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REVIEW ARTICLE

Applications of Chromatographic Methods in Standardization and Analysis of Herbal Drugs

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ABSTRACT

Herbal medication is accepted as necessary therapeutic agents for treating many diseases. Flavourer medicative products are dietary supplements that folks desire improves their health. The event of authentic analytical strategies that may faithfully estimate the Phyto-chemical composition, together with quantitative analyses of bioactive compounds and different constituents, could be a major challenge to scientists. Pharmacognostical analysis of medicinal herbs remains challenging, as herbs are an advanced system of mixtures. Developed Analytical separation techniques, for example-ultra high-performance liquid natural action (UHPLC), gas chromatography (GC), High-Performance thin layer chromatography (HPTLC), Hydrophilic interaction chromatography (HILIC) et. among the foremost standard methods of selection used for internal control of staple and finished flavourer product.

Keywords: High Performance Thin Layer Chromatography (HPTLC), Hydrophilic Interaction Chromatography (HILIC), Gas Chromatography (GC), Ultra-High Performance Liquid Chromatography (UPLC).

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INTRODUCTION

In the existing era, conventional fashion has been shifted from artificial to natural medication, i.e., "Return to Nature".[1] Ayurveda is a time-tested, relied on global plants primarily based totally device of drugs[2] which is advanced through everyday existence studies with the mutual courting among mankind and nature.[3] According to WHO, there are three types of natural drugs: uncooked plant fabric, processed plant fabric, and natural medicinal products.[4] Herbal drugs are complicated chemical combinations acquired from a plant extensively utilized in healthcare in each advanced and growing country.[5] It is not any surprise that the world's one-fourth population uses conventional drugs to remedy numerous ailments.[6] However, one of the impediments withinside the popularity of the ayurvedic or herbal drugs is the shortage of preferred exceptional manipulated profiles.[7] Due to the complicated nature and inherent variability of the chemical materials of the plant-based drugs, it's miles tough to set up exceptional manipulate parameter.[8] Quality warranty of natural medication is a crucial element and primary requirement for natural drug enterprises and different drug improvement organization.[9] numerous issues impact the exceptional of natural drugs.

- Variable sources of the raw material: The chemical ingredients of herbs and natural merchandise might also additional range relying on the degree of collection, elements of the plant collected, harvest seasons, plant origins (local status), drying strategies and different factors.[10]
- Extracts are normally combos of many ingredients.
- The energetic principle(s) is (are), in maximum instances unknown.

There will be no commercial availability of selective analytical methods or reference substances.[11,12,13] All pharmacopoeias set requirements for the best, purity, strength, and consistency of those merchandise–important to the general public health. USP–NF includes about 4500 monographs for drug substances, dosage forms, excipients and different therapeutics. Today, USP proposes the primary 23 elements to be blanketed withinside the new Herbal Medicines Compendium (HMC).[14] The IP 2007, which turned into made powerful from remaining July, has already over 1600 monographs.[15] The British Pharmacopoeia 2012 includes about 3375 monographs for substances, arrangements and articles used withinside the exercise of medicine.[16] Standardization is a vital step for the status quo of constant organic activity, a constant chemical profile, or truly the best warranty software for manufacturing and USP production of a natural drug.[17] It is the technique of growing and agreeing upon technical requirements. Specific requirements are labored out via way of means of experimentation and observations, which might result in the technique of prescribing a fixed of traits exhibited via way of means of the precise natural medicine. Hence standardization is a device withinside the best manipulation technique.[18]

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Chromatographic Techniques in Standardization of Herbal Drug Analysis

