipients. It should also include a generic description of any particular controls forming the basis for standardization of the product (such as control of the agricultural or harvesting process, control of marker content, etc.), without revealing confidential details. Finally, where customers have concerns with respect to specific issues (e.g., the presence of allergens or solvent residues; use of organic or Kosher materials; etc.), the manufacturer should provide the relevant information upon request.

29 See section 8(a) below.

30 Good manufacturing practices (GMPs) are regulations promulgated by the Food and Drug Administration. They establish general requirements that manufacturers in various industries must meet to ensure the quality, safety, efficacy, and/or suitability of their products for their intended purpose.

7. TYPES OF PRODUCTS

Botanical products in general, and standardized botanical products in particular, form a diverse and heterogeneous group. To understand the nature of any given product thoroughly, there are at least three characteristics of the preparation that should be considered. The potential importance of these characteristics should not be overlooked.[31]

a. Degree of Purification

Botanically derived preparations can be purified to various extents, forming a continuum from crude plant materials through extracts to isolated chemicals.

At one extreme are products consisting of crude plant material, which may have been physically altered (as by drying or grinding) but not chemically altered; the material's chemical composition comprises the entirety of the plant part. At the other extreme are purified chemicals isolated from the plant.[32] In between these extremes are two general types of extracts: wide spectrum extracts, which comprise a broad range of the constituents native to the plant, and narrow spectrum extracts, which comprise a limited range of constituents native to the plant. Wide spectrum extracts are typically made using relatively non-selective solvents and manufacturing processes.[33] For example, an extract of St. John's wort made by macerating the herb in a mixture of water and ethanol will contain diverse constituents such as sugars, tannins, flavonoids, procyanidins, phloroglucinol derivatives, hypericins, etc. Narrow spectrum extracts, on the other hand, are made using a selective solvent for the initial extraction and/or additional purification steps thereafter, to separate the target constituents from the remainder of the material.[34] For example, milk thistle fruit extract containing ca. 80% silymarin has usually had its silymarin content increased 80-fold over the original plant material. Concomitantly, about 80% of the other native components have been removed.

Wide spectrum extracts that are dried into a powder are usually characterized by relatively low concentration ratios (usually around 5:1 or lower),[35] which are due to high levels of native extractives. For example, if about 25% of the constituents in an herb are extracted into a mixture of water and ethanol, then the extract will have a ratio of approximately 4:1 after drying. Dried narrow spectrum extracts are usually characterized by high extract ratios (usually on the order of 10:1 to 100:1; sometimes as high as 1000:1); for example, the silymarin extract described above must be concentrated roughly 100:1 in order to raise the silymarin content from

31 In a previous publication, "Guidance for Manufacture and Sale of Bulk Botanical Extracts," the Botanical Extracts Committee used terms such as "traditional-style extracts" and "semi-purified extracts" to refer to various kinds of extracts. This terminology is now seen to be an oversimplification that does not adequately represent the diversity of extracts in the marketplace. Section 7.0 of the current document expands upon the previous discussions and clarifies the Committee's current thinking.

32 Isolated chemicals may be botanically derived, as opposed to synthetic, but they are generally considered distinct from botanical extracts.

33 Extracts containing a wide variety of constituents are often called "broad spectrum" extracts. They are also sometimes called "full spectrum" extracts, although this term might more properly be reserved for extracts comprising virtually 100% of the soluble components of the herb, which can be made (for example) by extracting the botanical repeatedly with diverse solvents and combining the resulting fractions.

34 Narrow spectrum extracts made using a selective solvent are often called "selective" extracts. Narrow spectrum extracts made by purifying an initially broader spectrum extract are often called "semi-purified" extracts.

