PHARMACEUTICAL APPLICATIONS OF THIN-LAYER AND PAPER CHROMATOGRAPHY

Edited by

KAREL MACEK

3rd Medical Department
Medical Faculty, Charles University
Prague, Czechoslovakia

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Collaborators

V. BETINA, Department of Microbiology and Biochemistry, Faculty of Chemistry, Slovak Polytechnical University, Bratislava (Czechoslovakia)

J. DAVÍDEK, Department of Food Science and Analysis, Chemical Technical University, Prague (Czechoslovakia)

I. M. HAIS, Faculty of Pharmacy, Charles University, Hradec Králové (Czechoslovakia)

K. HILLER, Department of Pharmacy, Humboldt University, Berlin (G.D.R.)

J. JANÁK, Institute of Instrumental Analysis, Czechoslovak Academy of Sciences, Brno (Czechoslovakia)

G. KATSUI, Research Division, Eisai Co., Ltd., Tokyo (Japan)

B. P. LISBOA, Universitäts-Frauenklinik Eppendorf, Hamburg (G.F.R.)

M. LUCKNER, Department of Pharmaceutical Biology, Martin Luther University, Halle/Saale (G.D.R.)

L. NOVER, Institute for Biochemistry of Plants and Department of Pharmaceutical Biology, Martin Luther University, Halle/Saale (G.D.R.)

V. RÁBEK, Research Institute for Pharmacy and Biochemistry, Prague (Czechoslovakia)

G. SZÉKELY, Central Research, J.R. Geigy AG, Basel (Switzerland)

H.-D. WOITKE, Department of Pharmacy, Humboldt University, Berlin (G.D.R.)

Preface

The majority of methods originates from the needs of a certain field of science. Paper chromatography arose in the forties from the requirements of biochemistry. However, later on it spread to other branches of science as well, for example synthetic organic chemistry, drug analysis, etc. Thin-layer chromatography was first used by ISMAILOV AND SHRAIBER for the analysis of alkaloids in plant extracts. Its rediscovery by STAHL arose from the needs of pharmaceutical laboratories. On following the applications of both these chromatographic techniques in various branches of science we cannot but see the increasingly growing number of pharmaceutical applications. The number of references, from the year of the discovery of paper chromatography up to the present, exceeds 10,000, and with respect to the procedures for single types of drugs, for many workers the situation becomes difficult to survey. Therefore, in my opinion, the edition of a monograph surveying the possibilities of paper and thin-layer chromatography in pharmaceutical analysis as well as the basic literature (about 2,500 references) should be useful*.

After the compilation of the literature it became clear that it would be impossible to give detailed procedures for the analysis of single drugs, especially in the case of combined preparations, because such a book would be of only limited use. We therefore chose another approach, presenting in the early chapters an introduction to the technique, evaluation, and application of paper and thin-layer chromatography, and in special chapters selected procedures are presented in the form of a general discussion on the preparation of samples, detection, choice of solvent systems and sorbents, and the principles of quantitative analysis. These chapters are provided with rich tabulatory material. In the appendix recipes for the preparation of more than 160 detection reagents are also given. On the basis of these data anyone who has read the first chapters and understood the principles of both techniques can solve all analytical problems he may meet in drug analysis.

The reason we did not give preference to one of the two flat-bed techniques only, as is usual in the literature, is that both techniques have been consistently and successfully used for drug analysis, and each of them has its advantages and disadvantages. The reader can choose one or the other technique for his particular problems, depending on the equipment in his laboratory, or else he can apply the

* There is only one monograph in the literature which is devoted to the application of TLC in pharmacy (M. ŠARŠÚNOVÁ *et al.*, *Chromatografia na tenkých vrstách vo farmácii a v klinickej biochémii*, Obzor, Bratislava, 1968). This book, however, is written in Slovak and covers literary data only up to 1964.

experience gained with one of these techniques to the other. It is to be expected that future developments will efface the boundaries between the two techniques.

The question of which substances should be included in a monograph on pharmaceutical applications also presented a certain problem. It is evident that practically all groups of organic compounds should be treated in a work aiming at the complete coverage of such a subject as drug analysis. However, by doing so this monograph would cover the same ground as general monographs on PC or TLC, which surely would not be useful. In the case of plant components it was also necessary to consider those groups of substances which are not commonly included among drugs, although their function in the form of extracts is not quite clear from the pharmacological point of view. Therefore we endeavoured to avoid extremes and devoted our attention to those types of drugs most often used. The greatest attention was given to synthetic drugs which were not given so much notice by earlier authors of books on PC or TLC.

