High-Performance Thin-Layer Chromatography (HPTLC)

ManMohan Srivastava
Editor

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Editor
ManMohan Srivastava
Professor
Department of Chemistry
Dayalbagh Educational Institute
Agra-282110
India
smohanm@rediffmail.com

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About the Book

HPTLC: High-Performance Thin-Layer Chromatography

MM. SRIVASTAVA
EDITOR

The present edited book is the presentation of 18 in-depth national and international contributions from eminent professors, scientists and instrumental chemists from educational institutes, research organizations and industries providing their views on their experience, handling, observation and research outputs on HPTLC, a multi-dimensional instrumentation. The book describes the recent advancements made on TLC which have revolutionized and transformed it into a modern instrumental technique HPTLC. The book addresses different chapters on HPTLC fundamentals: principle, theory, understanding; instrumentation: implementation, optimization, validation, automation and qualitative and quantitative analysis; applications: phytochemical analysis, biomedical analysis, herbal drug quantification, analytical analysis, finger print analysis and potential for hyphenation: HPTLC future to combinatorial approach, HPTLC-MS, HPTLC-FTIR and HPTLC-Scanning Diode Laser. The chapters in the book have been designed in such a way that the reader follows each step of the HPTLC in logical order.

About the Editor

Dr. MM. Srivastava is Professor in Department of Chemistry of Dayalbagh Educational Institute, Agra, India and has extensive experience of twenty six years of teaching and research in Analytical and Environmental Chemistry. Prof. Srivastava is actively engaged in the research under the domain of Green Chemistry and delivered lectures in National Research Council, University of Alberta, Canada, University of Illinois, Chicago, Wisconsin and Maryland, USA. He has more than 100 research papers in journals of repute. Prof. Srivastava is recipient of Department of Science and Technology Visiting Fellowship and has recently been elected as Fellow of Royal Society, London, UK (FRSC) and Fellow of Indian Society of Nuclear Techniques in Agriculture and Biology (FNAS). He has edited books on Recent Trends in Chemistry, Green Chemistry: Environmental Friendly Alternatives and Chemistry of Green Environment.

Preface

Thin-layer chromatography is without doubt one of the most versatile and widely used separation methods in chromatography. The concept of TLC is simple and samples usually require only minimal pretreatment. It has been frequently used in pharmaceutical analysis, clinical analysis, industrial chemistry, environmental toxicology, food chemistry, pesticide analysis, dye purity, cosmetics, plant materials, and herbal analysis. The previous image of TLC regarding low sensitivity, poor resolution, and reproducibility made it stagnant and forgotten technique few years back. Now, it is the most used chromatographic technique and likely to remain so for times to come.

Today, most stages of this technique are automated and operated instrumentally in the form of modern high-performance thin-layer chromatographic system that allows the handling of a large number of samples in one chromatographic run. Speed of separation, high sensitivity, and good reproducibility result from the higher quality of chromatographic layers and the continual improvement in instrumentation. It is now capable of handling samples with minimal pretreatment, detecting components at low nanogram sensitivities and with relative standard deviations of about 1%. HPTLC is now truly a modern contemporary of HPLC and GC and continues to be an active and versatile technique in research with large number of publications appearing each year.

This edited book is the presentation of 18 in-depth national and international contributions from eminent professors, scientists, and instrumental chemists from educational institutes, research organizations, and industries providing their views on their experience, handling, observation, and research outputs on this multidimensional instrumentation. The book describes the recent advancements made in TLC which have revolutionized and transformed it into a modern instrumental technique HPTLC. The book addresses different chapters on HPTLC fundamentals, principle, theory, understanding, instrumentation, implementation, optimization, validation, automation, and qualitative and quantitative analysis; applications of HPTLC separation with special reference to phytochemical analysis, biomedical analysis, herbal drug quantification, analytical analysis, finger print analysis; and HPTLC future to combinatorial approach, potential for hyphenation, HPTLC–MS, HPTLC–FTIR, and HPTLC–scanning diode laser. The chapters in the book have

been designed in such a way that the reader follows each step of the HPTLC in logical order.

Our greatest ambition for editing this book has been to familiarize and popularize the theoretical and practical aspects of working and applications of a recent, modified, versatile analytical instrument HPTLC system among students, researchers, academicians, analysts, and chemists involved in various areas of research. We wish to place on record our appreciation to Prof. VG Das, Esteemed Director, Prof. LD Khemani, Head, Department of Chemistry, Prof. Satya Prakash, Professor Emeritus, Dayalbagh Educational Institute, Dayalbagh, Agra, and all the contributors for their cooperation and encouragement extended to me. Without their enthusiasm and timely submission of their articles, this work would have not been possible. Although the bulk of material is original and/or taken from sources that the authors have been directly involved with, every effort has been made to acknowledge materials drawn from other sources.

Editor trusts that his apology will be accepted for any error, omission, and editing mistake in the manuscripts.

