World Journal of Pharmaceutical Science and Research
www.wjpsronline.com
Review Article
ISSN: 2583-6579
SJIF Impact Factor: 5.111
Year - 2025
Volume: 4; Issue: 4
Page: 370-378

THE FUTURE OF PLANAR CHROMATOGRAPHY: A REVIEW ON HPTLC INSTRUMENTATION AND INNOVATION

Aakanksha Sinha* and S. J. Daharwal

University Institute of Pharmacy, Pt. Ravishankar Shukla University, Raipur (C.G.).

Article Received: 24 June 2025 | | ***Article Revised: 15 July 2025*** | | ***Article Accepted: 08 August 2025***

***Corresponding Author: Aakanksha Sinha**
University Institute of Pharmacy, Pt. Ravishankar Shukla University, Raipur (C.G.).
DOI: https://doi.org/10.5281/zenodo.16888877

How to cite this Article: Aakanksha Sinha and S. J. Daharwal (2025) THE FUTURE OF PLANAR CHROMATOGRAPHY: A REVIEW ON HPTLC INSTRUMENTATION AND INNOVATION. World Journal of Pharmaceutical Science and Research, 4(4), 370-378. https://doi.org/10.5281/zenodo.16888877

ABSTRACT

High Performance Thin Layer Chromatography (HPTLC) method is a high-tech and automated version of thin-layer chromatography (TLC) with improved and superior separation efficiency and the detection limits and it is a perfect substitute for GC and HPLC. Uses of HPTLC are the phytochemical and biomedical evaluation, quantification of herbal drug, quantification of active ingredient, fingerprinting of formulations, and test for adulterants in the formulations. HPTLC effective in the detection of substances of forensic interest. Different advanced techniques in terms of HPTLC such as hyphenations in HPTLC-MS, HPTLC-FTIR and HPTLC-Scanning Diode Laser have transformed HPTLC into a potent analytical tool in the analysis field. Specialists are the belief that HPTLC future to combinatorial approach and the use of instrumental HPTLC towards the analysis of drug formulations, bulk drugs, and natural products will grow in the future.

KEYWORD: HPTLC, TLC, HPTLC-MS, HPTLC-FTIR.

INTRODUCTION

High-Performance Thin Layer Chromatography (HPTLC) is a newer and more advanced version of conventional thin layer chromatography (TLC), extensively utilized for qualitative and quantitative determination in pharmaceutical, biomedical, and herbal products. In contrast to simple TLC, HPTLC provides increased resolution, higher sensitivity, and better reproducibility because it uses high-quality precoated plates, automated sample spotting, accurate development chambers, and densitometric scanning. The method is inexpensive, fast, and can analyze multiple samples simultaneously, which is particularly useful in routine stability testing and quality control.[1-3]

Comparative Evaluation of TLC and HPTLC: Advancements in Planar Chromatography[4]

Parameters	HPTLC	TLC
Chromatographic plate used	Pre-coated	Hand made
Sorbent layer thickness	100-200µm	250µm
Pre-washing of plates	Must	Not followed
Sample volume	0.2-5µl	1-10µl
Analysis time	Greatly reduced	Slow
Shape	Spot/band	Spot
Spots size	0.5-1mm	2-4mm
Efficiency	High	Slow
Development chamber	Less amount of solvent is required	More amount of solvent is required
Scanning	Densitometer	Not possible

Principle of HPTLC[5]

HPTLC relies on the principle of differential migration-based separation of analytes on a stationary phase under the action of a mobile phase. The components of a sample mixture migrate at varying velocities based upon their affinity with the stationary phase (adsorbent) and solvent capacity in the mobile phase (solvent).

Advantage of HPTLC[6]

i) Samples in minute amounts like in nano-gram range
ii) Reduced handling and human errors as a result of automation,
iii) Increased precision and sensitivity.

Limitations of HPTLC[7]

Cumbersome equipment, huge space occupancy, several folds costly, demands strict condition of operation such as dust-free conditions and temperature-controlled environments, and technically qualified personnel with the expertise to operate the system.

