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# Preparative Chromatography Techniques

## Applications in Natural Product Isolation

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## Applications in Natural Product Isolation

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# Preface

Over the last few years several new instrumental techniques have been introduced for chromatographic separations. In addition, rapid developments in existing methods, such as preparative HPLC, have taken place. Despite these advances, however, a handbook covering the various preparative aspects of the new separation techniques does not exist. This book is an attempt to fill the gap and to present a compilation of modern separation techniques that will be useful for researchers faced with day-to-day preparative problems. Numerous examples of separations have been selected in order to show the possibilities (and also the limits) of each technique treated. These are often either applications from our own laboratory or else they reflect the approach we have been following for the isolation of natural products from plant sources. Owing to the large number of published papers and the diversity of secondary plant constituents, an exhaustive survey of the literature has not been undertaken. We hope, however, that the examples selected will suggest to the reader which technique(s) and which conditions to choose for a particular isolation problem in the field of natural products.

For invaluable help in the preparation of the manuscript for this book, we would like to thank Corinne Appolonia and Christine Marston.

Lausanne, November 1985

K. Hostettmann
M. Hostettmann
A. Marston

# Foreword

Although not many people realize this, isolation and purification of biologically active materials is becoming increasingly crucial. This is because the rapid progress in spectroscopic methods and X-ray crystallography have made structure determination a rather routine step. Chromatographic methods have also advanced explosively, but the choice of chromatographic method, column support, particle size, reverse phase or normal phase, solvent, etc. is never routine. Even HPLC supports of the same type and particle size behave differently depending on the manufacturer.

It is not exaggerating to state that, very frequently, the success or failure of studies with bioactive factors depends solely on whether one succeeds in the isolation, especially when the factor exists in minute quantities or is labile; and, indeed, most of the challenging problems these days are associated with the characterization of precisely such factors. Once the isolation has been achieved (which may take over ten years), the structure can usually be readily determined; structural elucidation then allows us to advance towards clarification of the mode of action, etc. on a concrete structural basis rather than guessing from graphs and tabulated data with no structures.

The importance of proper isolation and purification can never be sufficiently emphasized. Spectral data analysis may be more appealing to the research scientist, readers of a paper, or audience at a lecture because there is logic to it.

For contrast, chromatography is a much more delicate art and requires a tedious and patient trial and error approach. The details of an isolation process is usually of no interest to the general audience unless they are working with the same type of compounds. Nevertheless, isolation is crucial because it is the first step if one is interested in understanding a phenomenon on a structural basis, one of the important future directions of bioorganic chemistry.

I am delighted that Kurt and Maryse Hostettmann, together with Dr. Andrew Marston, have published a practical book covering all the up-dated techniques of chromatography with numerous practical examples. No team could be better for writing such a book. The Hostettmanns are the best analytical-minded organic chemists that I have encountered. During their two-year stay with us at Columbia University, 1976–1978, I was pleasantly shocked to see them efficiently check HPLC solvent composition, type of support, etc. and exploit the newly-arrived droplet counter-current chromatography system. They have been immensely successful in dealing with difficult separation projects. I have no hesitation in recommending this book, written by practical experimentalists with ample experience, to all scientists engaged in any field where isolation plays a role, analytical or preparative.

Columbia University, New York
February 1986

K. Nakanishi

# Contents

# 1 Introduction

With the increasing interest in plants and plant-derived compounds, the use of various chromatographic techniques is necessary for the isolation of milligram to gram quantities of pure substances for structural studies, bioassays, pharmacological tests, reference substances and standards for quantitative determinations. When one realises that a plant may contain thousands of chemical constituents, the choice of these chromatographic techniques becomes very significant. Review articles on applicable chromatographic methods abound and publications on the latest developments appear in the "Journal of Chromatography" and similar periodicals. A biennial review on HPLC is published in "Analytical Chemistry". All the same, a general reference work with the function of grouping together preparative separation techniques is required. This becomes all the more necessary with the array of newly-introduced chromatographic techniques which are now commercially available.