Agra, India ManMohan Srivastava

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List of Contributors

Abdul Moheman Department of Applied Chemistry, Faculty of Engineering and Technology, Aligarh Muslim University, Aligarh 202002, India

Ajai Prakash Gupta Public Health Engineering Department, IIIM-CSIR, Jammu & Kashmir, India

Ali Mohammad Department of Applied Chemistry, Faculty of Engineering and Technology, Aligarh Muslim University, Aligarh 202002, India

Ariane Nimptsch University of Leipzig, Medical Department, Institute of Medical Physics and Biophysics, Härtelstr, 16/18, D-04107 Leipzig, Germany

Arun Sharma Food Technology Division, Bhabha Atomic Research Centre, Mumbai 400085, India

Beate Fuchs University of Leipzig, Medical Department, Institute of Medical Physics and Biophysics, Härtelstr, 16/18, D-04107 Leipzig, Germany

Bharat G. Patel A. R. College of Pharmacy and G. H. Patel Institute of Pharmacy, Sardar Patel University, University of Leipzig Gujarat, Vallabh Vidyanagar 388 120, India

Claudia Cimpoiu Faculty of Chemistry and Chemical Engineering, Babes Bolyari University, Cluj Napoca, Romania

Devanand B. Shinde Department of Chemical Technology, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad 431001, India

G. Subramanian Department of Pharmaceutical Quality Assurance, Manipal College of Pharmaceutical Sciences, Manipal, Karnataka 576104, India

Gunawan Indrayanto Plant Biotechnology Research Group and Assessment Service Unit Faculty of Pharmacy, Airlangga University, Surabaya 60286, Indonesia

Janki N. Thakker Department of Biotechnology, PD Patel Institute of Applied Science, Charutar University of Science & Technology, Education Campus Changa, 388421 Gujarat, India

Joachim Franzke ISAS—Institute for Analytical Sciences, Bunsen-Kirchhoff-Straße 11, 44139, Otto-Hahn-Straße 6b, 44227 Dortmund, Germany

Jurgen Schiller University of Leipzig, Medical Department, Institute of Medical Physics and Biophysics, Härtelstr, 16/18, D-04107 Leipzig, Germany

Kathrin Nimptsch University of Leipzig, Medical Department, Institute of Medical Physics and Biophysics, Härtelstr, 16/18, D-04107 Leipzig, Germany

Kristin Teuber University of Leipzig, Medical Department, Institute of Medical Physics and Biophysics, Härtelstr, 16/18, D-04107 Leipzig, Germany

Lukasz Ciesla Department of Inorganic Chemistry, Faculty of Pharmacy, Medical University of Lublin, Lublin, Poland

M.A. Khan Centre for Transgenic Plant Development, Department of Biotechnology, Faculty of Science, Jamia Hamdard, Hamdard Nagar, New Delhi 110062, India

M.Z. Abdin Centre for Transgenic Plant Development, Department of Biotechnology, Faculty of Science, Jamia Hamdard, Hamdard Nagar, New Delhi 110062, India

Machindra J. Chavan Department of Pharmacognosy, Amrutvahini College of Pharmacy, Sangamner, S.K. Dist-Ahmednagar (M.S) 422 605, India

ManMohan Srivastava Department of Chemistry, Faculty of Science, Dayalbagh Educational Institute, Dayalbagh, Agra 282110, India

Mauji Ram Centre for Transgenic Plant Development, Department of Biotechnology, Faculty of Science, Jamia Hamdard, Hamdard Nagar, New Delhi 110062, India

Michael Edler ISAS—Institute for Analytical Sciences, Bunsen-Kirchhoff-Straße 11, 44139 Otto-Hahn-Straße 6b, 44227 Dortmund, Germany

Monika Waksmundzka Hajnos Department of Inorganic Chemistry, Faculty of Pharmacy, Medical University of Lublin, Lublin, Poland

Mrunali R. Patel Indukaka Ipcowala College of Pharmacy, Sardar Patel University, New Vallabh Vidyanagar, 388 121 Gujarat, India

N. Vadera Department of Pharmaceutical Quality Assurance, Manipal College of Pharmaceutical Sciences, Manipal, Karnataka 576104, India

Norman Ahlmann ISAS—Institute for Analytical Sciences, Bunsen-Kirchhoff-Straße 11, 44139 Otto-Hahn-Straße 6b, 44227 Dortmund, Germany

P. Musmade Department of Pharmaceutical Quality Assurance, Manipal College of Pharmaceutical Sciences, Manipal, Karnataka 576104, India

Paweł K. Zarzycki Section of Toxicology and Bioanalytics, Koszalin University of Technology, Śniadeckich 2, 75-453 Koszalin, Poland

Pinakin Dhandhukia Ashok and Rita Patel Institute of Integrated Study & Research in Biotechnology and Allied Sciences, New Vallabh Vidyanagar, 388 121 Gujarat, India

Prabhakar Jha Department of Botany, Faculty of Science, Jamia Hamdard, Hamdard Nagar, New Delhi 110062, India

Prasad S. Variyar Food Technology Division, Bhabha Atomic Research Centre, Mumbai 400085, India

Pravin S. Wakte Department of Chemical Technology, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad 431 001 (M.S), India

