WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 11, Issue 9, 1230-1243. Review Article ISSN 2277– 7105

APPLICATIONS OF HTPLC IN ANALYSIS OF PHYTOPHARMACEUTICALS: REVIEW

V. R. Biradar*, R. D. Chakole and M. S. Charde

Post Graduate Department of Pharmaceutical Chemistry, Government College of Pharmacy, Vidyanagar, Karad, District- Satara, Maharashtra, India 415124.

Article Received on
18 May 2022,

Revised on 08 June 2022,
Accepted on 28 June 2022

DOI: 10.20959/wjpr20229-24666

***Corresponding Author**
V. R. Biradar
Post Graduate Department of Pharmaceutical Chemistry, Government College of Pharmacy Vidyanagar, Karad, District- Satara, Maharashtra, India 415124.

ABSTRACT

Most of the plants provide various pharmacological activity including categories tannins, resins, essential oil, alkaloids, anthraquinones, cardiac glycoside. These all are categorized phytochemicals detected by various chromatographic method like HPTLC, GC, HPLC. The review included various applications HPTLC technique for phytopharmaceuticals and quality control of herbal botanical formulations; It also included various precise and accurate HPTLC parameters like mobile phase, flow rate, RF values, PH conditions, stationary phase for estimation of various phytopharmaceuticals. HPTLC is sophisticated, automated, and improved form of TLC. The checklist of botanical plants along with their specific validation is still biased. The tradition method is less scientific and poor. The HPTLC method was optimized to resolve the major compound from other components. This technique most popular due to multiple sample handling, visual chromatogram, and best separation technique. The phytopharmaceuticals are plant derived compound having various pharmacological action. HPTLC is helpful to identify and quantify the active constituents with minimizing cross contamination and time. It is also advanced in reproducible analysis, visual evaluation which are help to researcher to analysis of phytopharmaceuticals.

KEYWORDS: Phytopharmaceuticals, HPTLC, Herbals.

INTRODUCTION

In India several medicinal treatments created from plants, they have been used to treat ailments. These medicinal treatments successful with the help of many plant-based drug as

various alternative medicinal system like ayurveda, siddha, unani, homeopathy etc.[1] Phytopharmaceuticals are herbal medicine that rely on one or more plant components for their effectiveness. Some examples of phytopharmaceuticals includes theobromine, berberine, diosgenin, sitosterol, carotenoids, alkaloids etc.

HPTLC is powerful method equally suitable for quantitative and qualitative analysis. It is also known as planer or flatbed chromatography. HPTLC fingerprint is pattern of TLC plate of separated compound generated according to their highly specific Rf value, capable of providing specific and characteristics identity.[2] HPTLC is generally based on adsorption phenomenon. HPTLC is best quality assessment tool for the evolution of botanical materials. It allows broad number of compounds. Herbal Drugs is whole, fragmented or cut, algae, lichen usually in dried form. It involves plant or plant part that have been converted into phytopharmaceutical by means of simple process involving harvesting, drying and storage, it is also include other crude product derived from plant, which is no longer show any organic structure, such as essential oils, fatty oils, resins and gum.[3] medicinal Herbal product are plant derived material or product with therapeutic or other human health benefits which contain either raw or processed ingredients from one or more plant. Phytopharmaceutical drugs are purified and standardized fraction with defined minimum four bioactive compounds.as per D and C act 1940, these include purified and standardized fraction with defined minimum four bioactive or phytochemical compounds (qualitatively and quantitatively assessed) of an extract of a medicinal plant or its part, for internal or external use of human beings or animals for diagnosis, treatment, mitigation or prevention of any disease or disorder but does not include administration by parenteral route.

High performance thin layer chromatography

It is highly standardized methodology and advanced form of thin layer chromatography having higher efficiency, accurate sample application, reproducible chromatogram development and software control evaluation.[5]

Key features of HPTLC over other techniques- HPLC, Gravimetry, titrimetric and spectroscopy.

