**ABSTRACT**

* Herbal drugs have been employed for centuries to cure various health ailment, but their inherent variability and multifaceted chemicals nature create immense challenges in guaranteeing uniform quality and safety. High performance liquid chromatography (HPLC) is a highly effective analytical method that is vital in the standardization of herbal drug. This work examines the utility of HPLC in determining, quantifying, and measuring the purity of bioactive compound in herbal remedies. Using HPLC, producers can properly identify the active ingredients concentration, including alkaloids, flavonoids, and terpenoids, to confirm that every batch of herbal remedy is of set potency and quality. HPLC is also crucial in identifying impurities, contaminants, and adulterants, further confirming the safety of herbal remedies. The technique also facilitates stability testing, which aids in the determination of shelf life and storage conditions. In spite of difficulties such as the high cost of equipment and the intricacy of herbal preparations, HPLC is still a vital tool for guaranteeing the safety, efficacy, and uniformity of herbal medicines, play a crucial role in the overall standardization process the project emphasize the role of HPLC in contemporary herbal pharmaceutical markets and its continuing utility in safe supply of herbal preparation to the consuming public.
* There is growing recognition and overall acceptability of the employees of herbal drugs in contemporary medical practice. While most of these usage is non-traditional, it is however a established fact that more than 80% of the global population rely on herbal drug and product for healthy existence. This growth in the consumption of herbal product has also resulted I the growth of numerous forms of abuse and contamination of the product leading to consumer’s and manufacturers dismay and in some cases, to lethal effect. The challenges is myriad and vast renderings the globe herbal market unsafe. This review aims to inform stakeholders I herbal medicine of the necessity to set quality standards for collection handling, processing and production of herbal medicine and to use such standards in upholding the safety of the global herbal market. Good quality assurance and standardization process of herbal products and medicines were also addressed.
* The application of the use of herbal drugs in the treatment and prevention of different health conditions is in existence since time immemorial. Usually it is thought that there is very less risk from herbal drugs, but serious reactions report on pointing towards the necessity for the construction of effective markers system for isolations and identification of the individual constituents. possible, but challenging to achieve. In addition, regulation of these medicine is not consistence between nation. There are differences in the approaches used between medicines systems and nation in achieving stability and quality control. The currents study makes an endeavor to determine the progression of technological standards in production and the regulation guideline formulation for commercialization of herbal drugs

 **KEYWORDS:**

* herbal drug, standardization, herbal formulation, quality control

 **INTRODUCTION**

* Herbal drug are plant material or parts there of which have gone through simple drying, storage and basic harvesting operation have been processed into phyto pharmaceuticals. Also incorporate other plant crude product made from plant but now lacking any organic structure like essential oil, fatty oils, resin and gum as additional useful constituent of the term for quality control of raw material and finished herbal product, separation analytical technique like high performance liquid chromatography, gas chromatography, and mass spectrometry were among the most frequently used. Due to its simplicity and reliability, the method of fingerprinting analysis using high performance thin layer chromatography has come to be the most effective tool for quality control of herbal medicines. It may be used as tool for identification, authentication, and quality control of herbal drug.
* Plant product are increasingly sought after as medicines, nutraceuticals, and cosmeceuticals. They can be bought over the counter in pharmacies and healthy food shops for self-medication or as drug prescribed by non-allopathic physicians. Human being values quality above everything else in every sphere of life it is imperative that human drug is of superior quality since they are used to guarantee they well-being of human beings. The quality control of drug prepared from synthetically produced chemicals is regulated by tough rules and regulations. They have to pass many test and quality standards ton be sold and consumed by patients and consumers.
* The medicinal use of herbs is the oldest known form of healthcare to mankind and has been utilized by all cultures through history. It is extremely crucial that, a system of standardization is developed for each plant medicines available in the market, because the potential for variation in
* various batches of medicines is immense. The content of phytochemicals in plant material may differ depending upon location and time of harvest, which untimely influence therapeutic efficacy. Various environmental
* conditions and cultivation practices also are important factor for production of specific medicinal plant. This suggest the requirement of appropriate quality control test for whole preparation in order to assure quality of the product. Since commercialization of the herbal drug in process, safe assurance, quality and efficacy become a matter of concern. The herbal raw material is susceptible to much variability due to various factor, the significant one being, the identity of the plant and seasonal variation (which has an influence on the collection time), the ecotypes, genotypes and chemo types variations, drying and conditions of storage and existence of xenobiotic standardization, American herbal product associations. Standardization means the body of information and control required to the product material of decent consistency. Achieved by reduction of the unavoidable variation of the natural product compositions by quality assurance procedures applied in agricultural and production process.
* Determining the consistency of the active ingredients and making sure the herbal product contains the anticipated therapeutic components without hazardous contaminants are two steps in the process of standardizing herbal drugs to ensure that they meet specified quality specifications in terms of their content, potency, and safety.
* **The standardization of herbal drugs is vital for:**

