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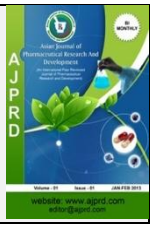

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Review Article

Analysis of Herbal Drugs by HPTLC: A Review

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A B S T R A C T

Densitometric evaluation through HPTLC is very useful method for standardization of medicinal plants and other natural products, especially those used in different system of medicine. It has been approved as an authenticated method of analysis in several pharmacopeias, including the USP and the IP. Result from a number of medicinal plants studied in the laboratory have shown that, TLC- densitometry is more advantageous strategy than HPLC or GLC. Improvement in resolution, HPTLC is valuable tool for the investigation of herbal products with respect to different aspects of their quality, sensitivity and reproducibility are the fundamental attributes. HPTLC plays an important role in the characterization of marker compounds for the development and standardization of herbal medicines. The HPTLC approach is particularly well suited to the examination of herbs and herbal preparations because it allows for easy comparison of samples using fingerprints and quantitative determination using scanning densitometry. Herbs and herbal mixtures are particularly challenging to standardise. Many of the current medications we use today for various ailments are based on plants and plant-based products. This review focused on the various HPTLC methods used to analyse herbal drugs. For each drug, extraction processes as well as the HPTLC analytical method have been optimised.

Key words: HPTLC, Densitometric evaluation, Medicinal plants, Herbal medicines, Quantitative determination.

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INTRODUCTION

Chromatographic techniques help in the separation of compounds in which stationary phase over which the mobile phase migrates in a directional manner. In planar chromatography the sorbent is spread on a planar surface. HPTLC is one sort of planar chromatography and the most advanced form of instrumental TLC, which is widely used as a cost-effective method for rapid analysis of sample mixture. In HPTLC sample application, chromatogram development, and detection are independent and widely used to standardize the methodology based on a validated method. The relative independence of sample application chromatogram development, and detection in time and location make possible the parallel analysis of many samples on the same plate. HPTLC is useful in the development of qualitative and quantitative evaluation techniques for the components present in any sample. It includes cutting-edge instruments controlled by a coordinated software programming, which ensures enhanced utility, reliability and reproducibility of the information produced. It is a flexible screening procedure with which both qualitative and quantitative analyses can be performed. HPTLC uses high-performance adsorbent layers (e.g., Silica gel with refined uniform particles, approximately 5 µm in diameter, as compared to 12µm in TLC).

Main Features of HPTLC:

The stationary phase used in this technique is disposable, that is the HPTLC plates. We can analyze several samples concomitantly through this technique, which results in its high test throughout. It permits more adaptability and ease in sample assessment due to the likelihood of sequential detection by corresponding strategies post chromatographic derivatization and quantification.

TLC and HPTLC are similar to each other in several aspects. HPTLC gives better efficiencies, better mass-exchange properties, and higher working speeds. HPTLC is a modern adaptation of TLC with better and more advanced separation efficiency and detection limits.

Fingerprint analysis by HPTLC is one of the most potent methods for linking botanical identity to a plant's chemical constituent profile. The fingerprint technology allows for a quick and easy identity check. It's also utilised to see whether there are any adulterations in the raw ingredients. A number of marker compounds can be chosen from the ingredient profile to indicate the quality of the herbals. Quantitative determination of these marker chemicals is also done using HPTLC.

The production of most herbal preparations includes some extraction process. It is essential for quality assurance that this extraction standardized. The quantity of marker compounds assayed by HPTLC is the principal method of monitoring the production process. When choosing marker compounds for a particular herb or herbal preparation, it is of critical importance that chemically well characterized standards are available for their quantification. It is often impossible to separate all components of a plant extract completely. Therefore, it must be proven with an independent method that a given marker compound in the extract is not coeluting with any other substance.[1,2]

Table 1: Major Differences between TLC & HPTLC-

Parameters	TLC	HPTLC
Technique	Manual	Instrumental
Efficiency	Less	High
Layer	Lab made	Precoated
Mean particle size	10-12 µm	5-6 µm
Layer thickness	250 µm	100µm
Plate height	30 µm	12 µm
Solid support	Silicagel, Alumina, Kiesulguhr	Silica gel-Normal Phase C8 and C18-reverse phase
Spotting of sample	Manual (Capillary/Pipette)	Syringe
Volume of sample	1-5µL	0.1-0.5 µL
Shape of sample	Circular (2-4nm)	Rectangular (6mm×1mm)
Separation	10-15 cm	3-5 cm
Separation time	20-200 min	3-20 min
Analysis time	Slower	Storage migration distance and the analysis time is greatly reduced
Scanning	Not possible	Use of UV/visible/fluorescence scanner

Various steps involved in TLC/HPTLC/planar chromatography:

Selection of TLC/HPTLC plates and sorbent.