- ✓ Capability to identify multicomponent samples.
- ✓ Simultaneous processing of sample and standard-
- ✓ Better analytical precision and accuracy less need for internal standard.
- ✓ Visual recognition is possible because it is an open method.

- ✓ Low maintenance cost compares to other instruments.
- ✓ Several samples can be separated on same plate resulting in a high output, time saving and high accuracy analysis.
- ✓ Sensitivity range of analysis usually occur at the pictogram (pg) and nanogram (ng)
- ✓ HPTLC method may help to eliminate exposure risk of toxic organic effluents and reduces it disposal problems and decreasing environmental pollution.

Table 1: Difference between TLC & HPTLC.

Parameter	**TLC**	**HPTLC**
Chromatographic Technique	Manual	Instrumental
Plate used	Handmade	Precoated
Mean particle size	10-12µm	5-6µm
Layer thickness	250µm	100-200µm
Solid support	Silica gel, alumina, kieselguhr.	Silica gel-normal phase C8 &C18- reverse phase
Shape of sample	Spot	Spot/band
Sample volume	1-10µl	0.2-5µl
Efficiency	Low	High
Analysis time	Slow	Greatly reduced
Development chamber	More amount of solvent	Less amount of solvent
Spot size	2-4mm	0.5-1mm
Scanning	Not possible	Use of UV/visible/fluorescence scanner(densitometer)

Steps involved in HPTLC

1. Selection of chromatographic plate
2. Layer pre-washing
3. Activation of precoated plates
4. Sample preparation & application
5. Selection of mobile phase
6. Preconditioning
7. Chromatographic development and drying
8. Detection& visualization
9. Documentation of chromatographic plate

Detection limit in nanogram range with UV absorption detection and in picogram range with fluorometric detection.

Large number of theoretical plates in minimum area of plates. Higher efficiency due to smaller particle size(5µm).

HPTLC Techniques

Techniques are classified on the basis of particle size distribution and thickness of sorbent layer.[5] Mainly there are four techniques that is ultra, high performance, preparative and classical etc.

Table no. 2: Technique of HPTLC.

Sr. no.	Technique	Size (µm)	Layer thickness (µm)
1	High performance	5	200
2	Ultra	1-2	10
3	Classical	12	250
4	Preparative	25	1-2

Material and Methods (overview)

1. Extraction of plant material (Phytopharmaceuticals)

- ✓ Infusion
- ✓ Decoction
- ✓ Digestion
- ✓ Maceration
- ✓ Percolation
- ✓ Successive solvent extraction
- ✓ Supercritical fluid extraction
- ✓ Steam distillation
- ✓ Headspace

2. Phytochemical screening

Extract of given sample is tested for presence of active compounds such as terpenoids, saponins, alkaloids, flavonoids, tannins, phenols and lipids by biochemical testing and after that confirmed by high performance thin layer chromatography (HPTLC).[5,12]

Table 3: chemical test for phytochemicals.

Sr. No.	Compounds	Test
1	Tannins	Goldbeater's test Gelatin test Braymer's test Test with bromine water

2	Saponins	Foam test Froth test
3	Terpenoids	Salkowski test Copper acetate test
4	Flavonoids	NaOH test
5	Phenol	Ferric chloride test Indophenol test
6	Alkaloids	Dragendorff's test Mayer's test Wagner's test Hager's test
7	Lipids	Sudan's test

Steps in phytopharmaceutical extraction & testing.

Extraction of crude drugs

↓

Phytochemical screening

↓

The extract is analyzed for their fingerprinting by HPTLC method.[11,12]

Procedure of HPTLC

1. Selection of chromatographic plate

Now a day handmade plates which is made up of cellulose or other material is not used. The plates with different support materials and sorbents layer with different format and thickness are used for qualitative and quantitative analysis and that plate is called as precoated plates. Smaller particle size of sorbents materials leads to greater resolution and sensitivity.

Support materials- aluminum, glass, polyethylene.