Automation of HPTLC

Contemporary TLC is generally referred to as HPTLC, and it can be carried out on precoated layers only, with the help of equipment and primarily for the determination of quantity. The terms TLC and HPTLC, therefore, are employed interchangeably. For educational purposes in imparting the chromatography principle, nearly everywhere in the world, TLC is employed. The primary reasons for this selection are visualization of the sample while chromatography occurs, extremely low-cost equipment for demonstration purposes, and ease to conduct. Its method for enhancing resolution under capillary flow-controlled conditions is through the utilization of multiple developments. Its one-dimensional or two-dimensional separations are achievable in planar chromatography. Mobile-phase velocity may also be controlled by external processes, including in forced-flow development.[8]

HPTLC is the most rapid chromatography technique. Chromatography of samples is carried out in parallel. Every step of the process is executed independently, which makes HPTLC not only more rapid but accommodating enough for HPTLC System to analyze multiple samples in parallel. Stationary phase and mobile phase usage are proportional to the number of analyzed samples.[9]

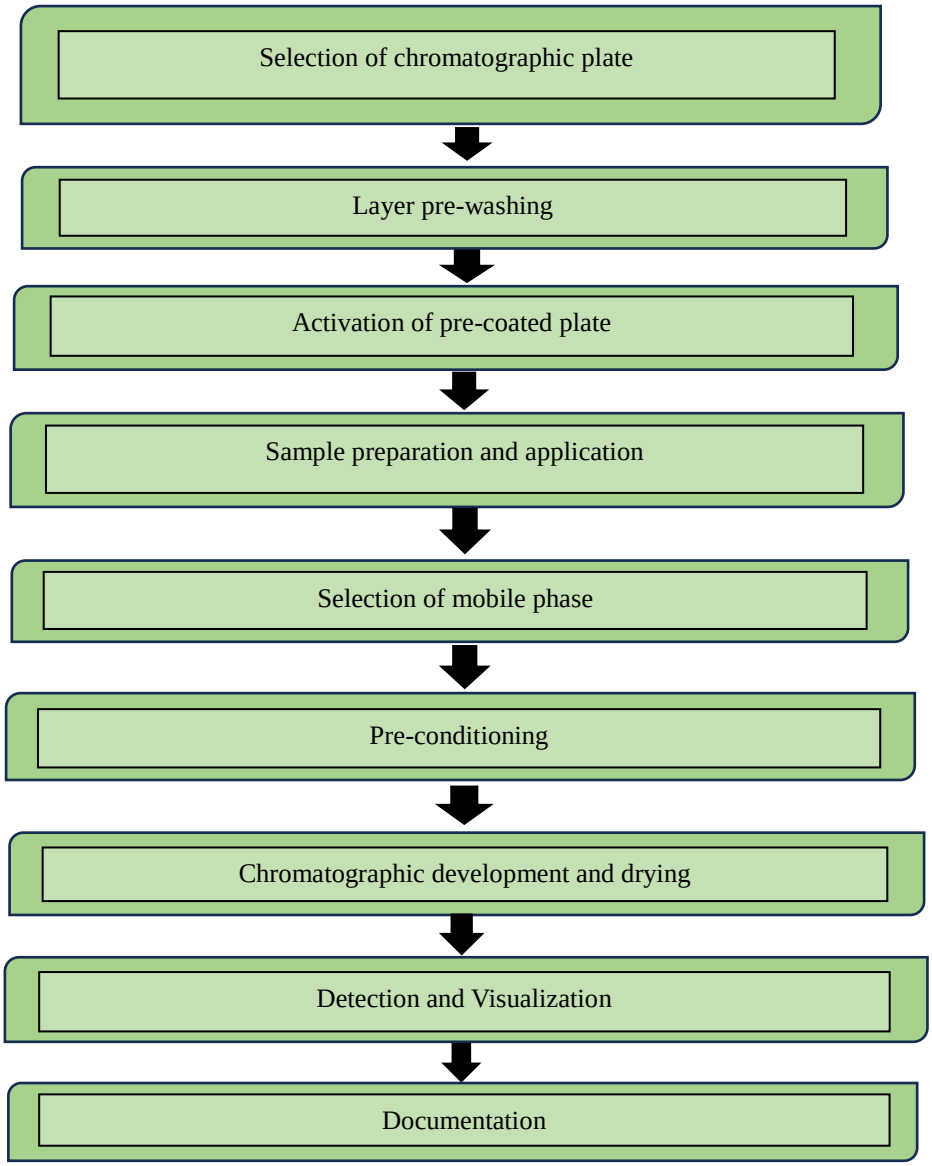

Figure 1: Steps involved in HPTLC.

