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Review on the Usefulness of Advanced Chromatographic Analytical methods for Determination of Herbal Molecules

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Abstract

The pursuit of natural products and herbal medicines has garnered significant attention in recent years due to their potential therapeutic benefits and minimal side effects. To harness the full potential of these traditional remedies, rigorous analytical techniques such as Gas Chromatography-Mass Spectrometry (GC-MS) and High-Performance Liquid Chromatography (HPLC) have emerged as indispensable tools. In this gripping abstract, we present a comprehensive review of studies that have employed GC-MS and HPLC to analyze herbal molecules and essential oils, shedding light on their chemical complexity and pharmacological activities.GC-MS, with its unparalleled capability to identify and quantify volatile and semi-volatile compounds, has been instrumental in the analysis of essential oils. Through its application, an array of bioactive components, including terpenes, phenols, and fatty acids, have been revealed. Studies utilizing HPLC have identified and measured various phytochemicals, including flavonoids, alkaloids, and polyphenols, elucidating their role in conferring medicinal properties to these plant-derived preparations. The validated HPLC methods have become crucial in assessing the stability and safety of pharmaceutical formulations containing herbal compounds, ensuring their efficacy and reliability.As the search for novel therapeutics continues, these cutting-edge analytical techniques will undoubtedly continue to shape the landscape of natural products research, promoting evidence-based herbal medicine practices and advancing the frontiers of modern medicine.

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INTRODUCTION

Pharmaceutical compounds encompass a diverse array of natural and synthetic bioactive substances, with varying definitions in scientific literature [1]. These bioactive compounds exhibit a wide range ofpharmacological properties, including potential anti-cancer effects, making them vital in the development of novel drugs and more effective pharmaceutical formulations for treating various diseases. Their applications extend to pharmaceuticals, nutraceuticals, dietary supplements, and cosmetics. In the realm of chemical analysis, the assessment of purity and quantification of biologically active substances, whether of natural or synthetic origin, in pharmaceutical preparations holds immense significance [2].

Chromatographic techniques have emerged as indispensable tools for analyzing bioactive compounds present in pharmaceutical preparations and extracted from medicinal plants. These methods encompass both planar techniques and high-performance liquid chromatography (HPLC) and gas chromatography (GC). The versatility of chro-

matography allows for the systematic analysis of the qualitative and quantitative composition of individual plant substances, facilitating comparisons based on their unique compositions. Unlike traditional separation techniques like crystallization, extraction, or distillation, chromatography enables compound separation without requiring in-depth knowledge of the substances' types, quantities, or relative proportions in the mixture [3].

The versatility and resolving power of chromatographic systems have rendered them indispensable in scientific, industrial, and medical fields. Chromatography not only serves as a fundamental element in numerous scientific studies but also plays a crucial role in environmental monitoring and the production of pure compounds in the pharmaceutical and chemical industries. It finds applications in various domains, including pharmaceutical analysis, biochemical and clinical chemistry, environmental protection, food and cosmetics, inorganic substances, and others. Additionally, chromatography is widely employed in numerous analyses outlined in various pharmacopoeias.

This article aims to provide a comprehensive review of modern approaches that utilize advanced equipment and validation parameters in liquid and gas chromatography techniques to identify and quantify various bioactive compounds present in pharmaceutical products and medicinal plant extracts. The primary focus of this review paper is to discuss chromatographic methods for the complex analysis of pharmaceutically active compounds in diverse dosage forms, encompassing tablets, capsules, drop solutions, injectable powders, creams, and herbal formulations. To compile this review, relevant content was sourced from various databases, including PubMed, Embase, and Google Scholar, using targeted keyword searches such as 'pharmaceuticals AND plant extracts AND LC analysis' and 'pharmaceuticals AND plant extracts AND GC analysis'.

HTPLC and TLC

HPTLC and TLC are two chemical techniques utilized to separate non-volatile components in mixtures. The primary distinction between TLC and HPTLC lies in the absorbent material; TLC plates consist of large particles, whereas HPTLC plates contain very small particles of the adsorbent material. TLC, or Thin Layer Chromatography, offers advantages such as availability, rapid analysis of multiple samples on the same chromatographic plate, and the ability to detect marked substances through various methods, including UV light or specific visualizing reagents. TLC is well-suited for dealing with complex and diverse matrices. However, it also has some limitations, as its separation results depend on numerous parameters that are challenging to standardize. For optimal reproducibility, chromatogram development in sandwich chambers and automatic chromatogram development (ACD) chambers are recommended.