Sorbents materials- silica gel 60F 254 (20×10cm), aluminum oxide, silica gel chemically modified with amino and CN group, cellulose.[6,7]

2. Pre-washing

It is purification steps. It involves to remove impurities which include water vapors, volatile substances and iron impurity in silica 60F.

Methods for washing are ascending, descending, continuous.

Solvent used for prewashing are

- ✓ Methanol
- ✓ Chloroform: methanol (1:1)
- ✓ Chloroform: methanol: ammonia (90:10:1)

Activation of plates

If plates are highly humid then activation is required. The plates are activated by placing the plates in oven at 110- 120°C for 30 mins. During activation of plates there is risk of sample being decomposed.[6,7]

3. Sample application

Sample application is first step in chromatography and it affect the quality of result at the end process. Sample application technique are spot application and spraying on sample, and degree of automation. The selection of sample application technique and device to be used depends on sample volume, No. of sample to be applied and required precision. Spot wise sample application using fixed volume of capillary is easiest way. Spraying on sample as narrow band of larger volume is best resolution that can be achieved with chromatographic system selected.[8] The most critical step for obtaining good resolution for quantification by HPTLC.

Applicators used for spotting are

- ✓ Capillary tubes
- ✓ Micro bulb pipette
- ✓ Automatic sample applicator
- CAMAG nanomat
- CAMAG linomat
- CAMAG automatic TLC Sampler

4. Plate conditioning

After sample application place the plate for 45 mins in suitable desiccator containing a saturated solution of magnesium chloride.

5. Development and Drying

Plates are spotted with sample and air dried and placed in developing chamber. Methods for development of chamber are 1. Ascending 2. Descending 3. 2-dimensinal 4. Horizontal 5. Multiple overrun 6. Gradient 7. Multimode. Forced flow planner chromatography. Different chambers are used like simulations chamber, round chamber, nano chamber and HPTLC chamber.[6] HPTLC chamber is ideal for the development of HPTLC 5×5cm plates.[8] Drying of chromatogram should be done in vacuum desiccator with protection from light and heat.

6. Detection and Visualization

Detection under UV light is first choice and it should be non-destructive. Florescent compound spot detects at 254nm (shorter wavelength) or 366nm (longer wavelength). Non-UV absorbing compound are dipping plates in 0.1% iodine solution.[6]

When individual compound doesn't respond to UV then derivatization required for detection.

7. Derivatization

Derivatization is performed by mainly two ways

Automatic dipping device– immersion type 5cm/s and no dwell time.

Automatic spraying device – 2-4ml of reagent used.

8. Documentation

30 minutes after derivatization step, take image of derivatized plate under UV 366nm. The recorded things are types of plate, chamber system, mobile phase, running time, supplier name, item number, batch no, individual plates and all data recorded using photo-documentation.

Application HPTLC technique for phytopharmaceuticals

HPTLC is applicable in identification of marker compounds, percentage of purity and minimum content of information. HPTLC plays important role as to establish the pharmacopeial standards of various herbals, ayurvedic, siddha's formulations by HPTLC fingerprinting profile as major quality control aspects.[9] The modern HPTLC technique used to avoid many problems like difficulty in quality control, variation in chemical components etc. Modern HPTLC technique used simultaneously in comparison with both reference and sample can work as quality control tool apart from identification.[10] HPTLC technique widely used for quantitative estimation of phytopharmaceuticals due to accurate and simple technique.

HPTLC also applicable for preliminary analysis of adulterants and substitutes, phytochemical properties and pharmacogenetic test as quantitative test.

1. **Leaf Extracts of pisonia aculeata**[18]

Stationary phase	Mobile phase	phytochemicals	Spots/ Rf values
pre-coated silica gel 60F254 aluminium sheets (5 x 10 cm) with the help of Linomat 5 applicator attached to CAMAG HPTLC system	n-Hexane: ethyl acetate (3.5:1.5)	alkaloids, triterpenes, tannins, saponins, glycosides, phenolic compounds and flavonoids.	0.03 to 0.95 The Rf values and finger print data were recorded by WIN CATS software after mice were sprayed with anisaldehyde sulphuric acid as part of a study at the University of Bristol's National Catastrophic Acoustics Centre (NCAC) to improve understanding of cat behaviour.

