1 Introduction

Thin-layer chromatography (TLC) is a very old method of analysis that has been well proven in practice. For more than thirty years, it has occupied a prominent position, especially in qualitative investigations. With the development of modern precoated layers and the introduction of partially or completely automated equipment for the various stages of operation of TLC, not only are highly accurate quantitative determinations now possible, but also the requirement that the work should comply with the GMP/GLP guidelines can be fulfilled.

Following the widespread use of high-performance liquid chromatography (HPLC), the importance of TLC, mainly measured by the work rate of the method, has been forced into the background. This is reflected in the unfavorable treatment of TLC as taught in universities, higher technological teaching establishments, technical colleges and industry. In addition to this, the restructuring of the chemical industry begun some years ago and the consequent job losses have led to considerable loss of specialist know-how in the use of TLC.

For these reasons, it is hoped that the present book will point towards good practical methods of performing TLC. Special attention is paid to possible sources of error. Theoretical aspects are not placed in the foreground, but emphasis is rather placed on the current state of the technology and the scope of modern TLC. The arrangement of the book strictly follows the individual operating steps of TLC, so that the user will be able to locate these various steps with ease.

This book is mainly intended for the younger scientific generation. For teachers it tries to encourage a form of teaching close to practical "real-life" TLC analysis, and the many practical tips also offer invaluable support for the less experienced users in industrial and official laboratories. Last but not least, it can be used by the analyst in a pharmaceutical laboratory as a work of reference.

1.1 What Does TLC Mean?

Chromatography means a method of analysis in which a mobile phase passes over a stationary phase in such a way that a mixture of substances is separated into its components. The term "thin-layer chromatography", introduced by E. Stahl in 1956, means a chromatographic separation process in which the stationary phase consists of a thin layer applied to a solid substrate or "support" [1, 2]. For some years, TLC has also been referred to as planar chromatography. However, apart from the fact that paper

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chromatography, which is also a planar method, is now hardly used, I do not think that this term will ever be widely accepted because the abbreviation PC could easily be confused with the abbreviation for personal computer.

The term TLC is now used to describe the method in all its forms, including manual or semiautomatic operation on conventional, high-performance or modified layers.

1.2 When Is TLC Used?

An essential precondition is that the substances or mixtures of substances to be analyzed should be soluble in a solvent or mixture of solvents.

TLC is used if

- the substances are nonvolatile or of low volatility
- the substances are strongly polar, of medium polarity, nonpolar or ionic
- a large number of samples must be analyzed simultaneously, cost-effectively, and within a limited period of time
- the samples to be analyzed would damage or destroy the columns of LC (liquid chromatography) or GC (gas chromatography)
- the solvents used would attack the sorbents in LC column packings
- the substances in the material being analyzed cannot be detected by the methods of LC or GC or only with great difficulty
- after the chromatography, all the components of the sample have to be detectable (remain at the start or migrate with the front)
- the components of a mixture of substances after separation have to be detected individually or have to be subjected to various detection methods one after the other (e.g. in drug screening)
- no source of electricity is available

1.3 Where Is TLC Used?

Pharmaceuticals and Drugs
Identification, purity testing and determination of the concentration of active ingredients, auxiliary substances and preservatives in drugs and drug preparations, process control in synthetic manufacturing processes.

Clinical Chemistry, Forensic Chemistry and Biochemistry
Determination of active substances and their metabolites in biological matrices, diagnosis of metabolic disorders such as PKU (phenylketonuria), cystinuria and maple syrup disease in babies.

Cosmetology
Dye raw materials and end products, preservatives, surfactants, fatty acids, constituents of perfumes.

Food Analysis
Determination of pesticides and fungicides in drinking water, residues in vegetables, salads and meat, vitamins in soft drinks and margarine, banned additives in Germany (e.g. sandalwood extract in fish and meat products), compliance with limit values (e.g. polycyclic compounds in drinking water, aflatoxins in milk and milk products).

Environmental Analysis
Groundwater analysis, determination of pollutants from abandoned armaments in soils and surface waters, decomposition products from azo dyes used in textiles.

Analysis of Inorganic Substances
Determination of inorganic ions (metals).

Other Areas
Electrolytic technology (*meta*-nitrobenzoic acid in nickel plating baths).

