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THE FATE OF PESTICIDES IN AQUATIC ENVIRONMENT. III. THIN LAYER CHROMATOGRAPIC METHOD FOR THE DETERMINATION OF ABATE

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A method for the quantitative thin layer chromatographic determination of Abate is described. Fluorescence quenching was used as a mode of detection. The method was tested on samples of natural waters. The procedure of solvent ratio variation was applied to remove the interferences. The ambiguous clean-up procedure was thus avoided. A very sensitive TLC-EI technique was developed only for the detection of Abate but it was not suitable for the quantitative determination because of poor reproducibility.

Abate, a thiophosphoric acid ester, is a pesticide which is mostly used in mosquito control. Its toxicological properties which have been thoroughly investigated (1—5) clearly indicate that Abate is one of the lowest hazardous larvicides for mammals developed so far. It is a weak inhibitor of cholinesterases.

For the quantitative determination of Abate in plants, soil and water colorimetric (6), gas chromatographic (7—10) and high pressure liquid chromatographic methods have been developed (11). Although sensitive, these methods are time-consuming mostly because of ambiguous clean-up procedures.

The only known simple method is the thin layer chromatographic (TLC) determination (12), but it is not sufficiently sensitive. In this work we describe a sensitive TLC method for the quantitative determination of Abate in which fluorescence quenching is used as a mode of detection.

Quantitative evaluation is carried out by direct densitometric measurement. The procedure was applied for the determination of Abate in surface waters. An even more sensitive TLC technique based on acethylcholinesterase inhibition (TLC-EI) is used only for detection because in the case of Abate the quantitative evaluation of thin layer chromatograms has been found poorly reproducible.

MATERIALS AND METHODS

0,0,0',0'-tetramethyl-0,0'-thiodi-p-phenylene phosphorothioate (Abate, Biothion Insecticide) 90.3% was obtained from WHO, Geneva, Switzerland. Abate 1% granular formulation was a gift from Mr. V. Vuković, Institute of Public Health of Croatia, Zagreb, Yugoslavia.

We prepared standard stock solutions by dissolving a weighed amount of Abate in acetone. The same solvent was used for further dilution.

Abate was extracted from water samples by means of chloroform (12). The water to extractant v/v ratio was 10:1. In the extraction of Abate from natural waters the centrifugation at 2000 r. p. m. for two minutes was necessary to achieve a complete separation of layers (Centrifuge Typ S-51, H. Janetzki Maschinenbau, Engelsdorf, Leipzig, Germany). The chloroform layer was evaporated in a rotary evaporator (Rotavapor-R, Büchi, Flawil, Switzerland) until about 1 cm^3 of the solution remained. This volume was diluted to 2 cm^3 with chloroform and Abate was quantitatively determined by TLC.

Pre-coated 20 x 20 cm TLC plates with silica gel 60 F_{254} layer 0.25 mm thick with a fluorescence indicator, manufactured by Merck, Darmstadt, Germany were used for the quantitative determination. The samples were applied with 10 μl graduated micropipettes. The thin layer chromatograms were developed by means of solvent system ethylacetate and n-hexane. The v/v ratio was 1:3. The spots were visualized by fluorescence quenching in UV light (254 nm) as dark violet spots against a bright intensely fluorescent background. The thin layer cromatograms were quantitatively evaluated by direct densitometric measurement with a Camag-T Scanner (Camag, Müttenz, Switzerland) supplied with UV_{254} lamp, primary filter 810 ($\lambda = 254$ nm) and secondary filter 816 ($\lambda = 415$ nm). The areas under curves were calculated according to Monte Carlo method (13).

TLC-EI technique was used only for detection (15). The TLC plates, application of sample and development were the same as described previously. After the development and evaporation of solvent, the plates were exposed to bromine vapours for three minutes. Adhering bromine was aired before spraying. The plates were placed into the moisture chamber for 30 minutes immediately after spraying with enzyme solution. The enzyme solution was a 10% aqueous solution of rat plasma diluted with Sörensen's phosphate buffer pH = 8.0 to volume ratio 1:1.

The spots were finally visualized by spraying with substrate solution and heated at 37°C for 20 minutes. The substrate solution was prepared just before spraying with one milligram indophenylacetate dissolved in each millilitre of absolute ethanolic solution. The spots were pale yellow against a pink background.

