PREPARATIVE-LAYER CHROMATOGRAPHY OF AN EXTRACT OF ALKALOIDS FROM *Fumaria officinalis*

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SUMMARY

A strategy has been established for preparative-layer chromatography of the alkaloid fraction extracted from the plant *Fumaria officinalis*. The extract, obtained by maceration and percolation of ground plant material with 0.5 mol L^{-1} aqueous acetic acid, was re-dissolved in methanol and fractionated on alumina by column chromatography. Two fractions, containing medium and high-polarity alkaloids, respectively, were used in the experiments. The effects of mass and volume overloading and the mode of sample application (use of an automatic sample applicator and application to the edge of the layer by use of the mobile-phase distributor of the chromatographic chamber) were investigated, as also was the effect of layer thickness on band resolution. The role of derivatisation in the location of the separated alkaloid bands for isolation was also considered.

INTRODUCTION

Preparative-layer chromatography (PLC) is an effective and easy means of obtaining small quantities of compounds from natural mixtures, which can then be used for different purposes – for example determination, by spectroscopic methods, of the structure of the compounds isolated or investigation of their biological activity [1].

It should be also remarked that PLC can be used not only for isolation but also for on-line purification of plant extracts rich in non-polar (lipids, chlorophylls, waxes) or polar (tannins, sugars) ballast [2,3], as a method of sample preparation when purification in one step is not sufficient for isolation of a fraction before GC, HPLC, or TLC analysis [4–10]. Preparative-layer chromatography can be also used as a pilot technique for preparative column chromatography, in which both optimization of system

selectivity and determination of the effects of overloading are important [3,11].

There are many examples of the use of PLC for isolation of alkaloids from plant material, for example quaternary alkaloids from *Chelidonium majus* [12], tobacco alkaloids from *Arachus hypogea* and *Corylus avellana* [5], *Lycopodium* alkaloids [13], protoberberine alkaloids from *Fissistigma balansae* [14], anthranille alkaloids from *Ticorea longiflora* [15], diterpenoid alkaloids from *Aconitum leucostomum* [16], benzylisoquinoline alkaloids from *Anisocycla jollyana* [17], alkaloids from *Strychnos icaja* [18], pyrolizidine alkaloids from *Heliotropium crassifolium* [19], alkaloids from *Hernandia nymphaeifolia* [20], and tazeline-type alkaloids from *Galathus species* [21].

The most important aspect of optimisation of preparative-layer chromatography is, of course optimisation of the chromatographic system. The best chromatographic system depends on the chemical properties of the compounds being separated. The mobile phase should consist of volatile solvents of low viscosity which are easily removed. Buffers, ion-pair reagents, and other components difficult or impossible to evaporate should be eliminated. For basic compounds it is usually necessary to use aqueous ammonia or short-chain amines as additives. The adsorbent should not react irreversibly with the components being separated. The stationary phases used in PLC are similar to those applied in analytical TLC, although cost is also important. Normal-phase systems are preferred for preparative purposes.

To achieve satisfactory separation in preparative-layer chromatography the effects of a few strategic conditions must be investigated. These include the kind of overloading (volume or mass) and the method of introduction of large volumes of sample to the adsorbent layers.

The objective of the work discussed in this paper was optimization of preparative-layer chromatographic separation of alkaloids extracted from *Fumaria officinalis*, by investigation of the effects of the conditions described above. The effect of layer thickness on the separation of neighbouring bands and the effect of derivatisation on the location of the bands to be isolated were also investigated.

EXPERIMENTAL

Plant Extract

Plant material purchased from Herbapol (Poland) was macerated in aqueous acetic acid [22] for 24 h then extracted by percolation with the

same extractant at ambient temperature. The solvent was evaporated to dryness under vacuum and the dry residue was extracted several times with methanol to remove inorganic ballast. Both the percolate and the methanol extracts were tested for alkaloid content by use of Dragendorff's reagent. Both processes were repeated until testing, by derivatization on a strip of paper, gave a negative result. The solvent was evaporated under vacuum at 60°C and the dry residue was extracted with methanol. The extract contained 50 mg dry weight in 1 mL solution.

