



# Natural Products

Trade Science Inc.

*An Indian Journal***Review**

NPAIJ, 3(3), 2007 [111-117]

## Chromatographic fingerprint analysis-an approach for herbal medicines

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Received: 30<sup>th</sup> October, 2007 ; Accepted: 4<sup>th</sup> November, 2007

### ABSTRACT

Quality, safety and efficacy of herbal drugs have to be ensured to provide sound scientific footing to enhance consumer confidence and to improve business prospects for herbal medicines globally. Therefore, very efficient and selective methods, including the extraction techniques are required for identification and quantitative analysis of the active compounds or drug standardization. Chromatographic fingerprinting represents main technique applied in this field due to their powerful separation efficiency combined with sensitive detection. © 2007 Trade Science Inc. - INDIA

### KEYWORDS

Herbals;  
Quality;  
Safety;  
Efficacy;  
Fingerprinting.

### INTRODUCTION

Herbal drugs have been used since ancient times as medicines for the treatment of a range of diseases. Medicinal plants have played a key role in world health. In spite of the great advances observed in modern medicine in recent decades, plants still make an important contribution to health care<sup>[1]</sup>. Medicinal herbs are moving from fringe to mainstream use with a greater number of people seeking remedies and health approaches free from side effects caused by synthetic chemicals. Recently, considerable attention has been paid to utilize eco-friendly and biofriendly plant based products for the prevention and cure of different human diseases. Considering the adverse effects of synthetic drugs, the western population is looking for natural remedies, which are safe and effective. It is documented that 80% of the world's population has faith in traditional medicine, particularly plant drugs for their primary healthcare.

India is sitting on a gold mine of well- recorded and traditionally well- practiced knowledge of herbal medicine. This country is perhaps the largest producer of

medicinal herbs and is rightly called the botanical garden of the world<sup>[2]</sup>. Because of poverty and lack of access to modern medicine, about 65-80% of the world's population which lives in developing countries depends essentially on plants for primary health care. Currently, the major pharmaceutical companies have demonstrated renewed interest in investigating higher plants as source for new lead structures and also for the development of standardized phytotherapeutic agents with proven efficacy, safety and quality<sup>[1]</sup>.

With the widespread use of traditional medicine (TM) as well as complementary/alternative medicine (CAM) and the rapid expansion of international herbal medicine markets, the development of national policies and regulations on TM/CAM has become an important concern for both health authorities and the public. National policies and regulations on TM/CAM could ensure the safety, quality and efficacy of herbal therapies and products, and function as important steps towards integrative health- care systems<sup>[3]</sup>.

In order to promote global acceptance of herbal drugs, there is an urgent need to evaluate the therapeutic

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tic potential of the drugs as per regulatory guidelines. Ironically, not many herbal products are available in standardized form, which is the minimum requirement for introducing a product in the global market<sup>[2]</sup>.

The single and most important factor, which stands in the way of wider acceptance of herbal drugs, is the non-availability or inadequacy of standards of checking and ensuring their quality. Quality is wholesome covering statutory requirements, technical experience and market expectations. Technical experience ensures quality standardization, quality production, quality testing, quality monitoring and quality assurance.

It is a misconception that all the naturals are safe as there is a likelihood of innate toxicity, poor quality product, mistaken use of wrong species, adulteration with other medicines and even potent chemical substances. There could be incorrect dosing, interaction with other medicines and even misuse of herbal drugs by the health care providers and consumers<sup>[4]</sup>.

Ayurvedic therapy presents numerous potential safety concerns. One serious problem is that many ayurvedic herbs have never undergone a formal safety evaluation and those that have been evaluated have not necessarily proven harmless.

Botanical and herbal preparations are medicinal preparations, containing a single plant or a mixture of two or more different types of medicinal plants. For botanicals and herbal preparations, there is a need to approach scientific proof and clinical validation with chemical standardization, biological assays, animal models and clinical trials. Quality assurance of botanicals and herbal preparations is the prerequisite of credible clinical trials. According to the draft guidelines stated by the United states food and drug administration and the European agency for the evaluation of medicinal products, various aspects of analysis must be performed for the purpose of certification of botanical drugs and herbal preparations<sup>[5]</sup>. Currently, there is a common practice among natural products analysis to select one or more compounds as either active or markers for purposes of identification and quality assessment. This problem is compounded when one substance that contains a specific class of compounds is combined with others containing the same or different classes of compounds. It is necessary to develop a type of quality assessment system that adequately meets the complex

characteristics of herbals. Chromatographic fingerprint analysis by which multiple compounds in single herbal drugs and finished herbals can be identified represents a rational approach for the quality assessment of herbals.

