High Performance Thin Layer Chromatography (HPTLC): A Modern Analytical tool for Biological Analysis

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Abstract: Among the modern Analytical tools HPTLC is a powerful analytical method equally suitable for qualitative and quantitative analytical tasks. HPTLC is playing an important role in today analytical world, not in competition to HPLC but as a complementary method. This article describes HPTLC features and basic steps involved in instrumentations.

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Introduction

Among the modern Analytical tools HPTLC is a powerful analytical method equally suitable for qualitative and quantitative analytical tasks. HPTLC is playing an important role in today analytical world, not in competition to HPLC but as a complementary method. One of the most obvious orthogonal features of the two techniques is the primary use of reversed phases in HPLC versus unmodified silica gel in HPTLC, resulting in partition chromatography and adsorption chromatography respectively. Unlike other methods, HPTLC produces visible chromatograms complex information about the entire sample is available at a glance. Multiple samples are seen simultaneously, So that reference and test samples can be compared for identification. Similarities and differences are immediately apparent and with the help of the image comparison. Several chromatograms can be compared directly, even from different plates. In addition to the visible chromatograms, analog peak data are also available from the chromatogram. They can be evaluated either by the image based software Videoscan or by scanning densitometry with TLC Scanner, measuring the absorption and/or fluorescence of the substances on the plate. TLC is an offline technique: the subsequent steps are relatively independent, allowing parallel treatment of multiple samples during chromatography, derivatization and detection. Some of the steps can be repeated independently of others, for example in post chromatographic derivatization, some reagents can be applied in sequence allowing multiple derivatization and thus multiple detection of the same sample. In view of the above article describes the key features of traditional thin layer chromatography and modern HPTLC advantages.

Key feature of HPTLC

1. Simultaneous processing of sample and standard - better analytical precision and accuracy less need for Internal Standard.
2. Several analysts work simultaneously.
3. Lower analysis time and less cost per analysis.
4. Low maintenance cost.
5. Simple sample preparation - handle samples of divergent nature.
6. No prior treatment for solvents like filtration and degassing.
7. Low mobile phase consumption per sample
8. No interference from previous analysis - fresh stationary and mobile phases for each analysis - no contamination.
9. Visual detection possible - open system.

On UV absorbing compounds detected by post-chromatographic derivatization 10.

HPTLC- High Performance Thin Layer Chromatography is a sophisticated and automated form of TLC.

Main Difference of HPTLC and TLC - Particle and Pore size of Sorbents.

	HPTLC	***TLC***
Layer of Sorbent	• 100µm	• 250µm
Efficiency	• High due to smaller particle size generated	• Less
Separations	• 3 - 5 cm	• 10 - 15 cm
Analysis Time	• Shorter migration distance and the analysis time is greatly reduced	• Slower
Solid support	• Wide choice of stationary phases like silica gel for normal phase and C8 , C18 for reversed phase modes	• Silica gel , Alumina & Kiesulguhr
Development chamber	• New type that require less amount of mobile phase	• More amount
Sample spotting	• Auto sampler	• Manual spotting
Scanning	• Use of UV/ Visible/ Fluorescence scanner scans the entire chromatogram qualitatively and quantitatively and the scanner is an advanced type of densitometer	• Not possible

Steps involved in HPTLC :

Selection of chromatographic layer

Precoated plates - different support materials - different Sorbents available
80% of analysis - silica gel GF · Basic substances, alkaloids and steroids - Aluminum oxide Amino acids, dipeptides, sugars and alkaloids - cellulose
Non-polar substances, fatty acids, carotenoids, cholesterol - RP2, RP8 and RP18
Preservatives, barbiturates, analgesic and phenothiazines- Hybrid plates-RPWF254s

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

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 110-120ºc for 30' prior to spotting
Aluminum sheets should be kept in between two glass plates and placing in oven at 110-120ºc for 15 minutes.

Application of sample and standard
Usual concentration range is 0.1-1µg /µl
above this causes poor separation
Automatic applicator- nitrogen gas sprays sample and standard from syringe on TLC plates as bands
Band wise application better separation high response to densitometer

Selection of mobile phase
Trial and error
Ones own experience and Literature based

Normal phase
Stationary phase is polar
Mobile phase is non polar
Non-polar compounds eluted first because of lower affinity with stationary phase
Polar compounds retained because of higher affinity with the stationary phase

Reversed phase
Stationary phase is non polar
Mobile phase is polar
Polar compounds eluted first because of lower affinity with stationary phase
Non-Polar compounds retained because of higher affinity with the stationary phase
More than five component mobile phase should be avoided
Multi component mobile phase once used not recommended for further use and solvent Composition is expressed by volumes (v/v) and sum of volumes is usually 100
Twin trough chambers are used only 10 -15 ml of mobile phase is required
Components of mobile phase should be mixed introduced into the twin - trough chamber

Pre- conditioning (Chamber saturation)
Un- saturated chamber causes high Rf values
Saturated chamber by lining with filter paper for 30 minutes prior to development - uniform distribution of solvent vapors - less solvent for the sample to travel - lower Rf values.

Chromatographic development and drying
After development, remove the plate and mobile phase is removed from the plate - to avoid contamination of lab atmosphere
Dry in vacuum desiccators - avoid hair drier - essential oil components may evaporate

Detection and visualization
Detection under UV light is first choice-non destructive and spots of fluorescent compounds can be seen at 254 nm (short wave length) or at 366 nm (long wave length) Spots of non fluorescent compounds can be seen - fluorescent stationary phase is used - silica gel GF Non UV absorbing compounds like ethambutol, dicylomine dipping the plates in 0.1% iodine solution When individual component does not respond to UV- derivatisation required for detection.

Quantification
Sample and standard should be chromatographer on same plate-after development chromatogram is scanned TLC scanner III scan the chromatogram in reflectance or in transmittance mode by absorbance or by fluorescent mode - scanning speed is selectable up to 100 mm/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 RSD report automatically concentration of analyte in the sample is calculated by considering the sample initially taken and dilution factors.

Documentation

Plates with imprinted identification code supplier name. Item number, batch number and individual plate number - avoid manipulation of data at any stage - coding automatically gets recorded during photo documentation.

Multiple Detection
Separation of Herbal sample and reference substances, white light (left), UV 254 nm (center), UV 366 nm (right) in addition to the evaluation of chromatograms via images there is a broad array of other detection modes available. Detection is a very flexible and independent step. Multiple detection is possible without repeating the chromatography.

Scanning densitometry
Allows measuring the absorption and/or fluorescence of underivatized or derivative substances at wavelengths between 200 and 800 nm. Up to 31 wavelengths can be evaluated and spectra of any peak can be recorded. Following integration densitometric data can be quantitatively evaluated. Biological tests can be performed directly on the HPTLC plate. Bacteria, enzymes, yeast, fungi, etc. can be used as test organisms.

HPTLC-MS allows the hyphenation of a high resolution planar separation with modern mass spectrometers for identification and quantitation of substances. Technologies for available interfaces include elution and desorption approaches. Multi-wavelength scan evaluation of UVspectra toxicity screening with Vibrio fischeri (Bioluminex TM, Chromadex) and identification by HPTLC-MS.

Conclusions: In case of biological sample matrix is very complex most of biological sample chemical constituent are not know even constituents know variability of naturally occurring samples may very considerable. Furthermore, the HPTLC fingerprint is also suitable for rapid and simple authentication and comparison of the suitable difference among samples with identical plant resource but different geographic locations.

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