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Note

Determination of Abate in environmental water samples by densitometry on preadsorbent reversed-phase thin-layer plates

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Abate (temephos) (O,O,O',O'-tetramethyl-O,O'-thiodi-*p*-phenylene phosphorothioate) is an organophosphorus insecticide that is used as a mosquito, black fly, and midge larvicide in natural waters. Abate has been determined in water, fish, and mud using gas chromatography with flame photometric detection[1]. Extracts of water were analyzed directly while mud required partition cleanup and fish silica gel column cleanup. High-performance liquid chromatography on C_{18}-[2] and phenyl-[3]bonded reversed-phase columns was used to determine Abate in water samples. In ref. 2, Abate was concentrated on and eluted from a Sep-Pak C_{18} cartridge prior to analysis, while in ref. 3 an extract with added emulsifier was directly analyzed. A UV-absorption detector was employed in both analyses.

High-performance thin-layer chromatography (TLC) coupled with densitometric scanning has recognized advantages of speed, simplicity, versatility, accuracy, and precision for quantitative analyses of a wide variety of samples[4]. Abate has been qualitatively determined in mud, meat, and water using silica gel TLC. The sensitivity of detection with bromophenol blue–$AgNO_3$ chromogenic reagent was only at the 1-μg level[5]. Silica gel TLC has also been used for the semi-quantitative determination of Abate in water and settled sewage with bromine vapor–N,N-dimethyl-*p*-phenylazoaniline detection reagent. Sensitivity was again at the microgram level[6]. We now report a quantitative TLC determination of Abate residues in water using chemically bonded C_{18} reversed-phase plates with a preadsorbent spotting area. Detection is made with the sensitive and selective N,2,6-trichlorobenzoquinoneimine (TCQ) reagent for organophosphorus pesticides. The accuracy and precision of the method is demonstrated by recovery studies of fortified distilled and natural (surface, pond, lake) waters.

EXPERIMENTAL

Standard Abate was obtained from the American Cyanamid Co. (Princeton, NJ, U.S.A.) or from the Pesticide Repository of the U.S. Environmental Protection Agency, Research Triangle Park, NC, U.S.A. A stock standard solution was prepared in acetonitrile at the 1 mg/ml level and diluted 1:20 to give a solution containing 50 ng/μl.

Analyses were carried out on 20 × 20 cm Whatman $LKC_{18}D$ chemically bonded reversed-phase plates containing a 3-cm-wide preadsorbent spotting area along the bottom below the analytical sorbent layer. The layer is scored into 19

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channels 0.9 cm in width. The plates were prewashed by development with chloroform–methanol (1:1) and dried in a fume hood before use. The principles and practice of reversed-phase TLC were described in a recent manual[7].

Volumes of standards between 4 and 25 μl (200–1250 ng) were applied by streaking across the preadsorbent areas of the lanes using a 25-μl Drummond Dialamatic microdispenser. The streaking was confined to an area just below the layer interface and 5 mm above the bottom edge of the plate. The spotting area was completely dried with a stream of air from a hair drier after application.

Plates were developed for a distance of 10 cm beyond the layer interface in a paper-lined, saturated (10 min), rectangular glass TLC chamber with acetonitrile–water (80:20). Chromatograms were dried in a ventilated chromatography oven and then sprayed with 5% magnesium chloride in methanol, air dried, and again sprayed with 0.3% TCQ in hexane. The plate was heated for 5–10 min at 110°C in a ventilated chromatography oven. A fine-mist sprayer such as the Kontes Chromaflex should be used to assure uniform application of reagents, or application can be made by dipping.

The Abate zones were scanned with a Kontes Model 800 fiber optics scanner in the single-beam, transmission mode using the B (8 mm) head and the white phosphor (440 nm peak, 300 nm band width). Peaks on the recorder chart were measured using the formula height × width at one-half height (both in mm), and area × attenuation *vs.* ng per spot was plotted to produce calibration curves.

