Gas Chromatographic Determination of Abate using Flame Photometric and Electron-Capture Detectors

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A gas chromatograph equipped with a flame photometric detector has been used for the development of a highly sensitive and specific method for the determination of Abate (*O,O,O',O'*-tetramethyl-*O,O'*-thiodi-*p*-phenylene phosphorothioate) in water. Since only phosphorus or sulfur is determined by this detector, no clean-up is required. Therefore, *n*-hexane extracts of water samples were concentrated to a suitable volume and aliquots were injected directly into the gas chromatograph. The recovery of Abate from water averaged 98.5 ± 1.3%. The method is sensitive to 2 ng. of Abate as phosphorus and to 30 ng. of Abate as sulfur. The method can also be used with gas chromatographs equipped with electron-capture detector, and possibly other detectors. However, with the electron-capture detector, the method is only sensitive to 8 ng. and lacks the specificity of the flame photometric detector.

Abate (*O,O,O',O'*-tetramethyl-*O,O'*-thiodi-*p*-phenylene phosphorothioate) is one of the most effective new compounds for the control of mosquito larvae. It is lethal to *Aedes aegypti* larvae in concentrations below 0.005 p.p.m. and is reported to have an oral LD_{50} to rats and mice in excess of 4000 mg. per kg. (Gaines *et al.*, 1967). In addition to its use in a conventional manner in natural waters, it has been proposed as an additive in drinking water in cisterns, drums, and other storage containers to prevent breeding of *Culex* and *Aedes* mosquito larvae in tropical areas (Brooks and Schoof, 1965; Jakob, 1965).

Approval was granted by the Puerto Rico Department of Health for a field trial in which 1 p.p.m. of Abate was applied to drums and other small reservoirs of drinking water in an experimental area in Puerto Rico to control *Aedes aegypti* (Laws *et al.*, 1968). To ensure that the maximum permissible dosages are not exceeded, samples of water from the test area are taken monthly for analysis.

Several methods have been proposed for determination of Abate residues, none of which are completely satisfactory for determination at the low levels encountered in the treatment of potable waters. A colorimetric method for determination of Abate residues by Blinn and Pasarela (1966) was based on the reaction of the hydrolysis product, 4,4-thiodiphenol, with 4-aminoantipyrine and periodate and subsequent determination at 485 mμ. In the method of St. John and Lisk (1968), the methylated alkyl phosphate hydrolytic product of Abate was determined, using a gas chromatograph equipped with a thermionic detector. A recent gas chromatographic method using flame ionization detection was reported for Abate in water (Wright *et al.*, 1967). Although the method is sensitive to 0.05 p.p.m., it requires extensive clean-up and the average recovery is only 70%.

Until recently, we have determined Abate residues in water by extraction of the samples with chloroform, concentration of the extract, and separation of the Abate on a silica gel thin layer plate eluted with 1-to-1 hexane-ether solution. An accurate measurement was made by removal of the spot, oxidation of the Abate with perchloric acid and determination of the total phosphorus by the molybdenum blue colorimetric method (Chen *et al.*, 1956). A much more rapid, sensitive, and highly specific gas chromatographic method has now been developed. This method utilizes a gas chromatograph equipped with a flame photometric detector (Brody and Chaney, 1966) which is highly specific for phosphorus- and sulfur-containing compounds. The new method has proved most satisfactory for determination of Abate in potable water.

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MATERIALS AND METHODS

Reagents. Abate, analytical standard, purity 99.9% (American Cyanamid Corp., Princeton, N. J.).

Cotton, U.S.P., extracted with acetone overnight in a Soxhlet extractor. Air-dried until there was no odor of acetone and then heated for 2 hours at 100° C.

Apparatus. Gas chromatograph MicroTek MT-220 equipped with a Melpar flame photometric detector (F.P.D.) with interference filters for spectral isolation of phosphorus emission at 526 mμ and sulfur emission at 394 mμ. The flame photometric detector was modified as described below to permit operation at 250° C. The chromatograph was also equipped with an electron-capture detector, 130 mc. tritium source.

Digital readout system Infotronics Model CRS-11HS-B/A with a Victor digit-matic printout.

Strip chart recorder Westronic Model LD11B.

Filter tube Fisher 8-261.

Preparations of Standards. Weigh accurately 0.030 gram of analytical standard Abate into a 25-ml. volumetric flask and make to volume with acetone.

By serial dilution with redistilled *n*-hexane, prepare standard solutions to contain 1.0, 12.0, and 60.0 ng. of Abate per μl.