Selection of chromatographic plate[10]

- Handmade plates consisting of cellulose and other materials which are no longer extensively used these days.
- Pre-coated plates: Plates with various sorbent layer and support materials of varying format and thickness are employed for qualitative and quantitative analysis.
- Support materials employed in plates: Glass, Polyester /polyethylene, Aluminium.
- The Sorbents employed in plates are silica gel 60F, aluminium oxide, cellulose, silica gel chemically altered –a) Amino group (NH2) b) CN group.
- The Smaller particle size of silica facilitates higher resolution and sensitivity.

Layer pre-washing[11]

- In this process of purification, the main aim of the pre-washing process is to eliminate impurities which are water vapours and volatile compounds from the atmosphere when they are exposed in the lab setting.
- Some of the usual methods employed for prewashing are-Ascending, Dipping, Continuous.

- The Solvents employed in pre-washing: Methanol, Chloroform: Methanol (1:1), Chloroform: Methanol: Ammonia (90:10:1).

Activation of Pre-coated Plate[12]

- The newly opened box of HPTLC plates does not require activation.
- If longer time plates are exposed to high humidity, then activation is asked.
- The plates are activated by heating in the oven at 110-1200C for 30min, where this process is utilized to drive off water physically adsorbed onto the surface on the sorbent layer.

Optimizing Sample Preparation and Application -

1. Sample preparation[13]

- The Sample and reference substances must be dissolved using the same solvent to have equal distribution at starting zones.
- It requires very little amount of sample high concentrated solution has to be applied.
- Then the plates were dried and kept in dust free environment.

2. Sample application[14]

- General concentration range recommended for HPTLC is 0.5-5µL.
- This sample spot applied should not be more than 1mm in diameter.
- Overloading problem can be resolved by using the sample in the form of a band.
- Applicator to be used is decided based on sample volume and quantity of samples to be applied.
- Some of the applicators employed for the application of the sample include Micro syringes, Linomat etc.

Figure 2: Camag Linomat 5 Sample Applicator used in HPTLC.

3. Selection of mobile phase[15]

- The choice of the mobile phase is determined by the adsorbent material employed as stationary phase and is further a function of physical and chemical character of the analyte.
- The peaks of interest must be solved between Rf value of 0.15 and 0.85.
- Power of elution of the mobile phase is based upon a characteristic known as eluent strength which is dependent upon the polarity of the components of the mobile phase.

- The more nonpolar the molecule, the sooner it will elute (or less time retain on stationary phase) and the more polar the molecule the slower it will elute (or more time retain on the stationary phase).
- Less amount of mobile phase is needed compared to TLC.

Table 1: Few examples of Common Solvents with their respective Elution strengths.

S. No.	Solvent	Eluent Strength
1.	N- Pentane	0.00
2.	Hexane	0.01
3.	Cyclohexane	0.04
4.	Carbon tetrachloride	0.18
5.	Toluene	0.29

4. Pre-conditioning (Chamber saturation)[16]

- Un-saturated chamber results in high Rf values.
- Saturation of Mobile phase must be carried out in chamber by lining with filter paper for 30min before development indicates uniform distribution of solvent vapours leads to low Rf values.
- For low polarity mobile phases, saturation is not required but saturation is required only for high polar mobile phases.

5. Developmental Techniques[17]

- Plates are spotted with sample and air dried and put in the developing chambers.
- The various development techniques employed are – Ascending Descending Horizontal
- Typically, saturated twin-trough chambers fitted with filter paper have the best reproducibility.

