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AN *IN SITU* FLUORIMETRIC METHOD FOR THE DETECTION AND QUANTITATIVE ANALYSIS OF FENITROTHION, ITS BREAKDOWN PRODUCTS AND OTHER AMINE-GENERATING COMPOUNDS

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SUMMARY

A procedure is described for the *in situ* detection of primary amine-generating pesticides on thin layers of silica gel. The technique is based on the reaction of fluorescamine with primary amines to yield highly fluorescent derivatives. Nitro compounds are first reduced to their corresponding primary amines.

Pesticides studied were aminotriazole, fenitrothion, parathion and parathion-methyl. Possible breakdown products of fenitrothion were also included. Detection limits were in the low nanogram range.

The method is simple, rapid and very selective and can also distinguish between primary amines and nitro compounds. An example of a typical chromatographic separation is presented and quantitative aspects are discussed.

INTRODUCTION

Fenitrothion [O,O-dimethyl O-(3-methyl-4-nitrophenyl)phosphorothioate] has been determined by colorimetry and spectrophotometry[1–7], thin-layer chromatography[8–13] and gas chromatography[14–23]. Most of these techniques, however, cannot be used for the simultaneous determination of fenitrothion and its degradation products. The quantitative analysis of fenitrothion and its oxon and cresol derivatives can be performed by gas chromatography using an electron-capture detector[19] but the method lacks specificity. A sensitive and specific response is obtained for fenitrothion, fenitro-oxon and aminofenitrothion when using a flame-photometric detector[19] but 3-methyl-4-nitrophenol and 4-amino-3-methylphenol are not detected and a separate column with an electron-capture detector has to be used.

It was intended in this study to develop an *in situ* fluorimetric method that would permit the simultaneous detection of fenitrothion and some of its major breakdown products and that could eventually be used for their quantitative evaluation in environmental samples.

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EXPERIMENTAL

Chemicals

Fluorescamine (Hoffmann-La Roche, Nutley, N.J., U.S.A.) was purchased from Fisher Scientific (Montreal, Canada). A solution was prepared containing 25 mg of the reagent in 100 ml of acetone. A solution of tin(II) chloride was prepared by dissolving 0.5 g of the compound in 5 ml of concentrated hydrochloric acid and diluting to 120 ml with a solution of 50 ml of water plus 65 ml of acetone. This solution was always freshly prepared.

Fenitrothion (Folithion) and fenitrooxon (Folithion oxygen analogue) were obtained from Chemagro (Kansas City, N.Y., U.S.A.). Parathion, parathion-methyl and aminotriazole were supplied by American Cyanamid Co. (Princeton, N.J., U.S.A.). 3-Methyl-4-nitrophenol and 4-amino-3-methylphenol were purchased from Pfaltz & Bauer (Flushing, N.Y., U.S.A.). Aminofenitrothion was synthesized according to Zitko and Cunningham[1]. Individual stock solutions were prepared at a concentration of 1 $\mu g/\mu l$ in acetone, except for the two phenols, which were dissolved in ethanol. Dilutions were made in *n*-hexane or ethanol.

Layers of silica gel (20 × 20 cm) were prepared 250 μm thick using a mixture of 30 g of silica gel H (Brinkmann Instruments, Rexdale, Canada) and 80 ml of 0.1 *M* potassium dihydrogen orthophosphate solution. The plates were dried in air and were not activated before use.

Apparatus

A Turner Model III fluorimeter (G. K. Turner Assoc., Palo Alto, Calif., U.S.A.) equipped with a Camag TLC scanner was used for all quantitative fluorimetric measurements. A 7-60 excitation filter (360 nm) and a 2A secondary filter (>415 nm), both available from Corning Glass Works (Corning, N.Y., U.S.A.) were utilized. For measuring the fluorescence spectra, a Farrand spectrophotofluorimeter (UV–VIS Chromatogram Analyzer, Farrand Optical Co., New York, N.Y., U.S.A.), equipped with a xenon lamp and grating monochromators supplemented by a Corning 7-54 excitation filter (230–420 nm) and a 3-73 emission filter (>405 nm), was used.

Brinkmann spray guns using freon propellant cans and atomizer heads were utilized for the spraying of the chromatograms.

Methods

For chromatographic separation, the compounds were spotted 2 cm from the bottom of a chromatographic plate and the development was made to a height of 10 cm in an appropriate solvent system. Fenitrothion and its related compounds were separated in *n*-hexane–acetone (2:1), parathion-methyl and parathion were separated in *n*-hexane–acetone (4:1), while for aminotriazole the plate was developed in *n*-hexane–acetone (1:2).

For the detection of compounds bearing a primary amino group, the plate was first sprayed lightly with water–acetone (1:1) solution, then with the fluorescamine reagent followed by a second water–acetone spray until the plate was just moist. The chromatogram was dried in a stream of cold air and examined under long-wavelength UV light (360 nm).

