Determination of Oxamyl and Methomyl by High-Performance Liquid Chromatography Using a Single-Stage Postcolumn Derivatization Reaction and Fluorescence Detection

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A method is described for the determination of oxamyl and methomyl residues in apples, oranges, soybean leaves, insects, and river water by high-performance liquid chromatography. This method uses matrix solid-phase dispersion isolation, a single-stage postcolumn derivatization reaction, and fluorescence determination. Recovery data for both oxamyl and methomyl were obtained by fortifying the raw agricultural commodities (RACs) at levels ranging from 20 to 1000 ng/mg and the water at levels ranging from 5 to 50 ng/mL. The recoveries ranged from 72 to 129%. The limits of detection, calculated as 4 times the baseline noise, were 5 mg/mL for water and 20 ng/g for the RACs tested.

INTRODUCTION

Du Pont Vydate (oxamyl) insecticide/nematicide is a broad-spectrum product that is effective on a wide variety of important plant pests including insects, mites, and nematodes. Insect and mite control is normally achieved by foliar sprays. Because Vydate is highly systemic in plants, application to the soil allows absorption by the roots and movement up to growing points and leaves. Soil applications of Vydate are moderately effective on pests that inhabit the soil.

Du Pont Lannate (methomyl) insecticide is a broad-spectrum product that is effective on a wide range of insects as an ovicide, larvicide, and adulticide. Lannate is most effective by direct contact with pests. Although residual activity is short-term, insects that ingest treated foliage are intoxicated.

The active ingredients in Vydate and Lannate are oxamyl, *S*-methyl *N'*,*N'*-dimethyl-*N*-[(methylcarbamoyl)oxy]-1-thiooxamimidate, and methomyl, *S*-methyl *N*-[(methylcarbamoyl)oxy]thioacetimidate, respectively. Both are *N*-methylcarbamate pesticides. The chemical structures of oxamyl and methomyl are shown in Figures 1 and 2, respectively.

The chemical and physical properties, efficacy, crop tolerance, and toxicology of oxamyl and methomyl have been reported by Du Pont in the "Vydate Technical Bulletin" (Du Pont Agricultural Products, 1989) and the "Lannate Technical Bulletin" (Du Pont Agricultural Products, 1991), respectively.

Matrix solid-phase dispersion (MSPD) isolation technology was first introduced by A. R. Long and his coworkers (Long et al., 1990). This technique involves blending a small amount of biological matrices with C_{18} (octadecylsilyl derivatized silica) followed by a small amount of solvent washing and elution to extract a wide range of compounds. This technology has been successfully used to extract several pesticides (e.g., organophosphates, sulfonamides, cephalosporins, and benzimidazoles) from liver, muscle tissue, kidney, milk, and fat (Long et al., 1990).

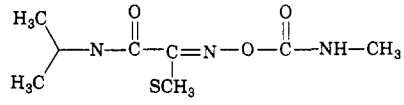

Figure 1. Chemical structure of oxamyl.

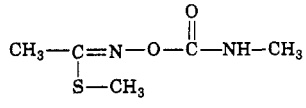

Figure 2. Chemical structure of methomyl.

The high-performance liquid chromatography (HPLC) method for postcolumn derivatization and fluorescence determination of residues, developed by Moye et al. (1977), was widely recognized for its sensitivity and selectivity in determining residues of *N*-methylcarbamate pesticides in the late 1970s and early 1980s. The two-stage postcolumn reaction converts the carbamate into a fluorescent derivative which is then determined by a fluorescence detector (DeKok et al., 1987). In the first stage, the carbamate molecules are hydrolyzed, causing the release of methylamine. In the second stage, the released methylamine is derivatized with *o*-phthalaldehyde (OPA) and 2-mercaptoethanol or 3-mercaptopropionic acid to form a highly fluorescent substituted isoindole. The method has been adapted as Environmental Protection Agency (EPA) methods 531.2 and 531.5 by the U.S. EPA (Foerst, 1985).

Nondek et al. (1983a,b) explored the possibility of using a catalytic solid-phase reactor packed with an anion-exchange resin to hydrolyze the carbamates. Later, McGarvey (1989) mixed the hydrolysis and derivatization reagents together and then reacted them with the carbamate at an elevated temperature (130–150 °C). Both groups demonstrated the possibility of eliminating one postcolumn reagent-delivering pump and so avoided mixing and flow pulsation problems. In addition, the same researchers demonstrated that a single-stage reaction can eliminate band broadening from analyte dilution in the mobile phase.

