Journal of Advances and Scholarly Researches in Allied Education
Vol. 22, Issue No. 01, January-2025, ISSN 2230-7540

DOI: https://doi.org/10.29070/2415sk26

Pharmaceutical Analysis and Standardization of Herbal Medicines

Falah Shynan Alqahtani [1] * , Zainab Ibrahim Alshabeb [2] , Meshal Muflih Aljohani [3] , Nahla Faleh Alotaibi [4] , Abdulrahman Buzaya Alshammari [5]

1. Pharmacy Technician, PSMMC, Riyadh , SA
falah_050@hotmail.com ,
2. Pharmacist, PSMMC, Riyadh , SA ,
3. Pharmacy Technician, PSMMC, Riyadh, SA ,
4. Pharmacy Technician, PSMMC, Riyadh , SA ,
5. Pharmacy Technician, PSMMC, Riyadh, SA

Abstract: This paper provides a comprehensive overview of the criteria used to test and standardize medicinal plants and components in the pharmaceutical sector. Natural or herbal therapies are becoming increasingly popular as alternatives to commercially produced drugs due to their low risk of side effects. As herbal therapeutic items become more popular, concerns regarding their effectiveness, safety, and quality arise. These problems stem from the lack of herbal therapy standardization criteria. To maintain quality, herbal medicinal plant components must be standardized using rigorous standards and analytical methods. Herbal treatments are tested and standardized using several physical, chemical, and biological approaches. These methods determine if herbal plant materials and formulations are safe, pure, and effective and constitute the basis of quality control. Analytical methods like TLC, HPTLC, GC, LC-MS, UPLC, UHPLC, UPLC-MS, and UHPLC-MS are used to quantify herbal drugs and their formulations. This assessment emphasizes these methods. Standardized assessment criteria are necessary to guarantee herbal medical product active ingredients are dependable and effective. These analytical methods can standardize and test herbal medicines. This preserves herbal medicine quality and reliability. If these guidelines are followed, herbal medicines will meet safety, efficacy, and consistency criteria.

Keywords: Standardization, Herbal, analysis, natural, Pharmaceutical

X

INTRODUCTION

Over the course of the last several decades, the popularity of herbal therapy has exponentially increased. The growth in popularity of this substance, which is impacting both developed and emerging countries, may be attributed to its natural origin as well as the fact that it has minimal adverse effects. Traditional vaidyas would adjust their therapies to the specific requirements of each individual patient and construct drugs in accordance with those requirements. Herbal medicines are now mass-produced in pharmaceutical units, where they confront various problems, such as a lack of standards, an insufficient methodology for standardizing specific pharmaceuticals and formulations, and a lack of quality control criteria. However, the situation has changed, and herbal medicines are now mass-produced.

Through the course of human history, Ayurveda lays an emphasis on the interconnectedness that exists between people and plants. As a consequence of individuals turning to herbal medicines as an alternative to the hazardous and often deadly side effects of traditional allopathic treatment, there has been a significant increase in the manufacture of herbal medicines. [1] Since the beginning of time, herbal treatments have been the main therapeutic approach used in traditional medicine. Both the biomedical benefits and the cultural beliefs that are prevalent in many parts of the world guarantee that the practices are being practiced

today, and they have made a significant contribution to the preservation of human health. [2]Medical items labeled as herbal remedies comprise active compounds, aerial or subterranean plant parts, or a mix of these, according to the World Health Organisation (WHO). [3]

Fundamental medical requirements focused on the healing properties of plants and animals

By conducting their own exams, Rishis, Vaidyas, and Hakims, the ancient medical systems have all taken into consideration the implementation of quality control measures. However, according to the current way of thinking, they need to make adjustments to their plan in order to put into practice a genuine method of quality control that takes into consideration the development of modern ideas. As a result, the evaluation of medicinal plants and herbal formulations that have been utilized historically in contemporary times has moved its attention to quality assurance.

The most impressive example of the unique phenomena of symbiosis may be found in nature, which acts as a gold standard in this respect. As more and more people in the West become aware of the risks associated with synthetic drugs, they are searching for alternatives that are based on natural ingredients and take a more uncomplicated approach to the healing process. Historically, natural substances produced from plants, animals, and minerals have served as the basis for the treatment of human illnesses. According to the most recent estimates, over eighty percent of the world's population continues to rely on traditional medicine, which is primarily focused on the healing properties of plants and animals, to meet their fundamental medical requirements.

Effectiveness of plants as medicines on the chemical components

Alternative medicine is becoming more popular in today's society. There is a growing need for herbal medicines, and the popularity of these remedies is only likely to continue to increase. Because there is a large quantity of raw materials that are easily available, the World Health Organisation (WHO) strongly recommends the use of traditional herbs and therapies in the healthcare sector. Their low levels of toxicity make them a very safe option. The majority of the villagers continue to depend on herbal remedies since, in the thoughts of the common people, natural medicines will magically treat them without the detrimental consequences that are associated with allopathic drugs. However, the effectiveness of plants as medicines is dependent on the chemical components of those plants, which could change depending on the age of the plant, the location it is grown in, and the harvesting techniques used. Standardizing herbal medicines is of the highest importance because of problems such as the presence of pesticide residue, the presence of microbiological adulteration, and the improper authentication of herbs.

The herbal preparation does not yet have any criteria that are accepted. The majority of the tests that are conducted by the manufacturers are simply preliminary; nevertheless, those that are conducted include the establishment of their own criteria and the testing of formulations. The process of determining whether or not a composition really includes all of the ingredients that are listed is becoming more challenging. Consequently, it is of the utmost importance to devise a metric that is capable of identifying the existence of every component. It is possible to do this by analyzing the physicochemical qualities, experimenting with various chromatographic and spectrophotometric techniques, or by doing both of these things. The quantitative estimation of certain chemicals or groups of compounds that possess bioactive qualities, such as alkaloids, flavonoids, polyphenolic components, and so on, may be accomplished by the use of these methodologies wherever they are suitable.

If polyherbal Ayurvedic medicines were to be evaluated, it would be very difficult to determine the chemical makeup of each individual component. The few major components that are associated with the therapeutic activities that are listed on the label should be quantitatively estimated, since this is also suggested. Performing this action in combination with other metrics that may be used to authenticate the presence of all compounds is something that should be done. This is something that can only be accomplished via the collaborative efforts of scientists from different fields.

OBJECTIVES

1. To standardize analytical processes that utilize HPLC, HPTLC, LC-MS, and GC to identify and quantify herbal product active components to ensure their efficacy, safety, and purity.

2. To address raw material variability and ensure that herbal medicines meet worldwide pharmaceutical standards for therapeutic use by providing comprehensive regulatory standardization and quality control procedures.