35 See the glossary for an explanation of solvent ratios. In contrast to dried extracts, for liquid products the extract ratio never correlates to whether the product is wide or narrow spectrum; both very low dilution ratios (e.g., 1:2) and very high dilution ratios (e.g., 1:1000) are equally possible.

l% in the crude herb to 80% in the finished extract.[36, 37] However, not all narrow spectrum extracts exhibit a high ratio; some are manufactured to a low ratio by diluting a highly concentrated intermediate extract with inert materials, such as if a 100:1 milk thistle extract were diluted with filler to a ratio of 4:1. In such preparations, a narrow range of native constituents is present even though the extract ratio is low.[38]

The various types of preparations falling in between crude plant material and isolated chemicals cannot be organized into true categories; there are too many gray areas in between. For example, it would be difficult to say whether modern ginkgo leaf extracts should be characterized as "wide spectrum" (since they contain roughly 24% flavonol glycosides, 6% terpene lactones, and 70% miscellaneous other constituents) or "narrow spectrum" (since they are concentrated roughly 50:1 and are manufactured using a variety of selective solvents and purification processes). These products are clearly not conventional broad spectrum extracts, but neither are they as highly concentrated as other semi-purified extracts. They fall somewhere in the middle of the spectrum. This is just one example of the diverse possibilities that defy easy categorization.

b. Degree of Novelty or Uniqueness

Botanical preparations range from those with a long history of use ("historical preparations"), sometimes going back thousands of years, to those which are very recent inventions ("novel preparations"), along with every possible time frame in between.

Historical preparations generally represent the cumulative experience of a culture; centuries of experimentation with botanicals reveals those which have beneficial properties and a range of conditions under which each can be safely and efficaciously used. In many cultures, this knowledge is still transmitted orally; in others, it has been recorded in written forms such as pharmacopoeial monographs. Common historical preparations include the crude plant material in fresh or dried form, juices, and extracts made with a variety of food-grade solvents (water, ethanol, vinegar, glycerin, oil). Extractions, where performed, use straightforward techniques such as maceration or percolation. There are usually no post-extraction steps that alter the chemical composition of the native extract after the initial extraction, although solvents may be removed and/or excipients may be added. For some botanicals, special procedures for cultivation, harvest, removal of toxins, or other purposes may have also been developed.

In modern times, a wide variety of novel preparations[39] have come into the marketplace. These may be made, for example, with a new cultivar of the plant, a plant part different from the usual one, or an extraction process that differs significantly from what was historically used. Such extraction processes may

36 The concentration ratio would theoretically be 80:1, but since the extraction yield is always less than 100% the actual ratio must be somewhat higher.

37 The preceding discussions assume the finished extract consists of 100% native extract, with no added excipients. If excipients are added the concentration ratio of the finished extract will be lowered.

38 In order to compare dried extracts accurately, it can be useful to consider the plant-to-native extract ratio as well as the plant-to-finished extract ratio, so that the effect of any excipients can be seen.

39 In Europe, novel extracts are often called "special" extracts to differentiate them from the corresponding generic, pharmacopoeial preparations of the botanical.

include special purification steps (e.g., the use of selective solvents, preparative chromatography, or precipitation) to remove constituents the manufacturer considers undesirable, or to concentrate constituents the manufacturer considers desirable. The creation of novel preparations may be driven by scientific information or technical concerns (e.g., the desire to elevate levels of a pharmacologically active compound, to enhance product safety by removing a known toxin, or to improve product stability by denaturing destructive enzymes), or by marketing or legal issues (e.g., the desire to differentiate a product in the marketplace or achieve patent protection).

Information concerning historical preparations, especially those that have been documented in written form, is available in the public domain. Furthermore, historical preparations can be made using technologies that have been readily available for centuries. Therefore, historical preparations are commonly made by a variety of different manufacturers in more or less similar forms, all of which share a similar common heritage. In contrast, detailed information concerning novel preparations is usually kept proprietary, and such preparations are made with materials and/or processes unique to each manufacturer. Therefore, each such preparation must be considered unique. This is true even when different proprietary extracts are manufactured to the same marker content; although the content of the marker is the same from one manufacturer to the next (assuming each manufacturer uses the same test method), the remainder of the native extract will usually vary due to differences in the manufacturing processes.

