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. The other differences are

	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

“ Features 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
10. Non UV absorbing compounds detected by post-chromatographic derivatization

Steps involved in HPTLC

1. Selection of chromatographic layer
2. Sample and standard preparation
3. Layer pre-washing
4. Layer pre-conditioning
5. Application of sample and standard
6. Chromatographic development
7. Detection of spots
8. Scanning

9. Documentation of chromatic plate

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:!0:1), 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
· Linomat IV (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
- one's own experience and Literature
- 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
- 3 - 4 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 vapours - 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 desiccator - avoid hair drier - essential oil components may evaporate

Detection and visualization

· Detection under UV light is first choice - non destructive
· 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 etc - 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 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 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 or CI report automatically
· Concentration of analyte in the sample is calculated by considering the sample initially taken and dilution factors

Documentation

E - Merck introduced plates with imprinted identification code - supplier name. Item number, batch number and individual plate number - Avoid manipulation of data at any stage - coding automatically get recorded during photo documentation

Validation of analytical method

All validation parameters such as precision, accuracy, LOD, LOQ, Ruggedness, Robustness can be performed