Using cyanoethylpolysiloxane on Gas Chrom P, TURNER *et al.*[3] also reported promising results in the estimation of pregnanediol in pregnancy urine. Their conditions did not separate pregnanediol from pregnanolone however. Some preliminary data on urinary pregnanediol separations have been given by PATTI *et al.*[4] with SE 52 columns.

The results presented above indicate that gas chromatography can be used for rapid and accurate analysis of pregnanediol in pregnancy urine, following a very simple hydrolysis and extraction procedure. Further work is in progress to establish fully the specificity, accuracy and reproducibility of the procedure as a standard analytical technique for pregnanediol estimation.

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Received July 19th, 1963

Notes

Devices for continuous development and sample application in preparative thin-layer chromatography

The resolution of closely related substances by thin-layer chromatography may often be improved by decreasing the polarity of the solvent system to a point where the R_F values are less than 0.1. Either repeated or continuous development is then required to obtain appreciable mobilities and complete separation. Repeated development has the advantage of being technically simple and, because the lower parts of the zones are readied first by the new solvent front, tailing effects are reduced. However, a disadvantage is the long drying time required between developments when relatively thick layers are used for preparative work.

In paper chromatography, continuous development is usually carried out by the descending overflow technique. Methods for descending development of thin-layer chromatograms have been described[1,2], but the zones are broader than those obtained by ascending development[2] and special apparatus is required. Two methods of continuous development based on evaporation of the solvent from the terminal edge of the plate have been reported[2,3]. BRENNER AND NIEDERWIESER[3] used a hori-

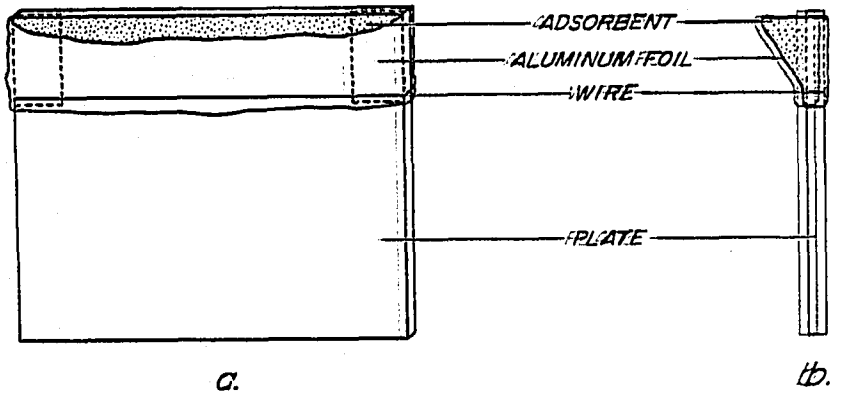

Fig. 1. Device for continuous development of thin-layer chromatograms, (a) front view; (b) side view.

zontal development technique in which solvent is fed to the plate by a paper wick, evaporation from the surface of the plate being prevented by an overlaid glass plate. This method does not require a developing chamber and it has been used successfully with preparative plates, but the development time is about twice that required for ascending development[4]. ZÖLLNER AND WOLFRAM[2] used ascending development in a chamber kept partly open to allow evaporation from the top edge, while preventing evaporation in the lower part of the plate with solvent-soaked paper. This arrangement leads to changes in the composition of solvent mixtures due to differential evaporation and is therefore best suited for use with a single solvent.

We have been using a method of continuous development which requires no

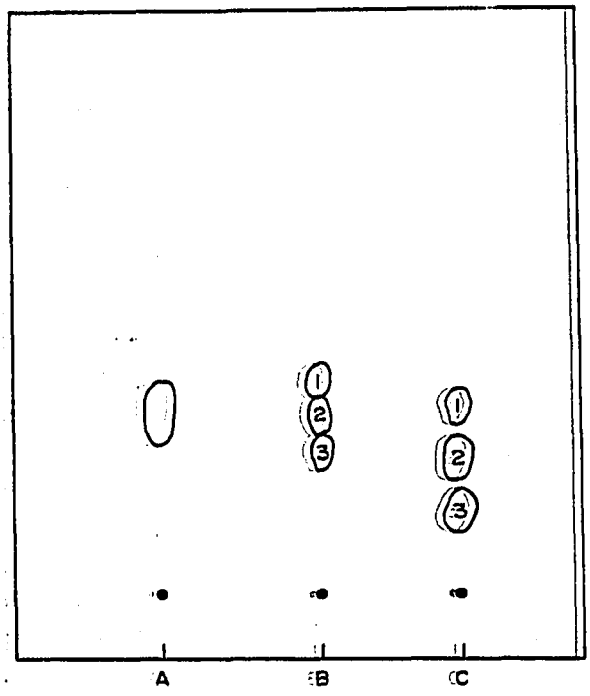

Fig. 2. Separation of 1 μg each of β-sitosterol acetate (1), cholesterol acetate (2), and stigmasterol acetate (3), by (A) single 10 cm development with hexane–ether (80:20) for 9 min; (B) repeated development (five times) with hexane–ether (94:6) for 60 min; (C) continuous development with hexane–ether (97:3) for 120 min; on Anasil B plates, 275 μ thick.

special apparatus, can be used with plates of different sizes, and is adaptable to either analytical or preparative thin-layer chromatography. It consists essentially of a trough of aluminum foil, filled with loose adsorbent, which is attached to the top of the plate. The trough is tied to the plate with a thin wire and the sides of the aluminum foil are folded around the edges of the plate (Fig. 1). Enough adsorbent can be placed in the trough to allow Silica Gel G* plates to be developed overnight, although 6–8 h is usually sufficient. The adsorbent can, of course, be reused after drying.