The importance of these distinctions becomes apparent when attempting to compare the safety and/or efficacy of one preparation to another. The extent to which two products will exhibit a similar physiologic effect depends largely on the extent to which they share a common chemical composition; and the extent to which they share a common chemical composition depends on the similarities or differences in the materials and manufacturing processes used. The more closely a preparation resembles those traditionally used, the more likely it will share the safety and efficacy of the historical preparations. Conversely, the more a preparation differs from those historically used, the more its safety and efficacy may need to be individually established.

c. Relation of Particular Constituents to the Activity of a Botanical

A small proportion of the botanicals in the marketplace has been studied to identify the physiologically active constituents. These efforts have experienced varying degrees of success. In some cases, a clear picture of the active compound has emerged; frequently the data suggests that multiple compounds or types of compounds contribute to the activity. In many cases, the identity of the active compounds remains completely unknown. Understanding the state of the science in relation to individual constituents can be crucial to evaluating the quality of any given preparation. In general, constituents can be divided into three main categories, although the placement of any particular compound into any particular category may be controversial and may change over time as new data emerge.

i. Active Compounds. Active compounds are compounds or classes of compounds that have been tested at similar levels both in isolation and as part of a botanical preparation, and have been proven to exhibit similar therapeutic activity in both cases. Such compounds also exhibit a dose-dependent response. Once this has been definitively proven (a process requiring extensive research), the observed activity of the botanical preparation can be attributed wholly to that compound or class of compounds. In such cases, the composition of the remainder of the preparation has a reduced significance, at least for the biological activity in question.[40] In addition to their direct correlation with physiologic activity, active compounds can also be used for the same technical purposes as marker compounds (see iii below). An example of compounds in this category are sennosides in senna.

ii Co-Active Compounds. Co-Active compounds are compounds or classes of compounds which have been shown to be biochemically active, either *in vivo* or *in vitro*, but which have not been scientifically proven to exhibit the same activity both in isolation and as part of a botanical preparation. In other words, if the compounds are tested both in isolation and as part of the corresponding botanical preparation, the isolated compounds are expected to exhibit less activity than those in their natural matrix. In such cases the activity of the botanical preparation cannot be attributed solely to that compound or class of compounds. (The name "co-active" derives from the fact that two or more types of compounds work together to produce the effect.) The composition of the remainder of the preparation remains highly significant, and the native extract in its entirety should properly be considered the "active component." In addition to their partial correlation with physiologic activity, co-actives can also be used for the same technical purposes as marker compounds (see iii below). Examples of compounds in this category are hypericin and hyperforin in St. John's wort and salicins in willow.

iii. Marker Compounds. Marker compounds are compounds or classes of compounds used for technical purposes in the manufacturing process. Both biochemically active and inactive compounds may be used as markers, although in the strictest sense, the term "marker compound" refers to those with no relevance to the preparation's efficacy.[41] Markers may be used to confirm identity; monitor stability; validate proper blending; evaluate content uniformity; etc. Marker compounds are also used to demonstrate that a manufacturing process performs reproducibly, (i.e., each batch produced contains marker levels consistent with historical levels). No part of the activity of the botanical preparation can be attributed to compounds that function only as markers. Therefore, the composition of the remainder of the

40 Many botanicals exhibit more than one type of biological activity. St. John's wort, for example, has been studied for both anti-viral and anti-depressant effects. Different aspects of the plant's activity may rely on different chemical constituents in the plant. Therefore, even when research establishes that a single class of constituents is responsible for a particular therapeutic effect, other therapeutic effects may still depend on diverse components of the preparation. Furthermore, the safety profile of an isolated bioactive compound may be different than that of the full spectrum of constituents in the botanical preparation.

41 In the U.S. it is common to refer to all quantified constituents, both active and inactive, as "marker compounds" since the regulatory environment precludes acknowledgement of most therapeutic activities in products other than drugs. In Europe the term "marker compound" is more commonly reserved for compounds that are not therapeutically active.

preparation is of primary importance, and the native extract in its entirety should properly be considered the "active component." An example of compounds in this category is agnuside in chaste tree.