2. **Aloe Vera extract**[16]

Stationary phase	Mobile phase	Phytochemicals	Spots/ Rf values
Precoated silica gel 60 F254	Ethyl acetate: methanol: water (81:11:8) n-propanol: formic acid: water (90:1:9) toluene: ethyl acetate: formic acid (7:5:1)	Tannins, flavonoids, steroids, glycosides, alkaloids Quantitative analysis of berberine Gallic acid	0.21 0.48 0.57 0.96

3. **Essential oils in plant organs of annona muricata L.**[19]

Stationary phase	Mobile phase	phytochemicals	Spots/ Rf values
Aluminium HPTLC plates (10 × 10 cm) silica gel 60 F254	Toluene: ethyl (9.3:0.7)	Essential oils	0.52 (rind) 0.67 (pulp) 0.82 (leaf) 0.52 (roots) purple, or brownish spots after spraying with the vanillin-sulphuric acid reagent.

4. **Tiliae flos**[12]

Stationary phase	Mobile phase	Phytochemicals	Spots/ Rf values
Precoated silica gel 60 F254	Tetrahydrofuran: dichloromethane: formic acid: acetic acid: water (4:9:4: 2:3)	Caffeic acid Astragalin Linarin Isoqueracetin	0.87 0.67 0.39 0.53

5. Careya arborea Roxb (bark)[15]

Stationary phase	Mobile phase	Phytochemicals	Spots/ Rf values
pre-coated silica gel 60F254 aluminium sheets (5 x 10 cm) with the help of Linomat 5 applicator attached to CAMAG HPTLC system	Toluene: ethyl acetate: diethyl amine: methanol	Alkaloids Anthracene derivatives Arbutin derivatives	0.83 0.60 0.72 0.67

6. Polyathia longifolia thwaites

Stationary phase	Mobile phase	phytochemicals	Spots/ Rf values
Precoated silica gel 60 F254	Methanol: glacial acetic acid: formic acid: water (3:0.9:0.9:0.5)	Rutin Ethanolic extract	0.78

7. Chemical fingerprinting of andrographis paniculate

Stationary phase	Mobile phase	Extracts	Spots/ Rf values
Aluminium HPTLC plates (10 × 10 cm) silica gel 60 F254	Chloroform: toluene: methanol: (60:25:15)	Methanol extract (neo andrographolide) Water extracts	0.14, 0.27, 0.37 0.40, 0.54, 0.69

8. Asplenium aethipicum (Burm. F.) Becherer (Phytochemical profile study)

Stationary phase	Mobile phase	Phytochemicals	Spots/ Rf values
Precoated silica gel 60 F254	Toluene: acetone: Formic acid (4.5:4.5:1) Toluene: ethyl acetate: formic acid: methanol (3:3:0.8:0.2)	Flavonoids Tannins	0.16 to 0.83 0.1, 0.36, 0.52, 0.75

9. Methanol leaf extracts of randiaspinosa[20]

Stationary phase	Mobile phase	Significant study result
Aluminium sheets Silica Gel 60F254 Coated : (10 × 20 cm)	N-6-Hexane: Ethyl acetate (8:2)	Scanned by CAMAG® TLC Scanner4.The methanol and ethyl acetate fractions have shown different phytoconstituents compared to n-hexane fraction. The duplicates of different concentrations of each fraction were visualized under UV 366 nm. Results confirm that there was a single major band in n-hexane fraction, which has been confirmed by HPTLCL bands.

