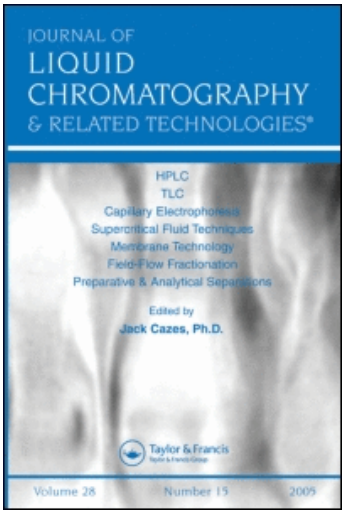

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Triangular Thin-Layer Chromatography[1,2]

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ABSTRACT

This study illustrates the use of triangular plates in thin-layer chromatographic separations. A comparison between triangular, conventional and circular thin-layer chromatography clearly indicates the superiority of triangular plates in terms of sensitivity, detection limits and savings in plates and solvent.

INTRODUCTION

Thin-layer chromatographic (TLC) separations are normally carried out on rectangular or square plates of different sizes depending on the amount of sample to be separated. The early use of thin-layer chromatography employed

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glass microscope slides with a 2 mm layer of alumina. One drop of the mixture to be separated was placed in the center, and solvent was added until the components separated into rings (1). Today, the principle of circular development is used in high performance thin-layer chromatography (HPTLC) which uses a 0.1 mm thick adsorbent layer on 10x10 cm or 5x5 cm glass plates (2). In circular chromatography, the sample mixture is spotted at, or around, the center of the plate and the solvent (with the aid of a pump and capillary action) flows from the center to the circumference of the plate where the sample components are separated into diffused arcs. In anticircular TLC, a principle introduced by Kaiser (3), the sample is spotted at the circumference of the plate and the solvent moves from the circumference to the center separating the components of the mixture into elongated spots. In conventional TLC, where the plate is square or rectangular, the spots after development are diffused circles. The longer the development, the more diffused the spots, which results in less concentrated sample per unit area (molecules/unit area). One way to restrict diffusion is to restrict the area into which the spots can move. Anticircular chromatography is based on this principle, although the resulting spots are elongated and some loss of resolution may occur. The higher the R_f value, the more elongated the spots and the poorer the resolution. A relatively expensive device is also needed for development.

This paper presents a simple, inexpensive method by which diffusion is restricted and the spots, after development, are more concentrated (molecules/unit area) than with conventional or circular plates. The method employs triangular plates which can easily be made from Bakerflex plates, silica gel impregnated fiber (ITLC-SG) plates or glass plates. Its advantages are discussed and it is compared with circular TLC.

EXPERIMENTAL

Reagents and Materials. Bakerflex silica gel plates and silica gel impregnated glass fiber (ITLC-SG) plates (Gelman Instrument Company, Ann Arbor,

MI) were used. All solvents were previously distilled in glass (Burdick & Jackson, Muskegon, MI). Triangular plates were cut from square plates with a paper cutter, or a sharp razor blade, to sizes of 5x20 or 5x10 cm. Standard developing tanks were used. HPTLC silica gel plates were from Merck (Darmstadt, Germany).

Procedure. Five µl of dye mixture (V-tech Corp., New Berlin, WI.) were spotted at the base of the 5x20 cm triangular plate and developed in benzene: ethyl acetate (90:10). After development, the plate was removed, dried, and developed again in the same solvent system until the solvent front passed the highest spot on the plate. In antitriangular chromatography, the sample is spotted 1 cm from the peak of the triangle and developed by dipping the peak in the solvent. With 5x10 cm triangular plates, only 1 µl of sample solution was spotted. When HPTLC plates were used, round spots were obtained after the plate was developed once.

RESULTS AND DISCUSSION

Long development in conventional (square or rectangular plates) or circular TLC does not necessarily give better resolution of the spots because of problems with diffusion.

Resolution is defined by the following equation:

$$R = X/0.5(d_1+d_2)$$

where X = distance between two spots

d_1 = diameter of spot 1

d_2 = diameter of spot 2

Diffusion is proportional to the square root of the distance traveled by the spots. The longer the distance, the greater the diffusion, the less the resolution, and the less concentrated (molecules/unit area) the spots.

From these considerations, it would appear that in conventional and circular TLC (including anticircular), the higher the R_f (i.e., the longer the distance traveled by the spot) the more diffuse the spots. The lower the R_f, the more concentrated the spots. This is especially true in conventional and circular development. In anticircular TLC, the spots move from the circumference to the center of the circular plate, a smaller area where radial diffusion is restricted. As a result, after development the spots are elongated, especially at higher R_f's, but the starting and finishing widths are the same. In his discussion of anticircular TLC, Kaiser (3) concluded that the mobile phase front moves with constant speed and that the available development area decreases quadratically with the phase front, giving rise to a mechanical compression from both sides which increases toward the center. The higher the R_f the more pronounced is this lateral compression and the more elongated are the spots. In triangular thin layer chromatography the sample is spotted at the base of the triangle. The plate is then developed in a cylindrical or rectangular container. During development, the spots move from a large to a small area, where the mechanical effects are similar to those present in anticircular TLC. As a result, the spots at high R_f values are elongated but very narrow. A second development (which takes approximately 3 minutes for a 5x10 cm plate) in which the solvent front passes the highest spot, transforms the spots at higher R_f's into circular spots, and the spots at lower R_f's into more compact and concentrated spots (Figure 1). Triangular TLC is very easy to perform and requires no special equipment; thus it is cheaper than circular and anticircular TLC. It is also cheaper since two triangular plates are obtained from one rectangular plate, and less solvent is needed for development (Figure 2).