HERBAL DRUG STANDARDISATION

When components are standardized, it ensures that they will have the same quantity, quality, and therapeutic effect in every dose that they were originally intended to have. [4] . The scientific validity of a herbal product is contingent upon the genuineness and characterization of the medicine that was investigated. This is necessary in order to guarantee that the manufacturing process can be repeated reliably. In addition, there have been several instances of adverse consequences that have been dangerous and even deadly. These adverse effects include allergic reactions, interactions with herbal drugs, direct toxic effects, and impacts from pollution. [5] The medicinal effect of a herbal preparation is derived from the photochemical components that are present in the preparation. For scientists, one of the most major challenges is the development of dependable analytical methods for phytochemical composition profiling. These methods should include quantitative investigations of marker/bioactive compounds as well as other essential components. Now that we have that out of the way, standardization is an essential stage in the process of developing a quality assurance program for the development and manufacture of herbal medications, as well as in the process of establishing a consistent chemical profile and biological activity characterization. [6] . The detection of adulterants from genuine medicinal plants and the authentication of herbal pharmaceuticals are both crucial for pharmaceutical firms as well as for public health, and they are also necessary for ensuring that the quality of herbal medicine can be reproduced. [7]

Typical herbal formulation standardisation procedures

Data from raw plant passports, botanical authentication, microscopic and molecular examination, identification of chemical composition via a variety of chromatographic techniques, and overall plant biological activity are all components of the herbal raw medicine standardization process. The chemical profile of herbal materials, as well as their macroscopic and microscopic inspection, has been recorded by a number of researchers for the goal of quality control and standardization. [8-10] Microscopy involves a comparative microscopic inspection of powdered herbal medicine, while macroscopic identification of medicinal plant materials depends on sensory evaluation criteria such as shape, size, color, texture, smell, and taste. Microscopy compares and contrasts the appearance of powdered herbal medicine. In addition, the use of light and scanning electron microscopes (SEM) in the process of standardizing herbal medications has resulted in an enhancement of the precision and capabilities of microscopy as a technique

for detecting herbal crude materials. [19] . In addition, the standardization of herbal remedies today makes use of a range of advanced processes, including chromatographic, spectrophotometric, electrophoresis, polarography, and the use of molecular biomarkers in fingerprints. [19-23].

Herbal formulation standardisation

In order to achieve and maintain consistency in herbal formulation, it is necessary to adhere to Good Manufacturing Practices (GMP). It is also very important to do research on the chemical profiles, self-life, toxicity evaluation, dose, stability, pharmacokinetics, and pharmacodynamics of herbal formulations. The presence of pesticide residue, aflatoxine concentration, heavy metal contamination, and Good Agricultural Practices (GAP) are all important additional issues that are taken into account throughout the process of standardizing herbal medicine. [24]

Polyherbal formulation standardisation

As a result of the fact that polyherbal formulations are combinations of more than one herb in order to produce the desired therapeutic effect, standardization is an essential component in the process of preserving and monitoring the standards of quality and safety for these products. [25] . In the process of standardizing the polyherbal hyperlipdemia formulation, aspects such as organoleptic, physical, and physicochemical properties have all been taken into consideration. [26] . The formulation and standardization of Artrex®, a polyherbal formulation that combines four botanicals for the treatment of arthritis, has been granted a patent in the United States of America. For the purpose of carrying out this procedure, modern scientific procedures and established markers were used. [27]. The standardization of various marketed herbal and polyherbal formulation There are eight different herbal ant diabetic medications that are included in Madhumehari Churna (Baidynath), which includes seeds, syzigium, trigonella, leaves, fruits, rhizomes, leaves, Gymnema, and heart-wood.[28] , Those suffering from gastrointestinal disorders have found relief with Pancasama Churna. [29] , Physiological processes are normalised after childbirth with the use of Dashamularishta, a traditional formulation. [30], Megni, Jawarish-e-Darchini, Gokshuradi Churna [31-33] to have occurred. However, many polyherbal compositions still need standardization since they are often used only for their ethanobotanical purposes. [34] . The safety, effectiveness, purity, and acceptability of the polyherbal formulations are ensured by standardization, which minimizes batch to batch variance. [35] . It has been suggested that Methiorep Premix, a mixture of herbs including Cicer arientinum, Phaseolus mungo, Mucuna pruriens, Triticum sativum, and allium cepa, as a safer alternative to synthetic methionine in poultry rations and as a supplement to basal diets on a regular basis, is an excellent source of protein. [36] . TLC and HPTLC fingerprint profiles were used in order to ascertain the qualities of the polyherbal formulation, including its identification, purity, and potency. In addition, the specifications for this Ayurvedic composition were established by using these methods.

METHODOLOGY

The methodology is based review based on quality of products and the concentration of plant chemicals can vary greatly depending on a number of factors. These factors include environmental factors such as sunlight, rainfall, altitude, temperature, soil, storage conditions, harvesting methods, time and method of collection, manufacturing processes such as selecting, drying, purifying, and extracting, with genetic variability also playing a role. Both the chemical composition of plants and the secondary metabolites that

they produce are vulnerable to environmental conditions such as the presence of microbial illnesses and the presence of insects that feed on them. There is also a difference in the concentration of chemical components between the different parts of the same plant, such as the roots, the stem, and the leaves. Seasonal shifts and diurnal fluctuations (such as paclitaxel and opium alkaloids) are two factors that contribute to the wide range of variations that may be found in herbal medicines.

Need for Standardisation

Traditional vaidyas would adjust their therapies to the specific requirements of each individual patient and construct drugs in accordance with those requirements. A significant number of traditional medical systems have incorporated quality control into their practices by conducting inspections of their Rishis, Vaidyas, and Hakims. These days, the difficulties stem from the costs of manufacturing on an industrial scale, the shelf life of the product, and the distribution of the product across enormous distances. This is in contrast to the days when traditional practitioners were responsible for making and verifying the effectiveness of herbal treatment. Because of this, it has become necessary to develop new criteria that are objective in order to evaluate the quality, safety, and efficacy of these treatments. More and more individuals are becoming aware of the potentially harmful consequences as well as the power of the substance. Researchers, manufacturers, and regulatory agencies need to implement stringent scientific methods in order to ensure the quality and consistency of traditional herbal medicines from one lot to the next. This is necessary in order to gain the trust of the general public and include herbal products into the modern health care system from the beginning. [37]. In a nutshell, this is why herbal product quality control and standardization are essential:

1. When traditional medicines were developed technology and concept of standardization was quite different.

2. During past thousand years dynamic process of evolution may have changed the identity of plant material.

3. Due to commercialization, supply of genuine raw material has become a challenge.

4. Properties of botanicals may have undergone change due to time and environmental factors [38]

There are a variety of variables that might give rise to significant variations in herbal raw materials. These include, but are not limited to, plant identity, seasonality (which affects when they are collected), ecotypic, genotypic, and chemotypic variations, drying and storage conditions, and the presence of xenobiotics. [39]. The quality of products and the concentration of plant chemicals can vary greatly depending on a number of factors. These factors include environmental factors such as sunlight, rainfall, altitude, temperature, soil, storage conditions, harvesting methods, time and method of collection, manufacturing processes such as selecting, drying, purifying, and extracting, with genetic variability also playing a role. Both the chemical composition of plants and the secondary metabolites that they produce are vulnerable to environmental conditions such as the presence of microbial illnesses and the presence of insects that feed on them. There is also a difference in the concentration of chemical components between the different parts of the same plant, such as the roots, the stem, and the leaves. Seasonal shifts and diurnal fluctuations (such as paclitaxel and opium alkaloids) are two factors that contribute to the wide range of variations that may be found in herbal medicines. There are a variety of therapeutic and toxic components that may be found in the plant, depending on the sections of the plant and the stage of development. [40]. It is impossible to regulate every

variable that impacts the chemical makeup of plants, and there is a large amount of variation across products of various manufacturers. [41-42]. The intricacy and inherent unpredictability of plant-based medicinal components make it difficult to define quality control measures for these components. However, new analytical methods should be able to aid in overcoming this issue. In addition to this, the components that are intended to have a therapeutic effect are often either not recognized at all or are only defined in a limited manner. The vast majority of herbal treatments, especially those that are contained in the classical formulae of traditional medicine, contain more than one plant. There are a great number of preparations that are either liquid or semisolid by nature. The process of establishing quality control standards for mixes of this kind is quite difficult. Even standards that have been published by the government are not available. Due to the peculiar processing techniques that are utilized in their creation, the separate pharmaceuticals are changed into a highly complicated mixture.

This makes it exceedingly difficult to isolate, identify, and study the components that make up the medication. In herbal product standardization, there are two primary types: active constituents extracts, in which the biochemical principles contain therapeutic value, and marker extracts, in which the active principle is unknown but a characteristic compound is used as a marker to evaluate the presence of other biochemical compounds with therapeutic value. Both types of extracts are used to determine whether or not the herbal product contains therapeutic value. [43]. The fact that standardizing only takes into account the individual chemicals that are present in a plant, rather than the plant as a whole, which may have additive or buffering characteristics that reduce the negative effects of the chemicals, is one of the fundamental limitations of standardization.

Conventional medicine is standardized in every aspect, beginning with the acquisition of fundamental components and continuing all the way to the most stringent clinical application. The therapeutic efficacy of traditional medicine is a direct outcome of the high number of substances that are used in the treatment process. Therefore, the quality and purity of the medicine take into account the whole of its profile, rather than focusing on a single one of its qualities. Because of this, traditional medicine cannot be standardized without using an approach that incorporates several aspects. The name of the drug, its botanical origin, its geographical origin, its organoleptic properties, its morphological and anatomical characteristics, its physical properties, its chemical properties, and its biological activity should all be taken into consideration in this multi-dimensional approach. As stated by the World Health Organisation (WHO), the characterization of samples, the quantification of biomarkers and chemical indicators, and the creation of fingerprint profiles are all areas in which quantitative and qualitative techniques are equally significant. It would be the most reasonable line of action to quantify the primary active component, assuming that such a component does in fact exist. It is essential to standardize botanical preparations to ensure that they include just the active components that are known to contribute to the efficiency of therapeutic treatments. In situations in which the active components of the botanical are unknown, it is possible to make use of an analytical marker material that is specific to the plant.

The pharmacopoeia provides references that demonstrate the authenticity, quality, and purity of any herbal treatment that is being used. By publishing both traditional and alternative therapeutic uses of plants, these books provide the foundations for clinical therapy and thereby offer the foundation for clinical treatment. In a herb's monograph, you will find information on its botanical characteristics, analytical findings, medicinal indications, and interactions with other medications. Medications are recommended in the pharmacopoeia with regard to their structure, analysis, and physical characteristics, in addition to numerical values. [44]

RESULT

Markers' Function

A fragment of DNA or a gene that is connected to a particular attribute and whose exact positioning on a chromosome is known is referred to as a genetic marker within the scientific community. This is a variation that may be the consequence of alterations or mutations in the genetic loci that are apparent to the naked eye. Minisatellites are instances of long genetic markers, while the DNA sequence around a single base-pair mutation (also known as a single nucleotide polymorphism, or SNP) is an example of a short genetic marker. Several genetic indicators are employed in the practice of contemporary medicine. Among them are the following: RFLP, AFLP, RAPD, VNTR, STR, SFP, and Micro satellite polymorphism-SNP genotyping. It is possible to further categorize them. [45]. In order to distinguish between neem accessions sourced from various parts of the world, RAPD-based molecular markers have shown to be effective. [46]. A lot of work has also gone into germplasm analysis as a means of studying genetic variation. Rice, wheat, chickpeas, pigeon peas, pearl millet, and many more crops are being fingerprinted. [47]. Using techniques such as sequence-characterized amplified region (SCAR), amplification-dependent polymerase chain reaction (AP-PCR), radial phasing-amplitude polymorphism (RAPD), and RFLP, these plants have been successfully distinguished and replaced by other species that are closely related. P. quinquefolius, sometimes known as American ginseng, is a frequent alternative to the plant known as P. ginseng.[48]. A series of micro propagated Piper longum plants have been selected for preservation using RAPD markers.

The TLC/HPTLC method for herbal drug analysis

Before the development of instrumental chromatography methods like as GC and HPLC, thin-layer chromatography (TLC) was the approach that was the most widely used and versatile for herbal examination. The transfer of a solute from a liquid mobile phase to an adsorbent stationary phase is the process that is involved in thin-layer chromatography. High-performance thin-particle chromatography, often known as HPTLC, takes full advantage of all of the characteristics that make thin-layer chromatography (TLC) so unique. It is the most effective method of separation in terms of adaptability, dependability, and cost-effectiveness. There are a number of advantages to employing TLC/HPTLC to produce herbal medicine fingerprints, one of which is the simplicity of sample preparation. Additional benefits include its ease of use, wide range of applications, rapid velocity, exceptional sensitivity, and dependability. As a result, TLC is a very useful device for determining the quality of herbal products and determining whether or not they have been adulterated.. [49-51]

Herbal medicines and GC–MS

The examination of volatile oils using gas chromatography (GC) provides a number of benefits. To begin, the gas chromatography (GC) analysis of the volatile oil provides a reliable "fingerprint" for identifying it. As a result of the plant-specific character of its composition and the relative quantity of organic components, impurities in volatile oil are easily discernible. Second, GC-MS analysis makes the procedure of extracting volatile oil more simpler and more straightforward, and it can be standardized and is uncomplicated to comprehend. One of the most obvious advantages of gas chromatography is that it has a high sensitivity of detection for almost all volatile chemical substances. The GC-MS analysis and the FID detection are two areas in which this is especially true. [52-61] When a hyphenated GC-MS instrument is used, it is possible to get information that is particularly trustworthy about the identification of the

compounds. A few of the advantages of GC-MS are as follows: (1) A capillary column coupled with gas chromatography-mass spectrometry (GC-MS) has the potential to produce a chemical fingerprint of exceptional quality and has exceptional separation capabilities. (2) Mass spectrometry and the accompanying mass spectral databases have the ability to link qualitative and partly quantitative aspects of spectroscopy. Learn about the chemical composition of the plant if you are interested. The information that is produced by GC-MS will be of great use in directing future research that have the objective of determining the precise nature of the connection that exists between the chemical components of herbal medicines and the therapeutic benefits that they have. It is necessary to do further research in the area of pharmacology. [62-65]

High-performance liquid chromatography–DAD, HPLC–MS, etc.

