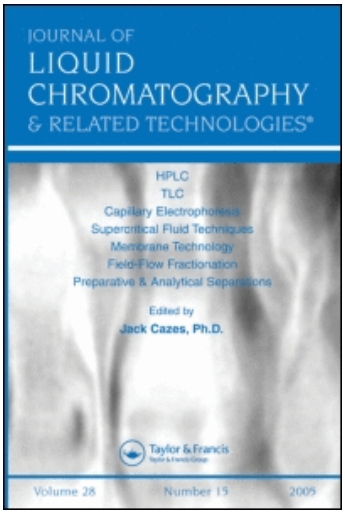

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Haleem J. Issaq[a]
[a] Chemical Carcinogenesis Program NCI Frederick Cancer Research Center, Frederick, MD

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A SIMPLE AND ECONOMICAL APPARATUS FOR DEVELOPING THIN-LAYER CHROMATOGRAPHY PLATES IN THE ANTICIRCULAR MODE

Haleem J. Issaq
Chemical Carcinogenesis Program
NCI Frederick Cancer Research Center
Frederick, MD 21701

ABSTRACT

An improved method for developing thin layer chromatography plates in the anticircular mode is described. The apparatus consists of two glass dishes arranged concentrically. The outer dish has a diameter of 98 mm x 15 mm deep, and the inner one a diameter of 92 mm x 13 mm deep. The inner dish rests on four small pieces of glass (5 x 5 x 2 mm). A paper wick, (16 mm wide x 1.5 mm thick) sits between the two dishes and transfers the solvent from the outer dish to the thin layer plates. The plate sits on the paper wick with the adsorbent facing down. To ensure an even solvent flow, the dishes are situated on a platform which has adjustable legs, and an horizontal level. Up to 50 samples can be analyzed on one 10 x 10 cm plate in 5 minutes. Less than 10 ml of solvent are needed.

INTRODUCTION

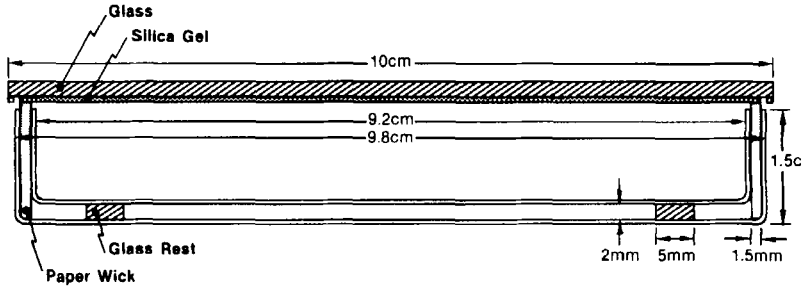
Anticircular thin-layer chromatography (TLC) was introduced in 1970 by Van Dyk (1). In this method, the sample is applied at the circumference of a circular plate and elution proceeds towards the center. Later, a simple apparatus was developed consisting of a turntable on which the plate was placed while the solvent was introduced from a stationary syringe to a felt ribbon surrounding the plate (2). The system needed to be protected from drafts to prevent the development of irregular circles. In 1978 Kaiser (3) introduced a high performance anticircular TLC system, consisting of a modified U-chamber. The solvent

was fed on to the plate by capillary action from a narrow channel. Kariko and Tomasz (4) used a system in which the solvent was placed in a petri dish. The solvent flowed on to the round plate through a cylindrical filter paper strip with feathered edges. This system, although quite simple, gave reasonably good results, but they were not as reproducible as those obtained with the modified U-chamber. An apparatus was developed which is simple, reliable, and easy-to-construct and operate. This apparatus combines the speed and reproducibility of the modified U-chamber with the economy of the petri dish approach.

EXPERIMENTAL

TLC silica gel plates (10 x 10 cm) were purchased from Whatman, Inc. Solvents were "glass distilled" (Burdick & Jackson). A blunt head 10 µl Hamilton syringe with a special dispensing device was used for spotting 0.5 µl of solution. Hexane:ethyl acetate (2:1) was used as the mobile phase.