Rashmin B. Patel A. R. College of Pharmacy and G. H. Patel Institute of Pharmacy, Sardar Patel University, Vallabh Vidyanagar, 388 120 Gujarat, India

Rosmarie Süß University of Leipzig, Medical Department, Institute of Medical Physics and Biophysics, Härtelstr, 16/18, D-04107 Leipzig, Germany

Shipra Ahuja Department of Pharmaceutics, Jamia Hamdard University, Hamdard Nagar, New Delhi 110062, India

Song Peng ISAS—Institute for Analytical Sciences, Bunsen-Kirchhoff-Straße 11, 44139 Otto-Hahn-Straße 6b, 44227 Dortmund, Germany

Tomasz Tuzimski Department of Physical Chemistry, Faculty of Pharmacy, Medical University of Lublin, Lublin, Poland

Part I
Introduction

Chapter 1
An Overview of HPTLC: A Modern Analytical Technique with Excellent Potential for Automation, Optimization, Hyphenation, and Multidimensional Applications

MM. Srivastava

Abstract High performance thin layer chromatography (HPTLC) is a sophisticated instrumental technique based on the full capabilities of thin layer chromatography. The advantages of automation, scanning, full optimization, selective detection principle, minimum sample preparation, hyphenation, etc. enable it to be a powerful analytical tool for chromatographic information of complex mixtures of inorganic, organic, and biomolecules. The chapter highlights related issues such as journey of thin-layer chromatography, basic principle, protocol, separation, resolution, validation process, recent developments, and modifications on TLC leading to the HPTLC, optimization, process control, automation, and hyphenation. It explains that HPTLC has strong potentials as a surrogate chromatographic model for estimating partitioning properties in support of combinatorial chemistry, environmental fate, and health effect studies.

Analytical chemists work to improve the reliability of existing techniques to meet the demands for better chemical measurements which arise constantly in our society. They adapt proven methodologies to new kinds of materials or to answer new questions about their composition. They carry out research to discover completely new principles of measurement and are at the forefront of the utilization of recent discoveries for practical purposes. Modern analytical chemistry is dominated by instrumental analysis. Analytical chemists focus on new applications, discoveries and new methods of analysis to increase the specificity and sensitivity of a method. Many methods, once developed, are kept purposely static so that data can be compared over long periods of time. This is particularly true in industrial quality assurance, forensic, and environmental applications. Analytical chemists are also equally concerned with the modifications and development of new

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MM. Srivastava
Department of Chemistry, Faculty of Science, Dayalbagh Educational Institute, Dayalbagh, Agra 282110, India
e-mail: smohanm@rediffmail.com

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instrument. The types of instrumentation presently being developed and implemented involve analytical tools including vibrational, rotational, optical, absorption, colorimetric and scattering spectroscopy, mass spectrometry, chromatography, electro chemicals, acoustics, laser, chemical imaging, light-induced fluorescence, light scattering, etc.

At this point, we will talk about chromatographic techniques. Chromatography, defined as the group techniques used for the separation of a complex mixture of compounds by their distribution between two phases, was invented in 1901 by Russian botanist Mikhail Semyonovich Tswet, during his research on plant pigments. No other separation method is as powerful and applicable as in chromatography. It is the most versatile and widespread technique employed in modern analytical chemistry. The fact has genuine reasons. First, very sensitive methods of detection are available to all types of chromatography and small quantities of material can be separated, identified and assayed. Second, chromatographic separations are relatively fast and an analysis can be completed in a short interval of time. Another advantage of chromatography is its relative simplicity and ease of operation compared with other instrumental techniques. Finally, if the established procedure is well controlled and the apparatus is well maintained, good accuracy and precision can be achieved.

Thin-layer chromatography, among various chromatographic techniques, score high over other chromatographic techniques where altogether a new problem, one might not have encountered or solved. It is a valuable tool for reliable identification providing chromatographic fingerprints.

The feature that distinguishes TLC from other physical and chemical methods of separation is that two mutually immiscible phases are brought in to contact while one phase is stationary and the other mobile. A sample is loaded on the stationary phase and is carried by the mobile phase. Species in the sample undergo repeated interaction between the mobile and stationary phase. When both phases are properly selected, the sample components are gradually separated into bands or zones. Figure 1.1 explains the facts involving the separation of the sample.

The common method of development in thin-layer chromatography employs capillary forces to transport the mobile phase through the layer. These weak forces arise from the decrease in free energy of the solvent as it enters the porous structure of the layer. For fine particle layers, capillary forces are unable to generate sufficient flow to minimize the main sources of zone broadening. Firstly, the mobile-phase velocity varies as a function of time and migration distance. Secondly, the mobile-phase velocity is established by the system variables and is otherwise beyond experimental control. This results in a slow and variable mobile-phase velocity through the layer with separation times that is longer than required. Separated zones are broader than they would be for a constant and optimum mobile-phase velocity and the zone capacity limited by the useful range of mobile-phase velocities. Multiple developments with an incremental increase in the development length and a decreasing solvent strength gradient is the basis of separations by automated multiple developments (AMDs). Results from phenomenological models indicate that further improvements over those already realized are

