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Significance of various chromatographic techniques in herbal drug analysis

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ABSTRACT

Herbal drugs are accepted as important therapeutic agents for the treatment of many diseases. Herbal medicinal products are dietary supplements that people take to improve their health. The development of authentic analytical methods which can reliably estimate the phyto-chemical composition, including quantitative analyses of bioactive compounds and other major constituents, is a major challenge to scientists. Pharmacognostical analysis of medicinal herbs remains challenging, as herbs are a complicated system of mixtures. Developed Analytical separation techniques, for example-ultra high performance liquid chromatography (UHPLC), gas chromatography (GC), High Performance Thin Layer Chromatography (HPTLC),Hydrophilic interaction chromatography(HILIC) etc. among the most popular methods of choice used for quality control of raw material and finished herbal product.

Keywords: Ultra-High performance Liquid Chromatography (UHPLC), High performance Thin Layer Chromatography (HPTLC), Gas Chromatography (GC), Hydrophilic Interaction Chromatography (HILIC), Two-dimensional Chromatography

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INTRODUCTION

People on all continents have used hundreds to thousands of indigenous plants for treatment of ailments since prehistoric times. Many plants synthesize substances that are useful to the maintenance of health in humans and other animals. These include aromatic substances, most of which are phenols or their oxygen-substituted derivatives such as tannins [1].

Sick animal tend to forage plants rich in secondary metabolites, such as tannins and alkaloids. Since these phytochemicals often have antiviral, antibacterial, antifungal and anthelminthic properties, a plausible case can be made for self-medication by animals in the wild [2].

According to an estimate of the World Health Organization (WHO), about 80% of the world population still uses herbs and other traditional medicines for their primary health care needs. Herbal medicine products are dietary supplements that people take to improve their health and are sold as tablets, capsules, powders, teas, extracts and fresh or dried plants. Herbals are traditionally considered harmless and increasingly being consumed by people without prescription [3].

Despite its existence and continued use over many centuries, and its popularity and extensive use during the last decade, traditional medicine has not been officially recognized in most countries. Consequently, education, training and research in this area have not been accorded due attention and support. The quantity and quality of the safety and efficacy data on traditional medicine are far from sufficient to meet the criteria needed to support its use world-wide. The reasons for the lack of research data are due to not only to health care policies, but also to a lack of adequate or accepted research methodology for evaluating traditional medicine". Hence every single herb needs to be quality checked to ascertain that it confirms to quality requirement and delivers the properties consistently. Standardization assures that products are reliable in terms of quality, efficacy, performance and safety [4].

CHROMATOGRAPHIC TECHNIQUES IN HERBAL DRUG ANALYSIS

Chromatography represents the most versatile separation technique and readily available. Chromatography is defined as technique of isolation and identification of components or compounds or mixture of it's into individual components by using stationary phase and mobile phase. Plant materials are separated and purified by using various chromatographic techniques. Herbal medicine is a complicated system of mixtures. Thus, the methods of choice for identification of' botanical drug' are mainly intended to obtain a characteristic fingerprint of a specific plant that represent the presence of a particular quality defining chemical constituents.

Chemical fingerprints obtained by chromatographic technique and especially by hyphenated chromatography, are strongly recommended for the purpose of quality control of herbal medicines, since they might represent appropriately the "chemical integrities" of the herbal medicines and therefore be used for authentication and identification of the herbal products. Thin layer chromatography (TLC) and High Performance Thin Layer Chromatography (HPTLC) are valuable tools for qualitative determination of small amounts of impurities. Also many analytical techniques such as Volumetric Analysis, Gravimetric Determinations, and Gas Chromatography (GC), Column Chromatography (CC), High Performance Liquid Chromatography (HPLC) and Spectrophotometric methods are also frequently used for quality control and standardization [5].

Thin Layer Chromatography

Thin layer chromatography is simply known as TLC. It is one of the most popular and simple chromatographic technique used of separation of compounds. In the phytochemical evaluation of herbal drugs, TLC is being employed extensively for the following reasons:

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

In TLC fingerprinting, the data that can be recorded using a high performance TLC (HPTLC) scanner includes the chromatogram, retardation factor (Rf) values, the color of the separated bands, their absorption spectra, λ max and shoulder inflection/s of all the resolved bands.