Column Chromatography

Column chromatography was performed on a classic 20 cm long × 2 cm diameter glass column packed with 90 g alumina (Aluminiumoxid 60 HF_{254} basisch, type E; Merck, Darmstadt, Germany). The methanol solution of the extract (20 mL) was applied to the column by use of a pipette and the column was eluted sequentially with 10% propan-1-ol in dichloromethane (fraction I) then methanol (fraction II). Each fraction collected was tested for alkaloids by use of Dragendorff's reagent. The fractions were evaporated to dryness and the residues were dissolved in 5 mL methanol.

Preparative-plates Chromatography

Chromatography was performed on 50 mm × 100 mm glass plates precoated with 0.25, 0.5, or 2 mm layers of silica gel Si 60 HF_{254} (Merck). Samples were applied by use of a Desaga (Heidelberg, Germany) AS 30 automatic applicator or were applied to the edge of the layer by use of the mobile phase distributor of the DS chamber [1,23].

Plates were developed face-down, to a distance of 8 cm, in a horizontal Teflon DS chamber (Chromdes, Lublin, Poland) after conditioning for 15 min with mobile phase vapour. After development the mobile phase was evaporated to dryness and plates were sprayed with Dragendorff's reagent by use of a TLC sprayer (Merck).

Plates were scanned by use of an Desaga CD 60 densitometer controlled by CD 60 version 5.3 software. Videoscans were obtained by use of a Desaga VD-40 videoscanner controlled by ProViDoc software.


RESULTS AND DISCUSSION

To obtain an extract rich in alkaloids the method published in pharmacopoeias and used in practice [22] was applied. Use of a dilute aqueous solution of acetic acid enables extraction of the alkaloids with a small amount

of ballast, most of which was removed by evaporation of the aqueous acetic acid and re-extraction with methanol.

Optimisation of purification and fractionation of the alkaloid fraction was performed by thin-layer chromatography on alumina plates. Use of this adsorbent enables purification from remaining inorganic ballast, which was strongly adsorbed on the bed, and separation of extract into two fractions. The first fraction was eluted with 10% *n*-propanol in dichloromethane and the second, containing mainly strongly polar quaternary alkaloids, was eluted with methanol. This experiment enables transfer of such methods for column chromatographic separation of the alkaloid extract into two fractions.

The most important aspect of preparative-layer chromatography is the mode of application of large volumes of sample to the adsorbent bed. The effect of the method of sample introduction on the separation of neighbouring bands was examined. Our previous experience was with use of the mobile phase distributor for application of the sample to the plate from the edge of the layer. Although the starting zone obtained in this way is relatively wide, separation of the bands was satisfactory [23], because in this method the preliminary arrangement of the starting bands is in order of their subsequent retention. Introduction of the sample by use of an automatic applicator gives very narrow band of sample. After elution, six zones are partly separated (Fig. 1a) of which five are separated almost to baseline. When application to the edge of the layer was used, resolution of the bands was much poorer – in the densitogram shown in Fig. 1b only four bands separated to baseline can be isolated. Similar conclusions can be drawn from comparison of Figs 2a and 2b, obtained after separation of the same amounts of fraction II of the alkaloids. When the automatic applicator was used seven partly separated zones were obtained (with five zones separated to baseline) whereas introduction from the edge of the layer resulted in only three partly separated zones (Fig. 2b).