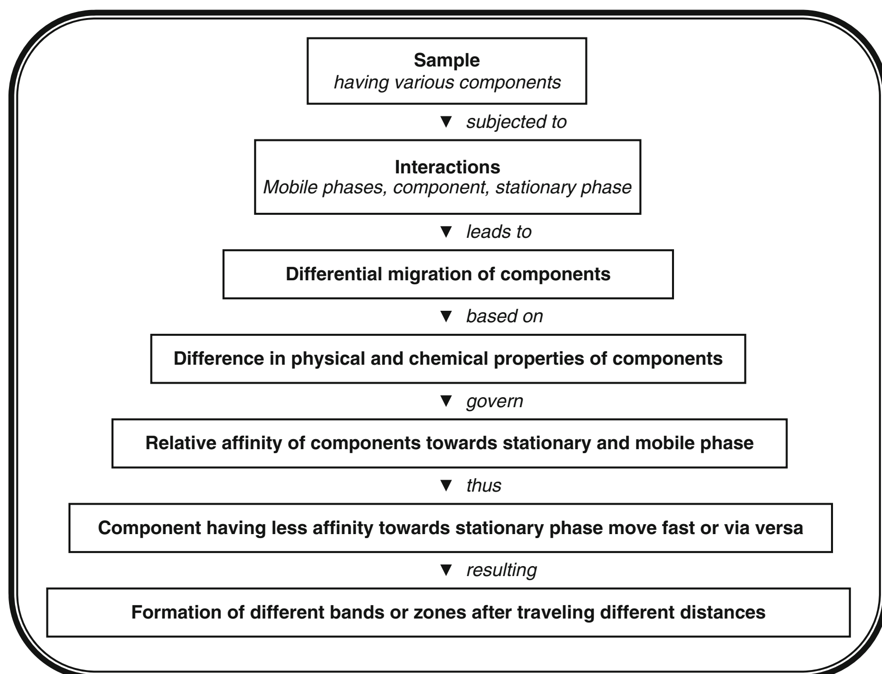

Fig. 1.1 Separation of bands on thin-layer chromatographic plate

unlikely for capillary flow systems and there is no solution to the significant increase in separation time. The magnitude and range of capillary flow velocities fundamentally limit separations in thin-layer chromatography. Faster separations with an increase in zone capacity require a higher mobile-phase velocity than in capillary flow as well as a velocity that is independent of the solvent front migration distance.

The attractive features of TLC are low-cost analysis of samples requiring minimal sample clean up and allows a reduction in the number of sample preparation steps. TLC is also preferred for the analysis of substances with poor detection characteristics requiring post-chromatographic treatment for detection. Thin-layer chromatography retains a historic link with the characterization of dyes and inks and the control of impurities in industrial chemicals. It is used for the identification of drugs and toxic substances in biological fluids, unacceptable residue levels, maintaining a safe water supply by monitoring natural and drinking water sources for crop projecting agents used in modern agriculture, and confirmation of label claims for content of pharmaceutical products. It remains one of the main methods for class fractionation, speciation and flavor potential of plant materials. It continues to be widely used for the standardization of plant materials used as traditional

medicines. It is frequently selected as the method of choice to study the metabolism and fate of radiolabeled compounds in the body and environment.

Journey of Thin-Layer Chromatography

In order to separate inorganic ions, Meinhard and Hall (1949) used a starch binder to give some firmness to the layer and described as surface chromatography. Advances were made by Kirchner et al. (195l) who used the now conventional ascending method using a sorbent composed of silicic acid. Reitsema (1954) used much broader plates and was able to separate several mixtures in one run. However, from 1956 a series of papers from Stahl appeared in the literature introducing thin-layer chromatography as an analytical procedure. Since then, silica gel nach Stahl became well known as a stationary phase. Plaster of Paris (calcium sulfate) was used as a binder and TLC began to be widely used. First book on thin layer chromatography was published by Kurt Randerath (1962), followed by those of Stahl and co-workers and second edition of Stahl's book (1969). These authors showed the wide versatility of TLC and its applicability to a large spectrum of separation problems and also illustrated how quickly the technique had gained acceptance throughout the world. Stahl (1965) could quote over 4,500 publications on TLC works. Stahl's publication highlighted the importance of factors such as the controlling of the layer thickness, the layer uniformity, the binder level, and the standardization of the sorbents as regards pore size, volume, specific surface area and particle size. Commercialization of the technique began in 1965 with the first precoated TLC plates and sheets. TLC quickly became very popular with about 400–500 publications per year appearing in the late 1960s. It was recognized as a quick, relatively inexpensive procedure for the separation of a wide range of sample mixtures. It soon became evident that the most useful sorbents was silica gel, particularly with an average pore size of 60 Å. Modifications to the silica gel began with silanization to produce reversed-phase layers. This opened up a far larger range of separation possibilities based on a partition mechanism, compared with adsorption. Until to this time, quantitative TLC was fraught with experimental error. However, the introduction of commercial spectro densitometric scanners enabled the quantification of analytes directly on the TLC layer. Initially, peak areas were measured manually, but later, integrators achieved this automatically.