These distinctions must be considered when evaluating the significance of levels of particular components in a preparation. A product deficient in a therapeutically active compound will certainly suffer reduced efficacy, while a product with excessive levels might be toxic. In contrast, higher or lower levels of an inactive marker compound have no direct effect on a product's safety or efficacy.[42]

The quantification of constituents has, in and of itself, caused more confusion in the U.S. marketplace than perhaps any other development in botanical products. The presence or absence of a particular constituent on a label does not necessarily reveal much about the nature, quality, safety, or efficacy of the preparation. Just because a compound is quantified on the label does not mean that compound is essential to the preparation's quality or efficacy; it may be only a marker. Nor does it mean the preparation has been manipulated to elevate or isolate that constituent. In many cases, there is no substantive difference in the composition of one extract that is standardized for particular constituents and another that is not, as long as the raw materials and manufacturing process are similar; the only difference is that one product has been chemically characterized to a greater degree than the other.

Conversely, just because a compound is not quantified on the label does not mean the compound is not in the product; it may only mean the manufacturer did not quantify it, or did not want to list the quantity on the label. Some manufacturers feel there are more important ways to standardize their products than by testing one or a few individual components. Others avoid listing constituents on their labels to avoid confusion of their wide spectrum extract with narrow spectrum, targeted extracts. In such cases, the levels of the compounds may still be quantified for internal, process control purposes. The levels may also be evaluated qualitatively, rather than quantitatively; for example, the presence of hypericins in St. John's wort can be determined visually, and the presence of alkylamides in echinacea can be determined by taste.[43]

Each of these ways of categorizing botanical products is useful in its own way, and should be understood as distinct from the others. Much confusion has occurred by conflating one or more categories together. Each botanical preparation must be evaluated individually with respect to its degree of purification, its degree of novelty, and the chemical basis for its activity, if known; only then can conclusions about its nature, quality, safety, or efficacy, or about its comparability to other preparations, be drawn.

42 The elevation or reduction of marker compound levels may, however, have an indirect effect on product safety or efficacy, to the extent that more important aspects of the preparation are neglected or ignored in favor of focusing excessive attention on the marker.

43 This assumes that the crude botanical was properly identified to begin with, otherwise the presence of undeclared chemical or herbal materials can yield erroneous results.

8. Product Development and Process Validation

There are two distinct approaches that can be taken to the development of a new product. In some cases, manufacturers seek to create products containing the natural or usual level of native constituents. In other cases, manufacturers seek to alter the product, raw materials, or manufacturing process based on the levels of certain constituents. Each of these approaches must be undertaken only in the context of a thorough understanding of the botanical and of pre-existing preparations of the botanical.

a. General Product Development and Process Validation

In order to ensure the safety and efficacy of their products, manufacturers should review the information available and carefully consider what is known about each botanical before devising a new preparation. Particular attention should be paid to the nature of any historically or scientifically proven preparations, any special practices traditionally used, any potential for toxicity, what is known about the chemical basis for activity, and other considerations as appropriate for the particular botanical in question. This type of review will provide valuable information to guide the product development process.

Once the manufacturer has identified the factors to be considered and the goals to be accomplished for the product, process validation of a new standardized product can range from the relatively simple to the extremely complex. Factors influencing the raw material supply and the production process may need to be investigated, as appropriate for the product.

After the basic nature of the raw material supply and manufacturing process is established, comprehensive specifications must be developed for the formula, raw materials, manufacturing process, and finished product. Specifications are often established based on the results of research and development batches. Alternatively, appropriate specifications or control limits[44] for characteristics such as viscosity, color, marker content, or marker recovery can be developed by tracking these parameters over the course of the first few batches manufactured. Specifications or limits for marker or active compounds often establish the minimum content to be expected, but for constituents with possible safety concerns it is prudent to establish a maximum as well.

Studies may also be necessary to determine the preparation's safety and efficacy. Evidence of safety and efficacy may be established in one or more of several ways: (a) on the basis of historical use and experience; (b) on the basis of *in vivo* and *in vitro* studies; (c) on the basis of use in clinical practice, in which patient outcomes can be directly, if anecdotally, correlated to use of the preparation; (d) on the basis of clinical trials. The further a manufacturer intends to deviate from preparations whose safety and efficacy have been previously established, the more important it may be to examine the safety and efficacy of the new preparation.

