Journal of Chromatography, 121 (1976) 161–164

CHROM. 8986

Note

Determination of metamidophos and acetamidophos in formulations by reversed-phase high-pressure liquid chromatography

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(Received November 26th, 1975)

Metamidophos (O,S-dimethyl phosphoroamidothioate, Tamaron*) and acetamidophos (O,S-dimethyl N-acetylphosphoroamidothioate, Orthene**) are systemic insecticides which have recently attained widespread usage and acceptance owing to their effectiveness as pesticides.

Currently, metamidophos is marketed as a liquid formulation with a label guarantee of 50%. On the other hand, acetamidophos, which is the acetylated metamidophos, is sold as a solid formulation with a label guarantee of 75%. It was of interest to us to utilize high-pressure liquid chromatography (HPLC) for the determination of these compounds, as both have water solubilities of 65% or greater and the technique would allow the use of water as the solvent in reverse-phase liquid chromatography. The simple process of dissolving the sample in water is more rapid for quality control purposes than gas chromatographic methods, which may require extraction with chloroform.

EXPERIMENTAL

Apparatus

The high-speed liquid chromatograph used was a Waters Assoc. (Milford, Mass., U.S.A.) Model ALC-202. The unit was equipped with a refractive index detector (Model R-401) and a 6000 p.s.i. solvent delivery system with constant-flow capabilities. The separations were made on a 1 ft. × 1/4 in. O.D. precision-bore stainless-steel column packed with micro-Bondapak/*n*-C_{18} (Waters Assoc.). A computerized reporting integrator (Hewlett-Packard, Model 3380A) was used for area and concentration measurements.

Operating conditions

The eluting solvent was 10% (v/v) methanol in distilled water; the flow-rate was 1.4 ml/min at a pressure of 1900 p.s.i.; the column temperature was ambient; and the injection volume was 7 μl for metamidophos samples and 3 μl for acetamido-

* Tamaron is a trademark of Bayer Chemical Co., Leverkusen, G.F.R.

** Orthene is a trademark of Chevron Chemical Co., Richmond, Calif., U.S.A.

phos samples. Analyses were performed with an attenuation setting of $\times 8$ ($192 \cdot 10^{-6}$ R.I. units full scale).

Preparation of standard solutions

Weigh 0.8 g of analytical-grade metamidophos (Bayer) into a 50-ml calibrated flask and dilute to volume with the eluting solvent. For the analysis of acetamidophos, prepare a standard solution containing 0.1–0.15 g of analytical-grade acetamidophos (Chevron) in 50 ml of eluting solvent. Volumes of 1.0–7.0 μl of the solutions obtained are injected in order to construct the calibration graph.

An internal standard solution is prepared by weighing 1.3 g of 2-methyl-2-propanol (analytical standard, PolyScience Corp., Niles, Ill., U.S.A.) in a 100-ml calibrated flask and dilute to volume with the eluting solvent. This internal standard solution, used in the analysis of metamidophos, is used to determine a calibration factor by mixing in a vial 5.0 ml of the internal standard solution and 5.0 ml of the standard metamidophos solution.

Sample preparation

Weigh samples to contain 0.8 g of metamidophos in a 50-ml calibrated flask, using the eluting solvent as diluent. Pipette 5 ml of the sample solution and 5 ml of the internal standard solution into a vial and use this solution for injection. In the analysis of acetamidophos, weigh 0.8 g of the commercial formulation into a 100-ml calibrated flask and dilute to volume with the eluting solvent. The solution is turbid and requires a settling time of 1 h prior to injection. Alternatively, an aliquot of the solution can be withdrawn with a 1-ml syringe provided with a 0.4-μm Millipore filter. The solution withdrawn can be injected immediately.

Determinations

The computer integrator is programmed to yield answers directly as percentage of metamidophos by using the following equation:

$$\text{metamidophos } (\%) = \frac{A_m \cdot R_s \cdot W_s}{A_s \cdot R_y \cdot W_{sm}} \cdot 100$$

where A_m and A_s are the areas of the metamidophos and standard peaks, respectively, and R_y and R_s are the response per unit weight of metamidophos and the internal standard, respectively, which were determined from the mixed standard solution; W_{sm} and W_s are the weight of sample and standard, respectively. The acetamidophos is determined by an absolute calibration where the computer integrator is used to calculate, by a least-squares analysis, the equation of the straight line. A typical equation obtained is $Y = 21.726\,W - 856$ where W (μg) is the weight of acetamidophos in the volume injected. The correlation coefficient is 0.9997. The area integrated varies from 20,000 to 160,000 units.

Analysis of dilute solutions

The insecticides were weighed and dissolved in distilled water according to the instructions of the label for preparing solutions for foliar applications. The resulting concentrations were 579 and 880 ppm for metamidophos and acetamidophos,

respectively. Six standards of each insecticide were prepared, containing 400, 500, 600, 700, 800 and 900 ppm. These standards were used for constructing calibration graphs. With metamidophos, the standard solutions contained 600 ppm of the internal standard.

RESULTS AND DISCUSSION

The separations obtained are shown in Figs. 1 and 2 for metamidophos and acetamidophos, respectively, under the same chromatographic conditions. Fig. 1 shows the separation of the components of the metamidophos formulation. Peak 3 corresponds to metamidophos, while peak 4 corresponds to the internal standard (2-methyl-2-propanol), which is free from interference from any component present in the formulation. The remaining peaks 1, 2 and 5 are formulation components of unknown identity. Fig. 2 shows the separation obtained for acetamidophos (peak 2) from an unknown formulation component (peak 1). Both insecticides must be detected with a refractive index detector, as both have very small absorptivities in the range 210–280 nm.