Halpaap (1973) was the first to recognize the advantage of using a smaller average particle size of silica gel (5–6 mm) in the preparation of TLC plates. He compared the effect of particle size on development time, R_f values and plate height. Commercially the plates were first called nano-TLC plates but soon changed to the designation HPTLC plates with the recognition that HPTLC has added a new dimension to TLC. It was demonstrated that less amount of mobile phase, precision (tenfold) and reduction in analysis time (similar factor) could be achieved. The first major HPTLC publication was made by Zlatkis and Kaiser (1977). Halpaap and Ripphahn described their comparative results with the new 5.5-cm HPTLC plates

versus conventional TLC for a series of lipophilic dyes. Reversed-phase HPTLC was reported by Halpaap et al. (1980). Jost and Hauck (1982) reported an amino (NH2−) modified HPTLC plate which was soon followed by cyano-bonded (1985) and diol-bonded (1987) phases. The era of 1980s also saw improvements in spectro-densitometric scanners with full computer control including options for peak purity and the measurement of full UV/visible spectra for all separated components. AMD made its appearance because of the pioneering work of Burger (1984). This improvement enabled a marked increase in the number and resolution of the separated components.

Recent Developments

The multiple developments and its combination with other analytical techniques have dramatically increased the use of thin-layer chromatography for the characterization of complex mixture. TLC has strong potential as a surrogate chromatographic model for qualitative and quantitative analysis. To convert these opportunities in to the practice, several modifications have been carried out on the conventional TLC system.

Over-Pressured Layer Chromatography

Forced flow separations in the overpressured development chamber involves the sealing of the layer on its open side by a flexible membrane under hydraulic pressure and a pump is used to deliver the mobile phase to the layer. A constant mobile-phase velocity independent of the solvent front migration distance is obtained as long as the hydraulic pressure applied at the membrane maintains an adequate seal with the layer. When a solvent is forced through a dry layer of porous particles sealed from the external atmosphere, the air displaced from the layer by the solvent usually results in the formation of a second front (β front). The space between the α and β fronts is referred to as the disturbing zone and consists of a mixture of solvent and gas bubbles. In practice, the disturbing zone can be eliminated or minimized by predevelopment of the layer with a weak solvent in which the sample does not migrate. The solvent dislodges trapped air from the layer before starting the separation and consists of a mixture of solvent and gas bubbles.

Planar Electrochromatography

Electro-osmosis provides a suitable alternative transport mechanism to pressure driven flow in open tubular and packed capillary chromatography. Electro-osmotic

flow in packed capillary columns is the basis of capillary electrochromatography. The plug-like flow profile reduces *trans*-axial contribution to band broadening as well as providing a constant and optimum mobile-phase velocity. In addition, the mobile-phase velocity is independent of column length and average particle size up to the limits established by double-layer overlap. The general interest created by the rapid development of capillary electro chromatography as a useful separation method has trickled over to thin layer chromatography. Electroosmotically driven flow could provide an effective solution to the limitations of capillary flow. The current status of electroosmotically driven flow in thin-layer chromatography is probably more confusing. Recent studies have brought some enlightenment to this technique. Enhanced flow is caused by forced evaporation of the mobile phase from a solvent-deficient region at the top of the layer. Because of drainage in vertically mounted layers, electrical resistance is highest at the top of the layer and the increase in heat production drives the evaporation of solvent, pulling additional solvent through the layer. In an open system like thin-layer chromatography, evaporation of mobile phase from the layer surface competes with electro osmotic flow along the layer. The voltage, pH, and buffer concentration must be optimized to minimize either excessive flooding or drying of the layer to avoid degradation of the separation quality. These processes are probably better controlled by enclosing the layer and improving the thermostating of the system. Since high pressures are not involve, mechanisms for enclosing the layer could be relatively simple compared to pressure-driven forced flow and new approaches suggest that effective temperature control is possible. Thinner layer may also help to contain temperature gradients in combination with adequate thermostating.

Image Analysis

Slit-scanning densitometry is the dominant method of recording thin-layer separations for interpretation and quantification. This technology is now relatively mature although limited to absorption and fluorescence detection in the UV–visible range. It has adequately served the needs of thin-layer chromatography for the last two decades. Evolution of slit-scanning densitometry is now largely progressive and major changes in operation and performance seem unlikely. A possible exception is the development of scanners employing a fiber optic bundle for illumination of sample zones and collection of reflected light in conjunction with a photodiode array detector for simultaneous multi-wavelength detection and spectral recording. This approach simplifies data acquisition for some applications and affords the possibility of facile application of modern chemometric approaches for data analysis. This approach may improve the quality of available data from thin-layer separations, but does not overcome the principal limitations of slit-scanning densitometry.

For video densitometry, optical scanning takes place electronically, using a computer with video digitizer, light source, monochromators, and appropriate

optics to illuminate the plate and focus the image onto a charge-coupled device (CCD) video camera. The main attractions of video densitometry for detection in thin-layer chromatography are fast and simultaneous data acquisition, a simple instrument design without moving parts, increase in sensitivity, longer acquisition times and compatibility with data analysis. Video densitometry cannot compete with slit scanning densitometry in terms of sensitivity, resolution and available wavelength-measuring range.