10. AervalanataL.[21]

Stationary phase	Mobile phase	Phytochemicals	Spots/ Rf values
Aluminium sheets Silica Gel 60F254 Coated : (10 × 20 cm)	N-6-Hexane: Ethyl acetate (7.2:2.8)	steroids, terpenoids, flavonoids, alkaloids, glycosides, sugar, carbohydrate, proteins, ash content and amino acids. Terpenoids	captured the images under white light, UV light at 254 and 366 nm. 0.6 to 0.97

11. HPTLC fingerprinting of Nicotiana tabacum leaf[22]

Stationary phase	Mobile phase	phytochemicals	Spots/ Rf values
n pre-coated silica gel 60F254 aluminium sheets (3 x 10 cm) with the help of Linomat 5 applicator attached to CAMAG HPTLC system,	Hexane: ethyl acetate (5:1)	alkaloids, phenols, flavonoids, phytosterols, triterpenoids, tannins and carbohydrates	0.11, 0.29, 0.43, 0.98 The chromatograms were scanned by densitometer at 550 nm after spraying with anisaldehyde sulphuric acid

12. Analysis of taraxerol in clitoriaternatea

Stationary phase	Mobile phase	Phytochemicals	Spots/ Rf values
Aluminium HPTLC plates (10 × 10 cm) silica gel 60 F254	hexane and ethyl acetate (80:20 v/v).	Taraxerol	0.53 scanned with a Camag TLC scanner 3 using a detector at 420 nm wavelength

13. Determination of galanthamine in amaryllidaceae plant extracts[23]

Stationary phase	Mobile phase	Phytochemicals	Spots/ Rf values
silica gel 60F254 thickness (0.25mm) (20 × 10 cm)	0.1% trifluoroacetic acid,	Galanthamine	0.72 viewed under a 288 nm UV light

14. Separation of free radicals of curcuma longa[24]

Stationary phase	Mobile phase	Phytochemicals	Spots/ Rf values
glass plates Kieselgel 60 F 254 (10 × 10 cm; Merck, Darmstadt,	n-hexane: ethyl acetate: glacial acetic acid (80:15:5)	Curcuminoids	0.89 Detect by UV absorption spectra (λ = 200–700 nm)

15. Ruellia tuberosa L. (Phytochemical studies)[25]

Stationary phase	Mobile phase	Phytochemicals	Spots/ Rf values
Aluminum precoated with silica gel 60F254 Applicator: CAMAG Linomat IV	Toluene: ethyl acetate (6:2)	Tannins terpenoids phenol flavonoids triterpenoids	Visualization: under UV 254 & 366nm, scanned under deuterium lamp Major peaks at Rf 9.45, 8.30 & 8.46 At moderate concentration Rf 4.19, 3,82, 4,97

16. Piper nigrum linn fruits[26]

In preliminary testing phenol is detected by UV spectroscopy while flavonoids are detected by calorimetry.

Stationary phase	Mobile phase	phytochemicals	Spots/ Rf values
Precoated silica gel HPTLC aluminium plates 60F-254 (20 cm×20 cm, 0.2 mm thicknesses, 5–6 µm particle size, E-Merck. CAMAG-HPTLC system of Switzerland with a Linomat 5 sample applicator was used to obtain HPTLC fingerprinting.	toluene: diethyl ether: dioxane (6:2:1).	Flavonoids, phenols	Spectra recording and data of peak area of each band were recorded. Absorption/remission was then measured at a scan speed of 20 mm/s. Chromatograms were recorded at the wavelength of 254 and 366 nm. The retention factor (Rf) value of each compound was calculated.

17. Phytochemical and biological activities of Silene viridiflora extractives

Stationary phase	Mobile phase	phytochemicals	Spots/ Rf values
pre-coated with silica gel 60 F254, 0.20 mm layer thickness Merck	THF: toluene: 1 mM TFA in MeOH: H2O (16:8:2:1)	ecdysteroid, 20-hydroxyecdysone	A TLC scanner 3 (Camag,Muttenz, Switzerland) was used in the reflectance absorbance mode at.n250 nm for this study. VisionCATS 2.4 software was used for instrument control and data evaluation. Rf- 0.43

CONCLUSIONS

The present review revealed that HPTLC technique analyze different phytopharmaceuticals in the crude drugs. Several sample can be separated parallel to each other on sample plate resulting high output, zero impurities, rapid cost analysis and time saving.it will helpful to standardize and quality control of herbals and phytopharmaceuticals. This review on HPTLC technique will be useful for the analysis of phytopharmaceuticals which help in understanding of available applications.

