SEMI-AUTOMATIC THIN-LAYER CHROMATOGRAPHY SAMPLE APPLICATOR*

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INTRODUCTION

One reason for the considerable increase in the use of thin-layer chromatography (TLC) over the last few years is that it provides a relatively fast, easy, and effective means of separating and cleaning up highly complicated mixtures such as those derived from samples of biological origin[1]. Thus it is generally possible to concentrate the compounds of interest in narrow sections of a TLC plate. These can then be removed and quantitatively analyzed by a variety of methods.

The efficiency of the procedures involved in locating the exact boundaries of these regions is entirely dependent on the sharpness of their separation. However, because TLC plates have a limited capacity, incomplete separation of adjacent spots may occur with relatively small loads. Thus with the currently used 0.25 mm layers, spots should not contain much over 1 μg of material. One is led therefore, to the repeated spot loading of dilute solutions to ensure both a sharp separation and the recovery of enough material for subsequent analysis. Ideally, the volume of test solution containing the required amount of compound should be applied over a stretch of the starting line long enough to ensure a relatively low density per cm. The resulting chromatogram should show neatly separated horizontal bands. Attempts to achieve this result are usually frustrated when the solution is applied by hand. Unavoidably irregular loading of the test solution then leads to jagged, distorted bands: the very purpose of a time-consuming operation is thereby completely defeated.

The instrument described below provides an answer to this problem. An adaptation of the PB 600-1 Auto-Delivery unit (Hamilton Co., Los Angeles, Calif.), it was designed for the precision repetitive loading of TLC plates.

Sensitive quantitative methods of analysis (GLC, U.V. and I.R. spectroscopy, etc.) seldom require more than 10 μg of any test compound for optimal accuracy. On 0.25 mm TLC layers, bands from 4 to 5 cm long which contain this amount remain narrow enough after development to ensure neat separations (Fig. 1). The present instrument (Fig. 2 and Fig. 3) was designed to produce such bands by the spotwise application of 50 μl volumes of test solution over 4 cm lengths of the base line. Three samples can be applied on a single 20 × 20 cm TLC plate by filling each of the syringes with a different sample solution. The samples can be applied almost simultaneously

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by actuating in quick succession each Auto-Delivery unit, once for every position of the TLC plate. By shifting this position by regular increments, an evenly distanced series of overlapping spots is obtained under each syringe. These overlapping spots approximate very regular 4 cm long solid lines and produce thin parallel bands upon development (Fig. 1)

