



HPTLC- A VERSATILE SEPARATION METHOD

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ABSTRACT

Analysis of pharmaceutical, natural compounds and newer drugs is mandate route used in all the stages of drug discovery and development process. High-performance thin layer chromatography is one of the classy instrumental techniques based on the full capabilities of thin layer chromatography. HPTLC is the most versatile, dependable, safest and fastest chromatographic technique for the quality control of drug components. The benefits of selective detection principle, minimum sample preparation, hyphenation, scanning, automation, full optimization and so on enable it to be a potent analytical tool for chromatographic information of complex mixtures of pharmaceuticals, natural products, clinical samples, food stuffs etc.

INTRODUCTION

Quality is important in every product or service but it is vital in medicine as it involves life. Unlike conventional consumer goods there can be no “second quality” in drugs. Science and technology have not ever been so promising nor have delivered so many opportunities to improve health and extend lives, but continued investments are being invested in both the public and private sector, in spite of the current economic climate. Increasing pharmaceutical industry success rates and delivering more medicines are very challenging, but very few prophetic scientific and analytical tools are available.^[1-4]

The number of new drugs is constantly developing. This requires new methods for controlling their quality. Modern pharmaceutical analysis must comply the following requirements.

1. The analysis should take a least time.
2. The accuracy of the analysis must meet the demands of Pharmacopoeia.
3. The analysis should be cost-effective.
4. The method should be precise and selective.

All above requirements are met by the physico-chemical methods of analysis, a virtue of which is their universal nature that can be employed for analyzing organic compounds with a assorted structure of them, visible Spectrophotometry is generally preferred especially by small scale industries as the equipment cost is least and the maintenance problems are minimal.^[11-13]

HPTLC METHODS OF ANALYSIS FOR DRUGS IN COMBINATIONS

The term “thin-layer chromatography”, introduced by E.

Stahl in 1956, means a chromatographic separation process in which the stationary phases consists of a thin layer applied to a solid substrate or “support”.^[4,15-17]

Pharmacopoeial standards are regularly used by industry as a basis for meeting QC requirements and current good manufacturing practices (cGMPs).

- Layer of sorbent: 100µm
- Efficiency: High due to smaller particle size generated
- Separations: 3-5cm
- Analysis time: Shorter migration distance and the analysis time is greatly Reduced.
- Solid support: Wide-ranging choice of stationary phases like silica gel for normal phase and C₈/ C₁₈ for reversed phase modes.
- Development chamber: New type that require less amount of mobile phase.
- Sample spotting: Auto sampler
- Scanning: Use of U.V./Visible/Fluorescence scanner scans the entire chromatograms qualitatively and quantitatively and the scanner is an advanced type of densitometer.

ANALYTICAL METHOD VALIDATION: THE REGULATORY PERSPECTIVE^[2,3,8,18,22]

In US, there was no mention of the word validation in the cGMP's of 1971, and precision and accuracy were stated as laboratory controls. It was only in the cGMP guideline of March 1979 emphasize on the need for validation. It was done in two sections: (1) Section 211.165, where the word ‘validation’ was used and (2) section 211.194, in which the proof of suitability, accuracy and reliability was made compulsory for

regulatory submissions. Another guidance on validation of chromatographic methods was released by CDRE on 1st Nov. 1994.

The WHO published guidelines under the title, 'Validation of analytical procedures used in the examination of pharmaceutical materials'. It appeared in the 32nd report of the WHO expert committee on 'specifications for pharmaceutical preparations' which was published in 1992.

The international Conference on Harmonization (ICH), which has been on the forefront of developing the harmonized tripartite guidelines for adoption in the US, Japan and EC, has issued two guidelines under the titles- 'Text on validation of Analytical procedures (Q2A) and validation of Analytical procedure Methodology (Q2B)'.

Among the pharmacopoeias, USP XXII 1225 (1995) carries a section which describes requirements of validation of compendial methods. The British Pharmacopoeia includes the definition of method validation in 15 latest editions, but the term is completely missing from the Indian Pharmacopoeia. (1996).

RECENT PAPERS ON TLC AND HPTLC^[12-17]

HPTLC is one of the most widely applied methods for the analysis in pharmaceutical industries, clinical chemistry, food, forensic chemistry, biochemistry, cosmetology and drug analysis, environmental analysis, and other areas. Primarily due its numerous advantages, for example, it is the only chromatographic method offering the option of presenting the results as an image. Other advantages include simplicity, low costs and parallel analysis of samples, high sample capacity, rapidly obtained results, possibility of multiple detection > etc.

Ford MJ et al. evaluated a HPTLC for Quantitative thin layer chromatography/mass spectrometry analysis of caffeine using a surface sampling probe electrospray ionization tandem mass spectrometry system.

Morlock G, et al. estimated HPTLC for Determination of isopropylthioxanthone (ITX) in milk, yoghurt and fat by HPTLC-FLD, HPTLC-ESI/MS and HPTLC-DART/MS.

Kertesz V, et al. determine HPTLC for Automation of a surface sampling probe/electrospray mass spectrometry system.

Sudberg S, et al. evaluated a HPTLC Fingerprint analysis and the application of HPTLC to the determination of identity and quality of botanicals, from an industry perspective.

Shah NJ et al. evaluated a HPTLC technique for multicomponent analysis Development and validation of a simultaneous HPTLC method for the estimation of

olmesartan medoxomil and hydrochlorothiazide in tablet dosage form.