A graphical representation of the distribution of TLC publications among the most important fields of application during the years 1993 and 1994 is given in Fig. 1 [3]. However, this diagram does not give any indication of the distribution of the actual use of the technique. Reliable information on this subject is difficult to obtain. Information on quantities of materials used for TLC must mainly come from the manufacturers, but they are unwilling to release this on grounds of industrial secrecy. Our own research in northern and southern Germany has revealed that 40 % of the precoated layers go to universities and other higher educational establishments for use in the areas of pharmacy, medicine and biology, while a further ca. 40 % are used in the pharmaceutical industry, including use by pharmacists, and the remainder is divided between official investigative organizations (e.g. food monitoring, police and customs) and private institutions. This leads us to conclude that the majority of TLC users work in the area of pharmaceutical investigations. Recent polls confirm this distribution.

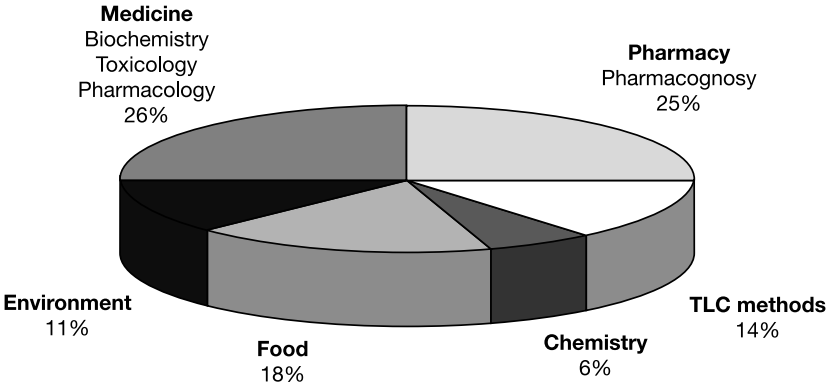

Figure 1. Fields of application of thin-layer chromatography (TLC/HPTLC) over the years 1993–1994

1.4 How is the Result of a TLC Represented?

Please do not expect a profound treatment of chromatographic parameters at this point. As beginners in TLC you should not be frightened off at the very beginning of this book. Any reader interested in the theory of TLC should read books devoted to this subject, the two by Geiss being especially recommended [4, 5].

The subject of TLC has its own special parameters and concepts, the most important of these for practical purposes being described below.

1.4.1 Retardation Factor

The position of a substance zone (spot) in a thin-layer chromatogram can be described with the aid of the retardation factor $\boldsymbol{R_f}$. This is defined as the quotient obtained by dividing the distance between the substance zone and the starting line by the distance between the solvent front and the starting line (see Fig. 3):

$$R_f = \frac{Z_S}{Z_F - Z_0}$$

where

R_f = retardation factor
Z_S = distance of the substance zone from the starting line [mm]
Z_F = distance of the solvent front from the solvent liquid level [mm]
Z_0 = distance between the solvent liquid level and the starting line [mm]

From this formula, one obtains an "observed" R_f value, which describes the position of a spot in the chromatogram in a simple numerical way. It gives no information about the chromatographic process used or under what other "boundary conditions" this result was obtained. This calculated R_f is always ≤ 1. As it has been found to be inconvenient in routine laboratory work always to write a zero and a decimal point, the R_f value is multiplied by 100, referred to as the **hR**$_f$ value,[1)] quoted as a whole number, and used for the qualitative description of thin-layer chromatograms.

In the calculation of hRf values as described in the literature, the distance Z_S is measured from the starting line to the mid-point of the substance zone. In general, this is correct and is also accurate enough for small spots. However, in purity tests on pharmaceutical materials, amounts of substance up to and even exceeding 1000 µg/spot are used, and this can lead to hRf value ranges up to ca. 18. If, in addition, limit-value amounts of at least 0.1 % of the same substance are applied and chromatographed on the same plate, these ideally lie exactly in the calculated central point of the main spot. However, this does not always happen. They are more likely to deviate from this position and be distributed over the whole hRf value range. Here, the term **"hRf value range"** means the imaginary hRf value range from the beginning to the end of a substance spot. In Fig. 2a–c, the chromatogram of purity tests of three active substances are given in which the position of the small amount of substance is respectively at the top end, approximately in the center, and at the bottom end of the hRf range.

▶ **Figure 2:** see Photograph Section.

☞ **Practical Tip** for calculation of the hRf values:

- In purity tests, always quote hRf values as a range extending from the beginning to the end of a substance spot.