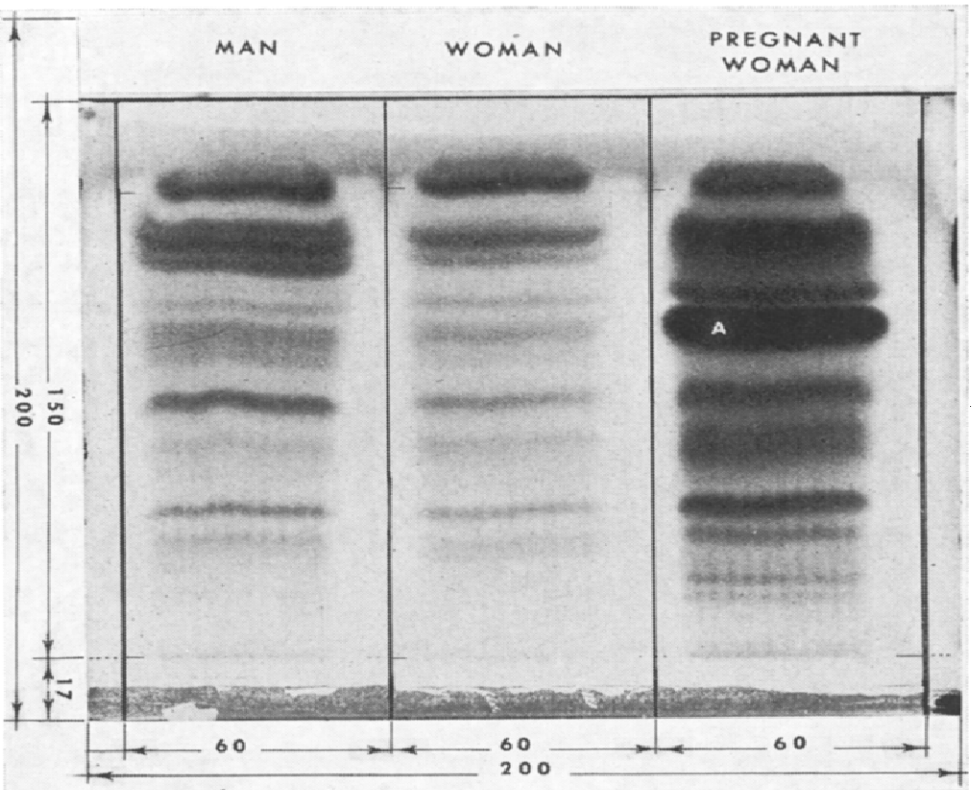

Fig. 1. Dimensions in mm. Photograph of plate showing the separation of urinary steroids. Band A shows considerable spread due to overloading: this band contains 50 μg of pregnanediol. Note parallelism and sharp separation of bands of lower content. Conditions for TLC: *cf.* ref. 1, solvent system I.

DESCRIPTION OF THE INSTRUMENT

The sample applicator (Fig. 2 and Fig. 3) consists of 4 main parts: syringe stand, A; base plate, B; plate carrier, C; and carrier motion mechanism, D.

Syringe stand A

This is made of hard, polished steel and consists of three uprights, 1, fastened to a horizontal shaft, 2. A vertical adjustment sleeve, 3, slides over each upright. To each sleeve, a Hamilton Auto-Delivery unit, 4, is fastened by screws. The sleeves can be locked in position by vertical adjustment screws, 5. Adjustment of the sleeves brings all syringe needle tips to the same level (see below). Each Auto-Delivery unit carries a 50 μl Hamilton syringe (705 N), 6, held in locking collar, 7. Each time the impulse knob, 8, is depressed, the syringe piston, 9, will advance 1/50th of its total course, each time expelling a 1-μl drop. The piston is linked by setscrews, 10, to the piston advance rack, 11, of the Auto-Delivery unit. Micrometric adjustment screw, 12, permits the precise location of all needle tips in relation to the surface of any TLC plate (see below).

Base plate B

This is made of anodized aluminum and is connected to syringe stand, 1, by bearings, 13, in which stand shaft, 2, can rotate when the pressure of lockscrews, 14, is released. The stand can then be made to rest backwards at 30° from the horizontal (Fig. 3). In this position the syringes are fully accessible for filling or cleaning. Forward movement of the stand is arrested at a position close to the vertical (operating position) depending on the adjustment of micrometric screw, 12. This adjustment, by slightly modifying the inclination of the stand in its upright position brings all syringe needle tips to the desired level (see below). The stand can be locked firmly in operating position through screws 14.

Plate carrier C

The beveled sides of aluminum plate carrier C slide smoothly in grooves of the base plate. The bottom edge of TLC plate 15 is held against a ridge in the carrier by the pressure of spring 16. Thus a lateral displacement of the carrier will bring about a corresponding displacement of the TLC plate.