One distinct problem is the method of overloading – mass or volume. The first method results in asymmetric zones with back-tailing; the second leads to rapid broadening of the bands [24]. Figure 3 shows densitograms obtained after chromatography of increasing masses of extracts I and II. The amounts of sample introduced, by use of the autosampler, were 2, 3, 4, and 5 mg. With such overloading, increasing the amount of sample affects the resolution of bands to a limited extent only. It is apparent from the densitograms (Figs 3a and 3b) and from the videoscans (Fig. 4) that introduction of 2.5 times more extract does not markedly affect band
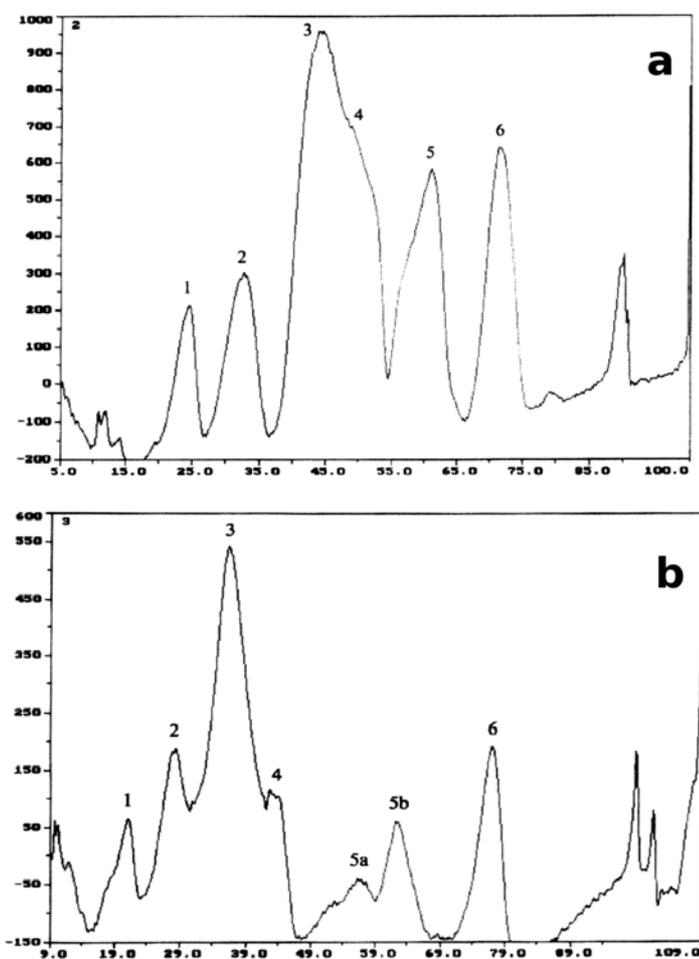
Fig. 1

Densitograms of preparative-layer chromatograms obtained after application of 5 mg fraction I by means of an automatic applicator (a) or by introduction of 5 mg fraction I dissolved in 100 μL methanol from the edge of the layer by means of the mobile phase distributor (b). The stationary phase was silica gel and the mobile phase AcOH–PrOH–CH_2Cl_2, 1:4:5. The plates were developed three times then scanned at 520 nm after derivatisation with Dragendorff's reagent

resolution – six partly separated alkaloid bands were still obtained. When volume overloading is used, however, increasing the volume of sample introduced severely affects band resolution. When the sample was introduced from the edge of the layer by use of the mobile phase distributor, increasing
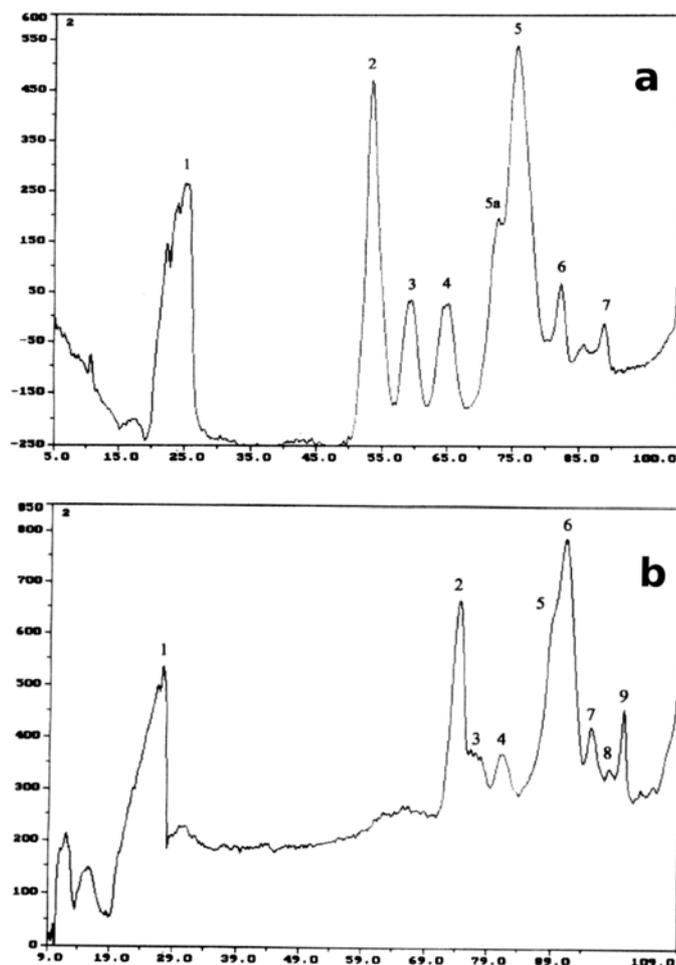
Fig. 2