  **Ensuring consistency** across batches.

  **Guaranteeing safety** by ensuring the absence of harmful substances.

  **Verifying efficacy** through the correct dosage of bioactive compounds.

  **Complying with regulatory requirements** set by health authorities.

* The percentage of active ingredients in herbal medications may naturally vary because they are made from plants. Because of this, standardization becomes difficult, which is where advanced analytical methods like HPLC are useful.

 **ROLE OF HPLC IN HERBAL DRUG**

* One chromatographic method for separating, identifying, and quantifying each component in a mixture is high-performance liquid chromatography (HPLC). It is especially helpful for examining complicated mixes of active ingredients, including terpenoids, alkaloids, flavonoids, and phenolic chemicals, in the case of herbal medications. In high-performance liquid chromatography (HPLC), a sample is run under high pressure through a column filled with a solid stationary phase. The components of the sample are separated according to how they interact with the stationary phase. A detector (often UV/Vis, fluorescence, or mass spectrometry) finds and measures the separated chemicals. Because it is easy to learn and use and is not impacted by the sample's stability or volatility, HPLC is a commonly in utilized method for medicinal plant analysis. The most widely utilized columns for the analytical separation of herbal medicines are reversed-phase (RP) columns. Nowadays, practically all analytical labs worldwide employ HPLC

**A.** **Identification of Active Constituents**

* Finding the active chemical components in herbal medications is one of the main applications of HPLC in herbal drug standardization. Numerous bioactive substances are found in plants, and some molecules are frequently responsible for their therapeutic benefits. Herbal medications can be examined for the presence of these substances using HPLC. For examples

  **Alkaloids** in Echinacea or Ephedra.

  **Triterpenes** in Ganoderma lucidum

  **Flavonoids** in Ginkgo biloba

* These components can be precisely identified by HPLC utilizing spectrum data or by comparison with established standards. Manufacturers can make sure that the right chemicals are present in enough amounts by identifying the essential components.

**B**. **Quantification of Bioactive Compounds**

* Determining the precise quantity of active components in the finished product is a common step in the standardization of herbal medications. When it comes to precisely measuring bioactive chemicals, HPLC is quite successful. This is necessary to guarantee that the herbal product has the intended medicinal benefits. For example, St. John's Wort can be standardized in terms of its hypericin content since the active ingredient, hypericin, can be measured using HPLC. Likewise, HPLC may be used to measure the amount of curcumin present in turmeric extracts.

**C**. **Quality Control**

* HPLC is an indispensable tool in **quality control** during the manufacturing process. It allows manufacturers to:

 **Ensure batch-to-batch consistency:**

* Manufacturers can verify that the concentrations of active ingredients stay constant by comparing HPLC profiles from several batches, guaranteeing consistency in the therapeutic effects.

**Detect impurities and contaminants:**

* Unwanted materials like pesticides, heavy metals, or other harmful components can be found in herbal products using HPLC

 **Ensure the absence of adulteration:**

* it helps detect the presence of adulterants or substitutes that might be introduced in herbal product

**D**. **Stability Testing:**

* Additionally, HPLC is used in stability testing to track the changes in the active components in herbal products over time under different storage circumstances, such as exposure to light, humidity, and temperature. This guarantees that the product will remain safe and effective for the duration of its shelf life. Manufacturers can ascertain the product's expiration date with the aid of routine HPLC testing.

**E.** **Standardization of Extracts:**

* Many herbal items are processed into extracts rather than being utilized in their full plant form. Depending on the extraction technique, plant source, and environmental conditions, the concentration of active compounds in these extracts might vary substantially. These extracts are standardized with the aid of HPLC by:

Ensuring that they contain the right amount of active compounds**.**

**F.** **Development of Herbal Formulations**

* By ensuring that mixtures of several herbs or active components are properly prepared, HPLC aids in the development of herbal formulations. HPLC can assist in figuring out how various active ingredients interact and how effective they are when combined in poly herbal compositions.

**G. Regulatory Compliance**

* In order to ensure adherence to pharmacopoeia standards, such as those established by the European Pharmacopoeia (EP) or the United States Pharmacopoeia (USP), HPLC is essential. These standards frequently call for a particular active component content, which HPLC can ascertain. Before herbal goods are put on the market, it makes sure they fulfil the necessary requirements for quality, safety, and efficacy.