Figure 3 gives a graphical representation of the parameters and terms used in this book to describe a thin-layer chromatogram. Explanations of other terms are given in Section 1.4.3.

Because of the often poor reproducibility, especially when TLC plates prepared in-house are used and the conditions necessary for a good chromatographic result are in consequence not complied with, the so-called R_{St} value, based on a standard substance, was formerly often also given. This is defined as

$$R_{St} = \frac{Z_S}{Z_{St}}$$

where

Z_S = distance from the substance zone to the starting line [mm]
Z_{St} = distance of the standard substance from the starting line [mm]

[1)] Because of the formatting difficulties associated with subscripts in the computer age, the term **hR**f value has become established and is used throughout this book.

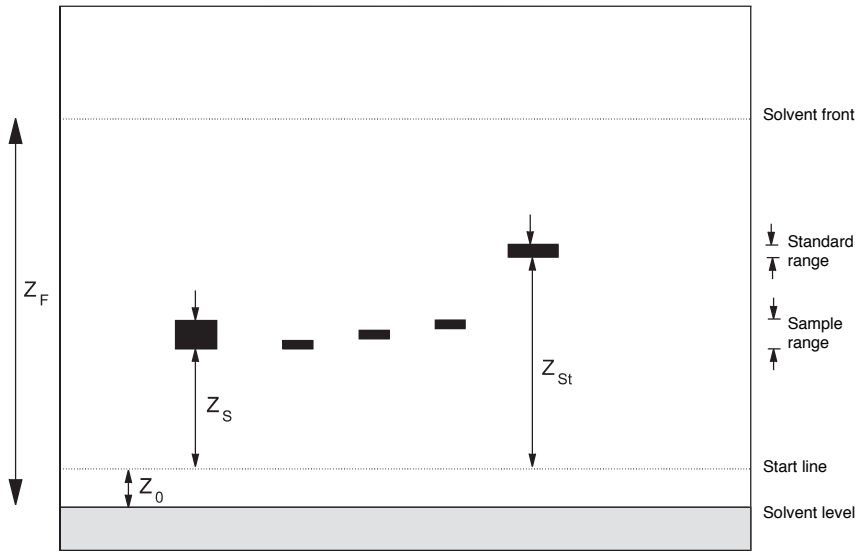

Figure 3. Terms used to describe a thin-layer chromatogram

According to Geiss [4, p. 65], it is not a good principle to quote R_{St} values as they are practically worthless and only give the appearance of certainty. In pharmacopoeias also, the still common linking of samples to standard substances with known R_f values has been shown to be of doubtful value as routine laboratory practice. Therefore, only the hRf value is used to evaluate results in this book.

1.4.2 Flow Constant

The flow constant or velocity constant (κ) is a measure of the migration rate of the solvent front. It is an important parameter for TLC users and can be used to calculate, for example, development times with different separation distances, provided that the sorbent, solvent system, chamber type and temperature remain constant. The flow constant is given by the following equation:

$$\kappa = \frac{Z_F^2}{t}$$

where

κ = flow constant [mm^2/s]
Z_F = distance between the solvent front and the solvent level [mm]
t = development time [s]

The following example illustrates the usefulness of the flow constant in laboratory work. In a TLC, if the development time for a migration distance of 10 cm was 30 min and the Z_0 distance is 5 mm, the κ value is 6.125 mm^2/s.

Question: How much time is required to develop a 15-cm migration distance if the sorbent, solvent system, Z_0 and laboratory temperature remain constant?

Answer: 65.4 min.

This means that more than twice the development time is required for a migration distance which is only 5 cm longer!

It should be mentioned here that the flow constant is influenced by other effects also, e.g. the surface tension and viscosity of the solvent system. In general, the greater the viscosity and the smaller the surface tension of the solvent system, the smaller is the migration rate of the front.

1.4.3 Other TLC Parameters

In the TLC literature, different terms are often used for the same characteristic values and parameters. As this can lead to confusion, especially for beginners, the most commonly used terms are listed below, those used in this book being in bold type.