Densitograms of preparative-layer chromatograms obtained after application of 5 mg fraction II by means of an automatic applicator (a) or by introduction of 5 mg fraction II dissolved in 100 μL methanol from the edge of the layer by means of the mobile phase distributor (b). The stationary phase was silica gel and the mobile phase AcOH–H_2O–MeOH–CH_2Cl_2, 5:2.5:20:72.5. The plates were developed twice then scanned at 520 nm after derivatisation with Dragendorff's reagent

the volume of sample resulted in rapid loss of resolution. Figure 5a shows band profiles obtained after elution of 200 μL sample; six partly separated alkaloid zones can be isolated. Increasing the volume introduced to 400 μL, however, results in loss of resolution – only three partly separated bands are apparent on the chromatogram (Fig. 5b).
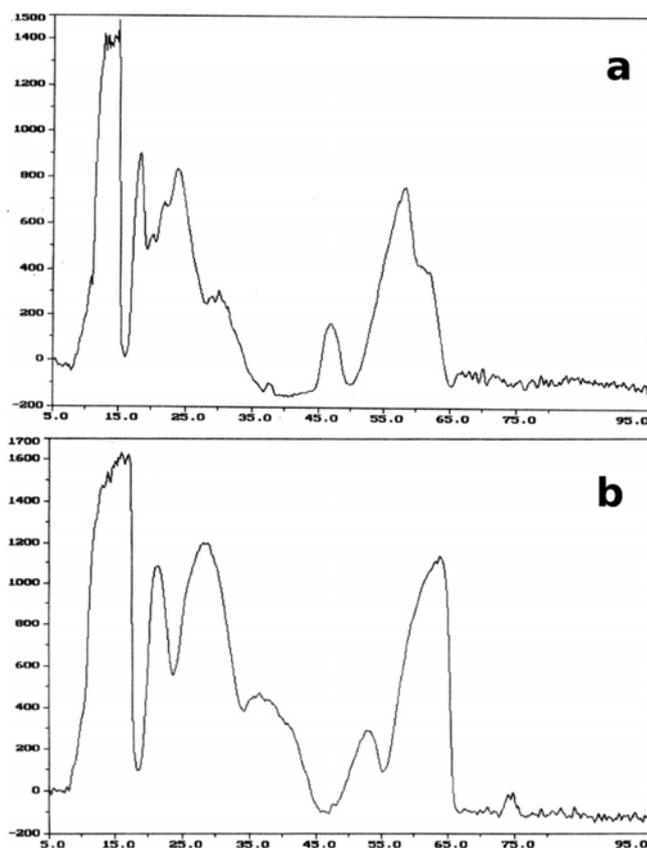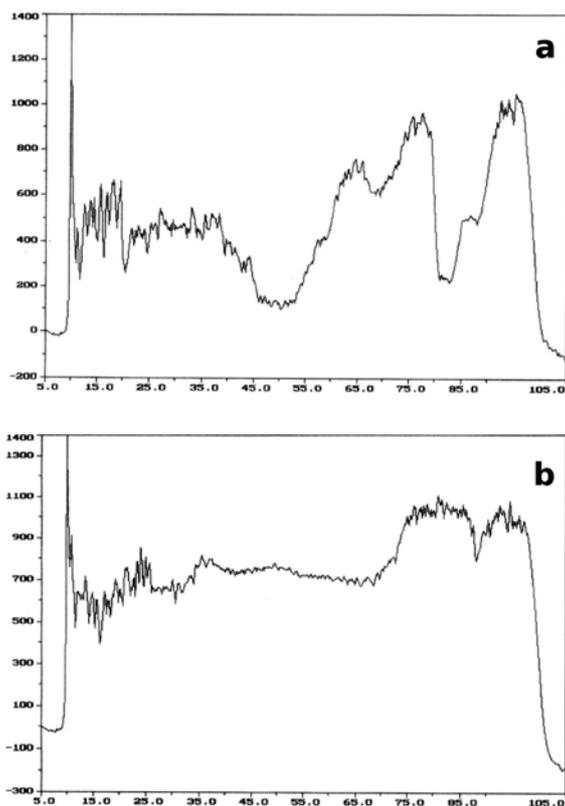
Fig. 3