**H. Monitoring of Degradation Products**

* Herbal drugs can undergo degradation over time, particularly when exposed to light, heat, or moisture. This degradation can lead to the loss of bioactivity or the formation of harmful by-products. HPLC can be used to monitor these degradation products, ensuring that the herbal drug remains within its shelf life and is safe for consumption

 **BENEFITS OF USING HPLC FOR HERBAL DRUG STANDARDIZATION**

 **Supports Regulatory Compliance**

* Herbal goods must meet certain requirements set by regulatory authorities such as the FDA, WHO, and other national bodies. HPLC helps manufacturers meet these standards by offering a straightforward, scientifically verified method for assessing the safety and quality of their products.

**Application in Pharmacokinetic Studies**

* The absorption, distribution, metabolism, and excretion (ADME) of herbal substances in the human body is another area of research that uses HPLC. Understanding the pharmacokinetic characteristics of herbal medications is essential for creating safe and efficient herbal formulations.

 **Non-destructive Testing**

* Herbal samples can be analysed by HPLC without being destroyed, enabling additional testing and guaranteeing that important components in the herbs are kept for future study if necessary.

###  ****Establishing Standardization Criteria****

* For regulatory agencies, the capacity to create standard profiles of herbal medications using HPLC data is essential. This enables producers to standardize their goods based on particular indicators or chemical signatures that show the herb's strength and purity.

 **LITERATURE SURVEY**

**GUIDELINES FOR HERBAL DRUG STANDARDIZATION(WHO)**

* The WHO's criteria can be summed up as follows: The topic of herbal medication standardization is extremely broad and complex.
* in reference to the drug's identity. Assessing plants by sensory characteristics, foreign organic materials, microscopy, histology, histochemistry, quantitative studies, and
* referring to the drug's physicochemical properties. Identification via physical and chemical characteristics, chromatographic fingerprints, moisture content, ash and extractive values, volatile oil and alkaloidal testing, and quantitative estimation procedures
* The pharmacological parameters, biological activity profiles, bitterness values, haemolytic index, astringency, swelling factor, foaming index, and so on are all included
* Details of toxicity include heavy metals, pesticide residues, pathogens as Salmonella, E. Coli, P. Aeruginosa, S. aureus, and Enterobacteria as well as microbiological contamination

**REGULATORY APPROACHES FOR HERBAL MEDICINES**

* In 1986, the WHO International Conference on Drug Regulatory Authorities was the first to incorporate herbal medicines. WHO created draft guidelines for evaluating herbal medicines in 1991, and the 6th International Conference on Drug Regulatory Authorities (ICDRA) endorsed them. This covers the fundamental standards for the effectiveness, safety, and quality of herbal medications. National regulatory bodies, scientific associations, and manufacturers can all benefit greatly from these principles as they evaluate submitted material. WHO continued to create pharmaceutical monographs on herbal medicines in 1991 in response to the recommendations of the 6th ICDRA. These monographs were based on the following guidelines for the evaluation of herbal medicines: Part I: Botanical Characteristics, Major Active Constituents, and Quality Control (QC); and Part II: Summaries of Clinical Applications, Pharmacology, Precautions, and Adverse Reactions. The documentation of herbal medicine should include information on the technologies, cultivation, and harvesting processes, such as plantation development and processing techniques; the prior validation of products used in herbal medicine; the properties of synthetic products that are identical to or related to the active constituent(s) of the medicine; the chemistry of herbs thought to be responsible for the activity; the outcomes of any clinical trials conducted on the product and aspects of marketing and trading; and legal issues, including intellectual property rights. Only a small portion of the hundreds of thousands of plant species have been thoroughly studied in the lab, making this an enviable task.
* Herbal items can only be sold as dietary supplements in the US. The U.S. Food and Drug Administration (FDA) must approve some health claims. According to the European Guidelines for the Assessment of Herbal Medicines, unless there is scientific proof to the contrary, a substance's historical use can be used to document its safety and effectiveness. The European approach has two characteristics: There is no inherent prejudice against complex plant substances; they are deemed safe and effective. Herbal medications are less expensive, approved more quickly, and one can apply the doctrine of reasonable certainty," which does not compromise safety.
* Providing scientific knowledge on the safety, effectiveness, and quality control of medicinal plants, as well as facilitating the appropriate use of herbal medicines, were the goals of the WHO monographs.