An added advantage of triangular plates is that they can be used in preparative separations of small quantities of materials such as metabolites and drugs in body fluids. The sample is streaked at the base of the plate. Depending on their positions on the plate, the developed streaks are narrower

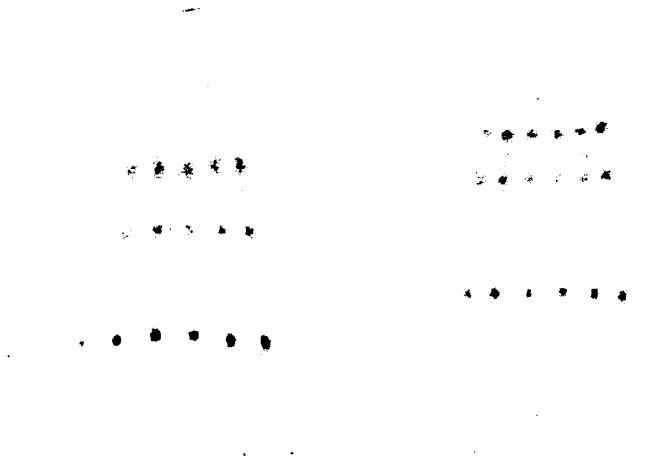

Figure 1. Thin-layer chromatography using 5x20 cm triangular plates. The plate to the left was developed once while that to the right was developed twice in the same solvent system.

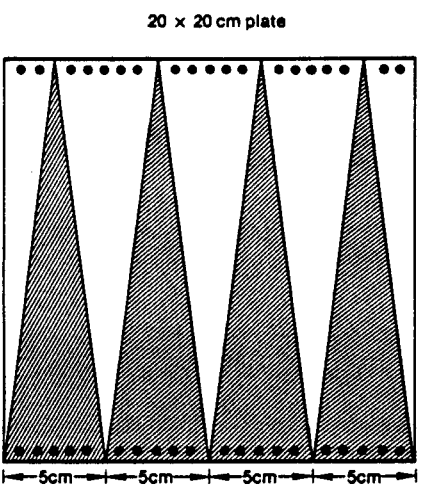

Figure 2. Division of a 20x20 cm plate into seven 5x20 triangular plates.

than the original streak at the base of the plate (Figure 3). This means that: (a) the streaks are more concentrated, up to 100%, than if they were spread on a conventional or circular plate; and (b) the streaks require less solvent to elute from the triangular plate than from the conventional and circular plate, resulting in a more concentrated solution suitable for further analysis by ancillary techniques such as NMR, MS, IR, and UV-VIS. A comparison of TLC separations using conventional (rectangular), circular and triangular plates is shown in Figure 4. The compactness of the spots using triangular plates as can be seen clearly.

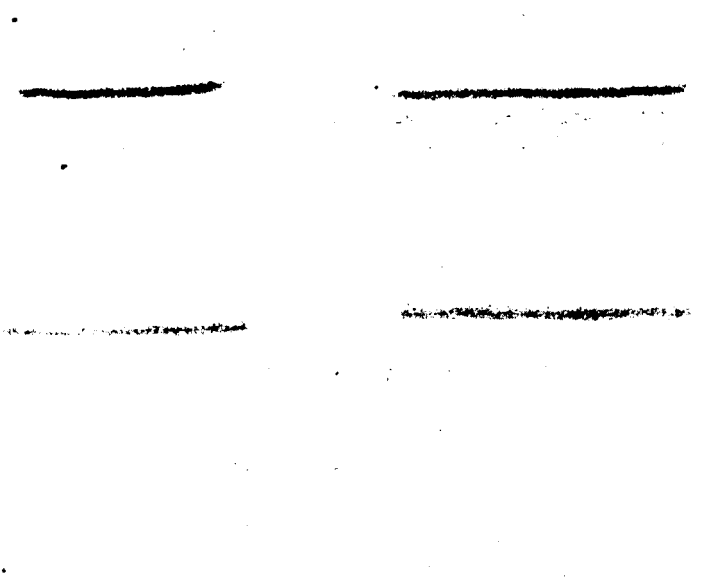

Figure 3. Comparison of the separation of streaks of dye on triangular and rectangular 5x20 cm plates.

Figure 4. Comparison of TLC separations using triangular, circular and rectangular plates.

CONCLUSION

The use of triangular plates for TLC separations offers many advantages.

(1) A 50% savings in plate use is achieved. A 20x20 cm plate gives seven 5x20 cm plates and adequate separation of micro samples (1 µl spotted) is possible on 5x10 cm plates.

(2) Restricted diffusion. Since the sample is spotted at the base of the plate, the movement of the solvent front is from a wide to a narrow area which restricts the diffusion of the spots. As a result, the spots are compact and more concentrated, (molecules/unit area).

(3) Increased sensitivity. Since the spots (streaks) are more concentrated after development compared with other TLC modes, less sample is needed for the separation, which means that detection limits are lower.

(4) Preparative separations. Triangular plates are useful in preparative separations for structural characterization by ancillary techniques such as NMR, IR, MS, or UV-VIS. The sample is streaked at the base of the triangle. When the sample is developed, it is concentrated in a smaller area and requires less solvent to elute. The higher the R_f the more concentrated the zone, and less solvent is needed for elution. This is especially important in

the separation and identification of metabolites at the microgram or submicrogram level.

(5) No special developing units are needed. Unlike HPTLC and anticircular chromatography, which require a special developing apparatus, triangular TLC uses regular developing tanks or any glass jar of convenient size.

Antitriangular TLC gave results which are similar to those obtained by circular chromatography.

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