PharmD IIIrd Yr

Pharmaceutical Analysis

HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY

-By Rupam Swain

INTRODUCTION

- **Chromatography** is a physical method of separation in which the components to be separated are distributed between two phases, one of which is stationary (stationary phase) while the other (the mobile phase) moves in a definite direction.

- **HPTLC** is a well known and versatile separation method which shows a lot of advantages and options in comparison to other separation techniques.

- The method is fast and inexpensive. It does not require time consuming pretreatments.

- The basic difference between conventional TLC and HPTLC is only in particle and pore size of the sorbents.

- It is very useful in quantitative and qualitative analysis of pharmaceuticals.

ADVANTAGES

- High resolution of zones due to higher number of theoretical plates.
- Shorter developing times
- Less solvent consumption
- Enormous flexibility
- Parallel separation of many samples with minimal time requirement
- Simplified sample preparation due to single use of the stationary phase.

PRINCIPLE

- Principle Same theoretical principle of TLC.
- *The principle of separation is <u>adsorption.</u>*

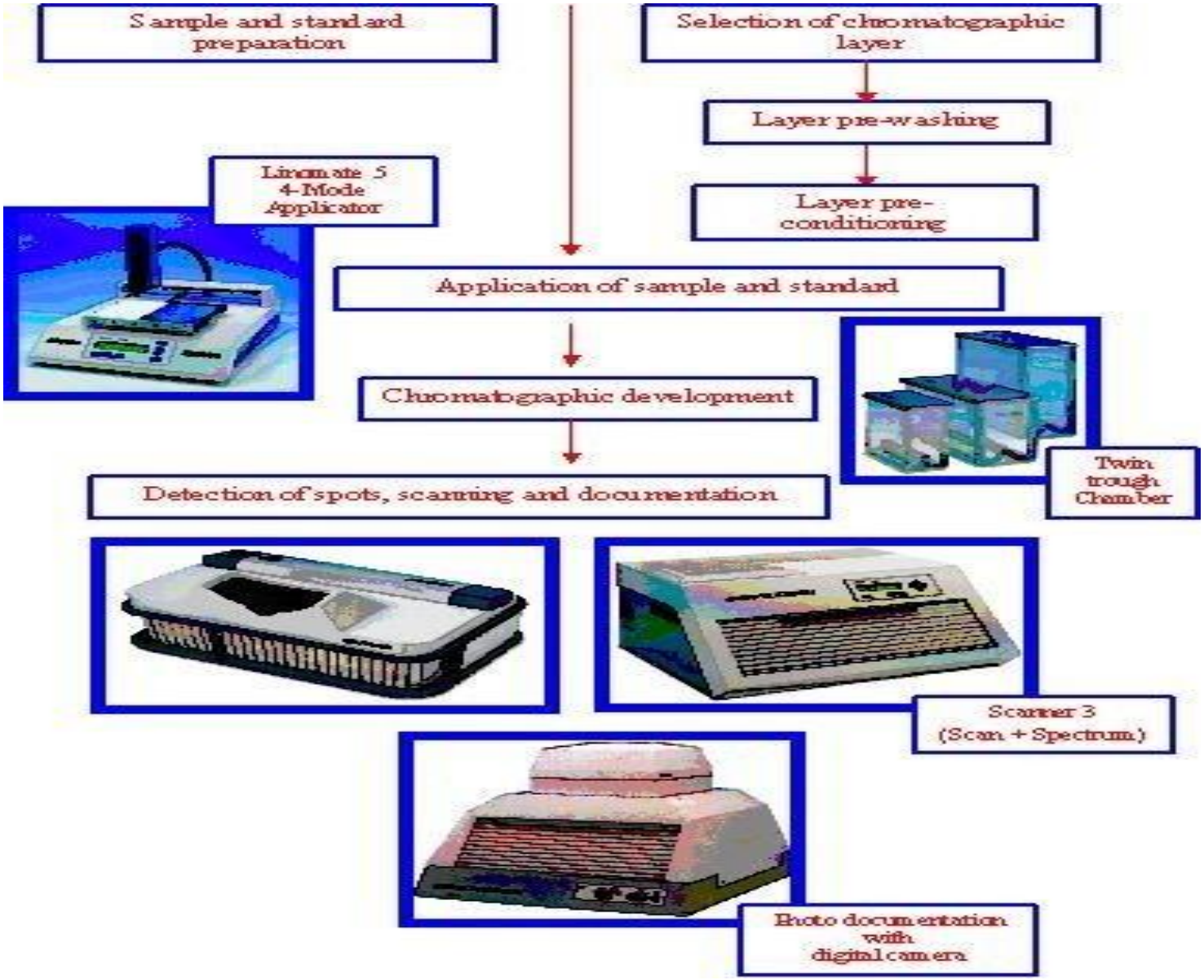
Major difference between TLC and HPTLC

Parameter	TLC	HPTLC
Mean particle size	10-12µm	5-6µm
Distribution	5-20µm	4-8µm
Layer thickness	250µm	200µm
Plate height	30µm	12µm
Separation time	20-200min	3-20min
Sample volume	1-5µL	0.1-0.5µL