Two-Dimensional Separations

Multidimensional separations employing two or more coupled orthogonal separation systems represent the preferred approach in chromatography to obtain a high peak capacity for the separation of complex mixtures. Two-dimensional separations are easily performed using planar separation systems. Even capillary flow separations can be expected to afford a zone capacity of a few hundreds rising to a few thousands for forced flow developments. In most cases, the two-solvent systems differ only in their intensity for a given set of intermolecular interactions and are not truly complementary. Such systems are responsible for the low success of two-dimensional separation systems to provide a significant increase in the separation potential apparent in many applications. Recent reports are encouraging and recognize the importance of the orthogonal nature of the retention mechanisms if a high separation capacity is to be achieved. Bilayer plates with a smaller reversed-phase strip along one edge of the plate adjacent to a larger silica gel layer have provided the most popular approach for the implementation of two-dimensional separations with a high separation capacity. Chemically bonded layers can also be used in the reversed-phase and normal phase mode and allow the use of buffers as a further means of adjusting selectivity. The awaited breakthrough in general detection for two-dimensional planar separations is likely to come from video densitometry. Data acquisition is straight forward since the whole plate is imaged simultaneously, but a problem remains with quantification that has still to be addressed.

High-Performance Thin-Layer Chromatography (HPTLC)

HPTLC allows fast, inexpensive method of analysis in the laboratory as well as in field. Modern quantitative HPTLC, when properly performed by well-trained analysts, can be advantageous compared to high-performance liquid-column chromatography in many analytical situations. The modern HPTLC technique, combined with automated sample application and densitometric scanning, is sensitive and completely reliable, suitable for use in qualitative and quantitative analysis. HPTLC is a valuable tool for reliable identification because it can provide chromatographic fingerprints that can be visualized and stored as electronic images. To

fully take advantage of this unique feature inherent to HPTLC, reproducible results and images must be ensured. Special advantages of HPTLC include high sample throughput and low cost per analysis; multiple samples and standards can be separated simultaneously, and sample preparation requirements are often minimal because the stationary phase is disposable. Other advantages include static, off-line detection of zones using a great variety of complementary post-chromatographic universal and selective detection methods that are often applied sequentially, and storage of the separation, containing all sample components, on the layer for identification and quantification at a later time by in situ or elution methods (Fig. 1.2). At the present time, all steps of the TLC process can be computer controlled.