Discussion on the theory behind several of the methods described is kept to a strict minimum because a) this is often described in great detail elsewhere, and b) it is sometimes difficult to apply the theory — for example, the most important parameters in preparative HPLC (sample capacity, loading capacity, column overloads, stationary phase capacity) are often hard to define. Instead, emphasis is placed on the applications, especially the most recent applications, of the chromatographic processes concerned. Separations involving quantities of material ranging from the milligram to the multigram scale are covered here. Most of the examples come from the areas of secondary metabolites and general natural product chemistry because the rôle of preparative chromatography is so important for the isolation of the substances concerned. However, the separation of biopolymers is not covered in any great detail (electrophoresis, affinity chromatography, gel and ion-exchange chromatography may be more important for these molecules) and the use of preparative GC for volatile materials is not included

In addition to the modern separation techniques detailed, some well-established procedures, such as Sephadex LH-20 gel filtration and preparative thin-layer chromatography, are touched upon since they play an important part in many isolations of natural products.

Finally, the choice of combinations of different chromatographic steps is frequently of vital importance to the successful separation of pure substances. There is no simple magic chromatographic technique but a carefully considered strategy is of inestimable value. This theme and some of the combinations of separation techniques employed in the field of natural products are described at the end of the book.

# Sample Preparation and Purification

Of vital importance before undertaking complex separation and purification procedures is the preparation of the sample. This factor is of special regard when dealing with preparative HPLC on expensive columns. A correct pre-treatment of the sample can save much time and effort in subsequent steps and make isolation considerably easier. Whether the probe is of biological origin with contaminating proteins, or from industrial processes and contains residual catalyst, or from a plant source with interfering matrix elements, a simple preliminary step is often useful to remove most of the undesired material.

Straightforward *solvent partition* methods remove a large proportion of extraneous constituents and, especially when used in conjunction with a bioassay, fractions enriched in the sought-for constituent are rapidly obtained. For example, such solvent partition schemes have been used while searching for antitumour agents from plants (Wall et al. 1976). Pettit et al. (1983) were able to concentrate P-388 lymphocytic leukaemia (PS) activity of *Pimelea prostrata* (Thymelaeaceae) by the scheme shown in Fig. 2.1. The final active dichloromethane fraction was chromatographed to give the pure antileukaemic substances.

In the separation of saponins from plant material, a single butanol-water partition step often suffices to concentrate the saponins in the butanol fraction and provide a preliminary cleaning-up step (Hostettmann 1980). Thus a methanol extract of *Xanthocera sorbifolia* (Sapindaceae) fruits was partitioned between n-butanol and water before the butanol fraction was further purified (Chen et al. 1985).

Multiple partition steps provide another possibility for the preliminary purification of samples which are to be separated by liquid chromatography. A Craig counter-current distribution, generally with a restricted number of transfers (e.g. the isolation of cocarcinogenic phorbol esters from *Euphorbia cooperi* (Euphorbiaceae); Gschwendt and Hecker 1973) or a droplet counter-current separation (e.g. in the isolation of steroidal glycoside sulphates from starfish; Riccio et al. 1985) is useful in this respect, especially when separating lipophilic from more polar constituents.

*Filtration* provides the easiest and most obvious method of sample preparation, necessary for counter-current chromatographic, low-, medium- and high-pressure separations. This can take the form of the passage of a sample solution through a filter paper or sintered glass funnel, in order to remove particulate and insoluble material. A further degree of purity can be achieved by filtering the solution through a short column of silica gel or other suitable packing material. This has the effect of removing strongly adsorbing contaminants which may prove awkward during column chromatography (Hostettmann et al. 1977). The requirements are even more rigorous for HPLC and here the filtration of particulate matter, often using membranes of carefully controlled pore size (Millex HV filter units etc.) or commercially available cartridges is essential.

