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HPTLC Solutions

Tech Tip

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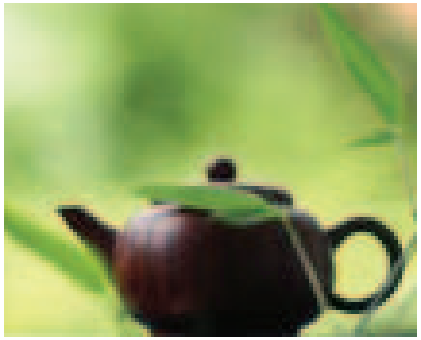

Parameters of the HPTLC Process

An inherent advantage of planar chromatography is its enormous flexibility derived from the off-line principle and interaction of many parameters. It is helpful to know these parameters for each step of the process and to understand how they can affect the overall result in order to utilize the advantages for unlocking the full potential of high performance thin-layer chromatography (HPTLC) This issue of *HPTLC Solutions* is dedicated to the first part of the HPTLC process which includes plate handling, sample application, and chromatogram development. The next issue will cover the subsequent steps of HPTLC: detection of chromatograms, documentation, and quantitative evaluation by scanning densitometry.

Handling of plates

There is a great variety of stationary phases used in planar separations. This issue focuses on the one most frequently used in HPTLC: glass-backed plates pre-coated with silica gel 60 F_{254} [1].

HPTLC's off-line principle requires manual handling of the chromatographic plate between the steps. Because the active layer of the plate is quite delicate special care must be taken to avoid any damage or contamination. Impurities on the plate may still accumulate from the laboratory atmosphere and from packing material. While for most qualitative analyses plates are typically used "out of the box" without any pre-treatment, it is important to consider a standardized cleaning procedure if the analytical method has to be validated (stability test, quantification) and reproducible results are required.

Upon storage and handling silica gel will adsorb water vapour which affects the activity of the plate and thus the Rf value of the analyte as well as the selectivity of the separation (Figure 1). To overcome these effects, automatic development chambers may be used, which make chromatogram development more reproducible and independent of environmental factors and manual handling. With such an instrument the plate is automatically lowered into the mobile phase present in the conventional developing tank. The development

Figure 1

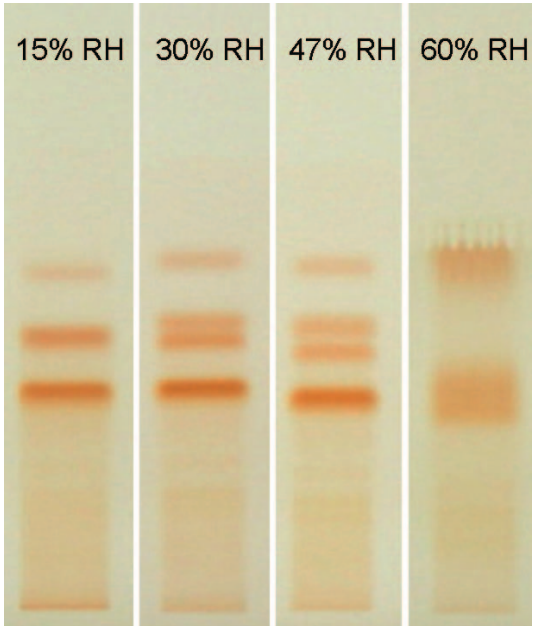

Figure 1: Influence relative humidity (RH) on the chromatographic results. Separation of Green Tea [2]