- **Solvent system** Developing solvent, mobile phase, eluent (only used in OPLC)
- **Migration distance** Run distance, run height, developing distance
- **Developing time** Run time
- **Derivatization reagent** Detection reagent

Other terms commonly used in TLC are:

- **Fluorescence quenching.** If a TLC plate has a layer which contains a fluorescence indicator, UV-active substances cause the fluorescence to be totally or partially extinguished and can be seen as dark spots on a bright background (see also Section 2.2.3 “Additives”).
- **Separation efficiency** describes the spread of the spots caused by chromatographic effects in the chosen system.
- **System suitability.** This is an expression used in the German Pharmacopoeia (Deutsche Arzneibuch, DAB), and describes a method of testing a system whereby two or more substances have to be separated from each other on a TLC plate prepared in-house, in order to establish whether samples under investigation can in fact be analyzed using the system.
- **Selectivity** describes the varying strengths of the interactions between the sample substances to be separated and the stationary phase (ΔhRf) in the chosen TLC system.

1.5 What Kinds of Reference Substances Are Used in TLC?

Because of their great importance to TLC, the various types of reference substances are described in the following Section. These are often known as "standards" and must only be used if they are of suitable quality for the intended application. These levels of quality are of especial importance in the field of pharmacy. All the relevant requirements must therefore be controlled in an SOP (standard operating procedure, see Chapter 9 "GMP/GLP-Conforming Operations in TLC").

1. Pharmacopoeia Substance (PS)

This term indicates a commercially available substance that meets the requirements of the relevant pharmacopoeia. For example, the American pharmacopoeia is indicated by the suffix **USP**, the British by **BP**, and the European by **CRS**. The possible use of a **PS** is specified by the relevant institutions, and is terminated by a change in the LOT number in the suppliers' catalogs. The Commission of the USP lists so-called "official distributors", of which the company LGC Promochem is a member (see Section 12.5 "Market Overview"). Care must be taken when ordering a substance listed in a pharmacopoeia to use the precise term for the substance. Although it is extremely rare, it does happen that the related compounds (rel. c.) of a substance have different names in the DAB and CRS lists.

It is especially confusing if, for example, the "rel. c. A" of a substance (e.g. ranitidine HCl) in the USP list appears as "rel. c. B" in the BP list, "rel. c. A" in the BP list bears the name "rel. c. C" in the USP list, and the "rel. c. B" in the USP list does not appear at all in the BP list.

2. Primary Reference Substance (PRS)

This term denotes a substance referred to as a Class 1 Standard by suppliers (analysis certificate with, e.g., at least two assays performed by different methods) or defined by the user's own tests without reference to other substances.

3. Secondary Reference Substance (SRS)

A tested and accepted batch of a substance which, after comparison with a **PS** or **PRS**, is declared as a "house standard". Can also be termed a **working standard**.

4. Related Compound (rel. c.)

This is usually a substance obtained from a supplier, but may be a substance produced by the user for purity testing, which is not a **PS** or a **PRS** and does not require information about its concentration. Such substances are in most cases decomposition products or intermediates in the synthesis process, and can be linked to a particular active substance.

5. Identity Substance (IS)

This is usually a commercially obtained substance with high chromatographic purity, although the content of the desired substance need not be known exactly if it is only to be used for identification purposes, e.g. for determining the hRf value and/or the documentation of the color or the fluorescence.

☞ **Practical hints** for ordering reference substances:

- In view of the existence of different descriptions of a substance in the lists in pharmacopoeias, it can be useful to make a note of the structural formula or to ask for help from an experienced person. Both names should then be written on a data base label indicating the relevant pharmacopoeias. Using the substance glibenclamid as an example:

  In the DAB literature, this is named

  5-Chlor-2-methoxy-N[2–4-sulfamoyl-phenyl]benzamid CRS,

  and in LGC Promochem's list "Reference Substances from the European Pharmacopoeia" it can be found under

  4-[2-(Chloro-2-methoxybenzamido)ethyl]-benzene-sulphonamide.

- A large number of reference substances that are constituents of plants can be obtained from the companies Phytolab and Carl Roth (see Section 12.5). When placing an order, the question of an analysis certificate should be always be raised.

1.6 The Literature on TLC

The first edition of the laboratory handbook "Dünnschicht-Chromatographie" edited by E. Stahl was published by Springer in 1962. Many subsequent publications can now be regarded as obsolete because of the developments in methodology and technique. I hope that the personal comments on the publications listed below will help the user in his or her choice.