Chromatography represents the maximum flexible separation method and with ease available. Chromatography is described as a method of isolation and identity of additives or compounds or combination of it's into person additives via the use of desk bound segment and cellular segment. Plant substances are separated and purified via way of means of the use of numerous chromatographic strategies. Herbal medication is a complex machine of mixtures. Thus, the strategies of preference for the identity of 'botanical drug' are meant to gain a function fingerprint of a selected plant that constitutes the presence of a specific pleasant defining chemical constituents. Chemical fingerprints received via way of means of the chromatographic method and specifically via way of means of hyphenated chromatography are strongly encouraged for the motive of pleasant manage of natural drug treatments. Seeing that, they could constitute accurately the "chemical integrities" of the natural drug treatments and consequently be used for authentication and identity of the natural Thin layer chromatography (TLC) and High-Performance Thin Layer Chromatography (HPTLC) are precious gear for qualitative dedication of small quantities of impurities. Also, many analytical strategies along with Volumetric Analysis, Gravimetric Determinations, Gas Chromatography (GC), Column Chromatography (CC), High Performance Liquid Chromatography (HPLC) and Spectrophotometric strategies also are often used for pleasant manage and standardization.[19]

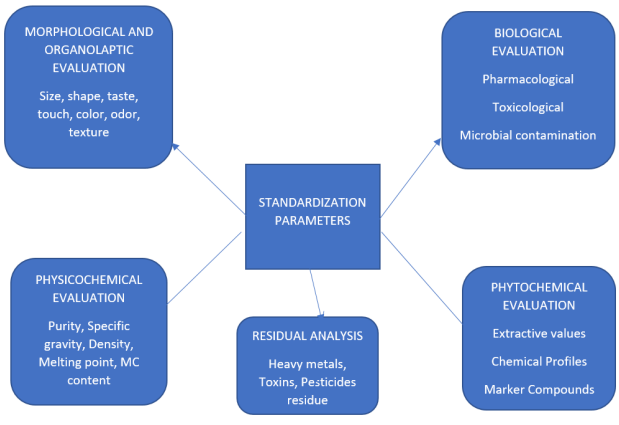

Figure 1: Different method of standardization of Herbal Drugs

Thin Layer Chromatography

Thin layer chromatography is, without a doubt, called TLC. It is one of the most famous and easy chromatographic methods used to separate compounds. In the phytochemical assessment of natural drugs, TLC is being hired significantly for the subsequent reasons:

1. It allows speedy evaluation of natural extracts with minimal pattern clean-up requirement,
2. It offers qualitative and semi-quantitative data of the resolved compounds.
3. It allows the quantification of chemical constituents. Fingerprinting the usage of HPLC and GLC is likewise done in precise cases.

In TLC fingerprinting, the statistics that may be recorded for the usage of an excessive overall performance TLC (HPTLC) scanner consists of the chromatogram, retardation factor (Rf) values, the satiation of the separated bands, their absorption spectra, λ max and shoulder inflection/s of all of the resolved bands.

All of these, collectively with the profiles on derivatization with exclusive reagents, constitute the TLC fingerprint profile of the pattern. The data so generated has a capacity software withinside the identity of a true drug, except the adulterants and in keeping the nice and consistency of the drug.

TLC turned into the not unusual place technique of preference for natural evaluation earlier than instrumental chromatography techniques like GC and HPLC had been established.

Table 1: Some examples of analytes evaluated by using TLC

S. No.	*Analytes*	*TLC system parameter*	*References*
1	Harhra' (Terminaliachebula and Gallic acid)	Stationary phase: Silica gel Mobile phase: Toluene – ethyl acetate – formic acid, 5:5:1	20
2	Azadirachta indica, Catharanthus roseus and Momordica charntia	Stationary phase: Silica gel Mobile phase: Dichloro methane–methanol, 2:8	21
3	Mushroom extracts	Stationary phase: Silica gel Mobile phase: Dichloromethane – ethyl acetate-methanol, 3:1:1	22
4	Strychnos nux vomica	Stationary phase: Silica gel Mobile phase: Chloroform–ethyl acetate– diethyl amine, 0.5:8.5:1	23
5	Constituents from the fruit of Piper chaba (Piperine, piperamine, Piperlonnguminine, and methyl piperate)	Stationary phase: Silica gel Mobile phase: n-hexane-ethylacetate, 1:1	24
6	Quinones	Stationary phase: Silica gel 60 Mobile phase: dichloromethane-n-hexane, 8:2	25

