Acknowledgement

The authors are indebted to Prof. RAYMUNDO MONIZ DE ARAGÃO for suggesting this research.

*Biochemistry Department, National School of Chemistry, University of Brazil, Rio de Janeiro (Brazil)**

NORMA O. DE SOUZA
ANITA PANEK

[1] E. STAHL AND U. KALTENBACH, *J. Chromatog.*, 5 (1961) 351.
[2] M. GEE, *J. Chromatog.*, 9 (1962) 278.
[3] N. NELSON, *J. Biol. Chem.*, 153 (1944) 375.
[4] E. J. BOURNE, N. HAWORTH, A. MACEY AND S. PEAT, *J. Chem. Soc.*, (1948) 924.

Received January 8th, 1964

* Address: Laboratorios de Microbiologia, Escola Nacional de Química, Av. Pasteur 404, Rio de Janeiro, Brasil.

Sample application in preparative thin-layer chromatography

The successful resolution of mixtures on preparative thin-layer chromatoplates requires uniform application of the sample with respect to both concentration and shape of the starting zone. Uniformity of concentration has been attained by application of a solution of the mixture from a travelling syringe[1]. Recently a much simpler device was described[2] but this suffers from the drawback that a maximum volume of *ca.* 0.3 ml of solution can be added with a single application. Despite careful handling of the applicator, it is not possible to avoid slight irregularities in the shape of the starting zone on repeated application to the same position on a plate. These irregularities become exaggerated as the zones migrate so that separation of compounds with $\Delta R_F < 0.3$ is seldom efficient, especially with quantities greater than 20 mg of mixture per plate (20 × 20 cm). We have found that uniform migrating zones, and efficient separation of compounds with ΔR_F as low as 0.1, are obtained from the narrow, straight-edged bands prepared by the following procedure:

Two parallel cuts (A, Fig. 1), *ca.* 3 mm apart, are made through the adsorbent

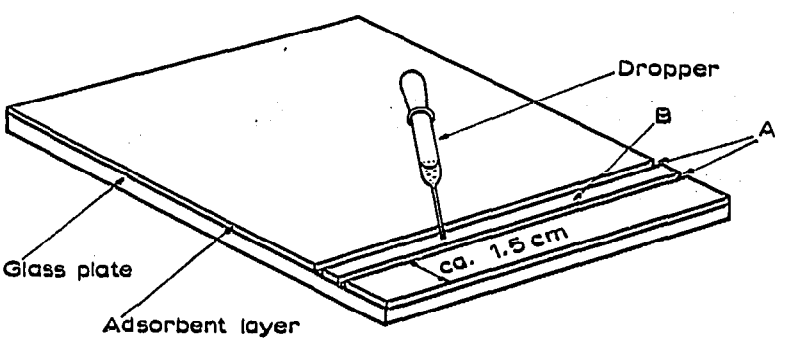

Fig. 1.

layer by means of a thin knife-blade. If these cuts are made by lightly drawing the blade over the adsorbent three or four times, and not by a single stroke, the ridge (B, Fig. 1) will not break. Blow out any loose material in the cuts and apply a solution of the mixture to be resolved, in any convenient solvent, to the ridge with a fine-tipped dropper as uniformly as possible. The ridge should be allowed to dry out thoroughly after each application of solution. The cuts are then filled with *dry* adsorbent, using an aluminium foil mask (M, Fig. 2) with a 1 mm slit (S, Fig. 2) which is

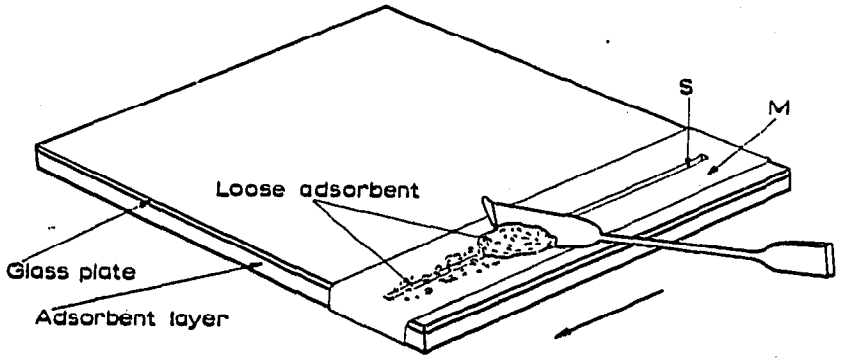

Fig. 2.

placed over each cut in turn. The adsorbent is packed by firmly drawing the back of a large spatula over the slit in the mask (Fig. 2). No special care is needed in handling these layers—the "loose" adsorbent will not fall out when the plates are placed vertically in a chromatographic tank. The chromatograms are run in the usual manner.

By this method, 50 mg crude 7-ketocholesteryl acetate, m.p. 156–158°, $[\alpha]_D$ —98°, prepared by oxidation of cholesteryl acetate with *tert.*-butyl chromate, on a 1 mm layer of silica impregnated[2] with Rhodamine 6 G and developed with benzene, gave, in less than one hour, 48 mg pure 7-ketocholesteryl acetate, m.p. 158–159°, $[\alpha]_F$ —103°, showing a single spot on an analytical thin-layer chromatogram. Material of the same physical constants, but still showing a second weak spot on an analytical thin-layer chromatogram, was obtained from the crude acetate only after six crystallisations from acetone.

The Chemistry Department, University College, Dublin (Ireland)

J. P. CONNOLLY
P. J. FLANAGAN
R. Ó. DORCHAÍ
J. B. THOMSON

[1] F. J. RITTER AND G. M. MEYER, *Nature*, 193 (1962) 941.
[2] R. D. BENNETT AND E. HEFTMANN, *J. Chromatog.*, 12 (1963) 245.

Received January 20th, 1964