This paper describes an HPLC method with a single-stage reaction that measures the recovery of oxamyl and methomyl from raw agricultural commodities and insects after extraction through a matrix solid-phase dispersion isolation. The method also measures recoveries of these

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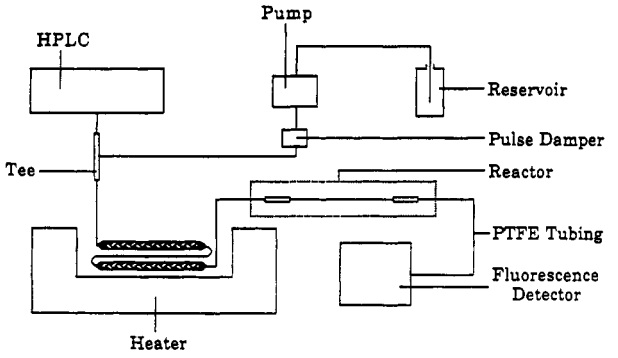

Figure 3. Single-stage postcolumn reactor.

compounds from river water filtered through a 0.2-μm Acrodisc filter.

EXPERIMENTAL PROCEDURES

Chemicals and Reagents. The matrix solid-phase dispersion used C_{18} sorbent (J. T. Baker, Phillipsburg, NJ). The derivatization reagent was prepared by adding 10 mg of *o*-phthalaldehyde in 1 mL of methanol to 400 mL of 0.01 M KOH solution in HPLC grade water. The solution was degassed under vacuum with magnetic stirring, and 0.05 mL of 2-mercaptoethanol was added.

The carbamate quantitative test mixture with 12 carbamate insecticides was purchased from Pickering Laboratories, Inc., Mountain View, CA (cat. no. P/N 1700-0063). This mixture contained aldicarb sulfone, aldicarb sulfoxide, oxamyl, methomyl, 3-hydroxycarbofuran, aldicarb, propoxur, carbofuran, 1-naphthol, carbaryl, methiocarb, and BDMC.

Apparatus. Analyses were performed with a Hewlett-Packard 1090L liquid chromatograph (Hewlett-Packard, Avondale, PA) equipped with an autosampler injector. A Zorbax ODS column (25 cm × 4.6 mm i.d., Macmod Analytical, Inc., Chadds Ford, PA) was used. Hydrolysis and derivatization reagents were pumped by a Kratos URS 051 pump (Kratos Analytical Instruments, Ramsey, NJ). The pulse damper connected to the pump was an SSI Model LP-21 (Scientific Systems, Inc., State College, PA).

The single-stage postcolumn reactor, illustrated schematically in Figure 3, was configured as follows. The mixture of hydrolysis and derivatization reagents was delivered from a reservoir by a Kratos URS 051 pump at a rate of 0.6 mL/min. The pump was connected to a pulse damper to reduce pump noise. The reagent mixture flowed to a Supelco zero-volume tee (Supelco Inc., Bellefonte, PA), where it mixed with the flow stream from an HPLC column. The two streams were then mixed in two knitted PTFE delay tubes (3 m × 0.5 mm i.d.), immersed in series in a Briskheat heater (Briskheat Corp., Columbus, OH) set at 140 °C. From the delay tubes within the heater, the mixture flowed into a single-bead string reactor (Supelco) to ensure complete component reaction. The mixture was cooled in 1 m × 0.5 mm i.d. PTFE tubing before entering the flow cell of an HP 1046A programmable fluorescence detector (Hewlett-Packard). Oxamyl and methomyl were detected in the flow cell with the excitation wavelength set at 340 nm and the emission wavelength set at 455 nm. The chromatogram was recorded and integrated using Multichrom on a VAX system (VG Laboratory Systems, Cheshire, England).