1.6.1 General Literature

1.6.1.1 Books and Information Sheets in German

Wintermeyer, U.: **Die Wurzeln der Chromatographie**, GIT Verlag, Darmstadt 1989.
This book is recommended for anyone who would like to know how chromatography began. It goes up to the mid-1970s and also describes the development of CHROMart (the artistic use of chromatography).

Stahl. E. (ed): **Dünnschichtchromatographie,** Ein Laboratoriumshandbuch, 2nd edn, Springer-Verlag, Berlin 1967.
This book is popularly known as "thick Stahl" to distinguish it from the earlier thinner edition. It is a standard work, providing a good basic knowledge and including a large number of practical examples which are still valid, although modern stationary phases are not included.

Geiss, F.: **Die Parameter der Dünnschichtchromatographie**, eine moderne Einführung in Grundlagen und Praxis, Vieweg Verlag, Braunschweig 1972.
This is the most frequently cited work when the theory of TLC is under discussion: it is **the** treat for formula freaks.
English edition: **Fundamentals of Thin Layer Chromatography** (Planar Chromatography), Huethig Verlag, Heidelberg 1987, ISBN 3-7785-0854-7.

Kaiser, R.E. (ed): **Einführung in die Hochleistungs-Dünnschicht-Chromatographie HPDC**, published by the Institut für Chromatographie, Bad Dürkheim 1976.
The now usual English abbreviation HPTLC for high-performance thin-layer chromatography becomes a mixture of English and German for this book title (high-performance Dünnschicht-Chromatographie). It describes the fundamental principles of quantitative TLC, and (almost) all the great names among the founders of TLC have contributed.

Pachaly, P.: **DC-Atlas, Dünnschicht-Chromatographie in der Apotheke**, Wissenschaftliche Verlagsgesellschaft, Stuttgart, 4th Supplement 1999.
A supplement in loose-leaf form (following the bound volume published in 1982), which has the great advantage, for non-pharmacists, of giving comprehensibility to the rather unusual style of the German Pharmacopoeia (DAB); an extremely impressive model of the chromatographic system in the form of a shopping street; alphabetical sequence of monographs; clear and well-organized description of the process of research; color plates of the chromatograms.

Wagner, H.; S. Bladt, E.M. Zgainski: **Drogenanalyse**, Springer-Verlag, Berlin/Heidelberg 1983, ISBN 3-540-11867-5.
The bible for all plant analysts arranged according to drug classes, although the search for chromatographic parameters and detection reagents can sometimes be laborious. The color plates of the chromatograms are very good.
English edition: **Plant Drug Analysis**, Thin Layer Chromatography Atlas, 2nd edn 1996, ISBN 3-540-596-76-8.

Jork, Funk, Fischer, Wimmer: **Dünnschicht-Chromatographie**, **Reagenzien und Nachweismethoden**, Vol 1a, 1989 and Vol 1b, 1993, VCH Verlagsgesellschaft, Weinheim.
The absolute must in every TLC laboratory! Many formulae of reagents and reaction equations, descriptions of the practical performance of reactions with tested examples, and some scanned chromatograms and color plates of chromatograms.
English editions**: Thin-Layer Chromatography, Reagents and Detection Methods**, Vol. 1a, 1990, ISBN 3-527-27834-6 and Vol 1b, 1994, ISBN 3-527-28205-X.

Frey, H.-P., K. Zieloff: **Qualitative und quantitative Dünnschichtchromatographie**, Grundlagen und Praxis, VCH Verlagsgesellschaft, Weinheim 1993, ISBN 3-527-28373-0.
This book answers the principally theoretical questions about TLC which are not discussed in the present practically oriented volume.

Kraus, Lj., A. Koch, S. Hoffstetter-Kuhn: **Dünnschichtchromatographie**, Springer Laboratory Manual, Berlin 1996.
Building on his "Kleines Praktikumsbuch der Dünnschicht-Chromatographie", whose first edition appeared in 1985 and 4th edition (published by DESAGA, Heidelberg) in 1992, Kraus (who died in 1994) has attempted to give a comprehensive account of the theory and practice of TLC. The examples given show that this book is mainly intended for pharmaceutical biologists. However, the theoretical principles are also quite suitable for other TLC students.

1.6.1.2 Books in English

Most of the publications listed in Section 1.6.1.1 have also appeared in English, usually after a short delay, but nevertheless describe the most recent state of the technique at their publication date. The following books published after and including 1994 have not appeared in German.