Our apparatus (Fig 1) consists of two glass dishes arranged concentrically. The outer dish has a diameter of 98 mm x 15 mm deep, and the inner one a diameter of 92 mm x 13 mm deep. The inner dish rests on four small pieces of glass (5 x 5 x 2 mm). A paper wick, (16 mm wide x 1.5 mm thick), sits

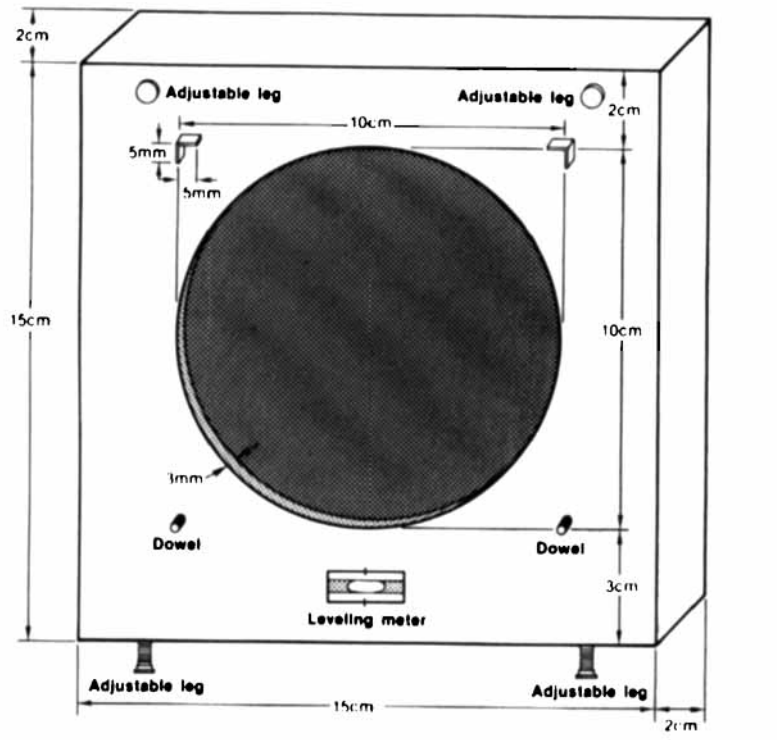


Anticircular Developing Apparatus Chamber Assembly

Figure 1. Schematic of anticircular apparatus.

between the two dishes and transfers the solvent system from the outer dish to the thin layer plates. The plate sits on the paper wick with the adsorbent facing down. A circle 10 cm in diameter is scored on the 10 x 10 cm plate with a sharp spatula. The sample is spotted on the plate at a distance 5 mm inside the scored circle. The eluting solvent (10 ml) is placed in the outer dish; the inner dish contains either 2 ml of the eluting solvent for equilibration or saturation, or any conditioning reagent required. When the plate is positioned absorbent down on the paper wick, development is initiated.

To ensure an even flow of the solvent the dishes are placed on a special platform which has adjustable legs and a horizontal leveling meter (Fig 2).



Platform for Anticircular Developing Apparatus

Figure 2. Schematic of platform used with the anticircular apparatus.

The platform is equipped with two corner pieces 10 cm apart on one side of the platform (Fig 2). Opposite the corner pieces, at a distance of 10 cm, two dowels are situated. The platform has a circular groove 10 cm in diameter x 3 mm deep into which the outside dish fits. The corner pieces, the dowels, and the groove, ensure that the plate always occupies the same position on the platform. A glass or plastic lidless box 11 x 11 x 2 cm covers the dishes to eliminate drafts. When development is complete, the plate is removed and a dish with the same dimensions as the outside dish is used to cover the system to retain the saturated atmosphere. When 20 x 20 cm plates are developed, larger sized dishes are used.

RESULTS AND DISCUSSION

Thin layer chromatography is a simple and economical analytical technique which gives reasonably reproducible results if plate-to-plate variations are eliminated, and solvent and vapor effects are controlled. Anticircular TLC is the most efficient mode for the simultaneous development of large numbers of samples. It has been shown (3) that the anticircular mode is superior to both circular and linear modes in terms of sensitivity, speed of analysis, and amount of solvent required.

The anticircular U-chamber (3) gives reproducible results at high cost. The unit described by Kariko and Tomasz (4), on the other hand, while cheap lacks certain features which would make it more versatile. The feathered strips do not always give uniform transfer of the solvent because the filter paper is not held firmly in place, and there are gaps between the feathered strips. With neither of these systems is it possible to condition the plates during development with a second solvent system or other reagents, such as sulfuric acid (to control humidity), or ammonia (to control streaking).

