

A method for sample application in preparative thin-layer chromatography*

The resolution obtained by thin-layer chromatography (TLC), and the speed and simplicity of TLC have prompted many attempts to use it for preparative purposes. Successful preparative TLC requires uniform application of the sample as a band of narrow width at the starting line. Several methods for sample application have already been described¹⁻⁵. A simple method for rapid and controlled application to one plate of more than 1 ml of solution without damaging the layer has not been reported previously and is described below. This method is inexpensive, easy to use and requires components which are readily made.

The key component used in the present method is a needle guide. Fig. 1 shows the needle guide secured by a set screw to a hypodermic needle the tip of which was ground flat. The needle is connected to a 2 to 5 ml syringe with Luer-Lok tip and inserted in a slot (6.5 mm wide \times 200 mm long) milled in a spotting plastic template (available from Research Specialties Corporation). The position of the needle guide on the needle is adjusted so that the tip of the needle is about 1 mm above the surface of the layer. The needle is thus prevented from direct contact with the layer.

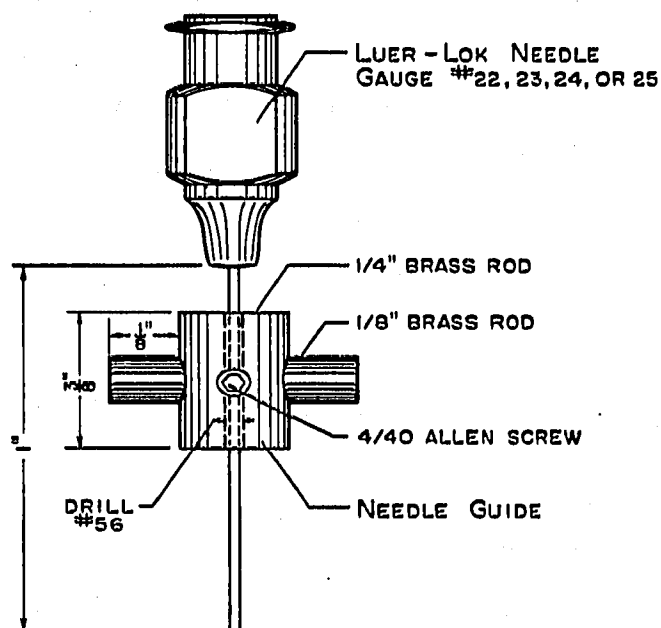


Fig. 1. Needle guide with needle attached.

The syringe is filled and placed in position with the arms of the needle guide resting on the template 2-3 cm from one edge of the plate. The syringe is slanted at 45° from vertical, in the direction of application, and moved at an even rate across the plate while even pressure is exerted on the plunger with the index finger. A fine jet of solution is thus placed on the plate as in the method of RITTER AND MEYER⁶. The arms of the needle guide must be in contact with the template and the body of the needle guide must remain in the slot at all times during sample application, to steady the needle. Application of the solution is stopped 2-3 cm from the edge of the plate and resumed on the opposite side after the solvent has evaporated from the layer.

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Failure to wait for solvent evaporation results in an increase in the width of the sample band. The rate of solvent evaporation may be increased by heating the plate during sample application and by blowing warm air over the surface of the layer.

The method of sample application described has been used successfully for over one year with 20 cm × 20 cm plates of silica gel G of 2 mm thickness. An example is the separation of the naturally occurring pigments javanicin and fusarubin⁷ which involved a difficult problem of sample application because of the lack of solubility of the pigments. Six milliliters of a saturated solution of 100 mg of pigments in chloroform was applied to each plate. A further example is the separation on one plate of a 200 mg mixture of equal amounts of (a) azobenzene, (b) *p*-methoxyazobenzene, (c) Sudan Yellow, and (d) *p*-aminoazobenzene. The time required to apply the solution (2 ml) using a gauge 24 needle was 5 min. The sample band was 15 cm long and 7.0 to 8.3 mm wide. The mixture was separated into four components by ascending development using 2 passes of benzene as developer. The position of the horizontal bands in cm from the starting line was as follows: (a) 13.7–15.2, (b) 9.9–12.3, (c) 4.3–7.3, (d) 1.6–4.3.

It is suggested that the technique described for sample application be used to streak preparative thin-layer chromatograms with an appropriate reagent in order to visualize colorless bands.

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Detection of minute traces of blood by thin-layer chromatography

A thin-layer chromatographic method has been developed for the rapid detection of traces of blood in the microgram range. The method is described below.

The material under test and suspected of containing blood is eluted with distilled water or an 0.85 % solution of NaCl. 0.005–0.01 ml of this aqueous solution is applied directly on to the thin layer.

Experimental details

Adsorbent: Kieselgel G (Merck).

Layer thickness: 250 μ .

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