Actual analyses were demonstrated by spiking distilled and natural (lake, pond) water samples at the 0.50 μg/l level (0.50 ppm) by adding the appropriate amount of an acetonitrile solution of Abate with vigorous magnetical stirring. The fortified samples were acidified with 5 ml of 1 *N* hydrochloric acid, filtered through glass wool if significant solids were present, and vigorously extracted twice with 200 ml of chloroform. The chloroform layers were combined, filtered through Whatman phase-separating paper to remove traces of water, evaporated to 5 ml in a rotary vacuum evaporator, transferred to a 12-ml centrifuge tube, and evaporated just to dryness under a stream of room-temperature nitrogen in a water bath at 60°C. The residue was dissolved in 50 μl of acetonitrile, and 35 μl were spotted for TLC analysis. This volume would contain 350 ng if recovery was 100%. Bracketing standards (*e.g.*, 250, 350, 500, and 750 ng) were applied to adjacent lanes. After development, detection, and scanning, the best linear calibration curve was drawn through the standard areas. The amount of pesticide in the sample spots was interpolated from the standard curve, and recovery levels were calculated by comparison to the theoretical 350-ng level.

RESULTS AND DISCUSSION

Thin-layer plates with preadsorbent spotting area have great advantages for quantitative TLC[8]. Sample application can be carried out rapidly, and the preadsorbent area automatically produces sharp, narrow bar- or streak-shaped zones of constant size, even though different sample volumes are used. Accurate, precise, and sensitive densitometry requires that initial zones of samples and standards have small, uniform dimensions[9].

The R_F value of Abate was 0.39 using acetonitrile–water (80:20) in a saturated

chamber, and the zone was in the form of a narrow streak across the lane. This R_F value is within the optimum range of 0.3–0.7 required for accurate and precise densitometric quantitation[9].

Abate was detected as a bright red-orange zone with the $MgCl_2$–TCQ reagent. This reagent is selective for organophosphorus compounds so that many other classes of pesticides or impurities would not be detected and cause interference. The sensitivity limits were *ca.* 200 ng for precise scanning and *ca.* 100 ng for visual detection. Other organophosphorus insecticides that would be detected if present in the sample were developed in the acetonitrile–water (80:20) mobile phase and gave the following R_F values (and colors with TCQ; r-o = red-orange, y = yellow): fenthion, 0.56 (r-o); ethyl parathion, 0.56 (r-o); dichlorvos, 0.77 (y); diazinon, 0.45 (r-o); phosphamidon, 0.86 (y); methyl parathion, 0.75 (r-o); methyl oxydemeton, 0.88 (y); malathion, 0.66 (y); disulfoton (dy-syston), 0.48 (y); dimethoate, 0.89 (y); and fensulfothion, 0.68 (r-o). Based on these R_F values, only diazinon and disulfoton would be possible interferences in the quantitation of Abate. The heating step in the detection procedure was carried out until the background just began to turn from pure white to off-white; the background darkened with prolonged heating and on storage of the plate. Differences in color among the various phosphate pesticides (yellow compared to red-orange, as well as various shades of red-orange for different compounds) would serve as an aid in identification by comparison of samples and standards.

A typical calibration curve between 200 and 1250 ng is illustrated in Fig. 1. The curve became non-linear above 1250 ng, leveling-off toward the x-axis. Slope and intercept values typically were within 10% from plate to plate, and regression analysis gave degrees of linearity usually >0.98. To correct for variations, several bracketing standards should always be chromatographed on the same plate with samples.

Various modes of scanning were evaluated, and single-beam transmission with the white phosphor proved best in terms of signal-to-background ratio. Reproducibility of the procedure was checked by applying four 350-ng zones to separate lanes