Fig. 2 shows the differences in separation of three sterol acetates by the usual ascending technique, by repeated development, and by continuous development. It will be observed that, although continuous development causes the spots to become more diffuse, the separation is better than by the other two methods. It is interesting that cholesterol acetate and β-sitosterol acetate, differing only by an ethyl group in a saturated side chain, can be separated by adsorption chromatography on Anasil**, but not on Silica Gel G[5].

We have found this method of continuous development especially useful in preparative thin-layer chromatography, based on the procedures of HONEGGER[4] with the following modifications. Rhodamine 6 G*** (0.1 mg per plate) is dissolved in the water used to prepare the slurry of Silica Gel G. The plates, usually 1 mm thick, are first developed with acetone to move extractable material to the top edges. The solvent systems (100–250 ml) are allowed to equilibrate for 30–60 min in the developing chambers**** (30.5 × 9.9 × 27.6 cm), which are lined completely with Whatman No. 3 MM paper. After development and drying, the bands are located and marked under short-wave ultraviolet light. Less than 1 mg of compound spread across a 200 × 200 mm Silica Gel G plate, 1 mm thick, can be detected by this method. The bands are scraped off the plate, placed in a chromatographic column, and eluted with acetone. Enough of the dye is eluted to produce a light yellow color, but this is easily removed in the further purification steps.

In many cases a micro pipet is satisfactory for the application of the sample solution along the starting line. However, if the mobilities of the compounds are markedly dependent on their concentrations, the zones will be irregular, as it is very difficult to achieve even distribution across the plate by this method. We have found an easily constructed applicator to be useful in such instances. This device, shown in Fig. 3, is assembled as follows. One side of a 75 × 50 × 1 mm microscope slide is covered lengthwise with pressure-sensitive tape§ to a thickness of about 0.5 mm, except for an area of about 18 × 75 mm. Another slide is placed over it and secured by tape, making certain that the lower edges of both slides are exactly even and free of imperfections. Two such applicators are mounted in a holder, prepared as follows. Tape is wound around the ends of two glass rods, 200 mm long, to a thickness of 2 mm. The rods are then bound together at the ends with tape. The applicators are supported in this holder by metal clips in such a way that they can move freely up and down but cannot fall through; a narrow piece of tape wound around the middle of the holder keeps them separated.

** Brinkmann Instruments Inc., Great Neck, New York.
*** Analytical Engineering Laboratories, Inc., Hamden, Conn.
**** Fisher Scientific Co.
§ Time Tape, Professional Tape Co. Inc., Riverside, Ill.

The lower edges of the applicators are now dipped into the solution of sample to be applied. The solution is taken up by capillary action, which may be regulated by varying the distance between the slides with a suitable number of layers of tape. The applicator is then positioned just above the starting line on the plate and the

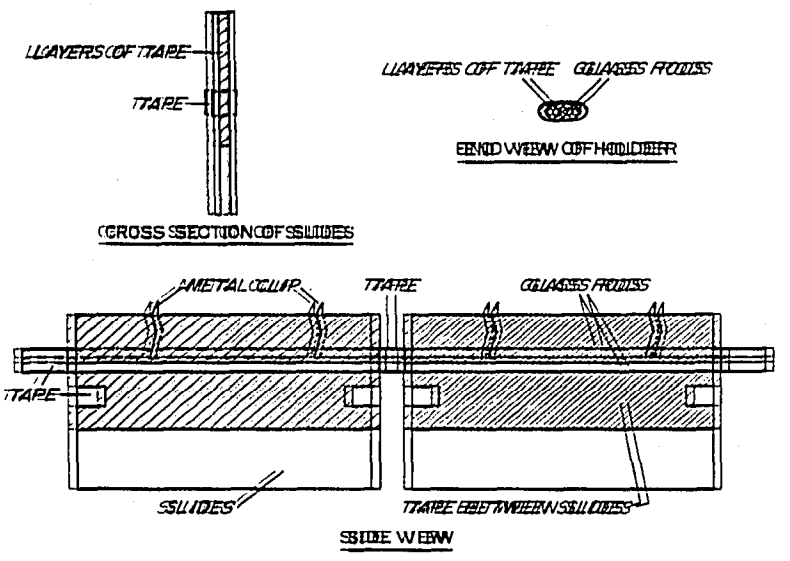

Fig. 3. Sample applicator for preparative thin-layer chromatography.

glass rods, held by the ends, are *rapidly* dropped so that the lower edges of the slides rest flat on the plate. If this operation is carried out rapidly enough, the edges will rest evenly on the plate and the solution will flow out in two even bands. There is little or no disturbance of the adsorbent surface, even with the more fragile Anasil layers. This operation can be repeated as many times as necessary to transfer all of the sample to the plate.

To test the efficiency of the application and elution steps, 50 mg of cholesterol acetate was applied to a 200 × 200 mm Silica Gel G plate, 1 mm thick, using the apparatus described above. After development with dichloromethane and drying, the zone was located, scraped off the plate, and eluted with acetone, 99 mole %, pure* (about 50 ml). Evaporation of the acetone left 48 mg of cholesterol acetate, homogeneous by analytical thin-layer chromatography.

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Received February 25th, 1963

* Fisher Scientific Co.