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Organic-Solvent-Free Extraction Method for Determination of Carbamate and Carbamoyloxime Pesticides in Soil and Sediment Samples

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Abstract This study evaluated the usefulness of monochloroacetic acid buffer (MCAAB) for extracting several carbamate/carbamoyloxime pesticides from a silt-loam soil and sediment, and an organic clay soil. The MCAAB extraction method, relative to acetonitrile and methanol extractants, was more accurate and precise for extraction of aldicarb, aldicarb sulfoxide, aldicarb sulfone, oxamyl, methomyl, carbofuran, 3-hydroxy-carbofuran, and propoxur; with recoveries ranging from 78.8% to 121.1%. Recoveries of carbaryl and methiocarb ranged from 0% to 64.1%. The MCCAB extraction method did not perform well for extraction of most compounds from the organic-clay soil, with recoveries ranging from 0% to 66.7%.

Keywords MCAAB · Monochloroacetic acid · Organic-clay · Silt-loam

The carbamate and carbamoyloxime insecticides have historically been important tools for the agricultural and horticultural industries. They are also important tools in landscape maintenance. Both groups are acetylcholinesterase inhibitors, and are biologically active by contact or ingestion. Several carbamate/carbamoyloxime pesticides of interest include aldicarb, carbaryl, carbofuran, methomyl, methiocarb, propoxur, and oxamyl. All of these pesticides are labeled for use in various types of agriculture/horticulture/landscape maintenance throughout much of North America and other countries. Within Florida, the Florida Department of Agriculture and Consumer Services reported statewide usage of each from 2003 to 2006 was: aldicarb – 113,475 kg, carbaryl – 51,834 kg, methomyl – 91,540 kg, and oxamyl – 21,863 kg (Shahane 2008). Usage for propoxur, methiocarb, and carbofuran was not available, but is not expected to be as high as the others due to more crop restrictions.

Much effort has focused on the development of methods for extraction and analysis of carbamates/carbamoyloximes in soil, water and food samples (Nollet 2006; Coly and Aaron 1998; Sanchez-Brunet et al. 2003; Torres et al. 1996; Williams et al. 1976; Chin et al. 1975; Bleidner et al. 1978; Austin and Briggs 1976). Current methods for extraction from soil or other matrices, including U.S. EPA method 632 and EPA 8318A, usually employ methylene chloride, methanol, ethyl acetate, or acetonitrile as extraction solvents (USEPA 1993, 2000). These solvents are often more toxic than the target pesticides themselves (Sanchez-Brunet et al. 2003; Torres et al. 1996). Given the increased attention on environmental stewardship and the toxic nature of commonly used organic solvents, development of an organic solvent-free soil extraction method could provide a safer alternative for characterizing the presence of these pesticides in soils, allowing for more ecologically friendly environmental risk assessments to be conducted.

Several analytical methods have been proposed for the separation and quantification of carbamate/carbamoyloxime residues in environmental samples (Bassett et al. 2003; Nollet 2006). Two sensitive methods available for analysis are EPA 531.1 and 531.2 (USEPA 1989, 2001). These methods describe analytical conditions for direct injection

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analysis of the compounds in finished drinking water using high performance liquid chromatography (HPLC) with post-column derivatization and fluorescence detection. Compared to UV detection, the fluorescence detector coupled with post-column derivatization offers at least an order of magnitude improvement in detection sensitivity (Coly and Aaron 1998). Coupling of this sensitive analytical method with an organic-solvent free extraction method could provide risk assessors with a very sensitive, efficient, and useful method for screening carbamate/carbamoyloxime pesticide concentrations in soils. This project evaluated the potential usefulness of an organic-solvent-free extraction method for rapid and sensitive analysis of the carbamate and carbamoyloxime insecticides, aldicarb, oxamyl, methomyl, propoxur, and carbofuran in soil samples. Additionally, the breakdown products of aldicarb (aldicarb sulfoxide and aldicarb sulfone) and carbofuran (3-hydroxy-carbofuran) were also evaluated.

Materials and Methods

Soils for these studies were collected from a citrus grove (Fort Pierce, FL) and sugarcane field (Belle Glade, FL), each representing two major soil types (silt loam and organic clay) in Florida. In addition to the soils, silt loam sediments were collected from Ten Mile Creek (TMC; Fort Pierce, FL). The physical and chemical properties of the soils and the sediment are listed in Table 1. The soils were collected from the upper 0–20 cm in the fields. The sediment was collected from the bottom of ten mile creek (TMC) using a stainless steel Eckman dredge. The samples were brought back to the laboratory and air dried at room temperature. Once dry, samples were passed through a sieve with 2 mm i.d. pore size before using in these experiments.