Analytical Procedures. *Extraction and Purification of Samples.* A batch of C_{18} sorbent (40-μm average particle diameter) was washed, 20 g at a time, with 60 mL each of hexane, methylene chloride, ethyl acetate, and methanol in sequence in a 75-mL reservoir with frits. The sorbent was dried under vacuum with a Visiprep vacuum manifold (Supelco). Samples (10–15 g) were preprocessed by grinding them with dry ice to form a powder before weight for extraction. One-half gram of processed tissue (RAC or insect) was fortified with 50, 25, 10, or 1 μL of 10 μg/mL oxamyl and methomyl standards. Fortified tissue and 2.5 g of washed C_{18} sorbent were ground in a 1-oz glass mortar with pestle. (The amount of tissue can be increased if necessary; however, C_{18} sorbent should be increased proportionally if this is done.) The tissue and C_{18} sorbent mixture were then packed in a 25-mL reservoir with a frit on the top of the packed bed. The column was first washed with 10 mL of hexane, and then oxamyl and methomyl were eluted from the column with 10 mL of methylene chloride. The eluent was then dried under a stream of nitrogen gas with an N-Evap (Organomation Associates, Inc., South Berlin, MA) and reconstituted with 1 mL of pH 3 water. Before samples were injected into the chromatograph, they were sonicated for 5 min and filtered through a 0.2-μm Acrodisc filter (Gelman Sciences, Ann Arbor, MI).

Table I. Standard Curves of Oxamyl and Methomyl[a]

photomultiplier tube setting (PMT)	slope[b]	
	oxamyl	methomyl
10	466.7	556.39
13	5140.28	5869.57
14	9048.12	10366.67
15	16640.92	17856.79

[a] Peak height (μV)/ng. [b] This linear regression curve represents a 95% confidence range.

One gram (1 mL) of water sample (obtained from the Brandywine River next to the Du Pont Experimental Station in Wilmington, DE) was fortified with 50, 25, 10, or 5 μL of 1 mg/mL oxamyl and methomyl standards filtered through a 0.2-μm Acrodisc filter before injection into the chromatograph.

HPLC Analysis. Oxamyl and methomyl were determined by HPLC (equipped as reported under Apparatus) by comparing the chromatographic peak heights of oxamyl and methomyl in sample solutions with the corresponding peak heights of standard solutions containing known quantities of the respective test substances. Chromatographic conditions for oxamyl and methomyl analysis were as follows: injection volume, 200 μL; solvent system, 16-min continuous gradient of 80:20 water (pH 3.0)/acetonitrile to 20:80 water (pH 3.0)/acetonitrile followed by a 21-min column wash and return of gradient to 80:20 H_2O/acetonitrile; flow rate, 1 mL/min; column temperature, 40 °C; excitation wavelength, 340 nm; emission wavelength, 455 nm.

RESULTS AND DISCUSSION

A matrix solid-phase dispersion (MSPD) method has been successfully used to isolate oxamyl and methomyl from animal matrices and milk (Long et al., 1990, and references cited therein). An ongoing Du Pont study has confirmed that MSPD is very effective in extracting oxamyl from both fresh and aged spiked plant leaf tissue (data not shown; Du Pont Agricultural Products study AMR-1825-90). Accordingly, the method described here was used to extract oxamyl and methomyl from all matrices spiked at various concentrations.

To address the oxamyl and methomyl concentration ranges of interest, four sets of standard curves were generated. These curves used four different photomultiplier tube (PMT) settings (i.e., 10, 13, 14, and 15 with increasing sensitivity) on the fluorescence detector, corresponding to three concentration ranges (i.e., 0–400 ng for 10, 0–40 ng for 13 and 14, and 0–10 ng for 15). Slopes plotting peak height (microvolts) nanograms of oxamyl or methomyl injected were derived from linear regression of standard curves (Table I). These slopes were used to calculate the concentration of compounds in the extracts.

Table II shows the fortification (recovery) data of oxamyl in plant and insect tissues and river water spiked with known amounts of oxamyl. Recoveries ranged from 72 to 129%, which is within the recommended acceptable range set by the EPA Pesticide Assessment Guideline, Subdivision O (Nelson and Griffith, 1986). The corresponding recovery data for methomyl, shown in Table III, ranged from 80 to 126%. Data presented in these tables clearly show that MSPD is suitable for oxamyl and methomyl extraction from all matrices tested, and the single-

Table II. Summary of Oxamyl Fortification (Recovery) Data in Plant and Insect Tissues and Standing Water

test matrix	fortification, ppm added	oxamyl, ppm	recovery, %
apple fruit	0.02	0.025	127
	0.20	0.193	97
	0.50	0.600	120
	1.00	0.840	84
orange fruit	0.02	0.022	109
	0.20	0.259	129
	0.50	0.520	104
	1.00	1.160	116
soybean leaves	0.02	0.023	116
	0.20	0.220	110
	0.50	0.361	72
	1.00	0.960	96
house flies	0.02	0.022	110
	0.20	0.203	101
	0.50	0.645	129
	1.00	1.230	123
standing water	0.005[a]	0.0051	101
	0.010	0.0073	73
	0.025	0.0292	117
	0.050	0.0492	98

[a] Detection limit level—two samples gave same peak height as the water blank.