**TRADITIONAL APPROACHES FOR STANDARDIZATION OF HERBAL MEDICINE**

* Herbal medicine quality control techniques include sensory inspection, which includes both microscopic and macroscopic analyses. When comparing the macroscopic identification of botanical materials to a standard reference material, factors such as shape, size, colour, texture, surface features, fracture characteristics, odour, taste, and other organoleptic aspects are taken into consideration. Comparative microscopic examination of broken, powdered, crude, and botanical materials is part of microscopy. Analytical examination is done using instrumental techniques such thin layer chromatography, HPLC, GC.MS, LC.MS, near infrared (NIR), and spectrophotometry, among others. Analytical HPLC differs from preparative HPLC in that it considers both the level of solute purity and the quantity of compound that may be produced in a given amount of time, or throughput or recovery.

 **Adoption of HPLC for Herbal Drug Analysis (1980s - 1990s**

* The pharmaceutical sector, which includes herbal medicine, started to see the potential of HPLC for quality control and standardization by the late 1980s and early 1990s. Given the complexity of plant materials, which contain a wide range of bioactive chemicals, traditional methods of testing herbal remedies were frequently insufficient.
* 980s: The isolation and identification of bioactive phytochemicals, including alkaloids, flavonoids, terpenoids, and essential oils, was the main emphasis of the first HPLC uses in herbal medicine.
1990s: The use of HPLC in the examination of herbal medications rose as interest in ethno pharmacology and phototherapy grew. As knowledge of the pharmacological significance of active components grew, it became imperative to guarantee that herbal medications included the right amounts of these active substances. HPLC techniques for analysing active markers in herbs including ginseng, ginkgo, Echinacea, and St. John's Wort gained popularity in both industry
**INTEGRATIONS OF HPLC WITH MODERN TECHNIQUES (2010s)**
* HPLC technology developed in tandem with the growing complexity of herbal formulations and the growing demand for herbal medicine. In the 2010s and beyond, HPLC became a standard tool for more complex studies that combined nuclear magnetic resonance (NMR), mass spectrometry (MS), and multi-dimensional chromatography to obtain a more thorough profile of herbal compounds, as well as for the analysis of individual active ingredients.
* 2010s: Researchers were able to more precisely identify unknown substances in herbal medications thanks to the combination of HPLC and mass spectrometry (HPLC-MS), which improved purity and potency assessments and allowed for the full characterization of herbal extracts. For example, techniques like HPLC-DAD (Diode Array Detection) and HPLC-UV have become widely utilized to identify polyphenolic chemicals in plants like green tea (Camellia sinensis) and turmeric (Curcuma longa).
* 2010s–2020s: Studies on sustainability and bioavailability gained attention, and HPLC remained a key component of these efforts. To learn more about the clinical effectiveness of herbal remedies, researchers started examining how the body absorbs, metabolizes, and excretes herbal chemicals. Research on substances such as flavonoids and curcumin benefited from the accurate, high-resolution measurements that HPLC offered.

**PROCEDURE**

**CHROMATOGRAPHY METHODS**

* It is possible to identify a herbal material or extract with the use of chromatographic and chemical procedures. For fingerprinting, spectroscopic techniques like IR, NMR, and UV as well as chromatographic techniques like HPLC, TLC, GC, and capillary electrophoresis may be employed. DNA fingerprinting has been extensively employed in numerous species, such as Panax species and their adulterants. Identifying herbal materials, establishing raw material requirements, standardizing botanical preparations throughout all manufacturing processes, and obtaining stability profiles are all possible with the use of marker compounds. A list of phytoconstituents used for the examination of crude pharmaceuticals or herbal formulations incorporating these crude drugs was provided by the Central Council for Research in Ayurveda and Siddha.

**HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)**

* both analytical and reparative The pharmaceutical sector makes extensive use of HPLC to separate and purify herbal components. There are essentially two kinds of preparative HPLC: high pressure HPLC (pressure >20 bar) and low pressure HPLC (usually < 5 bar). Resolution, sensitivity, and quick analysis time are crucial factors to take into account. Several components of a medical mixture made up of multiple crude medicines have been analysed using HPLC. HPLC is the most widely used analytical technique for standardizing Indian herbal medicines because of its accuracy, adaptability, and affordability, which is an improvement over the one or two marker quantitative approach. One of HPLC's primary benefits is that it may be connected with a variety of detectors, including UV, DAD, ELSD, FLD, RID, MS, and NMR, among others, providing a greater number of options for detecting various

**THIN LAYER CHROMATOGRAPHY**

* TLC is a straightforward, affordable, adaptable, and targeted technique for identifying herbal medications. The distinctive feature of TLC's picture-like image provides an easy-to-understand visible profile. 24 These days, HPTLC is a standard analytical method. It has been widely documented that using less mobile phase than in HPLC allows for the simultaneous running of many samples. Additionally, it has been reported that HPTLC can be performed with mobile phases that have a pH of 8 or higher. The ability to repeatedly detect (scan) the chromatogram under the same or different circumstances is another benefit of HPTLC. As a result, HPTLC has been studied for the simultaneous testing of several components in a formulation with multiple components. This method makes it feasible to authenticate diverse plant species and assess the durability and uniformity of their preparations from various manufacturers.

