

Non-destructive zone developing in thin-layer chromatography

The separation of compounds by column chromatography fails at times; however, thin-layer chromatography¹ has been found to fail only rarely*. When thin-layer chromatography is used to separate reaction mixtures to isolate the products, the location of the various compounds is a problem, for there is neither a foolproof nor a convenient non-destructive way to locate the developed zones.

We have overcome this problem in these laboratories by employing the simple technique of *spraying the developed thin-layer chromatoplates with water*. When the water-moistened plate is held against a dark background, it is found that most of the chromatoplate is translucent; however, there are bands which are not translucent, but white. These regions have been found to contain the components of the mixture. Clearer zones are sometimes obtained by first saturating the chromatoplate with water and then letting the plate dry until the zones are distinctly visible. The process may be repeated if it is necessary.

This technique has proved to be very valuable when larger amounts of compounds were to be separated by spotting the entire edge of a large (20 × 20 cm) chromatoplate. Up to 40 mg of a mixture has been separated on a plate covered with silica gel G, followed by evaporation of the solvent, location of the bands with the water spray, circumscription of the areas with a stylus, evaporation of the water by drying in an oven, removal of the absorbant and compounds by scraping the appropriate bands from the chromatoplate with a spatula, and extraction of the absorbant with an appropriate solvent.

The following mixtures were successfully separated and are illustrative:

(1) 3 mg of a cholestanone and α -cholestanol mixture, developed by a 20:20:60 pentane-ether-benzene solution.

(2) 40 mg of a 3-octyl- α -cholestan-3-ol, 3-octyl- β -cholestan-3-ol, α -cholestanol, and β -cholestanol mixture, developed by benzene first and then 30:70 ether-benzene solution.

(3) 25 mg of a *cis*-1-octyl-4-*tert.*-butylcyclohexanol, *trans*-1-octyl-4-*tert.*-butylcyclohexanol, *trans*-4-*tert.*-butylcyclohexanol, and *cis*-4-*tert.*-butylcyclohexanol mixture, developed by benzene first, and then 80:20 benzene-ether solution.

It has been found that the compounds can be water soluble and still give a white spot; however, they must not be water wetting.

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* We have not been able to separate α - and β -cholestanol from cholesterol.