These studies were conducted twice, using three replicates for each treatment. Subsamples of the dried and screened soils/sediment were spiked at 4, 20, and 40 ng/g-soil, and incubated for 2 h at room temperature under dark conditions before the extraction process. BDMC (4-Bromo-3.5-dimethyl phenyl-*N*-methyl carbamate; Chem Service Inc., West Chester, PA, USA) at 5 ng/g-soil was added to all spiked samples as a surrogate.

For the extraction, 5 g of each spiked subsample were placed into a 40 mL amber borasilicate glass bottle. Twenty milliliters of 0.015 M monochloroacetic acid buffer (MCAAB) solution was then added to each bottle. The bottles were capped with a Teflon-lined lid, loaded into a reciprocating shaker (Eberbach Corporation Inc, Ann Arbor, MI, USA), and shaken for 2 h at low speed settings. After shaking, the extracts were centrifuged for 30 min at 716 g_n using a Marathon 21/BR centrifuge (Fisher Scientifics, Pittsburgh, PA, USA). Two milliliters of the extracts were collected from the bottle after centrifugation, followed by filtration into a 1.8 mL GC amber-glass-vial (National Scientific, Rockwood, TN USA) using a 13 mm Millex^R-GV PVDF syringe-driven filter unit (pore size: 0.22 μm, Millipore Corporation, Billerica, MA, USA). The filtered extracts were stored at 4°C until analysis.

For comparison, subsamples of the same soil used for the MCAAB extraction were spiked at 20 ng/g-soil and extracted with methanol (Bleidner et al. 1978) and acetonitrile (EPA method 8318A). These studies were also conducted twice, using three replicates for each treatment, but only at the middle concentration (20 ng/g) used for the MCAAB trials. For the methanol extraction method, 5 g of each soil or sediment sample were extracted with 20 mL of methanol using the same procedures previously described for the MCAAB extraction method. However, after centrifuging 10 mL of the extracts were collected into a 25 mL concentrator-tube (Kimble-Kontes, Vineland, NJ, USA) from the top layer of methanol phase. Next, 100 μL of pesticide grade ethylene glycol was added into the concentrator tube. The methanol was then evaporated in a RapidVap evaporation system (Model 79000-02, Labconco Corporation, Kansas City, MO, USA) until only the ethylene glycol containing the extracted compounds remained. The extract was then brought to a final volume of 2 mL in methanol and cleaned up by passing through a 3 mL, 500 mg C-18 reverse-phase cartridge (Fisher Scientific, Pittsburgh, PA, USA). The C-18 cartridge was pre-washed with 4 mL methanol before being used. After passing through the C-18 cartridge, the compounds were eluted using 5 mL of methanol. The eluted extracts were quantitatively transferred into another clean concentrator tube and evaporated to dryness as previously described. Finally, the extracts were re-dissolved in 2 mL of 0.015 M MCCAB buffer and filtered into GC vials with 13 mm Millex^R-GV PVDF syringe-driven filter units. For the

Table 1 Collection site, pH, organic matter, sand, silt, and clay percent (%) contents for soil and sediment evaluated in these studies

Soil	Collection site	pH	Organic matter content	Sand	Silt	Clay
Silt loam	Ft. Pierce, FL	7.21	1.26	37	53	10
Silt-loam sediment	Ft. Pierce, FL	6.98	2.17	19	57	24
Organic-clay	Belle Grade, FL	7.06	32.98	3	22	75

acetonitrile extraction method, spiked sub-samples were extracted 3× with acetonitrile as described in EPA Method 8318A. The only deviation from EPA 8318A was the use of MCAAB solution to dissolve the final extracts instead of acetonitrile.

The extracts of carbamates from the soil and sediment samples obtained by the MCAAB, methanol and acetontrile extraction methods were analyzed using a Waters 2695 high performance liquid chromatograph (HPLC) equipped with a Waters 474 scanning fluorescence detector, Waters post-column reaction module, and reagent managers (Waters, Milford, MA, USA). The detector excitation and emission wavelengths were 339 and 445 nm, respectively. The HPLC was equipped with a Waters carbamate analysis column (150 × 3.9 mm i.d.) and a Waters C18 guard column (Waters, Milford, MA, USA). The HPLC column oven and post-column reaction module temperatures were maintained at 30 and 80°C, respectively. The sample injection volume was 400 µL. The mobile phase consisted of water, methanol, and acetonitrile (EPA method 531.2). The flow rate used was 1.5 mL/min. The flow rates for the post-column reaction module and reagent managers were 0.5 mL/min. Analytical-grade calibration standard stock solutions (100 µg/mL) were purchased from Supelco (Sigma Aldrich, PA USA). The detector response was linear from 0.5 to 8 ng/mL. The correlation coefficients

Table 2 Retention times (RT), method detection limits (MDL), practical quantification limits (PQL), and calibration range/R^2/model equations used for quantifying carbamate and carbamoyl-oxime concentrations

