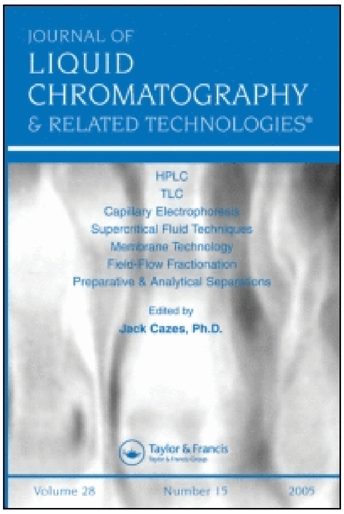

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Joseph C. Touchstone[a]; Sidney S. Levin[a]
[a] School of Medicine University of Pennsylvania, Philadelphia, Penna.

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SAMPLE APPLICATION IN THIN LAYER CHROMATOGRAPHY

Joseph C. Touchstone and Sidney S. Levin
School of Medicine
University of Pennsylvania
Philadelphia, Penna. 19104

ABSTRACT

Resolution in thin layer chromatography depends on the ability to properly apply the sample to the origin. Thin lines or streaks are recommended. Preabsorbent layers facilitate sample application since thin lines result. Automatic sample applicators are prerequisite if preabsorbent layers are not used and more than 15 μl of the solution is to be applied.

INTRODUCTION

The spot size or the width of the sample zone at the origin of thin layer chromatograms (TLC) has a great influence on the separation characteristics and resolving power of the chromatograms. Wide application points defeat the resolution in conventional TLC and ruins the high separation power of high performance TLC (HPTLC) (1). It is important to keep the application zone as small as possible. The solution to the problem of sample application is the key to greater resolution in TLC.

This report describes two methods by which the application zones of the chromatograms can be kept small; one using commercial automatic sample applicators and the other utilizing the "preabsorbent" zones in the preabsorbent thin layer plates now available commercially. Both of these methods give narrow starting zones which contribute to the success of the TLC.

Materials and Methods

All solvents used in the experiments to follow were Burdick and Jackson redistilled in glass solvents or the equivalent.

The thin layer plates were L-K5 (Whatman, Clifton, N.J.) or LHPTLC (Whatman) and the equivalent without the preabsorbent layer obtained from Whatman. The dye mixture "dye standard" containing 5 different dyes was obtained from Whatman.

An Analytical Instrument Specialties (Libertyville, Ill.) automatic spotter with provision for 19 syringes of various sizes was used in this work. The spotter was set up for slow delivery of the sample to the layer.

The plate was heated and a hot air blast from a hair dryer was used to facilitate the evaporation of solvent during sample application. The hair dryer was positioned in such a way that the air blast uniformly hit the layer and did not blow the sample from the surface.

The thin layers were routinely scored into lanes of 10 mm width using a Schoeffel scoring device. Both the layers containing the preabsorbent and those without were treated in this manner. A Schoeffel model 3000 spectrodensitometer was used in this work.

EXPERIMENTS AND RESULTS

Automatic versus manual spotting

The automatic spotters made by Analytical Instrument Specialties allow application of up to 10 or 19 samples simultaneously depending on the model chosen. The glass syringes provided accommodate either 1,5,50 µl or 100 µl and consist of a blunt tipped needle coated with Teflon to prevent sample residue creep-back and deposition on the tip of the needle during delivery. Samples are drawn up into the syringes, which then are placed in the instrument with the tips of the syringes aligned over the origins of the lanes of the TLC plate. At the opposite end of the syringe a motor driven bar slowly depresses the plungers of all syringes simultaneously, and the rapidity of sample delivery is regulated by the speed with which the plungers are depressed. The spotter is equipped with a heater so that the temperature of the layer can be regulated during sample application, in order to facilitate solvent evaporation. An air blast from the dryer can also be used to aid rapid drying. The contents of a 100 μl syringe can be delivered over periods varying from 2-30 minutes, allowing spotting with any type of solvent. Unattended use of the instrument will result in formation of circular spots on the layer. Streaking may be achieved by slightly shifting the plate several times during sample application such that the tips of the syringe deliver the sample to several spots across the origin in each lane. The coefficient of variation of this automatic sample applicator was less than 5%.