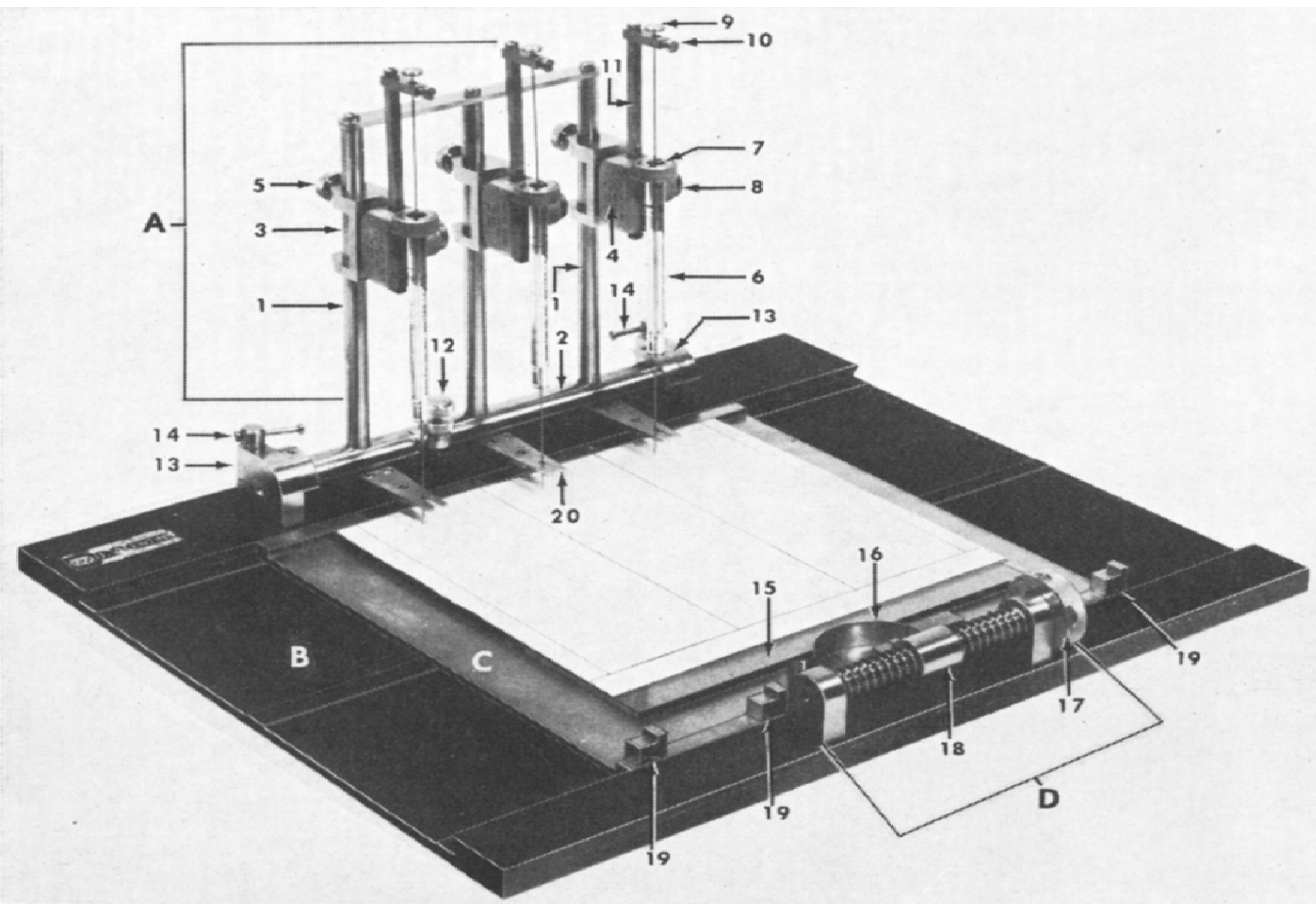

Fig. 2. TLC applicator: operating position of syringe support.