Even nowadays, TLC is often used to evaluate natural drugs because numerous pharmacopoeias including American Herbal Pharmacopoeia (AHP), Chinese drug monographs and evaluation, Pharmacopoeia of the People's Republic of China etc. nonetheless use TLC to offer first feature fingerprints of herbs. Rather, TLC is used as a less difficult technique of preliminary screening with a semi-quantitative assessment collectively with different chromatographic techniques.

High Performance Thin Layer Chromatography (HPTLC)

HPTLC approach is extensively hired in pharmaceutical enterprise in manner development, identity and detection of adulterants in natural product and enables in the identity of pesticide content, mycotoxins and exceptional manage of herbs and fitness Food. It has been nicely pronounced that numerous samples may be run concurrently via means of using a smaller amount of cell segment than in HPLC. It has also been pronounced that pH eight and above cell levels may be used for HPTLC. Another benefit of HPTLC is the chromatogram's repeated detection (scanning) with equal or specific conditions. Consequently, HPTLC has been investigated for simultaneous assay of numerous additives in a multi-component formulation. With this approach, authentication of diverse plant species is possible, in addition to the assessment of balance and consistency in their arrangements from specific manufacturers. Various employees have evolved HPTLC approach for phytoconstituents in crude capsules or natural formulations inclusive of bergenin, catechine and gallic acid in *Bergenia cilliata* and *Bergenia lingulate*.[26]

High-Performance Liquid Chromatography (HPLC)

Over the beyond decades, HPLC has acquired the maximum full-size software withinside the evaluation of natural medicines. Reversed segment (RP) columns can be the famous maximum columns used withinside the analytical separation of natural medicines. Preparative and analytical HPLC are broadly utilized in pharmaceutical enterprises to separate and purify natural compounds. There are essentially styles of preparative HPLC: low-stress HPLC (normally beneath Neath five bar) and high-stress HPLC (stress >20 bar). The crucial parameters to be taken into consideration are resolution, sensitivity and speedy evaluation time in analytical HPLC while each the diploma of solute purity and the quantity of compound that may be produced in keeping with unit time, i.e., throughput or healing in preparative HPLC.

In preparative HPLC (stress >20 bar), large stainless-steel columns and packing materials (particle length 1030μm are needed. Examples of everyday segment silica columns are Kromasil 10 μm, Kromasil sixteen μm, Chiralcel AS 20 μm, while for the opposite segment are Chromasil C18, Chromasil C8, YMC C18. The intention

Table 2: List of herbal drugs extract analyses by HPLC.[27]