Cholesterol in a constant concentration of 10 ng/ul was spotted in varying volumes by manual methods using Drummond microcapillaries, and by automatic (AIS) spotter. Development was carried out in hexane:ether:acetic acid (86:13:2); to 2 cm from the top. After development, charring (spraying with 3% cupric acetate in 8% phosphoric acid followed by heating at 180^{o} for 10 minutes) was performed. Then scanning with a Schoeffel model 3000 spectrodensitometer at 400 nm was performed (2). The response was channelled into a Schoeffel SDC 3000 density computer recorder in order to provide a continuous recording of the photomultiplier response. Quanitation can be carried out using the area of peaks so obtained.

The results of comparison of manual and automatic sample application are shown in Figure 1. With manual application up to 15 μl of

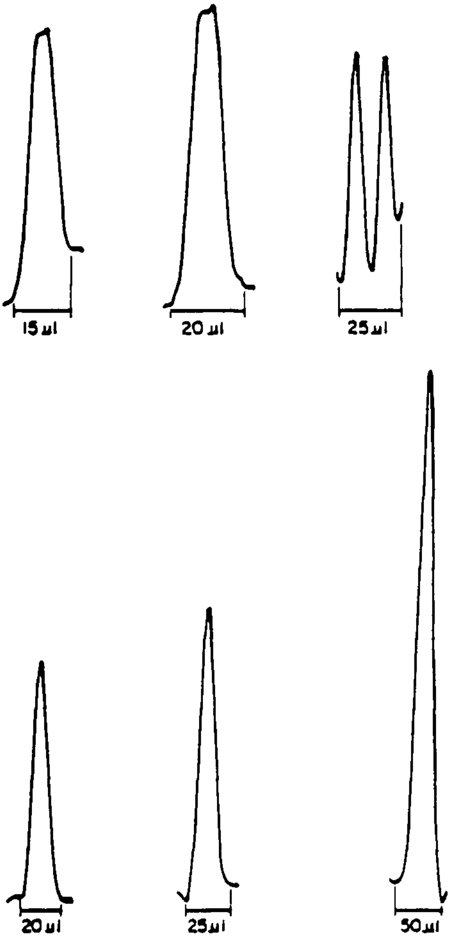

Figure 1. Results of scans of chromatograms of a cholesterol solution applied manually (upper panel) and by automatic (AIS) spotter (lower panel). The cholseterol was developed in a solution of hexane-ether-acetic acid (86:13:2) on a Whatman HPTLC plate.

sample could be applied to give a linear relationship between the amount of cholesterol in the sample and the recorder response. Above 15 µl of the scans showed "double peaking", indicating that the spots had migrated as a circle. That is, the sample had diffused to the periphery of the circular spots and migrated as 2 bands. In contrast, up to 250 µl could be safety applied using the AIS automatic spotter, still retaining a single peak on scanning, indicating that the component in the sample had migrated as a single band as seen in Figure 1. It is not generally appreciated that larger sample volumes are best applied with use of an automatic device, and small volumes can be applied manually using the nanopipet described by R.E. Kaiser (1). This study indicates the importance of selecting the proper method of sample application depending upon the volume to be applied.

The sample applicator produced by Kontes, Instruments Division (Vineland, N.J.) essentially consists of large-bore glass tubes fitted with syringe needles. The needle is positioned just above the layer to deliver the solute gradually to the layer by capillary action. The needle size determines drop size and flow rate. The essential feature is that the needle is surrounded by a template with orifices that permit compressed gas flow over the layer to evaporate the solvent as the solution percolates into the layer. The gas (air, nitrogen, etc.) can be cooled or heated, or the plate can be heated. Because of this, regardless of the volume of solvent, a small spot will result. This type of sample applicator can be very useful when dilute samples must be applied. Up to six samples can be performed simultaneously. As much as 5 ml of solution can be applied by this method. It is widley used in pesticide work where clean up results in dilute samples.