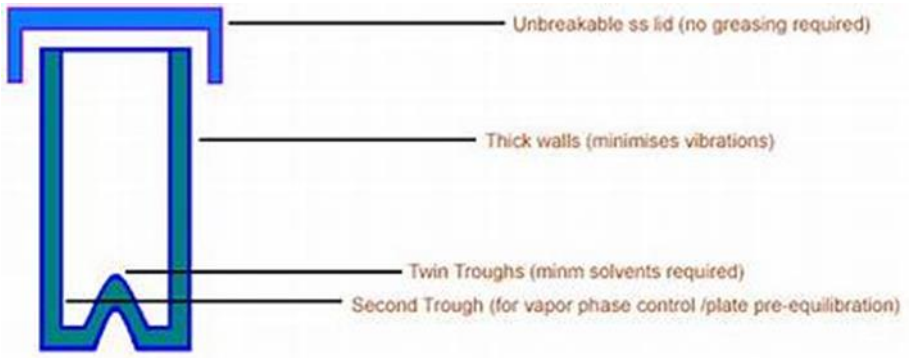

Figure 3: Camag Twin Trough chamber.

6. Detection[18-22]

The Detection of separated compounds on the sorbent layers is facilitated through quenching of fluorescence by UV light (ranged normally at 200-400 nm). This process is popularly referred to as Fluorescence quenching.

Visualization at UV 254 nm

F254 can be explained as quenching phosphorescence. It is extremely short lived, but longer than 10 seconds. The fluorescent indicator F254 is excited by UV wavelength at 254 nm and gives green fluorescence. The compounds that absorb 254 nm radiation suppress the emission on the layer, and dark violet spot on a green background is seen where the zones of the compound are present.

Visualization at UV 366 nm

F366 can be termed Fluorescence quenching. In this case the fluorescence doesn't persist when the source of excitation is withdrawn. The quenching is exhibited by anthraglycosides, coumarins, flavonoids, Phenol carboxylic acids, certain types of alkaloids (Rauwolfia, Ipecacuanha alkaloids).

Visualization in white light

Zone consisting of separated compounds can be identified by observation of their natural colour in daylight (White light).

7. Derivatisation[23]

- Derivatization can be termed as procedural method which mainly alters analytes function in a way that enables chromatographic separations.
- Derivatization is done by immersing the plates or by spraying with appropriate reagent on plates. Immersion is more preferred to achieve better reproducibility as derivatization method.

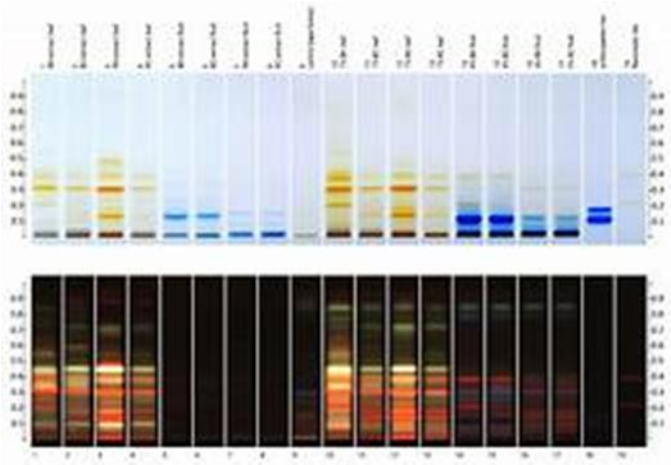

Figure 4: Derivatizing Plate.

8. Quantification[24]

Scanning Densitometry

Enable the measurement of absorption and/or fluorescence of underivatised or derivative compounds at wavelength in the range 200 to 800 nm. Wavelengths of up to 800nm may be tested and spectra of any peak may be recorded. The Biochemical tests may be carried out directly on the HPTLC plate.

Digital Camera-Based Image Documentation[25]

The UV Cabinets are being replaced with better design and UV Cabinets which permitted digital camera to be set fixed for capturing images of the plate. were as in now a days, HPTLC is a main requirement for any lab engaged in herbal analysis for identification of plant extracts by comparison with Botanical Reference Material (BRM) extracts to identify the substitutes or adulterants and the formulation studies etc. Forensic analysts has already mentioned that the starting points are the microscope for physical examination and TLC for chemical examination.

Software-Induced Scanning[26,27]

This can be applied to quantification in the absorbance and fluorescence modes and capture UV Vis in the absorbance spectra. Depending on the end-user needs, the gradient chamber and/or a photo documentation unit and bioluminescence detector can be added or an automated system can be purchased. Hyphenation methods with MS or IR or NMR can be obtained with an appropriate commercially available interface. A new device is recently available to interface HPTLC with MS. This interface will transfer the selected fraction which is eluted from the layer and directly introduce it into the MS. The output from LC–MS analysis can be significantly enhanced, when combined to TLC or HPTLC. Any predefined fraction of the plate can be analyzed. Other fractions that are available may be left out. TLC can be used to optimize the MS parameters for a given molecule.

