A Gas Chromatographic Method for the Determination of Low Concentrations of Abate in Water

by M. T. SHAFIK[1]

Abate (0,0,0',0'-tetramethyl-0,0'-thiodi-p-phenylene phosphorothioate) is a larvicide suggested for the control of mosquito larvae in natural waters.

Data are needed for Abate residues in water in order to determine the levels which will result from the routine use of this compound in drinking waters.

Abate is determined by its hydrolysis to 4,4'-thiodiphenol which in turn can be determined spectrophotometrically in the ultraviolet region or can be reacted with 4-aminoantipyrine to give a color to be used as the basis for a colorimetric procedure (1). Abate was also determined using a flame ionization detector and a DC-11 column maintained at 270°C (2). The colorimetric methods lack sensitivity, and the gas chromatographic method lacks both sensitivity and reproducibility, as Abate and its hydrolysis product 4,4'-thiodiphenol have a low vapor pressure which discourages the use of gas liquid chromatography.

The injection of a large number of Abate samples in the gas chromatograph contaminate the instrument with the result that Abate peaks appear with the solvent injection only.

The following investigation is concerned with the quantitative conversion of the low vapor pressure 4,4'-thiodiphenol to a product of high vapor pressure using silylating reagents.

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The replacement of the active hydrogens in the two hydroxyl groups with Si $(CH_3)_3$ groups enhance the passage of the compound through the gas chromatograph.

Materials and Methods

Reagents.

Trimethylchlorosilane - Analabs, Hamden, Conn.[2/]
Hexamethyldisilazane - Analabs, Hamden, Conn.
Chloroform, redistilled
n-Hexane, dry, redistilled
Methanol, redistilled
Acetone, redistilled
Aqueous potassium hydroxide solution 15N
Aqueous sodium hydroxide solution 0.1N
Sulfuric acid solution 1N and 6N
Sodium sulfate, anhydrous

Apparatus

Gas chromatograph model MT 220 equipped with a flame photometric detector, sulfur filter and a U-shaped aluminum column, ¼" o.d. x 4' packed with 80/100 mesh Chromosorb W coated with 2.5% E 301 and 0.25% Epon 1001 (w/w). The temperatures are; inlet 190°C, column 190°C, detector 165°C. The flow rates are, nitrogen 100, hydrogen 150, oxygen 15 ml./minute. On column injection was used in this work.

Analytical Procedure

Preparation of standard curve. Dissolve 0.1000 gram of the 4,4'-thiodiphenol standard in one liter of dry benzene. Pipet 0.02, 0.04, 0.06 and 0.08 ml of the standard solution in 25- ml graduated concentrator tubes

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2/ Commercial sources and trade names[2] are provided for identification only. Their mention does not constitute endorsement by the Public Health Service or by the U.S. Department of Health, Education and Welfare.

corresponding to 2,4,6 and 8 micrograms respectively. Add dry benzene to the 1-ml mark. To the 4,4'-thiodiphenol add 0.25 ml dry pyridine, 0.15 ml hexamethyldisilazane, 0.05 ml trimethychlorosilane; stopper, mix and heat at 50°C for 10 minutes with caution.* Allow tubes to stand overnight at room temperature. Add approximately 5 ml water then 5 ml 1N sulfuric acid to make the solution acidic and shake vigorously. After separation of the two layers, aspirate off the water layer. Repeat the water washing of the n-hexane or benzene layer two more times. Adjust the volume of the remaining n-hexane or benzene so that 10 microliters will contain 2-20 or 20-80 nanograms 4,4'-thiodiphenol. Plot the response or peak height versus concentration on regular or log log graph paper.

Extraction. Filter a 500 ml sample of water into a liter separatory funnel. Rinse the filter and container with two 15 ml portions of acetone and add to separatory funnel. Add 100 ml chloroform via the filter. Shake the funnel vigorously for one minute. Transfer the chloroform layer to a second separatory funnel. Wash the chloroform with 25 ml of 0.1N sodium hydroxide solution. Dry the chloroform extract by passing through a column of anhydrous sodium sulfate into a Kuderna-Danish concentrator fitted with a 15-ml round bottom test tube. Repeat the extraction, washing and drying steps with two additional portions of fresh chloroform and combine the extracts in the Kuderna-Danish concentrator. Concentrate the extract on a steam bath, then evaporate the last trace of chloroform using a gentle stream of dry nitrogen, and save residue for the hydrolysis step.

