CHEMICAL FINGERPRINTS OF MEDICINAL PLANTS- HPTLC PROFILING

Acharya Balkrishna

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Chemical Fingerprints of Medicinal Plants - HPTLC Profiling

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PREFACE

Herbs have been used for medicinal purposes since ancient time. A large number of populations in the world rely on herbs and herbal extracts for different ailments. The Indian traditional medicine system – "Ayurveda" also recognizes the importance of the herbs and their authenticity.

In the words of Sage Acharya Charaka,

Tasyāpīyaṃparīkṣā- Idamevaṃprakṛtyevaṃguṇamevaṃprabhāvamsmindeśe

jātamasminnṛtāvevaṃ gṛhītamevaṃnihitamevamupaskṛtamanayā ca mātrayā

CarakaSaṃhitāVimānasthāna 8:87

"Meaning - for selection of herbs Organoleptic, physical and chemical characteristics, habitat, season, method of collection, method of preservation, processing, and dosage should be considered"

With the increasing reliance of the worldwide population on herbs, the selection of the right quality of herbs, identification, and authentication are the need of time. In the modern day, different techniques like taxonomy, biological and chemical fingerprinting are in use to validate the procedure for the selection of the right quality herb.

In the present book, we have developed and compiled the High-Performance Thin Layer Chromatography (HPTLC) fingerprints of some of the commonly used medicinal plants. HPTLC fingerprint provides a scientific approach for the identification and authentication of herbal material as it provides a unique phytochemical distribution pattern which can be seen in the form of bands and can further be used for the purpose of qualitative and quantitative checks.

I am sure that this book will be immensely helpful as a reference book for the scientific community working in this area throughout the nation and world over.

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CHAPTER 1

Introduction to High-Performance Thin Layer Chromatography (HPTLC)

Abstract: This chapter discusses chromatography, classification of chromatography, thin layer chromatography (TLC) and how high-performance thin-layer chromatography (HPTLC) is an advanced version of TLC. In brief, this chapter also discusses the advantages and the areas where the HPTLC technique can be used.

Keywords: Chromatography, Densitometry, HPTLC, TLC, TLC fingerprint, UV 254 nm, UV 366 nm, Visualization.

INTRODUCTION

Chromatography is a technique or a process that is used to separate compounds from a mixture. It consists of two different phases: one is stagnant which is the stationary phase. The other is the mobile phase which moves through the stationary phase and separates out the components on the basis of their interactions with the mobile and stationary phases. The stationary phase can be solid (silica gel) or liquid, and mobile phase can be in liquid, gas, and supercritical form.

Chromatography can be classified on the basis of the mobile phase into liquid chromatography and gas chromatography. It is also classified on the basis of separation mechanism: ion exchange, size exclusive and adsorption, and can also be categorised by the shape of the bed into column chromatography and planer chromatography (TLC).

THIN LAYER CHROMATOGRAPHY (TLC)

TLC is a chromatographic technique that is used to elute out compounds on a thin layer of stationary phase generally prepared from silica gel.

For performing TLC/ HPTLC analysis, first, we dissolved the sample in a suitable solvent. The prepared sample is then applied on the TLC plate either manually or by autosampler (in HPTLC) in the form of a band or a spot. This TLC plate is then transferred to the chamber containing the mobile phase for development.

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As the mobile phase passes through the stationary phase, it allows the compounds to be separated on the basis of their polarity or affinity with both phases. As the mobile phase migrates toward the marked solvent front, the TLC plate is removed and dried. The developed TLC plate is then visualized under UV 254 nm, 366 nm, and white light with or without derivatization on the basis of the molecule to be detected [5].

HPTLC is the mechanized and advanced form of TLC as it allows the application of samples through the automatic sampler, with complete documentation of chromatographic data using software, and also with an auto scanner which helps in the identification of compounds. It provides both qualitative and quantitative analysis which is not possible in manual TLC methods.

The use of HPTLC allows proper separation with high resolution, repeatability, analysis of multiple samples together, visual inspection of chromatograms, and fast delivery of results.

TLC/HPTLC can successfully be hyphenated with other analytical techniques like High-performance liquid chromatography (HPLC), Mass spectroscopy (MS) and Fourier transform infrared (FTIR), which can give new dimensions to the research works [5].

Advantages of TLC/HPTLC

TLC offers several advantages:

- Visual representation and documentation of results.
- Parallel analysis of samples for fast screening.
- Flexibility.
- Detection of multiple samples.

Visual Representation and Documentation of Results

TLC provides the option to visualize the chromatograms in the form of the TLC plate image. TLC plate can be visualized under UV 254 nm and 366 nm for UV active compounds and some compounds can also be documented after derivatization using different spraying reagents on the basis of the class of compounds. Compounds are visualized in the form of bands and provide a fingerprint of that particular sample Fig. (**1**)

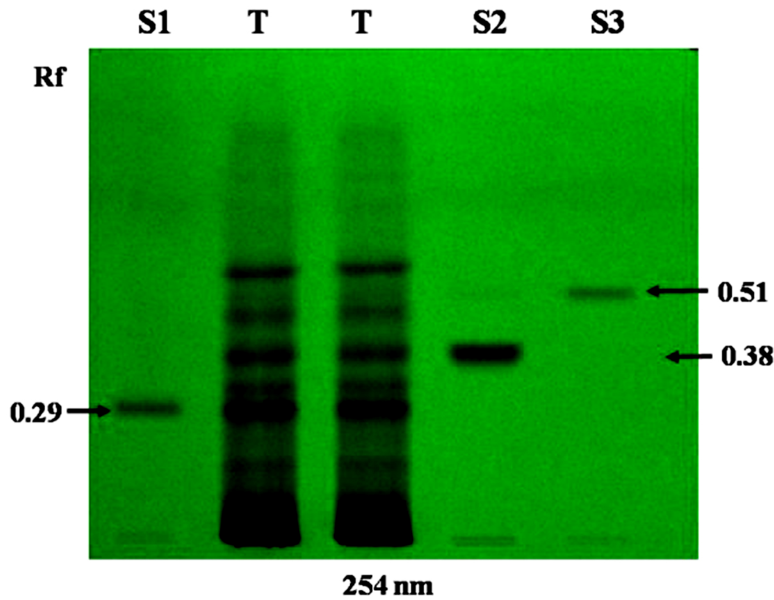

Fig. (1). HPTLC fingerprint of *Withania somnifera* (L.) Dunal at 254 nm.
Mobile phase (MP): Toluene: ethyl acetate: formic acid (5: 5: 1 v/v/v). S1: Witheferin A (Rf 0.28), T: *Withania somnifera* (L.) Dunal, S2: Withanolide A (Rf 0.20), S3: Withanolide B

Parallel Analysis of Samples for Fast Screening

This technique enables parallel analysis of multiple samples. In one TLC plate, we can apply approximately 18 different samples or same samples of different batches. TLC plate is developed under same chromatographic conditions and visualized, and it provides fast screening of multiple samples at one time Fig. (**2**).