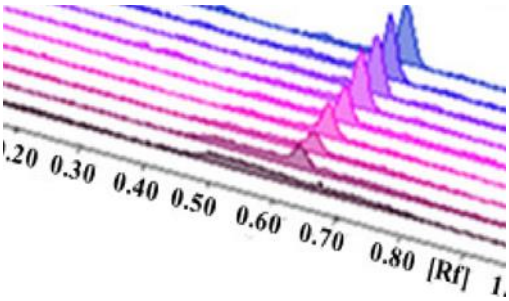

Figure 5: Chromatograms by Densitometer.

Pharmaceutical Applications of HPTLC[28-30]

1. Department of Quality control
2. Determination of purity
3. Clinical uses: In metabolism research, Drug screening etc.
4. Forensic Science: In cases of Poisoning investigation, assaying radiochemical impurities of radiopharmaceuticals, detection and identification of Pharmaceutical raw materials, drugs and their metabolites in biological fluids.
5. Cosmetic Analysis: In Hydrocortisone determination in lanolin ointment.
6. Natural products Detection: Glycosides in herbal medicines, piperine in piper longum etc.
7. Drug analysis in blood.
8. HPTLC was applied to routine quality control of topiramate, dutasteride, pharmaceutical drug products.
9. In herbal medicinal products, HPTLC is also employed as a perfect screening for adulterations and is very suitable for evaluation and monitoring of cultivation, harvesting, and extraction stages and stability testing.

CONCLUSION

HPTLC technique serves as vital tool in qualitative and quantitative estimation in Phytochemical analysis, analysis of a broad array of compounds, e.g., herbal and botanical dietary supplements, nutraceuticals, traditional western medicines, traditional Chinese medicines and Ayurvedic (Indian) medicines, pesticide or insecticide detection in food; forensic analysis of the dye composition of fibres. Due to its vast use, it has become a powerful analytical tool in the field of Pharmaceutical Analysis. It is noteworthy to Utilize the HPTLC instrumentation toward the analysis of drug formulations, API's, Natural products, Food stuffs and much more in the future prospects.

