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ANALYSIS OF TRIAZINE HERBICIDES BY COMBINED THIN-LAYER CHROMATOGRAPHY AND FLUORIMETRY

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SUMMARY

The analysis of a number of *s*-triazine herbicides by combined thin-layer chromatography and fluorimetry is investigated. The method involves hydrolysis of the triazines in 1 *N* HCl to yield cyanuric acid and the corresponding alkylamines. The amines are coupled with Dns chloride (5-dimethylaminonaphthalene-1-sulfonyl chloride) to yield highly fluorescent compounds which are separable by thin-layer chromatography. The derivatives are quantitatively evaluated by fluorimetry after removal of the spots from the plate. A variety of food samples is successfully analysed at concentrations as low as 0.05 ppm. The method also proves useful for the confirmation of triazines which have been analysed by other techniques, such as gas chromatography.

INTRODUCTION

Thin-layer chromatography (TLC) has been used by a number of workers for the analysis of triazine herbicides[1–4]. These methods involved the use of spray reagents for visualization of the compounds after separation. The major drawback of this approach is the lack of sensitivity which is seldom better than 1 μg per spot. Also, the spraying process and the subsequent reaction on the plate are often difficult to reproduce, causing difficulty in quantitation by spectrophotometry. TLC and fluorimetry have been used successfully for the low-level detection of a number of pesticides including methylcarbamates[5,6], phenylcarbamates and ureas[7–9], organophosphates[10], hydroxybiphenyls[11], and chlorophenols[12]. These methods involved the formation of fluorescent derivatives which were separated either by TLC and quantitatively analysed *in situ* by fluorimetry[5,7,8,11,12] or by high-speed liquid chromatography with fluorescence detection[6,9,10]. Detection limits were often in the 1–5-ng range.

The present work describes the results of a similar TLC–fluorimetric investigation of triazine herbicides in a variety of crops.

EXPERIMENTAL

Reagents

Analytical-grade 5-dimethylaminonaphthalene-1-sulfonyl chloride (Dns-Cl) (Aldrich, Milwaukee, Wisc., U.S.A.) was dissolved in glass-distilled, residue-free acetone at a concentration of 1 mg/ml. The triazines were atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine), simazine (2-chloro-4,6-bis(ethylamino)-*s*-triazine) and propazine (2-chloro-4,6-bis(isopropylamino)-*s*-triazine) prepared in pesticide-grade methanol at a concentration of 1 mg/ml. Deionized distilled water was used throughout. All other organic solvents were glass-distilled and residue free. The crops examined were beets, carrots, corn, parsnips, peas, potatoes and turnips.

Extraction procedure

One hundred grams of crop material were placed in a Waring blendor and blended with 150 ml of methanol for 5 min. The macerate was suction filtered through a 600-ml medium-porosity sintered glass funnel. The filtrate was warmed to room temperature and an aliquot equivalent to 10 g of sample was placed into a 500-ml separatory funnel containing 200 ml of distilled water and 50 ml of chloroform. The contents were shaken and the chloroform layer removed after the phases had separated. This step was repeated with second and third volumes of chloroform. The combined organic extracts were reduced to about 1 ml by rotary vacuum evaporation at 30°. The concentrate was quantitatively transferred to a 15-ml test tube with PTFE-lined screw cap and evaporated to dryness at 30° under a gentle stream of nitrogen.

Reaction procedure

One millilitre of 1 *N* HCl was added to the test tube containing the residue. The cap was tightly placed and the test tube heated at 150° overnight (16–18 h), cooled and carefully opened. A 1.0-ml volume of 1 *N* NaOH was added to the test tube followed by 2.0 ml of 5% borax solution. The tube was then shaken and 1 ml of Dns-Cl solution added. The cap was tightly replaced, the test tube shaken again and placed in a water-bath at 45° for 15 min. After this time the test tube was cooled and opened. A 0.5-ml volume of benzene was added and the test tube shaken. The phases were allowed to separate. The benzene layer was removed and the process repeated