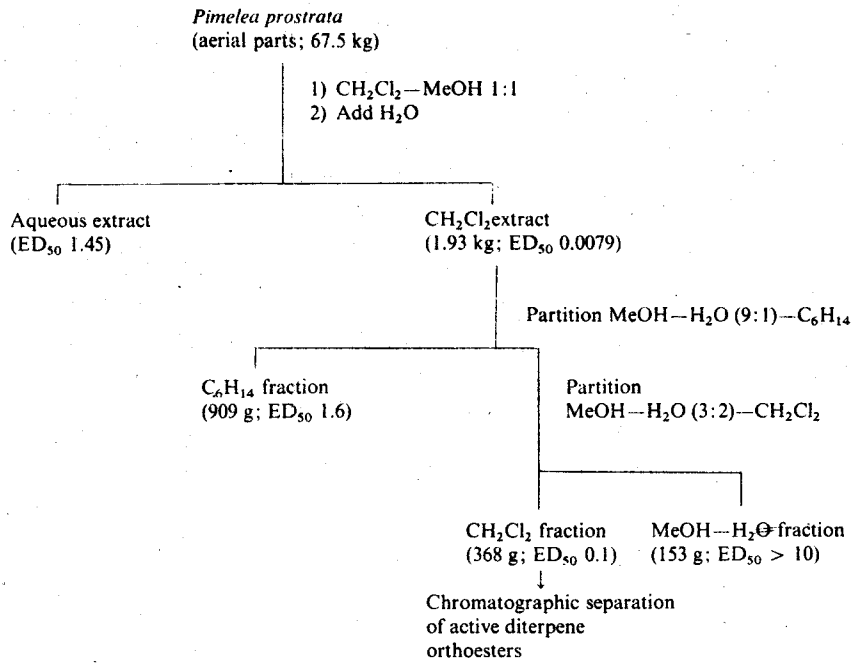



**Fig. 2.1.** Solvent partition of a *Pimelea prostrata* tumour growth-inhibitory extract. $ED_{50}$ of fractions in PS test expressed in μg/ml. (Reprinted with permission from Pettit et al. 1983)

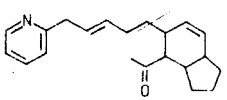
When the sample that is to be introduced onto a chromatographic column (flash, dry-column, vacuum liquid chromatography etc.) is not very soluble in the eluent, a *solid introduction* may be carried out. The material is dissolved in a suitable solvent and mixed with about five times its weight of deactivated adsorbent (or Celite). The mixture is evaporated in a rotary evaporator at 30–40 °C and the resulting powder is distributed on the top of the column (Loev and Goodman 1967). This may then be covered with a shallow layer of sand or glass beads before elution.

Another preliminary purification of samples is by means of *precipitation*. This is a method very often employed in work on saponins: a concentrated methanol solution of an extract containing saponins is poured into a large volume of diethyl ether. The precipitated saponins are collected by filtration or centrifugation. For better results, the precipitation can be repeated several times (see, for example, Wagner et al. 1984).

*Preparative high-performance liquid chromatography*: sample preparation is not too important a problem when employing a silica gel stationary phase, with techniques such as medium-pressure LC or Jobin-Yvon systems, since the packing material is generally rejected after the separation and impurities left on the stationary phase are consequently also disposed of. However, in the case of preparative HPLC or in work using reversed-phase packing material, the columns are very expensive and

careful sample preparation is necessary to avoid contamination with slow-running impurities. This may take the form of an off-line clean-up or an on-line clean-up, the former method involving preliminary purification by e.g. open-column LC, simple filtration through coarse silica gel (Burton et al. 1982) or cartridges (Sep-Pak, Bond Elut etc.). Pre-packed cartridges operate on the principle of liquid-solid extraction and may be used in one of two modes: a) the interfering matrix elements of a sample are retained on the cartridge, while the components of interest are eluted or b) the components of interest are retained while interfering matrix elements are eluted. In the latter case, a concentration effect can be achieved. The required compounds are then eluted from the cartridge by changing the solvent. Cartridges with a variety of packings, both normal- and reversed-phase, are obtainable (Sep-Pak: Waters Millipore, Milford, Massachusetts. Bond Elut: Analytichem International, Harbor City, California. Alltech/Applied Science Micro-Cleanup columns. Hamilton Chrom-Prep cartridges).

Preliminary purification on a C-18 Bond Elut cartridge was carried out on an extract of the mollusk *Philinopsis speciosa* (Cephalaspidea) before preparative HPLC separation of the $C_{16}$-alkadienone-substituted 2-pyridine *1* (Coval and Scheuer 1985).




1

Similarly, prior to a separation of flavonoid glycosides from *Dryas octopetala* (Rosaceae), a silica gel cartridge was used to eliminate tannins from an ethanol extract and then chlorophyll was removed by elution on a C-18 cartridge (De Bernardi et al. 1984).