ACKNOWLEDGEMENTS

The authors are thankful to Principal, Government College of Pharmacy, Karad & also to AICTE for providing financial assistance in the form of stipend during my M. Pharm tenure.

REFERENCES

1. Moulishankar, A., Ganesan, P., Elumalai, M. and Lakshmanan, K., Significance of TLC and HPTLC in Phytochemical Screening of Herbal Drugs. *J. Glob. Pharma Technol*, 2020; *13:* 30-45.
2. Syed, M.H., Yasmeen, A., Hussain, M.S. and Subramanian, N.S., Preliminary phytochemical screening and HPTLC fingerprinting of leaf extracts of Pisonea aculeata. *Journal of Pharmacognosy and Phytochemistry*, 2013; *2*(1).
3. Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K.M. and Latha, L.Y., Extraction, isolation and characterization of bioactive compounds from plants' extracts. *African journal of traditional, complementary and alternative medicines*, 2011; *8*(1).
4. Ingle, K.P., Deshmukh, A.G., Padole, D.A., Dudhare, M.S., Moharil, M.P. and Khelurkar, V.C., Phytochemicals: Extraction methods, identification and detection of bioactive compounds from plant extracts. *Journal of Pharmacognosy and Phytochemistry*, 2017; *6*(1): 32-36.
5. Shivatare, R.S., Nagore, D.H. and Nipanikar, S.U., HPTLC'an important tool in standardization of herbal medical product: A review. *J Sci Innov Res*, 2013; *2*(6): 1086-1096.
6. Palve, A., Shetty, P., Pimpliskar, M. and Jadhav, R.N., Study on antibacterial and antifungal activities of Sterculia lychnophora extracts [J]. *Int J Curr Microbiol Appl Sci*, 2015; *4*(11): 336-341.
7. B. Meier & A. Spriano, Zurich university of applied science, "Standard operating procedure for HPTLC" Sonia, K. and Lakshmi, K.S., HPTLC method development and

validation: An overview. *Journal of Pharmaceutical Sciences and Research*, 2017; *9*(5): 652.