Sample preparation including any clean up and pre-chromatographic derivatization.

Application of sample

Development (separation)

Detection including post-chromatographic derivatization

Quantitation

Reviews on HPTLC Methods for Herbal Drugs:

H Singh *et al.* (2018). performed the isolation characterization and quantification of marker compound (salicylic acid) from Cleome viscosa L seeds. HPTLC - Densitometric method has been developed and validated for bioactive compound (salicylic acid) from ethyl acetate extract using the solvent system of ethyl acetate: acetonitrile (1.1: 0-9, v/v). Precoated TLC glass plates with Silica gel F_{366} (Stationary Phase) was used. In terms of precision, reproducibility, and accuracy, this approach was validated using ICH principles. Salicylic acid showed a linearity range of 1050 ng/spot, and the quantity of salicylic acid was 0.9 - 0.04 ug/g of seed. Salicylic acid's limit of detection (LOD) and limit of quantitation (LOQ) were 4 ng and 8 ng, respectively.[3]

Sarfaraz Ahmed *et al.* (2019) extracted miquelignin from Euphorbia schimperi aerial parts and found to have antioxidant and anti-diabetic properties. A validated HPTLC method was used to quantify miquelianin, as well as kaempferol 3-0-glucuronde and quercetin 3-0-hamnoide, which were all separated from the same source. Chemical methods were used to measure antioxidant activity in terms of ABTS radical cation and DPPH radical scavenging activity. Miquelianin showed significant scavenging activity in both ABTS and DPPH assays as compared to standard BHA. In ABTS method IC_{50}values of miquelianin and standard BHA was found to be 58.90 ±3.40 ug/ml and 28.70 ± 5.20 ug/ml respectively while in DPPH assay IC_{50} values of miquelianin and standard BHA is 47.20 ± 4.90 µg/mi and 30.50 ± 6.20 µg/ml respectively**.** The inhibitory activity of α-amylase and α-glucosidase was used to investigate the anti-diabetic impact. The strong binding sites of miquelianin were also supported by the mechanistic approach through molecular modelling, which showed significant α-amylase and α -glucosidase inhibitory activities with IC50 values of 128.34 ±12.30 and 89.20± 9.20 ug/ml, respectively, as compared to agarose ±ug/ml. The concentration of miquelianin was determined to be 16.39 g/mg, and the concentrations of 3-0-glucuronid and 3-0-rhamnoside were found to be 3.92 and 14.98 ug/ml of dried extract, respectively, according to validated RP-HPTLC tests**.**[4].

N Deattu *et al.* (2013) used HPTLC and GC-MS to chromatographically analyse a polyherbal extract and formulation. Saraca indica, Symplocosracemosa, Hemidesmus indicus, Aloe vera, Asteracantha longifolia, Erythrina indica, and Tribulus terrestris were all used to make the polyherbal extract. After adding the needed excipients, the polyherbal extract was crushed into tablets. HPTLC study of extract and polyherbal formulation was carried out to ensure the correlation between them. Rf values of 0.03, 0.33, 0.48, 0.63 and 0.76 were detected in the chromatogram of both the extract and formulation. It was observed that the chromatogram of the formulation matched exactly with that of the extract. Thus HPTLC studies confirmed that there was good correlation between extract and formulation.The phytochemicals present in the formulation and the extract were identified by GC-MS method. Several peaks were visible on the GC-MS chromatogram of the extract and formulation. By comparing the NIST library data of the peaks and mass spectra of the peaks with those described in literature, the chemicals pertaining to the peaks were identified. The chemicals identified were found in both the extract and the formulation, demonstrating a strong link between the two.[5].

