Separation and Detection of Submicrogram Quantities of Pesticides by an Improved TLC Technique

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In the last few years thin-layer chromatography has developed into one of the most important techniques available for the separation and identification of components of a mixture. It has paralleled gas chromatography in importance and period of development.

The spreading and diffusion of spots, particularly at increased Rf values is often a problem with thin-layer and paper chromatography. This paper describes an approach to a procedure in which the developing spots are prevented from spreading; thereby permitting a more sensitive means of detection.

In 1962, Gamp, et. al. (2) described a procedure using ribbed glass plates for thin-layer chromatography. Such plates are not always available and they suffer from the disadvantage of being difficult to prepare with standard equipment. Uneven layers in each groove make solvent migrations uneven and

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comparisons difficult. Adjustment of the width of the strip is not possible with the fixed rib plates.

We have chosen to call the new procedure "thin-strip thin-layer chromatography" (TSTLC). The idea is to prepare plates composed of thin parallel strips of adsorbent. Each strip then functions as a separate thin-layer column or plate, yet all of the strips are equal in resolving power, as they are all developed at the same time under the same conditions.

A group of organo-phosphorus pesticides was chosen to demonstrate the technique and to show that small quantities were detectable as compared to a standard plate. An alternate procedure that will produce a similar plate, but with much thicker strips can be followed. This will provide hemi-cylindrical columns of adsorbent that are capable of carrying greater quantities of materials to be separated, yet retaining the characteristics of the previously described plates.

Experimental

Several approaches were made in the development of the final procedure described. A process now being considered is to use a cylindrical piece such as a section of large bore glass tubing in place of the glass plate. The thin strips may then be applied vertically or in a spiral up the cylinder in order to lengthen the column. A piece of tubing eight inches long by 2.5 inches diameter would provide opportunity for as many strips as an 8 x 8 inch plate, but longer strips if applied as a spiral.

The thin-layer plates (8 x 8 inch) were prepared from Silica-Gel H, by mixing 30 grams of the dry powder with 33 ml. of 0.1 N hydrochloric acid and 33 ml. of absolute methanol and applying the material to the glass plates with a Desaga-Brinkmann applicator set for a thickness of 250 microns. The plates were allowed to air dry for 15 minutes followed by oven drying at 110° C for 45 minutes.

The plates were stripped by means of a modified window cleaner as illustrated in Fig. 1.

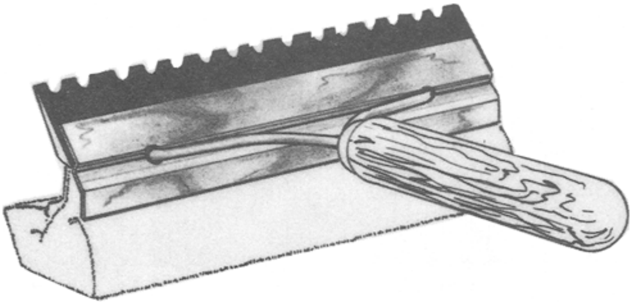

Fig. 1. Tool for preparing TSTLC chromatographic plates.

The tool was made from an 8 inch rubber squeegee, notched with a small circular file. The notches may be made by freezing the rubber at sub-zero temperatures in a dry ice-acetone bath and notching with a file upon immediate withdrawal from the bath.

By resting one edge of the plate and stripper against a smooth object such as a board, a smooth even stroke of the stripper drawn across the plate results in uniform and straight strips. Fig. 2 presents a demonstration of the appearance of plates with developed chromatograms, comparing a standard TLC plate with a TSTLC plate.