44 See glossary for a discussion of control limits.

Whenever active, co-active, and/or marker compounds are measured during product development and process validation, each should be chosen appropriately for its intended purpose. For example, blend or content uniformity validations can be performed using any compound that is reproducibly quantifiable in the relevant matrix. On the other hand, to study extraction efficiency or product stability it is usually more pertinent to use active or co-active compounds where such are known. Moreover, for the purposes of safety or efficacy studies, materials should be characterized with respect to their content of known toxins, actives, or co-actives, if such are known.

Bioassays may also be advantageously used during product development and process validation, usually in conjunction with quantification of certain constituents. As with constituent quantification, bioassays must be carefully chosen for their intended purpose; see section 5(e) above.

b. Constituent Quantification Used as a Guide to Product Development

Some manufacturers seek to alter or manipulate the composition of their product based on the content of one or more constituents. In such cases, the quantification of specific constituents is not just a process control providing evidence of batch-to-batch reproducibility, but as a guide for product development. This can be a useful approach, but it must be undertaken with a thorough understanding of the nature of the botanical preparation and the constituents. Manufacturers must use information about individual constituents only in addition to the more general information discussed above, and only in the broader context of controlling the overall composition of the preparation. Manufacturers should avoid altering the content of particular constituents in the preparation, or the proportions between various constituents, without carefully considering the possible impact on safety and efficacy.

In particular, there is the tendency to believe that "more is better" and that therefore higher levels of constituents are beneficial. In fact, this is not always – or even usually – the case. If the constituent in question is merely a marker, with no relevant biochemical activity, then there is obviously no therapeutic benefit in a product with elevated levels of the compound. Even compounds with biochemical activity should generally not be elevated at the expense of other constituents, because those other constituents may have important direct or indirect activities of their own.[45] Furthermore, excessive levels of a constituent, whether on an absolute basis or in proportion to other components, may change the safety profile of the preparation.

The importance of such considerations becomes especially apparent in the manufacture of extracts. For example, there is an inherent economic incentive to reduce the quantity of botanical material used to make an extract. Using selective solvents, purification technologies, and/or new plant varieties containing higher

45 As has been mentioned several times above, the total physiologic effect of a botanical preparation usually depends on the total chemical composition of the preparation. Even components with no direct physiologic effect can influence the pharmacodynamics and pharmacokinetics of other constituents. Therefore the complete native extract is relevant to product quality, not just one or a few constituents. The nature and content of various excipients can also have important effects that must be considered.

levels of selected constituents, it is possible to reduce the amount of crude botanical used during production while maintaining the same content of the target constituents in the finished extract. Such processes result in a product with a reduced content of native extract even though the content of the target constituent is preserved.[46] The resulting extract may appear nominally the same as other extracts of the same species and plant part, but it will have a different native extract content. The product will thus be fundamentally different from the others in ways which may alter its safety or efficacy.

Both the nature and the content of the native extract should be controlled in a standardized product. Where adjustment of the product to a particular level of constituents is desired, this should be accomplished through blending of raw material or extract batches rather than through the addition of fillers, and any excipient ranges specified in the product formula should be appropriately narrow. This will maintain the batch-to-batch reproducibility in the content of native extract. These guidelines may be relaxed in cases where the existence of a sole therapeutic active has been proven. With these considerations in mind, appropriate specifications for the formula and for any blending processes should be developed.

Bioassays are also sometimes used to guide product development and process validation, and to establish parameters for the adjustment of batches to a particular activity. The same concerns apply to this procedure as apply to alteration of the product based on constituent content. In particular, it must be borne in mind that *in vitro* activity may not correlate well with *in vivo* potency, and appropriate caution used.