Pesticide	RT (min)	MDL (ng/g)	PQL (ng/g)	R^2	Equation
Aldicarb sulfoxide	4.136	0.50	1.99	0.995	Y = 13800x − 550
Aldicarb sulfone	4.962	0.30	1.20	0.999	Y = 15100x − 2680
Oxamyl	5.615	1.12	4.47	0.997	Y = 15000x − 6250
Methomyl	6.579	0.34	1.36	0.999	Y = 19400x − 5110
3-Hydroxy-carbofuran	9.893	0.28	1.12	0.999	Y = 15400x − 372
Aldicarb	11.767	0.21	0.84	0.992	Y = 8770x − 444
Propoxur	14.986	0.50	1.99	0.999	Y = 15900x − 421
Carbofuran	15.581	0.36	1.44	0.999	Y = 13800x − 2390
Carbaryl	18.139	0.40	1.60	0.999	Y = 24900x − 9210
Methiocarb	22.410	0.30	1.20	0.998	Y = 12200x − 78.1

Table 3 Summary of carbamate/carbamoyloxime pesticide recovery using MCAAB extraction from a silt-loam soil and sediment, and an organic clay soil fortified at 4, 20, and 40 ng/g

Carbamate	Recovery (%, mean ± SD)								
	Silt loam soil			Silt loam sediment			Organic-clay		
	Fortified level (ng/g)			Fortified level (ng/g)			Fortified level (ng/g)		
	4	20	4 0	4	20	40	4	20	40
Aldicarb sulfoxide	114.5 ± 12.2	108.8 ± 6.0	105.1 ± 4.8	99.9 ± 1.9	96.6 ± 3.9	90.3 ± 1.9	91.6 ± 6.9	78.8 ± 2.9	77.1 ± 3.5
Aldicarb sulfone	121.1 ± 9.9	103.6 ± 5.3	100.8 ± 3.7	93.8 ± 1.2	93.8 ± 4.5	87.1 ± 2.0	52.8 ± 13.1	66.7 ± 4.9	64.9 ± 0.8
Oxamyl	111.0 ± 5.7	94.3 ± 3.3	93.82 ± 3.4	86.8 ± 18.1	95.1 ± 10.1	86.8 ± 8.1	62.3 ± 27.1	48.1 ± 3.7	50.36 ± 7.9
Methomyl	109.6 ± 9.2	91.6 ± 5.4	91.6 ± 2.2	0	83.2 ± 3.4	80.2 ± 2.4	93.9 ± 7.7	20.6 ± 3.4	27.3 ± 16.8
3-Hydroxy-carbofuran	112.4 ± 8.4	103.4 ± 5.6	101.7 ± 2.6	98.5 ± 7.5	92.7 ± 5.2	86.2 ± 2.6	42.2 ± 10.9	26.1 ± 0.3	25.0 ± 9.2
Aldicarb	107 ± 8.0	100.0 ± 4.0	99.6 ± 1.1	121.0 ± 5.4	95.9 ± 1.8	94.0 ± 3.5	54.3 ± 8.5	36.9 ± 2.7	40.7 ± 6.9
Propoxur	111.0 ± 11.3	94.3 ± 8.3	92.3 ± 5.9	90.3 ± 14.6	85.2 ± 2.0	80.4 ± 3.1	0	25.1 ± 0.7	26.1 ± 8.0
Carbofuran	109.9 ± 17.8	90.7 ± 8.0	88.8 ± 6.6	89.4 ± 10.5	79.9 ± 1.7	78.8 ± 2.9	0	22.3 ± 0.5	21.9 ± 8.7
Carbaryl	0	26.6 ± 12.6	31.9 ± 4.6	64.1 ± 1.1	39.1 ± 2.7	49.7 ± 7.8	0	0	0
Methiocarb	40.9 ± 38.0	18.8 ± 8.9	25.7 ± 1.4	0	17.7 ± 2.2	28.6 ± 10.1	0	0	0.4 ± 0.1

n = 6

(R^2) for the standards ranged from 0.992 to 0.999, and the method detection limits (MDL) of the MCAAB method ranged from 0.03 to 1.12 ng/g soil (Table 2).

Results and Discussion

A summary of the recoveries at the 4, 20, and 40 ng/g spiking levels is shown in Table 3. Recoveries of compounds from the different soil and sediment samples varied depending on the chemical and soil type. The recoveries of aldicarb, aldicarb sulfoxide, aldicarb sulfone, oxamyl, methomyl, carbofuran, 3-hydroxy-carbofuran, and propoxur from the silt loam soil and sediment ranged from 78.8% to 121.1%. Recoveries from the 4 ng/g spiking level tended to be slightly higher than for the 20 and 40 ng/g levels for most compounds. Recoveries of carbaryl and methiocarb were low, ranging from 0% to 64.1%, and tended to be more variable. Recoveries of the compounds from the organic clay soil were low, ranging from 0% to 66.7%; with the exception of aldicarb sulfoxide (77.1%–91.6% recovery) and methomyl (93.9% recovery for the 4 ng/g spiking level).