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*It is necessary to handle the silylation reagents in a well-ventillated hood.

Hydrolysis. Dissolve the Abate residue from a standard or from a water extract in 4 ml methanol. Add 1 ml 15N potassium hydroxide solution. Place a one-ball Snyder column in the top of the tube and heat at 100°C until all the methanol has evaporated. Remove the Snyder column and continue to heat at 100°C for one hour. Cool and dissolve the residue in 5 ml of water; then transfer the hydrolysate to a 50-ml separatory funnel with the aid of 10 ml of chloroform. Acidify the aqueous layer with 5 ml 6N sulfuric acid and extract with a total of two 20-ml portions and one 10-ml portion of chloroform. Dry the combined chloroform extracts by passing through anhydrous sodium sulfate into a Kuderna-Danish evaporator fitted with a 25-ml evaporative concentrator tube. Concentrate the extract to a volume of about 5 ml on a steam bath. Evaporate the last 5 ml of chloroform with the aid of a gentle stream of dry nitrogen and a room temperature water bath.

Dissolve the residue in 1 ml of dry n-hexane or dry benzene. Stopper and heat the tubes at 50°C for about 10 minutes to dissolve the hydrolysate "4,4-thiodiphenol."

Silylation reaction. Add to the dissolved Abate hydrolysate, 0.25 ml dry pyridine, 0.15 ml hexamethyldisilazane, 0.05 ml trimethylchlorosilane; stopper, mix and heat at 50°C for 10 minutes with caution. Allow tubes to stand overnight at room temperature. Add approximately 5 ml water then 5 ml 1N sulfuric acid to make the solution acidic and shake vigorously. After separation of the two layers, aspirate off the water layer. Repeat the water washing of the n-hexane or benzene layer two more times. Adjust the volume of the