"Spotting" versus "streaking"

The individual success of high performance thin layer chromatography is very dependent on the origin configurations of the applied sample. As seen in Figure 2, the application of the sample as a "spot" decreases resolution as compared to the same amount of sample a-

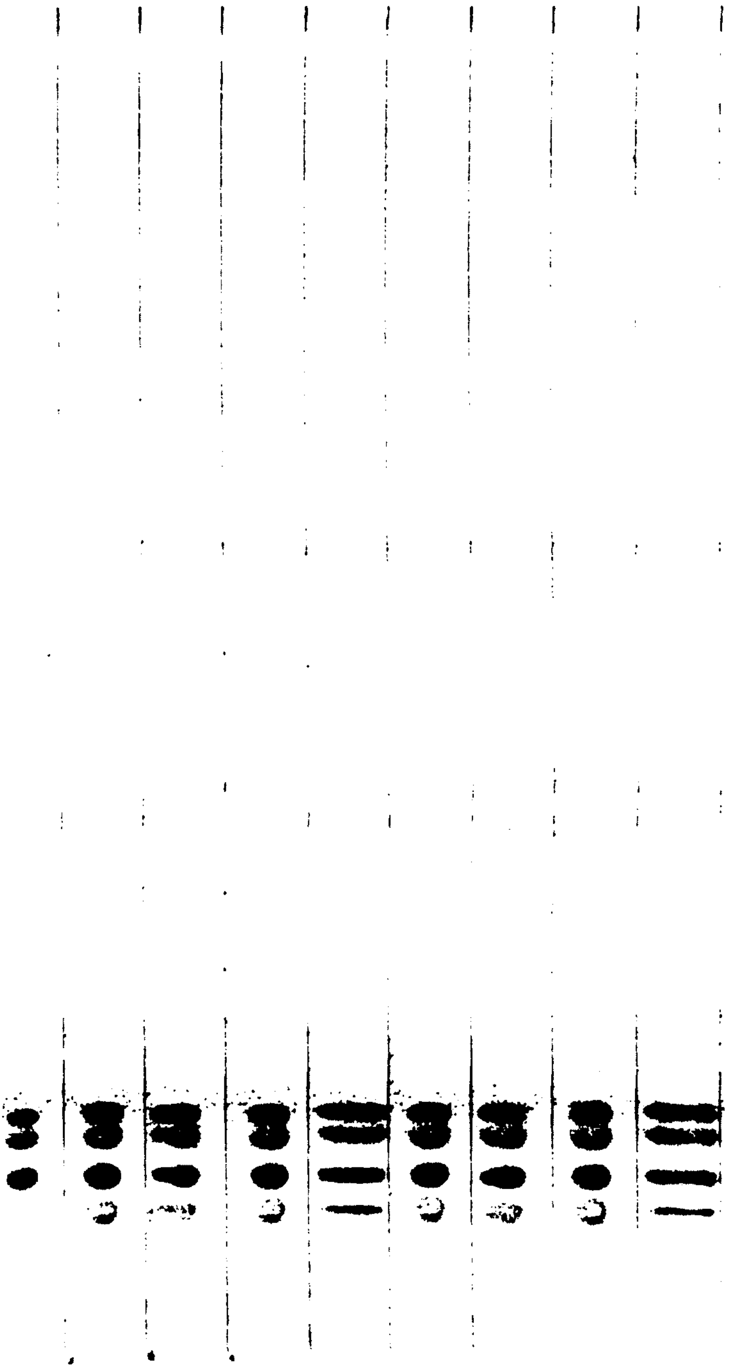

Figure 2. Results of "streaking" versus "spotting" on a conventional K-5 (silica gel) (20 X 20 cm) TLC plate. The five component dye mixture was developed with a mobile phase of toluene-chloroform (1:1) for 2 cm distance of front movement.

pplied as a "streak". The reason for this can be seen in the expression for resolution (3).

$$\text{Resolution} = \frac{2D}{W_2 + W_1}$$

Where D is the distance between the apex of two peaks and W_1 and W_2 are the baselines of the two peaks obtained by scanning the thin layer chromatogram by densitometry.