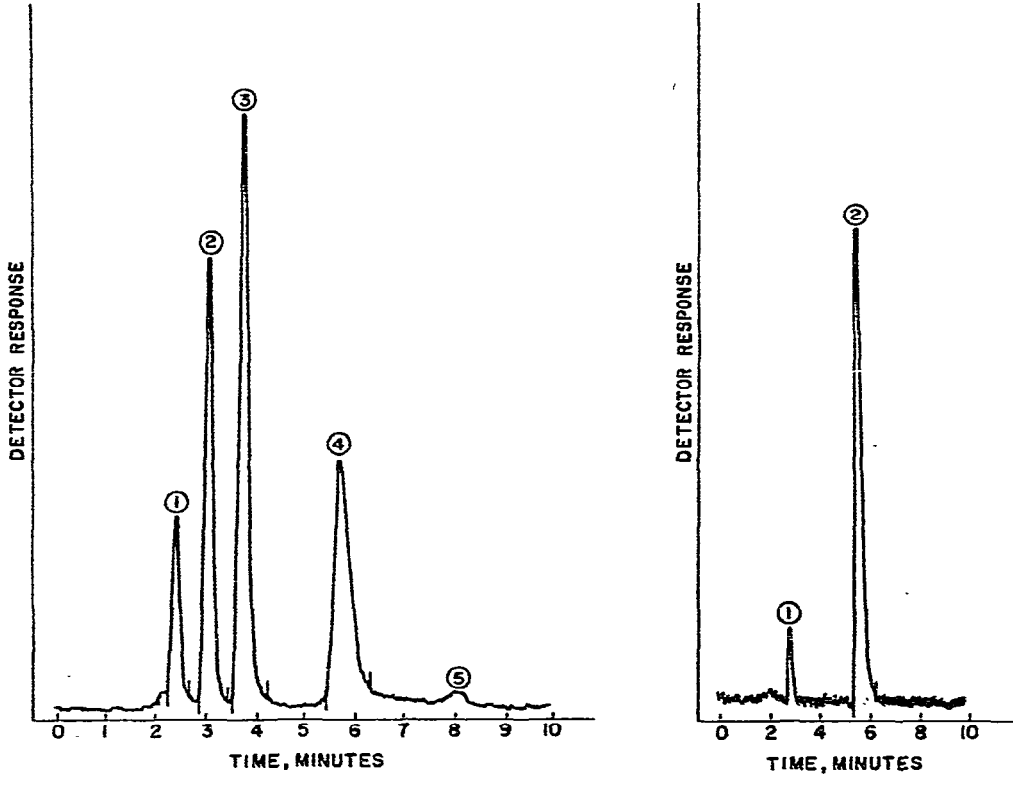

Fig. 1. Chromatogram of a metamidophos formulation. Identity of components: 3 = metamidophos; 4 = 2-methyl-2-propanol; 1, 2 and 5 = unknowns.

Fig. 2. Chromatogram of an acetamidophos formulation. Identity of components: 2 = acetamidophos; 1 = unknown.

Table I shows the comparative results obtained with HPLC and gas–liquid chromatography (GLC). In all instances there is good agreement between the two techniques, as the largest difference is 2.2%. The GLC analyses used in this work were adapted from published techniques for the residue analysis of these two insecticides[1,2]. Further, an indication of the good linearity and accuracy is the fact that dilutions performed with metamidophos followed by HPLC analysis reveal differences of not more than 2.5%. It was also found that good agreement is obtained between the HPLC and GLC results of the analysis of acetamidophos.

TABLE I

ANALYSIS OF METAMIDOPHOS AND ACETAMIDOPHOS BY HPLC AND GLC

Compound	*Active ingredient by label or dilution (%)*	*Amount by analysis (%)*	
		*HPLC**	*GLC**
Metamidophos	50	50.34 (0.04)	50.33 (1.18)
		51.77 (0.78)	51.03 (0.52)
		50.60 (0.41)	50.90 (0.97)
	29.10	28.43 (0.28)	28.13 (1.36)
	39.67	38.69 (0.11)	37.85 (1.42)
	0.0579	0.0572 (0.0008)	—
Acetamidophos	75	76.11 (0.63)**	77.68 (1.90)
	0.0880	0.0802 (0.0009)	—

* Average of triplicate analyses; values in parentheses are the standard deviations.
** Average of seven determinations, each injected in triplicate.

The detectability of these two insecticides was determined by preparing individual solutions containing 1 $\mu g/\mu l$ of the insecticide. The solutions were injected until a change of refractive index equal to three times the noise level was obtained. The detector could not be used below a sensitivity setting of $24 \cdot 10^{-6}$ R.I. units. Under these conditions, it was possible to detect 1 μg of each insecticide.

Table I also shows the results of the analysis of formulations in water to be used in foliar applications. There is good agreement between the concentration of the carefully prepared formulation according to the label instructions and that obtained by HPLC for metamidophos and acetamidophos. Again, with metamidophos the internal standard technique was used. The agreement is also good for acetamidophos. These dilute solutions are difficult to analyse by GLC owing to the incompatibility of water with many of the GLC columns, and extraction techniques are difficult owing to the high solubilities of the insecticides in water.

The column was found to be stable for over 200 injections of either insecticide. After approximately this number of injections, the retention times increase and resolution is lost. The column was regenerated by the passage of 100 ml of methanol.

With the method described, it is possible to analyze commercial preparations of these insecticides (containing 50–75% of the insecticide) in the concentration range 400–800 ppm.

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