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*chromatography
of alkaloids*

part A: thin-layer chromatography

*A. Baerheim Svendsen
and R. Verpoorte*

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- Volume 23A Chromatography of alkaloids. Part A: Thin-Layer Chromatography, by A. Baerheim Svendsen and R. Verpoorte

Dedicated to the memory of
Friedrich Wilhelm Sertürner
who was the first to isolate an alkaloid (morphine).
On the occasion of the 200th anniversary of his birth.

LIST OF ABBREVIATIONS USED FOR DESCRIPTION OF TLC SYSTEMS

AcOH	acetic acid
AmOAc	amyl acetate
AmOH	amyl alcohol
BuNH ₂	butylamine
BuOH*	butanol
Bu ₂ O	dibutyl ether
cell	cellulose
DEA	diethylamine
DMFA	dimethylformamide
EtOAc	ethyl acetate
EtOH	ethanol
Et ₂ O	diethyl ether
FMA	formamide
imp	impregnated
isoAmOH	isoamyl alcohol
isoPrOH	isopropanol
(isoPr) ₂ O	diisopropyl ether
Me ₂ CO	acetone
MeEtCO	methyl ethyl ketone
MeOAc	methyl acetate
MeOH	methanol
MePrCO	methyl propyl ketone
lt. petr.	light petroleum
PrOH	propanol
Pr ₂ O	dipropyl ether
THF	tetrahydrofuran
TrEA	triethylamine
two-dim	two-dimensional

*In the Tables the prefix *n*, *sec* or *t* is added if it was mentioned in the original literature.

PREFACE

In the analysis of alkaloids, thin-layer chromatography (TLC) has so far been applied more than any other chromatographic technique, as can be seen from the hundreds of papers published.

In this part of *Chromatography of Alkaloids* TLC is dealt with. The alkaloids have been classified in groups according to their chemical skeleton (indole, isoquinoline, piperidine, etc.). In some instances, however, alkaloids of the same botanical origin (opium alkaloids, ipecacuanha alkaloids) and alkaloids of importance from a special point of view (drugs of abuse) have been treated separately. The papers published have been examined chronologically and the references are listed at the end of each chapter in the same way to give the reader the opportunity of tracing the development that has taken place in the TLC analysis within each group of alkaloids.

In a number of published investigations the aim has been to develop a systematic approach to the identification of alkaloids by means of TLC. Other studies deal with newly developed TLC systems which are useful for various alkaloids. Publications of this kind have been dealt with separately.

Data collected in the literature on the separation of the many representatives of a group of alkaloids, *viz.*, solvent systems, adsorbents and the aim of the TLC analysis, have been listed in tables. A selection of such TLC separation systems which proved to be the best for solving a series of specific analytical problems has been made, and they are presented in other tables together with the R_F values obtained for the alkaloids analysed. Detection reagents for the various groups of alkaloids are described, together with the colours obtained with the various reagents. The compositions and the preparation of the most important TLC spray reagents for alkaloids are given in the Appendix.

Sample preparation is less important in TLC than in GLC and HPLC, as a TLC plate is used only once. In TLC it is not necessary to the same extent as in GLC and HPLC to remove non-alkaloidal compounds from the sample to be analysed prior to the analysis, provided that they do not interfere in the separation. However, for the analysis of extremely small amounts of alkaloids in mixtures with other compounds, such as in biological material, extensive purification and removal of other compounds from the sample may be necessary. Isolation and clean-up procedures for such analyses are given in Chapter 12.

Quantitative analysis in TLC can be performed either directly on the plate (*in situ*) or indirectly, after elution of the alkaloid from the spot on the plate. For the direct, densitometric determinations the necessary data, wavelengths,

etc., are given. For indirect determinations all kinds of quantitative methods can be applied after the elution of the alkaloid to be analysed from the plate. Quantitative TLC is discussed in detail in papers by Shellard¹ and Touchstone and Sherma².

Special techniques involved in the sample preparation prior to or in connection with the application of the alkaloids on the TLC plate, e.g., the TAS technique and reaction chromatography, are described in a separate section for each group of alkaloids.

Lists of abbreviations used in the tables for alkaloids, etc., are given in each chapter. The abbreviations used for organic solvents are listed on page VII.

INTRODUCTION

"No other discovery has exerted as great influence and widened the field of investigation of the organic chemist as much as Tswett's chromatographic adsorption analysis. Research in the field of vitamins, hormones, carotinoids and numerous other natural compounds could never have progressed so rapidly and achieved such great results if it had not been for this new method, which also disclosed the enormous variety of closely related compounds in nature". (Karrer³.)

The application of chromatographic techniques in the isolation, purification and fractionation of alkaloids has also created new possibilities in the important field of alkaloid research, which started with the isolation of morphine by Sertürner in 1806. The introduction of many groups of important alkaloids in therapy, such as the curare alkaloids, the *Rauwolfia* alkaloids and the *Catharanthus* alkaloids, was made possible to a great extent owing to the application of chromatographic separation methods.

In addition to the isolation and purification of alkaloids from plant material, investigations on alkaloids are also performed for several other reasons: for studies of the distribution of alkaloids in various parts of the plant as a function of the development of the plant, the plant growth conditions and time of harvesting, for the quantitative determination of total and individual alkaloids, for the control of crude drugs and pharmaceutical alkaloid-containing preparations, for international control of traffic in narcotics and other alkaloid-containing drugs, for toxicological investigations and for many other chemical, pharmaceutical, plant physiological, pharmacological and toxicological reasons.