The apparatus we have developed (Fig 3) overcomes these disadvantages. The solvent flows from the dish to the plate via capillary action from the paper wick which is held firmly in place by the inner dish. The paper wick is

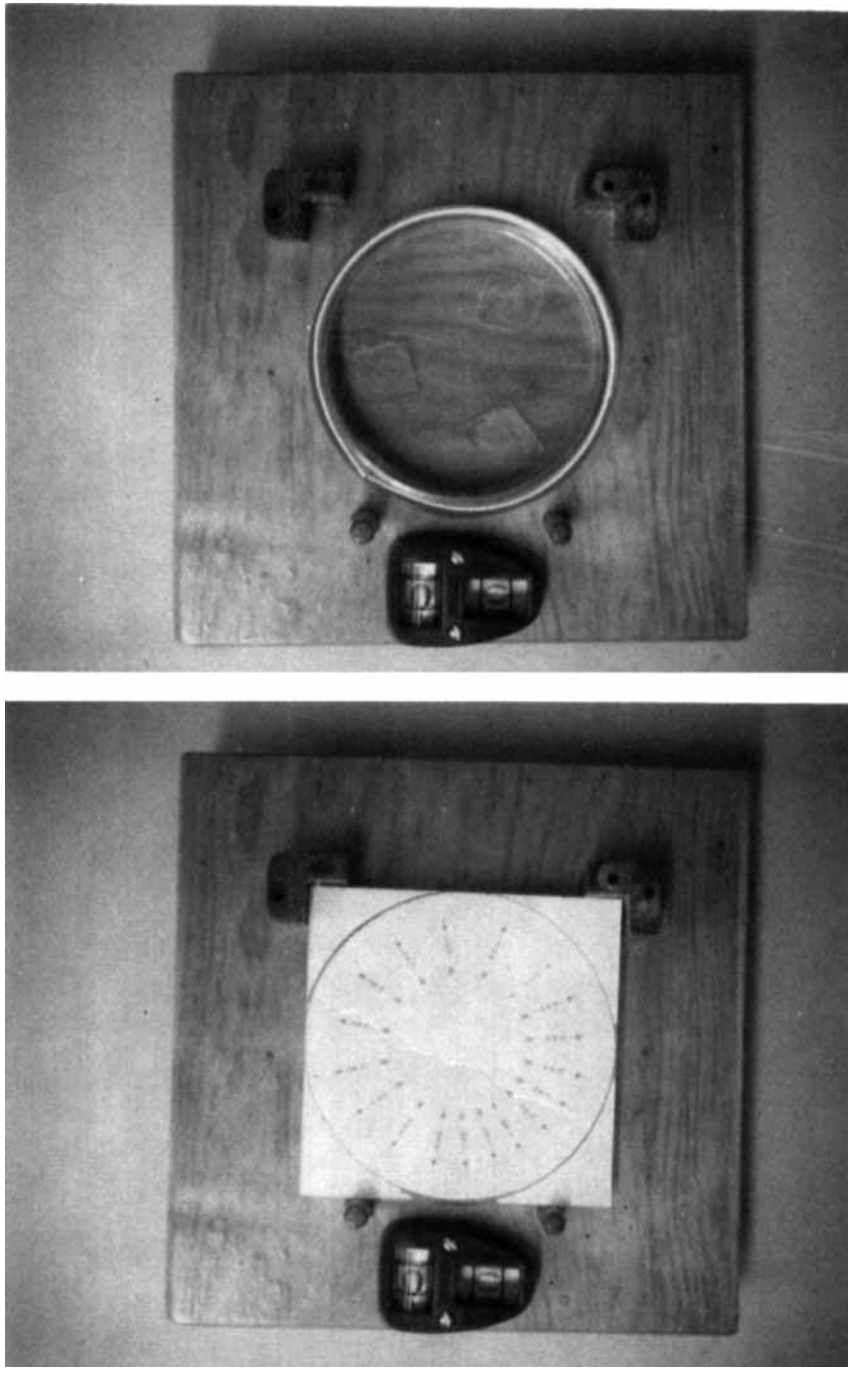


Figure 3. Prototype of anticircular apparatus and platform (a) without plate and (b) with plate in place.