Figure 2

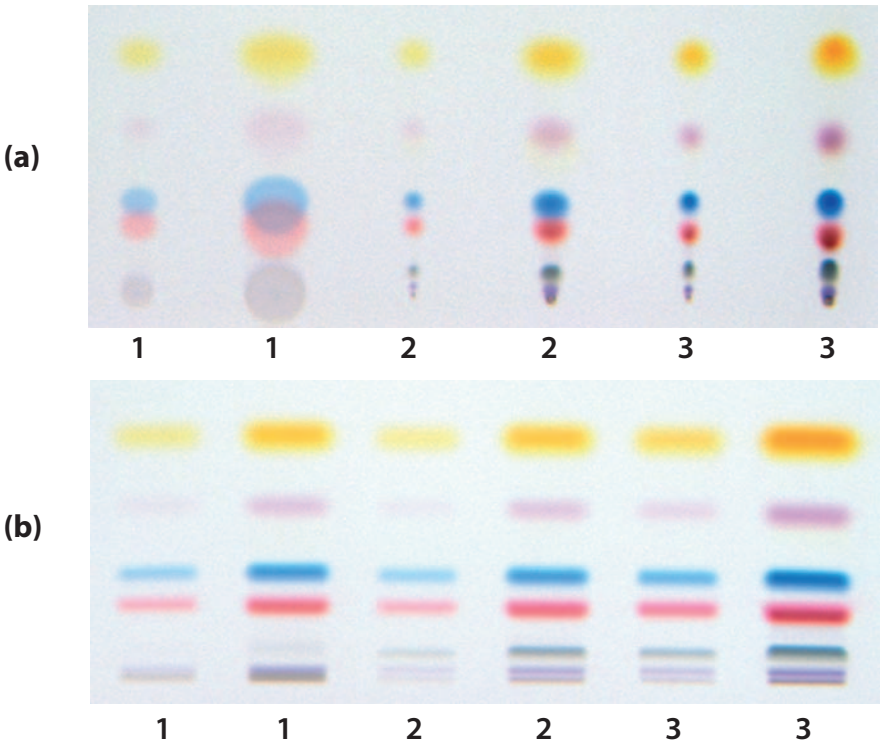

Figure 2: Effects of the solvent and the type of sample application on the chromatogram: Image of the developed plate after (a) contact application of spots and b) spray-on application of bands. Sample: test dye mixture (0.5 and 5 µL) dissolved in (1) methanol, (2) toluene and (3) hexane.

process is controlled by automatic measurement of the migration distance. After completion of chromatography the plate is automatically removed from the developing tank and homogenously dried. The ultimate benefit of automatic systems is the efficient adjustment of the relative humidity surrounding the plate and thus affording a defined activity of the stationary phase.

Sample application

Sample application is the first important step in HPTLC because it highly influences the resolution of the chromatogram. For maximum separation power of the chromatographic system, the application zone should be as small as possible in the direction of chromatography. During contact spotting the solvent of the sample may disturb the homogenous distribution of the sample components throughout the spot and may lead to broad spots or even concentric rings (Figure 2).

Resolution and detection limits of a given HPTLC system can be further improved if samples are applied as sharp bands using the spray-on technique.

For reproducible results and comparability of data from plate to plate it is important that the plate layout is defined. This includes distances between samples and plate edges as well as applied volumes and width of applied bands (Figure 3).

Figure 3

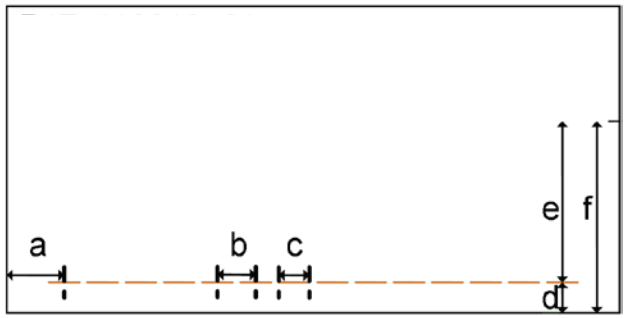

Figure 3: Layout for an HPTLC plate with position of samples (orange) and labels. (a) 20 mm; (b) min. 10 mm; (c) 8 mm; (d) 8 mm; (e) 62 mm; (f) 70 mm. The resulting maximum number of tracks per plate is 15.

Chromatogram development

Chamber type and chamber saturation

HPTLC differs from all other chromatographic techniques in the fact that in addition to a stationary and mobile phase a gas phase is present which can influence the separation. The "classic" way of developing a chromatogram is to place the plate in a chamber containing the mobile phase. Driven by capillary action the mobile phase moves up the layer until the desired running distance is reached and chromatography is interrupted.

For adsorption chromatography on silica gel, four partially competing processes occur in the closed developing chamber (Figure 4).

- Between the components of the developing solvent and their vapour phase equilibrium is being established eventually (1 = saturation).
- While still dry, the stationary phase adsorbs molecules from the gas phase (adsorptive saturation). This way particularly the polar components will be withdrawn from the gas phase (2 = pre-conditioning).
- Simultaneously, the part of the layer which is