INSTRUMENTATION

1. *Plate coaters*
2. *Drying racks*
3. *Plate cutters*
4. *Immersion device*
5. *Plate heater*
6. *Sample application*
7. *Development chamber*
8. *Derivatization devices*
9. *Scanning densitometer*

Flow diagram of HPTLC Instrumentation:



1.Plate coater ,

a.Hand operated

- The manual plate coater functions in the same manner as the automatic coater , except with this model the plates are pushed through by hand , one after the other and lifted off on the other slide.

b.AUTOMATIC PLATE COATER

- The glass plates to be coated are conveyed underneath a hopper filled with the adsorbent suspension.

- The plates are moved by a motorized conveying system at a uniform feeding rate of 10cm/s, to ensure a uniform speed.

2.Drying Rack

•The Drying Rack consists of ten individual aluminum trays.

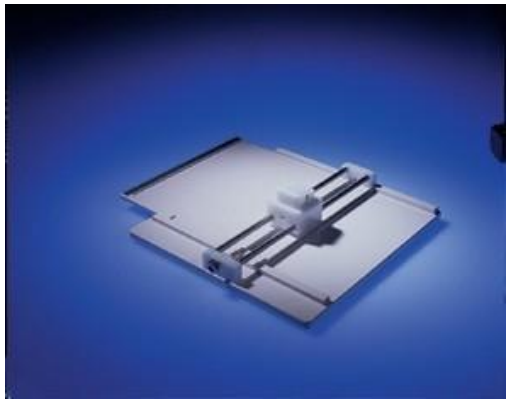
•A tin box for storing the trays and two wire handles , to move the stack while hot ,are supplied.

•The drying rack is convenient to use , particularly when TLC plates are prepared with the automatic plate coater in large runs.

3.PLATE CUTTER

- Used to cut HPTLC plates easily and more precisely.

•Cuts plates with a thickness up to 3mm.



- Does not damage the sensitive layer.

•Easy to handle .Read the required size from the scale directly.

•Helps saving costs on pre-coated plates of high quality by preventing off cuts.

4.Immersion Devise

•For proper execution of the dipping technique , the chromatogram must be immersed and withdrawn at a controlled uniform speed.

Key features

- Uniform vertical speed
- Immersion time selectable between 1 and 8 seconds.

•The device can be set to accommodate 10cm and 20cm plate height.

- Battery operated , independent of power supply.

5.Plate Heater

•The TLC plate heater is designed for heating TLC plates to a given temperature , while ensuring homogenous heating across the plate.

•The TLC plate heater has a heating surface which is resistant to all common reagents and is easily cleaned.

•Programmed and actual temperature are digitally displayed.

•The temperature is selectable between 25 and 200^{0}c.

- The plate heater is protected from overheating.

6.Sample Application

Usual concentration of applied samples 0.1 to 1 µg / µl for qualitative Analysis and quantity may vary in quantitation based on UV absorption 1 to 5 µl for spot and 10 µL for band application.

- MANUAL , SEMI-AUTOMATIC , AUTOMATIC APPLICATION

  Manual with calibrated capillaries

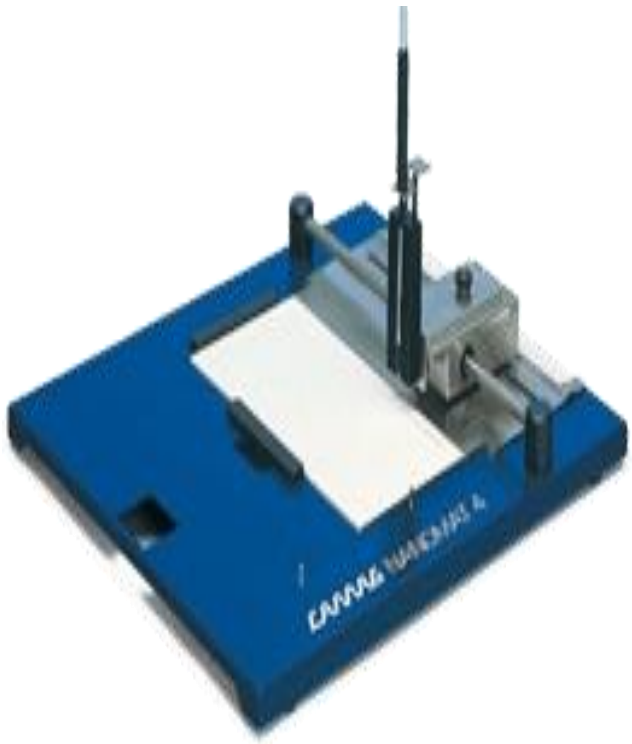
  Semi and auto-application through applicators

- Applicators use spray on or touch and deliver technique for application.

a. Manual Sample Applicator

•The Nanomat serves for easy application of samples in the form of spots onto TLC and HPTLC layers .