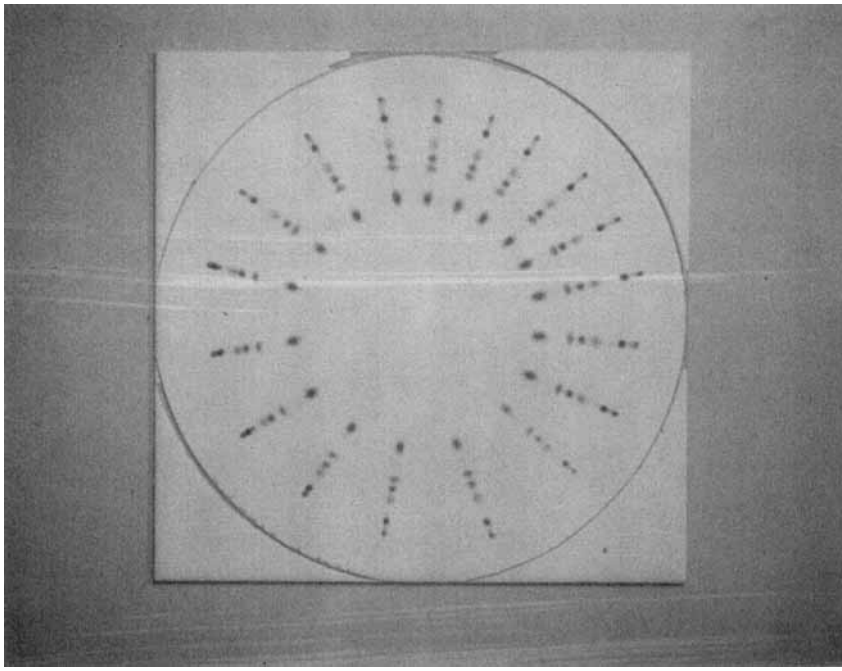


Figure 4. Separation of test dye mixture using the anticircular apparatus.

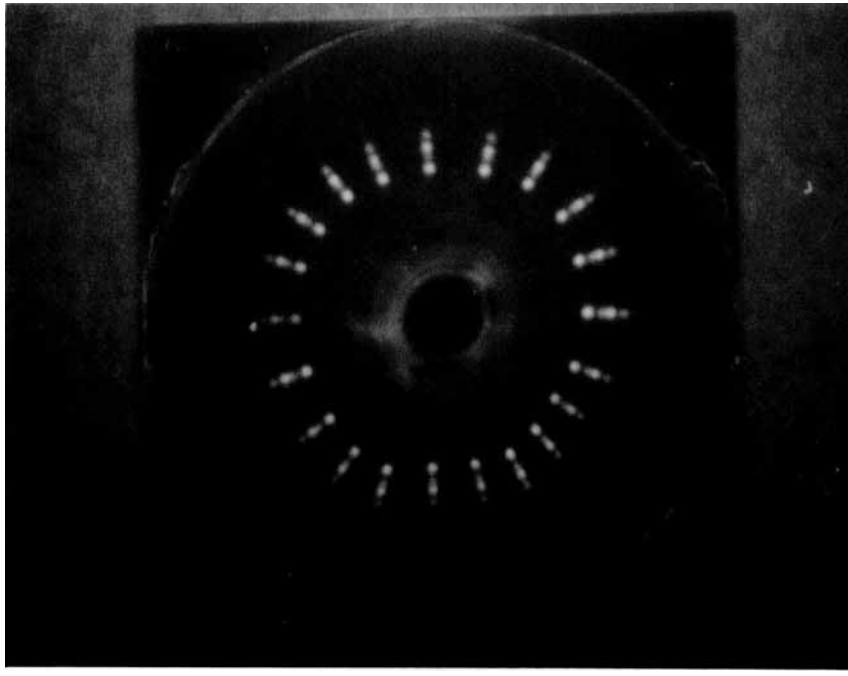


Figure 5. Separation of aflatoxins B_1, B_2, G_1, and G_2.