 **Table 1. Quantitative applications of HPLC method for identification of herbal drugs**

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##  **quantitative application of HPLC method with, UV detector for identification of herbal drug**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Sr.No.** | **Name of Drug/ Chemical** | **Parameters for Validation** | **Evaluation Parameters of Standardization** |  |
| 1 | *Cannabis sativa*Linn. | 1. **HPLC:**

**Stationary Phase:** LaChrom Elite System consists of LaChrom Elite L-2200 | 1. Chromatographic Analysis
2. Extraction
3. Method development and validation
 |  |
|  |  | autosampler, LaChrom Elite L-2130 pump, | D) Stability |  |
|  |  | LaChrom Elite L-2350 column oven and | E) Quantification |  |
|  |  | LaChrom Elite L-2420 UV-VIS detector | F) Loss on drying |  |
|  |  | **Mobile Phase:** Gradient Elution with pure- |  |  |
|  |  | water and Acetonitrile (both with 0.1% Formic |  |  |
|  |  | acid) |  |  |
|  |  | **Column:** Phenomenex Kinetex XB-C18 |  |  |
|  |  | column (150 × 4.6mm;2.6 µm) |  |  |
|  |  | **Retention Time:** 15min |  |  |
|  |  | B) **UHPLC:** |  |  |
|  |  | **Stationary Phase:** HITACHI ChromasterUltra |  |  |
|  |  | UHPLC system consists of 6270 autosampler, |  |  |
|  |  | a 6310-column oven, a 6170 binary pump and |  |  |
|  |  | a 6430 Diode Array Detector |  |  |
|  |  | **Mobile Phase:** Gradient Elution with pure- |  |  |
|  |  | water and Acetonitrile (both with 0.1% Formic |  |  |
|  |  | acid) |  |  |
|  |  | **Column:** Phenomenex Kinetex XB-C18 |  |  |
|  |  | column (150 × 2.1mm; 1.7 µm) |  |  |
|  |  | **Retention Time:** 15min |  |  |
| 2 | *Cuscuta chinensis*Lam. | **Stationary Phase:** Waters Alliance system equipped with a vacuum degasser, quaternary detector**Mobile Phase:** Linear gradient with O- phosphoric acid 0.25% - acetonitrile **Column:** ACE C18 (4.6 × 250mm, 5µm)**Retention Time:** 42min | 1. Determination of Flavonoids
2. Method development and Validation
 |  |
| 3 | *Panex notoginseng*(Burkill) | **Stationary Phase:** HPLC system consists of a quaternary solvent delivery system, a linedegasser, an auto sampler, a column | 1. Optimization of HPLC separation
2. HPLC-UV fingerprinting of the XST injection
 |  |
|  |  | temperature controller and UV detector**Mobile Phase:** A) Deionized water: Acetic acid (100:0.1v/v) | 1. Identification of characteristic peaks
2. Determination of main saponins in the XST injection
 |  |
|  |  | B) Acetonitrile: Acetic acid (100:0.1v/v) |  |  |
|  |  | **Column:** UltimateTM XB-C18 column |  |  |
|  |  | (250mm× 4.6mm,5 µm) |  |  |
|  |  | **Retention Time:** 85min |  |  |

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 **standardization of herbal medicine**

 **SUMMARY**

* Standardization evaluation parameters can help assure the activity and efficacy of phytoconstituents in herbal products and aid in quality control.
The improvement of herbal medication items will be facilitated by the application of analytical techniques. A globally recognized set of guidelines for the quality monitoring of herbal medicinal plant material and its formulation is necessary for the growth of the herbal industry. The quality and, eventually, effectiveness of the herbal medicinal compositions can be supported by the set of standards. A variety of techniques, including TLC, HPTLC, HPLC, GC, UPLC, and other hyphenated techniques like GC-, are used to fix the set of standards.
HPLC-UPLC employs a variety of detectors, including UV, PDA, and DAD detectors.
These methods are helpful for determining the calibre of herbal medications or their derivatives. Different chemicals found in herbal plants are identified and separated using analytical techniques.

Alkaloids, glycosides, tannins, sennosides, coumarins, flavonoids, and other compounds are detected by chromatographic methods. Isoflavonoids etc.