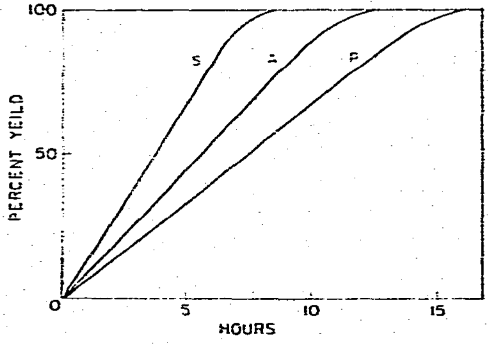


Fig. 1. Hydrolysis of simazine (S), atrazine (A) and propazine (P) in 1 *N* HCl at 150°.

twice more. The combined benzene extracts were dried with anhydrous sodium sulfate and evaporated to 1 ml.

Thin-layer chromatography

2–5 μl of the concentrated benzene extract were spotted on a silica gel TLC plate (Eastman, Rochester, N.Y., U.S.A.: 6061 silica gel without fluorescent indicator). The plate was developed in a paper-lined chromatography chamber with hexane–chloroform (3:1) to a 15-cm solvent front, removed and air dried. The plate was then chromatographed in the same direction a second time with the same solvent system. The plate was dried in a stream of air and sprayed until visibly moist with cyclohexanone or triethanolamine[7]. It was then placed again under a stream of air until excess of solvent had evaporated.

Analysis

The sprayed chromatoplate was observed under a UV light (Ultra-Violet Products Inc.) at long wavelength ($\approx$366 nm). Quantitative analysis was carried out by cutting out the spot of interest and washing it with two 1-ml volumes of acetone to remove the derivative from the silica gel. The mixture was suction filtered through a 25-ml medium-porosity sintered glass funnel. The filtrate was evaporated to dryness under a stream of nitrogen and brought to 5 ml with hexane. The fluorescence intensity of the solution was measured on a Perkin-Elmer spectrophotofluorimeter MPF-3 and compared to standards carried through the same reaction procedure.

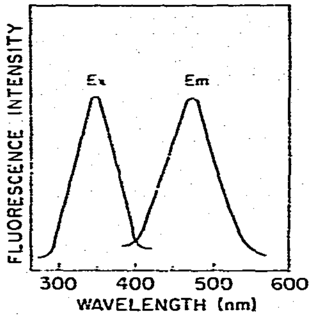
RESULTS AND DISCUSSION

Reactions

The hydrolysis of the triazines by 1 *N* HCl was reported earlier for the determination of the *s*-triazine ring in organic molecules[13]. Fig. 1 shows the hydrolysis rates of the triazines determined by monitoring the formation of Dns amines resulting from the hydrolysis mixtures and comparing to authentic Dns-amine standards. Simazine was hydrolysed most rapidly under the given conditions and was complete in about 8 h. The hydrolysis rates were found to be dependent upon the structure of the alkylamino substituents. The ethylamino substituents of simazine were cleaved more rapidly than the sterically hindered isopropylamino groups of propazine. Ethylamine was also cleaved faster from atrazine than was the isopropylamine moiety. Hydrolysis for all compounds was complete in 16–18 h. However, when smaller quantities of acid were used, hydrolysis took longer. Simazine, atrazine and propazine, for example, were only 90, 55 and 35%, respectively, hydrolysed after 18 h when 0.3 ml of 1 *N* HCl were used. Higher acid concentrations did not appreciably increase hydrolysis relative to 1 *N* HCl.

Fluorescence measurements

The fluorescence spectrum of the Dns derivative of ethylamine in benzene is shown in Fig. 2. Excitation and emission maxima appeared at 346 nm and 475 nm, respectively. The fluorescence wavelengths and intensity were found to alter significantly with increasing dielectric constant of the solvent. Solvents such as hexane or benzene caused hypochromic shifts in emission wavelengths by as much as 50–60 nm



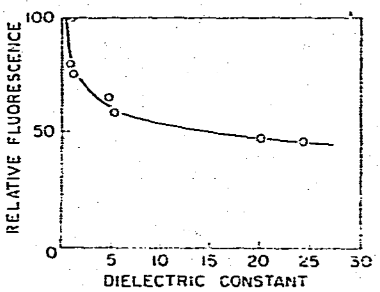


Fig. 2. Fluorescence spectrum of Dns-ethylamine at 200 ng/ml in benzene.