The wider the base line of peaks in the scans of the chromatograms, the lower the resolution. A survey was made to compare "streaking" and "spotting" as means of sample application in terms of this equation. As seen in Figure 2 , "streaking" of the sample results in sharper separations or narrower separation zones. This will result in higher resolution as verified by a series of studies using a five component dye mixture. The resolution for the separations resulting from the samples which were "streaked" was as much as 32% greater than that from the spotted samples. Thus, it must be emphasized that sample application is an important factor in resolution and that "streaking is the method of choice.

Use of "Preabsorben" Layers

The recent development of layers containing preabsorbent zones has facilitated the use of high performance thin layer chromatograms. It is difficult to manually apply samples so as to take advantage of high efficiency of HPTLC without the use of special devices developed for this purpose (4). The preabsorbent area of the plates which provide this option gives a means of producing a very fine line as a "streak" for the origin of the chromatogram. It also facilitates sample application since the sample can be applied rapidly. Here again the application must be made as a "streak" or line since spotted zones results in circular zones after development. Streaking across the lanes scored in the layer as seen in Figure 2 is recommended. Application fo the sample vertically in the middle of the lane also results in a circular concentrated zone which can decrease the efficiency of the sorbent. As seen in Figure 3, a very fine line results from the use of the "preaborbent" area of the chromatogram. The preabsorbent does not hold back the sample. Thus, when the mobile phase travels up the layer it deposits the sample as a narrow line in the junction between the preabsorbent and the sorbent. It is possible to obtain lines as narrow as 0.1 mm which thus facilitate the separation in the short time and distance under which the HPTLC is developed.

Effect of sample size on efficiency

A study was made on the effect of sample size on the efficiency of high performance thin layer chromatographic separations. Generally those practicing TLC tend to overload the capacity of the sorbent in use. Overloaded systems result in poor resolution and useless chromatograms. Larger sample loads naturally would result in larger areas. The formula for plate number N can be used to determine this effect

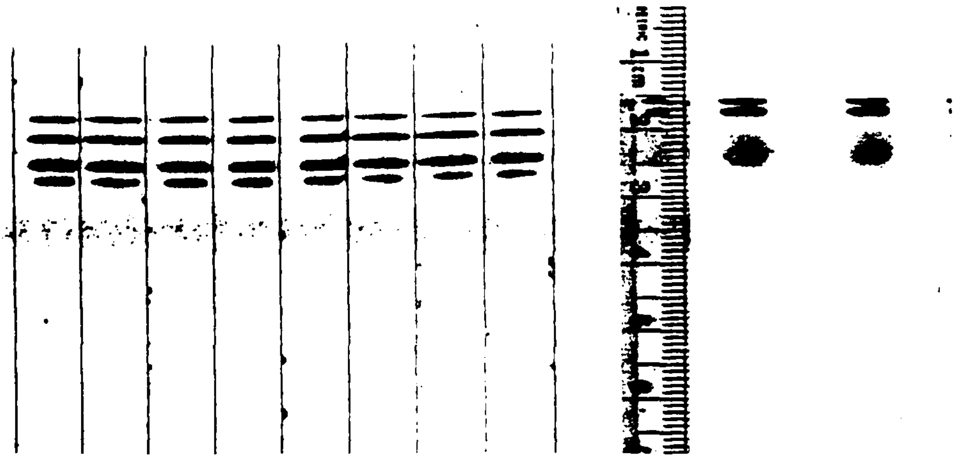

Figure 3. Chromatogram of dye mixture showing the utility of the preabsorbent layer in HPTLC. The origin of the chromatograms is less than 1 mm wide.

in TLC (6). Using the formula: $N = 4\left(\frac{t_r}{t_w}\right)^2$ a survey of the effect of sample loading on efficiency of LHPTLC plates was carried out. (t_w = width of the peak at 0.601 x height).