The high-performance liquid chromatography (HPLC) technique has become more popular for the examination of medicinal plants. This is mostly due to the fact that it is simple to use and does not have any limits regarding sample volatility or stability. The use of reversed-phase (RP) columns is the method that is most often used for the analytical separation of herbal medicines. At this point in time, the use of HPLC-DAD has spread to almost every analytical laboratory on the whole earth. Through the use of UV spectral data, qualitative analysis of intricate herbal medicine samples is made more straightforward than it was in the past. Since the beginning of the past few decades, there has been a discernible increase in the use of LC-MS and HPLC-DAD for the purpose of analyzing herbal medicines. For the purpose of researching the bioactive chemical components of plants and herbal medicines, high-performance liquid chromatography (HPLC), and more specifically hyphenated HPLC procedures, is the method of choice, as stated by a number of well-written assessments. LC-DAD-MS has shown to be an effective approach for the quick identification of components in herbals. This is because it eliminates the need to isolate all of the compounds that need to be identified, which is a waste of time. This was made feasible by hyphenation methods, which enabled one to identify the chromatographic peaks immediately online by correlation with recorded literature or with standard chemicals. This made it possible for this achievement to be accomplished. [66-69]

Table 1. Quantitative HPLC herbal drug identification

Herbal Drug	Target Compounds	Mobile Phase	Detection Wavelength (nm)	Column Used	Retention Time	Quantitativ Applicatio
Ginseng	Ginsenosides (Rb1, Rg1, Re)	Acetonitrile:Water (gradient)	203 nm	C18 Column	25-40 min	Quantificatic of ginsenoside for potency analysis

Turmeric (Curcuma longa)	Curcumin, Demethoxycurcumin, Bisdemethoxy	Methanol:Acetonitrile:Water (60:20:20)	425 nm	C18 Column	8-12 min	Curcumin content quantificatic for anti-inflammator studies
Green Tea (Camellia sinensis)	Catechins (EGCG, ECG, EGC)	Water:Acetonitrile (80:20)	280 nm	C18 Column	12-18 min	Quantificatic of catechins for antioxidant properties
Aloe Vera	Aloin A, Aloin B	Methanol:Water (70:30)	254 nm	C18 Column	10-15 min	Determining aloin conter for skin car formulation

Table 2. Quantitative HPTLC herbal drug identification

Herbal Drug	Target Compounds	Solvent System	Detection Wavelength (nm)	Stationary Phase	Rf Value	Quantitative Application
Ashwagandha (Withania somnifera)	Withanolides (Withaferin A)	Chloroform:Methanol (9:1)	530 nm	Silica gel 60 F254	0.45	Quantification of withanolides for adaptogenic effects
Neem (Azadirachta indica)	Azadirachtin	Toluene:Ethyl Acetate (7:3)	254 nm	Silica gel 60 F254	0.32	Quantification of azadirachtin for insecticidal properties

Amla (Phyllanthus emblica)	Gallic Acid, Ellagic Acid	Toluene:Ethyl Acetate:Formic Acid (6:4:0.1)	254 nm	Silica gel 60 F254	0.67 (Gallic Acid)	Measurement of gallic and ellagic acid for antioxidant activities
Tulsi (Ocimum sanctum)	Ursolic Acid, Oleanolic Acid	Toluene:Ethyl Acetate (9:1)	366 nm	Silica gel 60 F254	0.58 (Ursolic Acid)	Quantification of ursolic acid for anti-inflammatory and antimicrobial properties

Table 3. Quantitative LC-MS Herbal Drug Identification Applications

Herbal Drug	Target Compounds	Mobile Phase	Detection Mode	Retention Time	Quantitative Application
Ginkgo Biloba	Flavonol Glycosides (Quercetin, Kaempferol)	Acetonitrile:Water (70:30)	Negative Ion Mode	12-18 min	Quantification of flavonol glycosides for neuroprotective activity
St. John's Wort	Hypericin, Hyperforin	Methanol:Water:Formic Acid (60:40:0.1)	Positive Ion Mode	10-15 min	Measurement of hypericin and hyperforin for antidepressant effects
Milk Thistle (Silybum marianum)	Silymarin (Silibinin, Silychristin)	Methanol:Water (gradient)	Positive Ion Mode	15-20 min	Determining silymarin content for liver protection

Licorice (Glycyrrhiza glabra)	Glycyrrhizin	Methanol:Water (65:35)	Negative Ion Mode	10-12 min	Quantification of glycyrrhizin for anti-inflammatory effects
Echinacea (Echinacea purpurea)	Phenolic Acids (Cichoric acid, Caftaric acid)	Methanol:Acetonitrile:Water (50:30:20)	Negative Ion Mode	18-22 min	Measurement of phenolic acids for immune support properties

HPLC

High-performance liquid chromatography (HPLC) is often used in the pharmaceutical sector for the purpose of isolating and purifying compounds that are contained in herbs. Both high-pressure (often more than 20 bar) and low-pressure (typically less than 5 bar) high-performance liquid chromatography (HPLC) are used for preparative applications. For analytical high-performance liquid chromatography (HPLC), resolution, sensitivity, and the ability to perform analysis in a short amount of time are crucial aspects to take into consideration. On the other hand, for preparative HPLC, throughput (the amount of compound that can be produced in a given amount of time) and solute purity (the extent to which a compound can be manufactured) are important factors to take into account. By using high-performance liquid chromatography (HPLC), it was established that the concentration of vasicine, which is the primary bioactive alkaloid of Adhatoda vusica, was 18.1 mg/100 g in Shereeshadi Kashaya and 0.7 mg/100 g in Yastyadivati, which are both polyherbal medicine formulations. High-performance liquid chromatography (HPLC) analysis of Senna leaves was used to evaluate the presence of many different compounds, including sennoside, kaempferol 3-O-D-gentiobioside, aloeemodine 8-O-D-glucopyranoside, rhein 8-O-D-glucopyranoside, torachrysone 8-O-D-glucopyranoside, and isorhamnetine 3-O-D-gentiobioside. I concur, Mr. via the use of an acidic mobile phase and an RP18 column, the herbal composition known as Triphala, which is comprised of Emblica officinalis, Terminalia chebula, and T. belerica, was standardised via the application of the HPLC technology. The blend contains a significant amount of antioxidants. A combination of high-performance liquid chromatography (HPLC) and mass spectrometry (LC/MS) is considered to be the gold standard for determining whether or not traditional Chinese medicine is effective. Liquor, also known as Gan-Cao

