An *In Situ* Fluorometric Method for the Analysis of Bayrusil in Foodstuffs

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Bayrusil [O,O-diethylO-(2-quinoxalyl) phosphorothionate], for which the common name diethquinalphione has been proposed, was introduced in 1969 for the control of biting and sucking insects of field crops and fruits (SCHMIDT and HAMMAN 1969). A gas chromatographic procedure for the analysis of Bayrusil in foodstuffs has already been proposed (DRAGER 1969). The technique gives good quantitative results but additional steps are needed for the positive identification for the residues and therefore more time is required for the analysis. It was thought desirable to have a more simple method that could be used for the simultaneous identification and determination of residues of Bayrusil in plant materials at similar concentrations.

Much attention has been devoted during the past few years to the analysis of organophosphorous pesticides by in situ fluorometry. Earlier work was carried out with chelate spray reagents following treatment of the chromatogram with bromine vapours (FREI and MALLET 1971). This work led to the analysis of azinphosmethyl in blueberries (FREI et al. 1971).

The use of bromine vapour was eliminated when metal-fluorochromic indicators were discovered. BIDLEMAN et al. (1972) showed that organophosphorous compounds appeared as yellow fluorescent spots on thin-layer chromatograms when sprayed with a palladium-calcein complex. This method of detection was used by MACNEIL et al. (1974) for the analysis of dimethoate and malathion in lettuce. Very recently, BRUN and MALLET (1973) showed that some compounds could be made fluorescent on silica-gel layers simply by heating the chromatogram in an oven. This detection technique was used successfully to determine Bayrusil in water (BRUN and MALLET 1974). In this study, the same detection technique is applied to the analysis of Bayrusil in various fruits and field crops.

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EXPERIMENTAL

Apparatus: A Turner Fluorometer Model 111 (G.K. Turner Associates) equipped with a Camag TLC Scanner was used for quantitative work. The chromatograms were scanned at a speed of 2 in/min with a chart speed of 60 mm/min. The surface under the peaks was measured with a planimeter (A. Ott, Kempten, Bayern, G.F.R.). Excitation filter # 7-60 (360 nm) and secondary filter # 2-A (415 nm), both available from Corning Glass Work (New York, U.S.A.) were utilized.

Reagents: Bayrusil was obtained from Chemagro Corporation (Kansas City, Missouri) as an analytical standard (99.4%). A stock solution was prepared 1000 ppm (v/v) in n-hexane from which dilutions were made.

Florisil (60-100 mesh, Fisher Scientific Co., Montreal, Canada) was heated in an oven at 130°C for at least 48 hours, then deactivated with 3% water and stored in a dessicator until use. The thin layer chromatographic plates (20 x 20 cm^2) were prepared 250 microns thick from 30 g of SILICA-GEL H (Brinkmann Instruments Limited, Rexdale, Ontario) and 80 ml of 0.25 N KOH. The plates were left to dry at room temperature before use.

Extraction Procedure: 100 g of sample were homogenized for five minutes with 200 ml of acetonitrile in a Waring Blendor. The mixture was filtered with vacuum on a Büchner funnel through a Whatman No. 42 filter paper. The residue on the filter paper was then re-extracted with 100 ml of acetonitrile and the mixture was filtered as before. The Blendor jar was rinsed with acetonitrile (50 ml) which was then used to wash the filter cake. The combined extracts were transferred to a 1000-ml round-bottomed flask and the acetonitrile was removed on a rotary vacuum evaporator at 35°C. The remaining aqueous solution was transferred to a graduated cylinder and diluted to 100 ml with distilled water. It was then extracted twice with 125-ml portions of n-hexane in a 250-ml separatory funnel and the combined extracts were dried for five minutes with 30 g of anhydrous sodium sulfate. The hexane extract was transferred to a 500-ml round-bottomed flask and the solvent was evaporated to approximately 5-10 ml on the rotary evaporator at 35°C.

Florisil Column Cleanup: A plug of glass wool was placed into the bottom of a chromatographic tube (20 mm, i.d.). Anhydrous sodium sulfate (5 g) was added, followed by 10 g of Florisil. The Florisil layer was topped with 5 g of anhydrous sodium sulfate. The column

was then washed with 30 ml of hexane. The residue from the hexane evaporation was transferred to the column with small portions of n-hexane which was then drained from the column. 50 ml of 10% ether in n-hexane (v/v) were passed through the column and the eluate was discarded. Bayrusil was eluted from the Florisil using 100 ml of 25% ethyl ether in n-hexane. The eluate was evaporated to less than 0.1 ml, first in a 250-ml round-bottomed flask, and finally into a 15-ml graduated centrifuge tube. The volume was then made up to the 0.2 ml mark with n-hexane.

