e-ISSN:2321-0812
p-ISSN:2347-2340

Research and Reviews: Journal of Pharmaceutical Analysis

Critical Factor That Governs a Successful TLC/ HPTLC Analysis of Herbal Medicinal Products (HMPS).

Rahul Kasar[1]*, Ashish Gogia[2], Kartik Shah[2], Vetriselvan[1] and Chinmay Anand[2]

[1]Health Foods Research Lab, Phytochemistry, Research and Development, The Himalaya Drug Company, Makali, Bangalore 562 123, Karnataka, India.
[2]Formulation and Development Department, Cadila Pharmaceutical Limited, Ahmedabad, Gujrat, India.

Review Article

Received: 28/08/2013
Revised: 10/09/2013
Accepted: 17/09/2013

*For Correspondence

Health Foods Research Lab, Phytochemistry, Research and Development, The Himalaya Drug Company, Makali, Bangalore 562 123, Karnataka, India.

Keywords: Quality control, Phytoconstituents, TLC/HPTLC, Herbal industries.

ABSRACT

Chromatography is one of the fast emerging tools by which the quality control and fingerprint of herbs can be maintained. Using this technique, the identification of various chemical markers of the herbal drugs can be easily done and it also helps to identify the same herbs in combination. Application of TLC/HPTLC methodology in testing of phytoconstituents from individual herbal drugs and fingerprint characteristic of the herbal plants are reviewed in this paper. Popularity of TLC/HPTLC analytical method for analysis of herbal drugs due to economic, rapid, simultaneously screening of large number of herbal samples and less time consuming methods. The different mobile phase, spraying reagent, property of herbal drugs and its phytoconstituents, TLC/HPTLC plates, trouble shooting of HPTLC, different developing solvents and chromatograms are pointed out in this paper

INTRODUCTION

Many aqueous extract or alcoholic extracts, hydro alcoholic extracts are used in manufacturing Ayurvedic and herbal formulations. If the phytochemical profile of the plant or its part is known an appropriate kind of extract can always be used by selection for a particular purpose. A TLC or HPTLC profile of the phytochemical can be employed for the similarity or dissimilarity or to find out the presence or absence of the certain phytochemicals [1]. TLC/HPTLC has excellent resolution and, therefore, permits simultaneous identification of a wide range of substances in a single run. In this paper, application of TLC/HPTLC methodology in testing, investigation, advantages of HPTLC for analysis of Medicinal plants, General guideline for the analysis by HPTLC, Trouble shooting in HPTLC, Typical tasks in the quality control of medicinal plant, Documentation are reviewed as table no 1, 2, 3.

Parameters of Planar Chromatography

The articles in this series are dedicated to the important steps of planar chromatography and their parameters, which influence the chromatographic result. Hints for optimization are given to help the reader to use planar chromatography most efficiently. General methodology for HPTLC is a guideline for the analysis by HPTLC. This SOP provides general guidance for HPTLC.

TLC/HPTLC Methodology [5]

The components of TLC/HPTLC methodology for drug testing and investigation are 1) Selection of TLC material, 2) use of appropriate developing solvents, 3) use of appropriate color reagent and 4) interpretation of TLC/HPTLC result. Various aspect of TLC/HPTLC methodology is reviewed in this section.

Conversion of methods from TLC to HPTLC

Parameters	TLC	HPTLC
Application volume, µL	10-20 (50)	1-5 (10- 20%)
Band length, mm	10- 20	8
Developing distance, cm	10- 15 (18)	6

e-ISSN:2321-0812
p-ISSN:2347-2340

Table 1: Chrecterstics of Various Analytical Chromatographic Methods [2]

Method	Principle	Sensor	Matrix	Detection Limit	Processing Interferences requirements
Gas chromatography	Partition between mobile/ stationary phase	Thermocouple	Gases	10^{-2} -10^{-3} g	Not usually needed; good selectivity
		Flame ionization detector	Organic volatile compounds	10^{-4} -10^{-5} g	Not usually needed; good selectivity
		Electron capture detector	Halogenated volatiles	10^{-6} -10^{-7} g	Not usually needed; good selectivity
GC-MS	Ions /Species from GC mass analyzed (Ion from electron impact resolved by magnetic /electric field)	Mass analyzer (Quadrupole)	Gases/volatile liquids	10^{-10} g	May be needed ; highly specific, multi components capability
Liquid chromatography (HPLC)	Partition of (Organic) species between mobile/stationary phases	UV- absorption PMT	Aqueous /Non aqueous solution	10^{-3} -10^{-4} g	Not usually needed; good selectivity
		Amperometric detector	Generally aqueous electro active ions	10^{-3} g /ml	Sometimes needed; good selectivity
Ion chromatography	Ionic conductivity of analytes	Conductivity detector	Aqueous solutions of concentration	ppb level generally selective	Not usually needed
High Performance thin layer chromatography (HPTLC)	Partition of (Organic) species between mobile/stationary phases	UV- absorption, florescent detection	Aqueous/ organic solution of concentration	10^{-3} -10^{-4} g	Sometimes needed; good selectivity