REFERENCES

1. Sharma A, Shanker C, Tyagi LK, Singh M, Rao CV. Herbal medicine for market potential in India: an overview. Acad J Plant Sci, 2008; 1(2): 26-36.
2. Kadam PV, Yadav KN, Shivatare RS, Pande AS, Patel AN, Patil MJ. Standardization of gomutra Haritaki vati: An ayurvedic formulation. Int. J. Pharm. Bio. Sci., 2012; 3(3): 181-7.
3. Mukherjee PK, Wahile A. Integrated approaches towards drug development from Ayurveda and other Indian system of medicines. Journal of ethnopharmacology, 2006 Jan 3; 103(1): 25-35.
4. Ambati P, Bala Krishna P, Srinivasa Rao Y, Varaprasada Rao K, Deepthi R. Instrumentation and future prospects of HPTLC-A. World Journal of Pharmaceutical Research, 2021 Apr 7; 10(6): 650-61.
5. Bairy PS. A comparison study of HPLC and HPTLC: principles, instrumentations and applications. ASIO J. Anal. Chem.(Asio-JAC), 2015; 1(1): 20-8.
6. Kakde R, Gadpayale K, Qureshi MO. Stability indicating HPTLC method for determination of Moxonidine in pharmaceutical preparations. International Journal of PharmTech Research, 2012; 4(1): 358-63.
7. Koba M, Marszall M, Sroka W, Tarczykowska M, Bucinski A. Application of HPTLC and LC-MS methods for determination of topiramate in pharmaceutical formulations. Current Pharmaceutical Analysis, 2012 Feb 1; 8(1): 44-8.
8. Wadodkar SG, Wankhede SB, Gupta KR. A Validated HPTLC Determination of Stavudine in Capsules. INDIAN DRUGS-BOMBAY, 2004; 41: 300-2.
9. Reich E, Schibli A, DeBatt A. Validation of high-performance thin-layer chromatographic methods for the identification of botanicals in a cGMP environment. Journal of AOAC International, 2008 Jan 1; 91(1): 13-20.
10. Knapp DR. Handbook of analytical derivatization reactions. John Wiley & Sons; 1979 Sep 10.
11. Zotou A. An overview of recent advances in HPLC instrumentation. Open Chemistry, 2012 Jun 1; 10(3): 554-69.
12. Sonia K, Lakshmi KS. HPTLC method development and validation: An overview. Journal of Pharmaceutical Sciences and Research, 2017 May 1; 9(5): 652.
13. Jain A, Parashar AK, Nema RK, Narsinghani T. High performance thin layer chromatography (HPTLC): A modern analytical tool for chemical analysis. Current Research in Pharmaceutical Sciences, 2014 Mar 30: 8-14.
14. Dhandhukia PC, Thakker JN. Quantitative analysis and validation of method using HPTLC. InHigh-performance thin-layer chromatography (HPTLC) 2011 (pp. 203-221). Springer, Berlin, Heidelberg.
15. Camag planar chromatography catalog; CAMANG Scientific Inc: Wilmington, NC, 2010/11, http://www.camang.com
16. Camag parameters for planar chromatography- useful hints, CAMAG, Muttenz, Switzerland, http://www.camag.com.
17. Kalász H, Báthori M. Pharmaceutical applications of TLC. LC GC EUROPE, 2001; 14(5): 311-21.
18. Hussain SZ, Maqbool K, Naseer B. High performance thin layer chromatography: principle, working and applications. Int J Res Pharm Pharm Sci., 2019; 4: 83-8.
19. Renger B. Contemporary thin-layer chromatography in pharmaceutical quality control. Journal of AOAC International, 1998 Mar 1; 81(2): 333-40.
20. Sherma J. Thin layer chromatography. InAnalytical Instrumentation Handbook 2004 Nov 30 (pp. 1021-1040). CRC Press.

21. Kadam DK, Saudagar RB, Paliwal SK, Sharma S. Innovative chromatographic method for development and validation. RJLBPCS, 2018; 4(3): 64-93.
22. Gunjal Sanket B, Dighe PR. Analysis of herbal drugs by HPTLC: A review. Asian Journal of Pharmaceutical Research and Development, 2022; 10(2): 125-8.
23. Shewiyo DH, Kaale EA, Risha PG, Dejaegher B, Smeyers-Verbeke J, Vander Heyden Y. HPTLC methods to assay active ingredients in pharmaceutical formulations: A review of the method development and validation steps. Journal of pharmaceutical and biomedical analysis, 2012 Jul 1; 66: 11-23.
24. Middleditch BS, Sievers RE, Uden PC, Anton K, Berger C, Settle FA, Verrall MS, Westwood SA. 4.6. 5 Thin-layer Chromatography. Additives in Polymers: Industrial Analysis and Applications, 2005 Apr 8: 279.
25. Ambati P, Bala Krishna P, Srinivasa Rao Y, Varaprasada Rao K, Deepthi R. Instrumentation and future prospects of HPTLC-A. World Journal of Pharmaceutical Research, 2021 Apr 7; 10(6): 650-61.
26. Stahl E. Thin Layer Chromatography: A Laboratory Handbook, Academic Press, San Diego, CA, 1965; 485-502.
27. Nyiredy S. Planar chromatographic method development using the PRISMA optimization system and flow charts. J. Chromatogr. Sci., 2002; 40: 553–563.
28. Deshpande, M.M., Kasture, V.S., Gosavi, S.A., Application of HPLC and HPTLC for the simultaneous determination of cefixime trihydrate and ambroxol hydrochloride in pharmaceutical dosage form. Eurasian J Anal Chem, 2010; 5(3): 227-238.
29. Colleen, N.A., Salmon, Yvonne, A., Bailey-Shaw, Characterisation of cultivars of Jamaican ginger (Zingiber officinale Roscoe) by HPTLC and HPLC. Food Chemistry, 2012; 131: 1517–1522.
30. Dhaneshwar SR. Pharmaceutical applications of high-performance thin layer chromatography. InInstrumental Thin-Layer Chromatography 2015 Jan 1 (pp. 451-478). Elsevier.