Thin-Layer Chromatography: A 20 µl aliquot of the sample was spotted on the right-hand side at the bottom of a chromatographic plate. The plate was developed to 10 cm in a 5:1 mixture of n-hexane in acetone. Standards were then spotted on the free portion of the chromatogram atop the right-hand side and the plate was placed in an oven at 100^{0}C for 30 minutes. After cooling, it was developed 10 cm in a 100:10 mixture of carbon tetrachloride in methanol. After removal from the chromatographic chamber, the plate was dried with a stream of warm air and the spots were located under UV light, the fluorescence of the spots was then measured.

RESULTS AND DISCUSSION

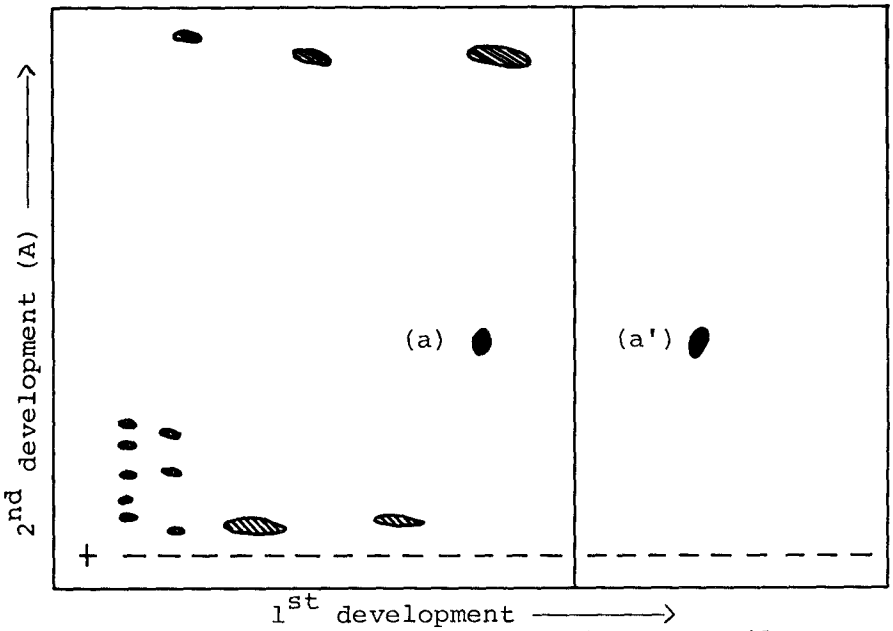
Various solvents were considered for extracting Bayrusil from the sample but acetonitrile was chosen because of its lower solubility towards waxes and fats (GETZ, 1962).

Although the water-hexane partitioning of the extract was very efficient in removing a large amount of co-extractives, it was not sufficient. Florisil column cleanup was deemed necessary in order to minimize substances which interfered with the instrumental detection. Even so, two-dimensional chromatography of the extract had to be done to separate Bayrusil from interfering substances. With the use of a polar solvent system, most of the interfering co-extractives are eluted to the top (Scheme 1). The impregnation of the adsorbent with the KOH was found to minimize spreading of the spots and background irregularities normally encountered when using sprays.

The fluorescence obtained from Bayrusil is quite stable (BRUN and MALLET 1974) and a decrease in fluorescence is not noticeable even after a few days exposure to daylight. As little as 6 ng per spot of the pesticide can be detected (BRUN and MALLET 1973)and

calibration curves are linear up to a concentration of 10 µg per spot (BRUN and MALLET, 1974).

Results obtained by analyzing for Bayrusil in various crops are given in Table I. Recoveries are better than those obtained by DRAGER (1969) who worked at a concentration of 0.1 ppm. Results higher or lower than 100% are within the precision of the technique which is of the order of ±5% (BRUN and MALLET, 1974). However, higher than normal recoveries may be



a - Bayrusil from sample extract; a' - Bayrusil standard

A - development after heat treatment

Scheme 1

Typical thin-layer chromatogram after heat treatment and development

Table I

Recoveries of Bayrusil in various crops at the 0.02 ppm level

Substrate:	apple	lettuce	tomatoes	cucumbers	beans
% Recovery:	105	109	96	98	101
	102	101	105	107	98

attributed in part to interferences of the background which causes some difficulty in recording the chromatogram properly at very low concentrations.

Reproducibility in the results is excellent compared to that reported by MACNEIL et al. (1974) in their analysis of dimethoate and malathion in lettuce extract. It is also important to note that the method described in this study is done at a much lower concentration and the pesticide is spiked before extraction.

When working at levels of 0.10 ppm or more the column chromatography step can be omitted. The method is then faster and more simple than that described by DRAGER (1969).

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