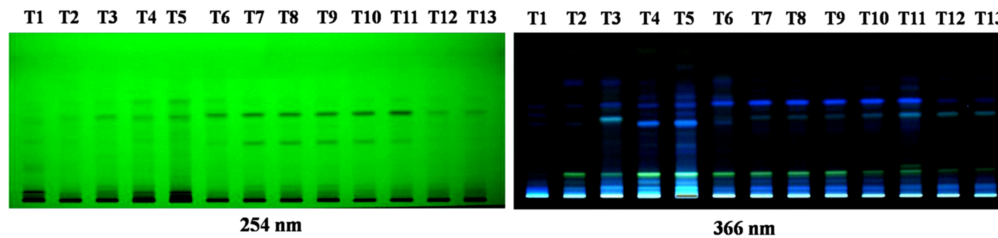

Fig. (2). Parallel analysis of multiple samples. MP: Ethyl acetate: toluene: formic acid (5: 5: 1 v/v/v).
A: 254 nm, B: 366 nm.

CHAPTER 2

High-Performance Thin Layer Chromatography Instrumentation

Abstract: HPTLC is one type of planar chromatography and the most advanced form of instrumental TLC. HPTLC equipment involves an autosampler for the application of a specific amount of sample on the plate, after spotting of sample the plate is developed in a TLC development chamber after development the plate is dried and visualized using visualizer. This chapter briefly discusses the equipment involved in the development of TLC.

Keywords: ATS 4, AMD 2, CAMAG, Densitometry, TLC.

INTRODUCTION

Sample Applicator

The quality of the chromatographic results depends on sample application. For quality results, samples must be positioned precisely, sample volume must be controlled and the layer must not be damaged.

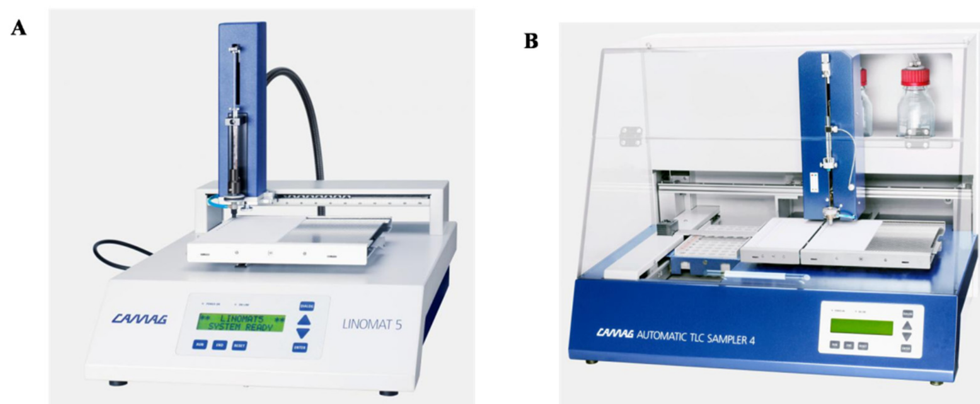

Fig. (6). Instrument for sample application. (**a**) CAMAG Linomate 5, (**b**) CAMAG Automatic TLC Sampler 4.

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In HPTLC, different instruments are available for semiautomatic and automatic sample application. The most widely used sample applicator featuring the spray-on technique is CAMAG Linomat 5 Fig. (**6A**) and CAMAG Automatic TLC Sampler 4 Fig. (**6B**). All application parameters are computer-controlled and programmed by CAMAG winCATS software. CAMAG Linomat 5 is semiautomatic in which the sample is loaded into the syringe manually. CAMAG ATS 4 is an automatic sample applicator as it allows the application of samples by both contact spotting and spray-on techniques [5].

TLC Developer

Chromatogram development is the key element of the process. There are different types of chambers that can be used for development: flat-bottom chamber Fig. (**7A**), twin-trough chamber Fig. (**7B**), automatic development chamber Fig. (**7C**) and automatic multiple development chamber Fig. (**7D**).

Fig. (7). Different types of development chambers. (**a**) Flat-bottom chamber, (**b**) Twin-trough chamber, (**c**) Automatic development chamber (ADC 2), (**d**) Automatic multiple development chamber (AMD 2).

A flat-bottom chamber permits the development of the plate under conditions of partial or complete saturation of the gas phase with solvent vapours.

Twin-trough chamber (TTC) is economic as well as flexible. A TTC is charged with a solvent to reach an initial level of 5 mm or 10 mm in the trough that receives the plate. The same amount is used in the rare trough if chamber saturation is established.

Automatic Development Chamber 2 (ADC 2,) is a fully automatic development chamber that generates reproducible results independent of handling errors. After placing the TLC plate into the device, the layer is automatically brought in contact with the mobile phase and development happens [4].

In automatic multiple development chamber (AMD 2), gradient mobile phase can be used. It is a fully automatic technique.

DERIVATIZER

Derivatization is performed to visualize fingerprints. This can be achieved through the gas phase and reagents. Different instruments used for derivatization in HPTLC/TLC are described below.

The glass reagent sprayer Fig. (**8A**) comes with a rubber pump but also be operated from a compressed air or nitrogen supply. CAMAG TLC sprayer Fig. (**8B**) is used for manual spraying of derivatization reagents onto TLC plates. CAMAG TLC spray cabinet Fig. (**8C**), ensures the complete removal of reagent mist while manually spraying onto the TLC plate. CAMAG Chromatogram immersion device Fig. (**8D**) is used for automatic dipping. HPTLC/TLC plates are immersed with uniform vertical speed into derivatization reagents. CAMAG TLC plate heater Fig. (**8E**), is designed for heating HPTLC/TLC plates at required temperature before and after derivatization [4].

VISUALIZER

TLC offers the advantage of visualizing chromatographic results directly in the form of fingerprints or chromatograms using UV light, at 254 nm and 366 nm and white light. CAMAG TLC Visualizer 2 Fig. (**9**) is used for imaging and documentation of TLC fingerprints (V) (https://www.camag.com).

CHAPTER 3

Process Steps for HPTLC Analysis

Abstract: This chapter briefly discusses the general aspects that can affect the result of TLC. Sample preparation, application of samples, preparation of development solvents, the steps involved in the development of TLC, visualization and derivatization, and detection of analyte are the key steps of chromatographic development. This chapter focuses on the process steps for HPTLC analysis.

Keywords: Derivatization, Detection, Stationary phase, Visualization.