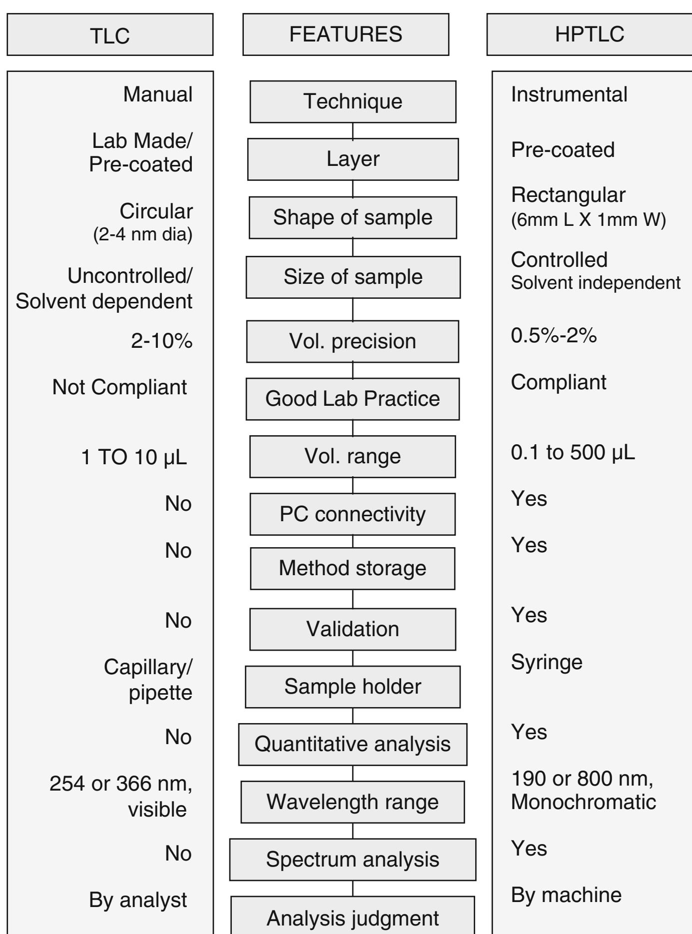

Fig. 1.2 Advancements made on TLC leading to the development of HPTLC

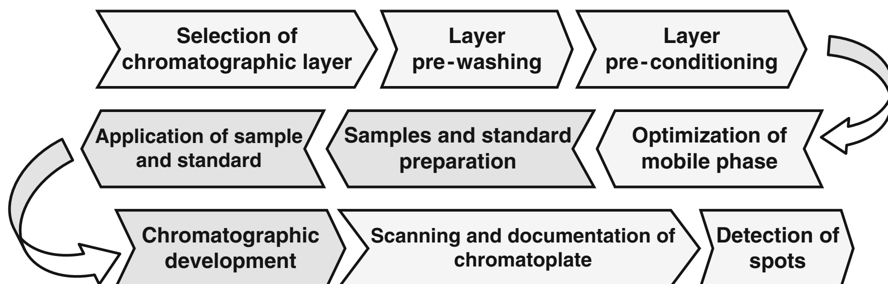

Fig. 1.3 Schematic procedure for HPTLC method development

The use of highly sensitive (CCD) cameras has enabled the chromatographer to electronically store images of chromatograms for future use and for direct entry into reports at a later date.

HPTLC-based separations involves several steps shown in Fig. 1.3. The details of each step have been discussed in the preceding chapters.

HPTLC: Separation and Resolution

To which extent various components of a formulation are separated by a given HPTLC system is the important factor in quantitative analysis. It depends on the following factors:

- Type of stationary phase
- Type of precoated plates
- Layer thickness
- Binder in the layer
- Mobile phase
- Solvent purity
- Size of the developing chamber
- Saturation of chamber
- Sample's volume to be spotted
- Size of the initial spot
- Solvent level in the chamber
- Gradient
- Relative humidity
- Temperature
- Flow rate of solvent
- Separation distance
- Mode of development

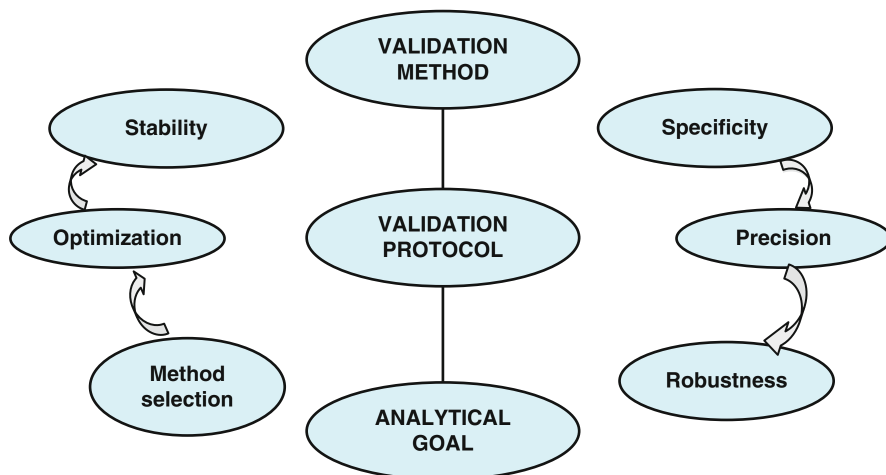

Fig. 1.4 Validation process involved in HPTLC

HPTLC: Validation Process

Validation should not be seen separately from the development of a method. The entire process can be visualized with the scheme in Fig. 1.4. It starts from a clearly defined analytical goal, method selection, optimization, and development, which is called prevalidation considerations before arriving at the elaboration of a validation protocol and is the starting point of the actual validation. After performing all the experiments described in the validation protocol, obtained data are evaluated and compared with the acceptance criteria. If all criteria are met, the method can be regarded as valid. In a less-formal approach, some validation data may be incorporated from experiments, which were conducted previously as part of the method development.

The above approach is widely accepted for validation of qualitative HPTLC methods for identification during routine use. It is possible that the validation method in different situations may require some changes in the standard validation protocol. Such changes may include restrictions with respect to relative humidity, waiting times, precision, etc. The validation protocol is a key instrument for structuring, regulating and documenting the validation processes, depending on the quality management system. The following elements must be included:

Selectivity

Ability of the developed analytical method is to detect analyte quantitatively in the presence of other components which are expected to be present in the sample matrix. Results are expressed as Resolution. If the expected impurities or related substances are available, they should be chromatographed along with the analyte to check the system suitability, retention factor, tailing factor, and resolution.

Sensitivity

Ability of the method within a given range to obtain test results in direct proportion to the concentration of analyte in the sample – calibration curve for the analyte.

Precision

Precision provides an indication of random error. Its results should be expressed as relative standard deviation (RSD) or coefficient of variation (COV). Precession is observed in terms of *replication*: precision under same conditions, same analyst, same apparatus, short interval of time and identical reagents using the same sample; *measurement of peak area*: RSD should not be greater than 1%, based on seven times measurement of same spot; *peak position*: RSD should not be greater than 2% based on seven times repositioning the instrument after each measurement; *sample application*: equal volume applied as seven spots and RSD should not be greater than 3% and under different conditions, different analyte, different laboratory, and different days and reagents from different sources using the same sample. RSD should not be greater than 10% within laboratory reproducibility.