Pachaly, P. (ed): **Simple Thin-layer Chromatographic Identification of Active Ingredients in Essential Drugs**, Gesundheitshilfe Dritte Welt, German Pharma Health Fund e.V., ECV, Aulendorf 1994. Brochure for the identification and purity testing of pharmaceutical agents in the Third World.

Sherma, J., B. Fried (ed): **Handbook of Thin-Layer Chromatography**, Chromatographic Science Series, Vol. 71, Marcel Dekker, New York 1996, ISBN 0-8247-9454-0.
In this textbook, theoretical aspects as well as a large number of practical examples, arranged by substance classes, are described. This second edition contains literature references up to and including 1995.

Sethi, P.D.: HPTLC, **High Performance Thin-Layer Chromatography, Quantitative Analysis of Pharmaceutical Formulations**, CBS Publisher & Distributors, New Dehli (India) 1996, ISBN 81-239-0439-8.
After a short introduction to TLC and quantitative HPTLC, ca. 200 examples of pharmaceutical materials are listed in order of substance class. Detailed examination has shown that, at least for some substances, the analytical methods are a little slipshod. In spite of this, the book has considerable value for its information in the field of pharmacy.

Fried, B., J. Sherma: **Thin-Layer Chromatography**, 4th edn, revised and expanded, Chromatographic Science Series, Vol. 81, Marcel Dekker, New York 1999, ISBN 0-8247-0222-0.
A good overview of the modern state of TLC is provided in two parts (general practical training in TLC and examples of various substance groups). The comprehensive list of references, which refers to publications (unfortunately only those that appeared in English) up to and including 1997, is especially valuable. The chapter on documentation in TLC is rather meager, and there are neither color plates of chromatograms nor advice on working according to the GMP/GLP guidelines. Also, new developments in sorbents, (e.g. the HPTLC LiChrospher Si 60 F_{254s} coated plates, which contain spherical silica gel) are not included in the book.

Wall, P.E.: **Thin-Layer Chromatography – A modern Practical Approach**, The Royal Society of Chemistry (RSC), Cambridge 2005, ISBN 0-85404-535-X.
The book derives from Wall's own long-term experience in TLC up to the year 2000. Chapters about the new precoated layers (Lux®, UTLC, ProteoChrom®) as well as documentation are missing. The print is in black and white only.

Kowalska, T., J. Sherma (ed): **Preparative Layer Chromatography**, CRC Press, February 2006, ISBN 0-8493-4039-X.
A good description of all important areas of preparative layer chromatography, theory and a wide range of applications (e. g. the use of PLC for isolation and identification of unknown compounds from the frankincense resin (Olibanum), strategies for finding marker substances).

1.6.1.3 Book in Another Language

Chinese

Xie Peishan (ed): *Chinese Pharmacopoea TLC ATLAS of Traditional Chinese Herb Drugs,* June 1993, ISBN 7-5359-1023-81/R.192.
A very elaborately produced book with exceptional color plates of the chromatograms. English translations of four monographs are provided, and these give a very good description of the derivation of the chromatographic parameters, with comprehensive data. Unfortunately, at least 95 % of the experiments were performed with TLC plates prepared in-house, so that full evaluation of these would be problematical. The English edition is in preparation.

1.6.2 Journals

No journal in the German language exists which deals exclusively with TLC. Many authors are of the opinion that only articles in English can provide the necessary wide distribution and thus attract enough attention to their research results.

1.6.2.1 German Language Journals Containing Articles on TLC (Selection)

Chrom View, Eigenverlag Merck KGaA, Darmstadt

Deutsche Apotheker Zeitung, (DAZ), Deutscher Apotheker Verlag, 70191 Stuttgart

Die pharmazeutische Industrie (pharmind), Editio Cantor Verlag, 88322 Aulendorf

Die Pharmazie, up to 1991 VEB Volk und Gesundheit, Berlin, from 1991 Govi-Verlag, 65760 Eschborn/Taunus

GIT Fachzeitschrift für das Laboratorium und *GIT SPEZIAL Chromatographie*, GIT-Verlag, Darmstadt

LABO, Kennziffer-Fachzeitschrift für Labortechnik, Verlag Hoppenstedt GmbH, Darmstadt

Laborpraxis, Vogel-Verlag, Würzburg

Naturwissenschaften im Unterricht Chemie (NiU-Chemie), E. Friedrich Verlag, 30917 Seelze