INTRODUCTION

Sample and standards

Appropriate sample preparation is an essential step for successful TLC analysis. A suitable solvent system was used for either dissolving or extracting a solid or liquid sample to make the target molecules present in the sample available for analytical procedures without any interference from the sample matrix.

Preparation of Sample Solution

The raw materials or extracts of the medicinal herbs were processed and extracted using the appropriate solvent system, *i.e.* water/ethanol/methanol/hydro-methanol/ethyl acetate/hexane. Approximately, 1000 mg (for raw material) or 500 mg (for extract) of the test sample was transferred to a 10 mL volumetric flask and 5 mL of the solvent was added and homogenized in an ultrasonic bath for 20 minutes. The volume of the solution was made up to 10 mL mark with the solvent. The samples were centrifuged for 10 minutes at 9000 RPM and the supernatant, devoid of any particles was transferred to the sample application tube.

Standard Solution Preparation

For the preparation of the standard solution, 10 mg of the commercially available standard was weighed and transferred into a 10 mL volumetric flask. The standard

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was dissolved in 5 mL methanol, sonicated for 5 minutes and the volume of the standard was made up to 10 mL with methanol to obtain a concentration of 1 mg/mL.

Stationary Phase

The stationary phase is an adsorbent, material layer, usually silica gel or aluminum oxide, coated onto a surface, which is typically a glass plate or aluminum. There are a number of inert materials that are available as sorbent in TLC. To achieve optimum separation, it is very important to choose the correct material. Silica gel and aluminum oxide is mostly used for a wide range of applications. Silica gel and aluminas can also be split into a number of distinct sorbents, depending on the pore size, particle size and pH. The selection of the sorbent depends upon the nature and the type of compounds to be separated. Polarity, solubility, and molecular weight are some of the characteristic features for deciding the separation mechanism [7].

TLC silica gel 60 F_{254} (Merck KGaA, 64271 Darmstadt Germany) aluminum backed plates with a dimension of 5 × 10 cm or 10 × 10 cm or 20 × 10 cm (width × length) were used as stationary phase [7].

Sample Application

Application of sample and/or reference solution is a very critical step for obtaining quality results. This is due to the fact that precise and uniform sample application determine the overall quality of resolution and eventually the quantification values.

Band-wise spray-on technique was used for sample application on TLC plates. Suitable volumes of sample and standard were applied as 8 mm narrow bands on TLC plates using CAMAG ATS4, equipped with a 25 µL Hamilton syringe programmed by winCATS software.

Preparation of Developing Solvent

The mobile phase or developing solvent is the liquid that flows through a chromatographic system, moving the material to be separated at different rates on the stationary phase [6].

Development solvents consisting of more than one component were prepared by measuring the required volume of each component separately and transferring them into a solvent bottle of appropriate size. The bottle is closed with a lid and shaken to ensure proper mixing of the content. For measuring volumes smaller than 1 mL, a suitable micropipette was used, and a graduated volumetric pipette

of suitable size was used for volumes up to 20 mL. A list of commonly used solvent systems is provided in Appendix Table A1.

Development of TLC Plate

TLC plates were developed in a saturated twin trough chamber (TTC) according to the following procedure:

1. Appropriate volume of developing solvent was prepared (10 mL for 10 × 10 cm, 20 mL for 20 × 10 cm TTC).
2. A piece of correct sized (10 × 10 cm, 20 × 10 cm) filter paper was placed inside the rare trough.
3. The solvent was transferred into a chamber so that the filter paper is thoroughly wetted and adheres to the rare wall of TTC.
4. Chamber was tilted to the side (about 45 degrees) to equalize the volume of solvent in both troughs.
5. The chamber was allowed to saturate for 10 to 15 minutes or more on the basis of method development.
6. The TLC plate was then placed inside the TTC and allowed to develop up to 70 mm followed by drying the developed TLC plate.

Visualization and Derivatization

After development, compounds can be visualized under white light, long UV light (366 nm), short wave UV light (254 nm), or in white light after derivatization Fig. (**11**). Derivatization is performed to make compounds visual that cannot be visualized under UV light. There are several derivatizing reagents, which can be used for the purpose based upon the nature of compounds [6]. A list of commonly used derivatizing reagents is provided in Appendix Table A2.

For derivatization, the developed TLC plates were sprayed with derivatizing reagents and heated using a TLC heater at 110°C for 5-10 minutes.

Detection

Detection can be performed in absorbance or fluorescent mode using a tungsten, deuterium or mercury lamp. Deuterium lamp offers a continuous spectrum of UV light from 190 to 400 nm, tungsten lamp at 370 to 800 nm, and mercury vapour lamp at 366 nm. Detection generates chromatograms that can be used for both qualitative and quantitative purposes Fig. (**12**).

CHAPTER 4

Stationary Phase in High Performance Thin Layer Chromatography (HPTLC)

Abstract: As discussed in the first chapter, Chromatography is a separation technique based on the different affinities of the components in a mixture of two immiscible phases. One phase does not move (stationary phase) and the other moves through it in a definite direction (mobile phase). This chapter discusses the adsorbent or stationary phase used in TLC and the properties of these materials.

Keywords: Aluminium, Adsorption, Stationary phase, Silica gel.

INTRODUCTION

In TLC, the stationary phase is a thin layer of adsorbing materials, mostly silica gel or aluminum oxide, coated upon an inert glass, plastic, or aluminium plate surface. From the point of view of chemicals, silica gel is silicium dioxide, where each silicium atom is bound with four atoms of oxygen [4].

There are a number of materials that can be used as adsorbent in TLC. Some adsorbing materials can be used for all types of compounds (silica gel), while some have a limited range of applications (silica gel impregnated with caffeine for polyaromatic hydrocarbons). When we select the sorbent for analysis, the focus should be given to the class of compounds to be analyzed or separated. The polarity, solubility, molecule weight, shape and size of the targeted molecule play an important role in deciding the mechanism of separation. The separation of components depends on both the adsorbing material and solvent system [5].

The most widely used sorbent is silica gel, followed by cellulose and alumina. With time, there is an evolution in silica gel also, which results in opening up new separation. Some sorbents that can be used on the basis of class of compounds are shown below (4) (Table **1**).

SILICA GEL

Silica gel is also known as silicic acid and kieselgel gel. From the chemical point, silica gel is silicium dioxide, where each silicium atom is bound with four atoms

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of oxygen. The process of formation of silica gel starts with sand. When sand reacts with sodium hydroxide or sodium carbonate, it is converted into soluble sodium silicate. When this silicate solution is acidified, it gets hydrolyzed to form silicic acid which condensates to very tiny polysilicic acid hydrosol. With time, the hydrogel gets aggregated, establishes siloxane bonds and takes the shape of a cake. This cake is pulverized and washed to remove soluble impurities and salts of sodium [5].