Table 2: Advantages of HPTLC for Analysis of Medicinal Plants [3]:

Parameter	Advantage
Analysis time	Comparatively short, many samples can easily be compared side by side on the same plate. This is particularly important for screening and inspection/ selection of raw materials, and for in process control during manufacture.
Fingerprint	Can be optimized for certain target compounds. Even if some components migrate with the solvent front or remain at the application position, the fingerprint always represents the sample in its entirety. Unlike in column chromatography, it is not problematic if portion of the sample are irreversibly adsorbed, because each plate is used only once. A complex sample can be analyzed in several different chromatographic systems focusing on different substance classes.
Sample preparation	Most of the time, little or no cleanup is necessary.
Lower resolution power	Although this would most likely be looked at as a downside, it may be advantage because small insignificant differences between samples due to natural variability may not show. It is therefore easier to define acceptance criteria for quality control.
Flexibility of detection	The convenience of specific derivatization and the possibility of multiple detection without repeating the chromatography are particularly useful for fingerprint analysis.
Visual data	HPTLC results are not only reported as peak data but can also conveniently be presented and communicated as images.

e-ISSN:2321-0812
p-ISSN:2347-2340

Table 3: General Guideline for the Analysis By HPTLC [4]

Procedures	Its application
Plate material	Rewashing HPTLC plates (silica gel 60 F 254) in methanol.
Sample application	With the help of Linomat, Automatic TLC sampler
Preparation and storage of developing solvents	The developing solvents are preparing by measuring the require volume of each component separately and transferring them into a solvent bottle of appropriate size. The bottle is close with lid and shakes to ensure proper mixing of the content. Developing solvents should be clear solution.
Developments	Plates are developed in a saturated Twin Trough Chamber
Derivatization	Spraying or dipping may accomplish transfer of reagent for derivatization of samples on a HPTLC plate. Dipping is preferred method. If derivatization includes heating, a plate heater is used.
Documentation of plates	Each developed plates is documented with an electronic documentation system under UV 254 nm (Short-wave UV light), UV 366 nm (Long-waver UV light) and white light. All images are labeled and listed in the project work sheet.
Labeling	Each plate images are given an identification number (ID), which will be written in pencil in the top right corner. The ID includes Project number, dash, Year, month, day, dash and a consecutive number each day. [Example:- A091-060322-02 and for image label is A091-060322-02-A254]
Quantitative evaluation	It is performed with the TLC Scanner 3 using WinCATS software. The analysis files are labeled to reflect the plate ID.
Documentation of work	All work performed is documented in a project worksheet.

Table 4: General HPTLC methods followed for typical class of phytochemical

Substance class	Mobile phase
Alkaloid drug	Toluene, ethyl acetate, diethyl amine or ammonia (70:20:10)
Purine drugs	Ethyl acetate, methanol, water (100:13.5:10)
Anthracene derivatives	Ethyl acetate, methanol, water (100:13.5:10) or n-propanol, ethyl acetate, water, acetic acid (40: 40:29:1)
Essential oils	Ethyl acetate or methanol and toluene or hexane in various concentrations, or neat dichloromethane
Flavonoids drugs	Ethyl acetate, formic acid, acetic acid, water (100:11:11:26) or formic acid, water, ethyl acetate in various concentrations, with or without ethyl methyl ketone.
Arbutin, hydroquinone derivatives	Ethyl acetate, methanol, water (70:30:10) Formic acid, water, ethyl acetate (6:6:88)
Saponins drugs	Chloroform, methanol, water (70:30:4) Acetic acid, water, 1-butanol (10:40:50) or ammonia, water, ethanol, ethyl acetate (1:9:25:65) or ethyl acetate, water, 1-butanol (25:50:100 upper phase)
Tannins	Formic acid, water and ethyl acetate in various concentrations, with or without acetic acid, or ethyl acetate, toluene (2:98) or acetic acid, ether, hexane, ethyl acetate (20:20:20:40)
Carbohydrates	Water, Acetonitrile (10:85) or sodium dihydrogen phosphate 1.6%, 1-butanol, acetone (10:40:50)
Amino acids	1-butanol, water, acetic acid, formic acid, formic acid (28:8:9:2)

