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Note

Semiquantitative determination of trace substances in the ng/g (ppb) range by means of thin-layer chromatography

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By increasing the application volumes from microlitres to 5–50 ml per spot it is possible to detect and determine semiquantitatively trace substances in the ng/g (ppb) range by means of thin-layer chromatographic (TLC) techniques.

EXPERIMENTAL

Apparatus and method

A simple device allows the application of relatively large volumes onto silica gel 60 F_{254} Merck (Darmstadt, G.F.R.) thin-layer plates (Fig. 1). This method is based on known procedures, but adapted to the needs of TLC[1–8]. The continuous procedure is carried out under a permanent air draught in a fume-hood. The size of the spot is intentionally influenced by the rate of air circulation (adjustment of fume-hood window). The silica gel layer is marked and scratched with a metal pointer as shown in Fig. 2. Up to one blank and three standard samples may be spotted onto a single 200 × 200 mm plate.

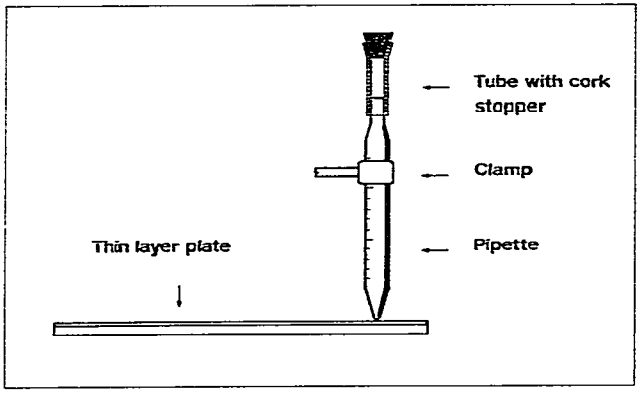

Fig. 1. Application of sample and reference solution, respectively.

Procedure

The marking lines on the plate are made by carefully and repeatedly scraping off the silica gel with a metal pointer (Fig. 2). The plate is then cleaned with a brush.

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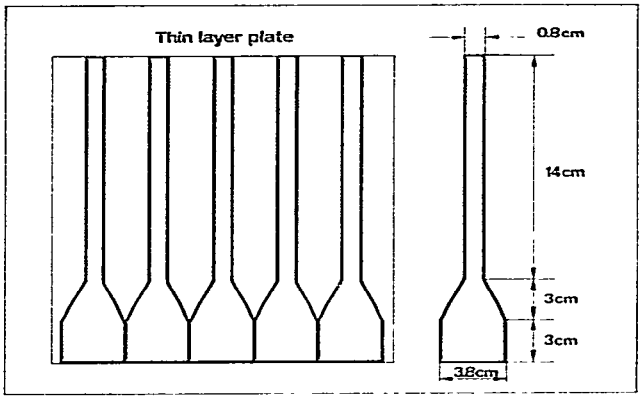

Fig. 2. Marking and separation of layer.

The sample is dissolved in a solvent with as low a boiling point as possible (*e.g.*, chloroform). The pipette is filled and closed at the top (Fig. 1). Excess of solution is removed by dispensing it onto a filter-paper until the pipette is filled to the mark.

The pipette is then placed at the start of the first track on the prepared thin-layer plate (Fig. 3). The pipette solution immediately runs out at a slow rate, so that the spotting of 20 ml of solution takes about 3–4 h in a self-controlled procedure. Constant surveillance is not necessary; however, occasional checking may be helpful. The spot must not be allowed to become too large otherwise substance is lost due to mass transport effects along the marking lines (Fig. 3). After spotting the plate, the spots are allowed to migrate once or twice with a polar mobile phase (*e.g.*, acetone) from the original into the second starting region (Fig. 3). Unwanted phenomena, such as formation of a ring of the concentrated substance and alteration of the R_F values due to impurities, are avoided by the following procedure.

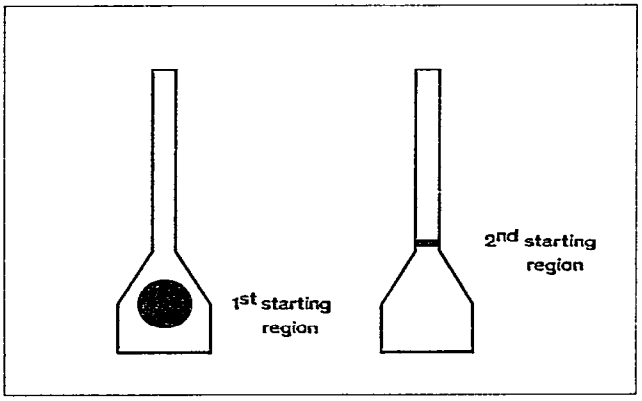

Fig. 3. Transfer of a single spot on a narrow silica gel strip.

After the substances are concentrated in the second starting region, both border lines near the spot are eluted with small quantities (*ca.* 10 μl) of the solvent used to concentrate the probes. With this method the tailing along the scratched lines

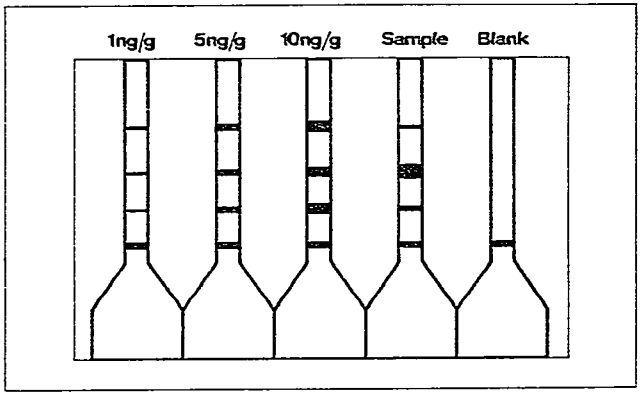

Fig. 4. Scheme of TLC separation with a mixture of dyestuffs. 1 = Irganol brilliant violet base; 2 = terasil brilliant red; 3 = cibazet violet; 4 = unknown.

is reduced or even completely avoided. After drying for a short time, the chromatogram is run in the usual way (Fig. 4). In order to ensure an optimum supply of mobile phase and to achieve a good separation, the plate must be immersed *ca.* 5 cm into the eluting solvent.

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