In the analysis of natural and synthetic bioactive compounds, both TLC and HPTLC have been applied. Investigations have focused on synthetic medicinal substances commonly found in pharmaceutical preparations, such as antidiabetic drugs, steroids (e.g., vitamins D and K), and various alkaloids and antihypertensive compounds. Additionally, the scope of research has extended to herbal medicine or phytotherapy, where raw materials and plant preparations are utilized. Natural bioactive compounds primarily found in plants, such as steroidal compounds (diosgenin, stigmasterol, ecdysteroids, β-sitosterol) and phenolic compounds (gallic acid), have been studied. Furthermore, analyses have encompassed anticancer substances (arctiin, arctigenin), tannins, saponins, coumarins, alkaloids (trigonelline, berberine, barbamine, palmitine, magnoflorine, jatrorrhizine, ephedrine), flavonoids (e.g., diosmin, rutin), triterpenes (betulinic acid), essential oils, and antidepressants (salidroside, rosavin, p-tyrosol, and hydroquinone).

The analyses were predominantly conducted using TLC in conjunction with densitometry, following the International Conference on Harmonization (ICH) guidance for validation. The validation process involved determining the linearity range, precision (intraday and interday precisions), recovery, specificity, limit of detection, limit of quantification, and robustness of the methods used. Several studies have demonstrated the utility of TLC for the separation, identification, and evaluation of bioactivity, including antioxidant activity and toxicity, of bioactive compounds obtained from various plant sources, such as red algae and members of the Silene family. HPTLC combined with high-resolution mass spectrometry (MS) has been applied for the detection, identification, and imaging (HPTLC/MSI) of ecdysteroids present in plant extracts.

Moreover, TLC has been used for phytochemical screening of extracts from different plants to identify various bioactive compounds, such as saponins, flavonoids, tannins, coumarins, and alkaloids. HPTLC has been employed for the analysis of essential oils in various plant parts and the composition of herbal remedies in capsules. Additionally, innovative approaches have been explored, like using smartphones to recognize patterns of planar chromatograms.

Liquid Chromatography

High-Performance Liquid Chromatography (HPLC) is a widely used technique in pharmaceutical and phytochemical analysis of bioactive compounds. It offers high sensitivity and accuracy for qualitative and quantitative analysis of pharmaceutically active compounds (APIs) in various samples, including synthetic drug products and plant materials (Corradini, 2000). HPLC procedures have been developed for simultaneous determination of multiple active ingredients in complex samples, including newly synthesized co-drugs. These methods are simple, cost-effective, and yield reproducible results, making them suitable for routine control of APIs and phytochemical constituents in different pharmaceutical dosage forms like tablets, capsules, drops, creams, and injection vials.

Literature reviews indicate that HPLC, in combination with UV, diode array spectrophotometry (DAD/PDA), and mass spectrometry (MS), provides accurate, reproducible, and stability-indicating assays for various APIs and phytochemical compounds, even in the presence of excipients or degradation products as impurities. HPLC has played a crucial role in the phytochemical analysis of plants rich in anticancer compounds, leading to the development of new potential anticancer drug candidates.Researchers have validated HPLC methods for the stability determination of quercetin and rutin in pharmaceutical formulations using a mobile phase consisting of ammonium acetate and acetonitrile. Other studies utilized HPLC to determine antioxidants in Crocus sativus L. flowers extract and quantified bioactive compounds with antioxidant activity in polyherbal Unani formulations. HPLC methods have been validated for the simultaneous determination of curcumin and quercetin in Allium cepa and Mentha arvensis extracts.

HPLC-MS/MS profiling has been applied to identify potential antioxidants, such as phenolic acids and flavonoids, in Salvia species extracts from Jordan [4]. Another study evaluated the antitumor activity of Japanese quince leaf extracts and identified 16 phenolic compounds using UPLC-ESI-MS/MS. HPLC-DAD was utilized to characterize paclitaxel-loaded micelles as an anticancer drug. Additionally, HPLC-DAD methods have been employed to quantitatively determine isoquinoline alkaloids in Sanguinaria canadensis plant extracts, as well as other alkaloids, such as columbamine, jateorhizine, palmatine, and berberine, in Mahonia plant materials. HPLC-ESI-MS/MS and HPLC-DAD methods have been applied for the analysis of aconite alkaloids in Aconiti kusnezoffii Radix extract. An HPLC-DAD method was established for the determination of five constituents in Maiwei Dihuang pills, a traditional Chinese medicine [5]. Moreover, size-exclusion chromatography (SEC) coupled to multi-angle light scattering (MALS) has been utilized to control the production process of biological drugs, such as recombinant anti-interleukin-23 monoclonal antibodies with potential anticancer properties [6].