The nitro compounds were reduced to their corresponding primary amines by the following procedure. The plate was sprayed to saturation with the tin(II) chloride solution, allowed to stand for 5 min and then dried in a stream of air. The acid on the plate was neutralized by spraying it lightly with 2 *M* sodium carbonate solution. Additional fluorescamine was sprayed and the chromatogram was examined under UV light to detect the nitro compounds.

For determining detection limits, measured aliquots of the compound being studied were spotted in a series of decreasing concentrations at the bottom of a chromatographic plate and, after migration of the spots in an appropriate solvent system, the fluorescence was formed according to the procedure described above. The spots were scanned on the Turner fluorimeter. The range selector (aperture) was set to its maximum position (30×) and the slit adjusted to 1 mm. A 10-mV recorder connected to the fluorimeter was used to record the fluorescence of the spots. As the baseline is free from noise, the lowest amount that gave a 1-cm deflection on the recorder under the experimental conditions was taken as the limit of detection.

RESULTS AND DISCUSSION

The procedure described here permits the simultaneous detection and determination of fenitrothion and some possible breakdown products on a thin-layer chromatogram. It is based on the reaction of fluorescamine with primary amines to give highly fluorescent derivatives[24,25]. It has already been shown to be reliable and sensitive for the analysis of amino acids[26–30], peptides[27,31], proteins[32], enzymes[33], polyamines[33–36] and other compounds of biological interest[37–39], both in solution and on thin-layer chromatograms. Lawrence and Frei[40] suggested the use of this reagent as a labelling compound for the detection of primary amine-generating pesticides. It was later used for the quantitative determination of Chloramben in vegetables[41].

Of the compounds investigated (Table I), only aminofenitrothion and 4-amino-3-methylphenol have a primary amino group that is capable of reacting with fluorescamine. Fenitrothion, fenitrooxon and 3-methyl-4-nitrophenol each possess a nitro group that can be reduced to the corresponding primary amine.

The *in situ* reaction of fluorescamine with the primary amines on thin layers of silica gel yielded derivatives that appeared as greenish yellow spots under long-wavelength UV light. A similar reaction and formation of fluorescence was observed with the nitro compounds after reduction to the corresponding amines. The excitation and emission maxima for each fluorescent derivative are given in Table II.

All of the compounds were detected by the fluorimetric procedure and the results in Table II indicate that the instrumental limits of detection are in the nanogram range. Under the experimental conditions used, 3-methyl-4-nitrophenol exhibits a weaker fluorescence than the other compounds at identical concentrations, but the fluorescence can be increased by spraying the plate lightly with a 10% solution of triethanolamine in ethanol. The phenol is also detected as a yellow spot immediately after spraying with the sodium carbonate solution. It is important to note that only a slight decrease in the fluorescence of the derivatives was observed after several days when the chromatogram had been treated with sodium carbonate solution, compared with almost complete disappearance of the fluorescence after 24 h for an untreated plate bearing the primary amines.

TABLE I

COMMON NAMES AND STRUCTURAL FORMULAE OF THE COMPOUNDS STUDIED

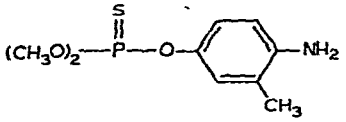
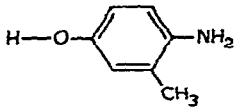
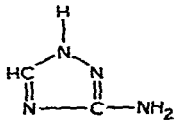
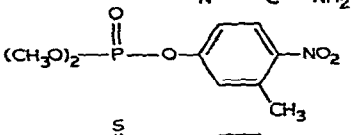
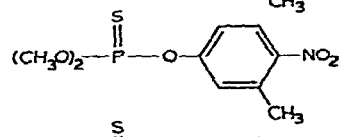
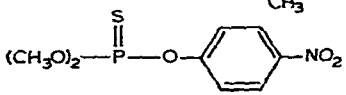
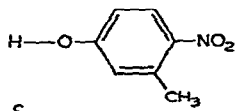
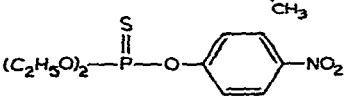
Compound	*Structure*
Aminofenitrothion	
4-Amino-3-methylphenol	
Aminotriazole	
Fenitrooxon	
Fenitrothion	
Parathion-methyl	
3-Methyl-4-nitrophenol	
Parathion	

TABLE II

FLUORESCENCE PROPERTIES OF THE FLUORESCAMINE DERIVATIVES ON THIN LAYERS OF SILICA GEL

Compound	*Excitation maximum (nm)*	*Emission maximum (nm)*	*Limit of detection (μg)*	*Upper limit of linear range (μg)*
Aminofenitrothion	382	500	0.01	0.5
4-Amino-3-methylphenol	382	492	0.02	0.5
Aminotriazole	382	488	0.01	0.5
Fenitrooxon	383	490	0.01	1.0
Fenitrothion	383	490	0.01	1.0
Parathion-methyl	390	510	0.01	1.0
3-Methyl-4-nitrophenol	385	490	0.08 (0.02*)	1.0
Parathion	390	510	0.01	1.0

* Limit of detection after spraying with 10% triethanolamine in ethanol.