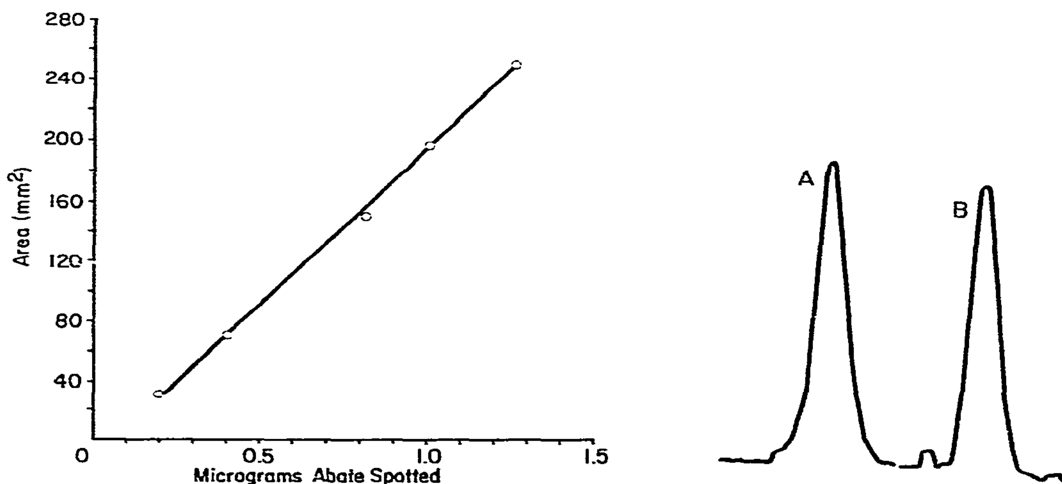

Fig. 1. Calibration curve for 200–1250 ng of Abate after chromatography, detection, and densitometric scanning. Areas were divided by 100 before plotting.

Fig. 2. Typical densitometer scans of Abate zones: A, 350-ng standard; B, fortified pond water extract chromatographed on the same layer, representing 89.0% recovery (350 ng theoretical). An attenuation setting of × 16 was used with the Kontes scanner; for other conditions, see text.

of a plate, followed by development, detection, and scanning. The relative standard deviation of the peak areas of the densitometer scans was 7.8%, which is acceptable precision for densitometry in trace analysis.

Recovery from distilled water using the extraction procedure described above averaged 96.0% for four trials, with a range of 93.0 to 98.9% and a coefficient of variation (C.V.) of 8.5%. Analysis of fortified blank pond and river waters averaged 88.1% and 83.7%, respectively, for four trials, with C.V. values of 8.7% and 9.9%. Fig. 2 illustrates a typical scan of a 350-ng standard Abate zone (A) and an adjacent zone (B) from a pond water analysis that represented 89.0% recovery when its area was interpolated from the calibration curve constructed from all standards chromatographed in parallel with the sample.

The combined selectivity of the TLC separation and the detection reagent allowed the natural water samples to be analyzed without cleanup. Potential interferences were left in the preadsorbent, were separated by TLC, or were not detected. Other samples might require appropriate cleanup of extracts prior to spotting, such as by column chromatography[1,2], to avoid irregularly shaped or non-resolved zones of Abate. Since the purpose of this research was to demonstrate the quantitative TLC method, isolation procedures for samples other than water were not studied.

Advantages of the TLC method described include simplicity, speed, and the ability to analyze multiple samples at the same time under identical conditions and to process standards in parallel. Selectivity, sensitivity, accuracy, and precision adequate for residue analysis at the low ppm level in water have been demonstrated. Application of the method to other sample matrices should be possible if any required cleanup steps are carried out.

REFERENCES

1 J. W. Miles, W. E. Dale and F. C. Churchill, *Arch. Environ. Contam.*, 5 (1976) 29.
2 K. F. Ivie, in G. Zweig and J. Sherma (Editors), *Analytical Methods for Pesticides and Plant Growth Regulators*, Vol. XI, Academic Press, New York, 1980, p. 66.
3 A. Otsuki and T. Takaku, *Anal. Chem.*, 51 (1979) 833.
4 D. C. Fenimore, *Anal. Chem.*, 53 (1981) 254A.
5 N. P. Biryukova, Yu. F. Moryakov and A. A. Nepoklonov, *Veterinariya (Moscow)*, No. 11 (1976) 103.
6 L. H. Howe, III and C. F. Petty, *J. Agr. Food Chem.*, 17 (1969) 401.
7 J. Sherma, *Practice and Applications of TLC on C_{18} Chemically Bonded Reversed Phase Plates* (*TLC Technical Series*, Vol. 1), Whatman Chemical Separation Division, Clifton, NJ, 1981.
8 J. Sherma, *Amer. Lab.*, 10, No. 10 (1978) 105.
9 J. C. Touchstone and J. Sherma (Editors), *Densitometry in Thin Layer Chromatography —Practice and Applications*, Wiley-Interscience, New York, 1979.