Guard columns inserted between the injector and chromatography column are recommended for the removal of particulate and/or strongly retained sample components. They are usually packed with a small volume of the same support used

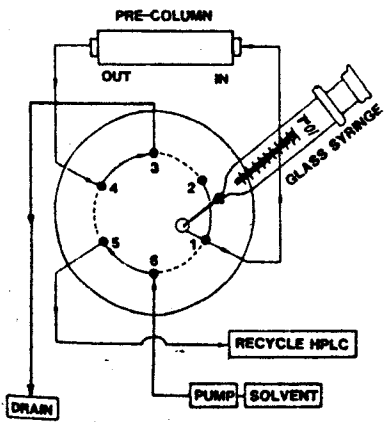



**Fig. 2.2.** On-line sample preparation by use of a pre-column. (Reprinted with permission from Okano et al. 1984)

in the main column and do not greatly decrease system efficiency, if correctly packed.

Silica pre-columns deal with problems caused by mobile phases containing buffer salts or bases. These dissolve the silica backbones of bonded-phase packing materials, often causing voids to form at the top of the column. The pre-column is placed between the pump and injector so that although the mobile phase becomes saturated with silica gel, no dead volume is introduced in the path of the sample.

On-line clean-up can also be accomplished by column switching, illustrated by the separation of 25-hydroxyvitamin $D_2$ (Okano et al, 1984). In this method, the pre-column attached to the sample injector (Fig. 2.2) was used to retain 25-hydroxy-vitamin $D_2$ while less polar components were eluted. The 6-port valve was then turned to the dotted position, the polarity of the solvent increased and the purified sample eluted into the recycle HPLC system. This method fulfils the same clean-up function as a Sep-Pak cartridge but is especially useful for loading a large volume of sample solution directly onto the HPLC column i.e. there is a *concentration* effect.

Other examples of column switching are given by Henschen et al. (1985) but it should be noted that with these on-line systems there are quite severe restrictions on the solvent systems that can be employed.

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# 3 Planar Chromatography

## 3.1 Preparative Thin-Layer Chromatography

One of the separation methods requiring the least financial outlay and using the most basic of equipment is preparative thin-layer chromatography (PTLC). Although gram quantities of material can be separated by PTLC, most applications (and there are many of these) involve milligram quantities. PTLC, in conjunction with open-column chromatography, is still to be found in the majority of publications covering the isolation of natural products, especially in work from those laboratories without access to modern separation techniques. However, as will be explained later, there are numerous inconveniencies connected with PTLC.

*Adsorbents*

Various studies have been carried out to investigate the effect of the thickness of adsorbent on the quality of separation (Stahl 1967) but the most frequently employed thicknesses are 0.5–2 mm. The format of the chromatography plate is generally $20 \times 20$ cm or $20 \times 40$ cm. Limitations on the thickness of the layer and on the size of the plates naturally reduce the amount of material that can be separated by PTLC. Silica gel is the most common adsorbent and is employed for the separation of both lipophilic and hydrophilic substance mixtures. A number of commercially-available adsorbents are recommended for the preparation of crack-free layers. Their particle and pore sizes approximate to those of the equivalent TLC grades.

PTLC plates can either be self-made or purchased with the adsorbent already applied (the so-called "Fertigplatten"). The advantage of preparing plates oneself is that any thickness (up to 5 mm) or composition of plates can be accommodated. Thus, silver nitrate, buffer substances etc. can be incorporated in the adsorbent. Application of the required adsorbent can be performed with one of a number of commercially-available spreaders e.g. from Camag, Desaga. Instructions for the preparation of these plates are supplied with the relevant adsorbent.

*Sample Application*

The sample is dissolved in a small quantity of solvent before application to the PTLC plate. A volatile solvent (hexane, dichloromethane, ethyl acetate) is preferred, since problems of band-broadening occur with less volatile solvents. The concentration of the sample should be about 5–10%. The sample is applied as a band, which must be as narrow as possible because the separation depends on the width of the band. Application can be by hand (pipette) but an automatic applicator (Camag, Desaga etc.) is preferred. For a band which is too broad, concentration can be achieved by allowing the migration of a polar solvent to about 2 cm above the applied band. The plate is then dried and eluted with the desired solvent (Stahl 1967). Special pre-coated plates with concentration zones are also available.

*Choice of Mobile Phase and Development of the PTLC Plate*

There are many variables in PTLC but as a general guideline, 10–100 mg of sample can be separated on a 1 mm thick 20 × 20 cm silica gel or aluminium oxide layer (Székely 1983). Doubling the thickness allows the application of *50%* more sample.

Choice of eluent is determined from a preliminary investigation by analytical TLC. Since the particle sizes of the adsorbents are approximately the same, the analytical TLC solvent is directly transferable to PTLC. The standard reference work on thin-layer chromatography by Stahl (1967) gives a large selection of solvent systems for many different classes of compounds.