Types of TLC plate

HPTLC analysis was performed on a Camag HPTLC system .The plate used was HPTLC 254 silica gel 60 (E. Merck). Camag HPTLC system equipped with a sample applicator Linomat IV, Twin trough developing chamber, Integration software system, CATS V.4.06, TLC scanner III in absorbance/reflectance mode

Spray reagent

After the plate is developed, it is sprayed with various reagents for the development of color often the color reaction is not confined to a single compound but is produce by several compounds belonging to a particular group. Therefore along with using migration rates, various constituents of a sample are also identified their response to

chemical treatment. The coloring reagents commonly used for drug testing are listed in table no.5 and the correlations of color response with particular aspect of drug structure are reviewed.

Table 5: Most common derivatization reagents [6]

Reagents	Substances classes
Sulfuric acid	General reagents (charring reaction), Sterols, Saponins, Terpenoids, Iridoids, Flavonoids, most of lipophilic compounds
Anisaldehyde/ vanillin with sulfuric or phosphoric acid	As sulfuric acid, but usually shows more colored zones
Fast blue salt R	Tannins, coumarins, cannabinoids, amines
Ammonia vapor	Alkaloids, Flavonoids, opiates, mycotoxins, sennosides, valepotriates, anthracenes
Iodine solution	Alkaloids, heterocyclic nitrogen compounds,
Dragendorff's reagent	
Aniline-diphenylamine-phosphoric acid	Sugar, glycosides
Iron (III) chloride	Phenols, Flavonoids, tannins, plant acids, ergot alkaloids, hop bitter principles, hypericin.

Table 6: Fingerprint of some medicinal plants by TLC/HPTLC method

Sr. No.	Herbal drug	Properties of phytoconstituents	Mobile phase	Spray reagents
1	*Garcinia campogia*	Used in Ulceration and dysentery	Benzene: Ethyl acetate: Methanol [9: 1.6: 0.8]	5% Sulphuric Acid reagent
2	*Salvadora persica*	Antibacteric, anti-oxidant	Ethyl acetate: Formic acid: Methanol: Acetic acid (4:6:1:0.5: 1)	5 % sulphuric acid
3	*Boswellic serrata*	Anti- inflammatory	Toluene: Chloroform: Methanol [6 : 4 :0.6]	5%Vanillin Phosphoric acid
4	*Centella asiatica*	Brain tonic, Sedative	n-butanol: Ethyl acetate: Water [4:1:5] {Upper layer taken}	Anisaldehyde Reagent
5	Triphala churna	Astringent, used in Hyperacidity	Toluene: Ethyl acetate: Methanol (3:3.2:0.6)	5 % sulphuric acid
6	Glycyrrhiza glabra	Expectorant, antispasmodic	For hydrolysis saponins Toluene: ethyl acetate: acetic acid (12:7:0.5)	1% cerric sulphate in 10% H_2SO_4
7	*Boswellic serrata*	Anti- inflammatory	Toluene: Chloroform: Methanol [6: 4 :0. 6]	5%Vanillin Phosphoric acid

Acceptances of Chromatographic Techniques in Pharmacopoeia as Quality Control Methods [6]

As primary source for TLC fingerprint method for identity of Herbal medicinal products (HMPs) and starting materials such as plants material, crude drugs and extracts, the *European Pharmacopoeia*, the *U.S. Pharmacopoeia/ National Formulary*, the *British Herbal Pharmacopoeia*, and the *Chinese Pharmacopoeia* may be consulted. Although none of these methods represent the state-of-the-art in modern HPTLC, they still have the status of validated methods.