Fig. 3. Effect of dielectric constant on fluorescence intensity of Dns-ethylamine at 200 ng/ml. Solvents used were hexane, dioxane, benzene, chloroform, ethyl acetate, acetone and ethanol in order of increasing dielectric constant.

when compared to solvents such as ethanol or acetonirile. Shifts in excitation wavelengths were in the range of 5–10 nm. Solvents with low dielectric constants also gave the highest fluorescence yields. Fig. 3 illustrates the effect of dielectric constant on fluorescence intensity of Dns-ethylamine at a concentration of 200 ng/ml. As low as 5 ng/ml of Dns-ethylamine or Dns-isopropylamine could be instrumentally detected.

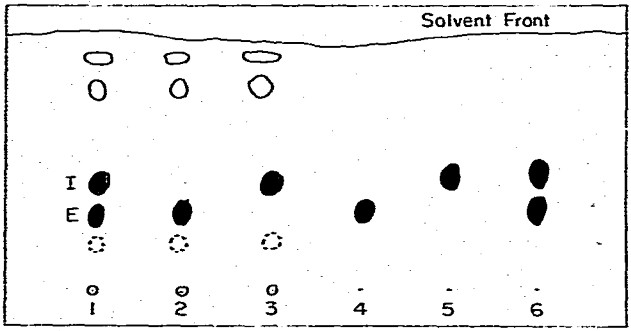


Fig. 4. Thin-layer chromatogram of Dns reaction products from (1) atrazine; (2) simazine; (3) propazine; (4) Dns-ethylamine standard; (5) Dns-isopropylamine standard; (6) mixture of 4 and 5. I = Dns-isopropylamine; E = Dns-ethylamine.

Analysis

Fig. 4 depicts a typical chromatogram of the reaction products of atrazine, simazine and propazine along with standards of Dns-ethylamine and Dns-isopropylamine. For quantitation, both TLC spots from atrazine were combined in one cuvette before fluorescence measurements were made.

A chromatogram showing the results of a corn analysis of simazine spiked at 0.2 ppm is illustrated in Fig. 5. The solvent system adequately separated the Dns derivatives from crop coextractives. Recoveries for the method including hydrolysis, Dns derivatization, TLC and quantitation averaged 85 ± 8% at 0.2 ppm for simazine.

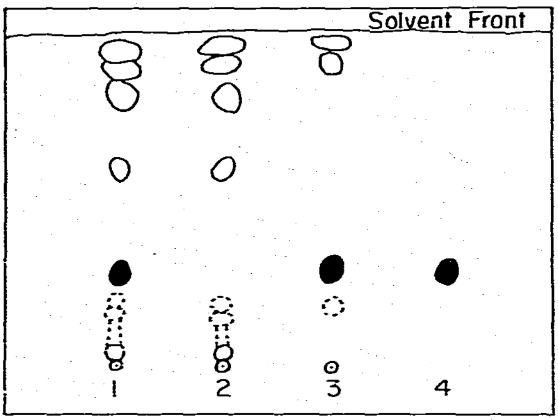


Fig. 5. Corn analysis containing 0.2 ppm simazine. 1 = Spiked sample; 2 = blank sample; 3 Dns-simazine standard; 4 — standard Dns-ethylamine.

CONCLUSIONS

The analysis of atrazine, simazine and propazine was successfully carried out in beets, corn, parsnips, peas and potatoes at concentrations as low at 0.05 ppm. Carrots and turnips had significant background interferences at these levels, however, the triazines could be visually detected at the 0.1-ppm level. This procedure is much more sensitive and quantitative than other TLC techniques for these compounds. The method is also applicable to other *s*-triazine herbicides.

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