Inject 1 to 10 μl. (1 to 120 ng.) of the 1.0- and 12.0-ng.-per-μl. standards into the gas chromatograph operated under the following conditions: Column temperature, 240° C.; inlet and outlet blocks, 250° C.; flame photometric detector, 526-mμ (phosphorus) filter, 240° C.; carrier gas, nitrogen, 150 cc. per min. at 50 p.s.i.; hydrogen, 150 to 200 cc. per min. at 20 p.s.i.; oxygen, 15 cc. per min. at 60 p.s.i.; attenuation, $10^2 \times 64$; column, aluminum tube, 1/4 inch, packed with 2 inches of 2% GE nitrite silicone gum XE-60, on 80/100-mesh Chromosorb

W, acid-washed, DMCS treated. Plot peak areas *vs.* quantities injected to obtain a standard curve which is linear from 2 to 120 ng.

To obtain a standard curve based on sulfur emission at 394 mμ, replace the 526-mμ (P) filter with a 394-mμ (S) filter and inject 30 to 240 ng. of Abate into the gas chromatograph, using appropriate standard solutions. Plot resulting peak areas *vs.* quantities injected on log-log paper to obtain a straight line.

For electron-capture detection of Abate, connect the column to an electron-capture detector and modify the operating conditions as follows: Detector, 200° C.; carrier gas, nitrogen, 77 cc. per min.; attenuation, $10^2 \times 2$. Inject 1 to 5 μl. (12 to 60 ng.) of the 12-ng.-per-μl. standard into the instrument and plot the resulting peak areas against quantities injected to obtain a linear standard curve.

Extraction. Place a 500-ml. sample of water in a 1000-ml. separatory funnel, add 1 ml. of concentrated HCl and extract with 50 ml. of hexane by shaking for three 1-minute intervals, being careful to allow the liquid phases to separate between each extraction period. Draw off the aqueous layer and discard. Place a small plug of cotton in a filter tube and wet with *n*-hexane. Pass the hexane extract through this tube and collect in a 250-ml. beaker. Rinse the separatory funnel twice with 5 ml. of *n*-hexane and pass these rinsings through the cotton. Finally, rinse the tube and cotton with 5 to 10 ml. of *n*-hexane. Evaporate the solvent from the extract to a volume of 2 to 5 ml. Transfer quantitatively with the aid of acetone to a 15-ml. centrifuge tube. Evaporate the acetone to complete dryness in a boiling water bath. Remove the tube immediately upon its going to dryness. Rinse down the walls of the tube with about 1 ml. of acetone, replace the tube in the water bath, and evaporate to dryness. Repeat the rinsing of the walls of the tube two times in order to concentrate the insecticide in the bottom of the tube. Make to an appropriate volume with isooctane and shake vigorously on a vortex mixer. Inject an aliquot into the gas chromatograph operated as described for the respective detector.

DISCUSSION

The manufacturer's recommended operating temperature for the flame photometric detector is 165° C. To determine Abate, it was necessary to operate the detector at 250° C. This was done by placing a water-cooled heat sink (Dale and Hughes, 1968) between the flame housing and the phototube. Owing to the short retention time of Abate, the improved re-ignition technique for the flame photometric detector described by Fetzer (1968) was used.

The column inlet and outlets on the MicroTek MT-220 are 3 inches apart; therefore, to install a 2-inch column, only the last 2 inches of a 10-$\times$ $^1/_4$-inch aluminum tube were packed. A small amount of glass wool was inserted into the tube and pushed in a distance of 2 inches. A small rod was then pushed in the other end of the tube until contact was made with the glass wool. This offered support to the glass wool plug during packing. The last 2 inches of the column were filled with the packing material and packed with the aid of a vibro-tool. Glass wool was inserted to secure the column packing. The column was then baked out overnight at 250° C. before being connected to the detector. No further conditioning was required.

The column was formed to fit the MT-220 oven, such that the packed portion of the column was connected to a low dead volume column adapter in the outlet block. The inlet of the column was connected in the on-column position. With the column connected as described, all column dead volume is ahead of the column packing. It is essential that the column be positioned as described.

The efficiency of the single extraction procedure was determined by adding Abate, in acetone, to water to give concentrations of 0.0036, 0.012, 0.06, 0.122, 0.61, and 1.22 p.p.m. The fortified samples were analyzed by the above procedure, and results are presented in Table I. The average recovery of Abate from water by this method was 98.5 ± 1.3%. These data are in good agreement with the partition distribution of Abate between hexane and methanol-water shown by Blinn and Pasarela (1966). Water fortified with Abate at 0.12 p.p.m. was also extracted as described with methylene chloride, chloroform, ethyl ether, and isopropyl ether. Recoveries obtained were 104, 100, 103, and 108%, respectively. The acetone rinsings described for transfer and concentration of the residues were essential to obtain quantitative recoveries.

The retention time for the Abate peak was 58 ± 1 second when using both the 394-mμ (S) filter and the 526-mμ (P) filter (Figure 1). As could be expected, with the slower carrier gas flow rate (77 cc. per minute) used with the electron-capture detector, the retention time for Abate was 109 ± 1 second. The retention volumes obtained for the peaks were 8700 and 8393, respectively, when using the F.P.D. and the E.C. This would indicate that the material detected by the three detection methods was the same.