Figure 4

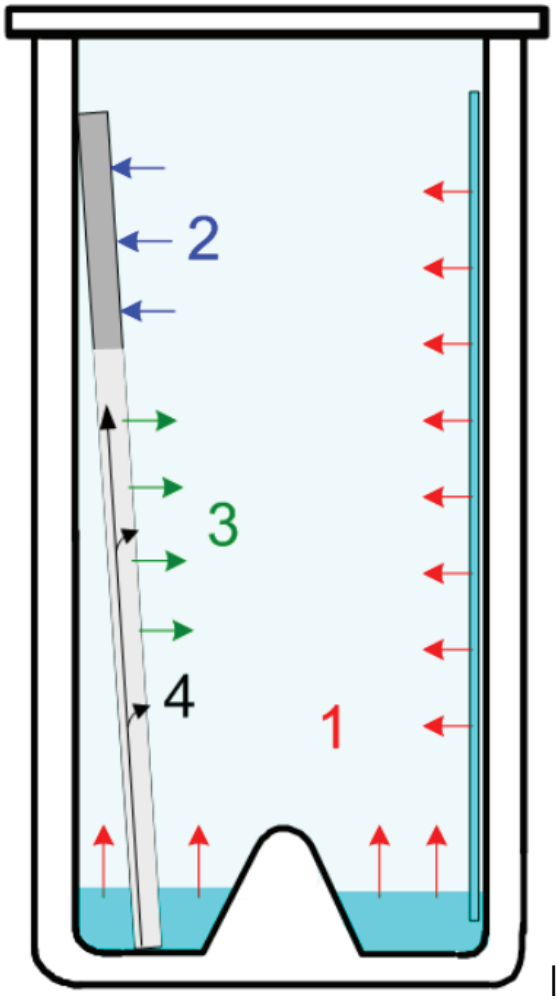

Figure 4: Processes in the developing chamber.

already wetted with mobile phase interacts with the gas phase and, especially, the less polar components of the liquid are given off in the gas phase (3 = evaporation).

- During migration, the components of the mobile phase can be separated by the stationary phase under certain conditions, causing the formation of secondary fronts (4 = formation of secondary fronts).

The processes (1) and (2) can be experimentally affected by chamber saturation (fitting the chamber with filter paper soaked with developing solvent and waiting a certain time before chromatography) or plate pre-conditioning (allowing the plate to interact with the gas phase prior to development without contact to the developing solvent). As a result Rf-values are lower in saturated chambers and particularly on pre-conditioned layers than in unsaturated chambers.

HPTLC in most cases proceeds in a non-equilibrium between stationary, mobile, and gas phase and, therefore, it is very difficult to mathematically describe the conditions in a developing chamber. In consequence reproducible chromatographic results can only be expected when all parameters are kept as constant as possible. Another consequence is that different developing chambers (size, geometry) or chamber setups (saturation) usually produce different results. Therefore, these parameters must be rigorously standardized for comparable and reproducible results to be obtained.

For further reading on the theoretical concepts of TLC and their significance for practical work see chapter 2 of the book "High-Performance Thin-Layer Chromatography for the Analysis of Medicinal Plants". [3]

On HPTLC plates the best resolution is obtained at a developing distance of 6 cm. Within a given chromatogram resolution is best in the Rf-range of 0.3–0.4. Therefore, the solvent strength of the mobile phase should be adjusted so that a critical substance pair is positioned in this range.

Summary

Proper handling of plates, defined sample application and controlled chromatogram development are essential pre-requisites if predictable HPTLC results are desired. It is useful to define all relevant parameters in a standard operating procedure.

However, if the flexibility of HPTLC is to be utilized all parameters can be easily modified to affect the results.

The next issue of *HPTLC Solutions* will reveal how results can be evaluated qualitatively and quantitatively.

References

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Featured Applications

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Rapid content uniformity test of 6 batches of Coenzyme Q10 in soft gel capsules by HPTLC

Company: Camag

Coenzyme Q10, found naturally in the body, is involved in the production of body energy. Thus heart, lung and liver have the highest concentration of Q10. The substance is active in many ways but primarily assumed to enhance the immune system and to work as an antioxidant protecting against free radicals that damage scells. Coenzyme Q10 is an expensive material. As dietary supplement it is mostly sold in soft gelatin capsules. For quality control of such products the CUT (content uniformity test) and the assay are of high interest.

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HPTLC Identification of Asian Ginseng

Company: Camag

Camag offers an application note describing the use of HPTLC for the identification of Asian Ginseng. The method uses silica gel 60 F254 HPTLC plates and a mobile phase of chloroform, ethyl acetate, methanol, water (15:40:22:9). Full chromatographic conditions and example plate images are provided.

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A rapid HPTLC method for detecting 5% of adulteration of *Cimicifuga racemosa* (*Actaea racemosa*) with *C. foetida, C. heracleifolia, C, dahurica*, or *C. americana*

Company: Camag

This poster presents a rapid and reliable HPTLC method for the identification of *Cimicifuga racemosa* and the detection of its most common adulterants by fingerprint profiles. With specific derivatization reagents, mixtures of *C. racemosa* with a minimum of 5% of one of the adulterant species *C. foetida, C. heracleifolia, C, dahurica,* or *C. americana* can be detected. The proposed can be used for quality control of black cohosh raw material. It was validated with respect to specificity, stability, precision and robustness.

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HPTLC Solutions

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