RESULTS AND DISCUSSION

In constructing the calibration curve for the quantitative determination of Abate we took the ratio between the sample peak area and the standard peak area as the ordinate to eliminate possible variations in experimental conditions. The peak areas were determined by Monte Carlo method. We compared the results with those obtained by the correlation of the sample spot area with the standard spot area, which we measured by copying the spots on a transparent squared paper and counting the number of square millimetres under spot areas. We also compared them with the results obtained by the correlation of the weights of the cut out pieces of paper which corresponded to the sample and standard spot areas (Fig. 1). Between 0.9 and 5 μg i. e. in the linearity range, all the three methods of evaluation show good agreement, but with larger quantities the results are somewhat dispersed. For the separation described, the development time was 30—45 minutes and R_f value for Abate was 0.31 ± 0.01. The least detectable quantity was 450 ng. The method is twenty times more sensitive than the TLC method described by *Howe* and *Petty* (12).

To achieve a higher sensitivity we used TLC-EI technique but the results showed a strong dependence on rat plasma which served as the enzyme source and could not be used for the quantitative determination because of poor reproducibility. However, the TLC-EI technique was applicable for the detection of Abate in quantities of about 3 ng.

With the described TCL method for the determination of Abate by means of fluorescence quenching we intended to solve the practical problems of monitoring the presence of Abate in surface waters. We studied the effects of interferences in the model system and set up the accumulation procedure.

Known quantities of Abate (10.54 mg, 0.45 mg and 0.045 mg of Abate in the form of acetone solution) were added to one litre of water samples of different origin. The extractions, carried out by means of chloroform, gave the same recoveries for all concentrations investigated regardless of the water origin (Table 1 and 2). The interferences were found to be insignificant.

Abate is usually added to surface waters in the form of granular formulations. The one we got was claimed to have 1% of Abate but the quantities of Abate found by extraction of granular formulation with chloroform, ether and acetonitrile were only 0.37, 0.28 and 0.33% respectively.

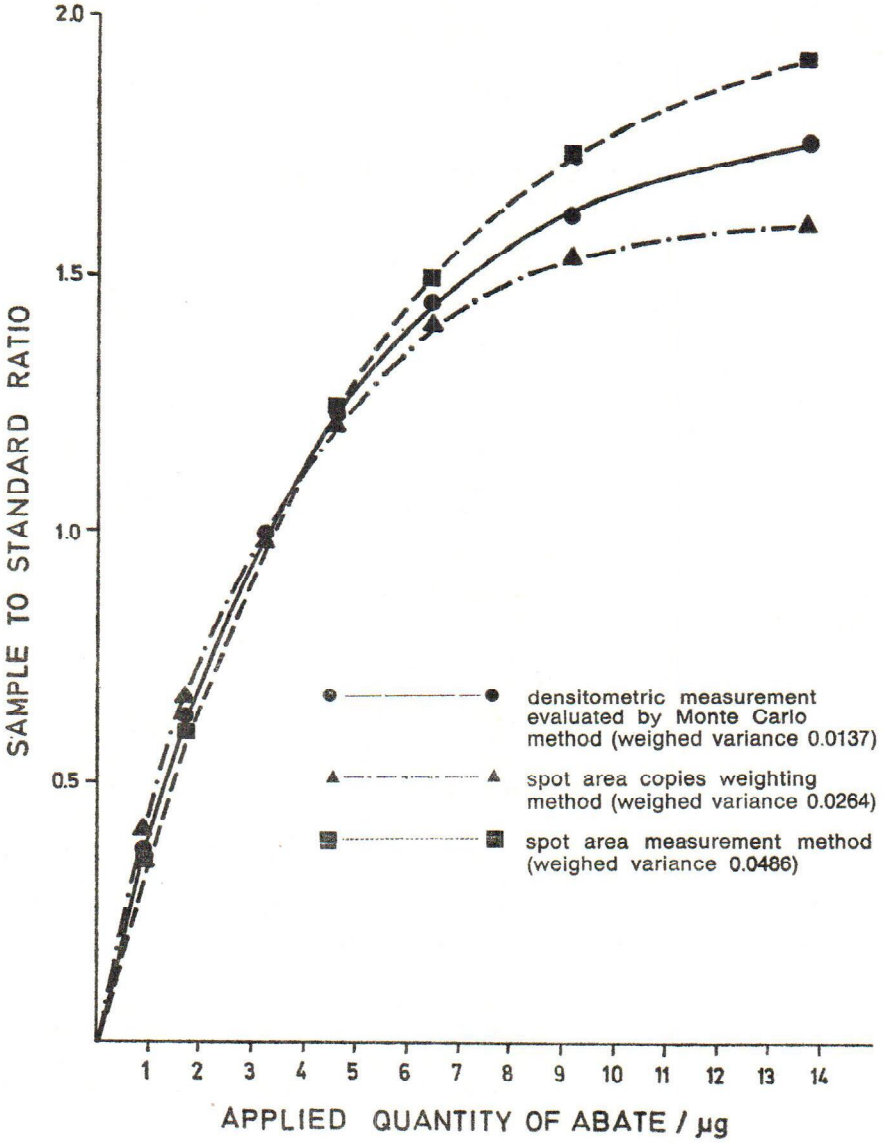

Fig. 1. *Calibration curves for quantitative thin layer chromatographic determination of Abate by fluorescence quenching as a mode of detection. The same thin layer chromatograms evaluated by three different methods (Plots of the fitted functions)*

Table 1.