Eric-Jovanovic S et al. evaluated a HPTLC technique for the determination of ceftriaxone, cefixime and cefotaxime in dosage forms

Fuchs B, Schiller J, Süss R, Nimptsch A, Schürenberg M, Suckau D. Capabilities and disadvantages of combined matrix-assisted laserdesorption/ionization time-of-flight mass spectrometry (MALDITOF MS) and high-performance thin-layer chromatography (HPTLC): Analysis of egg yolk lipids.

Many reports on studies related to clinical medicine have already been published in many journals. HPTLC is now strongly recommended in the analysis of drugs in serum and other tissues, also HPTLC-densitometry method for the quantification of pharmacologically active alkaloids in *Sceletium tortuosum* raw material and products.

STEPS INVOLVED IN HPTLC^[11,13,17,21-24]

- a) Selection of chromatographic layer
- b) Sample and standard preparation
- c) Layer prewashing
- d) Layer pre-conditioning
- e) Application of sample and standard
- f) Chromatographic development
- g) Detection of spots
- h) Scanning
- i) Documentation of chromatic plate

a) Selection of chromatographic layer

- Precoated plates- different support materials- different sorbents available.

b) Sample and standard preparation

- To avoid interference from impurities and water vapours
- Low signal to noise ratio – straight base line – improvement of LOD
- Solvents used are methanol, chloroform: methanol(1:1), ethyl acetate: methanol (1:1), chloroform: methanol: ammonia (90:10:1), methylene chloride : methanol (1:1), 1% ammonia or 1% acetic acid
- Dry the plates and store in dust free atmosphere

Following is the schematic procedure for HPTLC

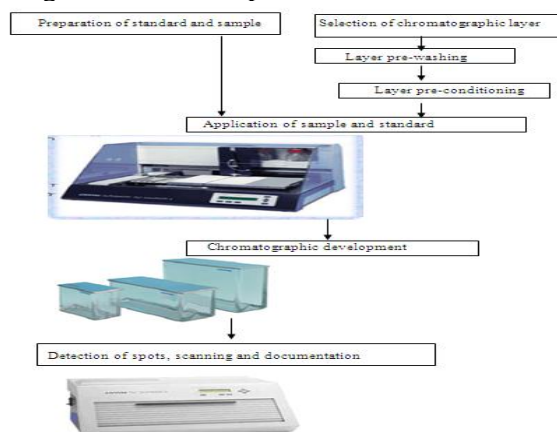


Figure 1 Schematic procedure for HPTLC

c) Activation of pre coated plates

- Freshly open box of plates do not require activation.
- Plates exposed to high humidity or kept on hand for long time to be activated.
- By placing in an oven at 110-120°C for 30 min prior spotting.

- Aluminum sheets should be kept in between two glass plates and placing in oven at 110-120°C for 15 minutes.

d) Application of sample and standard

- Usual concentration range is 0.1-1 µg/µl

Selection of mobile phase

- Trial and error.
- One's own experience and literature.

e) Detection and visualization

- Detection under UV light is first choice – non-destructive.
- Spots of fluorescent compound can be seen at 254nm (short wavelength) or at 366nm (long wavelength).
- Spots of non-fluorescent compounds can be seen – fluorescent stationary phase is used - silica gel GF.
- Non UV absorbing compound like ethambutol, dicyclomineetc – dipping the plates in 0.1% iodine solution.
- When individual components do not respond to UV derivatisation required for detection.

Table 1. Basically used derivatizing reagents for U.V. detection in HPTLC

Class of Compound	Derivatizing reagent
For detection of antioxidants, phenols, primary and secondary aliphatic amines, secondary and tertiary aromatic amines, aromatic hydrocarbons, pharmaceuticals, phenoxyacetic acid herbicides, etc	2,6-Dichloroquinone -4- chloroimide
For detection of alkaloids, aromatic hydrocarbons, e.g., antihypertensive drugs	Formaldehyde / sulfuric acid
For detection of amines, indole derivatives	p-Dimethylaminobenzaldehyde / hydrochloric acid reagent (Ehrlich's reagent)
For detection of nitrogen compounds, alkaloids, anti-arrhythmic drugs, surfactants	Dragendorff reagent
For detection of steroid alkaloids, steroid sapogenins and phenothiazine derivatives	Formaldehyde / phosphoric acid
Relatively unspecific universal reagent for many organic compounds	Iodine vapor
For detection of amino acids, amines, amino sugars.	Ninhydrin

Quantification

- Sample and standard should be chromatographed on same plate – after development chromatogram is scanned.
- Camag TLC scanner III scan the chromatogram in reflectance or in transmittance mode by absorbance or by fluorescent mode – scanning speed is selectable up to 100mm/s – spectra recording is fast – 36 tracks with up to 100 peak windows can be evaluated.
- Calibration of single and multiple levels with linear or non-linear regressions are possible.
- When target values are to be verified such as stability testing and dissolution profile single level calibration is suitable.
- Statistics such as R.S.D. or C.V. report automatically.

- Concentration of analyte in the sample is calculated by considering the sample initially taken and dilution factors.

CONCLUSION

This review describes the common technique of HPLC method development and validation of optimized method. HPTLC exhibits several advantages over HPLC and consequently it is sometimes preferred over it. HPTLC consumes by less amount of solvents and therefore can be regarded as more environment friendly as well as economic. In addition, it is a fast method of analysis permitting the simultaneous processing of large number of samples with no memory effect. Furthermore, it also allows the detection of those compounds even strongly retained on baseline. Undeniably, HPTLC does not require rich treatment or the sophisticated

experimental setup usually associated with HPLC methods of analysis.

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