### Major causes of inconsistency<sup>[2]</sup>

1. Adulteration in market samples due to regional linguistic nomenclature for the plant collectors.
2. Improper harvesting of the plants could also affect the quality. The medicinal properties of plants vary with respect to different seasons.
3. Degradation of medicinally valuable secondary metabolites of stored plant drugs due to fungal infestations has been reported.
4. The post harvesting practices such as the method of drying, garbling, packing and storage conditions influence the quality of raw material.
5. Lack of standardization and quality control of the herbal drugs.

### Challenges

Quality, safety and efficacy of herbal drugs have to be ensured to provide sound scientific footings to enhance consumer confidence and to improve business prospects for herbal medicines in India<sup>[6]</sup>.

The demand for high quality, safe, effective, and clean natural plant products and their formulations with various substances have been growing significantly in the industrial world. In the past, herbs and essential oil bearing plants were largely harvested from the wild and brought to the market without many questions asked about their origin, method of cultivation, botanical identity, purity, safety, and efficacy. With further improvement in communication and education, there has been a growing consciousness in industrialized countries about personal health, environmental safety, sustainable harvesting, and loss of genetic diversity<sup>[7]</sup>.

Compared with well- defined synthetic drugs, herbal medicines exhibit some marked differences, namely<sup>[4]</sup>:

- The active principles are frequently unknown;
- Standardization, stability and quality control are feasible but not easy;
- The availability and quality of raw materials are frequently problematic;
- Well controlled double- blind clinical and toxico-

logical studies to prove their efficacy and safety are rare;

- Empirical use in folk medicine is a very important characteristics;
- They have a wide range of therapeutic use and are suitable for chronic treatments;
- The occurrence of undesirable side effects seems to be less frequent with herbal medicines, but well controlled randomized clinical trials have revealed that they also exist;

Technical experience ensures quality standardization, quality production, quality testing, quality monitoring and quality assurance. The market expectations are very important, more realistic deciding the fate of the product and thus both the product and packaging standards play an important role. Safety is a fundamental principal of herbal medicines in health care and a critical component of their quality management<sup>[8]</sup>.

### Regulatory requirement for quality control of herbals

From the regulatory point of view, uniform criteria regarding the assessment of safety and efficacy do not currently exist.

WHO, in its resolution of WHA42.43<sup>[9]</sup> of 1989, urged member states to introduce measures for the regulation and control of medicinal plant products and for the establishment and maintenance of suitable standards.

WHO<sup>[10]</sup> drafted "Guidelines for the assessment of herbal medicines" in Munich in June 1991, which were adopted for general use by the 6<sup>th</sup> International conference of Drug Regulatory Authorities (ICDRA). The objective of these guidelines was to define criteria for the evaluation of quality, safety, and efficacy of herbal medicines and thereby to assist national regulatory authorities, scientific organization, and manufacturers.

In order to provide scientifically-based assistance for a harmonized assessment of herbal medicinal products, the European Scientific Cooperative on phytotherapy<sup>[11]</sup> (ESCOP) was founded in 1989 by six national scientific associations. The main objectives of this European scientific umbrella organization are to establish harmonized criteria for the assessment of herbal medicinal products, support scientific research, and contribute to the acceptance of phytotherapy on a Eu-

ropean level.

European committee in its directive 2004/24/EC<sup>[12]</sup>, March 31, 2004, describe the tendency around the world is the harmonization of basic concepts concerning safety, firstly, and, later, the efficacy of a plethora of products. The main points under discussion are:

1. The constant quality of botanicals to get a reproducible safety and efficacy profile.
2. The strict standardization of the extracts and their GMP production.
3. As the GMP is implemented, the local differences in manufacturing process will disappear and a uniform quality control will be possible nation wide. The GMP insists that the methods developed by the manufacturer for quality testing as well as those have mentioned in the ancient authoritative books have to be maintained to ensure the quality of the products.
4. Their genic safety and basic toxicity data.
5. Their reasonable tolerability based on safety information and drug interaction studies.

The dietary supplement, health and educational act (DSHEA) of United States<sup>[13]</sup> in its resolution in 1994, requires manufacturers to ensure that products placed on the market are safe.