These are two methods, separation methods and, detection methods are reunited to produce the hyphenated methods. These procedures are applicable in demonstrating both qualitative and quantitative study of a number of herbal medicine constituents. Chemical fingerprinting research of herbal medicinal products as well as quality control and standardization of herbal crude pharmaceuticals may also be enhanced by a range of hyphenated methodologies. A number of plant species, such as Cassia angustifolia, Paronychia argentea Lam, Salvia, Convolvulus pluricaulis, and Brown marine

They use macro algae. Different constituents present in these species have been made available through this route. Some plant species, like Oroxylum indica, Bridelia montana, and Tylophora indica, are used in the HPTLC technique to detect phytochemicals. For identification of an optimal solvent system resolution for Tylophora indica, some different solvent systems were tried employing hit-and-trial methods.

* Numerous plant species, including Cannabis sativa Linn., Cuscuta Chinensis Lam., and Panex Notoginseng, are employed in the HPLC-UV technique. This approach has been
was useful for fingerprinting the XST injection of Panex Notoginseng and identifying the flavonoids in Cuscuta Chinensis and saponins in Panex Notoginseng. Asparagus racemosus, Carica papaya, Panex notoginseng, Tribulus terrestris Linn., Curcuma aeruginosa, and Ascophyllum nodosum are all utilized in the LC-MS approach. This method is utilized to determine the bioactive component of Curcuma aeruginosa and to correlate the composition of metabolites with toxicity and antioxidant activity. Carpaine, saponin, and betaine analysis in Carica papaya, Asparagus racemosus, and Ascophyllum nodosum, respectively, are also performed using this method.
* As interest in natural therapies grows, standardizing herbal medicines is essential for addressing lifestyle problems. Because of bad lifestyle choices, lifestyle diseases like obesity, diabetes, heart disease, and high blood pressure are a global health concern. People are using herbal medicine as an alternative to traditional pharmaceutical therapies because of their shortcomings. This chapter emphasizes how important it is to standardize herbal medicine in order to guarantee the constant efficacy, safety, and quality of herbal medicines.

Herbal samples are characterized and important bioactive components are identified using both qualitative and quantitative techniques as well as fingerprint profiling. Concerns about the negative effects of synthetic drugs and a desire for sustainable and individualized healthcare solutions have led to a noticeable shift in the use of herbal therapy for lifestyle disorders in recent years. Research and technological developments have confirmed the therapeutic benefits of herbal medicine, increasing its legitimacy in conventional medical procedures. It is crucial to accurately identify and measure the active ingredients using analytical methods like spectroscopy and chromatography. Growing awareness of the effectiveness and safety of herbal treatments is reflected in their rising use.

* Chinese medicine and other forms of traditional medicine have special diagnostic techniques that need to be carefully taken into account while standardizing. Fuzzy logic stands out as one of the most effective methods for decision-making among the many different and difficult problems that artificial intelligence has recently demonstrated its ability to solve. The Medical Diagnostic System (MDS) uses fuzzy logic, an artificially intelligent technology, to identify a variety of ailments. It is regarded as one of the best methods for qualitative computation. For those looking for natural remedies, combining human cognitive processes with contemporary analysis is seen to be a complete and successful option.

**CONCLUSION**

* The quality of herbal plant material or its products is influenced by a variety of factors. The development in the analytical assessment may result in improvement in the standard of herbal remedies.

The process for guaranteeing the caliber and uniformity of medicinally active plants' active ingredients is called standardization. Standardization will therefore help to establish the quality control requirements, which can support the global growth of medicinal plants used in herbal remedies.

HPLC is a critical tool in the standardization of herbal drugs, facilitating accurate identification, quantification, and quality control of bioactive compounds and impurities. The capability to produce chemical fingerprints and provide batch-to-batch consistency is imperative to ensure the safety, efficacy, and quality of herbal products. With its substantial contributions to quality assurance and regulatory compliance, HPLC is vital for the ongoing development and safe consumption of herbal medicines in the international market.

High-Performance Liquid Chromatography (HPLC) is of essential importance in the standardization of herbal drugs by their quality, safety, and therapeutic potential. Since herbal medicines are of plant origin, the chemical constitution may be highly variable, making it difficult to keep them consistent and effective within batches. HPLC is a sensitive and accurate technique for the identification and quantitation of bioactive constituents, for detecting contaminants, and ascertaining the absence of toxic substances like pesticides and heavy metals.

via methods such as fingerprinting, HPLC enables the creation of a distinctive profile for every herbal drug, allowing for comparison between various batches to guarantee quality uniformity. This uniformity is crucial for consumer safety as well as the therapeutic efficacy of herbal products. HPLC also aids in regulatory compliance by satisfying the demands of health authorities, further guaranteeing the safety and efficacy of herbal drugs in the international market.

