International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 3 May-June 2023, pp: 828-833 www.ijprajournal.com ISSN: 2249-7781

# Standardization of Herbal Products by Various Chromatographic Techniques; a Review

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Submitted: 05-05-2023 Accepted: 15-05-2023

**ABSTRACT:**
In recent times more people throughout world are turning to use medicinal plant products in healthcare system. Worldwide need of alternate drug has resulted in growth of natural product markets and interest in traditional systems of drug. Herbal medicine technology is used for converting botanicals materials into drugs, where standardization and quality control with proper integration of modernistic scientific methodologies and traditional knowledge is methodologies like physical, chemical, natural is used for standardization and quality evaluation of herbal medicinal plants. These methodologies can act as a fundamental tool for the quality evaluation of herbal plant materials. Different parameters of standardization are the basic tool for estimating and assuring the quality of the herbal plant material and its products. In order to prove constant composition of herbal medications, acceptable analytical techniques the have to be applied similar as photometric analysis, and various chromatographic techniques like- thin layer chromatography (TLC), high performance thin layer chromatography (HPTLC), high performance liquid chromatography (HPLC), and gas chromatography (GC), etc. The set parameters can eventually lead to the quality and effectiveness of the herbal medicinal formulations. Analytical techniques and standardization can assure the quality and consistence of active constituents in herbal medicinal formulations.

**KEYWORDS:**Medicinal products, herbal medicine, standardization, quality control, chromatographic techniques, analytical techniques.

## I. INTRODUCTION:

Herbal medicines have been used since ancient times as drugs for the treatment of a range of illnesses. Medicinal plants have played a crucial function in world health. In spite of the great advances observed in modernistic drug in recent decades, plants still make an important contribution to health care [1]. Natural products have been our single most successful source of drugs. Each plant is like factory able of synthesizing unlimited number of largely complex and unusual chemical substances whose structures could else escape the imagination always [2]. There are at least 120 distinct chemical substances gathered from plants that are considered as important medicines presently in use in the world, while several other medicines are simple synthetic alterations of the natural products [3]. WHO has given some terms affiliated to herbal medicines, according to their definitions. Herbal drugs include herbs, herbal materials, herbal dosages and finished herbal products. In some countries herbal drugs may contain, by tradition, natural organic or inorganic active components that aren't of plant origin (e.g., animal and mineral materials).

Herbs include crude plant material, like as leaves, flowers, fruit, seeds, stems, wood, bark, roots, rhizomes or other plant parts, which may be entire, fragmented or powdered.

Herbal materials include, in addition to herbs, fresh juices, mucilage, fixed oils, essential oils, resins and dry powders of herbs. In some countries, these materials may be processed by various local procedures, like as steaming, roasting or stir- baking with honey, alcoholic beverages or other materials.

Herbal medications are the base for finished herbal products and may include milled or pulverized herbal materials, or extracts, tinctures and fatty oils of herbal materials. They're produced by extraction, separation, purification, concentration, or other physical or natural processes. They also include medications made by steeping or warming herbal materials in alcoholic beverages and/ or honey, or in other materials.

International Journal of Pharmaceutical Research and Applications
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Finished herbal products consist of herbal medications made from one or additional herbs. However, the If additional than one herb is used term “admixture herbal product” can also be used. Finished herbal products and admixture herbal products may contain excipients in addition to the active constituents. still, finished products or admixture herbal products to which chemically defined active substances have been added, including synthetic combinations and/ or separated ingredients from herbal materials, aren't considered to be herbal(WHO guidelines, 2000). So that it's necessary to maintain reproducible effectiveness and safety of phytopharmaceutical thus if phytopharmaceuticals have to regarded as rational medicine they should be standardized and pharmaceutical quality must be approved [4].

World Health Organization (WHO) stresses the significance of the qualitative and quantitative methodologies for characterizing the samples, quantification of the biomarkers and/ or chemical markers and the fingerprint profiles. If a principle active constituent is known, it's most logical to quantitate this compound. Where active constituents contributing to medicinal effectiveness are known botanical medications should be standardized to these mixtures. Where the active constituents aren't yet known a marker substance which should be specific for the botanical could be chosen for analytical purpose [5].