Herbal drug extract	*Active Compounds*	*Column*	*Mobile Phase*	*Flow rate*	*Gradient*	*Detector*	*Stop time*	*Inj Vol.*
Atropa Belladona	Atropine	4.6 × 75 mm Zorbax Eclipse XDB-C18,3.5 μm	A= 0.05m KH2P04 in water (pH- 3), B-acetonitrile	1.0 mL/min	At 0 min 10% B At 20 min 60% B At 23 min 60% B At 25 min 10% B	UV [diode array detector 210 nm/16 (ref. 360 nm/100), standard cell].	25 min	5 μL
Cortex Chinconae	Qunidine Quinine	4 × 125 mm Purospher RP 18 5 μm	A= 0.05m KH2P04 in water (pH- 3), B-acetonitrile	0.8 mL/min	At 0 min 4% B At 25 min 10% B At 45 min 30% B At 46 min 60% B At 49 min 60% B At 50 min 4% B	UV [diode array detector 210 nm/16 (ref. 360 nm/100), standard cell].	50 min	5 μL
Ephedra Sinica	Ephedrine Nonephedrine	4.6 × 75 mm Zorbax SB-C18,3.5 μm	A=0.025M KH2P04 in water (pH- 3), B-acetonitrile	1.0 mL/min	At 0 min 2% B At 10 min 10% B At 15 min 80% B At 18 min 80% B At 20 min 2% B	UV [diode array detector 210 nm/16 (ref. 360 nm/100), standard cell].	20 min	5 μL
Ginko Biloba	Quercetin kaempferol	4 × 125 mm Hpersil ODS, 5 μm	A= 0.5 %H 3P04 in water , B - methanol	2.0 mL/min	At 0 min 38% B At 12 min 48% B At 17 min 100% B At 25 min 10% B	Diode array detector 370 nm/16 (ref.off), standard cell	20 min	10 μL
Rbeum Palmatum	Rhein Emodin	4 × 125 mm Hpersil ODS, 5 μm	A= 0.05 M NH 40Ac in water (pH- 2.5), B-acetonitrile	1.0 mL/min	At 0 min 30% B At 10 min 80% B At 14 min 80% B At 15 min 30% B	Diode array detector 440 nm/16 (ref.off), standard cell	15 min	1 μL

is to isolate or purify compounds, while in analytical paintings, the intention is to get statistics approximately the sample. This may be crucial in the pharmaceutical enterprise these days because new products (Natural, Synthetic) must be brought to the marketplace as speedy as viable. Having to be had this type of effective purification method makes it viable to spend much less time at the synthesis conditions.[27]

HPLC vs HPTLC

High-performance thin-layer chromatography (HPTLC) is still finding its way into pharmaceutical analysis in various areas of the world. With improvements in the stationary phases and the introduction of densitometers as detection equipment, the method reaches accuracy and trueness equivalent to high-performance liquid chromatography for specific applications (HPLC).

Ultra High-Performance Liquid Chromatography (UPLC)

In recent years, UHPLC has been rising as a possible approach for the excellent manipulation of natural merchandise. UHPLC can face up to a strain of a maximum 8000 psi and it brings liquid chromatographic evaluation to every other degree through hardware adjustments of the traditional HPLC machinery. UHPLC makes it viable to carry out high-resolution separations advanced to HPLC evaluation by using strong segment debris of much less than 2 μm in diameter to obtain advanced sensitivity and resolution. Smaller particle length results in better separation performance, and shorter column length results in shorter evaluation time with little solvent consumption.[28] Within the duration of a previous couple of years, UHPLC fingerprints of natural merchandise have been advanced instead of the traditional HPLC approach.[29,30] In evaluation of HPLC, UHPLC analyses suggested a reduced evaluation time through a component up to 8 without lack of information. The effects acquired now no longer most effective confirmed reduced evaluation time; however, additionally proved a high-quality enhancement in selectivity in comparison to traditional HPLC evaluation.[31]

Hydrophilic Interaction Chromatography (HILIC)

HILIC has received interest in natural fingerprinting due to right separation great of hydrophilic compounds. Many of polar compounds of natural drug treatments are extracted through the usage of aqueous solution, which is probably higher separated by way of HILIC.[32]

HILIC changed into delivered as an opportunity for normal-segment liquid chromatography (NPLC); HILIC allows the separation of polar compounds on polar desk bound levels with aqueous cell levels. It is primarily based on the precept of partitioning among a water-enriched layer within the hydrophilic desk bound segment and a fantastically hydrophobic cell segment typically containing 5–40% water in the natural solvent. This approach is greater green than NPLC due to using water and polar natural solvents as cell segments. In addition, the polar compounds are greater soluble withinside the cell segment of HILIC.[33]

As HILIC is a fantastically latest approach, few papers reading natural merchandise have been posted yet. Most papers typically describe a method exploiting the orthogonal individual of the HILIC and reversed-segment liquid chromatography (RPLC) strategies for great control.[34]