It is also observed that most of the Pharmacopoeias such as *Indian Herbal Pharmacopoeia*, *European Pharmacopoeia*, the *U.S. Pharmacopoeia/ National Formulary*, the *British Herbal Pharmacopoeia*, and the *Chinese Pharmacopoeia* consist of standard quantitative and qualitative tests for phytoconstituents. The commonly used quantitative method for analysis is HPTLC e.g. Diosgenin from *Dioscorea composita*, Emodine & Crysophanol from *Rheum emodi*, Glycyrrhizine from *Glycyrrhiza glabra* and Ecdysterone. Test for identification of phytoconstituents are also mentioned in these pharmacopoeia e.g. podophyllotoxin from *podophyllum hexandrum*, Solasodine from *Solanum americanum*

Far any other TLC/HPTLC method used in quality testing the EU guidelines "Note for Guidance on Validation of Analytical Methods; Definitions and Terminology". Validation is required if Pharmacopeial method is

e-ISSN:2321-0812
p-ISSN:2347-2340

optimized or adapted for specific tasks in quality control or if a method is taken from other reliable sources such as the *American Herbal Pharmacopeia* (AHP), *the Indian Herbal Pharmacopoeia*, or Plant Drug Analysis: A Thin Layer Chromatography Atlas Of all the sources, only the AHP monographs feature modern HPTLC methods for identification.

Table 7: Screening of phytoconstituents by TLC/HPTLC method

Sr. no	Phytoconstituents in Medicinal Plant	Mobile phase	Spray reagents
1	Conessine [*Holarrhena antidysenterica*]	Ethyl acetate :Hexane : Diethyl amine [75:25:6]	Modified Dragendorff's reagent
2	Gymnemagenin [*Gymnema sylvestre*]	Toluene :Ethyl acetate : Methanol [9:0.8:1.6]	5%Anisaldehyde
3	Tannic acid, Catechin [*Emblica officinalis*] Piperine [*Pepper longum*]	Ethyl acetate: Acetic acid: Methanol [8:2:3]	Ferric chloride solution, Modified Dragendorff's
4	Betain [*Beta vulgaris]*	n-Butanol:Acetone: Glacial Acetic acid: Water: Methanol (7: 7: 2: 4: 0.5)	Modified Dragendorff's reagent
5	Shatavarine [*Asparagus* racemosus]	n- butanol: Ethyl acetate : Water [4:1:5] {Upper layer taken}	5%Vanillin Phosphoric acid
6	Ephedrine [*Ephedra sinica*]	Toluene: Ethyl acetate: Diethyl amine (7:2:1)	1% Ninhydrin Methanolic solution
7	Ursolic acid [*Ocimum sanctum*]	Chloroform: Methanol (9.5: 0.5)	5% Methanolic Vanillin hydrochloric acid
8	Diosgenin [*Dioscorea deltidea*]	Chloroform: Methanol (9.5: 0.5)	5% Methanolic Vanillin hydrochloric acid
9	± Jasmolic –acid [*Sugar cane*]	Chloroform: Methanol (14: 2)	Anisaldehyde Reagent
10	L-DOPA [*Mucuna pruriens*]	Butanol: Acetic acid: Water [8:2:4]	0.5% Ninhydrin reagent
11	Vasicine [*Adhatoda vasaka]*	Toluene: Chloroform: Methanol (3:6:2)	Modified Dragendorff's reagent
12	Piperine [*Piper nigrum]*	Toluene: Ethyl acetate (7:3)	Vanillin sulphuric acid
13	Reserpine [*Rauvolfia serpentine*]	Toluene: Ethyl acetate: Diethyl amine (7: 2: 1)	5% Methanolic Vanillin hydrochloric acid
14	Withaferin A [*Withania somnifera*]	Toluene: Methanol: Ethyl acetate [9: 1.6: 1.6]	Anisaldehyde reagent
15	Calcium sennosides [*Cassia angustifolia*]	n-Propyl alcohol; Ethyl acetate: water (4:4:3)	Ammonium hydroxide
16	Guggulosterone [*Comiphora mukul*]	Toluene: Ethyl acetate: Formic acid [8:2:0.5]	Vanillin sulfuric acid solution
17	Forskoline [*Coleus forskohlii*]	Benzene: Chloroform: methanol (9:1:0.4)	Vanillin sulfuric acid solution
18	Triacontanol [*Sugar cane]*	Hexane: Acetone: Benzene [16:4:1]	2 % Thymol blue reagent
19	Andrographolide [*Andrograph-oloide panuculata*]	Chloroform: methanol (7:1)	10% Sulfuric acid
20	Lecithin (phophotadylcholin) (Soybean oil)	Chloroform: methanol: water: acetic acid(65:24:4:2)	10% Sulfuric acid
22	Lycopene [*Calendula officinalis*]	Hexane: acetone (7:3)	
23	Sugangin A & B [*Mesua ferrea*](Coumarins der.)	Toluene: ethyl acetate: formic acid (6:3:1)	Methanolic KOH
24	Penicinillic acid, patulin, ocharaposin [Mycotoxin]	Benzene: chloroform: ethyl acetate: formic acid (6:4.5:4.5:1.5)	Natural product-polyethylene glycol reagent (NP/PEG)