Constituent quantification can, and frequently does, play a role in both of these approaches. In the one case, constituents are quantified primarily for the sake of process control; the composition of such products remains essentially the same as any comparable product made without quantification of constituents. In the other case, the nature of the raw materials, manufacturing process, and/or finished product are altered based on a heightened interest in a particular constituent. In these cases, the composition of the finished product is to a greater or lesser extent unique.

46 When such extracts are made using selective solvents or post-extraction purification processes, they are typically narrow spectrum extracts with a higher content of fillers than is usual in other preparations of the botanical. When such extracts are made using raw materials whose constituent content is elevated, they are typically wide spectrum extracts with a lower extraction ratio than is usual in other preparations of the botanical. In either case, the content of native constituents other than the target constituent is reduced.

CONCLUSION

Standardization involves a complex set of controls implemented over both raw materials and manufacturing processes in order to enhance the batch-to-batch reproducibility of a botanical product. The analysis of marker or active compounds is often employed as part of the standardization process. Although standardization reduces variability in the chemical composition of the preparation, it is not technically possible to eliminate all variability.

Raw material controls begin with selection of the appropriate botanical material (e.g., genus, species, variety, and plant part). They may also include controls over growth or cultivation conditions, harvesting, drying, storage, and other post-harvest practices. Manufacturing controls require specification of the formula and the nature and content of any excipients. They also include controls over milling, extraction, blending, packaging, and finished product storage, as appropriate for the product.

A wide variety of tests, examinations, and other data contribute to the creation of a standardized product. These can include organoleptic testing, macroscopic and microscopic examinations, fingerprinting, quantitative analyses of marker or active compounds, and bioassays. They may also include controls over a range of impurities, including extraneous matter, bacteria and fungi, contaminants such as heavy metals and pesticides, and naturally occurring toxins. Other parameters, such as the levels of moisture, ash, and extractives, may also require control.

Standardized products can be characterized based on their degree of purification from crude botanical to targeted extract. They can also be characterized based on their degree of novelty or uniqueness. Where particular constituents in the product are quantified, it should be noted whether those constituents are actives, co-actives, or markers. These characteristics must be carefully studied in the evaluation of a product's nature, quality, safety, or efficacy. They must also be considered during the development and validation of a new standardized product.

The term "standardization" has been frequently misunderstood in the U.S. marketplace, insofar as it is often equated with control or isolation of particular constituents. On the contrary, standardization is a complex, multifaceted process that relies primarily on appropriate controls of raw materials and the manufacturing process. Quantitative testing such as bioassays or measurement of specific constituents, may be used in addition to, but never in place of, these other measures, because analytical measurements by themselves can only confirm, not control, batch-to-batch consistency. Therapeutic reproducibility requires standardization of the product's composition as a whole, and this can only be achieved using reproducible raw materials and processes.

Strictly speaking, standardization refers only to enhancement of the preparation's reproducibility. It does not inherently reflect the quality of the preparation; it is equally possible, if not cheaper and easier, to standardize to poor quality than high quality. To avoid this danger, manufacturers must thoroughly understand the nature of the botanical, the nature of any historical or otherwise proven preparations, and the factors affecting their quality, safety, and efficacy.

Appendix I: Glossary

NOTE: Some of the definitions given below differ slightly from those published in previous documents of the Botanical Extracts Committee. They have been revised and updated for clarity, and to reflect current thinking.

Active Compound: A compound, or class of compounds, which has been tested both in isolation and as part of a botanical preparation, and has been proven to exhibit similar therapeutic activity in both cases. Such compounds also exhibit a dose-dependent response.

Active Marker: See Co-active compound.

Aflatoxin: A toxin produced by certain species of fungi.

Broad Spectrum Extract: An extract comprising a wide range of the constituents native to the plant.

> **NOTE:** Development of the AHPA Hemp Lexicon (2021) has added the following sentence to this definition:
>
> Broad spectrum extracts are made using relatively non-selective solvents and manufacturing processes so that both relatively hydrophilic and relatively hydrophobic types of botanical constituents are captured.

Cathartic: Having the effect of purging the lower intestinal tract.