This book is the result of my 20 years' work in the field of drug analysis using chromatographic methods. Therefore it will contain a number of unpublished data. It is my pleasant duty at this point to thank my collaborators who contributed to the development of new methods and to the checking of methods published in the literature. I have in mind especially Miss D. DOSEDLOVÁ, Mrs. M. JELÍNKOVÁ, Miss P. PECHMANOVÁ, Mrs. J. STANISLAVOVÁ, and Mr. S. VANĚČEK.

Finally, I should like to express my thanks to all who contributed in any way to the publication of this book, in the first place to my co-authors, as well as to the editors and publishers who gave us permission to publish their figures, and, finally, to those who contributed their expert advice on various aspects, including the translation into English: Dr. Z. DEYL, Dr. F. KAISER, Dr. Ž. PROCHÁZKA, Dr. Z. SVOJTKA, Dr. K. ŠUMBERA, and Prof. Dr. V. TRČKA.

Prague, October 1970 KAREL MACEK

Technical notes

Preparation of sample

In most cases only principles for the sample preparation and extraction procedures for various drug forms are given. Detailed procedures are given only in cases of key importance for drug analysis, as for example in the chapter on extracts from vegetable material.

Paper

Unless special requirements are stated, standard papers are meant, as for example Whatman No. 1 or Schleicher & Schüll 2043b, or such like.

Sorbents for TLC

Although sorbents used by the authors of original papers are sometimes mentioned,

this does not mean that sorbents from other producers, with other binders, or of different grain size could not be employed. If the substances analysed have sufficiently different R_F values on chromatograms, it should be mentioned that the above factors do not necessarily play a negative role in the separation; in the case of substances with closely similar mobility, these can influence their separation. Thus it is sometimes necessary to modify the mobile phase described, for example by the addition of a hydrophilic or a hydrophobic solvent.

Detection

In order not to overcrowd the text with recipes of detection reagents, the last chapter consists of a list of recipes for the preparation of the most important detection reagents. The references to single reagents in the text are indicated by the letter D followed by the respective number. In TLC the majority of substances can be detected by general reactions, as for example carbonisation with sulphuric acid or exposure to iodine vapours. As a rule, these general detections are not repeatedly mentioned for single groups of substances.

Chromatographic chambers and development technique

In this book, especially in tables of R_F values, the type of the chamber used, the development technique, or the type of chamber saturation are generally not given although they may have an appreciable effect on the separation. Relative humidity, especially, has a strong influence on TLC separations. The reason for some separations described in the literature not being reproducible should be sought in this factor.

Systems

Unless stated otherwise, when a system is described the stationary phase is always mentioned first and then the mobile phase (for example: formamide/chloroform). Numerical values, unless stated otherwise, refer to volume ratios. As a rule, in two-phase systems the non-aqueous phase is used for development. The solvents used should be chromatographically pure; they are best distilled and characterised (in larger laboratories) by gas chromatography.

Tables

R_F values multiplied by a hundred are given for greater clarity in the tables, *i.e.* the so-called hR_F values, and in some instances the relative R_X values are also given. These values should always be considered as only approximate, indicating the relative mobility of single substances when chromatographed in parallel.

Quantitative analysis

Single procedures are not described in detail because, when densitometry is employed, the experimental arrangement is dependent on the apparatus used, or, in the case of methods following elution the determination itself only depends on the

chromatographic separation indirectly, and a detailed description would exceed the frame of this monograph.

Applications

Applications are usually illustrated by several characteristic examples. For this reason, no great attention is devoted to toxicological applications.

References

References are given after each chapter. In the text individual papers are quoted by the name of the author or authors (if the paper was published by no more than two authors), or by the name of the first author followed by the abbreviation *et al.* if the article was published by three or more authors. If several papers of one author are listed they are differentiated by the addition of the year of publication and if the author published several papers in the same year they are differentiated by an additional small letter, a, b, c, etc. The references are given without any claim to completeness and usually disregard priority. The monograph should not and is not intended to serve as a bibliographic source of the applications of PC and TLC in pharmaceutical analysis, although it contains approximately 2,500 references concerning the most important papers published from the moment of the discovery of PC and TLC up to 1970. Other bibliographic data, in particular those on applications, may be found in corresponding bibliographic literature (K. MACEK AND I. M. HAIS, *Bibliography of Paper Chromatography 1944–1956*, Academia, Prague, 1960; K. MACEK *et al.*, *Bibliography of Paper Chromatography 1957–1960*, Academia, Prague and Academic Press, London, 1962; K. MACEK *et al.*, *Bibliography of Paper and Thin-Layer Chromatography 1961–1965*, Elsevier, Amsterdam, 1968; K. MACEK *et al.*, *Bibliography of Paper and Thin-Layer Chromatography 1966–1969*, Elsevier, Amsterdam, 1972; bibliographic data on more recent papers may be found in the bibliography section of the *Journal of Chromatography*).