Pharmazeutische Zeitung, (PZ), Govi-Verlag Pharmazeutischer Verlag GmbH, 95760 Eschborn/Taunus

Pharmazie in unserer Zeit, Wiley-VCH, Weinheim

Planta Medica, (Journal of Medicinal Plant Research), Georg Thieme Verlag, 70469 Stuttgart

Scientia Pharmaceutica, (Sci. Pharm.), Österreichische Apotheker-Verlagsgesellschaft mbH, Wien

1.6.2.2 English Language Journals on TLC

I am aware of only one journal that deals exclusively with TLC:
JPC – Journal of Planar Chromatography – Modern TLC, Published by the Research Institute for Medicinal Plants, P.O. Box 11, H-2011 Budakalász, in association with Springer Verlag, Hungary.

1.6.2.3 English Language Journals Containing General Articles on Chromatography (Selection)

Analytical and Bioanalytical Chemistry, Springer, Heidelberg, D

Chromatographia, Friedr. Vieweg und Sohn, Weisbaden, D

Journal of Agriculture and Food Chemistry, American Chemical Society, Washington DC, USA

Journal of Chromatography, Elsevier, Amsterdam, NL

Journal of Liquid Chromatography & Related Technologies, Taylor & Francis, London, UK

Journal of Separation Science, Wiley-VCH, Weinheim, D

LC-GC North America and *LC-GC Europe,* Advanstar, Iselin NJ, USA and Chester, UK, respectively

Pharmaceutical Biology, Taylor & Francis, London, UK

Phytochemical Analysis, John Wiley & Sons, Chichester, UK

Phytochemistry, Elsevier, Oxford, UK

1.6.3 Abstracts

CA Selects, Paper and Thin-Layer Chromatography, Chemical Abstracts Service, P.O. Box 3012, Columbus, Ohio 43210, USA

CBS – CAMAG Literaturdienst Dünnschicht-Chromatographie (CAMAG Bibliography Service) appears biannually in the form of yellow pages in the house journal of the company CAMAG, and gives an excellent review of articles on TLC published worldwide. It is arranged according to substance classes.

1.6.4 Pharmacopoeias

The first reference to TLC in a pharmacopoeia was as "Dünnschichtchromatografische Prüfung" ("thin-layer chromatographic testing") in the DAB7-DDR 1964 for the monograph "Oleum Menthae piperitae" (peppermint oil) [6]. It was first described as a stand-alone analytical method in the European Pharmacopoeia of 1974 (Ph. Eur. 1), where TLC was specified for the identification of 23 drugs. One year later, in the second issue of the DAB7, TLC identification was specified for a further 18 drugs.

TLC is now established in almost all the pharmacopoeias of the world, although only for identification and purity testing. The improvements achieved over 30 years in sorbents, solvent systems and detection reagents and the use of partially and fully automated equipment for sample application, development and densitometric determinations on substances are beginning to appear in the monographs of all pharmacopoeias, although rather slowly. Germany has proposed to the European Pharmacopoeia Commission a supplement to the Chapter "TLC" to include horizontal development and quantitative methods. The Commission accepted this recommendation and published it in Pharmeuropa [7] in 1994 as a draft for comment. A commentary to the draft monograph followed in 1996 [8]. A new section on the theme of TLC in the European Pharmacopoeia in the 1997 edition was an important step forward [9]. Precoated layers were first described under the heading "Reagents". Suitability tests were prescribed for the determination of the separation efficiency and selectivity, also for the effectiveness of fluorescence indicators in the precoated layers used. In the revised chapter on TLC, the quantitative determination of substances by means of a scanner in the UV-VIS and a counter for radioactive substances is now included under Method 2.2.27. In the DAB, the monograph on "Oil-Free Soya Lecithin" includes the world's first quantitative determination by means of a TLC scanner.

All modifications recommended in The European Pharmacopoeia 4th edition (English) 2002 with respect to the work method in TLC were also recommended on a European level but with a time delay. For the first time the documentation of chromatograms is required with photographs and/or data files.

Specialists demand a worldwide agreement on the analytical methods of TLC in the pharmacopeias. The latest editions are, however, far from it. Those previously mentioned improvements in Pharm. Eur. (Method 2.2.27) still are not present in 2006 in USP 29 with NF 24 (Method 621).