**REFERENCES**

1. **Bahulikar, A. S., Kashalkar, R. V., Pundlik, M., et al.** (2003). HPLC in Standardization of Herbal Drugs: Studies on Triphala Powder. *Asian Journal of Chemistry*,15(1),229–234.

https://asianpubs.org/index.php/ajchem/article/view/21599

1. **Gupta, P.** et al. (2018). Standardization of Herbs: An Essential Aspect for Drug *Natural Products Chemistry& Research*, https://[www.iomcworld.org/open-](http://www.iomcworld.org/open-) access/standardization-of-herbs-an-essential-aspect-for-drug-discovery-43770.html
2. **Desai, S., Tatke, P., et al.** (2019). High-Performance Liquid Chromatography (HPLC) as a Tool for Standardization of Complex Herbal Drugs. *Journal of AOAC International*,102(4),986–993. https://pubmed.ncbi.nlm.nih.gov/30558698/
3. **Rani, S., Rahman, K., Younis, P. M., et al.** (2015). HPTLC: An Emerging Technique in Herbal Medicine. *Research & Reviews: Journal of Herbal Science*, 4(2),25–37 https://sciencejournals.stmjournals.in/index.php/RRJoHS/article/view/716/0
4. **Balkrishna, A., Tomer, M., Joshi, M., Gujral, S., Mishra, R. K., Srivastava, J., Varshney,A., et al.** (2022). Standardization and Validation of Phytometabolites by UHPLC and High-Performance Thin Layer Chromatography for Rapid Quality Assessment of Ancient Ayurvedic Medicine, Mahayograj Guggul. *Journa* 45(10),1616–1635. https://analyticalsciencejournals.onlinelibrary.wiley.com/doi/10.1002/jssc.202100 935
5. **Gunjal, S. B., Dighe, P. R., et al.** (2019). Analysis of Herbal Drugs by HPTLC: A Review. *Asian Journal of Pharmaceutical Research and Development*, 10(2), 1–8. https://[www.ajprd.com/index.php/journal/article/view/1056](http://www.ajprd.com/index.php/journal/article/view/1056)
6. **Aravind, S. G., Arimboor, R., Rangan, M., Madhavan, S. N., Arumughan, C., et al.** (2008). Semi-Preparative HPLC Preparation and HPTLC Quantification of Tetrahydroamentoflavone as Marker in *Semecarpus anacardium* and Its Polyherbal Formulations. *Journal of Pharmaceutical and Biomedical Analysis*, 48(3),808–813.

https://[www.sciencedirect.com/science/article/pii/S0731708508004689](http://www.sciencedirect.com/science/article/pii/S0731708508004689)

1. **Shailajan, S., Patil, Y., Joshi, M., Menon, S., Mhatre, M., et al.** (2019). Simultaneous Quantification of Pharmacological Markers Quercetin and Berberine Using High-Performance Thin-Layer Chromatography (HPTLC) and High-Performance Liquid Chromatography (HPLC) from a Polyherbal Formulation Pushyanuga Churna. *Journal of AOAC International*, 102(4), 1003–1013. https://pubmed.ncbi.nlm.nih.gov/30563588/
2. **Ma, C., Oketch-Rabah, H., Kim, N. C., Monagas, M., Bzhelyansky, A., Sarma, N., Giancaspro, G., et al.** (2018). Quality Specifications for Articles of Botanical Origin from the United States Pharmacopeia. *Phytomedicine*, 45, 105–119.

https://[www.sciencedirect.com/science/article/pii/S0944711318300904](http://www.sciencedirect.com/science/article/pii/S0944711318300904)