All of these, together with the profiles on derivatization with different reagents, represent the TLC fingerprint profile of the sample. The information so generated has a potential application in the identification of an authentic drug, in excluding the adulterants and in maintaining the quality and consistency of the drug.

TLC was the common method of choice for herbal analysis before instrumental chromatography methods like GC and HPLC were established.

Even nowadays, TLC is still frequently used for the analysis of herbal medicines since various pharmacopoeias such as American Herbal Pharmacopoeia (AHP) , Chinese drug monographs and analysis, Pharmacopoeia of the People's Republic of China etc. still use TLC to provide first characteristic fingerprints of herbs .

Rather, TLC is used as an easier method of initial screening with a semi quantitative evaluation together with other chromatographic techniques.

Table 1: Example of analytes evaluated by TLC

Sr. No.	Analyte	TLC System parameters	Reference
1.	*Harhra' (Terminaliachebula and Gallic acid)*	**Stationary phase:** Silica gel **Mobile phase :**Toluene – ethyl acetate – formic acid, 5:5:1	[6]
2.	*Azadirachta indica, Catharanthus roseus and Momordica charntia*	**Stationary phase:** Silica gel **Mobile phase:** Dichloro methane–methanol, 2:8	[7]
3.	*Mushroom extracts*	**Stationary phase:** Silica gel **Mobile phase:** Dichloromethane – ethyl acetate-methanol, 3:1:1	[8]
4.	*Strychnos nux vomica*	**Stationary phase:** Silica gel **Mobile phase:** Chloroform–ethyl acetate– diethyl amine, 0.5:8.5:1	[9]
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	[10]
6.	*Quinones*	**Stationary phase:** Silica gel 60 **Mobile phase:** dichloromethane-n-hexane, 8:2	[11]

High Performance Thin Layer Chromatography (HPTLC)

HPTLC technique is widely employed in pharmaceutical industry in process development, identification and detection of adulterants in herbal product and helps in identification of pesticide content, mycotoxins and in quality control of herbs and health Food. It has been well reported that several samples can be run simultaneously by use of a smaller quantity of mobile phase than in HPLC. It has also been reported that mobile phases of pH 8 and above can be used for HPTLC. Another advantage of HPTLC is the repeated detection (scanning) of the chromatogram with the same or different conditions. Consequently, HPTLC has been investigated for simultaneous assay of several components in a multicomponent formulation. With this technique, authentication of various species of plant is possible, as well as the evaluation of stability and consistency of their preparations from different manufactures. Various workers have developed HPTLC method for phytoconstituents in crude drugs or herbal formulations suchas bergenin, catechine and gallic acid in *Bergenia cilliata* and *Bergenia lingulata*

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Table 2: Example of mobile phase used in HPTLC for herbal compounds [12] [13] [14]

Chemical compounds (Herbal)	Mobile phase
Polar Compounds Anthraglycosides, Arbutin, Alkaloids, Cardiac Glycosides, Bitter Principles, Flavonoids, Saponin	Ethyl Acetate: Methanol: Water [100:13.5:10]
Lipophilic Compounds Essential oils, Terpenes, Coumarin, Napthoquinons, Velpotriate	Toluene: Ethyl Acetate [93:7]
Alkaloids	Toluene: Ethyl Acetate: Diethyl Amine [70:20:10]
Flavonoids	Ethyl Acetate: Formic Acid: Glacial Acetic Acid: Water [100:11:11:26]
Saponin	Chloroform: Glacial Acetic Acid: Methanol: Water [64:32:12:8]
Cardiac Glycosides	Ethyl Acetate: Methanol: Water [100:13.5:10] OR [81:11:8]
Terpenes	Chloroform: Methanol: Water [65:25:4]
Triterpenes	Ethyl Acetate: Toluene: Formic Acid [50:50:15] Toluene: Chloroform: Ethanol [40:40:10]
Essential Oil	Toluene: Ethyl Acetate [93:7]
Lignans	Chloroform: Methanol: Water [70:30:4] Chloroform: Methanol [90:10] Toluene: Ethyl Acetate [70:30]

Table 3: List of common derivatization agents used in HPTLC [15] [16]