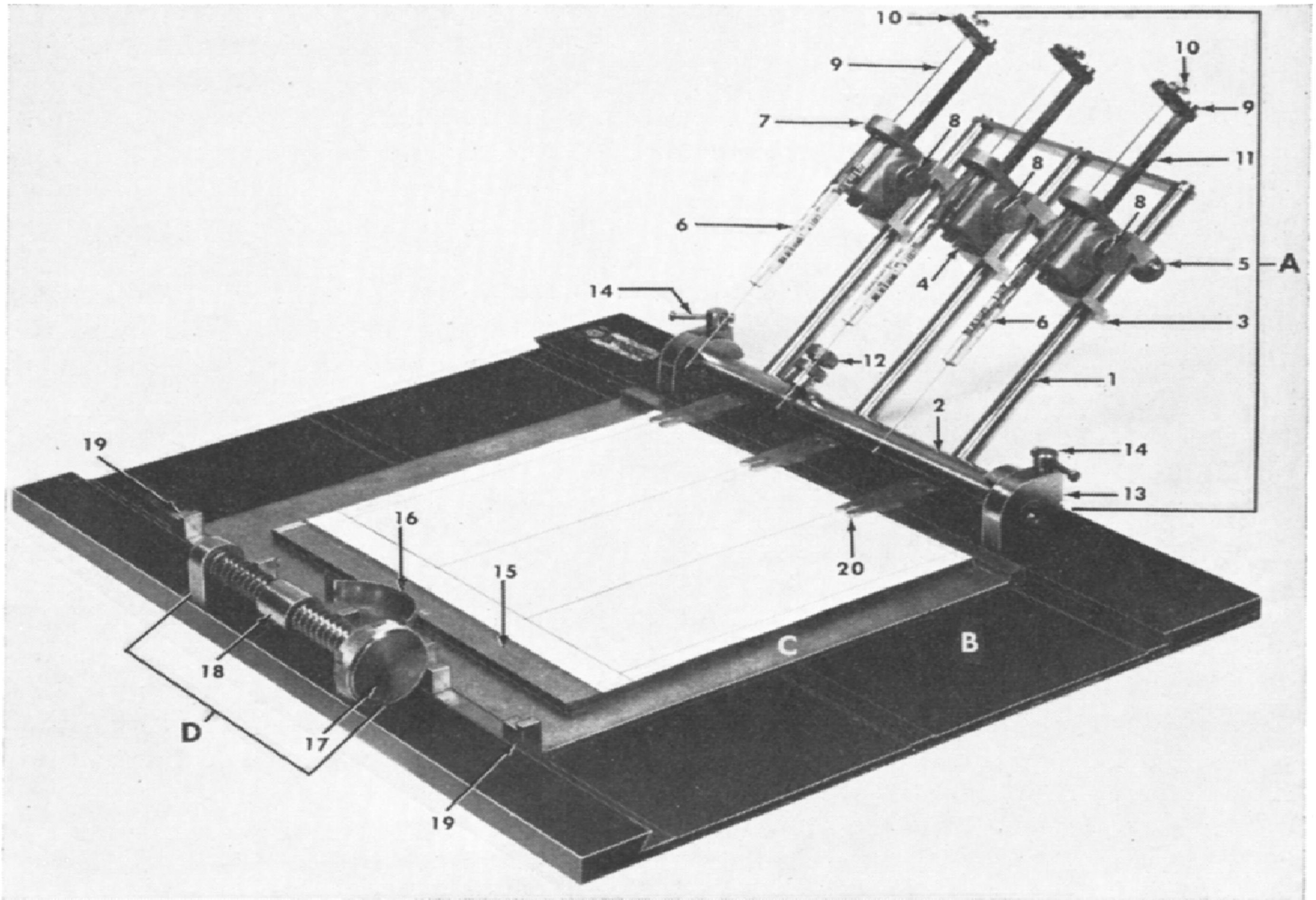

Fig. 3. TLC applicator: position of syringe support for filling (or rinsing) syringes.

Motion mechanism D

This consists of knurled knob, 17, actuating a screw. Rotation of knob 17 will impart a longitudinal motion to rider 18, and consequently to TLC plate 15, when rider 18 and one of carrier connector, 19, are engaged. Upon being rotated, knob 17 snaps in positions exactly corresponding to each 1/5th of a turn. Concomitantly, the figures 1, 2, 3, 4, 5, engraved on the periphery of knob 17, successively appear at the top. Each complete turn of the knob corresponds to a 4 mm displacement of the TLC plate.

Needle guides, 20, correct any deviation of the needles from the vertical and protect them from accidental displacement.

PROCEDURES

Selection and marking of plates

It is well known that commercial TLC glass plates are not of uniform thickness. This has led to the development of layering systems (Quickfit, Shandon, Kensco, and others) whereby plate uniformity is no longer an important factor. A selection of plates does not involve, however, considerable time and effort since precise measurements at the four corners of several dozen plates can be made inside of 1 hour with a micrometer. From the measurements marked on the plates it will then be observed that very few plates show 4 different measurements. For most of them, the thickness will be the same $\pm$0.01 mm, at the corners of opposite sides. Usually there is a low side of uniform thickness, say 3.75 $\pm$ 0.01 mm, and a high side, 3.90 $\pm$ 0.01 mm, for example. In any case it is possible to mark distinctly and permanently the 2 sides of uniform thickness and to use either of these as top or bottom sides in all subsequent operations. This simple selection greatly facilitates operations with the present instrument.

The marking of layered plates, such as indicated on Fig. 1, is a helpful process which is most quickly and conveniently achieved by the use of a plastic template. Four equally spaced vertical grooves and one horizontal groove are traced through the adsorbant layer; vertical grooves are traced at 1 cm from the edges of the plate, and the 18 cm interspace is divided in three 6 cm strips; the horizontal groove is traced at the level selected for the solvent front at the end of development.

Adjustment of needle tips

The first adjustment consists in bringing all needle tips to the same level. A bare TLC plate, the *thinnest* in the lot to be used, is positioned in plate carrier C and brought under the needle tip position. The plate must be placed with its low side parallel to the base line. Micrometric screw 12 is turned fully counterclockwise. The Auto-Delivery units are fastened high and syringe support A is locked by screws 14 in a vertical position. By unscrewing 5 and cautiously lowering the corresponding Auto-Delivery unit, each needle tip is made to just contact the glass plate; the unit is then locked by screwing 5. This adjustment is permanent for any given set of syringes.