Table 3: Comparison between HPLC and HPTLC

Parameters	*HPLC*	*HPTLC*
Type	Reverse Phase Chromatography	Straight Phase Chromatography
Stationary Phase	Liquid	Solid
Conditioning Phase	None	Gas
Separation by	Partition	Adsorption
Results	By machine	By machine + eyes
Analysis	On - line	Off - line
Resolution	Very high	Moderate to high
Chromatography System	Closed	Open
Separating medium	Tubular column	Planar layer (plate)
Strongly Retarded Fractions Seen As	Broad peaks	Sharp Peaks
Analysis in parallel	No. Only 1 at a time	Yes. Upto 100 samples.
High temp. / pressure	High pressure	None
Time per sample	2- 60 min	1-30 min
Data obtained from chromatography	Limited to very high	High to very high

Gas Chromatography (GC)

GC is a well-installed analytical method that characterizes, quantifies, and identifies unstable compounds. It may be used in many exceptional fields along with pharmaceuticals, cosmetics or even environmental toxins. Since the samples should be unstable, human breath, blood, saliva, and different secretion containing big quantities of natural volatiles may be effortlessly analyzed using GC. The effective separation performance and touchy detection make GC a beneficial device for evaluating important oils.[35] Despite its advantages, GC evaluation of natural merchandise is generally restricted to the necessity's oils due to viable degradation of thermo-labile compounds, and the requirement of unstable compounds makes GC flawed for plenty natural compounds.[36] The hyphenation of GC–MS lowers evaluation instances of important oils (40–one hundreds) and reduces detection limits. GC–MS evaluation of necessities oils, displaying quicker evaluation and excessive performance, made use of microbore capillary columns with decreased desk bound section movie thickness (10 m× one hundred μm I.D. and five m×50 μm I.D.) with speedy temperature programming (20 °C/s), speedy records acquisition through FID and excessive break up ratio. Finally, low-stress GC–MS, the use of mega-bore analytical columns (10 m×530 μm with 0.25–1 μm movie thickness) became investigated at the important oils and brought about a barely decreased performance; however, manifold reduced evaluation instance.[37-39]

Table 4: Gas chromatogarphy conditions and setting value

Condition	*Setting Value*
Flow rate of carrier gas	1.7 mL/min (high purity N_2)
Injection temperature	220°C
Injection volume	1 μL
Splitless time	0.75 min
Detector	Ni ECD
Detector temperature	280°C
Flow rate of makeup gas	60 mL/min (N_2)
Retention gap	Fused silica, methyl deactivated, 2.5 m, 0.53 mm (i.d)
Column	HP- 5, 30 m × 0.32 mm (i.d.) × 0.25 μm (film thickness)
Initial oven temperature	50°C
Initial programming rate	10°C/min
Second isotherm temperature	150°C
Second isotherm rate	3°C/min
Third isotherm temperature (period)	240°C (10 min)
Quantitative method	External standards, peak area

Two-Dimensional (2D) Chromatography

Before going to 2D chromatography, one must first recognize the distinction among multi-dimensional fingerprint generated through hyphenated detection strategies and 2D chromatography. In multi-dimensional fingerprinting, the hyphenated detector collects records from the eluting compounds simultaneously as a fingerprint is recorded. In 2D chromatography, fractions eluting from a primary chromatographic gadget are chromatographed on a 2d gadget having distinct separation residences ensuing in extended the height ability of complete separation. To monitor all traits of complicated HDs, 2D chromatography turned into proposed. With the improvement of 2D chromatographic systems, a brand-new generation in natural fingerprinting has been clicked. The predominant benefit of 2D chromatography over traditional one-dimensional chromatography is to gain the excessive top ability, which theoretically equals the fabricated from the height capacities of man or woman dimensions. However, the main hassle could be very long-term span had to attain this most ability. Literature assessment exhibits that 2D chromatography has been used with distinct strategies which include 2D TLC, 2D HPLC, 2D GC, and 2D chromatography combining size-exclusion and RPLC.[40-42]