Sr. No.	Color reagent	Chemical compounds (Herbal)	Color
1.	**Dragendroff Reagent** It forms complex reaction with some nitrogen containing compounds	Alkaloids	Red - brown Zone (vis)
2.	**Natural products** Polyethylene Glycol reagent i.e. Diphenylboric acid -2-aminoethyl ester forms complexes with 3-hydroxyflavones via condensation reaction	Flavanoids	Intense yellow, Orange and Green Fluorescent zones in UV 366 nm
3.	**Ninhydrin Reagent**	Amino acids, peptides, amines and amino-sugars	ellow, brown to pink and violet (vis)
4.	**Iodine** It produce iodine reaction possibly result in an oxidative products	Indole, quinolone derivative, thiols and all organic ompounds	Dark zone (UV 254)
5.	**10 % Ethanolic KOH**	Anthraquinones (Emodin, Rhein)	Red zones (vis) Red Fluorescence (UV 366 nm)
		Anthrones (Aloin, Cascarosides)	Yellow zones (vis) Yellow Fluorescence (UV 366 nm)
		Coumarins, Scopoletin, Umbelliferone	Bright blue Fluorescent zone (UV 366 nm)
6.	**Vanillin Sulphuric Acid OR** **Anisaldehyde Sulphuric Acid**	Bitter Principle	Red-brown, Yellow-brown, Dark green Zone (vis)
		Saponins	Coloured zones (vis)
		Essential oil	Blue, brown or red zones (vis)

High Performance Liquid Chromatography (HPLC)

Over the past decades, HPLC has received the most extensive application in the analysis of herbal medicines. Reversed phase (RP) columns may be the most popular columns used in the analytical separation of herbal medicines. Preparative and analytical HPLC are widely used in pharmaceutical industry for isolating and purification of herbal compounds. There are basically two types of preparative HPLC: low pressure HPLC (typically under 5 bar) and high-pressure HPLC (pressure >20 bar). The important parameters to be considered are resolution, sensitivity and fast analysis time in analytical HPLC whereas both the degree of solute purity as well as the amount of compound that can be produced per unit time i.e. throughput or recovery in preparative HPLC.

In preparative HPLC (pressure >20 bar), larger stainless steel columns and packing materials (particle size 1030µm are needed. The examples of normal phase silica columns are Kromasil 10 µm, Kromasil 16 µm, Chiralcel AS 20 µm whereas for reverse phase are Chromasil C18, Chromasil C8,YMC C18. The aim is to isolate or purify compounds, whereas in analytical work the goal is to get information about the sample. This is very important in pharmaceutical industry of today because new products (Natural, Synthetic) have to be introduced to the market as quickly as possible. Having available such a powerful purification technique makes it possible to spend less time on the synthesis conditions [17].

Table 4: List of herbal drug extract analyzed by HPLC [17]

Herbal drug extract	Active compounds	Column	Mobile phase	Flow rate	Gradient	Detector	Stop time	Inj vol.
Atropa Belladona	Atropine	4.6 x 75 mm Zorbax Eclipse XDB-C18, 3.5 µm	A = 0.05M KH2P04in 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 Cinchonae	Quinidine Quinine	4 x 125 mm Purospher RP-18, 5 µm	A = 0.05M KH2P04in water (pH = 3), B = acetonitrile	0.8ml/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 Norephedrine	4.6 x 75 mm Zorbax SB-C18, 3.5 µm	A = 0.025M KH2P04in 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 x 125 mm Hypersil 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 20 min 38 % B	Diode array detector 370 nm/16 (ref. off), standard cell	20 min	10 µl
Rheum Palmatum	Rhein Emodin	4 x 125 mm Hypersil ODS, 5 µm	A = 0.05 M NH 4 0Ac 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

Ultra-high performance liquid chromatography (UHPLC)

In recent years, UHPLC has been emerging as a feasible technique for the quality control of herbal products. UHPLC can withstand a pressure of at most 8000 psi and it brings liquid chromatographic analysis to another level by hardware modifications of the conventional HPLC machinery. UHPLC makes it possible to perform high-resolution separations superior to HPLC analysis by using solid phase particles of less than 2 µm in diameter to achieve superior sensitivity and resolution. Smaller particle size leads to higher separation efficiency and shorter columns size leads to shorter analysis time with little solvent consumption [18]. Within a period of last few years, UHPLC fingerprints of herbal products were developed instead of conventional HPLC approach[19] [20]. In comparison to HPLC, UHPLC analyses reported a decreased analysis time by a factor up to eight without loss of information. The results obtained not only showed decreased analysis time but also proved a great enhancement in selectivity compared to conventional HPLC analysis [21] [22].