These studies demonstrate the versatility and applicability of HPLC in pharmaceutical and phytochemical analysis, making it an essential tool in drug development and quality control processes.

Liquid chromatography, specifically reversed-phase thin-layer chromatography (RP-TLC) and reversed-phase high-performance liquid chromatography (RP-HPLC), is widely utilized to evaluate the lipophilicity of organic compounds, which is a crucial physicochemical property influencing their biological activity as potential drugs.RP-TLC and RP-HPLC are effective techniques for assessing the lipophilicity of compounds due to their ability to separate analytes based on their hydrophobicity. In both methods, the stationary phase contains hydrophobic groups, such as C18-bonded silica, which interact with lipophilic compounds, causing them to be retained longer in the stationary phase. In contrast, hydrophilic compounds are less retained and elute faster.

RP-TLC is often used as a rapid and cost-effective screening tool for lipophilicity evaluation. Researchers can spot a small amount of the compound on the RP-TLC plate, which is then developed with a mobile phase consisting of an organic solvent (often a mixture of water and an organic modifier like methanol or acetonitrile). The distance travelled by the compound on the plate is related to its lipophilicity, with more lipophilic compounds travelling a shorter distance.On the other hand, RP-HPLC is a more sophisticated and precise technique that provides quantitative data on lipophilicity. In RP-HPLC, the compound is injected into the HPLC system, and the retention time is measured. Longer retention times indicate higher lipophilicity. RP-HPLC can also be coupled with various detectors, such as UV, diode array detector (DAD), and mass spectrometry (MS), for compound identification and characterization.

The literature review conducted by Gackowski et al. [7] showcases the utility of both RP-HPLC and chemometric analysis for determining the pharmacological profile of 15 cytostatic drugs. The study involved analyzing the lipophilicity of nimustine, actinomycin D, irinotecan, daunorubicin, doxorubicin, idarubicin, melphalan, mitomycin C, vinorel-

Table 1: Herbal molecules analysed using HPTLC and TLC

Sl. no	Herbal Molecule	Analytical Technique	Biological Activity
1	Steroids from red algae Eucheuma cottonii and Chlorella sp.	TLC (HPTLC)	Antioxidant activity, toxicity
2	Ecdysteroids	HPTLC/DESI/IMS/MSI	Insect moulting hormones
3	Sulfated lactosyl archaeol (SLA) archaeosomes	HPTLC	Stability for vaccine adjuvant
4	Flavonoids, tannins, saccharides, phenols	TLC	Antioxidant activity
5	Phenolic compounds	TLC-DPPH test	Antioxidant activity
6	Alkaloids, flavonoids, polyphenols, tannins, saponins, steroids	TLC	-
7	Saponins, flavonoids, tannins, coumarins, alkaloids	TLC	Phytochemical screening
8	Essential oils	HPTLC	-
9	Salidroside, rosavin, p-tyrosol, hydroquinone	HPTLC	Potential antidepressant and anxiolytic properties
10	Herbal remedies from citrus fruits	HPTLC (micellar TLC)	-
11	Four herbs in Weikangling capsules	HPTLC	-

bine, pirarubicin, docetaxel, vincristine, vindesine, vinblastine, and etoposide using RP-HPLC. The combination of RP-HPLC with chemometric analysis allows for comprehensive data analysis and the establishment of relationships between lipophilicity and pharmacological activity of these drugs.

Gas Chromatography

Gas chromatography (GC) with various detection systems, such as flame-ionization detector (GC-FID) or coupled to single or tandem mass spectrometric approaches (GC-MS, GC-MS/MS), has proven to be a powerful tool for the determination of various bioactive compounds in pharmaceutical and phytochemical analysis. The published papers demonstrate the application of GC-MS in the analysis of essential oils from different plant sources. For example, Almeida et al. used GC-MS to determine the essential oils in Ocotea odorifea, while Zakaria Nabti et al. examined the essential oils in the Algerian Origanum glandulosum Desf. Boukhatem analyzed the essential oil of Algerian Lavandula stoechas, and Ul-Khazir et al. studied the essential oil of Abies pindrow. These studies revealed the presence of various bioactive compounds such as α-pinene, camphene, β-pinene, 1,8-cineole, linalool, and others, each with potential pharmacological activities.