To summarize the technical requirement for an extract of acceptable quality, we can say that the following points are necessary:

- The plant has to be cultivated according to GAP.
- A chromatographic fingerprint has to be established for vegetal material and extract.
- A quantitative ratio among the selected active principles and markers has to be fixed.
- A spectroscopic technique has to be introduced for the evaluation of unknown substances and stability.
- Solubility and bioavailability of the active principles have to be studied.
- GMP has to be used at production level.

Ministry of health and family welfares, Department of Indian systems of Medicines and Homeopathy<sup>[8]</sup>, in its Gazette under Part II section 3, sub-section iii, published requirement of Good manufacturing practice for traditional medicines to ensure:

- Raw material used in the manufacture of drugs is authentic, of prescribed quality and free from con-

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tamination.

- The manufacturing process is as has been prescribed to maintain the standards;
- Adequate quality control measures are adopted;
- The manufactured drug which is released for sale is acceptable quality;
- To achieve the objectives listed above, each licensee shall evolve methodology and procedures for following the prescribed process of manufacture of drugs, which should be documented as a manual and kept for reference and inspection.

### Aspects of standardization of Herbal Drugs<sup>[14]</sup>

To standardize and control of herbal medicines, a feasible approach and control system is necessary<sup>[15]</sup>. The establishment of perfect quality control profiles of herbal medicines based on physical, chemical and biological evaluation, backed with their stability and bioavailability parameters is the need to ensure the acceptance of phytopharmaceuticals as an integral part of modern drug therapy<sup>[16]</sup>.

Unlike the single chemical entity that forms the basis of modern pharmacology and drug development, the paradigm of traditional herbal medicine views the multi-compound, multi-ingredient preparations typical of traditional herbal medicines as representing the activity of the herbal drugs. Selection of individual analytical compounds for determining either efficacy or quality is contrary to traditional herbal medicine principles<sup>[17]</sup>. The common clinical use of traditional herbal medicines requires the combination of two or more herbals based on recipes and formulae derived from historical references and empirical evidence of ayurvedic practitioners. Herbal drugs, singularly or in combinations, contain a myriad of compounds in complex matrices in which no single active constituents is responsible for overall efficacy. This creates a challenge in establishing quality control standards for raw materials and the standardization of finished herbal drugs. It is necessary to develop a type of quality assessment system that adequately meets the complex characteristic of traditional herbal preparations<sup>[18]</sup>.

It seems to be necessary to determine most of the phyto-chemical constituents of herbal products in order to ensure the reliability and repeatability of pharmacological and clinical research, to understand their

bioactivities and possible side effects of active compounds and to enhance product quality control<sup>[19]</sup>.

Chromatographic fingerprint analysis by which multiple compounds in single herbal drugs and finished herbal materials can be identified represents a rational approach for the quality assessment of traditional herbal preparations. It utilizes chromatographic techniques, TLC, HPLC, HPTLC, and GC etc. to construct specific patterns of recognition for multiple compounds in herbal drugs<sup>[20]</sup>. Chromatographic fingerprint analysis of herbal drugs represents a comprehensive qualitative approach for the purpose of species authentication, evaluation of quality, and ensuring the consistency and stability of herbal drugs and their related products<sup>[21]</sup>.

By definition, a chromatographic fingerprint of a HM is, in practice, a chromatographic pattern of the extract of some common chemical components of pharmacologically active and or chemically characteristics. This chromatographic profile should be featured by the fundamental attributions of "Integrity" and "Fuzziness" or "Sameness" and "Differences" so as to chemically represent the herbal medicines investigated<sup>[22]</sup>.

For standardization of natural product drugs, single chemical entities, "marker compounds" may be used as potency standards in high performance liquid chromatography (HPLC) analysis. Using well-characterized marker compounds, conventional pharmaceutical manufacturing criteria for assay and content uniformity may be applied. These marker compounds may be used to help identify herbal materials, set specifications for raw materials, standardize botanical preparations during all aspects of manufacturing processes, and obtain stability profiles<sup>[23]</sup>.

For the establishment of the quality and purity of crude plant material and finished formulation the procedure should be as per GMP. Following protocols should follow in addition of already described procedures

### 1. Assessment of quality<sup>[24]</sup>

#### Crude plant material

The botanical definitions, including genus, species and authority should be given to ensure correct identification of a plant. A definition and description of the part of the plant from which the medicine is made should be provided. The active and characteristic constituents



should be specified.

Foreign matter, impurities and microbial content should be defined or limited.

### **Plant preparation**

Plant preparations include comminuted or powdered plant materials, extracts, tinctures, fatty or essential oils, expressed juices and preparations whose production involves fractionation, purification or concentration. The manufacturing procedure should be described in detail.