Recently, a method (the "PRISMA" model) based on Snyder's solvent selectivity triangle has been described to aid mobile phase optimisation (Nyiredy et al. 1985e, f).

The following binary mobile phases (in varying proportions) are very often applied to PTLC separations: n-hexane-ethyl acetate, n-hexane-acetone, chloroform-methanol. Addition of acetic acid or diethylamine in small amounts is useful for the separation of acidic and basic compounds, respectively.

Elution of PTLC plates generally takes place in glass tanks, which may hold several plates at one time. The tank is kept saturated with mobile phase by the presence of a sheet of filter paper dipping into the solvent.

The separation efficiency can be augmented by *multiple development*. When a PTLC separation has taken place, the plate is dried and then re-introduced into the development tank. Depending on the $R_f$ of the band, this process can be repeated several times, albeit with a corresponding time penalty.

*Isolation of Separated Substances*

Most PTLC adsorbents contain a fluorescent indicator which aids localisation of the separated bands, as long as the separated compounds adsorb UV light. One problem with some indicators, however, is that they react with acids – occasionally even with acetic acid.

For non-UV-absorbing compounds, there are several alternatives:

a) spraying the plate with water (e.g. saponins),
b) covering the plate with a sheet of glass and spraying one edge with a spray reagent,
c) addition of a reference substance.

The bands, having been localised, are scraped off the plate with a spatula or with a tubular scraper connected to a vacuum collector. This latter method is not very practicable for sensitive substances because the adsorbent, containing the purified product, is in constant contact with a stream of air and there is a risk of autoxidation. Whatever the collection method, the substance has to be extracted from the adsorbent – with the least polar solvent possible (ca. 5 ml solvent for 1 g adsorbent). It should be noted that the longer the substance is in contact with the adsorbent, the more likely is decomposition to occur. The extract is filtered through a porosity 4 glass frit and then through a 0.2–0.45 μm membrane.

*Impurities in Substances Separated by PTLC*

PTLC adsorbents contain binders and fluorescent indicators, the chemical compositions of which are not generally known. During extraction of substances separated as bands on the PTLC plates, these and other impurities are quite possibly extracted

as well. In fact, the higher the polarity of the extraction solvents, the greater the quantity of unwanted material. A further problem is that these extraneous substances often do not absorb UV light and are missed when carrying out a final TLC analysis of the purified product. Székely (1983) has carried out the gravimetric IR and $^1$H-NMR analysis of impurities extracted from blank silica gel plates and the presence of phthalates and polyesters was quite clearly shown. A final purification step by gel filtration on Sephadex LH-20 is therefore highly recommended.

*Overpressure Layer Chromatography (OPLC)*

In overpressure layer-chromatography, a horizontal thin-layer chromatography plate is covered by an elastic membrane. Pressure is applied to the membrane, such that separations are carried out on the TLC plate in the *absence* of a vapour atomosphere (Tyihak et al. 1979, 1981). The mobile phase is pumped across the plate. In the totally enclosed environment, different parameters — solvent flow, pressure and temperature — can be adjusted with high reproducibility.

It is claimed that OPLC combines the advantages of thin-layer chromatography and high-performance LC (Newman 1985). Preparative OPLC, which requires the collection of the mobile phase containing the separated components, supposedly combines the advantages of preparative TLC and preparative HPLC (Nyiredy et al. 1985g). However, preparation of the plates for preparative OPLC is a rather complex operation, involving impregnation of the plates with a polymer, scraping channels for the pumping in and collection of solvent, together with band application of the sample.

Separations of up to 1 g of sample can be carried out within 45 min — for example, capsaicin from a tincture of *Capsicum annuum* (Solanaceae), caffeine from *Camellia sinensis* (Theaceae) and furanocoumarins from *Heracleum sphondylium* (Apiaceae) (Nyiredy et al. 1985g).

## 3.2 Centrifugal Thin-Layer Chromatography

Classical preparative thin-layer chromatography suffers from several drawbacks, the main disadvantage being the removal of purified substance from the plate and its subsequent extraction from the adsorbent. When toxic products are being scraped from the plates, serious problems can arise (e.g. Adolf et al. 1982). Other drawbacks include the length of time required for a separation and the presence of impurities and residues from the plate itself remaining after solvent extraction of the zones containing the product (Székely 1983).