Hydrophilic interaction chromatography (HILIC)

HILIC has gained attention in herbal fingerprinting because of good separation quality of hydrophilic compounds. Many of polar compounds of herbal medicines are extracted by using aqueous solution, which might be better separated by means of HILIC [23].

HILIC was introduced as an alternative for normal-phase liquid chromatography (NPLC); HILIC enables the separation of polar compounds on polar stationary phases with aqueous mobile phases. It is based on the principle of partitioning between a water-enriched layer in the hydrophilic stationary phase and a relatively hydrophobic mobile phase usually containing 5–40% water in organic solvent. This technique is more eco-friendly as compared to NPLC because of the use of water and polar organic solvents as mobile phase. In addition, the polar compounds are more soluble in the mobile phase of HILIC [23] [24].

As HILIC is a relatively recent technique, few papers analyzing herbal products have been published yet. Most papers usually describe a methodology exploiting the orthogonal character of the HILIC and reversed-phase liquid chromatography (RPLC) methods for quality control [24] [25].

Gas chromatography (GC)

GC is a well-established analytical technique commonly used for the characterization, quantization and identification of volatile compounds. It can be used in many different fields such as pharmaceuticals, cosmetics and even environmental toxins. Since the samples have to be volatile, human breath, blood, saliva and other secretion containing large amounts of organic volatiles can be easily analyzed using GC. The powerful separation efficiency and sensitive detection make GC a useful tool for the analysis of essential oils[26]. Despite its advantages, GC analysis of herbal products is usually limited to the essentials oils because of possible degradation of thermo-labile compounds and the requirement of volatile compounds makes GC unsuitable for many herbal compounds[27].

The hyphenation of GC–MS leads to reducing analysis times of essential oils (40–100 s) as well as decreased detection limits. GC–MS analysis of essentials oils, showing faster analysis and high efficiency, made use of micro-bore capillary columns with reduced stationary phase film thickness (10 m×100 µm I.D. and 5 m×50 µm I.D.) with rapid temperature programming (20 °C/s), fast data acquisition by FID and high split ratio. Finally, low-pressure GC–MS using mega-bore analytical columns (10 m×530 µm with 0.25–1 µm film thickness) was investigated on the essential oils and led to a slightly reduced efficiency but manifold decreased analysis times[28] [29][30].

GC can be used to determine the identity of natural products containing complex mixtures of similar compounds. For example, the geographic source of crude oil or natural gas can be determined by the fingerprint or relative distribution of major and trace compounds in each oil. Naturally produced oil such as food products and fragrances, can be identified by GCFID or GCMS. For example,

Sample	Carrier	Column	Injection	Detector
0.25 cc natural gas sample	Helium (8.6ml/min @60 ^{0}C)	HP-PLOT Q, (30 m X 0.53 mm X 40 µm)	Split mode (100ml/min)	TCD 250 ^{0}C

Two-dimensional (2D) chromatography

Before going to 2D chromatography, firstly one has to understand the difference between multi-dimensional fingerprint generated by hyphenated detection techniques and 2D chromatography. In multi-dimensional fingerprinting, the hyphenated detector collects information from the eluting compounds while a fingerprint is recorded. In 2D chromatography, fractions eluting from a first chromatographic system are chromatographed on a second system having different separation properties resulting in increased the peak capacity of entire separation. To reveal all characteristics of complex HDs, 2D chromatography was proposed. With the development of 2D chromatographic systems, a new era in herbal fingerprinting has been clicked. The main advantage of 2D chromatography over conventional one-dimensional chromatography is to obtain high peak capacity, which theoretically equals to the product of the peak capacities of two individual dimensions. However, a major limitation is very long time span needed to reach this maximum capacity. Literature review reveals that 2D chromatography has been used with different techniques such as 2D TLC, 2D HPLC, 2D GC and 2D chromatography combining size-exclusion and RPLC [31-34].

