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QUANTITATIVE PAPER CHROMATOGRAPHIC ANALYSIS OF THE ORGANOPHOSPHORUS PESTICIDE "VÚAgT-3"

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SUMMARY

A method is described for the determination of the biologically active components of the organophosphorus pesticide VÚAgT-3 in technical products and commercial formulations. The procedure consists of chromatographic separation on formamide impregnated papers with *n*-hexane and toluene as solvent, and colorimetric determination of phosphorus in eluates of undetected spots.

INTRODUCTION

"VÚAgT-3" is an systemic organophosphorus insecticide, containing (O-methyl O-ethyl)-S-(2-ethylsulfinylethyl)-phosphorodithioate and a certain quantity of O,O-dimethyl- and O,O-diethyl-S-(2-ethylsulfinylethyl)-phosphorodithioate, depending on the synthesis used[1]. In the technical product some biologically inactive ballast resulting from oxidative and hydrolytic decomposition, respectively, may also be found, which is not easy to remove before analysis. Therefore, the specific determination of the individual active components mentioned above presents considerable difficulties.

The known methods of analysis of related compounds are lacking in specificity. So are the different methods based on the determination of phosphorus or sulphur, and hydrolysis, respectively[2,3]. The infrared absorption method[2] measuring the absorbance of the P =S group at 15.2 μ can also be regarded as non-specific.

With respect to the great similarity of analytical and physical properties of the compounds to be determined, we considered quantitative paper chromatography a suitable method of resolving this problem. The evaluation of chromatograms originally used in our laboratory, based on the visual comparison of spot areas and colour intensities was semiquantitative[4]. In the present work the determination consists of a paper chromatographic separation, elution of the active components, and colorimetric determination of phosphorus. Similar procedures are known in the chemistry of organophosphorus pesticide residues[5–8] and in certain cases in the analysis of technical and formulated products[9].

EXPERIMENTAL

Paper chromatography

Reagents and apparatus

Chromatographic system[10]. Immobile phase: 20 % (v/v) formamide in acetone. Mobile phase: *n*-hexane–toluene (2:1, v/v) saturated with formamide.

Chromogenic reagent[10]. 0.1 *N* silver nitrate solution is mixed with concentrated ammonia (1:1). The reagent is freshly prepared before use.

Concentrated ammonia

Hydrogen peroxide

Stock solution of sample. 15 mg/ml solution of a technical product or 30 mg/ml of a commercial formulation in benzene.

Chromatography tank. An all-glass tank with an upper section for descending development was used, obtained from Labora n.p., Prague, Cat.No.9000.

Paper. Whatman No. 1 papers, 13.5 × 40 cm, were used, cut perpendicularly to the machine direction and marked according to Fig. 1.

Procedure

Four marked papers are drawn successively through a solution of the immobile phase, in the direction from the front to the start, and then allowed to drain by hanging freely (the start being at the bottom) in air to evaporate acetone. The 4 cm lower zone is cut off (see Fig. 1).

4 μl (about 60 μg) of the stock solution of the sample is applied at each mark on the start line using a high-precision Hamilton microsyringe or a calibrated capillary micropipet. The four spotted papers are suspended in the trough in the chromatography tank, saturated 24 h beforehand with vapors of mobile phase. Then 150 ml mobile phase is added to the trough and the chromatogram is run until the mobile phase has reached the marked front line (about 70 min). The papers are removed and allowed to hang under a fume hood until there is no discernible odor of toluene.

2.5 cm pilot strips are cut off at the sides of each paper and visualised by spraying with the chromogenic agent. The strips are heated in an oven at 110° until they have turned dark (5–8 min); then they are immersed for about 30 sec in a mixture of concentrated ammonia and a few drops of hydrogen peroxide until dark spots are discernible against a white background. The excess of reagents is washed out with tap-water and the washed strips are allowed to dry in air. The separation of the components is outlined in Fig. 1.

The chromatograms obtained serve as a reference for cutting off the remaining undetected chromatograms.

Quantitative analysis

Reagents and apparatus

All reagents were reagent grade.

Chloroform

Perchloric acid, 70 %.

Molybdovanadate reagent[11]. 40 g ammonium molybdate are dissolved in 250 ml hot water, cooled and 450 ml perchloric acid is added. The molybdate solution is gradually added to the vanadate solution with stirring, and diluted to 2000 ml.

Standard phosphate solution. A stock solution of KH_2PO_4 containing 0.1 mg P/ml is prepared.

Digestion flasks. 40–50 ml pear-shaped Kjeldahl flasks, neck length 18 cm, bulb diameter 3 cm, are used.

Vacuum–rotary evaporator

Aluminium foil. 20 × 22 cm sheets are used.

Digestion apparatus. The apparatus consists of aluminium cylindrical block (diameter 12.5 cm, height 4.5 cm) with six vertical holes (diameter 3.3 cm, depth 2.5 cm) heated by the gas-burner. The heating is controlled by means of contact-thermometer reading to 300°.

Photometer. A Carl Zeiss VSU 1 spectrophotometer or similar apparatus.