Quinidine sulfate solutions in methanol were prepared in concentrations ranging from 0.05 to 3 ng/5 µl. Samples of 5 µl each were applied to alternating channels of a scored 20 x 20 LHPK plate. The chromatogram was developed with a mobile phase of ethylacetate-methanol-ammonia (90:7:3) until the front had travelled 8 cm. This was done in triplicate. The chromatogram was dried at 110°C for 5 min., then sprayed with 2N H_2SO_4 in ethanol and heated at 111°C for 3 min. The chromatograms were scanned in duplicate (360 nm, transmission mode) to give six sets of data for each point. The results are given in Figure 4.

The use of smallest amount of sample consistent with detectibility at the level desired should be the rule. This is also essential in keeping the sample concentration in the layer at such a level

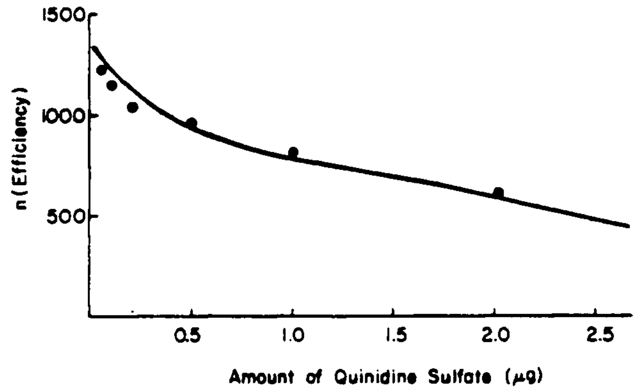

Figure 4. Effect of sample size on efficiency of separation of quindine sulfate.

as not to disturb mobile phase flow thru the matrix. Solvent tends to flow around zones which are too concentrated. This results in distorted spots.

DISCUSSION

The preabsorbent zone gives a means for sample application which avoids the tedious labor of manually trying to obtain a linear application in a small spot. However, care must be taken to avoid overloading the capabilities of the layer. High concentrations of sample cause a decrease in resolution since overloaded zones tend to be larger than normal properly applied samples. As the concentration of the sample in the layer increases, the efficiency and resolution will decrease. Therefore, it is recommended that samples be kept as small as is consistent with detectibility and conformation with principles of Beer's Law if densitometry is to be carried out (5). In view of the results consistently obtained, it is recommended that streaking rather than spotting be the method of choice for sample application. It is, however, possible to obtain a fine line if a number of "spots" are applied in a row. Thus, if automatic sample applicators are used, one should apply the sample as a series of spots rather than in one spot which may actually be too concentrated.

With improved sample applications and proper mobile phase selection, it is possible to obtain ultimate separation parameters.

REFERENCES

1. R.E. Kaiser in A. Zlatkis and R.E. Kaiser eds., J. Chromatogr. Lib. 9, 85 (1977).

2. J.C. Touchstone, M.D. Dobbins, C.Z. Hirsch, A.R. Baldino and D. Kritschevsky, Clin. Chem. 24, 1496 (1978).

3. B.L. Karger in J.J. Kirkland ed., Modern Practice of Liquid Chromatography, Wiley-Interscience, 1971, p. 12.

4. R.E. Kaiser in A. Zlatkins and R.E. Kaiser eds., J. Chromatogr. Lib. 9, 88 (1977).

5. J.C. Touchstone, S.S. Levin and T. Murawec., Anal. Chem. 43, 858, (1971)

6. R.P.W. Scott in A. Weissberger ed., Techniques of Chemistry, Vol II, Contemporary Liquid Chromatography, Wiley-Insterscience, New York 1976, p. 261.