Hyper-performance thin layer chromatography

Herbal analysis often uses TLC as its fingerprint technique. Using thin-layer chromatography (TLC), four different types of herbal remedies were resolved. [76] . The use of this procedure makes it possible to verify the species of Ginseng and Radix Puerariae, as well as to evaluate the consistency and consistency of their preparations from a variety of manufacturers. [77]. Despite the fact that high-performance thin-layer chromatography (HPTLC) fingerprints are often designated for the investigation of compounds with

mild to moderate polarity, Di et al. used automated multiple development in order to generate a fingerprint of fungal polysaccharide acid hydrolyzates. [78] . High-performance thin-layer chromatography, also known as HPTLC, is used in the pharmaceutical industry for a number of reasons, including the creation of processes, the detection of adulterants in herbal goods, the identification of pesticide and mycotoxin content, and the quality monitoring of herbal products and health foods. [79] . Four different Ashwagandha formulations were found to include Withaferin A and beta-sitosterol-dglucoside, which were both determined using the HPTLC method. [80]. Using high-performance thin-layer chromatography (HPTLC), the phytoconstituents of Syzygium jambolanum were quantitatively assessed, including the following: accuracy, repeatability, stability, and glycoside (jamboline), tannin, ellagic acid, and gallic acid. [81]. Bacoside A and B in Bacopa monnieria and its preparations were detected, monitored, and quantified using high-performance thin-layer chromatography (HPTLC). [82].

By measuring the THC concentration in urine samples using HPTLC, Cannabis stavia was standardized. [83] . Withaferine A, a component of Withania somnifera in herbal extract and polyherbal formulations, was estimated using high-performance thin-layer chromatography (HPTLC). [84] . Swetiamarin's quantitative estimate in several commercial polyherbal formulations and E. littorale fruit types (small, large, and fresh) has been documented using HPTLC. [85] . Standardized chandanasava was found to be an effective treatment for karsya, also known as malnutrition, through the use of organoleptic testing, physico-chemical analysis, thin-layer chromatography, and high-performance thin-layer chromatography. [86] . Ultra-performance liquid chromatography (UPLC) was used in order to evaluate the chemical alterations that occurred as a result of the decocting process, as well as the degree of chemical consistency that existed between traditional and dispensed granule decoctions. [87].[88]. The combination of chromatographic fingerprinting and metabolomics has the potential to accomplish the goal of determining the mechanism of action of traditional Chinese medicines (TCMs) and gaining further control over the intrinsic quality of these medications. The working mechanisms of TCMs were strengthened by rigorous investigation of chromatographic fingerprinting in combination with multivariate analytic techniques developed in bioinformatics and chemometrics. This was done in order to better control and increase the intrinsic quality of TCMs in a complete manner. [89].

Mass-spectrometry-liquid chromatography

At this point in time, LC-MS is the method of choice for many aspects of the drug development process. By utilizing LC-MS115 to perform chemical standardization on an aqueous extract of the mixture of twenty plants, twenty chemical components were obtained as reference markers. These components were used as reference markers. According to the findings of LC-MS study, aminoglycosides are removed by the kidneys at a rate of ninety percent, have a low affinity for plasma proteins, and are highly soluble in water. Furthermore, this technology makes it easier to do ion pairing chromatography analysis on plasma samples in order to determine the presence of aminoglycosides. For the purpose of determining the levels of aristolochic acid I and II in herbal medicines, two different high-performance liquid chromatography (HPLC) methods were developed. The first method used a photodiode array detector (LC/UV), whereas the second method utilised mass spectrometry (LC/MS). The LC/UV method included the use of a Cosmosil 5C18-MS column in combination with a gradient solvent system consisting of phosphate buffer-acetonitrile and a UV detector that was set to operate at a wavelength of 390 nm. For the LC/MS approach, a solvent solution consisting of acetonitrile and acetate buffer was used, and positive-ion electrospray ionization mass spectrometry was utilised. The fragment ions that are characteristic of aristolochic acid I

were selected at the mass-to-charge ratios of 359, 324.0, 298, and 296. On the other hand, the fragment ions of aristolochic acid II were selected at the mass-to-charge ratios of 329, 294, and 268.

Current Crude Drug Standardisation Regulations

There is a lot of knowledge that common Indians possess on the history of herbal medicine as well as its modern use. The size of the market for the traditional Indian system is difficult to provide a numerical value for. Given that the vast majority of practitioners are responsible for developing and administering their own formulas. On the other hand, the annual turnover of goods manufactured by big firms is believed to be somewhere about $300 million, which is far lower than the almost $2.5 billions that are spent on modern drugs. Researchers came to the conclusion that while there are some modern physicians who utilize Ayurvedic medications, the majority of them are not acquainted with them. When it comes to conditions such as the common cold, diarrhea, stomach difficulties, reproductive disorders, liver troubles, and skin illnesses, they are willing to test an Ayurvedic prescription as long as the effectiveness of the drug is backed by scientific proof. [90]

Ayurvedic drugs that are patent-exclusive are made available for purchase without the need for a prescription at pharmacies. It would seem that these products constitute a significant component of the traditional medicine that is commercially available in India. However, in order for practices such as Ayurveda to gain widespread acceptance and respect, they must first get the empirical support of modern medical science. More study is required to evaluate the advantages of traditional medicine systems in terms of their safety and efficacy. This is necessary in order to make better use of these supplemental medicine systems. The qualities and standards of a variety of plants and products generated from them have been detailed in monographs that have been issued by a number of pharmacopoeias on a worldwide scale. Several pharmacopoeias, including but not limited to

- Pharmacopoeia Committee
- Chinese Herbal Pharmacopoeia
- United States Herbal Pharmacopoeia
- British Herbal Pharmacopoeia
- British Herbal Compendium
- Japanese Standards for Herbal Medicine
- The Ayurvedic Pharmacopoeia of India (API)

Ensure the sustained high quality of herbs and herbal products in your nation by producing a monograph for them. In addition, the Indian government has issued Ayurvedic Pharmacopoeia India, which outlines 80 regularly used Ayurvedic herbal remedies and sets basic quality requirements for them.

Standardisation and Quality Control of Herbal Crude Drugs[91]

Physicochemical evaluation is a process that is defined by the World Health Organisation as the process of conducting a physicochemical evaluation of a crude drug. This process includes tasks such as selecting and handling the raw materials, evaluating the final product for safety, efficacy, and stability, documenting the

risks and benefits of the product based on previous experiences, informing consumers about the product, and promoting it.

- **Macro and Microscopic Examination:** In order to check for adulterants and identify the correct kind.

Foreign Organic Matter: Extraction of the medicinal compound from its natural environment.

Ash Values: In order to determine the authenticity and quality of crude drugs, certain criteria are used, such as total ash, sulfated ash, water-soluble ash, acid-insoluble ash, and so on.