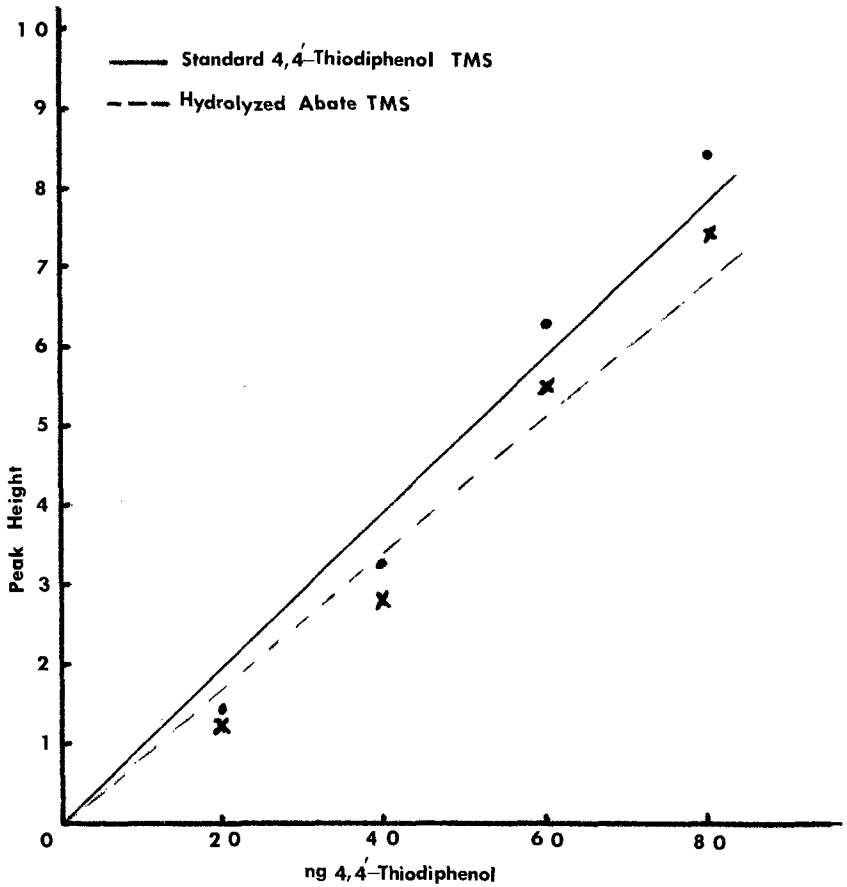


Fig. 1 Standard curves of 4,4'-thiodiphenol and hydrolyzed abate TMS

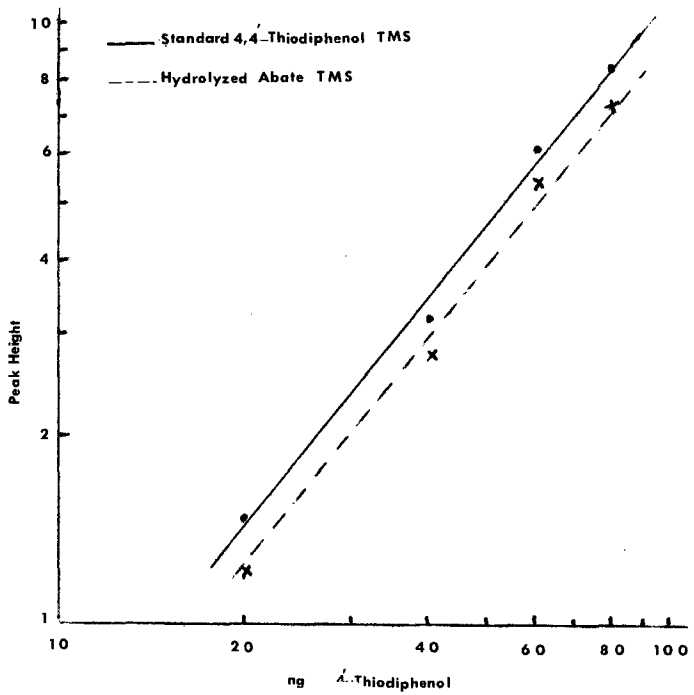


Fig. 2. Standard curves of 4,4'-thiodiphenol TMS and hydrolyzed abate TMS

remaining n-hexane or benzene so that 10 microliters will contain 2-20 or 20-80 nanograms 4,4'-thiodiphenol.

Results and Discussion

Four, 500-ml water samples were fortified with 2,3, 6, 16 micrograms of Abate. Each sample was carried through the entire analytical procedure. Standards of 4,4'-thiodiphenol were treated with the silylation reagents and analyzed by gas chromatography in order to determine the recovery of Abate from the fortified water samples. The results are shown graphically in Figures 1 and 2. The average recovery of Abate from the fortified water samples, when compared with the 4,4'-thiodiphenol standards, was 88%.

The hydrolysis of Abate with 15N potassium hydroxide using the procedure suggested by Blinn and Pasarela (1), is essentially complete. When aliquots of the hydrolysate were spotted, developed and detected on a silica gel fluorescein treated plate, using a 1:1 mixture of n-hexane and diethylether and an ultraviolet lamp, the R_f value was identical with that of pure 4,4'-thiodiphenol.

The chromatogram in Figure 3 shows the response of the flame photometric detector equipped with the sulfur filter to the hydrolysis product of Abate, 4,4'-thiodiphenol. Higher attenuations can be used depending on the noise level of the detector.