•The actual sample dosage performed with disposable capillary pipettes , which are precisely guided by the capillary holder.



The nanomat is suitable for

•Conventional TLC plates including self-coated Plates up to 20 × 20cm

- HPTLC plates 10 × 10 cm and 20 × 10 cm
- TLC and HPTLC sheets up to 20 × 20 cm

b.Semi automatic sample applicator

•The instrument is suitable for routine use for medium sample throughout . In contrast to the Automatic TLC sampler , changing the sample the Linomat requires presence of an operator.

•With the linomat , samples are sprayed onto the chromatographic layer in the form of narrow bands.

•During the spraying the solvent of the sample evaporates almost entirely concentrating the sample Into a narrow band of selectable length.

c.AUTOMATIC SAMPLE APPLICATOR

•Samples are either applied as spots
through contact transfer (0.1-5 micro lit)
or as bands or rectangles (0.5->50 micro lit)
using the spray on techniques.

•Application in the form of rectangles
allow precise applications of large volume
with out damaging the layer.

- ATS allows over spotting.

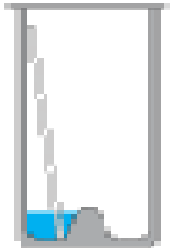
7. DEVELOPING CHAMBER

1.Twin trough chamber

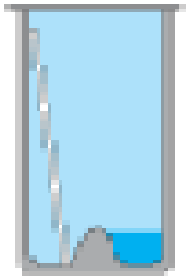
2.Automatic developing chamber

1.TWIN TROUGH CHAMBER

- **Low solvent consumption:**
  20 mL of solvent is sufficient for the development of a 20x20cm plate.
  This not only saves solvent , but also reduces the waste disposal problem



- **Reproducible pre –equilibrium with Solvent vapor:** For pre-equilibration, the TLC plate is placed in the empty trough opposite the trough which contains the pre-conditioning solvent. Equilibration can be performed with any liquid and for any period of time.



- **Start of development** : It is started only when developing solvent is introduced into the trough with the plate.

2.Automatic developing chamber (ADC)

- In the ADC this step is

fully automatic and independent of environmental effects.

- The activity and pre-conditioning of

the layer , chamber saturation ,developing distance and final drying can be pre-set and automatically monitored by ADC.

8. DERIVATIZATION DEVICE

a) Spraying

b) Dipping

c)Derivatization through gas phase

Reasons for Derivatization:

•Changing non-absorbing substance into detectable derivatives.

- Improving the detectability.
- Detecting all sample components.
- Selectivity detecting certain substance.
- Inducing fluorescence.

a) Derivatization by spraying

- It comes with a rubber pump but may also be operated from a compressed air or nitrogen supply.
- It also consists of a charger and a pump unit with two kinds of spray heads.
- Spray head type A is for spray solutions of normal viscosity , e.g. lower alcohol solution.
- Spray head type B is for liquids of higher viscosity , e.g. sulphuric acid reagent

b) Derivatization by Dipping

•For proper execution of the dipping technique the chromatogram must be immersed and withdrawn at a controlled uniform speed.

•By maintaining a well defined vertical Speed and immersion time , derivatization Conditions can be standardized and tide Marks , which can interfere with densitometry evaluation , are avoided.

c)Derivatization through gas phase

- It offers rapid and uniform transfer of the reagent.
- It is unfortunate that only few reagents are suitable they include I,Br,Cl, as well as volatile acids, bases and some other gases like H_2S , NO.
- In gas phase derivatization can be easily accomplished in twin trough chambers where the reagent is placed or generated in the rear trough , while the plate facing the inside of the chamber is positioned in the front trough.

9. Scanning densitometer

- The scanner is connected to a computer.

•The scanner features three light sources ,a deuterium lamp , a tungsten lamp and a high pressure mercury lamp.

•The scanning speed is selectable between 1 and 100 mm/s

APPLICATIONS

* Food analysis
* Pharmaceutical industry
* Clinical applications
* Industrial applications
* Forensic
*cosmetics

Factor affecting HPTLC :

- Type of stationary phase
- Mobile phase
- Layer thickness
- Temperature
- Mode of development
- Amount of sample
- Dipping zone & others

Product formulation	Mobilephase	Quantitation
Propanol hydrochloride	0.3M Sodium chloride, methanol-glacial acetic acid	Densitometry at 254nm or 275nm
Paracetmol, caffeine, Ascorbic acid	Dichloro methane-Ethylacetate –Ethanol	Densitometry at 254nm
Omeprazole	Methanol-water	Densitometry at 320nm
Ampicillin,Cloxacillin sodium	Methanol-Dipotassium Hydrogen phosphate	Densitometry at 490nm.