Moisture Content: The product may be preserved against deterioration by checking its moisture content.

Extractive Values: Here we may see the estimated amount of chemical components in crude medication..

Crude Fiber: The purpose of establishing purity standards is to identify cases with excessive woody material.

Qualitative Chemical Evaluation: The process of identifying and characterizing crude drugs in relation to their constituent phytochemicals is covered.

Chromatographic Examination: Make use of the principal chemical component as a marker for rudimentary drug identification.

Qualitative Chemical Evaluation: Standards for estimating the quantity of the primary component group.

Toxicological Studies: Reducing the impact of pesticide residue, possibly harmful components, and microbiological count on the final product.

CONCLUSION

Conducting pharmaceutical analyses and standardizing herbal medications is very necessary in today's increasingly globalised healthcare system. This is necessary in order to ensure that herbal medicines are of high quality, safe, and effective. It is necessary to establish stringent standardization procedures for herbal drugs in order to fulfill regulatory standards and guarantee the safety of consumers. Herbal medicines are in great demand because of the natural benefits that are considered to be associated with them and the low incidence of adverse effects. A battery of physical, chemical, and biological procedures are used in order to analyze the active components of herbal medicines. This is done in order to guarantee that the active components are consistent. To identify, quantify, and verify the consistency of these substances, advanced analytical methods like HPLC, HPTLC, TLC, GC, and LC-MS are necessary. It is vital to precisely identify and measure bioactive components such as alkaloids, flavonoids, glycosides, and terpenoids in herbal medicines in order to ensure that the therapeutic efficacy of these medicines is preserved. Pharmaceutical analysis helps to limit the amount of variation in raw materials and ensures that herbal formulations are effective, safe, and dependable. This is accomplished by adhering to stringent quality control procedures and developing standardized criteria for each herbal treatment. As the herbal medicine industry continues to expand, standardization and analytical approaches will become more important in order to facilitate the incorporation of herbal medicines into conventional pharmaceutical operations and healthcare systems all over the globe.

References

1. Agarwal, A., 2005. Critical issues in Quality Control of Herbal Products, Pharma Times, 37(6), Pp 09-11.
2. Sane, R.T., 2002. Standardization, Quality Control and GMP for Herbal drugs, Indian Drugs, 39 (3), 184-190.
3. Florey, H. W., Chain, E. and Florey, M. E., 1949. Vol. 1. The antibiotic, Oxford University Press. New York. P p. 576-628.
4. Zafar R, Panwar R, Sagar Bhanu PS. Herbal drug standardization: The Indian Pharmacist 2005; 4(36): 21-25.
5. Patra KC, Pareta SK, Harwansh RK, Jayaram Kumar K .Traditional approaches towards standardization of herbal medicines -Areview. J Pharm Sci Technol 2010; 2 (11): 372- 379.
6. Straus SE. Herbal remedies. New Engl J Med 2002; 347: 2046–2056.
7. Indian Herbal Pharmacopoeia, Indian Drug Manufacturers' Association, Mumbai, 2002.
8. British Herbal Pharmacopoeia, British Herbal Medicine Association, 1996.
9. Quality Control Methods for Medicinal Plant Materials, WHO, Geneva, 1996.
10. Bhutani KK. Herbal medicines enigma and a challenge for science and guidelines for new initiatives. J Nat Prod 2003;19(1): 3-8.
11. Mosihuzzaman M, Choudhary MI. Protocols on safety, efficacy, standardization, and documentation of herbal medicine. Pure Appl Chem 2008; 80(10):2195–2230
12. Seitz U, Bonn G, Oefner P, Popp M. Isotachophoretic analysis of flavonoids and phenolcarboxylic acids of relevance to phytopharmaceutical industry. J Chromatogr 1991;559, 499-504.
13. O'Shea TJ .Capillary electrophoresis/electrochemistry. Curr Sep 1995;14(1): 18-23.
14. Svicekova M, Havranek E, Novak V. Determination of heavy metals in samples of herbal drugs using differential pulse polarography. J Pharm Biol 1993; 42(2):68-70.
15. Anonymous: National Health Policy. New Delhi: Ministry of Health and Family Welfare, Government of India; 1983.
16. Indianmedicine.nic.in/writereaddata/.../2469101869- A%20to%20H.p
17. Pattanayak P, Behera M, Mohapatra P, Panda SK. Standardization and evaluation of laxative activity of a polyherbal formulation. Der Pharmacia Lettre 2011; 3(1): 276-286.
18. Patwardhan B, Warude D, Pushpangadan P, Bhatt N. Ayurveda and traditional Chinese medicine: A comparative overview. Evidence-Based Complementary and Alternative Medicine (eCAM) 2005; 2(4): 465–473.
19. Vijaya Kumar D, Raghavan KV. Novel chromatographic fingerprinting method for standardization of single medicines and formulations. Indian Institute of Chemical Technology, Hyderabad, WO 0246739-

EP2 0000991 991- 263397CSIR G01N30-88, 2002.

20. Mukherjee PK, Venkatesh M, Kumar V. An overview on the development in regulation and control of medicinal and aromatic plants in the Indian System of Medicines. Bol Latinoam Caribe Plant MedAromaticas. 2007; 6(4):129- 137.

21. www.who.int/medicinedocs/en/d/Js4928e/; accessed 28 Nov 2011. 30. www.who.int/medicinedocs/index/assoc/s7148e/ s7148e.pdf

22. Government of Canada. Natural Health Products Regulations; 2009. Available at: http://laws.justice.gc.ca/ e n /s h o w t d m / c r/ S O R- 2 0 0 3 - 1 9 6 / / ? s h o w t o c = & instrumentnumber=SOR-2003-196 [accessed November 22, 2009.

23. http://apps.who.int/medicinedocs/en/d/Js7916e/9.5.html

24. Bauer R. Quality criteria and standardization of phytopharmaceuticals: Can acceptable drug standard can be achieved?.J Drug Inform 1998; 32:101-110.

25. Sharma AK, Gaurav SS, Balkrishna A. A rapid and simple scheme for the standardization of polyherbal drugs. Int J Green Pharm 2009; 3:134-140

26. Satheesh Madhav NV, Kumud Upadhyaya, Bisht A. Phytochemical screening and standardization of polyherbal formulation for dyslipidemia. Int J Pharm Pharmaceut Sci 2011;3:56-59.

27. Patwardhan B. Amethod of treating musculoskeletal disease and a novel composition there of. US Patent 5494668, 1996.

28. Chandel HS, Pathak AK, Tailang M. Standardization of some herbal antidiabetic drugs in polyherbal formulation. J Pharmacog Res 2011;3(1):49-56

29. Meena AK, Rao MM, Panda P, Kiran, Yadav A, Singh U, et al. Standardisation of ayurvedic polyherbal formulation, Pancasama Churna. Int J Pharmacog Phytochem Res 2010;2(1):11-14.

