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Note

Identification and determination of triazine and diazine herbicides in water by thin-layer chromatography

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Many workers have tried to determine triazine herbicides, mainly using spectrophotometric^{1,2} and chromatographic methods³⁻¹². The diazine-based herbicide pyrazone has been determined in plants and soils by colorimetric methods and by thin-layer chromatography (TLC)¹³⁻¹⁶. At the present time, when environmental pollution has reached a high level, and also in view of the increasing use of combined pesticide preparations, multi-detection methods that permit the detection of as many of the compounds used as possible are required. Studies on the conditions for the extraction of six triazine derivatives and of pyrazone from water, purification procedures using adsorption chromatography and the identification of the compounds in the extracts with semiquantitative evaluation of thin-layer chromatograms are reported in this paper.

EXPERIMENTAL

Materials and equipment

Chloroform, methylene chloride, ethyl acetate, toluene and acetone, p.a. grade, rectified, were used as solvents.

The detection reagent was prepared by dissolving 250 mg of silver nitrate in 2.5 ml of distilled water, then adding 25 ml of 2-phenoxyethanol and three drops of hydrogen peroxide. Acetone was added to make the volume up to 500 ml and the solution was stored in a dark glass bottle.

Standard solutions were prepared by weighing 100 mg of each herbicide into 100 ml of solvent. The herbicides used were simazine (2-chloro-4,6-bisethylamino-*s*-triazine), atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine), propazine (2-chloro-4,6-bis-isopropylamino-*s*-triazine), desmetryne (2-methylthio-4-methylamino-6-isopropylamino-*s*-triazine), ametryne (2-methylthio-4-ethylamino-6-isopropylamino-*s*-triazine), prometryne (2-methylthio-4,6-isopropylamino-*s*-triazine) and pyrazone (1-phenyl-4-amino-5-chloropyridazone-6). The standard solutions were used to prepare a mixture of these compounds at lower concentrations.

Silufol plates (15 × 15 cm, layer 0.13 mm) were obtained from Kavalier Glassworks, Votice, Czechoslovakia. The other equipment used was a vacuum rotary evaporator, glass chromatographic chambers and a germicide lamp (wavelength 254 nm, 85 W).

Extraction

A water sample (500–1000 ml) was saturated with ethyl acetate and 20 g of sodium chloride were added (pH 1.5–2). Extraction was performed in a separating funnel with three 50-ml volumes of chloroform–ethyl acetate (1 + 1). The phases were allowed to separate completely (30 min). The combined organic extracts were filtered through anhydrous sodium sulphate into a round-bottomed flask and then concentrated in a vacuum rotary evaporator (temperature of the water-bath, 40–50°; vacuum, 300–400 torr). When required, the concentrated extracts (1–2 ml) were purified.

Purification of extracts

A piece of fat-free cotton-wool was placed at the bottom of a chromatographic column, 40 cm long and average I.D. 2 cm, fitted with a tap, and the column was then filled with 20 g of Woelm neutral alumina (Brockmann activity II) with a 5% water content. The column of adsorbent was washed with 30 ml of eluting solution (chloroform–methanol, 9:1). After the last portion of the eluting solution had been absorbed, the concentrated extract was applied to the top of the column and allowed to absorb. The herbicides were then eluted with 70 ml of chloroform–methanol (9:1). The eluate was collected into a round-bottomed flask, evaporated to a small volume, quantitatively transferred into a conical-bottomed tube and allowed to dry completely in a vacuum evaporator. The solids were then re-dissolved in a known volume of methylene chloride and the solution obtained was used for TLC analysis.

TLC analysis

A Silufol plate (15 × 15 cm) was activated for 30 min at 140° in a thermostat and washed twice with distilled water (level, 0.7–1 cm) in a chromatographic chamber (19 × 18 × 8 cm) fitted with a glass cover. The ascending technique of washing was used until the water reached the upper edge of the plate. It was then removed from the chamber, the front edge was placed downwards and it was allowed to dry in the air and then in a thermostat at 65°. After drying, mixtures containing various concentrations of the herbicides as well as extracts of water samples were placed at the top of the plate (2 cm from both the bottom and the edges of the plate). The spots should be placed at least 1 cm apart. The plate was developed in a chromatographic chamber with toluene–acetone (85:15)⁴. Repeated developments were carried out in order to separate the compounds more distinctly. Before detection, the plate was dried for 10 min at 65°. Detection was carried out by spraying the plate with 40 ml of the detection solution. After spraying, the plate was dried at 65° and irradiated with a germicide lamp (15 cm distance) until the spots representing the smallest amounts of the herbicides could be seen (10–15 min). The semiquantitative evaluation of the chromatogram was performed by comparing both the size and intensity of the spots obtained from water samples with the spots obtained from known concentrations of the herbicides in standard solutions.