Typical Tasks in the Quality Control Of Medicinal Plant [7]

Identification

Identification can be considered as the dominating application of TLC. Identification is established by comparison of a sample with a reference on a same plate. It is an advantage of TLC that not only the entire sample can be seen but also several samples can be easily compared at the same time. The prevailing value of HPTLC fingerprints is the visual impression, which can be further expanded by multiple detection (visualization or

derivatization). A broad spectrum of constituent can thus be detected and described without the need to know the chemical nature of each zone of the chromatogram. Finally fingerprint chromatograms with a visible pattern of bands provide fundamental data. Ideally, to achieve maximum information, several suitable methods should be used for fingerprinting different group of phytoconstituents.

In a cGMP environment, the identity of raw material must be documented. Electronic images of HPTLC fingerprints are conveniently generated and saved. They are typically evaluated based on number, color, sequence, and relative position of zones with respect to zone obtained on the same plate with chemical or botanical reference materials. General HPTLC methods followed for typical class of phytochemical are mentioned in Table no.4 and common spraying regent mention in Table no. 5. The methods used must be specific. That means only a sample properly identified will comply with the specification and any adulterant produces a significantly different fingerprint.

Semi quantitative assessments

In process control and stability tests, HPTLC fingerprints are often used during product and process development to establish proper extraction parameters, standardized extracts and detect any changes or degradation in the material during formulation. In a cGMP environment, it is essential to document how the raw material is converted/ preserved in the individual steps of the production process. Fingerprint of some medicinal plants [8] by TLC/HPTLC method are reported in table no. 6.

Quantitation of marker compound

Quantitation of marker compound is the most demanding application of TLC. Due to limited separation power of the technique, it is often not possible to obtained baseline separation of all components of such complex samples as medicinal plant materials. Therefore, most assays are typically based on HPLC. However, it often overlooked that with proper instrumentation and suitable methodology, quantitative determinations by HPTLC are no difficult. An example of quantitative determination [9] is shown in Figure 1 and screening of some phytoconstituents by TLC/HPTLC method reported in table No 7.

Documentation

Each developed plate is documented under UV light at 254nm, UV light at 365nm, and white light. If a type of light does not produce usable information, that fact must be documented. If plate is derivatized, images are taken out before and after derivatization. Image labels should include the plate number as well as the derivatization and the illumination mode. Video and digital documentation systems are widely used to document high-performance thin- layer chromatograms. The advantages of modern electronic documentation systems are instant images of the chromatogram/ fingerprint.

Trouble Shooting in HPTLC:

Fix the TLC problems before they cause trouble. Ideally all problems will be prevented by a good maintenance plan. One way to minimize problems is to anticipate them so they can be fixed at your convenience. Some problems, however, cannot be avoided, but good work practices will minimize the impact of such problems. Short remedies for trouble shooting in HPTLC are discussed in table no 8.

1. Quality control of herbal medicinal products (HMPs) is a challenging analytical task, because the entire herbal drug or herbal drug preparation is regarded as the active substances, regardless of whether constituents with defined therapeutic activity are known.
2. In herbal medicinal products the entire herbal drug or an herbal drug preparation is regarded as the active pharmaceutical ingredients
3. In quality control and stability testing of herbal medicinal products, fingerprint chromatograms are used as powerful tools to evaluate and compare the composition of compounds in such product[10]
4. Visualization (so- called fingerprint) of the entire pattern of compounds present in an herbal drug or preparation is therefore fundamental in the quality and stability testing of HMPs and the respective materials.
   High- performance thin-layer chromatography (HPTLC, Planar chromatography) is an ideal tool for analysis of medicinal plant and offers several advantages. In its traditional form, thin layer chromatography has a long record in almost all pharmacopoeias for its use in identification of botanical raw materials [11, 12]. However, HPTLC is not limited to identification. It can also be used for control of batch to batch consistency in stability testing of medicinal plants and for purposes of control throughout the entire manufacturing process of HMPs [12, 13, 14]. See Figure No. 1a. It helps to check quality control of batch to batch consistency. This can be done by well photodocumentation under light at 254 nm, 365 nm, and visible mode. Compare that scanned 3D chromatogram for further study.

e-ISSN:2321-0812
p-ISSN:2347-2340

RESULTS

The proposed HPTLC method provides an experimental procedure to identify the presence of phytochemical markers in the herbal dosage form and specifically to verify genuinity of plant material used which has direct correlation with the therapeutic efficacy of the finished product.