REFERENCES

[1] MR Hutchings; S Athanasiadou; I Kyriazakis; *I J Gordon. ProcNutr* Soc. May **2003**, 62(2), 361.
[2] E Cindy; M Houghton. Wild Health: How Animals Keep Themselves Well and What We Can Learn From Them, **2002**.
[3] BS Sekhon. *J Pharm Educ Res*., **2011**, 2(2), 55-56.

[4] General Guidelines for Methodologies on Research and Evaluation of Traditional Medicines, World Health Organization, Geneva, **2000**; 2001; 1.
[5] K Swatantra. *Archives of Applied Science Research*, **2010**, 2(1), 225-226.
[6] JK Lalla; PD Hamrapurkar; HM Mamania. *J. Planar Chromatogr*, **2000**, 13, 390.
[7] MY Habib; MS Islam; MA Awal; MA Khan. *J. Nutritn, Pak*., **2000**, 4, 17.
[8] J Lee; B Min; S Lee; M Na; B Kwon; C Lee; Y Kim; K Bae. *Planta Med.*, **2002**, 68, 745.
[9] MV Jadhav; SU Kedar; SB Gholve; VJ Kadam. *Int. J. Chem. Tech & Reser*., **2009**, 1-826.
[10] J Richter; K Kabrodt; I Schellenberg; X Yang; P Shang; X Hou; J Liu; W Sun. *J. Pharm. Anal.*,**2003**, 23 (3), 167.
[11] Article ID 732078, *BioMed Research International*, **2014**.
[12] MM Srivastava. Springer Verlag "High Performance Thin-Layer Chromatography (HPTLC), Berlin Heidelberg, **2011**, 32-60.
[13] H Wagner. Atlas "Plant Drug Analysis: A Thin Layer Chromatography", 2nd ed., Springer, **1996**.
[14*] Journal of Scientific and Innovative Research*, **2013**, 2(6), 1086-1096.
[15] D Knapp. Handbook of Analytical Derivatization Reactions, Wiley-Interscience, **1979**, 2 24, 449-453, 482.
[16] H Jork; W Funk. LC Reagents & Detection Methods –Physical & Chemical Detection Methods: Fundamentals, Wiley, **1990**, 15-40.
[17] Pharmaceutical Applications with HPLC, Agilent library, 2000.
[18] L Nováková; L Matysová; P Solich. *Talanta*, 68, **2006**, 908–918.
[19] W Kong; C Jin; W Liu. *Food Chem*., 120, **2010**, 1193–1200.
[20] CH Kuo; CW Lee; SC Lin. *Talanta*, 80, **2010**, 1672–1680.
[21] B Avula; YH Wang; RS Pawar. *J. Pharm. Biomed. Anal.* **2008**, 48, 722–73.
[22] Y Zhang; H Xu; X Chen. *J. Pharm. Biomed. Anal.* **2011**, 56, 497–504.
[23] Y Jiang; B David; P Tu *Anal. Chim. Acta*, 657, **2010**, 9–18.
[24] Y Jin; T Liang; Q Fu. J. *Chromatogr.*, **2009**, 1216, 2136–2141.
[25] Y Chen; W Bicker; JY Wu. J. *Chromatogr. A*, **2010**, 1217, 1255–1265.
[26] I Bombarda; N Dupuy; JP Da. *Anal. Chim. Acta*, 613, **2008**, 31–39.
[27] H Zhu; Y Wang; H Liang. *Talanta*, 81, **2010**, 129–135.
[28] F David; D Gere; F Scanlan. J. Chromatogr. A "Instrumentation and applications of fast high-resolution capillary gas chromatography", **1999**, 842, 309–319.
[29] AFL Godoi; W Vilegas; RHM Godoi. *J. Chromatogr. A* **2004**, 1027, 127–130.
[30] K Ravindra; AC Dirtu; A Covaci. *Anal. Chem.*, 27, **2008**, 291–303.
[31] X Li; DR Stoll; PW Carr. *Anal. Chem.*, 81, 2008, 845–850.
[32] Ł Cieśla; A Bogucka-Kocka; M Hajnos. *J. Chromatogr*.A, **2008**, 1207,160–168
[33] X Chen; L Kong; X Su. *J. Chromatogr. A* , **2007**, 43, 1721–1727.