30. Sanjay J, Sweta S, Rakesh B, Praveen K. Standardization of 'Dashamularishta': A polyherbal formulation. Pharmacog J 2009;1(3):54-57.

31. Kumar T, Chandrashekar KS, Tripathi DK, Nagori K, Pure S,Agrawal S, et al. Standardization of "Gokshuradi Churna" An ayurvedic polyherbal formulation. J Chem Pharm Res 2011;3(3):742-749.

32. Kasthuri KT, Radha R, Jayshree N, Austin A, Shanthi P. Development of GC-MS for a poly herbal formulationMEGNI. IntJ Pharm Sci 2010;2:81-83.

33. Meena R, Meena AK, Khan SA, Mageswari S. Standardization of Unani polyherbal drug - Jawarish-eDarchini. J Pharm Res 2010;7(3):11-12.

34. Rajani M, Kanaki NS .Phytochemical standardization of herbal drugs and polyherbal formulations. Bioactive Molecules and Medicinal Plants, (Ramawat KG, Mérillon JM (Eds.) Springer 2008; 349-369.

35. Ahmad I, Aqil F, Owais M. Turning medicinal plants into drugs. Modern Phytomed 2006; 384:67-72

36. Rajurker S, Rekhe DS, Maini S, Ravikanth K. Acute toxicity studies of polyherbal formulation

(Methiorep Premix). Vet World 2009; 2(2):58-59.

37. Wani M.S. Herbal Medicine and its Standarization.Pharma info, 5(6),2007, 1-6

38. Patwardhan B. Ayurveda the designer medicine: a review of ethnopharmacology and bioprospective research. Indian Drugs, 37 (5), 2000,2046-56

39. Dixit V.K. &Yadav.N.P. Recent approaches in herbal drug standardization. IntegrBiol,2 (3), (2008), 195-203.

40. Anna KD, Stephen PM. Safety issues in herbal medicine: implications for the health professions. Med J Aust166 (1997), 538-541.

41. Michael DR. Herbal medicine: a practical guide to safety and quality assurance. West J Med 171 (1999), 172-175.

42. Shinde VM, Dhalwal K, Potdar M &Mahadik KR. Application of quality control principles to herbal drugs. Int J Phytomedicine. 1(2009), 4–8

43. Hussain Khalid , Majeed M, Ismail Zhari, SadikunAmirin&Ibrahim Pazilah .Traditional and complementary medicines: Quality assessment strategies and safe usage ;Southern MedReview , 2 (1) (April 2009), 19-23

44. Quality control methods for medicinal plant materials (WHO) , Geneva, A.T.T.B.S Publishers and Distributers, Delhi. 2002

45. Raya Luis Gomez, Hanne Gro Olsen, FrodeLingaas, HelgeKlungland, Dag IngeVåge, Ingrid Olsaker, SeblewengelBekeleTalle, Monica Aasland, and Sigbjorn Lien. The Use of Genetic Markers to Measure Genomic Response to Selection in Livestock. Genetics. 2002,162, 1381-1388

46. Khanuja SPS, Shasany AK, Aruna V, Darokar MP, Kalra A, Bahl JR, Bansal, RP RAPD marking of three Pelargonium graveolens genotypes with chemotypic differences in oil quality., J. Med. Aromat. Plant Sci. 2002, 24: 729–32.

47. Ramakrishna W, Lagu M D, Gupta V S, Ranjekar P K., DNA fingerprinting in rice using oligonucleotide probes specific for simple repetitive DNA sequences., The Applied Genetics. 1994, 88,402-406

48. Lau.D. T., Shaw P. C,., Wang J., and Shaw P. C., Authentication of medicinal Dendrobium species by the internal transcribed spacer of ribosomal DNA, Planta Med.,2001, 67, 456–460

49. Ratnaparkhe M. B., Gupta V. S., Venmurthy M. R. and Ranjekar P. K. Genetic fingerprinting of pigeonpea (Cajanuscajan (L.) Millsp.) and its wild relatives using RAPD markers, The. Applied Genetics, 1995, 91, 893–98.

50. Shaw P. C. and But P. P. Authentication of Panax species and their adulterants by random-primed polymerase chain reaction. Planta Med. 61,(1995), 466–69

51. Harborne A.J. Phytochemical Methods A Guide to Modern Techniques of Plant Analysis. 3ed. New York; Springer Dordrecht; 1998. 110-130p.

52. Gong F, Liang Y, Xie P, Chau F. Information theory applied to chromatographic fingerprint of herbal medicine for quality control. J. Chromatogr. A. 2003;1002(1-2); 25-40

53. Wagner H, Bladt S, Rickl V. Plant Drug Analysis: A Thin Layer Chromatography Atlas. 2ed. New York; Springer Berlin, Heidelberg; 1996. 54-71p.

54. Brochmann-Hanssen E, Svendsen A. Gas Chromatography of Alkaloids, Alkaloidal Salts and Derivatives. J. Pharm. Sci. 1962; 51(11); 1095-1098

55. Soleas G, Diamandis E, Karumanchiri A, Goldberg D. A Multiresidue Derivatization Gas Chromatographic Assay for Fifteen Phenolic Constituents with Mass Selective Detection. Anal. Chem. 1997; 69(21); 4405-4409

56. Majlat P, Barthos E. Quantitative gas and thinlayer chromatographic determination of methyl paraben in pharmaceutical dosage forms. J. Chromatogr. A. 1984; 294; 431-435

57. Molyneux R, Mahoney N, Bayman P, Wong R, Meyer K, Irelan N. Eutypa Dieback in Grapevines: Differential Production of Acetylenic Phenol Metabolites by Strains of Eutypa lata. J. Agric. Food Chem. 2002; 50(6);1393-1399

58. Angerosa F, D'Alessandro N, Corana F, Mellerio G. Characterization of phenolic and secoiridoid aglycons present in virgin olive oil by gas chromatography-chemical ionization mass spectrometry. J. Chromatogr. A. 1996; 736(1-2); 195-203

59. Bunzel M, Ralph J, Marita J, Hatfield R, Steinhart H. Diferulates as structural components in soluble and insoluble cereal dietary fibre. J Sci Food Agric. 2001; 81(7); 653-60

60. El-Shazly A, Tei A, Witte L, El-Domiaty M, Wink M. Tropane Alkaloids of Hyoscyamus boveanus, H. desertorum, H. muticus and H. albus from Egypt. Z. Naturforsch. 1997; 52(11-12); 729-39

61. Ylinen M, Naaranlahti T, Lapinjoki S, Huhtikangas A, Salonen M, Simola L et al. Tropane Alkaloids from Atropa belladonna; Part I. Capillary Gas Chromatographic Analysis. Planta Med. 1986; 52(02); 85-7

62. Guetens G, De Boeck G, Wood M, Maes R, Eggermont A, Highley M et al. Hyphenated techniques in anticancer drug monitoring. J. Chromatogr. A. 2002; 976(1-2); 229-38