Table 1. List of some commonly used adsorbing materials used as stationary phases.

S. NO.	Adsorbing Material	Class of Compounds
1	Silica gel	All types of compounds: Phenols, alkaloids, carbohydrates, aromatic compounds.
2	Aluminium oxide	Alkaloids, amines, steroids, aromatic compounds, aliphatic hydrocarbons.
3	Cellulose	Amino acids, carbohydrates
4	Reversed phase silica gel	Fatty acids, lipids, tetracycline's

The size and distribution of silica gel are primary properties of TLC. Smaller particles with narrow size distribution yield highly efficient chromatographic beds. Silica gel used for TLC usually has a particle size of 15 to 20 µm. HPTLC plates are prepared with smaller particles of silica gel ranging from 5 to 7 µm.

Pore size plays an important role in the chromatographic performance of silica gel. TLC material has an average pore size of 60 Å or 6 nm that is why the term Si 60.

Silica is porous, hydrated silicon dioxide, where each silicon atom is surrounded with four oxygen atoms. Some of these bonds are present on the surface of the pores [4]. These structures under chromatographic conditions allow silica gel to interact with analytes of different types. Some following types of interactions are possible:

- Proton donor or acceptor interactions.
- Dipole dipole interactions.
- Interaction with dipoles induced by the stationary phase.
- Interactions based on dispersion forces.

Silica Gel Bonded Phases

Silica gel provides a wide range of possibilities to separate different classes of compounds on silica gel. However, there are some bonded phases that can be grouped into polar and non-polar phases (Table **2**).

Some non-polar and polar bonded phases:

Table 2. List of some polar and non-polar bonded phases.

S. No.	Name	Functionality	Polarity
1	RP_2	Dimethyl	Non polar
2	RP_8	Octyl	Non polar
3	RP_{18}	Octadecyl	Non polar
4	Whatman	Diphenyl	Non polar
5	Amino	3- Aminopropyl	Polar
6	Cyano	3- Cyanopropyl	Polar
7	Diol	Spacer bonded propanediol	Polar

These bonded phases can be produced easily. By boiling silica gel in water, number of free hydroxyl groups can be generated. The silica gel is than hydrolyzed using inert organic solvent for example C18 chain. On the basis of the type of silane, the bonding, the surface coverage and degree of cross linking also vary.

Some other commonly used stationary phase sorbents are aluminium oxide and cellulose. Aluminium oxide is prepared by dehydration of aluminium hydroxide at a high temperature. It contains acidic aluminium ions, basic oxide ions and polar AI-OH group. This is useful for the separation of alkaloids, amines, steroids, aromatic compounds, and aliphatic hydrocarbons.

Cellulose is available in microcrystalline or in fibrous form. As it is a carbohydrate, it works well with water and lower alcohols. It provides good separation when the solvent system is aqueous or water based. It can also separate very polar compounds, but its migration speed is very slow. It takes a lot of time to reach the solvent front. It is used for amino acids, and carbohydrates separation.

CHAPTER 5

Hyphenation of HPTLC with other Techniques

Abstract: Hyphenation techniques are those where two different analytical techniques are combined together to give synergy. The advantage includes high separation efficiency, and the detection of compounds with high sensitivity and specificity. HPTLC can be combined with liquid chromatography (LC), infrared (IR) spectroscopy, ultra violate (UV) spectroscopy, and mass spectroscopy (MS). This chapter briefly discusses the hyphenation of HPTLC with other analytical techniques, its uses, advantages and areas of application of HPTLC coupled with other analytical techniques.

Keywords: HPTLC, LC, IR, MS, MS-Interface, Hyphenation.

INTRODUCTION

A hyphenation is a combinational approach, which means combining one technique with another. The combination of two different techniques can lead to the production of a large number of compounds with a wide range of spectral diversity in a short span of time. HPTLC can be hyphenated with high-pressure liquid chromatography (HPLC), mass spectroscopy (MS), Fourier transform infrared spectroscopy (FTIR) and Raman spectroscopy.

High-performance Thin Layer Chromatography (HPTLC) Hyphenated with Mass Spectroscopy (MS)

HPTLC in combination with MS, brings an added level of information to the chromatographic results and it also makes the identification and detection of molecules much easier. HPTLC coupled with MS allows verification of the chemical structure of molecules. By using the HPTLC-MS-Interface Fig. (**13**), the analyte can be directly eluted out from the developed TLC plate.

Compounds are separated on the TLC plate, after that the targeted molecules are marked either under UV 254 nm, 366 nm or white light. TLC is manually positioned on HPTLC-MS, the piston is then lowered by pressing the push button. The piston should lock the marked or targeted zone. Pushing the lever starts the solvent flow.

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The solvent passes through the locked zone on TLC, and then the molecule is dissolved with the solvent that can directly be injected into MS or collected in a separate sample storage tube. Hyphenating HPTLC with MS provides new dimensions for the technique and makes it more prestigious from a scientific point of view. HPTLC-MS can be used for screening phyto-metabolites, identification of bio-active molecules, and detection and identification of unknown impurities present in samples [4].

Fig. (13). CAMAG HPTLC-MS-Interface.

In the field of medicinal plants, this technique can be very useful, as the herbal plant material and extracts contain a very complex matrix, there are a number of compounds present in herbal materials, and isolation of these compounds by column chromatography takes a lot of time. Moreover, the isolation of each compound is not possible. By using TLC, the compounds can be separated and separated molecules can be easily identified by HPTLC-MS Fig. (**14**).

High Performance Thin Layer Chromatography (HPTLC) Hyphenated with Infrared Spectroscopy (IR)

HPTLC in combination with IR can be a great technique for the identification of the molecule or the functional group present in the sample. IR works on the principle that when IR radiation passes through the sample, some of the radiation is absorbed, and the radiation that passes by the sample is recorded in the form of spectra. As different molecule has different spectra, and on the basis of spectra, the molecule can be easily identified.