Names of drugs

We endeavoured to use exclusively international non-proprietary names in the case of drugs. However, it is practically impossible to determine with certainty whether a particular name is registered or not as a trade name in some country or other so that we cannot guarantee that the names in this book are non-proprietary in all instances.

Abbreviations and symbols

In this book we tried to avoid using abbreviations and symbols. However, in cases where this was necessary the symbol used is either internationally recognised or explained at the beginning of the chapter. For brevity and convenience, however, the abbreviations PC and TLC (for paper chromatography and thin-layer chromatography) are used to a wide extent.

Contents

Chapter 1

Introduction

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Chromatography is a method which makes use of the heterogeneous equilibrium attained during the flow of the mobile phase (running) through the stationary one (fixed) for the separation of two or more substances. Either a solid or a liquid phase may serve as stationary phase, the latter usually being immobilised by fixing it on an inert carrier. The mobile phase can be either a liquid or a gas. According to the type of the phases used, chromatography can be classified into solid–liquid, liquid–liquid, gas–solid, and gas–liquid chromatography.

Although several types of chromatography exist, depending on the method of *formation of the chromatogram*, elution chromatography introduced by TSWETT is commonest. In elution chromatography the mixture to be separated is usually introduced at one end of the chromatographic system composed of the stationary phase (for example a tube filled with the solid phase), and the mobile phase is then allowed to flow through it. During this passage a repeated distribution of the chromatographed substance between the mobile and the adjacent stationary phase takes place, with the consequent movement of the chromatographed substance. This mobility is dependent on the distribution isotherm of the chromatographed substance in the given system of phases. If two or more substances are analysed, which have different distribution isotherms, they can be separated spatially. On the other hand, the mobility in a given system is characteristic of a substance qualitatively and therefore it can be utilised for identification purposes. The whole process is analogous to fractional distillation or countercurrent distribution, but it is much more effective. Among other methods of formation of chromatograms, frontal analysis and displacement chromatography should be mentioned because they can often interfere in a negative way with the elution development.

In addition to the classification based on the distinction of the phases used, individual chromatographic methods may also be classified according to *the character of the forces* influencing the separated substance in a system of two heterogeneous phases, or according to the nature of the distribution isotherm. If an equilibrium between two liquids is involved, we speak of partition chromatography in which the distribution isotherm is linear and follows Nernst's partition law. One of the phases is immobilised by fixation on a solid carrier which must be inert. This type of chromatography occurs mainly in gas–liquid chromatography, paper chromatography, and some types of thin-layer chromatography (cellulose or silica gel impregnated with a liquid as stationary phase). If we are performing a separation

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whereby the equilibrium between the surface of a solid stationary phase and a liquid or gaseous phase is the underlying principle of the separation, then we have adsorption chromatography. In this case the distribution isotherm is an adsorption isotherm which is not usually linear over the whole range of concentration. In both partition and adsorption chromatography the separation of the chromatographed substance takes place on the basis of non-bonding interactions with the system of phases used. The formation of hydrogen bonds plays a substantial role in many systems. Another considerable group of methods in which chemical interaction between the chromatographed substance and the system of phases employed is involved, is represented by ion-exchange chromatography. With respect to other types of chromatography precipitation, salting out, gel permeation chromatography, etc. should be mentioned. Even in cases when a certain type of chromatography is mentioned or discussed we should bear in mind that in many separations several types of forces are involved; sometimes secondary forces may interfere with the separations, but in other cases they may be welcome.

