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A method for the determination of Venzar (3-cyclohexyl-5,6-trimethyluracil) on silica gel layers*

The detection and determination of the herbicide Venzar (3-cyclohexyl-5,6-trimethyluracil) on silica gel layers is difficult because of the absence of specific reactions of this stable compound.

The localization of Venzar on a thin layer can be carried out with commonly used reagents, such as concentrated H_2SO_4, malachite green, rhodamine B, or by bromination and development under UV light[1]. Venzar can be determined by measuring the surface area of the spot, but this method is very inaccurate.

The method described in this paper consists in the chemisorption of rutine (quercetin-3-rutinoside) vapour on the spots containing Venzar on the silica gel layer, which results in yellow spots on a white background. These spots are easily visible in daylight and are highly fluorescent under UV light. As the spot size is proportional to the amount of Venzar present, this amount can be determined by determining the amount of rutine adsorbed.

Materials and procedure

Silica gel plates. Dimensions 20 × 20 cm, covered with a 0.75-μm layer of Kieselgel G (Merck, Darmstadt, G.F.R.). Prior to use, the plates were activated by heating them at 105° for 1 h.

Solvent system. Ethyl acetate–benzene (1:1).

Time of development. One hour.

Standard solution of Venzar. A 3-mg amount of Venzar was dissolved by heating in 5 ml of ethyl acetate and the solution was placed on a silica gel plate at a distance of 2 cm from the edge in duplicated amounts of 0.05, 0.1 and 0.2 ml per spot.

Method. A 6-mg amount of Venzar was suspended in 50 ml of tap water, and extracted with ethyl acetate (3 × 25 ml). The extract was evaporated to dryness on a water-bath at 60° and the residue was dissolved in 10 ml of ethyl acetate. A 0.15-ml volume of this solution was placed on the plate in triplicate, and the standard solution was placed on the same plate. The plate was dried under a hot air stream and sprayed with concentrated H_2SO_4, and then placed in a drying box at 140°.

Another plate covered with the same gel, and sprayed with a 0.5% solution of rutine in 96% ethanol, was placed in the same drying box. After drying for 10 min, the door of the drying box was opened so as to permit the evaporation of SO_2 fumes from the surface of the plate and to facilitate the adsorption of the rutine vapour by the silica gel. After a period of 10 min at 130–140°, bright yellow spots of $R_F = 0.48$ appeared on the plates at areas containing Venzar. The colour is stable for a few hours. After cooling, the plate was observed under a UV lamp to permit the contours of the highly fluorescent green–yellow spots to be observed.

The gel spots were then transferred quantitatively into test-tubes and 5 ml of

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96% ethanol were added to each. The tubes were allowed to stand for *ca.* 10 min, being stirred periodically. The eluates were separated from the gel by filtration and extinctions were measured at 270 nm with a Universal Spectrophotometer VSU-1 (Zeiss, Jena) and a Unicam SP-500 spectrophotometer.

Gel eluates, prepared from the same plate on which the determination was carried out, and with an area equal to that of the defined spot, were used as blanks.

Results and discussion

The graph of the concentration of Venzar on a thin layer and the extinction (E_{270}) of the eluates was a straight line (Fig. 1). When Venzar is determined, it should

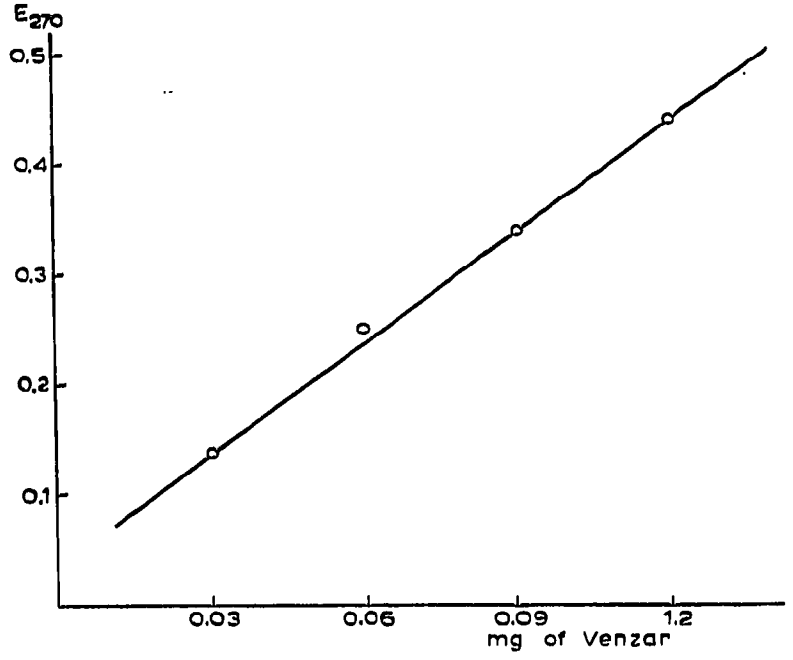

Fig. 1. Graph of extinction of the eluate *vs.* concentration of Venzar.

be placed on a plate in amounts of 0.03–0.12 mg per spot. The error of the method is about 10% and the detection limit is 0.010 mg of Venzar per spot. The sensitivity of the method depends to a great extent on the accuracy of the micropipette used for placing the Venzar on the silica gel layers, as well as on the standardization of the conditions used. Flavones are commonly used as fluorogenic spray reagents for numerous organic compounds. FREI *et al.*[2] used robinetine solutions for detecting organothiophosphorus pesticides on silica gel and cellulose layers. Similar investigations were carried out by MALLET AND FREI[3–5] with various flavone solutions used as fluorogenic spray reagents for pesticides such as S-triazines, carbamates, organophosphorus compounds and chlorinated hydrocarbons. The best results were obtained when using ethanolic solutions of fisetin, robinetin and flavonol.

Spraying the plates with rutine solutions gave no positive results. Our technique for the determination of Venzar in rutine vapour enables the presence of Venzar to be detected on a thin layer in daylight. Although the determination has a relatively high error of *ca.* 10%, nevertheless the method enables rapid determinations to be carried out and is applicable to serial determinations.

When using this method for the determination of Venzar in bacterial cultures, it has been found that the metabolites of bacteria do not affect the detectability of Venzar.

The replacement of the light absorption measurements by fluorescence measurements seems to be useful for greatly increasing the sensitivity of the method.

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