Efficiency of extraction of Abate added to distilled and surface waters

The concentration of Abate: 10,5 ppm

Sample	Recovery (%)			
	Distilled water	Water of the Sava river	Water of the Sljeme brook	Water of the Kutina puddle
1	88	93	88	95
2	75	91	88	95
3	97	106	94	87
4	87	88	91	88
5	87	86	89	92
6	93	92	93	92
7	102	88	94	80
8	—	93	86	84
9	—	89	93	90

Mean value 90% (SD = 6%)

Table 2.

Efficiency of extraction of Abate added to distilled and surface waters

Concentration of Abate	Recovery (%)			
	452 ppb		45 ppb	
Sample	distilled water	water of the Trnjanska Savica brook	distilled water	water of the Trnjanska Savica brook
1	86	96	91	68
2	89	100	82	91
3	92	93		
4	97	96		
5	84	93		
6	76	89		
7	86	78		
	Mean value 90% SD = 7%		Mean value 83%	

The solubility of Abate from granular formulations was tested in distilled water and in three surface water samples which were polluted under different environmental conditions: the Cmrok puddle was polluted almost only by natural organic material; the Zaprudje puddle was polluted by municipal wastes; and the slow Trnjanska Savica brook

was polluted by both. All the three surface waters are periodically treated with Abate during the mosquito season. To one litre of surface waters about 200 mg of 1% Abate granular formulation was added. Abate was not found in any of the surface waters before adding. After four hours the extraction was carried out and only 3.2% of added quantity was found dissolved in the water of the Zaprudje puddle, 15% in the water of the Trnjanska Savica brook and 16% in distilled water.

The dependence of Abate solubility on time was tested in the water of Trnjanska Savica brook (Table 3). Although Abate is claimed to be

Table 3.

Time dependence of Abate solubility tested in the water of the Trnjanska Savica brook

The quantity of 0.35% granular formulation of Abate added to one litre of water/mg	Time elapsed from Abate addition	Percentage of Abate found in water	Percentage of Abate found in precipitate	Total percentage of Abate found
228.5	4 hours	15	83	98
221.5	17 hours	15	85	100
228.0	7 days	24	44	68
233.0	26 days	12	28	40

very stable in natural waters (14) the total quantity of Abate after the extraction of water and precipitate, which included unsolved granular formulation of Abate, decreased with time. Moreover, in the water of the Cmrok puddle Abate was not found 26 days after it was added and in the precipitate formed with time only 57% of added Abate was found. The reason for such behaviour must be investigated and it is obvious that a continuous monitoring of Abate in natural waters is necessary.

In five litres of water taken from the Zaprudje puddle Abate was not found before the first official application in the same year. Seven days after the application eight ppb of Abate was found. When larger volumes of natural water are extracted the problem of interfering substances arises. This problem is usually solved by the use of different time consuming clean-up procedures. To avoid these, we removed the spots overlapping the spot of Abate by varying the ratio of n-hexane in developing system. For the Zaprudje puddle the ethylacetate and n-hexane v/v ratio 1:5 was found to be the best for removing the interferences. The method of the solvent ratio variation makes possible a direct determination of Abate in extracts if TIC procedure is used.

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Sažetak

SUDBINA PESTICIDA U VODENOM OKOLIŠU. III. METODA TANKOSLOJNE KROMATOGRAFIJE ZA ODREĐIVANJE ABATA

Opisana je metoda za kvantitativno određivanje Abata tankoslojnom kromatografijom. Za detekciju je upotrebljena metoda gašenja fluorescencije. Primjenljivost metode provjerena je na uzorcima prirodnih voda. Variranjem sastava eluensa uklonjene su supstancije koje interferiraju pri određivanju Abata čime je izbjegnuto čišćenje ekstrakta. Za detekciju nanogramskih količina Abata primijenjena je kombinirana metoda tankoslojne kromatografije i enzimske inhibicije. Iako osjetljivija od detekcije gašenjem fluorescencije enzimska se inhibicija u ovom slučaju ne može koristiti za kvantitativno određivanje Abata zbog loše ponovljivosti.

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