If any other substances are added during the manufacture in order to adjust the plant preparation to a certain level of active or characteristic constituents or for any other purpose.

A chromatographic fingerprint is necessary to ensure consistent quality of the preparation.

### **Finished products**

Manufacturing procedure and formula, including the amount of excipients, should be described in detail. A finished product specification should be defined. A method for identification, quantification of the plant material in the finished product should be defined.

A chromatographic fingerprint is necessary to ensure consistent quality of the preparation. The finished product should comply with general requirements for particular dosage forms.

### **Stability**

The physical and chemical stability of the product in the container in which it is to be marketed should be tested under defined storage conditions and the shelf life should be established.

## **2. Assessment of safety**

A review of the relevant literature should be provided with original articles or references to the original article. Long-term use without any evidence of risks may indicate that a medicine is harmless. Reported side effects should be documented according to normal pharmacovigilance practices.

### **Documentation of safety based on experience**

Documentation of a long period of use should be taken into consideration when assessing safety. When there are no detailed toxicological studies, documented

experience of long-term use without evidence of safety problems should form the basis of the risk assessment.

If any toxicological risk is known, toxicity data must be submitted. The assessment of risk whether independent of dose or related to dose, should be documented.

## **3. Assessment of efficacy**

### **Activity**

The pharmacological and clinical effects of the active ingredients and, if known, their constituents with therapeutic activity should be specified or described.

### **Evidence required supporting indications**

In the case of traditional medicines, the requirements for proof of efficacy should depend on the kind of indication.

### **Stability tests<sup>[25]</sup>**

Since the herbal drug or herbal drug preparation in its entirety is regarded as the active substance, a mere determination of the stability of the constituents with known therapeutic activity will not be sufficient. It must also be shown, by means of appropriate fingerprint chromatograms, that other substances present in the herbal drug or in the herbal drug preparation are likewise stable and that their proportional content remains constant.

Accelerated stability studies can be done for herbal preparations as in allopathic dosage forms by using appropriate fingerprint chromatogram (qualitative and quantitative), appropriate overall methods of assay and physical and sensory tests.

The performance of reported chromatographic fingerprinting constructed by single chromatogram sometimes turns out to be inadequate for complex herbal medicines, such as multi-herb botanical drug products. Multi chromatographic fingerprinting, which consists of more than one chromatographic fingerprint and represents the whole characteristics of chemical constituents of the complex medicine, is proposed as a potential strategy in these complicated cases<sup>[26]</sup>.

Pretreatment of chromatographic fingerprints are important for quality control of herbal medicines and they include data correction and data transformation. The data correction can reduce the variation of experimental procedures, and data transformation can put different weights on the different parts of the finger-

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prints. A new target peak alignment (TPA) procedure has been proposed to correct the retention time shifts, multiplicative scattering correction (MSC) has been introduced for response correction. Then the similarity of the fingerprints with mean and median fingerprints is used to evaluate the quality of herbal medicines<sup>27</sup>.

### CONCLUSION

Chromatographic fingerprint analysis is a rational and practical analytical strategy to assess the authenticity, quality consistency, and stability of herbal medicines. The information gathered from the fingerprint is more comprehensive than that provided from the typical approach of only focusing on the quantitation of individual markers or active constituents for identifying and quantitative assay. Chromatographic fingerprinting is gradually being applied in the quality assessment of traditional herbal medicines. It is currently required, by many of drug regulatory authorities, to ensure the quality control of injectable herbal preparations and is promoted for use in manufacture of oral preparations.

However, using the chemical fingerprints for the purpose of quality control of herbal medicines can only address to the problem of comparing the integrated sameness and/ or difference and controlling their stability of the available herbal products. The complex relationship between the chromatographic fingerprints and efficacy of the herbal medicines is not taken into account yet, which seems to be the most important aspect for the quality control of herbal medicines. More methodological validation work is required on more botanicals and botanical products. The fundamental requirement for developing a chromatographic fingerprint is specificity, reproducibility and applicability. Once an official chromatographic fingerprint is established and an acceptable allowance is given, all manufacturers should require meeting these specifications.

Thus, the researchers concerning the relationship between the chromatographic fingerprints and efficacy of the herbal medicines are urgent requirement for the quality control of herbal medicines. The work on possible contamination in herbal products, such as excessive or banned pesticides, microbial contaminations, heavy metals, chemical toxins, should be also conducted concurrently.

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