In order to overcome some of these problems, a number of approaches involving centrifugal chromatography have been attempted. The technique of centrifugal chromatography is, in principle, classical chromatography with an accelerated flow of the mobile phase produced by the action of a centrifugal force.

*Historical Description*

a) *Centrifugal Paper Chromatography*

The first mention of this method came from a report by Caronna (1955), who constructed a rotor consisting of two plexiglass plates sandwiching a circular sheet of chromatography paper, for the separation of inorganic and organic substances.

There have been several modifications, notably by McDonald et al. (1957), Herndon et al. (1962) and Dauphin et al. (1960), all summarised in a review by Deyl et al. (1964). All these methods employed horizontal rotors and did not meet with general acceptance because of numerous problems connected with the delivery of mobile phase, the flow of mobile phase through the paper, collection of eluent and restrictions on the amount of sample.

b) *The Chromatofuge*

Hopf introduced a centrifugally-accelerated apparatus for the separation of 100 mg quantities of substance in 1947 (Hopf 1947), which he named a "chromatofuge". This consisted of a perforated cylinder, filled with support material (aluminium oxide, barium carbonate etc.) and a central tube, down which sample and eluent were introduced. Radial migration of solvent in the basket was achieved by rotation around its axis. The method was modified by Heftmann et al. (1972), such that preparative separations could be more easily performed. Using this modification, the purines caffeine, theobromine, theophylline and xanthine were quickly separated in 5 mg amounts on silica gel (Heftmann et al. 1972). However, the shape of the collection vessel caused some re-mixing of the fractions. A scaled-up chromatofuge enabled the preparative separation of amino-acids on a g scale (Finley et al. 1978). The cylindrical basket was filled with ion-exchange resin, while spinning, and then a mixture containing 5 g each of alanine, histidine and tryptophan was separated at a rotor speed of 1000 rpm. Despite the satisfactory results, there was no improvement in resolution over conventional column chromatography.

c) *Centrifugal Thin-Layer Chromatography (CTLC)*

Although alternative centrifugal chromatographic methods have appeared (Deyl et al. 1964, Lepoivre 1972, Pfander et al. 1976), it was not until the introduction of two commercially-available devices, the Chromatotron (Harrison Research, Palo Alto, California) and the Hitachi CLC-5 Centrifugal Chromatograph (Hitachi Koki Ltd., Takeda Katsuta City, Japan), that centrifugal TLC enjoyed a sudden surge in interest. The two chromatographs overcome most of the difficulties encountered with the earlier methods and, in addition, their very simplicity of operation explains their widespread acceptance.

*Apparatus*

a) *Hitachi CLC-5 Centrifugal Chromatograph*

This apparatus is so constructed that a slurry of adsorbent in the eluting solvent is introduced into the space between *two horizontal* 30 cm diameter plates. Excess solvent is removed by centrifugal force and the sample mixture is introduced onto the still damp layer. Elution of bands is carried out in the normal fashion.

Applications of this instrument are less numerous than those for the Chromatotron, but one example, for instance, involves the determination of solasodine in fruits of *Solanum khasianum* (Solanaceae) (Nes et al. 1980). Before HPLC determination, an extract of the fruits was fractionated on the CLC-5, with a 3 mm spacer. Silica gel (Woelm, 50 g) was used as adsorbent and both the rotation speed and flow-rate were varied so as to keep the right amount of solvent between the plates.

Tanaka et al. (1982) reported the isolation of confusoside, a dihydrochalcone glycoside, from the leaves of *Symplocos confusa* (Symplocaceae) with the aid of the CLC-5 instrument. The thickness of the silica gel layer was 3 mm, rotation speed 600 rpm and solvents EtOAc–EtOH–$H_2O$ 100:8:1 and then EtOAc saturated with water.

The Hitachi Chromatograph has also been used in the isolation of neutral lipids from rice-bran oil (Shimasaki and Ueta 1983). A 3 mm layer of silica gel was eluted with $C_6H_{14}$–$C_6H_6$ 85:5, $C_6H_{14}$–$Et_2O$ 95:5, $C_6H_{14}$–$C_6H_6$–HOAc 94:5:1, $C_6H_{14}$–$Et_2O$ 85:15, 70:30, $Et_2O$ at 1000 rpm, to obtain the different lipid fractions. The flow rate was maintained at 15 ml $min^{-1}$.