The additional adjustment required with any plate from the lot is made as follows: lockscrews 14 are undone and micrometric screw 12 is given several turns clockwise. Syringe support A is moved backwards. The plate to be loaded is placed in operating position on carrier C. This involves depressing spring 16 with one finger and slipping the plate exactly in the middle of the carrier using a reference mark. The operator then moves the carrier to bring the farthest corner of the plate under the syringe position closest to him. This corner is cleaned from adsorbant and a small piece of 0.30 mm shim brass is placed upon it. Syringe support A is then brought forward and micrometric screw 12 is turned counterclockwise until the syringe needle tip just contacts the piece of shim brass. Syringe support A is then brought fully backwards.

Loading the syringes

With syringe support A fully backwards, as shown in Fig. 3, screws 10 are undone and racks 11 are pushed downwards by the pressure of the index finger until completely depressed. Small tubes containing sample solutions can be slipped over the needles and the syringes can be filled. In each case the meniscus is brought exactly to the 50 μl mark, rack 11 is pushed fully upwards (Figs. 2 and 3), and screw 10 is tightened.

Plate positioning

The carrier is made to occupy a position on the base plate indicated by a mark.

This position is obtained when the first syringe needle tip is about 1 cm inside the first 6 cm strip. Knob 17 is then turned until runner 16 can engage with the carrier connector 19 located near the end of the screw. With 16 and 19 engaged, knob 17 is rotated until figure 1 appears at the top (snapping action).

Loading operation

Syringe support A is brought forward as far as the setting of micrometric screw 12 will allow. It is locked in this position by screws 14. The Auto-Delivery units are then actuated in quick succession and knob 17 is made to rotate clockwise a full turn, *i.e.*, until figure 1 engraved on the knob again snaps in top position. The Auto-Delivery units are then actuated again in quick succession. For each complete turn of knob 17 another drop is laid under each syringe needle tip, forming a spot located 4 mm, center to center, from the preceding one. In all, 10 spots are laid under each syringe to complete the first pass. The carrier is brought back to its original position by turning knob 17 ten turns counterclockwise. However, Fig. 2 is now made to appear at the top and used as reference (full turn) to lay another series of 10 spots for each syringe, as described for the first pass. A third pass (Fig. 3) a fourth (Fig. 4), and a fifth (Fig. 5) complete the operation. In each pass the spots are made 0.8 mm ($1/5 \times 4$ mm) further than in the preceding one. In all, 50 regularly distanced spots (0.8 mm from center to center) are laid under each syringe.

DISCUSSION

In the procedure described, spots are laid 4 mm apart in 5 passes to avoid the distortion effect obtained when drops are laid on wet adsorbant, as would occur if they were laid too close in quick succession. The described procedure allows time for spots to dry before a new series is laid.

The complete operation including the preliminary adjustment of needle tips, the filling of syringes, the application of samples, and a final rinsing of the syringes with solvent can be made in less than 5 min. As demonstrated by the author[2,3], little manual dexterity or practice is required to operate this instrument.

The instrument can be used in a variety of ways. If a larger amount of material is required than what can be extracted from 4 cm bands, longer bands can be obtained by the use of 2 or 3 syringes filled with the same solution; the quantity can be further augmented by the use of longer preparative type TLC plates now commercially available. On the other hand, up to 6 different samples can be placed on the same 20×20 cm TLC plate by applying 25 μl of solution to 2 cm lengths of the base line. Also the 50 μl syringes can be replaced by Hamilton syringes of different capacity.

Contact with syringe needle tips with adsorbant may lead to plugging of the needle. This can be effectively avoided by slipping over each needle tip a 0.25 in. length of 28 gauge Teflon tubing (Hamilton) with about 1 mm of the tubing protruding. Teflon prevents damage to needle tips that may result from accidental contact with plates. Adsorbant, if picked up, is clearly visible and can be eliminated by removing and replacing the protective Teflon. In addition, Teflon helps in the formation of perfect droplets by preventing creeping of the solution over the needle. Levelling of needle tips must be made after placing the Teflon protection.

ACKNOWLEDGEMENT

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SUMMARY

A compact, versatile instrument for the rapid semi-automatic, precise strip loading of TLC plates is described.

REFERENCES

1 F. A. VANDENHEUVEL, G. J. HINDERKS AND J. C. NIXON, *J. Am. Oil Chemists' Soc.*, 42 (1965) 283.
2 F. A. VANDENHEUVEL, *Symp. Modern Lipid Methodology, Claremont, Calif., August 9–13, 1965.*
3 F. A. VANDENHEUVEL, *Intern. Course Methods Lipid Res., 2nd, Milan, September 16–25, 1965.*