Densitograms of preparative-layer chromatograms obtained from (a) 2 mg and (b) 5 mg fraction I, introduced by means of the automatic applicator. The stationary phase was silica gel and the mobile phase AcOH–PrOH–CH_2Cl_2, 1:4:5. The plates were scanned at 520 nm after derivatisation with Dragendorff's reagent

The next problem in preparative-layer chromatography is layer thickness. Plates with 2-mm layers are available but use of very thick (5, 10 mm) layers is also reported. Increasing the thickness of the layer should result in greater capacity, which should enable introduction of larger amounts of sample and more rapid isolation of mixture components. In our experiments commercial plates coated with 0.25, 0.5, and 2-mm layers were used. The same amounts – 5 mg fraction I – were introduced to the layers. It is apparent from Fig. 6a that the best separations were obtained when 0.25-mm layers were used – six partly separated zones can be isolated. In-
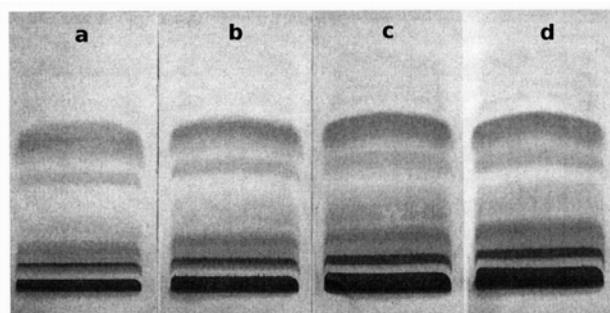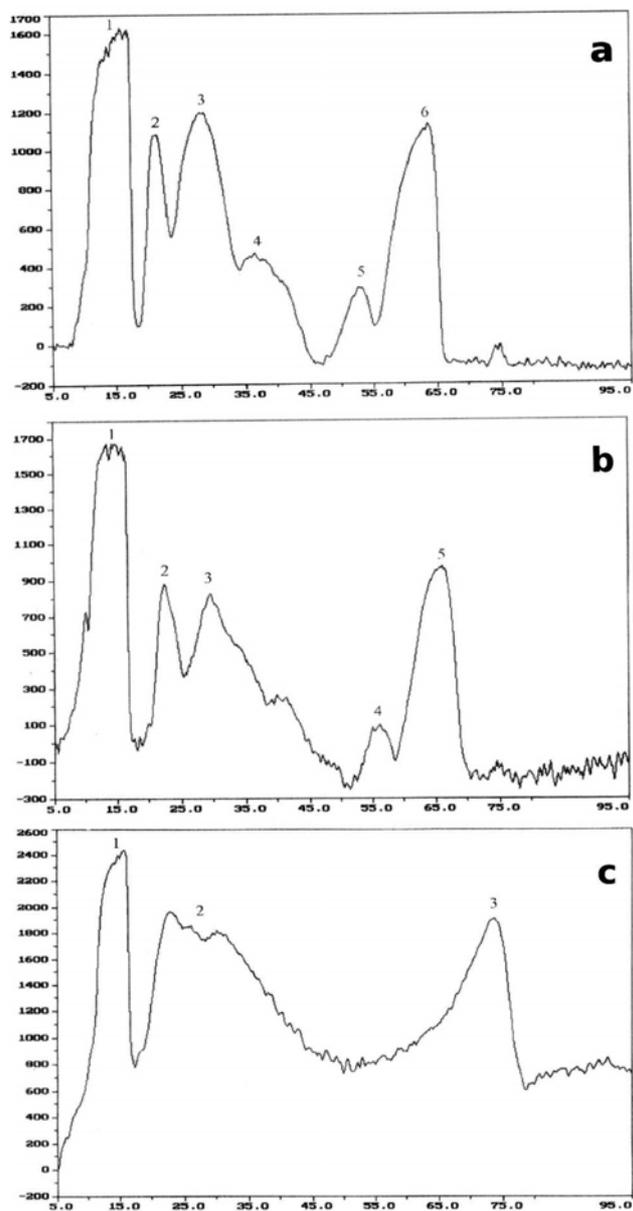
Fig. 4

Videoscans of preparative-layer chromatograms obtained from increasing masses of fraction I: (a) 2 mg, (b) 3 mg, (c) 4 mg, and (d) 5 mg. Other conditions as for Fig. 3a