The CLC-5 has approximately the same load capacity as the Chromatotron, for an equal layer thickness (Hunter and Heftmann 1983) but whereas the Chromatotron is limited to a 4 mm layer, the CLC-5 can be packed to a much greater thickness.

b) *Chromatotron*

The big difference between the Chromatotron and previous centrifugal TLC apparatus lies in the fact that the rotor is *inclined* and not horizontal (Fig. 3.1).

The heart of the apparatus is a 24 cm diameter circular glass plate which is covered with a suitable adsorbent, to provide the thin layer for preparative separations. Preparation of the thin layer is accomplished in the following manner: an aqueous suspension of the adsorbent, containing a bonding agent is poured onto the plate and allowed to spread out into a relatively uniform layer. The adsorbent is prevented from running over the edges by a collar of sticky tape around the circumference of the plate. When the uneven adsorbent is dry, the surface is removed by a scraping tool fixed to the centre of the plate, until a layer of the required thickness (1, 2 or 4 mm) is obtained. A small area is left free in the middle to allow introduction of eluent. For silical gel plates, the rotors are coated with a mixture of TLC-grade silica gel $GF_{254}$, calcium sulphate hemihydrate and water (Hostettmann et al. 1980). If the adsorbent needs to be more strongly bound to the plate, additional binder must be added.

The prepared plate is then screwed onto the hub of an electric motor and rotated at 800 rpm. Eluent is introduced onto the adsorbent-free centre of the plate via a

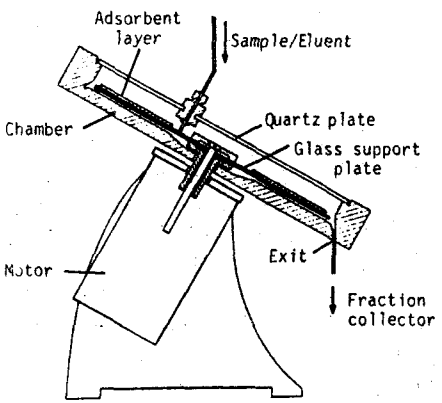


**Fig. 3.1.** Schematic view of the Chromatotron (Model 7924)

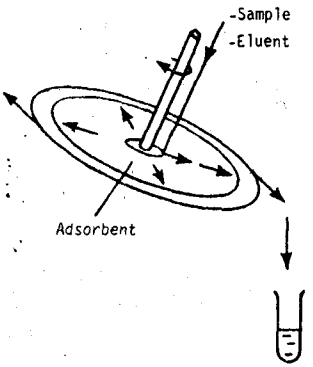


**Fig. 3.2.** The principle of the Chromatotron

piston pump capable of delivering 1–10 ml $min^{-1}$, and passes across the thin-layer under the influence of the centrifugal force. The rotor is thus washed for several minutes to remove impurities present in the adsorbent. Following this step, two options are available: direct introduction of the sample or preliminary drying of the plate before introduction of the sample. Elution is then continued, either with a solvent of constant composition or with a step gradient, at ca. 3–6 ml $min^{-1}$.

The rotor is housed in a chamber, covered with a quartz glass plate. This cover enables the observation of colourless but UV-active substance zones with the aid of a UV lamp. A steady flow of nitrogen is passed through the chamber, to prevent condensation of the eluent and to avoid oxidation of the sample.

Introduction of sample, followed by solvent elution gives concentric bands of the components (Fig. 3.2). At the periphery, the bands are spun off and collected through an exit tube in the chamber. Fractions of eluate thus obtained are analysed by TLC.

**Table 3.1.** Advantages and disadvantages of the Chromatotron

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|---|
| *Advantages* |
| 1) Simple operation |
| 2) Rapid — separations generally completed within 30 min |
| 3) No scraping of bands necessary |
| 4) Low consumption of solvent |
| 5) Coated rotors can be regenerated |
| 6) Less impurities extracted from adsorbent than preparative TLC |
| 7) Sample application straightforward |
| 8) Step gradients possible |
| 9) Oxidation of sensitive substances less likely than preparative TLC |
| 10) Higher recoveries of product than preparative TLC |
| *Disadvantages* |
| 1) Restricted choice of stationary phases |
| 2) Coated rotors not commercially available |
| 3) Resolution limited |
| 4) Limited range of detection methods |
| 5) Contamination of collection system |