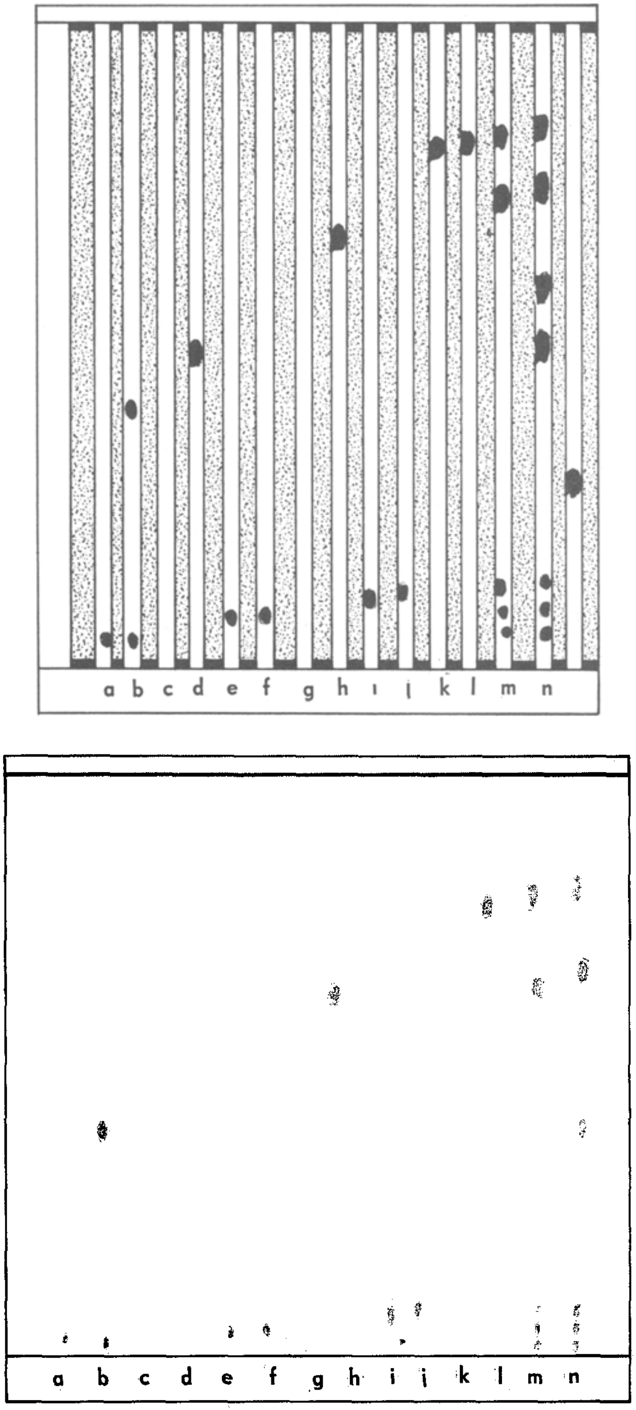

Fig. 2. Appearance of a TSTLC and a standard TLC plates with developed chromatograms. Identity of the compounds is given in the text and Table I.

A TSTLC plate and standard plate which was not stripped were each spotted with 1 and 2.5 microgram each of Systox, Parathion, Guthion, Thimet, Imidan, and Trithion respectively. The plates were then developed in a solvent mixture of 10 per cent n-hexane in toluene. Using the ascending technique the solvent front was permitted to travel 16 cm. from the original spotting. The plates were then dried by a stream of warm air and the separated spots were detected by spraying with a solution of 0.5 per cent 2,6-dibromoquinone-chlorimide w/v in methanol (3). After heating in an oven for 10 minutes at 70° C. the yellow to brown spots were easily detectable on the white background depending, of course, on the concentration of the specific pesticide. Table 1 presents the Rf values for each of these materials.

TABLE 1

Rf values for the pesticides used to demonstrate the method as described in the text and shown in Fig. 1.

Pesticide	Strips identified by letters as shown in figure 1	Rf
Systox	a, b	0.44
Parathion	c, d	0.53
Guthion	e, f	0.04
Thimet	g, h	0.73
Imidan	i, j	0.09
Trithion	k, 1	0.88

An alternate procedure for TSTLC was also utilized by spreading and stripping while the Silica Gel was wet. This technique was performed by spreading a heavy layer of the Silica Gel slurry at one end of a plate and, by utilizing the stripper mentioned previously, immediately stroking across the plate with a smooth even stroke. The plate may be restroked two or three times to give a more consistent and even strip from one end of the plate to the other.

Results and Discussion

In Fig. 3, two types of TSTLC plates are illustrated by a cross-section view of the thin strips.