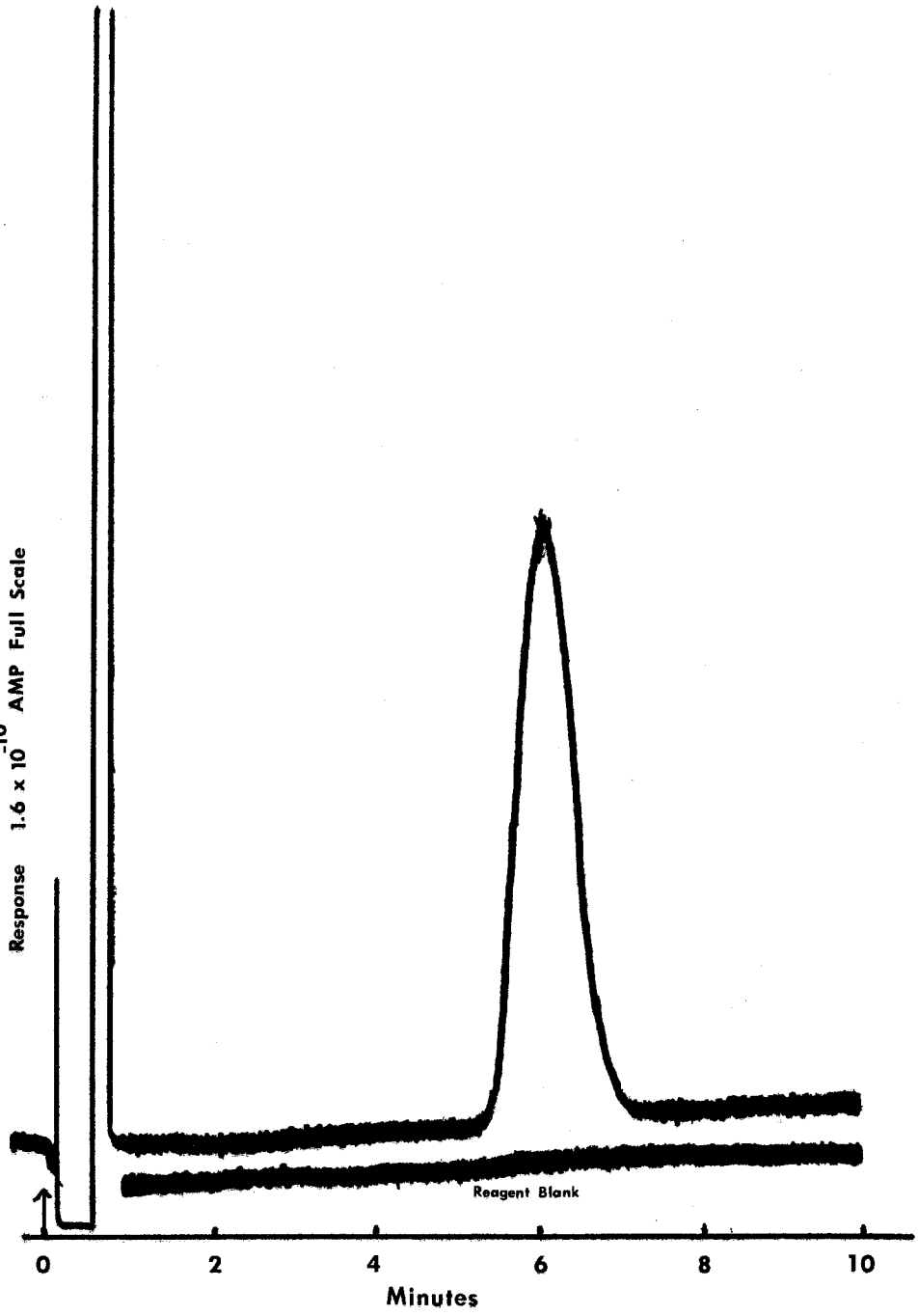
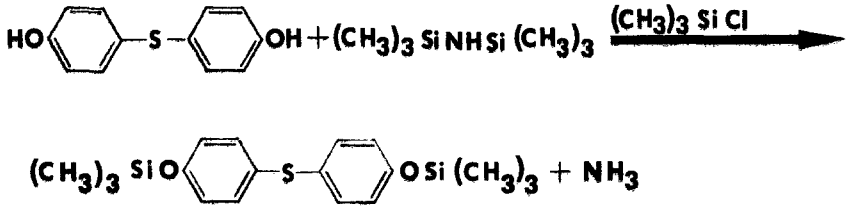


Fig. 3. Chromatogram of 10 ng of 4,4'-thiodiphenol TMS

The replacement of the active hydrogens in 4,4'-thiodiphenol with TMS groups can be represented by the following reaction:



The trimethylchlorosilane "TMCS" serves to catalyze the reaction and the hexamethyldisilazane "HMDS" is the silylating reagent.

It was not possible to inject this reaction mixture directly in the gas chromatograph, as the silylating reagents deposited on the windows on the detector. Such deposits decrease the intensity of the light transmitted to the photomultiplier tube which in turn lowers the sensitivity. In order to avoid this problem it is necessary to remove the excess reagents by acidifying and washing with water several times.

References

1. R. C. Blinn and N. R. Pasarela, J. Agr. Food Chem. 14, 152-6 (1966).
2. F. C. Wright, B. N. Gilbert and J. C. Riner, J. Agr. Food Chem. 15, 1038-9 (1967).