GC-MS has also been applied to determine the composition of essential oils in other medicinal plants, including Eucalyptus grandis $\times$ E. urophylla, Deverra tortuosa, and S. aromaticum. These studies identified the main compounds present in the essential oils and evaluated their pharmacological properties, such as antioxidant, antibacterial, antitrypanosomal, and antitumor activities.Additionally, GC-MS has been used for the determination of fatty acid content in herbal mixtures and carboxylic acid composition in leaves of different plant species. Such analyses provide valuable information on the chemical composition of these plants and their potential therapeutic applications.The sensitivity and ability to detect impurities at low levels make GC an essential tool for the analysis of bioactive compounds. The coupling of GC with mass spectrometry enhances the identification and characterization of individual compounds, providing valuable data for pharmacological profiling and drug discovery.

Integrated analytical systems

Integrated analytical systems, which combine multiple techniques such as HPTLC, HPLC, MS, NMR, and others, offer significant advantages in the analysis of complex samples and the identification of bioactive compounds. These techniques allow for the comprehensive profiling and structural eluci-

Table 2: Herbal molecules analysed using Liquid chromatography

Slno	Herbal Molecule	Analytical Technique	Biological Activity
1	Quercetin and rutin	HPLC-UV	Stability in pharmaceutical formulations
2	Bioactive compounds, including phenolics	HPLC-PDA	Antioxidant activity
3	Curcumin and quercetin	HPLC	Simultaneous determination in extracts and formulations
4	Phenolic acids and flavonoids	HPLC-MS/MS	Antioxidant activity
5	Polyphenolic compounds	UPLC-ESI-MS/MS	Potential anticancer activity
6	Paclitaxel-loaded micelles	HPLC-DAD	Cytotoxicity enhancement
7	Isoquinoline alkaloids	HPLC-DAD	Quantitative determination in Sanguinaria canadensis
8	Alkaloids	HPLC	Quantitative determination in Mahonia plant materials
9	Aconite alkaloids	HPLC-ESI-MS/MS, HPLC-DAD	Analgesic and anti-inflammatory phytocompounds
10	Deoxyschizandrin, γ-schizandrin, loganin, paeoniflorin, and paeonol	HPLC-DAD	Bioactive compounds in Maiwei Dihuang pills
11	Macromolecules (proteins and polymers)	SEC-MALS, HPLC	Control of production process of biological drugs

dation of various compounds in natural products, pharmaceuticals, and other matrices.In the study by Zekič, HPTLC and HPLC-PDA were used for the analysis of flavonoids and phenolic acids extracted from Solidago canadensis L. and Solidago gigantea Aiton. The combination of these techniques provided valuable information about the content of different phenolic compounds in the plant extracts.Similarly, Santos et al. applied an integrated approach involving UHPLC-DAD-ESI-HRMS/MS, molecular networking-based dereplication, and NMR for the identification of flavonoid-3-O-glycosides in the leaves of Casearia arborea. This method allowed for the characterization of bioactive compounds in the plant extract, including cytotoxic clerodane diterpenes, phenolics, flavonoids, and glycoside derivatives.

Cherfia et al. used an integrated LC-ESI-MS and MSn approach, combined with NMR analysis, for the isolation and structural identification of bioactive compounds from Calycotome spisosa (L.) Link leaves. This comprehensive approach facilitated the discovery and characterization of potentially valuable bioactive compounds in the plant extract [8]. In the study by Kalala et al., the HPLC-SPE-NMR technique was applied for the analysis of furanosesqui terpenoids from the bark exudates of Commiphora swynnertonni Burrt. This integrated approach allowed for the identification and structural elucidation of specific compounds in the plant extract, which has traditional medicinal uses.