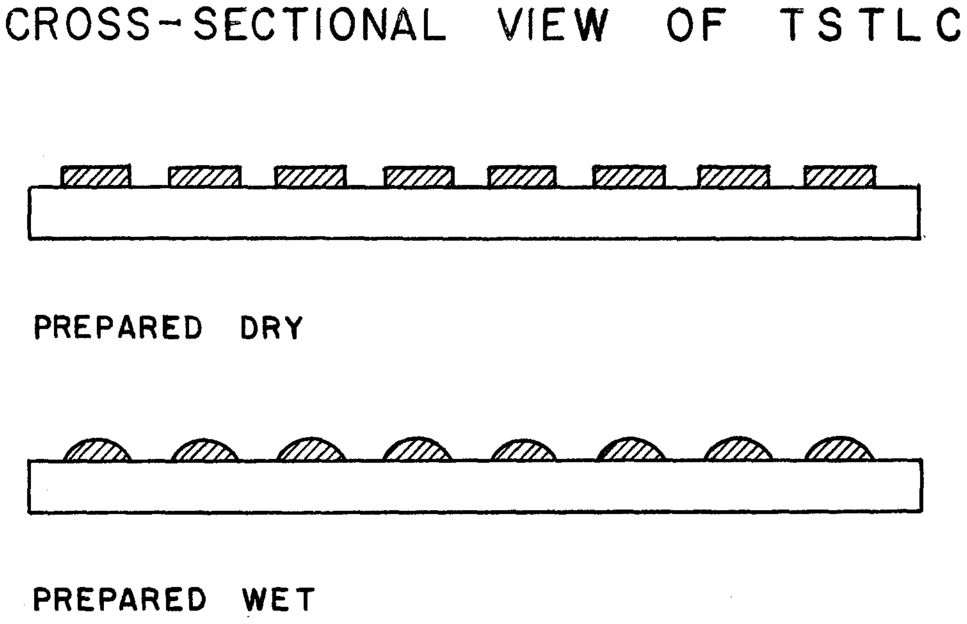

Fig. 3. Cross-section view of the glass plates showing the appearance of the silica gel strips prepared on a dry plate and as they would appear when prepared from a wet slurry.

On the left, the strips are flat since they were formed after the plate was dried, and on the right is the cross-section view of the plate made when the Silica Gel was wet. The wet slurry procedure requires more refinement in technique as the ratio of solution to adsorbent is critical to the point where over wetting will cause the strips to flatten out and insufficient wetting will cause the strips to be lumpy and not uniform. A degree of flexibility in the width of strips prepared may be obtained by using two equally grooved rubber plates that may be adjusted to control the opening for the size of the strip.

These micro strips on plates allow the separation and detection of submicrogram quantities of the compounds described earlier. The sensitivity of detection is the same regardless of Rf value as the narrow strips do not permit the spreading of the spots as they progress up the plate. The size of the strips can be adjusted to the desire of the investigator both in width and in thickness. However, if the strips are too narrow, the band of the developed area lengthens somewhat. This vertical spread will depend, to a large degree, on the type of compounds worked with as well as the amount of material to be chromatographed.

TSTLC permits many analyses to be carried out on a single plate depending, of course, on the width of the strips. In our investigation we found that strips 4 mm. wide were optimum and as many as 20 strips could be developed on one 8 inch x 8 inch plate. Extraction or removal of separated spots is more easily accomplished using TSTLC. One may spot and develop two strips in the same way using one for color development and the other for recovery of the compound at the corresponding Rf.

The compounds we selected for illustration in this paper were six difficult-to-separate organophosphorus pesticides. With the TSTLC technique and solvent system mentioned, we were able to separate all six organophosphorus compounds. An isomer of Systox was also separated.

A recent study involving the isolation and identification of products of parathion photodecomposition has allowed us to combine the use of this TSTLC technique and the sodium thermionic detector. Since the phosphorus sensitive detector on the gas chromatograph will respond to nanogram quantities of these compounds it is very useful in such a study. The use of larger amounts of compounds will allow identification of separated spots by infrared spectroscopy. This three-way detection system should aid greatly in the identification of the products being sought. The STD equipment in use is a conversion of a Wilkins Hi-Fy chromatograph and was described at a recent symposium on pesticide analysis. (1)

Literature Cited

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