8. Balekundri, A. and Mannur, V., Quality control of the traditional herbs and herbal products: a review. *Future Journal of Pharmaceutical Sciences*, 2020; *6*(1): 1-9.
9. Meier, B. and Spriano, D., Modern HPTLCA Perfect Tool for Quality Control of Herbals and Their Preparations. *Journal of AOAC International*, 2010; *93*(5): 1399-1409.
10. Sharma, M., Abid, R. and Sajgotra, M., Phytochemical screening and thin layer chromatography of Ficus carica leaves extract. *Pharmaceutical and Biosciences Journal*, 2017; 18-23.
11. Melnyk, N., Pawłowska, K.A., Ziaja, M., Wojnowski, W., Koshovyi, O., Granica, S. and Bazylko, A., Characterization of herbal teas containing lime flowers–Tiliae flos by HPTLC method with chemometric analysis. *Food Chemistry*, 2021; *346:* 128929.
12. Hilaire, V., Michel, G., Majoor, A., Hadji-Minaglou, F., Landreau, A. and Fernandez, X., New method for screening anti-Leishmania compounds in plants extracts by HPTLC-bioautography. *Journal of Chromatography B*, 2022; *1188:* 123061.
13. Mulaudzi, N., Anokwuru, C.P., Tankeu, S.Y., Combrinck, S., Chen, W., Vermaak, I. and Viljoen, A.M., Phytochemical Profiling and Quality Control of Terminalia sericea Burch. ex DC. Using HPTLC Metabolomics. *Molecules*, 2021; *26*(2): 432.
14. Gupta, P., Patil, D. and Patil, A., Qualitative HPTLC phytochemical profiling of Careya arborea Roxb. bark, leaves and seeds, 2019; *3*, *9*(8): 1-8.
15. Patel, D.K., Patel, K. and Dhanabal, S.P., Phytochemical standardization of Aloe vera extract by HPTLC techniques. *Journal of Acute Disease*, 2012; *1*(1): 47-50.
16. Rabel, F. and Sherma, J., New TLC/HPTLC commercially prepared and laboratory prepared plates: A review. *Journal of Liquid Chromatography & Related Technologies*, 2016; *39*(8): 385-393.
17. Syed, M.H., Yasmeen, A., Hussain, M.S. and Subramanian, N.S., Preliminary phytochemical screening and HPTLC fingerprinting of leaf extracts of Pisonea aculeata. *Journal of Pharmacognosy and Phytochemistry*, 2013; *2*(1).
18. Naik, A.V. and Sellappan, K., Chromatographic fingerprint of essential oils in plant organs of Annona muricata L.(Annonaceae) using HPTLC. *Analytical chemistry letters*, 2020; *10*(2): 214-226.
19. Mahabaleshwara, K., Chandrasekhar, N. and Govindappa, M., Phytochemical investigations of methanol leaf extracts of Randia spinosa using column chromatography, HPTLC and GC-MS. *Nat Prod Chem Res*, 2016; *4*(202): 2.

20. Yamunadevi, M., Wesely, E.G. and Johnson, M., Phytochemical studies on the terpenoids of medicinally important plant Aerva lanata L. using HPTLC. *Asian Pacific Journal of Tropical Biomedicine*, 2011; *1*(2): S220-S225.
21. Yamunadevi, M., Wesely, E.G. and Johnson, M., Phytochemical studies on the terpenoids of medicinally important plant Aerva lanata L. using HPTLC. *Asian Pacific Journal of Tropical Biomedicine*, 2011; *1*(2): S220-S225.
22. Kaushik, S., Sharma, P., Jain, A. and Sikarwar, M.S., Preliminary phytochemical screening and HPTLC fingerprinting of Nicotiana tabacum leaf. *J Pharm Res*, 2010; *3*(5): 144.
23. Abou-Donia, A.H., Toaima, S.M., Hammoda, H.M. and Shawky, E., New rapid validated HPTLC method for the determination of galanthamine in Amaryllidaceae plant extracts. *Phytochemical Analysis*, 2008; *19*(4): 353-358.
24. Pozharitskaya, O.N., Ivanova, S.A., Shikov, A.N. and Makarov, V.G., Separation and free radical-scavenging activity of major curcuminoids of Curcuma longa using HPTLC-DPPH method. *Phytochemical Analysis: An International Journal of Plant Chemical and Biochemical Techniques*, 2008; *19*(3): 236-243.
25. Arirudran, B., Saraswathy, A. and Krishnamurthy, V., Pharmacognostic and preliminary phytochemical studies on Ruellia tuberosa L.(whole plant). *Pharmacognosy Journal*, 2011; *3*(22): 29-34.
26. Ahmad, A., Husain, A., Mujeeb, M., Khan, S.A., Alhadrami, H.A.A. and Bhandari, A., Quantification of total phenol, flavonoid content and pharmacognostical evaluation including HPTLC fingerprinting for the standardization of Piper nigrum Linn fruits. *Asian Pacific Journal of Tropical Biomedicine*, 2015; *5*(2): 101-107.
27. Mamadalieva, N.Z., Böhmdorfer, S., Zengin, G., Bacher, M., Potthast, A., Akramov, D.K., Janibekov, A. and Rosenau, T., Phytochemical and biological activities of Silene viridiflora extractives. Development and validation of a HPTLC method for quantification of 20-hydroxyecdysone. *Industrial Crops and Products*, 2019; *129*: 542-548.