Chromatography: A technique for separating and detecting components from a mixture. Common chromatographic methods include HPLC (high performance liquid chromatography), GC (gas chromatography), and TLC (thin layer chromatography).

Co-Active Compound or Co-Active: A compound, or class of compounds, which has been shown to be biochemically active, either in vivo or in vitro, but which has not been scientifically proven to exhibit the same activity both in isolation and as part of a botanical preparation. In other words, if the compounds are tested both in isolation and as part of the corresponding botanical preparation, the isolated compounds exhibit less activity than those in their natural matrix. These compounds are known as "co-actives" since two or more types of compounds work together to produce the observed activity.

Compound Preparation: A preparation made from more than one species of plant or more than one plant part.

Control Limits: Limits establishing the normal range within which a process is expected to vary. Control limits are typically established based on historical data using more or less sophisticated statistical techniques. Deviations from established control limits indicate a problem in the manufacturing or testing process that should be investigated.

Crude Botanical: Raw plant material that usually has its cellular structures intact. The material may be fresh or dried, and in whole, cut, or powdered form.

Decoction: A liquid extract made by boiling the herb in water.

Enterotoxin: A toxin that attacks the gastrointestinal tract (i.e., stomach, intestines, etc.).

Excipient: Material, such as carriers, flow agents, preservatives, stabilizers, etc. that is added to the extract for technical purposes. Where the extraction solvent remains in the finished extract, it too constitutes an excipient.

Extract: The complex, multicomponent mixture obtained after using a solvent to dissolve components of the botanical material. Extracts may be in dry, liquid, or semi-solid form. Excipients may be added to extracts in order to adjust the concentration; enhance stability; limit microbial growth; and to improve drying, flow, or other manufacturing characteristics. Extracts are not the same as expressed juices, pure chemicals isolated from an herb, or synthetically modified plant constituents.[47]

Extract Ratio: The ratio between the quantity of dried botanical raw material that goes into the extraction process and the quantity of finished extract that comes out of the extraction process. For example, a 4:1 extract is one in which each kilogram (or other unit) of finished extract represents the extractives from four kilograms (or other unit) of dried botanical starting material. For liquid extracts this is usually a dilution ratio (e.g., 1:4) while for powdered extracts it is usually a concentration ratio (e.g., 4:1). The amounts of starting plant material and finished extract must be expressed in the same unit of measure except for liquid extracts, where an alternate notation of grams-to-milliliters (grams of starting material: milliliters of finished extract) is often used. Where fresh rather than dried starting material is used in determining the ratio, this must be disclosed.

Extractives: Soluble components removed from the botanical material during the extraction process.

Full Spectrum Extract: An extract comprising the complete range of soluble constituents native to the plant. (This term is also used, somewhat inaccurately, to refer to broad spectrum extracts.)

NOTE: Development of the AHPA Hemp Lexicon (2021) has amended this definition to the following:

Full Spectrum Extract means an extract that is especially complete, either chemically or botanically. The term is variously applied to products made by repeatedly extracting the same biomass using different solvents ranging from hydrophilic to hydrophobic or polar to nonpolar (thereby obtaining the complete range of soluble constituents native to the plant); extracting multiple parts of the same plant (e.g., extracting root, aerial parts, and flowers); extracting multiple species from the same genus; or by inclusion of crude (i.e., un-extracted) botanical along with the extractives.

Genotype: The genetic information an organism contains.

Hydrolysis: A chemical reaction in which one molecule is broken into two or more fragments by the addition of water.

Infusion: A liquid extract prepared by seeping or soaking the herb in water without boiling.

Juice: The liquid obtained by pressing botanical material without the addition of solvent.

Maceration: An extraction technique in which the botanical material is allowed to soak in the extraction solvent until the cellular structure of the herb is penetrated and the soluble portions are dissolved.

Macroscopic: Visible to the naked eye.

Marc: The spent botanical material that remains after the extraction process.

Marker Compound: A compound, or class of compounds, used for technical purposes in the manufacturing process. Both biochemically active and inactive compounds may be used as markers, although in the strictest sense the term, "marker compound" refers to those with no relevance to the preparation's efficacy.