From the point of view of the *experimental arrangement* the different types of chromatography may be divided into two groups: column chromatography, and chromatography on a flat bed, represented by paper and thin-layer chromatography. In column chromatography the solid stationary phase or the solid carrier with the stationary phase are packed into a tube. The chromatographed sample is introduced on to the top of the column thus formed, and a liquid or a gas is allowed to flow through it (liquid column chromatography, or gas chromatography, respectively). The substance to be separated either flows through the column, and after having left the column it is collected fractionally and detected directly in the eluate, or it is only allowed to separate on the column which is then cut into individual zones. The latter are then eluted separately. In the case of flat-bed chromatography the stationary phase is either a sheet of paper, usually made of cellulose, or a thin layer of a suitable sorbent which is fixed on a convenient support. In both cases the chromatographic procedure consists in the application on the start (near one end of the sheet) of a solution of the substance to be analysed; this end of the paper or of the layer is then immersed in a suitable solvent or solvent mixture (mobile phase) which then moves by capillary action through the material. When the solvent front reaches the end of the paper or the plate the development is stopped and the solvents are allowed to evaporate. The position of the chromatographed substance is detected either in UV light or by a suitable chemical colour reaction (detection). The position of the spot on the chromatogram characterises the substance analysed and it is usually expressed as the relative R_F value, *i.e.* the ratio of the distance of the centre of the spot *(A)* from the start, and the distance of the solvent front *(F)* from the start:

$$R_F = \frac{A}{F}$$

For greater clarity hundred times greater values, the so-called hR_F values, are usually published, in particular in tables. In certain cases, as for example in the case of the development where the solvent is allowed to run over the R_F values cannot be calculated. In such a case relative R_X values can be measured. R_X value is the distance of a given spot divided by the distance of a standard substance X (usually a faster one). If the distance of substance X from the start (after chromatography) is indicated by B, then

$$R_X = \frac{A}{B}$$

This book takes into consideration only flat-bed arrangements, since this is the arrangement most widely used in pharmaceutical analysis in view of its simplicity and its potentialities in analysis generally. As was already stressed above, both paper and thin-layer chromatography are considered here. The reason for this is the fact that the existence of one method does not exclude the use of the other one. In fact, both methods can be complementary if knowledge of them is sufficient. The technique in both methods is very similar: Application of the sample, development, detection, and quantitative analysis, all these operations are analogous. The two methods differ substantially only with regard to the character of the stationary phase: In paper chromatography the liquid phase is fixed on a carrier having the form of a sheet, *i.e.* paper, while in thin-layer chromatography the separation is carried out on a sorbent spread and fixed on a support having the form of a plate or foil. The sorbent may have either adsorptive properties (alumina), or it may function as the carrier of a liquid phase (in the case of cellulose), or both principles may be combined (silica gel). Each of the techniques mentioned has its advantages and disadvantages. The use of chromatographic paper is advantageous from the point of view of the simplicity of the preparation of the stationary phase, the attainment of reproducible results by simple means, the preservation of chromatograms, storing of the prepared system, and, last but not least, from the financial point of view. In the case of thin-layer chromatography the main advantages are rapidity of the analysis, the compactness of the spots permitting one to work as a consequence with lower concentrations of the samples, and the ease of the isolation of the spot from the layer. A number of advantages of thin-layer chromatography are due to the stationary phase, *i.e.* to its structure, which need not be fibrous, as in paper chromatography, in order to be cohesive. This is the reason why attempts were made recently (MACEK AND WOLF) to shorten the cellulose fibres or to use papers manufactured from cellulose (FIGGE) or glass fibres onto which a suitable sorbent could be introduced (for example silica gel) (for review see HAER). This latter type of paper is produced commercially under the somewhat unsuitable name "instant thin-layer chromatography material". However, according to the definition of single types of chromatography this is simply a modified paper. From these observations it follows that the boundaries between both methods are vague and

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it is quite possible that in the future they will disappear.

In view of the fact that this monograph is devoted to the utilisation of both methods in pharmaceutical analysis we shall omit here the exposition of the theory of chromatography because it would be outside the scope of this book. The reader keen to be more thoroughly acquainted with the theory of chromatography can find much more in specialised monographs (CASSIDY; GIDDINGS; HAIS AND MACEK, 1962, 1964; HEFTMANN; RACHINSKII; STAHL; SNYDER; and others). In contrast to this, some general rules following from the theory of chromatography, which can be used for the identification of substances or for the determination of optimum experimental conditions, or which are necessary for the comprehension of the chromatographic process, are described in the subchapter on the identification of substances (p. 138).

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Chapter 2

Techniques of Paper and Thin-Layer Chromatography

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