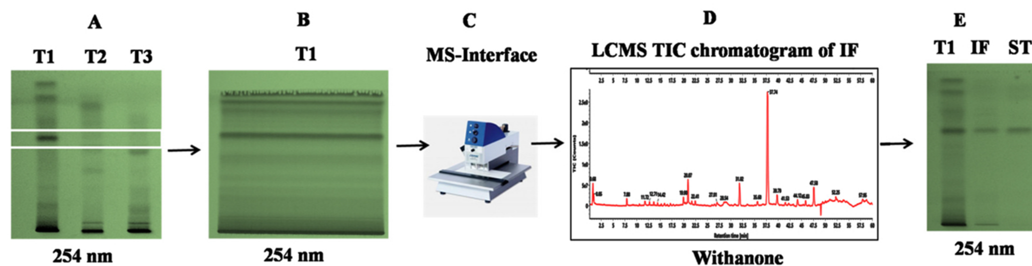

Fig. (14). Showing the application of HPTLC-MS-Interface in the field of medicinal plants for the identification of compounds. HPTLC fingerprint of *Withania somnifera* (Ashwagandha) at 254 nm along with the LCMS TIC chromatogram of isolated compound (by MS-Interface) and TLC of that isolated compound with withanone standard. M.P.: Ethyl acetate: toluene: formic acid (10: 1: 1 v/v/v). A: TLC fingerprint of T1: Ashwagandha mature leaves extract, T2: Ashwagandha juvenile leaves extract, T3: Ashwagandha whole plant extract. The marked compound is only present in T1 (Ashwagandha mature leaves extract), B: Preparative TLC of T1 (Ashwagandha mature leaves extract), C: MS-Interface for collection of the marked compound from TLC, D: LCMS TIC chromatogram of the collected compound (which is identified as withanone on the basis of its mass), E: HPTLC fingerprint of (T1) Ashwagandha mature leaves extract, (T2) Isolated fraction from T1 by MS-Interface, (ST) Standard withanone which is re-confirming the identified compound is withanone.

The HPTLC IR coupling can be categorized into two groups, direct and indirect hyphenation. Direct method is based on the direct hyphenation of HPTLC-FTIR, while in the indirect method, the targeted zone is collected from the TLC and transferred to IR. The spectra is then recorded. The HPTLC-FTIR, spectra make possible the detection and quantification of UV as well as non-UV compounds on the TLC plate.

Other analytical techniques that can be hyphenated with HPTLC are LC, UV spectroscopy and laser. Coupling of this technique can make the detection of molecules more simple and advanced. Coupling of HPTLC with other analytical techniques can be useful in food, chemical, biology, environmental and herbal or natural product areas.

CONCLUDING REMARKS

HPTLC is one of the most versatile techniques. It can easily handle various types of samples without consuming a lot of time. The hyphenation of HPTLC with other analytical techniques such as, UV, FTIR, LC and MS open various ways of the detection of molecules. It also gives new dimensions and makes hyphenation more prestigious from scientific point of view.

CHAPTER 6

Fingerprints of Medicinal Plants

Abstract: Medicinal plants are considered a rich source of phytochemicals, which can be used in drug development. For this purpose, the selection of correctly identified herbs is of significance as each herb has a unique phytochemical distribution pattern. High-Performance Thin Layer Chromatography (HPTLC) is one of the simplest yet robust techniques for the identification of herbs, as it can provide unique phytochemical fingerprints for each herb. However, process parameters of generating HPTLC fingerprints require rigorous optimization for each plant. This chapter provides HPTLC fingerprints for 108 medicinal plants together with their botanical name and common Hindi names. Besides providing optimized HPTLC conditions for generating these unique 108 fingerprints, this book also offers a list of solvent systems and derivatizing reagents for screening of phytochemicals. With classical taxonomical features and HPTLC fingerprinting methodology compiled, this chapter aims to serve as a guide for the identity verification of medicinal plants.

Keywords: Adsorbent, Canvas painting, HPTLC fingerprint, Medicinal Value, Solvent system.

INTRODUCTION

HPTLC fingerprint is a chromatographic pattern of phyto-active molecules that is specific for each and every medicinal plant. Chemical fingerprints provide an accurate method for the identification and authentication of herbal plant materials.

Achyranthes aspera L. (Common Name: Apamarga)

Plant Part: Whole plant.

Solvent System: Chloroform: methanol (9: 1 v/v).

Adsorbent: TLC silica gel 60 F_{254} (Merck).

Standard Solution Preparation: 10 mg of oleanolic acid was dissolved in 10 mL of methanol, and 10µL of the standard solution was applied on the TLC plate.

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Sample Solution Preparation: 100 mg of sample was dissolved in 10 mL methanol, sonicated and centrifuged, and 20 µL of the solution was applied on the TLC plate.

Derivating Reagent: Anisaldehyde sulfuric acid.

Medicinal Value: Useful in urinary ract disorders, indigestion and cold (1).

Conclusion: Rf 0.51 confirms the presence of bio-active marker oleanolic acid in Apamarga by HPTLC fingerprinting (Fig. **15a**, **b**).

Fig. (15a). Canvas painting of Apamarga illustrated by Patanjali Research Foundation, Haridwar, India.

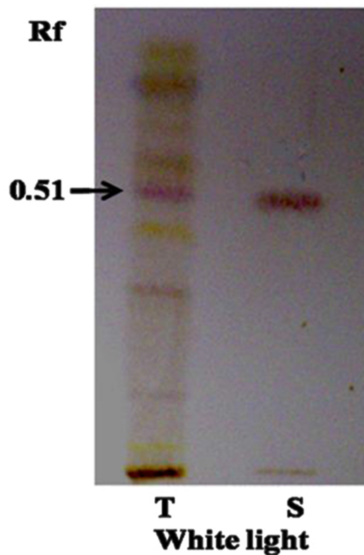

Fig. (15b). HPTLC fingerprinting of Apamarga: T: Apamarga whole plant, S: Oleanolic acid.

Acorus calamus **L. (Commmon Name: Vach)**

Plant Part: Rhizome.

Solvent System: Toluene: ethyl acetate: formic acid (5: 5: 1 v/v/v).

Adsorbent: TLC Silica gel $60F_{254}$ (Merck).

Sample Solution Preparation: 1000 mg sample dissolved in 10 mL of methanol, sonicated and centrifuged. 10µl of solution was applied on the TLC plate.

Derivatizing Reagent: Anisaldehyde sulfuric acid.

Medicinal Value: Used in skin diseases, respiratory and gastrointestinal diseases (3).

Conclusion: HPTLC fingerprint of Vach shows bands of phytochemicals under white light after derivatization (Fig. **16a**, **b**).

Fig. (16a). Canvas painting of Vach illustrated by Patanjali Research Foundation, Haridwar, India.

APPENDIX

Solvent Systems for Screening of Phytochemical Compounds of Herbal Plants by TLC/ HPTLC

Table A1. Solvent system for screening of phytochemical compounds of herbal plants by TLC/HPTLC.