1. **Pferschy-Wenzig, E. M., Bauer, R., et al.** (2015). The Relevance of Pharmacognosy in Pharmacological Research on Herbal Medicinal Products. *Epilepsy &B* 344–362. https://[www.sciencedirect.com/science/article/pii/S152550501500273X](http://www.sciencedirect.com/science/article/pii/S152550501500273X)
2. **Lee, J. S., Nam, Y. R., Kim, H. J., Kim, W. K., et al.** (2023). Quantification and Validation of an HPLC Method for Low Concentrations of Apigenin-7-O- Glucuronide in *Agrimonia pilosa* Aqueous Ethanol Extract Topical Cream by Liquid-Liquid Extraction.*Molecules*,28(2),713.https://[www.mdpi.com/1420-3049/28/2/713](http://www.mdpi.com/1420-3049/28/2/713)
3. **Osman, A., Chittiboyina, A. G., Avula, B., Ali, Z., Adams, S. J., Khan, I. A., et al.** (2023). Quality Consistency of Herbal Products: Chemical Evaluation. In *Progress in the Chemistry of Organic Natural Products* (Vol. 122, pp. 163–219). Springer. https://link.springer.com/chapter/10.1007/978-3-031-26768-0\_2
4. Kumar, P., Jha, S., Patel, D., & Alam, S. (2014). Validated HPTLC method for standardization of an Ayurvedic powdered formulation- Panchsakar Churna. International of Phytomedicine, 6(3),417–420. https://ijp.arjournals.org/index.php/ijp/article/view/354
5. Lal, U. R., Tripathi, S. M., Jakchak, S. M., Bhutani, K. K., & Singh, I. P. (2009). HPLC Analysis and Standardization of Arjunarishta – An Ayurvedic Cardioprotective Formulation. Scientia Pharmaceutica, 77(3), 605–616. https://[www.mdpi.com/2218-0532/77/3/605](http://www.mdpi.com/2218-0532/77/3/605)
6. Patel, P., Bhatt, J., Sureja, F., Dhoru, M., & Detholia, K. (2021). Herbal standardization of formulation containing curcuminoids, piperine, and ascorbic acid by dual detection mode densitometric analysis. Journal of Planar Chromatography Modern TLC 513–520. https://link.springer.com/article/10.1007/s00764-021-00139-z
7. Rao, A. S., &Ahmed, M. F. (2014). Simultaneous determination of phenolic compounds in Catharanthus roseus leaves by HPLC method. International Journal of Pharmaceutical Sciences and Research, 5(3), 977–981. https://[www.ijpsr.com/articles/5/3/977-981.html](http://www.ijpsr.com/articles/5/3/977-981.html)
8. Mundkinajeddu, D., Sawant, L. P., Koshy, R., Akunuri, P., Singh, V. K., Mayachari, A., Sharaf, M. H., Balasubramanian, M., Agarwal, A., et al. (2014). Development and validation of high performance liquid chromatography method for simultaneous estimation of flavonoid glycosides in Withania somnifera aerial parts. International Scholarly Research Notices, 2014, 1–6. https://[www.hindawi.com/journals/isrn/2014/789050/](http://www.hindawi.com/journals/isrn/2014/789050/)
9. Boligon, A. A., Jesus, R. D., Piana, M., de Brum, T. F., da Cruz, R. C., Mossmann, N., Athayde, M. L., et al. (2015). Development and validation of an HPLC-DAD analysis for flavonoids in the gel of Scutia buxifolia. Journal of Chromatographic Science,51557–1561. https://academic.oup.com/chromsci/article/53/9/1557/586100
10. Prasad, H. K., Hariprasad, R., & Rahman, S. H. (2019). Method development and validation for the simultaneous estimation of resveratrol and quercetin in bulk and pharmaceutical dosage form by RP-HPLC. Journal of Pharmaceutical Sciences 3777–3781 .https://[www.jpsr.pharmainfo.in/Documents/Volumes/vol11Issue12/jpsr11121934.](http://www.jpsr.pharmainfo.in/Documents/Volumes/vol11Issue12/jpsr11121934) pdf
11. Chaudhari, V. S., Borkar, R. M., Murty, U. S., & Banerjee, S. (2020). Analytical method development and validation of reverse-phase high-performance liquid chromatography (RP-HPLC) method for simultaneous quantifications of quercetin and piperine in dual-drug loaded nanostructured lipid carriers. Journal of Pharmaceutical and Biomedical Analysis, 186,113325.https://doi.org/10.1016/j.jpba.2020.113325
12. Kumar, A., Lakshman, K., Jayaveera, K. N., Tripathi, S. M., & Satish, K. V. (2010). Estimation of gallic acid, rutin, and quercetin in Terminalia chebula by HPTLC. Jordan Journal of Pharmaceutical Sciences, 3(1), 63–68. https://journals.ju.edu.jo/JJPS/article/view/100
13. Rakesh, S. U., Patil, P. R., Salunkhe, V. R., Dhabale, P. N., & Burade, K. B. (2009). HPTLC method for quantitative determination of quercetin in hydro alcoholic extract of dried flower of Nymphaea stellata Willd. International Journal of ChemTech Research, 1(4), 931–936. https://[www.sphinxsai.com/2010/ch\_vol3\_no1/chem/CT=3%281%29%](http://www.sphinxsai.com/2010/ch_vol3_no1/chem/CT%3D3%281%29%25)