63. Leung A, Gong F, Liang Y, Chau F. Analysis of the Water Soluble Constituents of Cordyceps sinensis with Heuristic Evolving Latent Projections. Anal. Lett. 2000; 33(15); 3195-3211

64. Gong F, Liang Y, Xu Q, Chau F. Gas chromatography–mass spectrometry and chemometric resolution applied to the determination of essential oils in Cortex cinnamomi. J. Chromatogr. A. 2001; 905(1-2); 193-205

65. Gong F, Liang YZ, Cui H, Chau FT, Chan BT. Determination of volatile components in peptic powder by gaschromatography – mass spectrometry and chemometric resolution. J. Chromatogr. A. 2001; 909(2); 237-247

66. Gong F, Liang YZ, Xu QS, Chau FT, Ng KM. Evaluation of separation quality in twodimensional hyphenated chromatography. Anal. Chim. Acta. 2001; 450(1-2); 99-114

67. Velasco-Negueruela A, Perez-Alonso MJ, Perez de Paz PP, Pala-Paul J, Sanz J. Analysis by gas chromatography–mass spectrometry of the essential oil from the aerial parts of Pimpinella junoniae Ceb. & Ort., gathered in La Gomera, Canary Islands Spain. J. Chromatogr. A. 2003; 1011(1-2); 241-244

68. Lazarowych NJ, Pekos P. Use of Fingerprinting and Marker Compounds for Identification and Standardization of Botanical Drugs: Strategies for Applying Pharmaceutical HPLC Analysis to Herbal Products. Drug Inf J. 1998; 32(2); 497-512

69. Li XN, Cui H, Song YQ, Liang YZ, Chau FT. Analysis of volatile fractions of Schisandra chinensis (Turcz.) Baill. using GC-MS and chemometric resolution. Phytochem Anal. 2003;14(1); 23-33

70. Tseng MC, Tsai MJ, Lin JH, Wen KC. GC/MS analysis on anorectics adulterated in traditional chinese medicines. J Food Drug Anal. 2020; 8(4); 315-330

71. Zhang H, Shen P, Cheng Y. Identification and determination of the major constituents in traditional Chinese medicine Si-Wu-Tang by HPLC coupled with DAD and ESI–MS. J. Pharm. Biomed. Anal. 2004; 34(3); 705-713

72. Chimezie A, Ibukun A, Teddy E, Francis O. HPLC analysis of nicotinamide, pyridoxine, riboflavin and thiamin in some selected food products in Nigeria. Afr J Pharm Pharmacol 2008; 2(2):29-36.

73. Saravanan J, Shajan A, Joshi NH, Varatharajan R,Valliappan K. A simple and validated RP-HPLC method for the estimation of methylcobalamin in bulk and capsule dosage form. IntJ Chem Pharm Sci 2010;1(2):323-324.

74. Rao Udaykumar B, Anna NP .Stability- indicating HPLC method for the determination of efavirenz in bulk drug and in pharmaceutical dosage form. Afr J Pharm Pharmacol 2009;3(12):643-650.

75. Anupam S, Krishan L, Handa SS. Standardization: HPLC determination of vasicine in polyherbal formulations. Pharm Biol 1992; 30(3):205-208.

76. Demirezer O, Karahan N, Ucakturk E, Kuruuzum-Uz A, Guvenalp Z, Kazaz C. HPLC fingerprinting of sennosides in laxative drugs with isolation of standard substances from senna leaves. J Nat Prod 2011; 5(4):261-270.

77. Singh DP, Govindarajan R, Rawat AKS. High-performance liquid chromatography as a tool for the chemical standardisation of Triphala an ayurvedic formulation. Phytochem Anal 2007; 19(2):164-168.

78. Vogel H, González M, Faini F,Razmilic I,Rodríguez J, Martín JS, et al. J Ethnopharmacol 2005; 97:97–100.

79. Xie P.S., Chen S.B., Liang Y.Z., Wang X.H., Tian R.T. & Roy Upton (2006). Chromatographic fingerprint analysis— a rational approaches for quality assessment of traditional Chinese herbal medicine J Chromatogr A 2006; 1112:171–

80. Di X, Kelvin K. C. Chan, Hei Wun Leung & Carmen W. Hui. (2003). Fingerprint profiling of acid hydrolyzates of polysaccharides extracted from the fruiting bodies and spores of Lingzhi by high-performance thin-layer chromatography. J Chromatogr A. 2003; 1018:85-95.

81. Soni K, Naved T. HPTLC- Its applications in herbal drug industry. The Pharma Review 2010:112-117.

82. Jirge SS, Tatke PA, Gabhe S. Development and validation of a novel HPTLC method for simultaneous estimation of beta-sitosterol- d-glucoside and Withaferin A. Int J Pharm Pharmaceut Sci 2011;3(Suppl 2):227-230.

83. Shanbhag DA, Khandagale NA. Application of HPTLC in the standardization of a homoeopathic mother tincture of Syzygium jambolanum. J Chem Pharm Res 2011; 3(1):395- 401.

84. Shahare MD and Mello PM .Standardization of Bacopa Monnieri and its formulations with reference to Bacoside A, by high performance thin layer chromatography. Int J Pharmacog Phytochem Res 2010; 2(4):8-12.

85. Priyamvada S, Srinivas Bharath MM, Pratima M. Qualitative high performance thin layer chromatography (HPTLC) analysis of cannabinoids in urine samples of Cannabis abusers. Indian J Med Res 2010; 132:201-208.

86. Mahadevan N, Rahul PK, Subburaju T and Suresh B .HPTLC analysis of Withaferine A from an herbal extract and polyherbal formulations. J Sep Sci 2003; 26:1707–1709.

87. Patel PM, Patel KN, Patel NM and Goyal RK .A HPTLC method for quantitative estimation of swetiamarin in marketed polyherbal antidiabetic formulations. Indian J Pharm Sci 2007; 69: 446-448.

88. Shankar K, Singh A. Standardization of polyherbal formulation: Chandanasava. Int J Res Ayur Pharm 2011; 2(2):665-669.

89. Li SL, Lai SF, Song JZ, Qiao CF, Liu X, Zhou Y, et al. Decocting-induced chemical transformations and global quality of Du–Shen–Tang, the decoction of ginseng evaluated by UPLC–Q-TOF-MS/MS based chemical profiling approach. J Pharm Biomed Anal 2010; 53946–957.

90. Li SL, Song JZ, Qiao CF, Zhou Y, Qian KD, Xu HX. UPLC– PDA–TOFMS based chemical profiling approach to rapidly evaluate chemical consistency between traditional and dispensing granule decoctions of traditional medicine combinatorial formulae.J Pharm BiomedAnal 2010; 52:468– 478.

91. Liang YZ, Xie PS, Chan K. Chromatographic fingerprinting and metabolomics for quality control of TCM. Comb Chem High Throughput Screen 2010; 13(10):943-953.