Positive identification of the Abate peak was made by preparing a $^1/_4$-inch $\times$ 1-foot column with the same packing material described above and placing in a Varian Model 1520 preparative gas chromatograph. When operated at 250° C. with carrier gas flow at 130 ml. per minute, retention time for Abate was 570 seconds. Multiple injections of a solution of Abate were made into the preparative instrument and fractions of Abate were collected as the compound emerged. The combined fractions were diluted with isooctane and a small portion was injected into the MicroTek Model 220 equipped with flame photometric detector and operated under the conditions described

Table I. Recoveries of Abate from Water with a Hexane Extraction

Abate Added, P.P.M.	Abate[a] Found, P.P.M.	Recovery[b] Av. %	Recovery[b] Std. Error
0.0036	0.0035	97	± 0.44
0.012	0.012	100	± 0.88
0.060	0.062	103	± 0.71
0.12	0.120	100	± 1.39
0.60	0.58	97	± 1.06
1.20	1.13	94	± 1.24
		$\bar{X}_6$ = 98.5	± 1.3

[a] All determinations were made with the flame photometric detector with phosphorus-sensitive (526-mμ) filter.
[b] Based on analyses of four 500-ml. aliquots of each Abate-fortified water sample.

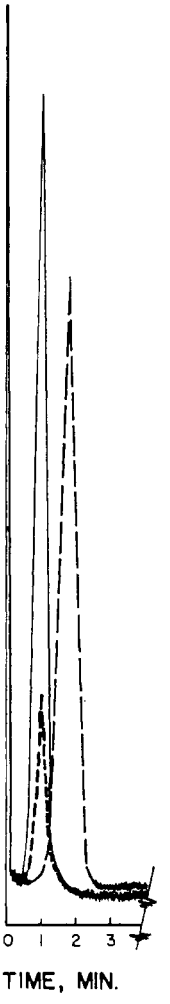

Figure 1. Gas chromatograms of Abate

— Flame photometric detector with phosphorus filter (526 mμ)
·· -- Flame photometric detector with sulfur filter (394 mμ)
– – Electron capture detector

above. The compound emerged with the same retention time as a known solution of Abate. Another fraction from the preparative instrument estimated to contain about 10 μg. of Abate was spotted on a thin layer plate of silica gel, which was then developed with a 1-to-1 solution of ether-hexane. The R_f value was the same as that for Abate. Finally, the spot was scraped from the thin layer plate and extracted with ether which was collected in a small agate mortar. Five mg. of KBr were added to the ether solution and ground with a pestle after evaporation of the solvent. The KBr was compressed into a pellet 1.5 mm. in diameter and the infrared spectrum recorded on a Perkin Elmer IR spectrophotometer Model 521 equipped with a beam condenser. The spectrum was compared with that of a pellet prepared in the same manner with 10 μg. of pure Abate (Figure 2) to confirm the identity of the GLC fraction.

The 2-inch column is highly specific for the determination of Abate under the operating conditions described. Abate was analyzed in the presence of approximately equal concentrations of 10 other commonly used organic phosphorus pesticides—namely, malathion, parathion, methyl parathion, methyl trithion, ethion, guthion, trithion, DDVP, bromophos, and dibrom. As was expected, all of these pesticides eluted from the column during the 10- to 15-second flame re-ignition period and did not interfere

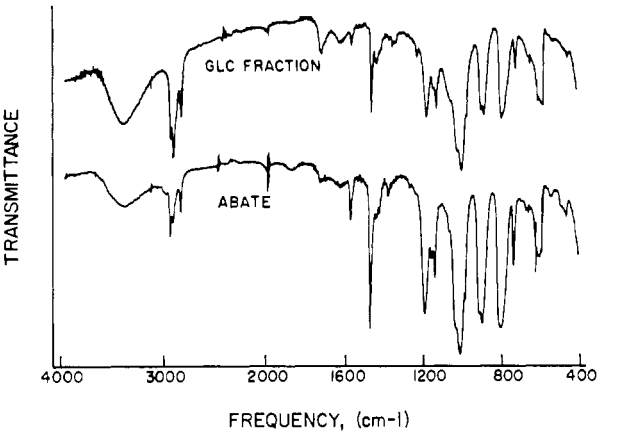

Figure 2. Infrared spectra of micro pellets made from pure Abate and fractions of Abate trapped from a gas chromatograph

with the analysis of Abate. A sample of the sulfoxide of Abate, which was reported as the principal metabolite of Abate by Blinn (1968), was obtained from the American Cyanamid Co., Princeton, N. J., and solutions were injected into the gas chromatograph. The sulfoxide derivative had a retention time of 240 seconds and was completely resolved from the Abate, which emerged at 58 seconds.

Once the efficiency and precision of the new method had been demonstrated, all water samples from the Puerto Rico field trial were run by this procedure. In a few isolated cases emulsions formed during the extraction step, and a second extraction was made to ensure quantitative recovery of the insecticide. The sulfoxide derivative of Abate was not found in any of the field samples.

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