Microscopic: Not visible without the use of a microscope.

Native Constituent: A compound occurring naturally in the plant.

Native Extract: Material consisting only of components native to the original plant or naturally formed during extraction, excluding any excipients or other added substances. In this document the term refers to an extract or that portion of a finished extract that is comprised solely of native components. (In other contexts the term is also used to refer to a concentrated liquid extract from which the added solvent has been removed.)

Organic Agriculture: Cultivation of crops without the use of chemical fertilizers, pesticides, or herbicides.

Organic Compound: A chemical compound containing carbon. Organic compounds are characteristic of animal and plant materials, as opposed to rocks or minerals.

Organic Solvent: A solvent whose molecular structure includes carbon and hydrogen. Most commonly used solvents, with the exception of water, are organic. Some organic solvents occur naturally (e.g., ethanol), but most are synthetic (e.g., acetone, hexane, methanol).

Organoleptic Testing: Evaluations made using the sense organs (e.g., eyes, nose, tongue).

Percolation: An extraction technique in which the botanical material is exhaustively extracted with fresh solvent until no further soluble components remain.

47 However, it should be noted that some chemical modifications may occur as the natural consequence of the extraction process, for example transesterification, hydrolysis, etc.

Pharmacodynamics: The study of the effects of drugs in the body.

Pharmacokinetics: The study of the absorption, metabolism, and excretion of drugs by the body.

Polymerase Chain Reaction (PCR): A technique for genetic analysis in which a small portion of genetic material is copied numerous times. This technique allows very tiny quantities of genetic material to be accurately analyzed.

Precipitation: A chemical process in which constituents in a solution become insoluble and crystallize into solid form, thereby allowing them to be separated from the solution.

Selective Extract: An extract made using solvents that selectively extract only a narrow range of the native constituents from the plant.

Semi-Purified Extract: An extract containing only a narrow range of the native constituents from the plant, which is made by partially purifying the desired components from an initially broader spectrum extract.

Solvent: The liquid used to extract the botanical material.

Solvent-Solvent Partitioning: A chemical process in which the constituents in a solution are separated based on their solubility in different solvents.

Spectroscopy: A technique for detecting chemicals based on their absorption of light and other electromagnetic radiation. Common spectroscopic methods include IR (infrared) and UV (ultraviolet–visible) spectroscopy.

Transesterification: A chemical transformation in which a type of molecule called an ester is converted into another ester by the addition of an alcohol.

Appendix II: Standardized Extracts in the European Union

The European Pharmacopoeia has recently proposed that extracts be divided into categories with the following terminology: (Note: This is based on a draft monograph (PharmEuropa Vol. 12, No. 4, October 2000)).

1. Type A extracts ("standardized" extracts) are adjusted to a defined range of proven therapeutically active compounds (as defined above in section 7(c)). Standardization is achieved by adjustment of the extract with inert material or by blending extracts.
2. Type B extracts ("quantified" extracts) are adjusted to a defined range of co-active constituents (as defined above in section 7(c)). Adjustment is achieved by blending of raw material or extract lots.
3. Type C extracts are defined essentially by the production process. Constituents considered to be relevant markers may be determined.

The Bundesverband der Arzneimittel-Herstller e.V. (German Medicine Manufacturers Association) has submitted comments suggesting the following:

- That Type A extracts are also known as "*normiert*," "*normatum*," "*eingestellt*," or "*titré*" (also "normalized" in English).
- That Types B and C should be grouped together as B1 and B2 (since they have similar manufacturing and validation concerns).
- That for Type A products, the amount of native extract may vary within a defined range, while for Type B1 and B2 products, the amount of native extract must be held constant.
- That for Type A products, standardization may also be achieved by blending of raw material lots (in addition to the other ways mentioned in the draft monograph).
- That it should be clarified that all types of extracts must be defined by the botanical raw material and the extraction process used (irrespective of whether any constituents are quantified).

Starting Materials of Herbal Origin. London: European Agency for the Evaluation of Medicinal Products, 2002.

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