RESULTS AND DISCUSSION

Herbicides were extracted with chloroform, methylene chloride and ethyl acetate. The best yield was obtained with a mixture of chloroform and ethyl acetate

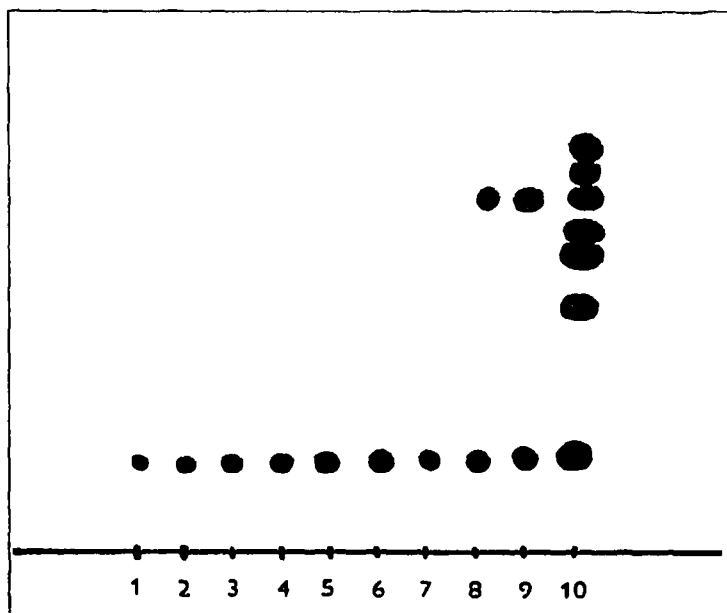


Fig. 1. Thin-layer chromatogram. Nos. 1, 2, 3, 4, 5 and 6 represent the pyrazone standards in amounts of 0.2, 0.4, 0.6, 0.8, 1.0 and 1.5 μg per spot, respectively. At positions 7, 8 and 9, three different amounts of extracts in chloroform-ethyl acetate from waste water (in which pyrazone and atrazine were identified) were placed. At position 10, a mixture of standard compounds (in the order pyrazone, desmetryne, simazine, ametryne, atrazine, prometryne and propazine from bottom to top) were placed (2.5 μg each).

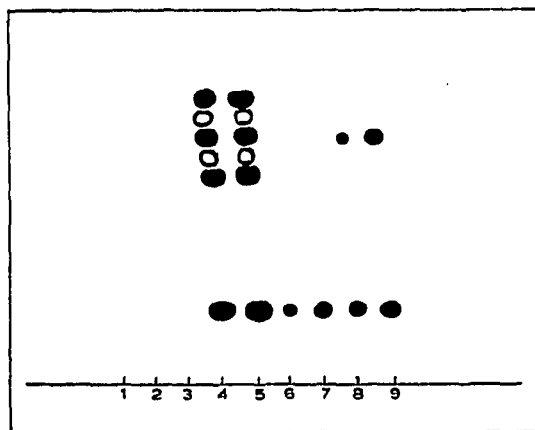


Fig. 2. Thin-layer chromatogram. Nos. 1, 2, 3 are various concentrations of a mixture of standards of α , β , γ and δ -BHC, DDE and DDT. Nos. 4 and 5 are mixtures of a standard solution of pyrazone and of six triazine herbicides (from bottom to top: pyrazone, desmetryne, simazine, ametryne, atrazine, prometryne and propazine). Nos. 6 and 7 are pyrazone standards in two different concentrations. Nos. 8 and 9 are two different amounts of purified extracts from waste water (in which pyrazone and atrazine were identified).

(1:1). When sodium chloride was added to the water, the yield increased because the solubility of the organic solvent in water decreased. The yield in the extraction with chloroform-ethyl acetate (1:1) with and without sodium chloride as well as the sensitivity of the detection in identifying pyrazone were tested (Fig. 1).

Interference by organochlorine pesticides in detection with silver nitrate solution with 2-phenoxyethanol was studied. As can be seen from Fig. 2, with toluene-acetone as eluting solvent the organochlorine insecticides follow the solvent front, while triazine herbicides and pyrazone are separated according to their respective R_F values in this system.

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