Savita Chewchinda *et al.* (2020) developed & validated a high performance thin- layer chromatography (HPTLC) densitometric method for the quantitative analysis of morin in Maclura cochinchinensis heartwood collected from different locations in Thailand. HPTLC analysis was carried out on an aluminium sheet of silica gel 60 F254 with a mobile phase of toluene, ethyl acetate, and formic acid (36:12:7, volume percent). At a wavelength of 410 nm, densitometric scanning was done. According to ICH guidelines, the HPTLC method was validated. Validation parameters for the proposed HPTLC method were found to be acceptable. Morin content ranged from 1.53 percent to 2.73 percent in M. cochinchinensis heartwood taken from eight different districts in Thailand. When compared to the HPLC method, this method had various advantages, including simplicity, speed, multiple sample handling, less solvent required, shorter analysis time, and lower cost per analysis [6].

Arti Gupta *et al.* (2013) identified ursolic acid in dichloromethane and ethyl acetate fractions of a methanolic extract of Ocimum gratissimum, as well as in a produced herbal hepatoprotective pill. Five fractions of three plant extracts, O. gratissimum, Butea monosperma, and Bauhina variegate, were used to create a hepatoprotective polyherbal formulation.O gratissimum is the only one of these three plants that contains ursolic acid. The mobile phase was petroleum ether: ethyl acetate; acetone (8.2:1.8:0.1 v/v/v) and the chromatographic separation was accomplished on silica gel HPTLC plates. After drying, the plates were sprayed with a 10% (v/v) ethanolic sulfuric acid solution and heated for 3 minutes at 120^{o}C. A computer-controlled densitometer was used to accomplish the quantification in absorbance/transmittance mode at a wavelength of 530 nm. For linearity 400-1200 (ng/spot), intraday precision percent CV (0.58-1.97), and interday precision percent C.V. (1.46-2.22). Correlation coefficient (r^2= 0 9960), the detection limits as well as recovery values (97.5 percent - 98.22 percent) were found to be adequate [7].

Prabhjot Kaur *et al.* (2019) simultaneously quantified oleanolic acid, ursolic acid, betulinic acid, and lupeol in distinct populations of five Swertia species. Oleanolic acid (OA), ursolic acid (UA), betulinic acid (BA), and lupeol (Lup) had retention factors of 0.25, 0.50, 0.58, and 0.74, respectively. When compared to the other standard methods, microwave aided extraction (MAE) utilising aqueous ethanol revealed to be the most efficient approach for extracting all of the examined triterpenoids [8].

Morteza Rouhani *et al.* (2019) used a probe type sonication technique using glycerol as the solvent to extract stevioside from dried Stevia Rebaudiana leaves. For stevioside detection and analysis, High Performance Thin Layer Chromatography (HPTLC) was employed as a very efficient, quick, and exact approach [9].

Saba Irshad *et al.* (2021) established a validated High Performance Thin Layer Chromatographic densitometric method for determining reserpine and ajmalicine in different seasons in *R. serpentina* and *R. tetraphylla*. In terms of instrument precision, range, linearity, and specificity, as well as limit of detection and limit of quantitation, the densitometric approach was validated. Using an adjusted mobile phase of toluene-ethyl acetate-diethyl amine (7:2:0.6 v/v/v), reproducibility of the peak of reserpine and ajmalicine was attained at Rf 0.56 and 0.79, respectively [10].

Sharda L. Deore *et al.*(2013) developed and validated HPTLC method for analysis of reserpine. Chloroform and acetone make up the solvent system (7:3). This system produced well separated reserpine dots. Reserpine Rf (0.28 ± 0.02), was analysed at a wavelength of 268 nm. The accuracy, precision, recovery, and robustness of the approach were all tested. The detection and quantification limits per spot were 109.85 and 292.10 ng, respectively.[11].

Duraisamy Gomathi et al.(2012) performed HPTLC fingerprinting of ethanolic extract of *evolvulus alsinoides (L.)* .The presence of steroids, terpenoids, and glycosides in an ethanolic extract of *E alsinoides* was discovered in the study[12].

CONCLUSION:

Plant materials are employed as home remedies, over-the-counter medicine products, and pharmaceutical raw materials in both developed and developing countries, and they account for a significant percentage of the global herbal drug market. As a result, it is critical to ensure that the quality of herbal products is repeatable. HPTLC is commonly utilised on precoated plates with an unaltered silica layer as the stationary phase and slit-scanning densitometry using UV-visible light as the detection method. The majority of the HPTLC methods developed and verified follow the general processes for the quantitative mode of this technology, according to this review.

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