Although Tswett^{4,5} is commonly credited with the first successful application of chromatography, no great success was made until its use by Kuhn et al.⁶ attracted attention. The application of chromatography in alkaloid research started when Karrer and Nielsen⁷ separated quinine and cinchonine on a Floridin XXF column by adsorption chromatography. Späth et al.⁸ isolated sanguinarine from *Sanguinaria canadensis* L. by adsorption on aluminium oxide, and Kondo et al.⁹, using the same adsorbent, isolated methylisochondrodendrine from *Cissampelos insularis* Makido. Stoll and Hofmann¹⁰ also applied adsorption chromatography on aluminium oxide to separate ergometrine and ergometrinine. In their studies on *Calebas curare* alkaloids, Wieland et al.¹¹ separated the alkaloids on aluminium oxide as reineckates and perchlorates. Pease and Elderfield¹² isolated α - and β -earleine from *Astragalus earlei* by adsorption chromatography of their picrates.

The development of partition chromatography by Martin and Synge^{13,14} created further new possibilities in the complex field of alkaloid research. Evans and

Partridge¹⁵ introduced the method into alkaloid analysis by using a Kieselguhr column impregnated with phosphate buffer as stationary phase and diethyl ether as mobile phase for the separation of hyoscyamine and scopolamine.

The introduction of partition chromatography brought about a revolution in alkaloid research - especially when partition chromatography was applied in the form of paper chromatography. This technique was introduced by Consden et al.¹⁶ and the first applications in alkaloid analysis were published by Munier and Macheboeuf¹⁷ on various groups of alkaloids, and by Foster et al.¹⁸ on ergot alkaloids.

In this work the available literature on the chromatography of alkaloids has been reviewed. However, because at the present time *thin-layer chromatography*, *gas-liquid chromatography* and *high-performance liquid chromatography* are the chromatographic techniques mostly applied in alkaloid research, we have dealt with these techniques only.

Review articles on the chromatography of alkaloids can be found in *Handbuch der Papierchromatographie* by I.M. Hais and K. Macek¹⁹; *Chromatography* by E. Lederer and M. Lederer²¹, *Dünnschicht-Chromatographie* by E. Stahl²¹ and *Chromatography* by E. Heftmann²².

To facilitate the use of the information available in the literature on the application of the three above chromatographic techniques in the various fields of alkaloid research, and because of the great amount of information available, it was decided to publish this work in two volumes, one for thin-layer chromatography and one for gas-liquid and high-performance liquid chromatography.

In each volume the alkaloids have been classified in groups according to their chemical skeleton. However, often a classification into subgroups according to the botanical origin of the alkaloids has been used.

There is generally no *a priori* "best solution" to any analytical problem: a number of solutions can be tried and one will emerge as the most acceptable for a number of reasons. Different scientists will probably prefer different methods, depending upon personal choice and experience, the instrumentation available, and so on.

These volumes of *Chromatography of Alkaloids* should be used as a source of information, ideas and methods tested in order to tackle a problem effectively.

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I. GENERAL PART

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I. GENERAL PART

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Chapter 1

ADSORBENTS, SOLVENT SYSTEMS AND TLC TECHNIQUES

1.1. ADSORBENTS

1.1.1. *Silica gel*

Silica gel is the most widely used adsorbent in the TLC analysis of alkaloids. With the commonly used 0.25 mm thick layer a reasonable linear adsorption isotherm is generally obtained for amounts of 5-50 μg of alkaloid. However, because of the weakly acidic properties of the silanol groups ($\text{pH} = 4-5$) chemisorption of basic compounds may occur. This is observed as pronounced tailing from the point of application of the alkaloid and its final spot after the chromatographic run. Chemisorption can be prevented by using basic mobile phases or by impregnating the silica gel with basic buffers or mineral bases*. As an alternative the alkaloids can be chromatographed as their salts by using polar, acidic mobile phases or ion-pair chromatography. A review of the latter technique was given by Tomlinson et al.⁴.

Neutral mobile phases in combination with silica gel layers may lead to the formation of double spots if basic compounds are applied as their salts. This phenomenon has been described by Wesley-Hadzya⁵ for fenfluramine and by Beckett and Choulis⁶ for some sympathomimetic amines on cellulose layers. The formation of double spots is explained as a partial deprotonation of the amine, resulting in separation of the salt (ion pair) and the base in a neutral solvent system. A review of double spot formation in TLC was given by Keller and Giddings⁷.

In the first period of TLC analysis, silica gel was always activated by heating at 110°C for 30 min prior to its use, and the plates were stored over drying agents. The advantage of such activation is, however, doubtful. According to Geiss⁸ activation is of little or no use, because during the application of the sample to be analysed the silica gel will adsorb moisture from the air; within 3 min an activated silica gel layer will then adsorb about 50% of its maximum water content. Therefore, more reproducible analytical results are obtained when the silica gel plates are stored in open air. In this way a relatively constant activity of the silica gel layers is obtained (under normal laboratory conditions).

*For more extensive discussions, see Refs. 1, 2 and 3.