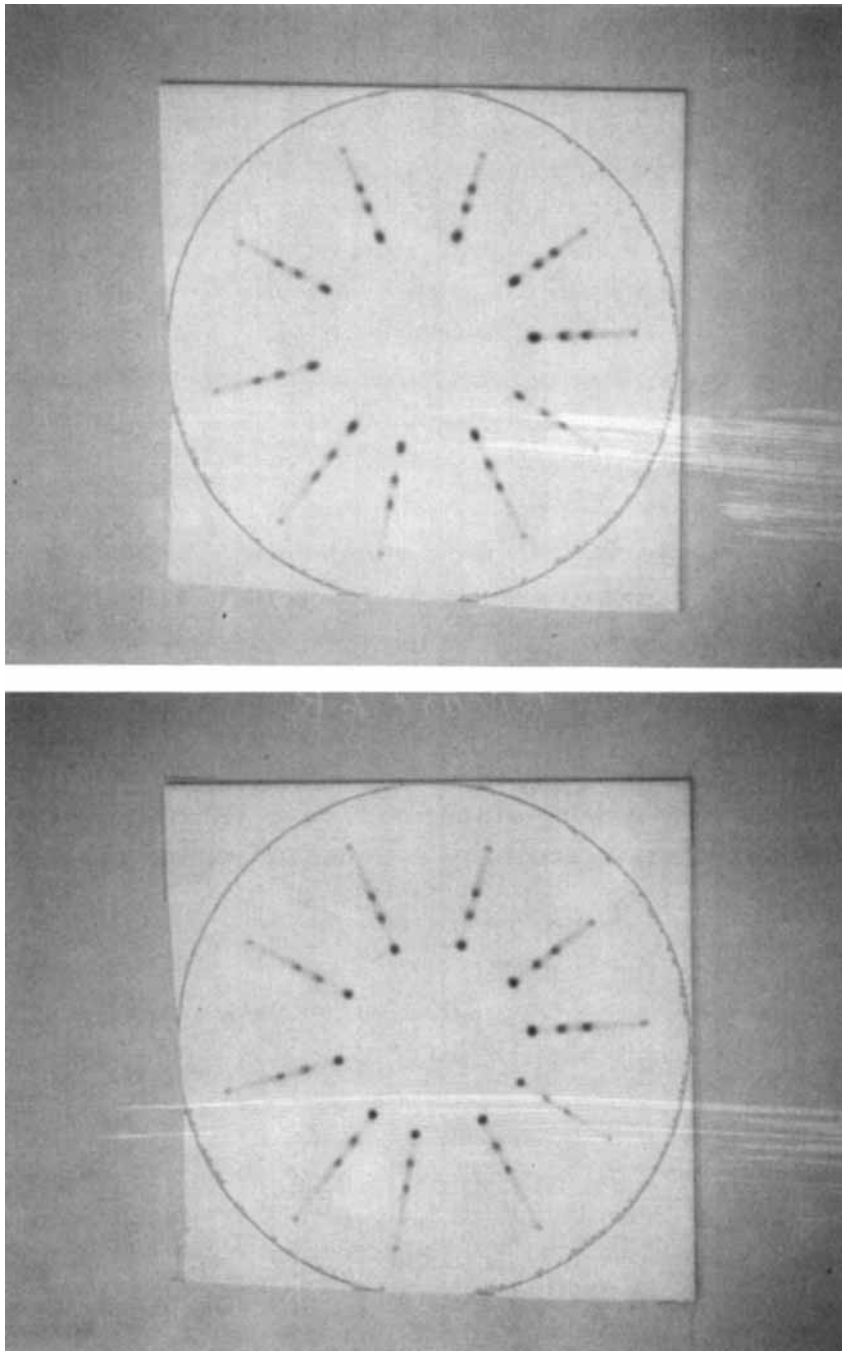


Figure 6. Test dye mixture developed (a) once and (b) twice in the same solvent system.

strong enough to provide good support for the plate while remaining in contact with the solvent. Any conditioning solution is placed in the inner dish, and the eluting solvent in the outer. If a conditioning solution is not needed, it is replaced by 2 ml of the developing solvent to aid in saturation of the atmosphere, reduce solvent evaporation off the plate, and assist in the production of compact and reproducible spots. The platform ensures even distribution of the solvent and reagent in the dishes, which results in regular and even development. The results of the development of a dye mixture on a 10 x 10 cm plate are shown in Figure 4. The apparatus was also employed in the separation of aflatoxins B_1, B_2, G_1 and G_2 (Fig 5).

Anticircular development tends to produce elongated spots at high R_f values. This is especially true when large sample volumes are spotted, and resolution can be affected. To overcome these problems, the plate is developed twice in the same same solvent system (Fig 6).

CONCLUSION

The system described is simple, versatile, and gives reproducible results at low cost. It can be assembled easily, and its operation requires no special skill. The two compartment apparatus allows the use of reagents without distrubing the the eluting solvent.

ACKNOWLEDGEMENTS

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REFERENCES

1. Van Dyk, H, Chimia 24, 234 (1970), (As reported in ref. 2-4).
2. Dyne, V..J.R. and Vetters, A.F., J. Chromatogr. 103, 177 (1975).
3. Kaiser, R.E., HRC and CC, 1, 164 (1978).
4. Kariko, K., and Tomasz, J., HRC and CC, 2, 247 (1979).