The integration of multiple analytical techniques in these studies offers several advantages, including enhanced sensitivity, accuracy, and repeatability of determinations. These integrated systems can detect and quantify compounds at very low levels, making them valuable tools for analyzing complex mixtures. However, the main limitation of these approaches is the high cost and specialized knowledge required to operate the instruments, which restricts their widespread use and makes them more suitable for research and specialized laboratories. Overall, integrated analytical systems play a

Table 3: Herbal Molecules analysed using Gas chromatography

Slno	Essential Oil	Analytical Technique	Major Components
1	Ocotea odorifea	GC-MS	α-pinene (0.3%), camphene (0.2%), β-pinene (0.1%), α-felandrene (1.9%), o-cymene (3.0%), 1,8-cineole (0.9%), camphor (0.4%), α-terpinol (0.3%), safrole (77.9%), eugenol (0.6%), (E)-caryophyllene (0.4%), γ-muurolene (0.3%), Δ-selinene (0.5%), bicyclogemacrene (1.1%), spathulenol (4.0%), and 11-selinen-4-alpha-ol (1.2%)
2	Algerian Origanum glandulosum Desf	GC-MS	Thymol (15.2–56.4%), carvacrol (2.8–59.6%), γ-terpinene (9.9–21.8%) and p-cymene (8.5–59.6%)
3	Algerian Lavandula stoechas	GC-MS	α-pinene, camphene, β-pinene, 1,8-cineol, linalool, pinocarveol, α-terpineol, myrtenal, arpmadendrene, cis-calamenene
4	Abies pindrow	GC-MS	Limonene (38.9%), α-pinene (36.5%), β-pinene (6.9%), α-selinene (4.4%)
5	Aloysia polystachya	GC-MS	R-carvone (91.03%)
6	Deverra tortuosa	GC-MS	Widdrol (14.64%), β-phellandrene (10.49%), piperitol (9.48%), cubedol (6.89%), (E)-10-heptadecen-8-ynoic acid methyl ester (6.52%), α-terpinene (6.21%), m-cymene (4.65%), citronellyl tiglate (4.58%)
7	Eucalyptus grandis $\times$ E. urophylla	GC-MS	α-pinene (17.02%), α-terpineol (13.63%), aromadendrene (11.08%), D-limonene (8.47%), endo-borneol (7.77%)
8	S. aromaticum	GC-MS	Eugenol (main compound)
9	Antidiabetic herbal mixture	GC-MS	Various fatty acids in different plant components
10	Leaves of four Iris species	GC-MS	Short-chain carboxylic acids, α-Linolenic acid
11	Clerodendrum serratum Linn roots	GC-MS	Squalene, methyl palmitate, hexadecenoic acid, stigmasterol

crucial role in advancing our understanding of complex natural products and their potential pharmacological activities. They provide researchers with a powerful set of tools for the identification and characterization of bioactive compounds, contributing to the development of new drugs and therapeutic agents.

CONCLUSION

In conclusion, the use of analytical techniques, particularly GC-MS and HPLC, has proven to be invaluable in the study of herbal molecules and essential oils. These advanced methods have enabled researchers to identify and quantify various bioactive compounds present in different plant materials. From the analysis of essential oils, a wide array of compounds such as terpenes, phenols, and fatty acids have been identified, highlighting the chemical

complexity and potential therapeutic properties of these natural extracts.The application of GC-MS and HPLC has facilitated the determination of specific bioactive compounds in herbal extracts, enabling researchers to assess their potential pharmacological activities. The identification and quantification of these compounds are essential for evaluating the quality and safety of herbal medicines and formulations. These techniques have been employed in various studies to assess the antioxidant, antibacterial, antitumor, and other biological activities of herbal extracts, supporting their traditional medicinal uses and providing evidence for potential new therapeutic applications. Moreover, the development and validation of analytical methods for the detection and quantification of herbal molecules have become essential in ensuring accurate and reliable data. Researchers have complied with international guidelines for method validation, such as ICH, to ensure the robustness and reproducibility of their findings.The integration of multiple techniques, such as HPLC-MS/MS, GC-MS, TLC, and HPTLC, has opened up new possibilities in the analysis of herbal molecules, allowing for a more comprehensive understanding of the complex chemical composition of plant extracts. These sophisticated approaches have also offered enhanced sensitivity, accuracy, and precision, leading to the detection of trace compounds and the identification of minor components that were previously overlooked. Overall, the studies reviewed demonstrate the significance of GC-MS and HPLC techniques in the analysis of herbal molecules and essential oils. These methods have facilitated the identification and quantification of a wide range of bioactive compounds, contributing to the exploration of their potential therapeutic applications and supporting evidence-based herbal medicine practices. As the field of natural products research continues to evolve, the continued utilization and advancement of these analytical techniques will undoubtedly play a crucial role in unraveling the complexity of herbal compounds and their potential benefits for human health.

Conflict of Interest

The authors declare that there is no conflict of interest.

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