Class of Compound	Mobile Phase	Derivatization
Alkaloids	Toluene: ethyl acetate: diethyl amine (7: 2: 1 v/v/v)	Dragendroff's reagent
	Toluene: methanol: diethyl amine (8: 1: 1 v/v/v)	
	Toluene: ethyl acetate: methanol: ammonia (25%) (3: 3: 1.5: 0.1 v/v/v/v)	
	Butanol: acetic acid: water (6: 2: 1 v/v/v)	
Essential oils	Toluene: ethyl acetate (9.3: 0.7 v/v)	Vanillin sulfuric acid
Flavonoids	Ethyl acetate: formic acid: glacial acetic acid: water (10: 1.1: 1.1: 2.3 v/v/v/v)	10 % methanolic sulfuric acid / Natural product reagent
	Toluene: ethyl acetate: formic acid (5: 5: 1 v/v/v)	
	Tetra hydrofuran: toluene: formic acid: water (1.6: 0.8: 0.2: 0.1 v/v/v/v)	
Glycosides	Ethyl acetate: methanol: water (20: 2.8: 2 v/v/v)	Alcoholic potassium hydroxide
Saponins	Chloroform: acetic acid: methanol: water (6.4: 3.2: 1.2: 0.8 v/v/v/v)	Anisaldehyde sulfuric acid
	Butanol: water: acetic acid (5: 4: 1 v/v/v)	
Steroids	Butanol: methanol: water (3: 1: 1 v/v/v)	Anisaldehyde sulfuric acid
Sterols	Ethyl acetate: chloroform (6: 4 v/v)	Anisaldehyde sulfuric acid
	Chloroform: methanol (9: 1 v/v)	

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Appendix I

Derivatization Reagents for Detection of Secondary Metabolites of Herbal Plants by TLC/HPTLC

Table A2. Derivatization reagents for detection of secondary metabolites of herbal plants by TLC/HPTLC.

Name of Reagent	Procedure	Examination	Detection of
Alcoholic iron chloride	2 g iron chloride is dissolved in 10 mL water and diluted to 200 mL with ethanol.	White light	Alkaloids, flavonoids, plant acids, phenols and tannins.
Alcoholic potassium hydroxide	5-10 g potassium hydroxide is dissolved in 10 mL of water and diluted to 100 mL with ethanol.	White light, UV 366 nm	Anthraquinones, coumarins and flavonoids.
Anisaldehyde sulfuric acid	Place 170 mL of methanol in 200 mL glass bottle and cool it down in water-ice cube bath. To the ice cold methanol, slowly and carefully add 20 mL glacial acetic acid and 10 mL sulphuric acid. Allow it to cool to room temperature, then add 1 mL para-anisaldehyde.	White light	Sterols, terpenoides and Saponins
Copper sulphate	20 g copper sulphate is dissolved in 180 mL water containing 16 mL ortho-phosphoric acid (80% in water).	White light	Lipids
Dregendroff's reagent	Solution A- 0.85 g basic bismuth nitrate is dissolved in 10 mL acetic acid and 40 mL water under heating. Solution B- 8 g potassium iodide is dissolved in 30 mL water. Just before spraying, 1 mL of each solution is mixed with 4 mL acetic acid and 20 mL water.	White light	Alkaloids
Natural product reagent	Solution A- 1 g of diphenylborinic acid aminoethylester is dissolved in 100 mL methanol. Solution B 5 g of polyethylene glycol 400 (macrogol) is dissolved in 100 mL ethanol.	UV 366	Flavonoids, carbohydrates, anthocyanines, plant acids
Ninhydrin reagent	0.6 g ninhydrin is dissolved in 190 mL isopropanol, then 10 mL acetic acid is added.	White light	Amino acid, ephedrins

(Table A2) cont.....

Name of Reagent	Procedure	Examination	Detection of
Vanillin sulphuric acid	1 g of vanillin is dissolved in 100 mL of 96 % ethanol add 2 mL of concentrated sulphuric acid.	White light, UV 366 nm	Terpenoids, ergot alkaloids, sterols

Appendix II

Table A5. Nkuv"of o gf kekpcri"planw0

S. No.	Botanical Name	Hindi Name	Sanskrit Name (Phonetics)
1	*Achyranthes aspera* L.	Apamarga	Apāmārgakaḥ kharamañjikaḥ
2	*Acorus calamus* L.	Vach	Ugragandhakaḥ vacaḥ
3	*Aegle marmelos (L.) Corrêa*	Bael	Bilvakaḥ tripatraḥ
4	*Albizia odoratissima* (L.f.) Benth.	Siris	Mṛdusumakam jāliśimbam
5	*Allium sativum* L.	Lehsun	Gandhālukaḥ rasonaḥ
6	*Aloe vera (L.) Burm.f.*	Aloe Vera	Majjapatrakam aṅgārasumam
7	*Anacyclus pyrethrum* (L.) Lag.	Akarkara	Ākārakarabhakaḥ chinnapatraḥ
8	*Andrographis paniculata* (Burm.f.) Nees	Kalmegh	Kālameghakaḥ tiktayavaḥ
9	*Areca catechu* L.	Supari	Pūgaphalakam vartulam
10	*Asparagus racemosus* Willd.	Shatavar	Śatāvarakaḥ adharakaṇṭaḥ
11	*Azadirachta indica* A.Juss.	Neem	Picumardakaḥ nimbaḥ
12	*Baccharoides anthelmintica* (L.) Moench	Kali jeera	Gandhimūlakam Kṛmighnam
13	*Bacopa monnieri* (L.) Wettst.	Brahmi	Brāhmikā medhyā
14	*Bauhinia variegata* L.	Kachnar	Yugmapatrakam bhinnadalam
15	*Berberis aristata* DC.	Daruhaldi	Pītadrukaḥ haridruḥ
16	*Berberis napaulensis* (DC.) Spreng.	Mahonia	Pītadrukaḥ daravalkaḥ
17	*Boerhavia diffusa* L.	Punarnava	Punarnavakaḥ raktakāṇḍaḥ
18	*Brassica juncea* (L.) Czern.	Lal sarson	Sarṣapakaḥrājikaḥ
19	*Cannabis sativa* L.	Bhang	Bhaṅgikā pītasapatrā
20	*Capsicum frutescens* L.	Mirch	Kaṭuvīrakā aṇḍapatrā
21	*Carica papaya* L.	Papita	Kumbhakarkaṭikā kṛṣṇerūḥ
22	*Carissa carandas* L.	Karonda	Karamardakaḥ pravṛttaphalaḥ
23	*Carum carvi* L.	Shah jeera	Jīrakam kapilam
24	*Cassia fistula* L.	Amaltas	Hemapuṣpakam kṛtamālam

(Table A3) cont.....