**STANDARDIZATION:**

Standardization of herbal formulations standardization is necessary for assessing quality pharmaceuticals based on active phytoconstituents concentrations, physical, chemical, phytochemical and in- vitro and in- vivo criteria. The three crucial criteria are necessary for the quality control of the herbal medicines viz. Authenticity, Purity and Assay. The evaluation of herbal formulation’s quality is critical in order to give reason for their acceptance in the modernistic medical system. The lack of stringent quality control parameters for herbal constituents and formulations is one of the primary issues confronting the herbal sector. The task of establishing a quality control standard for herbal crude medicines and their formulation entails natural evaluation of a specific ailment region, chemical profile of the material, and the establishment of a final product specification. As a result, in the case of herbal medicines and products, the term "standardization" should relate to the full field of study, from medicinal plant cultivation through clinical use.

Plant material and herbal treatments generated from it account for a significant share of the global market, therefore internationally recognised quality control morals are needed. Quality control of plant products is assured by WHO through the use of current technology and the use of applicable morals and standards. Other quality control measures must be researched in order to overcome certain essential demerits of the Pharmacopeial monograph. [6-8]

Herbal medicines standardization is a process by which a set of standard parameters for the characterization of the herbal medicines is specified. Because of the complex nature of plants-based medicines, the definite or dependable qualitative as well as quantitative characterization of herbal medicines is needed. All features that can contribute to the quality of medicinal herbs, similar as correct sample identity, organoleptic evaluation, pharmacogenetic evaluation, volatile matter, quantitative assessment like as ash values, extractive values, phytochemical analysis, test for the presence of foreign matter, microbial content, toxicity testing, and natural or medicinal action, should be considered in standardisation methodologies. The phytochemical profile is especially important because it directly influences the activity of herbal medications. The term "phytochemical standardisation" refers to the collection of all information about the chemical constituents found in herbal drugs.

The microscopic and macroscopic examinations, identifications, physicochemical properties, pharmacogenetic parameters, and other parameters reported for the first time, in accord with the process for formulation of standard herbal medicines in the pharmacopoeia and other standard texts, can play an important function for authenticating the herbs for future studies. The standardization parameters will capable to assure the quality, safety and effectiveness of the herbal medicines or the herbal products [9-10].

**STANDARDIZATION OF HERBAL FORMULATION:**

Standardization of herbal formulation requires execution of Good Manufacturing Practices (GMP) (WHO guideline, 1996) In addition, study of various parameters alike as pharmacodynamics, pharmacokinetics, dosage, stability, self- life, toxicity evaluation, chemical profiling of the herbal formulations is considered essential [11]. Heavy metals impurity, Good

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Agricultural Practices (GAP) in herbal medicine standardization are equivalently important [12].

**Guidelines For The Standardization Of Herbal Drugs:**

The guidelines set by WHO are:

Botanical characters, sensory evaluation, foreign organic matter, microscopic, histological, histochemical assessment, quantitative measures, Physical and chemical identity, fingerprints chromatography, ash values, extractive values, moisture content, volatile oil and alkaloids tests, quantitative estimation protocols, Estimation of natural activity, the values of bitterness, astringency haemolytic index, a factor lump, foaming index, Detail- toxicity pesticides residues, heavy metals, microbial impurity as feasible count sum total, pathogens alike as E. coli, Salmonella, P. aeruginosa, S. aureus, Enterobacteriaceae, Microbial impurity and radioactive impurity are followed [13].

**TECHNIQUES OF STANDARDIZATION:**

- **CHROMATOGRAPHIC FINGERPRINTING AND MARKER COMPOUND ANALYSIS:**

A chromatographic fingerprint of an Herbal Medicine (HM) is a chromatographic pattern of the extract of some common chemical elements of pharmacologically active and or chemical characteristics. This chromatographic profile should be featured by the basic attributions of “integrity” and “fuzziness” or “sameness” and “differences” so as to chemically represent the

HM researched. It's suggested that with the help of chromatographic fingerprints procured, the authentication and identification of herbal drugs can be exactly conducted integrity) indeed if the quantity and/ or concentration of the chemically characteristic components aren't exactly the same for different samples of this HM (hence, “fuzziness”) or, the chromatographic fingerprints could demonstrate both the “sameness” and “differences” between various samples successfully. therefore, we should universally consider multiple components in the HM extracts, and not separately consider only one and/ or two marker constituents for evaluating the quality of the HM products. still, in any HM and its extract, there are hundreds of unknown constituents and numerous of them are in low quantity. also, there generally exists variability within the same herbal materials. Hence, it's truly important to attain dependable chromatographic fingerprints that represent pharmacologically active and chemically characteristic constituents of the HM.

