## Dual-band preparative thin-layer chromatography for the separation of Diazinon and related compounds from plant material*

A project in this laboratory required the separation of Diazinon® [O,O-diethyl-O-(2-isopropyl-4-methyl pyrimidin-6-yl) phosphorothionate] and other compounds from plant co-extractives which interfere in the effective determination of these compounds. Although several clean-up procedures for organophosphate pesticides have been described[1-3], none of these proved to be entirely satisfactory for the purpose. The wood cellulose–Darco G60 column clean-up[4] was found to be the most effective for the isolation from spinach extract of Diazinon®, sulfatepp (tetraethyl dithiopyrophosphate) and oxo-Diazinon [O,O-diethyl O-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphate]. However, this procedure did not give satisfactory clean-up for optimum separation of mixtures by paper chromatography or thin-layer chromatography.

This paper describes a preparative thin-layer technique which makes use of two bands, each of a different adsorbing material, on a single glass plate. Extracts representing up to 50 g of spinach material have been purified by this method with good results. Average recoveries of 95 % for $^{35}S$-labeled Diazinon were found using autoradiography and liquid scintillation counting determinations.

*Experimental*

*Preparation of plates.* The adsorbent was prepared by mixing 12 g of Darco G60 and 10 g of Solka-Floc (prewashed with distilled $CHCl_3$, methanol, acetone and oven dried) in a dry state. Sixty ml of distilled methanol were added and the mixture was blended in an Omnimixer to ensure a fine, homogeneous texture. A separate mixture was prepared using 50 g of silica gel H and 160 ml of distilled methanol.

The plates were prepared with the help of an open reservoir applicator equipped with a 1.0 mm interchangeable gate**. In order to keep the two different adsorbents separated, an aluminium plate, tapered at the lower end, was inserted, crosswise, into the reservoir (See Fig. 1.)

*Sample application and development*

The spinach plant extract, prepared according to the LAWS AND WERLEY extraction procedure[5], was dissolved in chloroform and applied as a streak across the charcoal–cellulose band. Standards were spotted alongside the streaked sample and the plate was developed in distilled acetone.

*Detection.* After masking the side of the plate containing the streaked plant material, the standards were made visible by spraying with 0.5 % 2,6-dibromo-*n*-chloro-quinonimine in cyclohexane followed by 1 *N* HCl spray. The silica gel opposite the developed standards was transferred by scraping into a fine-sintered glass funnel and rinsed with 30 ml of distilled acetone.

*Recovery of 2-isopropyl-4-methyl-pyrimidin-6-ol.* For recovery of 2-isopropyl-4-methyl-pyrimidin-6-ol, a predicted hydrolysis product of Diazinon found in the

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** Materials for the preparation of the thin-layer plates were supplied by Kensington Scientific Corporation, Oakland, Calif.

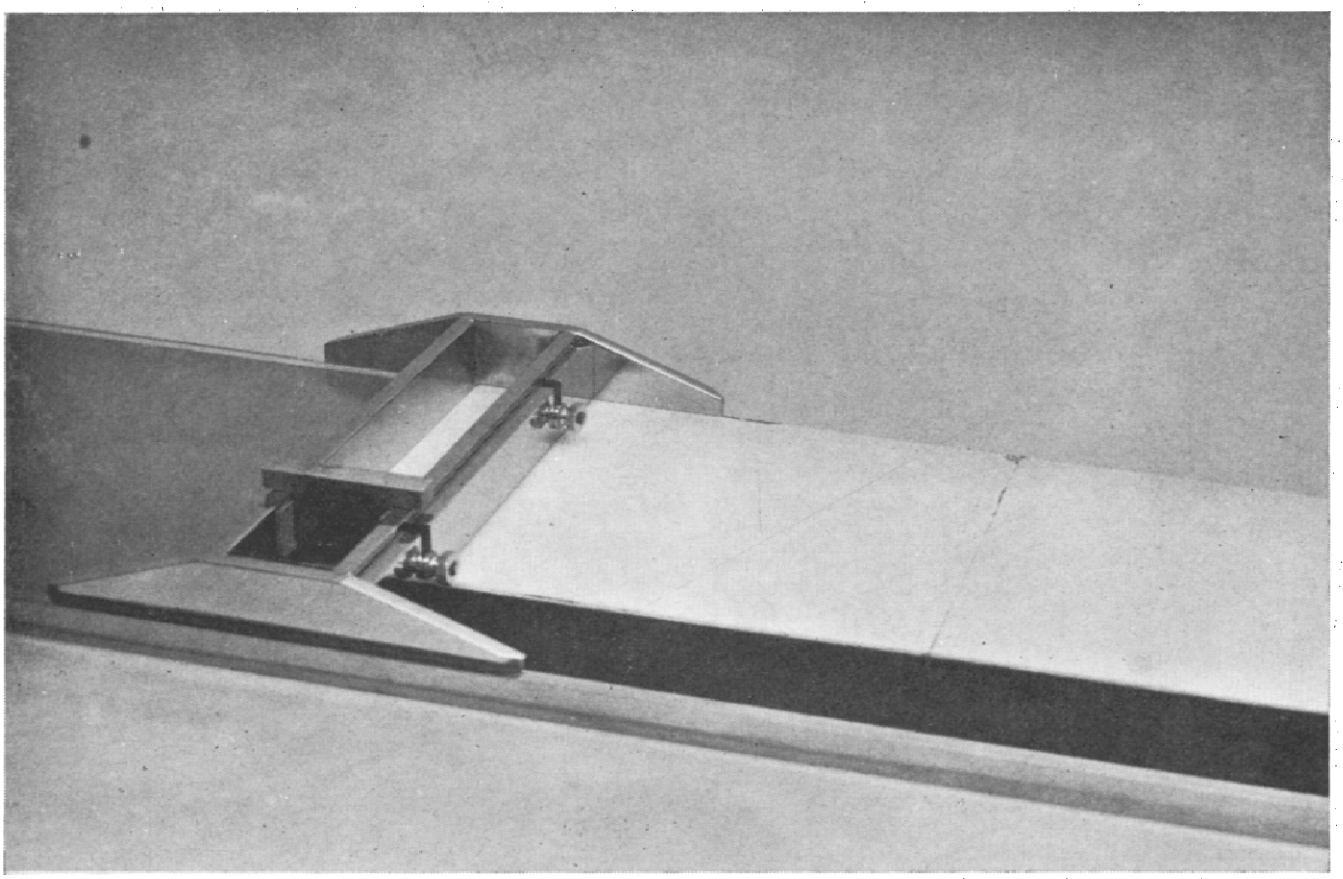

Fig. 1. Application of the adsorbents Darco G60–Solka-Floc and silica gel H.

water-soluble fraction of the extraction procedure, a modification of the previously described procedure, was required.

A gradient layer was prepared using a mixture of washed Solka Floc and Darco G60 (10:7, w/w) on the first 4 cm of the layer and alumina G on 16 cm. The adsorbent layer varied from a maximum thickness of 1.0 mm at the origin (carbon) to a minimum of 0.2 mm at the opposite end (alumina).

Further studies are being conducted to evaluate the application of this technique to the clean-up of other organophosphate and chlorinated hydrocarbon pesticides.

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