S. No.	Botanical Name	Hindi Name	Sanskrit Name (Phonetics)
25	*Catharanthus roseus* (L.) G.Don	Sadabhar	Sadāpuṣpakam pāṭalākṣam
26	*Cedrus deodara* (Roxb. ex D.Don) G.Don	Deodar	Devadrujaḥ dṛḍhakāṣṭhaḥ
27	*Celastrus paniculatus* Willd.	Malkangani	Kākāṇḍakaḥ gucchikramaḥ
28	*Chlorophytum borivilianum* Santapau & R.R.Fern.	Safed musli	Sitamuśalikā bhallapatrā
29	*Cinnamomum camphora* (L.) J.Presl	Kapoor	Gandhajātakam karpūram
30	*Cinnamomum tamala* (Buch.-Ham.) T.Nees & C.H.Eberm.	Tejpatta	Gandhajātakam tamālam
31	*Cinnamomum verum* J.Presl	Dalchini	Gandhajātakam tvak
32	*Cissus quadrangularis* L.	Hadjod	Vajravalī asthisaṁhārī
33	*Cocculus hirsutus* (L.) W.Theob.	Jaljamini	Śapherukaḥ mahāmūlaḥ
34	*Commiphora mukul* (Hook. ex Stocks) Engl.	Guggul	Guggulakaḥ devadhūpaḥ
35	*Convolvulus prostratus* Forssk.	Shankhpushpi	Kambumālakaḥ śaṅkhapuṣpaḥ
36	*Cordia dichotoma* G.Forst.	Lisoda	Śleṣmātakaḥ śeluḥ
37	*Coriandrum sativum* L.	Dhania	Dhāneyakaḥ kustumburuḥ
38	*Cornus capitata* Wall.	Thanboi	Cirāṅgaphalakam carmipatram
39	*Cressa cretica* L.	Rudanti	Rudantakaḥ caṇapatraḥ
40	*Crinum latifolium L.*	Sudarshan	Sudarśanakaḥ urupatraḥ
41	*Cullen corylifolium* (L.) Medik.	Bakuchi	Indulekhikā bākucī
42	*Cuminum cyminum* L.	Jeera	Ajājikā jīrakā
43	*Curcuma longa* L.	Haldi	Haridrakā pītāṅgā
44	*Cyperus scariosus* R.Br.	Nagarmotha	Jalamuk nāgaramustakaḥ
45	*Datura stramonium* L.	Datura	Dhattūrakaḥ kṛṣṇḥ
46	*Daucus carota* L.	Gajar	Garjarakaḥ nāraṅgaḥ
47	*Diplocyclos palmatus* (L.) C.Jeffrey	Shivlingi	Śivaliṅgikā karapatrā
48	*Eclipta prostrata* (L.) L.	Bhringraj	Bhṛṅgarājakaḥ keśyaḥ
49	*Elaeocarpus serratus L.*	Rudraksha	Rudrākṣḥ ārīpatraḥ
50	*Elettaria cardamomum* (L.) Maton	Elaichi	Elikā truṭiḥ
51	*Embelia ribes* Burm.f.	Viavidang	Viḍaṅgakaḥ kṛmighnaḥ
52	*Foeniculum vulgare* Mill.	Saunf	Miśreyakā śatāhvā
53	*Glycyrrhiza glabra* L.	Mulethi	Yaṣṭimadhukaḥ aroma
54	*Habenaria intermedia* D.Don	Riddhi	Ṛddhikārcī tuṣṭiḥ
55	*Hemidesmus indicus* (L.) R.Br.	Anantmool	Sārivakā sitaśirā

(Table A3) cont.....

S. No.	Botanical Name	Hindi Name	Sanskrit Name (Phonetics)
56	*Holarrhena pubescens* Wall. ex G.Don	Indrajao (Seeds)	Kuṭajakaḥ yavaphalaḥ
57	*Holarrhena pubescens* Wall. ex G.Don	Kutaj (Bark)	Kuṭajakaḥ yavaphalaḥ
58	*Lagenaria siceraria* (Molina) Standl.	Lauki	Kūpyālābukā pratumbī
59	*Lavandula stoechas* L.	Ustekhaddus	Phullakā rājisumā
60	*Lawsonia inermis* L.	Henna	Madayantikā rañjanā
61	*Leucas cephalotes* (Roth) Spreng.	Dronpushpi	Droṇapuṣpakam śīrṣakramam
62	*Lilium polyphyllum* D.Don	Ksheer kakoli	Lalāmakaḥ bahupatraḥ
63	*Mentha × piperita L.*	Mint	Pūtihākā tailapatrā
64	*Momordica charantia* L.	Karela	Kāravellakaḥ dīrghaphalaḥ
65	*Moringa oleifera* Lam.	Sahjan	Śigrukaḥ sitapuṣpaḥ
66	*Murraya paniculata* (L.) Jack	Gandhani	Kaiḍaryakaḥ pūgapuṣpaḥ
67	*Myristica fragrans* Houtt.	Javatri (Aril)	Gandhacolakaḥ raktaḥ
68	*Myristica fragrans* Houtt.	Jaiphal (Fruit)	Gandhacolakaḥ raktaḥ
69	*Nardostachys jatamansi* (D.Don) DC.	Jatamansi	Jaṭālakaḥ naladaḥ
70	*Nymphaea nouchali* Burm.f.	Neel kamal	Kumudakam nīlam
71	*Ocimum basilicum* L.	Tulsi	Sumañjarikā śyāmā
72	*Ocimum tenuiflorum* L.	Tulsi	Sumañjarikā rāmā
73	*Operculina turpethum* (L.) Silva Manso	Nisoth	Pīnaśalkam tāmrakali
74	*Phyllanthus emblica* L.	Amla	Āmalakaḥ dhātrīphalaḥ
75	*Picrorhiza kurroa* Royle ex Benth.	Kutki	Kaṭukā tiktā
76	*Piper longum* L.	Chhoti pippali	Kaṇikā pippalī
77	*Piper nigrum* L.	Marich	Kaṇikā maricā
78	*Piper retrofractum* Vahl	Badi pippali	Kaṇikā golasapatrā
79	*Pistacia chinensis subsp. integerrima (J.L.Stewart) Rech.f.*	Kakra singhi	Karkaṭaśṛṅgakam raktapallavam
80	*Putranjiva roxburghii* Wall.	Putrajeevak	Putraṇjīvakaḥ tigmāntaḥ
81	*Raphanus raphanistrum subsp. sativus (L.) Domin*	Mooli	Mūlakam hastidantam
82	*Rheum emodi Wall.*	Revandchini	Gucchikramakaḥ pītamūlaḥ
83	*Rosa indica* L.	Gulab	Taruṇikā pāṭalā
84	*Roscoea purpurea* Sm.	Kakoli	Kākolikā vṛntākā
85	*Saraca indica* L.	Ashok	Tāmrapallavakaḥ aṅgārakaḥ

(Table A3) cont.....