- **THIN LAYER CHROMATOGRAPHY (TLC):**

Thin layer chromatography is simply known as TLC. It's one of the most popular and simple chromatographic methodology used of separation of mixtures. In the phytochemical evaluation of herbal medicines, TLC is being employed considerably for the following reasons:

1. It enables rapid analysis of herbal extracts with minimum sample clean-up requirement.
2. It provides qualitative and semi quantitative information of the resolved compounds.
3. It enables the quantification of chemical constituents. Fingerprinting using HPLC and GLC is also carried out in specific cases.

In TLC fingerprinting, the data that can be recorded using a high- performance TLC (HPTLC) scanner includes the chromatogram, deceleration factor (Rf) values, the colour of the separated bands, their absorption spectra, λ max and shoulder inflection/ s of all the resolved bands. All of these, together with the profiles on derivatization with different reagents, represent the TLC fingerprint profile of the sample. The information so generated has a potential use in the identification of an authentic medicine, in eliminating the contaminants and in maintaining the quality and consistence of the medicine. HPLC fingerprinting includes recording of the chromatograms, retention time of individual peaks and the absorption spectra (recorded with a photodiode array detector) with different mobile phases. likewise, GLC is used for generating the fingerprint profiles of volatile oils and fixed oils of herbal medicines. likewise, the recent approaches of applying hyphenated chromatography and spectrometry similar as High-Performance Liquid Chromatography–Diode Array Detection (HPLC–DAD), Gas Chromatography–Mass Spectroscopy (GC – MS), Capillary Electrophoresis- Diode Array Detection (CE-DAD), High- Performance Liquid Chromatography–Mass Spectroscopy (HPLC – MS) and High- Performance Liquid Chromatography–Nuclear Magnetic Resonance Spectroscopy HPLC–NMR) could give the more spectral information, which will be genuinely helpful for the qualitative analysis and truly for the on- line structural explication [14].

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- **HIGH-PERFORMANCE THIN LAYER CHROMATOGRAPHY (HPTLC):**

HPTLC technique is extensively employed in pharmaceutical industry in process development, identification and spotting of pollutants in herbal product and helps in identification of pesticide content, mycotoxins and in quality control of herbs and health foods [15]. It has been well reported that several samples can be run contemporaneously by use of a lesser amount of mobile phase than in HPLC[16]. It has also been reported that mobile phases of pH 8 and over can be used for HPTLC. Another advantage of HPTLC is the repeated spotting and scanningof the chromatogram with the same or different conditions. Accordingly, HPTLC has been investigated for contemporaneous assay of several factors in amulti-component formulation[17]. With this methodology, authentication of various species of plant possible, as well as the evaluation of stability and consistence of their medications from different manufactures. Various workers have developed HPTLC methodology for phytoconstituents in crude medicines or herbal formulations similar as bergenin, catechin and gallic acid in Bergeniacilliata and Bergenialingulate[18].

- **HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC):**

Preliminary and analytical HPLC are extensively used in pharmaceutical industry for separating and cleansing of herbal combinations. There are principally two types of preparative HPLC low pressure HPLC (generally under 5 bar) and high pressure HPLC (pressure> 20 bar) [19]. The important parameters to be considered are resolution, sensitivity and fast analysis time in analytical HPLC whereas both the degree of solute purity as well as the quantity of mixture that can be produced per unit time i.e., throughput or recovery in preliminary HPLC [20]. In preliminary HPLC (pressure> 20 bar), larger stainless-steel columns and packing materials (particle size 10- 30 µm) are required. The examples of normal phase silica columns are Kromasil 10 µm, Kromasil 16 µm, Chiralcel AS 20 µm whereas for reversed phase are Chromasil C18, Chromasil C8, YMC C18. The objective is to separate or purify compounds, whereas in analytical work the aim is to get information about the sample. This is very important in pharmaceutical industry of present because new products (Natural, Synthetic) have to be introduced to the market as quick as possible. Having available such an influential purification methodology makes it possible to spend minor time on the synthesis conditions [21-22].

