

High performance Thin layer Chromatography

Introduction:

HPTLC

It is a most versatile technique and is known for uniformity, purity profile, assay values and precision and the accuracy of results. It can handle several samples of even divergent nature and composition. HPTLC is a modern analytical separation method with extensive versatility, although already much utilized, is still with great potential for the future techniques available. The scope of hyphenation of HPTLC with other analytical techniques appears to hold considerable promise for the analysts.

Principle:

Principle of HPTLC have similar approach and employ the same physical principles of TLC (adsorption chromatography) i.e. the principle of separation **adsorption**. The mobile phase solvent flows through because of capillary action. The

components move in according to their affinities towards the adsorbent. It is highly versatile separation of method in both of qualitative and quantitative.

Instrumentation:

1. Applicator.
2. Automatic Developing chamber.
3. Camera photo Digital to documentation
4. Scanner

Indicator:

Green fluorescent F254

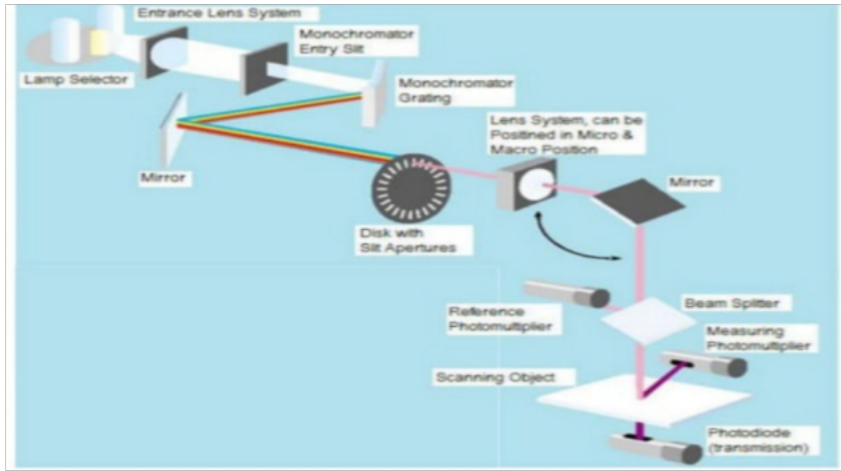
Blue fluorescent

Acid-stable F254s

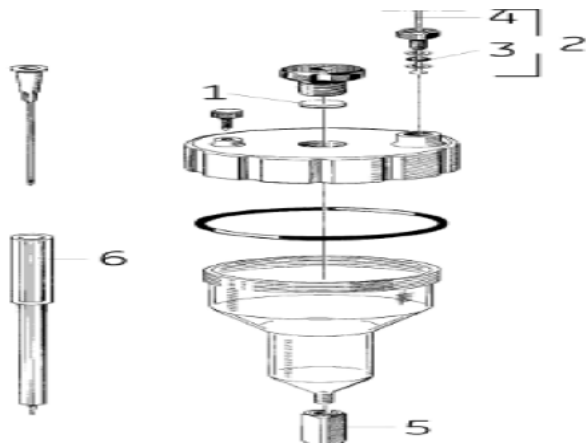
Plate size: 20×20cm 10×20cm

5×10cm 5×7.5cm

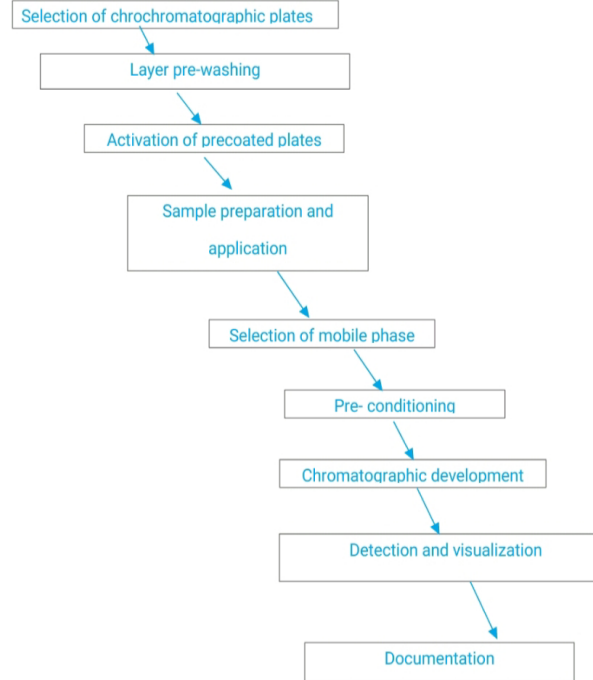
Instrumentation:



Sample Applicator:

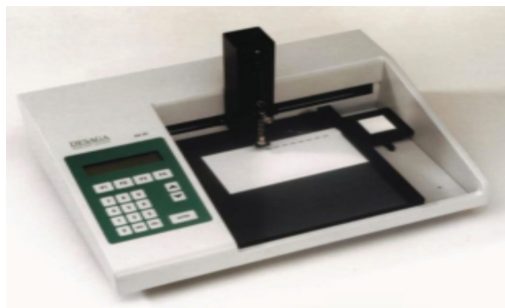


Steps involving in HPTLC



1.Applicator:

This principle permits motionless application of sample in the line in dots. Microcomputer controlled stepping motor and gas value clearly arranged menu system enables the input of up to ten methods which can be stored using battery backup memory.Used in combination with an automatic sample transmitter .



2.Automatic Developing chamber:

It is device for reproducible plate development step fully automatically and independent of environment effect. Activity and preconditioning of layer chamber saturation developing distance and final drying can be present and automatically monitored by automatically development chamber.



3.Camera photo Digital to documentation:

It is very important to observe developed HPTLC plates under UV light to distinguish different developed spots precisely. It is to document keep photograph of plates for ready reference keeping in mind a person has indigenously developed luminous.



4.Scanner:

The scanner 4 is scanning densitometer. It measures the reflection of separated compounds in absorption or fluorescence mode controlled by vision of CATS software the tlc scanner 4 enables quantitative

evaluation of generated distrometic data. Spectral range of light 190-900 nm is available for selecting single or multiple wavelength for scanning densitometry.



Detector:

UV cabinet diode- array detectors.

Lamp selector, Entrance lens slit.

Monochromator, entrance slit.

Grating. Mirror, Beam splitter.

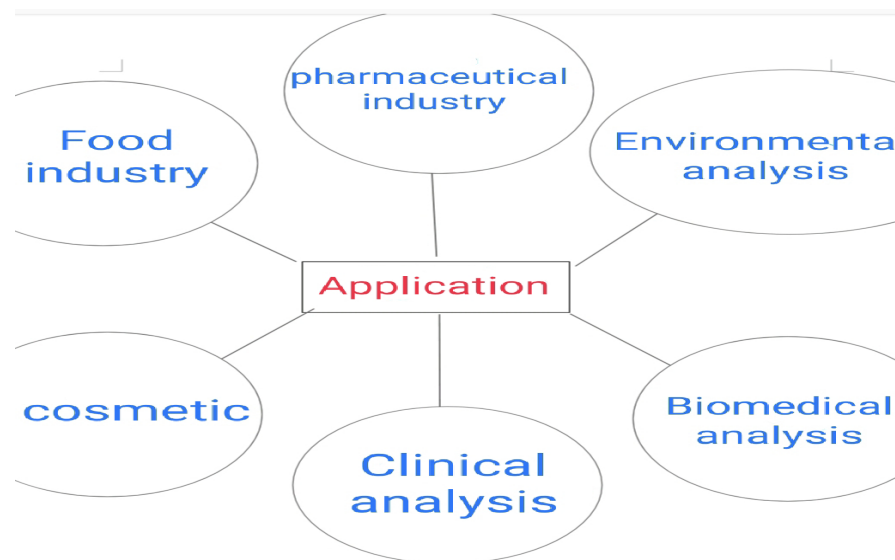
Reference photo multiplier.

Measuring photo multiplier.

Photo diode for transmission measurements.

Reference: Instrumental method of analysis Dr.PD Sethi, page no 4-50.

Instrumental method of analysis Kasture A.V. pageno 48-55.



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