S. No.	Botanical Name	Hindi Name	Sanskrit Name (Phonetics)
86	*Sauromatum venosum* (Dryand. ex Aiton) Kunth	Talia kand	Saraṭāṅkaḥ vṛntākaḥ
87	*Semecarpus anacardium* L.f.	Bhilawa	Bhallātakaḥ sphoṭalaḥ
88	*Senna alexandrina* Mill.	Senna	Cakramardakaḥ tanupatraḥ
89	*Senna occidentalis* (L.) Link	Kasundi	Cakramardakaḥ nāraṅgaśiraḥ
90	*Senna tora* (L.) Roxb.	Panwad	Cakramardakaḥ aṇḍadalaḥ
91	*Solanum lycopersicum* L.	Tamatar	Yukpañcakam rasapūram
92	*Solanum nigrum* L.	Makoy	Yukpañcakam kṛṣṇaphalam
93	*Solanum virginianum L.*	Chhoti kateli	Yukpañcakam pītaprakaṇṭam
94	*Swertia chirayita* (Roxb.) H.Karst.	Chirayita	Ghūrṇobhadalakam tiktam
95	*Syzygium aromaticum* (L.) Merr. & L.M.Perry	Laung	Jambukaḥ lavaṅgaḥ
96	*Terminalia arjuna* (Roxb. ex DC.) Wight & Arn.	Arjun	Vīrakaḥ arjunaḥ
97	*Terminalia bellirica* (Gaertn.) Roxb.	Bahera	Vīrakaḥ akaṣaḥ
98	*Terminalia chebula* Retz.	Harad	Vīrakaḥ harītakaḥ
99	*Tinospora cordifolia* (Willd.) Hook.f. & Thomson	Giloy	Saptaśirikā aromapatrā
100	*Trachyspermum ammi* (L.) Sprague	Ajwain	Yavānikā ugragandhā
101	*Trigonella foenum-graecum* L.	Methi	Methikā gandhibījā
102	*Urtica ardens* Link	Bichu ghas	Alipatrakam sajātam
103	*Viola odorata* L.	Banafsha	Vanapsikā nīlapuṣpā
104	*Vitex negundo* L.	Nirgundi	Nirguṇḍikā madhupapriyā
105	*Vitis vinifera* L.	Angoor	Drākṣikā gostanī
106	*Withania somnifera* (L.) Dunal	Ashwagandha	Aśvagandhakaḥ svāpakaraḥ
107	*Zanthoxylum armatum DC.*	Tambru	Tumburukaḥ kaṇṭikāṇḍaḥ
108	*Zingiber officinale* Roscoe	Sunthi	Ārdrakam sitauṣṭham

ABBREVIATIONS

ADC	=	Automatic development chamber
AMD	=	Automatic multiple development chamber
Fig	=	Figure
HPLC	=	High pressure liquid chromatography
HPTLC	=	High performance thin layer chromatography
Mg	=	Milligram
mL	=	Milliliter
mm	=	Millimeter
MP	=	Mobile phase
MS	=	Mass spectroscopy
nm	=	Nanometer
Rf	=	Retardation factor
RPM	=	Rotation per minute
S	=	Standard
T	=	Test sample
TLC	=	Thin layer chromatography
TTC	=	Twin trough chamber
UV	=	Ultraviolet
V	=	Volume
µm	=	Micrometer
254	=	Short wave length UV light, reflection
366	=	Long wave length UV light, reflection

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Medicinal plants are considered a rich source of phytochemicals for drug development. For this purpose, the selection of correctly identified herbs is significant, as each herb has a unique phytochemical distribution fingerprint. High-Performance Thin-Layer Chromatography (HPTLC) is one of the simplest yet robust techniques for herb identification, providing unique phytochemical fingerprints for each herb. However, the process parameters for generating HPTLC fingerprints require rigorous optimization for each plant. The book 'Chemical Fingerprints of Medicinal Plants - HPTLC Profiling' aims to meet this technical need in the present herbal medicinal world.

The HPTLC technique has been long in use for phytochemical identification. However, a systematic compilation of the process parameters for identifying different medicinal plants is missing. This book precisely fulfills those specific requirements and serves as an analytical guide for the identification and characterization of medicinal plants at large."

Professor Y. K. Gupta
MBBS, MD, FAMS, FNASc, FIPS, FIAN, FST

Acharya Balkrishna

Acharya Balkrishna, a person of humble demeanour, was born on July 25, 1972 to Nepalese parents – Shrimati Sumitra Devi and Shri Jay Vallabh in Uttarakhand, India. He serves as the Founder Secretary of Patanjali Yogpeeth Trust, holds the position of General Secretary of Patanjali Research Foundation and Vice-Chancellor at the University of Patanjali.

From a young age, he displayed a profound love for nature, turning it into a lifelong passion and dedicating his existence to the realm of Ayurveda. In recognition of his commitment to bringing Ayurveda to the global forefront, he received the UNSDG 10 Most Influential People in Healthcare Award at the United Nations (UN) headquarters in Geneva, Switzerland, on May 25, 2019. Additionally, Acharya Balkrishna has earned acknowledgment as one of the world's top 2% scientists in consecutive years 2022 and 2023 for his ground breaking research contributions to the field of Ayurveda.

Guided by Acharya Balkrishna's visionary leadership, the avant-garde Patanjali Research Foundation, nestled in Haridwar, stands as a beacon of pioneering research in the realm of evidence-based Ayurvedic medicine. The distinguished institution meticulously conducts ground breaking studies on Ayurvedic formulations, progressing from rigorous laboratory and animal trials to human trials, strictly adhering to international protocols.

Acharya Balkrishna's profound expertise serves as the cornerstone of inspiration driving all research pursuits at the Patanjali Research Foundation. Under his dynamic guidance, the foundation has contributed a wealth of knowledge to the global scientific community, boasting the publication of over 300 peer reviewed research articles in esteemed international scientific journals. Furthermore, his intellectual prowess is underscored by an impressive portfolio of 45 patents, a testament to his innovative contributions in the field.

His extensive literary legacy includes authoring more than 120 books on Yoga and Ayurveda, coupled with the meticulous scripting of over 25 unpublished ancient Ayurveda manuscripts. His extensive contributions extend to diverse projects, including Herbal Garden & Herbarium, the inaugural World Botanical Dictionary, Wealth of Food Crops, Identification of Rare Herbs with Medicinal Properties, Vegetation Survey of India, and the comprehensive multivolume World Herbal Encyclopaedia.

The book 'Chemical Fingerprints of Medicinal Plants – HPTLC Profiling' stands as a testament to their mission, bridging a technological gap with finesse. Through High-Performance Thin-Layer Chromatography (HPTLC), this literary masterpiece underlines a pivotal role in precisely identifying and characterizing medicinal herbs. Credit for this achievement lies with Acharya Balkrishna, whose profound analytical grasp of the 5000-year-old Ayurvedic system propels this endeavour. His expertise, a guiding light, ensures the meticulous analysis of medicinal plants, offering invaluable insights to practitioners of herbal medicine.