- **LIQUID CHROMATOGRAPHY-MASS SPECTROSCOPY (LC-MS):**

LC- MS has become system of choice in numerous stages of medicine development [23].Recent advances includes electrospray, thermos-spray, and ion-spray ionization methods which offer unique advantages of high spotting sensitiveness and particularity, liquid secondary ion mass spectroscopy, latterly laser mass spectroscopy with 600 MHz offers accurate determination of molecular weight proteins, peptides. Isotopes pattern can be detected by this method[21].

- **LIQUID CHROMATOGRAPHY-NUCLEAR MAGNETIC RESONANCE (LCNMR):**

LC- NMR improves speed and sensitiveness of spotting and establish useful in the areas of pharmacokinetics, toxicity studies, medicine metabolism and medicine discovery process. The combination of chromatographic separation method with NMR spectroscopy is one of the most potent and time saving system for the separation and structural illustration of unknown mixture and compounds, especially for the structure illustration of light and oxygen sensitive substances. The online LC- NMR method allows the continued registration of time changes as they appear in the chromatographic run automated data acquisition and processing in LC- NMR improves speed and sensitiveness of spotting. The recent preface of pulsed field gradient method in high resolution NMR as well as three- dimensional method improves use in structure illustration and molecular weight information. These new hyphenated methods are useful in the areas of pharmacokinetics, toxicity studies, medicine metabolism and medicine discovery process[24].

- **GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS):**

GC equipment can be directly affiliated with rapid scan mass spectrometer of various types. GC and GC- MS are unanimously accepted techniques for the analysis of volatile ingredients of herbal drugs, due to their sensitiveness, stability and high effectiveness. Especially, the hyphenation with MS provides dependable information for the qualitative analysis of the complex ingredients [25-26]. The inflow rate from capillary column is

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generally low enough that the column output can be fed directly into ionization chamber of MS. The simplest mass detector in GC is the Ion Trap Detector (ITD). In this instrument, ions are created from the eluted sample by electron impact or chemical ionization and stored in a radio frequency field; the trapped ions are further ejected from the depository area to an electron multiplier detector. The ejection is controlled so that scanning on the base of mass- to- charge ratio is possible. The ions trap detector is remarkably compact and less costly than quadrapole instruments. GC- MS instruments have been used for identification of hundreds of elements that are present in natural and natural system [27].

- **GAS CHROMATOGRAPHY-FLAME IONIZATION DETECTOR (GC-FID):**

A number of detectors are used in gas chromatography. The most common are the flame ionization detector (FID) and the thermal conductivity detector (TCD). Coupling capillary column gas chromatographs with Fourier Transform Infrared Spectrometer provides a potent means for separating and associating the elements of different compounds [28].

Both are sensitive to a wide range of elements, and both work over a wide range of concentrations. While TCDs are basically universal and can be used to detect any element other than the carrier gas (as long as their thermal conductivities are different from that of the carrier gas, at detector temperature), FIDs are sensitive primarily to hydrocarbons, and are more sensitive to them than TCD. still, an FID cannot detect water. Both detectors are also robust. Since TCD is non-destructive, it can be operated in- series before an FID (destructive), therefore delivering supplementary spotting of the same analytes [29].

- **SUPERCRITICAL FLUID CHROMATOGRAPHY (SFC):**

Supercritical fluid chromatography is a hybrid of gas and liquid chromatography that combines some of the best features of each. SFC permits the separation and determination of a group of mixtures that aren't accessibly handled by either gas or liquid chromatography. SFC has been applied to a wide variety of materials including natural products, medicines, food and pesticide. These mixtures are either non-volatile or thermally labile so that GC procedures are irrelative or contain no functional group that makes possible spotting by the spectroscopic or electrochemical methodology employed in LC[24].

## II. CONCLUSION:

The Indian herbal industry is growing in a tremendous rate. additional number of herbal products is arrived in the market. The safety and effectiveness of herbal products are dependent upon the standardization of these herbal medicines. The traditional approach towards standardization is inadequate for current herbal market and hence there's need for more advanced methods for is the protocol for assuring the quality and consistence of active principles of medicinally active plants. therefore, standardization will serve to set the quality control specifications, which can help the worldwide expansion of herbal medicinal plants. There are principally two methods used for standardization these are chromatographic fingerprinting and DNA fingerprinting. The chromatographic fingerprinting is based on the chromatographic separation and identification of marker compound from other elements. For these purpose TLC, HPTLC, HPLC, LC-MS, LC-NMR, GC-MS, GC-FID and SFC techniques are used.

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