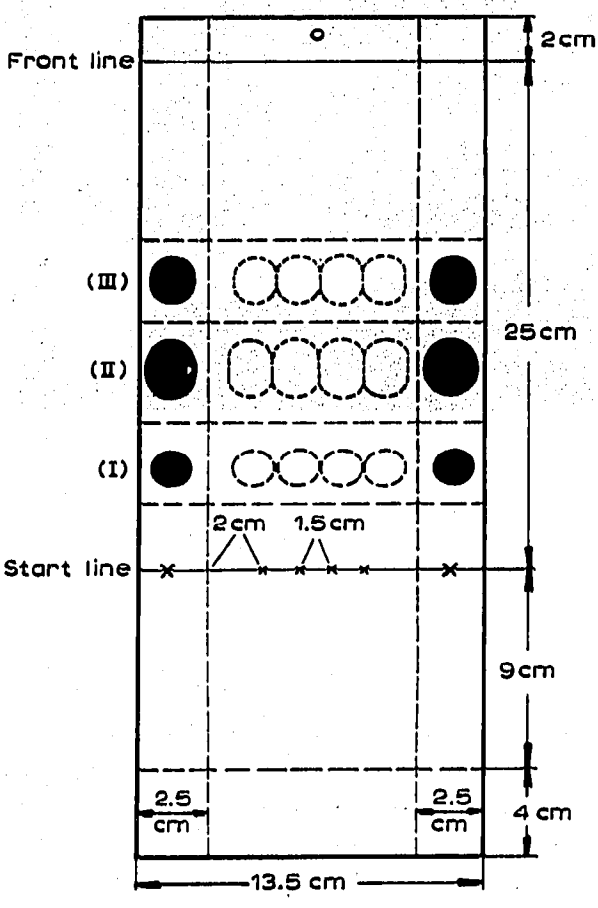

Fig. 1. Marking of the papers before impregnation and spotting.

Elution from the paper

The individual zones of the remaining undetected chromatograms are marked so that they correspond to the spots on the pilot chromatograms[12]. The marked zones are cut off, and identical zones of two parallel chromatograms are placed between two strips (5.5 × 20 cm) of Whatman No. 1 paper to avoid losses caused by handling, and wrapped tightly in aluminium foil to form a package approximately 1 cm wide. The packages are bound into a suitable form (see Fig. 2) and one end is immersed in a 250 ml beaker containing chloroform; 10 ml of the eluate is collected in the Kjeldahl flasks.