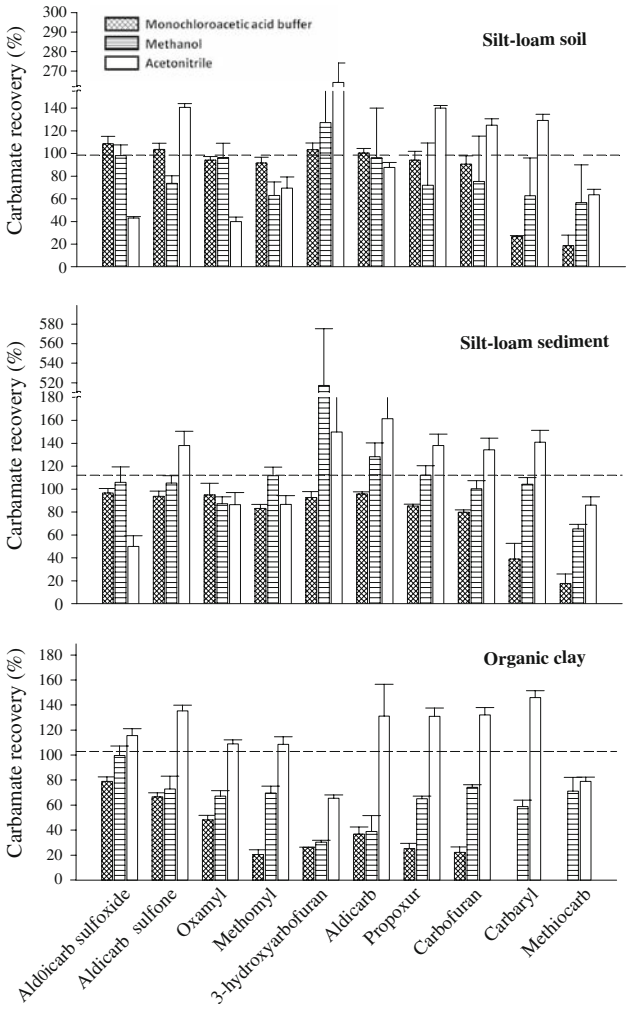

Fig. 1 Carbamate/carbamoyloxime pesticide recoveries from a silt-loam soil and sediment, and an organic clay soil using monochloroacetic acid buffer (MCAAB), methanol, and acetonitrile as extraction solvents. Soils were fortified at 20 ng/g

As with the MCAAB extraction method, recoveries of the compounds were variable with the methanol and acetonitrile extractions, depending on the soil type (Fig. 1). Recoveries from the methanol extraction ranged from 65.2% to 128% for most compounds extracted from the silt-loam soil and sediment. 3-Hydroxy carbofuran was the exception, with recoveries of 165% and 517% for the silt-loam soil and sediment, respectively. Recoveries from the organic clay were generally lower, ranging from 30.4% to 99.7%, depending on the compound. Recoveries from the silt-loam soil and sediment using acetonitrile as the extractant ranged from 39 to 140 and 50%–161%, respectively. Recoveries from the organic clay soil ranged from 65% to 146% using acetonitrile.

The results indicate that the MCCAB extraction method may be more accurate and precise, relative to the methanol and acetonitrile-based methods, for extraction of aldicarb, aldicarb sulfoxide, aldicarb sulfone, oxamyl, methomyl, carbofuran, 3-hydroxy-carbofuran and propoxur from silt-loam soil and sediment samples, but not for carbaryl and methiocarb. The MCCAB extraction method did not work well for extraction of the compounds from the organic-clay soil. For the organic-clay soil, extraction using acetonitrile provided better recoveries for all compounds evaluated. The lower recoveries of carbaryl and methiocarb extracted with MCCAB may be related to the solubility and sorption characteristics of the pesticides. Of all of the compounds evaluated, carbaryl and methiocarb are the least soluble in water (120 and 27 mg/L, respectively), and have the highest K_{oc} values (100–600 and 300–850, respectively) (Roberts and Hutson 1999).

A significant benefit of using the MCAAB extractant over the others is cleaner chromatography without the need for extensive cleanup. Chromatograms for extracts obtained using the MCAAB extraction method exhibited a smoother baseline and fewer non-target peaks than that of the extracts obtained by either of the other extraction methods. In addition to the cleaner chromatography, significant cost savings may also be realized by eliminating the need for C-18 cleanup, organic solvents, and the labor associated with cleanup. Increased environmental and human health safety due to lack of organic solvent use may also be realized. However, this method is not suitable for soils with significant organic carbon content, or for carbaryl and methiocarb in silt-loam soils.

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