CONCLUSION

These methods were also employed to analyze commercial samples to illustrate their application in qualitative ('fingerprint') and quantitative determination, demonstrating their feasibility in the quality control of phytoconstituents from mentioned Herbal drugs and formulations. This will help induced to come out uniform standard products, which will restore faith of product and Alternative herbal medicine therapy.

Table 8: Trouble shooting in HPTLC

Cause	Remedy
A] Poor Band quality [Linomat applicator]	
Gas flow not optimal	Check gas pressure and adjust to 2-5 bars
Wrong distance between needle tip/ TLC layer	Check distance and adjust to 1 mm
Damaged or clogged needle	Remove syringe and fill it with solvent. Force out by hand
B] Bad reproducibility	
Bad reproducibility	Limit switch for sample dosage syringe misadjusted
C] Poor accuracy [Linomat applicator]	
Damaged spray head due to wrong sample dosage syringe type	Replace the spray head
Leaky syringe because of destroyed glass barrel	It happen because of high pressure, replace the sample dosage syringe
D] Poor band quality	
Check gas supply for spraying	Check Regulating the gas flow
Check nozzle for clogs	Check Cleaning the spray nozzle
Check distances capillary/nozzle and capillary/object	Check Adjusting the capillary
The applied solution may be too concentrated. [Use low application speed for polar solvents such as methanol and water.]	Dissolve sample in more suitable solvent. [Use high application speed for volatile solvents such as ether and hexane]
Application position is overloaded with sample.	Apply less sample per zone
E] Leakage	
i. If air bubbles are formed, when the syringe is filled the syringe piston has a leak	Check and replacing the O-ring (gasket) as well as syringe piston.
ii. Another reason for leaks could be caused by the O-ring gasket in the connection between syringe and capillary	

REFERENCES

1. Indian Herbal Pharmacopoeia Vol-II, 93, IDMA, 1999.pp 93
2. PK Padmanabhan , over view of analytical instrumentation, National symposium Environment and pollution, September 2002, Nagpur, India.
3. Eike Reich, Anne Blatter. AOAC International, May/ June 2004, pp 14- 18.
4. Camag Bibliography service Planer chromatography, 89, September 2002, 22-24.
5. The European Pharmacopoeia 4 (2002) council of Europe, Strasbourg Codex, France.
6. Koll et al. Validation of Standardized High-Performance Thin-Layer Chromatographic Methods for Quality Control and Stability Testing of Herbals. Journal of AOAC Int. 2003;86:5:909- 915.
7. Blatter A, Reich E. Qualitative and Quantitative HPTLC Methods for Quality Control of Stephania tetrandra. J Liq Chromatogr Relat Technol. 2004;27:1-14.
8. Rahul P Kasar, K S Laddha, Development of quality control methods for polyherbal formulation, Chyawanprash. Natural Product Radiance. 2006;5(1):33-41
9. Kasar Rahul N, Mahadevan. HPTLC analysis of Withaferin A from an herbal extract and polyherbal formulations. J Sep Sci. 2003;26:1707-1709.

10. Keng-Yeow Sim, et al. J Nat Prod.1996;59(7):643-645
11. Chopra RN, Nayar SL and Chopra IC, Glossary of Indian Medicinal Plants, CSIR,New Delhi, 1956, 2,18,191.
12. Jork H, Funk W, Fischer W, Wimmer H. 1990, Thin Layer Chromatography, Volumes 1a and 2b, VCH Verlagsgesellchaft GmbH, Weineim, Germany
13. Wagner H, Bladt S. 1995, plant drug analysis- a thin layer chromatography atlas, 2nd Ed., springer, berlin, germany.
14. Dammertz W. Planar chromatography: a retrospective view for the third millennium, Sz. Nyiredy (Ed.), Springer, Budapest,Hungry, pp 234- 246.