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PHCOG MAG.: Chromatography tips

HPTLC – An Overview

Mueen Ahmed K K

Dept. of Pharmacognosy, Al-Ameen College of Pharmacy, Hosur road, Bangalore 560 027
e.mail : mueen.ahmed@phcog.net

PLANAR CHROMATOGRAPHY

Planar chromatography (PC) like any other chromatographic technique is a multistage distribution process. It is a form of liquid chromatography in which the stationary phase is supported on a planar surface rather than a column. Separations in PC occur because of differential migration velocities through the sorbent layer in a fixed separation time. High-performance thin-layer chromatography (HPTLC) has developed to the extent that separation and quantitation can provide results that are comparable with other analytical methods such as high-pressure liquid chromatography (HPLC).

HPTLC (HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY)

HPTLC is a modern separation technique which is accepted worldwide as an extremely flexible, reliable, and cost efficient method. Planar chromatography is operated in off-line mode and, its features are:

- Enormous flexibility
- Parallel analysis of many samples
- Unsurpassed clarity through simultaneous visual evaluation of all samples and sample components
- Simplified sample preparation because of the single use of the stationary phase
- Possibility of multiple evaluation of the plate with different parameters because all fractions of the sample are stored on the plate

IMPORTANT APPLICATIONS OF PLANAR CHROMATOGRAPHY

- Pharmaceutical industry
  Quality control, Content Uniformity Test (CUT), Identity/purity check, Phytopharmaceutical anlaysis.
- Food analysis
  Quality control, Additives, Pesticides, Stability testing
- Clinical applications
  Lipids, Metabolism studies, Drug screening, Stability testing
- Industrial applications
  Process development and optimization, In-process control, Cleaning validation,
- Forensic
  Detection of document falsifications, Poisoning investigations, Dye stuff analysis

BASIC STEPS IN HPTLC

Four Basic Steps in HPTLC Analysis, are as follows

- Sample Application,
- Chromatogram Development
- Derivatization
- Chromatogram Evaluation

SAMPLE APPLICATION

The samples are applied onto the separation layer, either as spots through contact transfer or as narrow bands using the spray-on technique. Precision of applied volume, small size of application zone and exact positioning are essential for the quality of the analysis. Bandwise sample application as available with Linomat 5 or Automatic TLC. Sampler offers the best separation results and highest flexibility regarding sample solvents and application volumes. With spray-on technique the applied volume can be easily adjusted to match the required detection limit of the analytical task. With the Linomat samples are sprayed onto the chromatographic layer in the form of narrow bands. This technique allows larger sample volumes to be applied than by contact transfer (spotting). While the solvent is almost completely evaporated in the process, the sample is concentrated on the layer surface into a narrow band of selectable length. Even of samples dissolved in rather polar solvents such as methanol or water, compact and narrow zones are formed. Starting zones sprayed on as narrow bands ensure the highest resolution attainable with any given planar chromatographic system

CHROMATOGRAM DEVELOPMENT

Chromatogram is drawn by capillary forces the developing solvent (mobile phase) migrates through the layer (stationary phase) over a defined distance. During this process the sample is separated into fractions (components). After evaporation of the mobile phase all fractions remain stored on the layer. Special features: Almost unlimited choice of mobile phase and large selection of stationary phases.

Automated Development (CAMAG)

The HPTLC plate is developed repeatedly in the same direction, Each successive run extends over a longer solvent migration distance than the one before and between runs, the solvent is completely removed from the developing chamber and the layer is dried under vacuum. Each successive run uses a solvent of lower elution strength than that of the one used before. In this way, a stepwise elution gradient is formed. The combination of focusing effect and gradient elution

results in extremely narrow bands. Their typical peak width is about 1 mm. This means that, with the available separation distance of 80 mm, up to 40 components can be completely resolved, i.e. with base line separation.

TWIN TROUGH CHAMBERS

Twin Trough Chambers (CAMAG) offer several ways to improve the results of TLC/HPTLC developing techniques Low solvent consumption. This not only saves solvent, it also reduces the waste disposal problem as there is almost no solvent left when the run is completed.

Reproducible pre-equilibration of the stationary phase with solvent vapor is done by placing the plate in the empty trough opposite the trough which contains the preconditioning solvent.

DERIVATIZATION

It is an inherent advantage of planar chromatography that fractions are stored on the plate and can be derivatized after chromatography. By derivatization, substances that do not respond to visible or UV light can be rendered detectable. In many cases, substances or classes of substances can be identified by specific reagents.

CHROMATOGRAM EVALUATION

The chromatogram is evaluated under ultraviolet or white light. Options range from visual inspection, electronic image processing, video densitometry and documentation to quantitative determination by means of monochromatic light in a classical densitometer, which additionally facilitates measurement of spectral information. Spectral information is available through the densitometer. Special features: Multiple evaluation of the chromatogram with different parameters and detection methods.

TLC SCANNER

The Camag TLC Scanner may be used, In which two continuum lamps, a deuterium and a tungsten halogen lamp, in combination with a monochromator generate light of 254/300 nm bandwidths in the spectral range from 190-800 nm. A third, high pressure mercury vapour lamp provides high energy for scanning by fluorescence. The lamps are selected and positioned automatically. Plates up to 20 cm X 20 cm are placed on a stage that is mechanically operated along the *x*- and *y*-axis. The scanning speed is variable to a max of 100 mm s^{-1}. All functions of the scanner as well as generation and processing of data are controlled by a personal computer using WinCATS software. A typical sequence of quantitative evaluation of a chromatogram includes raw data acquisition, data integration, calibration and calculation of results, and generation of the analysis report. peak data of the unknowns are related to those of the calibration standards. From several calibration functions, including single- or multi-level with linear or polynomial regression, the most suitable for the task is chosen. Multiwavelength scanning that initially acquires raw data of up to ten different wavelengths can be separately processed post-run in the integration and calibration routine. Therefore, each substance can be quantified at the wavelength of its maximum absorbance

In the Camag scanner both deuterium and halogen tungsten lamps remain powered, the whole time and are therefore well stabilized. The spectral data can be processed post-run for various purposes:

- To determine the optimum wavelength(s) for quantitative scanning.
- To identify individual fractions by comparison with spectra of authentic standards co-chromato-graphed on the same plate or stored in a spectrum library.
- To check identity by superimposing the spectra of all fractions within the same *R*F window.
- To check the purity of fractions by superimposing the spectra from different positions within a spot.

Future developments of instrumentation are likely to focus on improvement of the speed and flexibility of all automated individual steps. The future will see systems that combine the automated steps in sample application, chromatogram development and evaluation by video technology.