Supercritical Fluid Chromatography (SFC)

Supercritical fluid chromatography is a hybrid of gas and liquid chromatography that combines some of the best features of each. SFC permits the separation and determination of a group of compounds that are not conveniently handled by either gas or liquid chromatography. SFC has been applied to various materials, including natural products, drugs, food, and pesticide. These compounds are either non-volatile or thermally labile so that GC procedures are inapplicable or contain no functional group that makes possible detection by the spectroscopic or electrochemical technique employed in LC.[43]

Liquid Chromatography-Mass Spectroscopy (LC-MS)

LC-MS has become the method of choice in many stages of drug development. Recent advances include electrospray, thermo spray, and ion spray ionization techniques which offer unique advantages of high detection sensitivity and specificity, liquid secondary ion mass spectroscopy, and later laser mass spectroscopy with 600 MHz offers an accurate determination of molecular weight proteins, peptides. Isotope's pattern can be detected by this technique.[44]

Dna Fingerprinting

DNA analysis has been proved as a crucial tool in herbal drug standardization. This system is beneficial for identifying phytochemically indistinguishable genuine drug from substituted or adulterated drug. It has been reported that DNA fingerprint genome remains equivalent regardless of the plant structure used. At the same time, the phytochemical content will vary with the plant structure used, physiology and environment.[45] Deoxyribonucleic acid (DNA) is the fundamental building component of all living cells. Our characteristics, traits and physical features are determined by the precise arrangement of DNA base-pair sequences within the cell. This distinct arrangement of adenine, guanine, thymine and cytosine (called DNA nucleotides) regulates the assembly of specific proteins and enzymes via the Central Dogma Theory. Central Dogma theory is often defined because of biology's fundamental theory that genetic information flows from DNA to RNA to proteins.[46] This idea of fingerprinting has been increasingly applied within the past few decades to work out the ancestry of plants, animals and other microorganisms. Genotypic characterization of plant species and strains is beneficial as most plants, though belonging to an equivalent genus and species, may show considerable variation between strains. Additional motivation for using DNA fingerprinting on commercial herbal drugs is that the availability of intact genomic DNA from plant samples after they're processed. Adulterants are often distinguished even in processed samples, enabling the authentication of the drug.[47] The opposite useful application of DNA fingerprinting is the availability of intact genomic DNA specificity in commercial herbal drugs, which helps distinguish adulterants even in processed samples.[48]

CONCLUSION

To recapitulate, Herb provides specific treatment for the welfare of individuals. Have attained an enormous function in fitness care gadget everywhere in the world for people now no longer best with inside the diseased situation however additionally as capacity fabric for keeping right fitness. The natural enterprise could make tremendous strides with inside the world. In this, chromatographic techniques have a crucial role in determining the actual value of the content with proper demonstration. With the accelerated use of natural products, the destiny global labeling exercise must cope with nice aspects competently. Standardization of techniques and nice management records on protection and efficacy are required for expertise in using natural medicines. An important factor impeding the improvement of the medicinal plant-primarily based industries in growing nations has been the shortage of data at the social and financial blessings that might be derived from the commercial usage of medicinal plants. Further studies are needed to take advantage of the compounds answerable for the determined organic activity.

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ABBREVIATIONS AND SYMBOLS

TLC- Thin layer chromatography
HPLC- High-performance liquid chromatography
HPTLC- High-performance thin layer chromatography
GC- Gas chromatography
UPLC- Ultra performance liquid chromatography
HILIC- Hydrophilic interaction chromatography
LC-MS- Liquid chromatography-Mass chromatography
HMC- Herbal Medicines Compendium
WHO- World health organisation
USP- United states pharmacopoeia
SFC- Super critical fluid chromatography
MHz- Mega Hertz
DNA- Deoxyribonucleic acid
RNA- Ribonucleic acid

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