Table III. Summary of Methomyl Fortification (Recovery) Data in Plant and Insect Tissues and Standing Water

test matrix	fortification, ppm added	methomyl, ppm	recovery, %
apple fruit	0.02	0.019	95
	0.20	0.160	80
	0.50	0.539	108
	1.00	0.820	82
orange fruit	0.02	0.017	85
	0.20	0.240	120
	0.50	0.589	118
	1.00	1.200	120
soybean leaves	0.02	0.024	122
	0.20	0.217	109
	0.50	0.508	102
	1.00	1.070	107
house flies	0.02	0.024	120
	0.20	0.191	96
	0.50	0.566	113
	1.00	0.085	85
standing water	0.005[a]	0.0046	93
	0.010	0.0114	114
	0.025	0.0316	126
	0.050	0.0589	118

[a] Detection limit level.

stage postcolumn derivatization reaction and fluorescence detection technique described here is ideal to detect the magnitude of the residue of oxamyl and methomyl in various matrices.

As shown in representative chromatograms from each matrix tested (Figure 4), retention times of oxamyl and methomyl, which were easily distinguishable from other interference peaks, were about 6.5 and 7.5 min, respectively. No peaks were found at these locations in all matrix blank samples. To further illustrate the applicability of the system described here, 10 ng each of 12 carbamate pesticides (purchased from Pickering Laboratories) were injected into the system, and 12 easily distinguishable peaks were recorded (Figure 5). No attempt was made to identify all of these pesticides in this study.

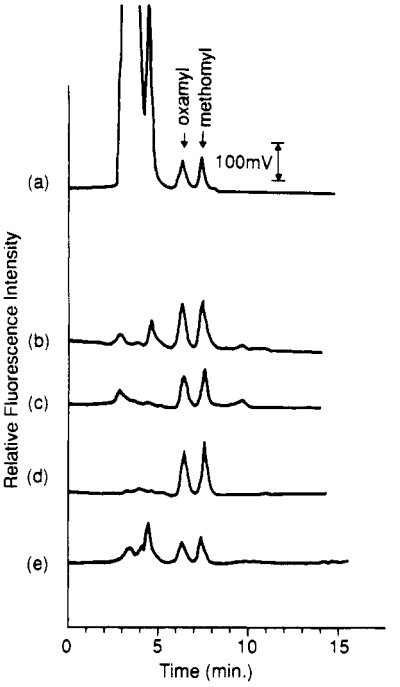

Figure 4. Chromatograms of apples, oranges, soybean leaves, insects, and river water. Chromatograms of oxamyl and methomyl extracted from apples (a), oranges (b), soybean leaves (c), and insects (d) spiked with 1 ppm each of oxamyl and methomyl before extraction; chromatogram of oxamyl and methomyl extracted from river water (e) spiked with 50 ppb of both compounds (PMT setting was 10 each for apples, oranges, soybean leaves, and insects and 13 for river water).

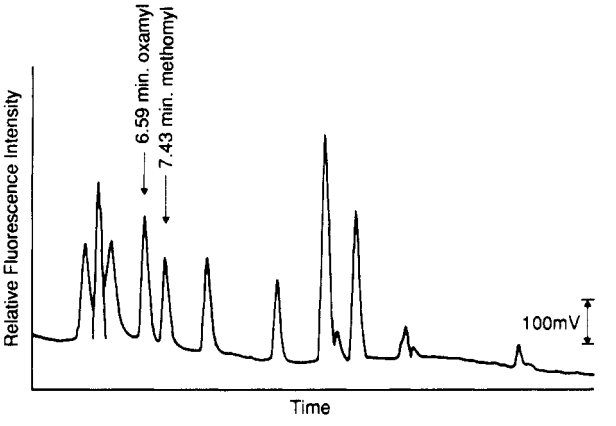

Figure 5. Chromatogram for carbamate pesticides (10 ng each).

CONCLUSIONS

The method described measures oxamyl, the active ingredient in Vydate insecticide/nematicide, and methomyl, the active ingredient in Lannate insecticide, with limited amounts of matrix (0.5 g) at 20 (crops) and 5 ppb (water) levels. The method, which demonstrates high sensitivity and selectivity for oxamyl and methomyl, is applicable to several crop matrices and standing surface water and groundwater.

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