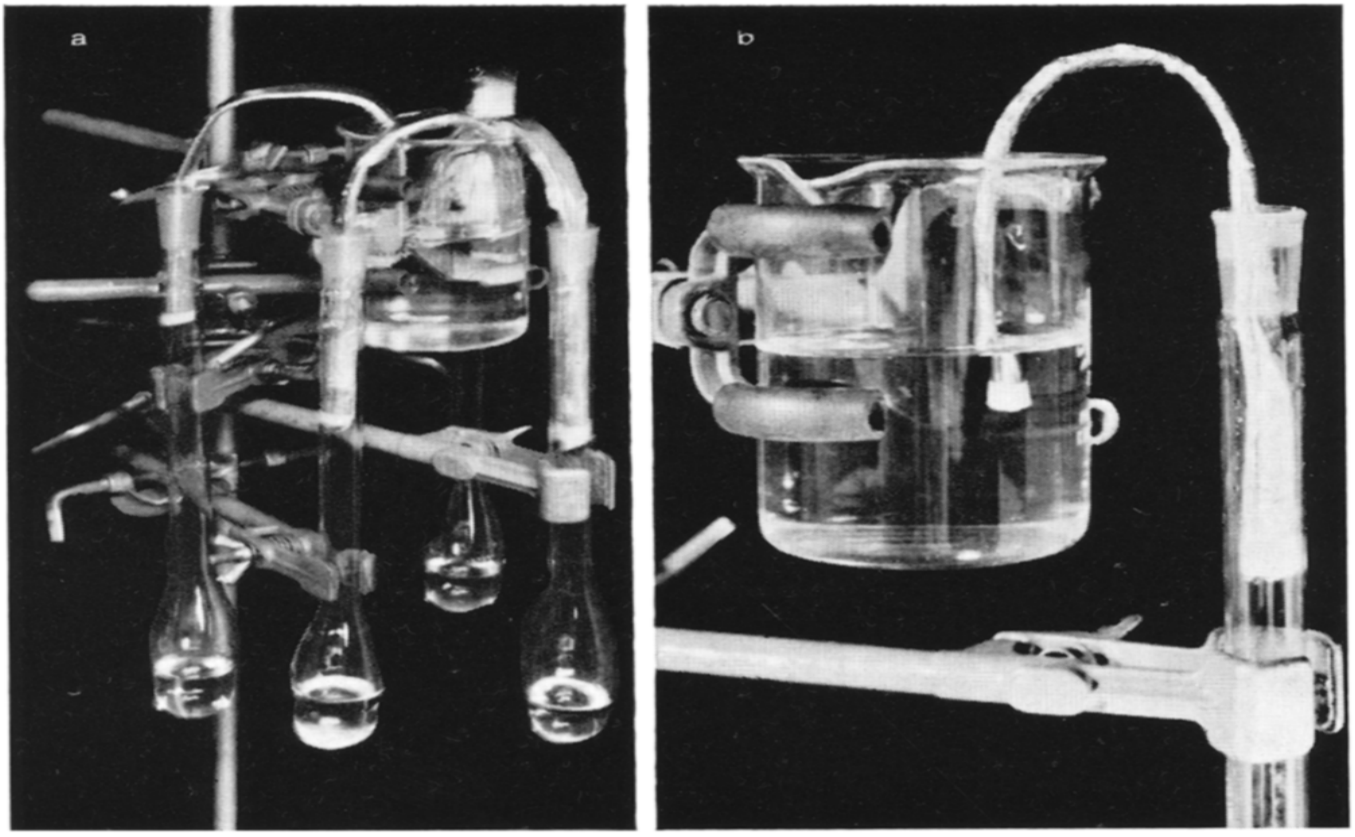

Fig. 2. (a) Elution of cut off zones; (b) a detailed view.

Determination of phosphorus

The Kjeldahl flask is attached to the vacuum–rotary evaporator and the chloroform evaporated nearly to dryness. The chloroform residues are removed by a stream of air. 1 ml of perchloric acid is added to each flask and the flasks are placed in the aluminium heating block. Digestion is at 250–260° for 1 h. The mixture is cooled before adding 5 ml of the molybdovanadate reagent, mixing thoroughly and transferring it to a 25 ml volumetric flask. The Kjeldahl flask is washed several times with water and the washings are collected in the volumetric flask. The volume is adjusted to the mark; the solution is mixed and transferred to a 4 cm cell when absorbance is read at 400 nm against a blank.

Standard curve. Aliquots of standard phosphate solution representing 10–50 μg of phosphorus were pipetted into 25 ml volumetric flasks. 1 ml perchloric acid and 5 ml of molybdovanadate reagent are added and the volume is made up to the mark, mixed thoroughly, and after 10 min the absorbance at 400 nm is read using 4 cm cells. Absorbance is plotted *vs.* μg P.

Blank. The blank is obtained from an unspotted chromatogram treated in the manner described.

Calculation. Per cent (p) of the individual components (I, II, III) are calculated according to the formula:

$$p = k \frac{P}{W} 100$$

where

k = conversion factor phosphorus to the component determined: 8.47 for I; 8.92 for II, and 9.38 for III.
P = amount (μg) of phosphorus read from the standard curve.
W = amount (μg) of a sample spotted on two chromatograms.

RESULTS AND DISCUSSION

R_F values of the components separated are given in Table I. The possible accompanying impurities do not interfere with the separation as their R_F values are higher than 0.7 or lower than 0.15.

The elution of the components I, II, and III from paper was studied quantitatively by means of a ^{32}P-labelled laboratory sample of VÚAgT-3. The results obtained showed that the losses due to the adsorption on the paper are less than 0.1 %. This

TABLE I

R_F VALUES FOR THE ACTIVE COMPONENTS OF VÚAgT-3

Compound	R_F
O,O-Dimethyl-S-(2-ethylsulfinylethyl)-phosphorodithioate (I)	0.18 ± 0.03
(O-Methyl O-ethyl)-S-(2-ethylsulfinylethyl)-phosphorodithioate (II)	0.35 ± 0.02
O,O-Diethyl-S-(2-ethylsulfinylethyl)-phosphorodithioate (III)	0.52 ± 0.02

TABLE II

ANALYSIS OF A LABORATORY SAMPLE OF ^{32}P-VÚAgT-3 BY THE DESCRIBED COLORIMETRIC METHOD AND MEASUREMENT OF RADIOACTIVITY

Compound	*Colorimetry* (%)	*Radio-analysis* (%)
I	32.6	30.0
	30.7	31.3
II	44.5	41.9
	43.6	41.4
III	17.9	16.2
	17.4	15.4

TABLE III

RESULTS OF SIX REPLICATE DETERMINATIONS BY THE PROPOSED METHOD FOR VÚAgT-3 COMPONENTS IN A 50% EMULSIFIABLE FORMULATION

Compound	*Quantity found* (%)
I	10.2
	10.2
	9.62
	9.63
	9.31
	8.9
II	25.5
	26.3
	24.4
	25.8
	25.4
	24.7
III	13.7
	14.0
	13.7
	13.1
	12.9
	13.5

was also confirmed by spraying the cut-off zones after the elution with the chromogenic agent. The quantity remaining on the paper was below the sensitivity of detection.

The ^{32}P-labelled sample was analysed by both the proposed procedure and by measurement of the radioactivity of the components eluted from the paper chromatograms. The results are given in Table II.

Results of replicate analyses of a commercial VÚAgT-3 formulation (50% emulsifiable concentrate) are summarized in Table III.

Considering the complexity of this pesticide the experimental results are satisfactory.

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