Accuracy

Accuracy of an analysis is determined by systematic error involved. It is defined as closeness of agreement between the actual value and mean analytical value obtained by applying the test method a number of times. The accuracy is acceptable if the difference between the true value and mean measured value does not exceed the RSD values obtained for repeatability of the method. This parameter is very important for formulated pharmaceutical dosage forms as it provides information about the recovery of the analyte from sample preparation and effect of matrix. It the recovery rate is found to be 100%, it implies that the proposed analytical method is free from constant and proportional systematic error. A blank matrix and known impurities must be available to test the accuracy of the method.

Ruggedness

This is one of the most important parameters for validation of HPTLC method. Experiments are usually recommended to evaluate ruggedness of a HPTLC method like *sample preparation*: composition, quantity of solvent, pH, shaking time, temperature and number of extractions; *sample application*: volume applied, spot shape and size, band and spot stability; *separation*: at least on three different plates; *chromatographic conditions*: chamber saturation, eluent composition, eluent volume, temperature, humidity and development distance; *spot visualization*: post-chromatographic derivatization, spraying, dipping, reaction temperature and time; *quantitative evaluation*: drying of plates, detection and wavelength.

Once the analytical method is developed, it should be performed independently by three analysts well conversant with practical aspects of the technique, analyzing the same sample under same experimental conditions to check reproducibility of the method.

Limit of Detection

Lowest amount of analyte that can be detected is not greater than 10% of the individual impurity limit. If this is not possible, then amount of analyte to be applied has to be increased. Limit of detection (LOD) is determined on the basis of signal to noise ratio. Mean of 15 noise peak areas and their absolute SD values are determined. LOD is the amount of applied sample producing a peak area which is equal to the sum of mean blank area and three times standard deviation.

Stability

Analyte should not decompose during development of the chromatogram and should be stable in solution and on the sorbent for at least 30 and 15 min, respectively. The intensity of the spot on the chromatogram should be constant for at least 60 min while optimization of the extraction/purification procedure and one must keep in mind the chemical properties and purity of the extraction solvent. Chemical reaction of the solvents and their impurities may produce extra spot/peak, thus leading to false assay values. Other important factor is pH of the aqueous phase used for extraction/purification which may lead to hydrolysis, oxidation and isomerization. The complete removal of organic solvent should be avoided.

HPTLC: Optimization and Process Control

A standard methodology is applied for optimization. Sample preparation, in most cases, a 5-min sonication with methanol, followed by centrifugation and using the supernatant as test solution, yields satisfactory results. Derivatization is optimized with the goal of convenience, safety, and reproducibility. Botanical Reference Materials (BRM) of known adulterants are used to ensure sufficient specificity of the method. Small modifications of the mobile phase composition are applied to fine-tune separation. Each step of the optimization process is documented for complete traceability. The optimization of the chromatographic mobile phase proved to be possible when the number of experimental determinations of separation parameters for each compound is obtained for more than one distinct compositions of mobile phase, at least equal with the number of variable use in the mathematical model. A mobile phase optimization program based on an original mathematical approach is to be developed for its performances by applying on three sets of compounds. The original optimization procedure starts from the idea that

into a mixture of three solvents the quantitative measure of the choused chromatographic parameter is dependent on composition of mobile phase through an equation of dependency with six or seven parameters, taking into consideration the molar fraction of the solvents. The optimization procedure is included in a program and applied on three sets of previously studied compounds through high-performance thin-layer chromatography with three solvents. The mobile-phase optimization process proved to be able to provide accurate, precise, and reproducible method of characterization and analysis of chromatographic parameters.

HPTLC: Automation

For the past 50 years, both automatic and automated instruments have been used to monitor and control process stream, such as density, viscosity, and conductivity. It is necessary to distinguish between the characteristics of automatic and automated devices. According to the current definitions of the International Union of Pure and Applied Chemistry (IUPAC), both devices are designed to replace, refine, extend or supplement human effort and facilities in the performance of a given process. The unique feature of automated devices is the feedback mechanism, which allows at least one operation associated with the device to be controlled without human intervention. An automatic photometer might continuously monitor the absorbance of a given component in a process stream, generating some type of alarm if the absorbance exceeds a preset value. By contrast, an automated system could transmit absorbance values to a control unit that adjusts process parameters (temperature and amount of additional reagent) to maintain the concentration of the measured component within preset limits. In spite, of this fundamental difference, the terms automatic and automated are often interchanged.

The use of automated sample processing, analytics and screening technology for profiling absorption, distribution, metabolism, excretion, and physicochemical properties is becoming more widespread. The use and application of these technologies is both diverse and innovative. High throughput screening technologies have been utilized enabling the profiling of an increased number of compounds. Although the drivers for using these technologies are common, different approaches can be taken. Control Systems, Safe, efficient, and economical operations of chemical processes are ever more dependent in the use of online analyzers. The use of analytical measurements of component properties in near real time for process control during manufacturing is becoming more common. The combination of online analyzers and advanced control technologies holds an enormous economic potential. As a result, the number of existing applications of HPTLC is growing steadily.

Advances in science and technology have raised an increasing demand for control analyses and posed various challenges to analytical chemists such as the need to develop new methods exhibiting as much selectivity, sensitivity, sample and reagent economy, throughput, cost-effectiveness, simplicity, and environmental