Fig. 5

Densitograms of preparative-layer chromatograms obtained from fraction I introduced from the edge of the layer: (a) sample volume 200 μL; (b) sample volume 400 μL. Other conditions as for Fig. 3a
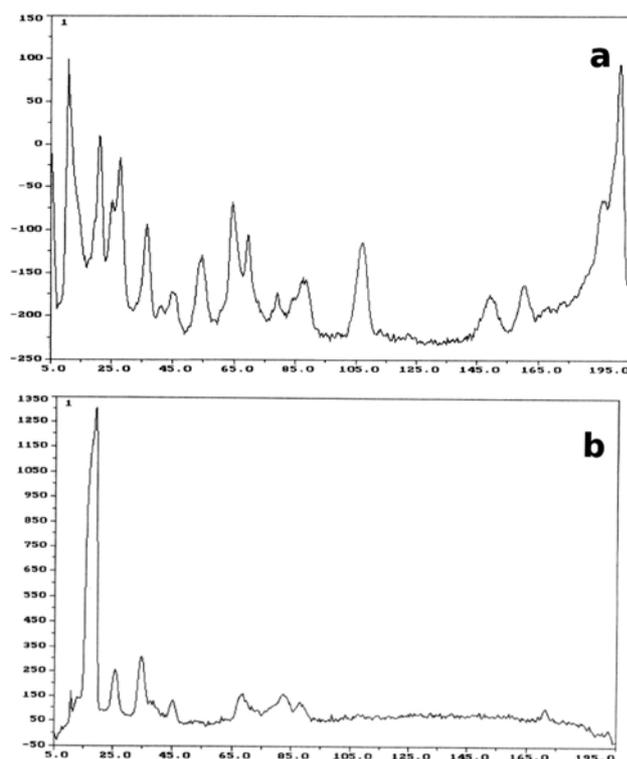
Fig. 6

Densitograms of preparative-layer chromatograms obtained from 5 mg fraction I introduced by use of automatic applicator: (a) 0.25-mm silica layer; (b) 0.5-mm silica layer; (c) 2.0-mm silica layer. The stationary phase was silica gel and the mobile phase AcOH–PrOH–CH_2Cl_2, 1:4:5. The plates were scanned at 520 nm after derivatisation with Dragendorff's reagent

creasing the layer thickness resulted in worsening of band resolution. In the densitogram obtained from the separation performed on the 0.5 mm layer only five bands are apparent (Fig. 6b) and when the 2 mm layers were used the resolution decreased markedly – only three partly separated zones can be observed (Fig. 6c). The cause of this effect could be the problem introducing an equal concentration of sample across the thick layer of adsorbent and/or problems with equilibration of the thick layer with mobile phase vapour.

The importance of derivatisation in thin-layer chromatography is widely known. Use of an appropriate reagent can be the first method of identification of compounds, most often group identification. In preparative-layer chromatography such methods of location of bands are rarely

Fig. 7

Densitograms of preparative-layer chromatograms obtained from 5 mg fraction II introduced by use of the automatic applicator. The stationary phase was silica gel and the mobile phase AcOH–H₂O–MeOH, 5:30:65. The plates were developed twice and scanned at (a) 366 nm and (b) 520 nm after derivatisation with Dragendorff's reagent

used because they usually change the chemical identity of the compounds being isolated. In preparative-layer chromatography UV light or UV–visible densitometry are therefore used for location of the separated bands. The bands can, however, be derivatised on the edge of the plate if the rest of layer is covered with the glass plate so the compounds remain in their initial state. Such a method can help in correct location of bands for isolation. Figure 7a shows the densitogram obtained from separated fraction II scanned at 366 nm. Several separated or partly separated bands can be located on the basis of this densitogram. Figure 7b shows the same plate scanned at 520 nm after derivatisation with Dragendorff's reagent (universal reagent for heterocyclic bases). It is clearly apparent that only seven of the bands are alkaloids.

CONCLUSIONS

During sample introduction to the adsorbent layer it is extremely important to obtain a narrow starting band. The best resolution of the separated bands was obtained when samples were introduced to the layer by use of the automatic applicator with simultaneous evaporation of the sample solvent.

With increasing of layer thickness the resolution of neighbouring bands deteriorates. Thus 0.25–0.5-mm layers are recommended for preparative purposes.

Derivatisation of compounds on the edge of the bands enables precise location of bands for isolation of mixture components of interest.

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