TABLE III

REPRODUCIBILITY OF THE TECHNIQUE

*Plate**	*Relative standard deviation (%)*	
	Fenitrothion	*Aminofenitrothion*
1	4.76	6.40
2	13.90	4.73
3	8.55	6.51
4	8.82	9.30
5	10.07	9.33
Average	9.22	7.25

* Five spots per plate.

Quantitative analysis is possible as calibration graphs are linear over a definite range for each compound. However, the slopes of the graphs may vary from one plate to another, and it is therefore recommended that standards be included in each series of analyses.

The reproducibility of the technique was evaluated for aminofenitrothion and fenitrothion using 0.4-μg spots. The relative standard deviations for each compound are given in Table III. The diffusion of the spots and greater background irregularities

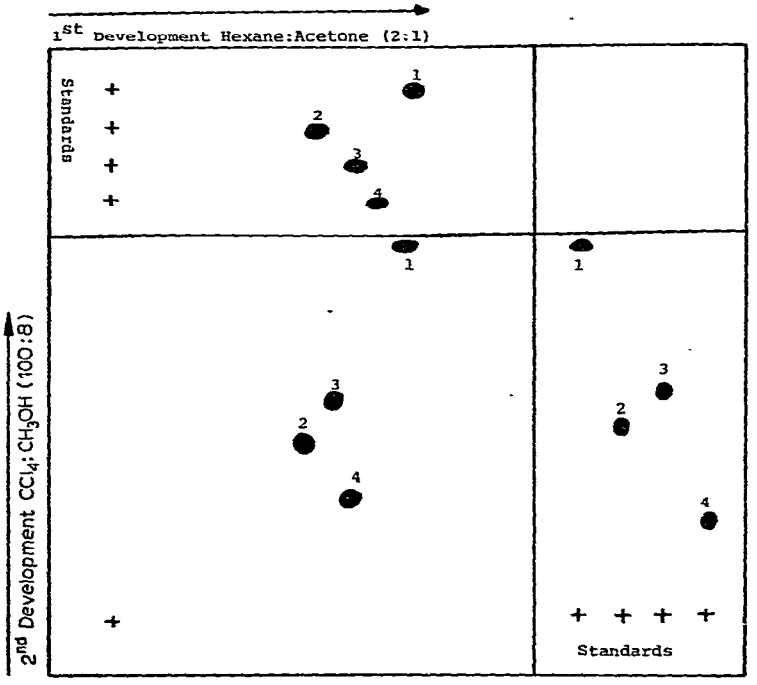


Fig. 1. Two-dimensional thin-layer chromatographic separation of fenitrothion and possible breakdown products. Spots: 1 = fenitrothion; 2 = fenitrooxon; 3 = aminofenitrothion; 4 = 3-methyl-4-nitrophenol.

on the chromatogram, caused by spraying with the inorganic reagents, explain the lower precision for fenitrothion compared with that for aminofenitrothion.

The amount of the sodium carbonate soluiton sprayed and the spraying technique used are important factors in obtaining reproducible results in the analysis of the nitro compounds because of the additional step involved. An excess of the reagent will cause flaking of the adsorbent and excessive diffusion of the spots. Good results were obtained when the plate was first sprayed lightly with the sodium carbonate solution, dried in a stream of air and re-sprayed in the same way.

Potassium dihydrogen orthophosphate was added to the layer in order to prevent the tailing of aminofenitrothion spots that was observed with certain developing solvents. Its use is not essential for rendering the spots visible by the fluorometric technique and untreated silica gel H can be used for most separations.

An example of a typical chromatographic separation of fenitrothion and some possible breakdown products is given in Fig. 1. The positions of the spots can be varied by changing the developing system. This figure clearly illustrates the feasibility of quantitatively analyzing more than one species on a single chromatogram. In fact, the technique is at present being used successfully in our laboratories for the determination of fenitrothion and some breakdown products in natural water samples.

CONCLUSION

The procedure described has been used successfully for the simultaneous detection and quantitation of breakdown products of fenitrothion in samples of natural water. Provided that the compounds to be measured are separated from the co-extractives, the *in situ* technique offers a simple and convenient method of analysis. It is possible to run more than one sample concurrently. When the separation of the compounds from the interfering material by one-dimensional thin-layer chromatography is not satisfactory, a two-dimensional separation of the extract can be carried out.

The technique is not limited to the compounds listed here, and could be used for the detection of other primary amino or nitro derivatives of fenitrothion or for the detection of several breakdown products of parathion and parathion-methyl. As the fluorogenic reagent is selective towards primary amines, the two-step procedure permits discrimination between amino and nitro compounds.

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