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A Rapid Carbamate Multiresidue Procedure for Vegetable Crops

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During this past decade carbamate insecticides have replaced many of the functions of the organochlorine pesticides. Carbamate insecticides, however, are very difficult to analyze by classical gas chromatographic methods which require elevated temperatures. With the practical development of liquid chromatography it became an efficient separation tool for the carbamates (Sparacino & Hines 1976). Then with application of post-column fluormetric labeling to the N-methyl carbamates, it is now possible to efficiently separate and quantitate carbamate pesticides (Krause 1978, Moye et al.1977). This paper will deal mainly with the sample preparation, cleanup, and concentration steps.

One of the physical properties common to the carbamates is their solubility in water which ranges from about 50 ppm for carbaryl to about 0.2% for propoxur. It was therefore decided to use water as the extracting solvent (keeping the pH at 7.00 or below to prevent degradation). Many approaches to the problem of blending or macerating the sample in water were investigated. This included denaturing the protein with heat, precipitating the protein with sodium oxalate and heavy metals, and digestion of the protein with papain. All of these techniques were abandoned because of their ineffectiveness or because they generated even further problems. Filter cell, the finest grade of diatomaceous earth supplied by Johns-Manville, was found to effectively trap the particulate matter and adsorb the proteinaceous material when mixed 2 parts sample to 1 part filter-cell. The slurry produced on blending is then filtered through Celite in a Buchner funnel. The clarified aqueous extract is passed through a reverse phase C_{18} Sep-pack as supplied by Waters Associates. The carbamates are adsorbed onto the Sep-pack allowing the water, salts, pigments, carbohydrates, etc., to pass through. The Sep-pack adsorbant is an approximate 40 micron LC preparative stationary phase contained in a polypropylene capsule. The carbamates may be eluted off the Sep-pack with as little as 2 mL absolute methanol. The sample at this point is ready for the determinative step.

MATERIALS AND METHODS

(A) Liquid chromatograph: model 3500 gradient HPLC (Spectra-Physics, Santa Clara, CA).

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(B) Injector: Manual sample injection valve Model CV-6-UHPa-N 60 10-μL loop (Valco Instruments Co., Houston, TX).

(C) Column: micro Bondapak C_{18} 10 micron octadecylsilane column, 30 cm x 4 mm ID (Waters Associates, Inc., Milford, MA) or C_{18} Ultrasphere, 15 cm - Altex.

(D) Carbamate hydrolysis chamber: 500-mL, two neck, round bottom flask fitted with reflux condenser, heating mantle and charged with water.

(E) Fluorescence Detector: Model FL-1A (Gilson Medical Electronics, Inc., Middleton, WI).

(F) Reaction Tubing: Teflon 0.8 mm ID

(G) Peristaltic Pump: Model Rabbit, 4 channel (Rainin Instrument Co., Inc., Boston, MA).

(H) Blender: Model Sorval Omni-Mixer (Ivan Sorvall Inc., Newton, CT).

(I) Water: distilled, filtered through 0.45 micro filter.

(J) Methanol: certified HPLC grade (Fischer Scientific, Fairlawn, NJ).

(K) Acetonitrile: certified HPLC grade (Fischer Scientific).

(L) Sodium Hydroxide Solution: Prepared from Baker Analyzed Reagent (J.T. Baker Chemical Co., Phillipsburg, NJ). Diluted to 0.05 N with water.

(M) o-Phthalaldehyde: Fluoropa Crystals (Pierce Chemical Co., Rockford, IL).

(N) 2-Mercaptoethanol: (Pierce Chemical Co.)

(O) Fluoropa Premix Diluent: (Pierce Chemical Co.)

(P) Reaction Solution: 500 mg o-phthaladehyde and 1 mL 2-mercaptoethanol in 10 mL methanol is added to 50 mL Premix Diluent and Diluted to 1 liter.

(Q) Pesticide Standards: Solutions were prepared from EPA pesticide reference standards.

(R) Filter Cell: diatomaceous earth produced by Johns-Manville, Celite Division, Greenwood Plaza, Denver, CO.

(S) Celite 545: (Johns-Manville)

(T) Sep-pack: C_{18} reverse phase (Waters Associates, Inc.).

(U) Hydroxylapatite: (Bio-Rad Laboratories, 32nd and Griffin, Richmond, CA)

Sample Preparation Extraction. Weigh 50 g sample into blender cup. Add 25 g filter-cell and 200 mL water. Blend sample, filter-cell and water 1.0 min then add to 75-mm Buchner funnel precoat prepared as follows: (1) pre-wet filter paper and seal to funnel, (2) slurry 10 g Celite 545 with water, (3) start vacuum, (4) add slurry to funnel rapidly, (5) break vacuum as last visual trace of water

disappears, (6) separate funnel from filter flask, discard water, reconnect and restart vacuum. The filtration of the sample is continued until the filter cake is semisolid. Three successive rinses of the blender, cup and filter cake are made with about 25 mL water each. Vacuum is continued until the filter cake has shrunk and pulled away from the sides of the funnel.

Hydroxylapatite. Hydroxylapatite, a form of calcium phosphate, is widely used in preparative biochemistry (Tiselius et al. 1956). It has the ability to sorb and desorb protein. This protein sorption action is controlled by the ionic strength of an eluting aqueous phosphate buffer system. The hydroxylapatite is purchased as an aqueous slurry and is slurry packed 2.5 cm deep in a 2.5 cm column. 25 to 50% Celite 545 may be added to the slurry to improve flow. The column is packed and eluted using 0.001 M sodium phosphate buffer at pH 6.5 - 7.0. After a 50 g sample has been extracted with water and filtered about 350 mL of extract will be recovered. This extract is buffered and applied directly to the hydroxylapatite column followed by a 50 mL 0.001 M phosphate buffer wash. The sample is now ready for the Sep-pack. Generally a little vacuum or pressure will need to be applied to the column to aid flow. The Sep-pack may be attached tandem to the base of the column thus combining two steps into one. Not all samples will require the hydroxylapatite cleanup. Artichokes, beets, berries, beans, and other crops high in pigments or protein may be improved by the use of the hydroxylapatite column. The column may be used for several samples then regenerated by eluting with 150 mL 0.6 M sodium phosphate buffer pH 6.5 - 7.0. The column should then be washed with the 0.001 M phosphate buffer prior to applying a new sample.

Concentration. The Sep-pack is activated by pumping 10 mL absolute methanol through it. Fill a 10 mL syringe with methanol and connect to long end of Sep-pack. Depress plunger to discharge methanol within 2-4 s. Set up 500-mL filter flask with long end of Sep-pack inserted in the base of a rubber stopper as shown in Figure 1. Vacuum is then applied and adjusted to provide a flow of about 30 mL/min. After the entire sample has been drawn through, the Sep-pack is flushed with 10 mL water and then a little air. The Sep-pack is finally eluted with no less than 2.0 mL absolute methanol, the elute being diluted to volume (5 - 10 mL) with water.

If lower minimum detectability is required the eluate direct from the Sep-pack may be injected without dilution. A larger injection loop may also be used.

The LC analytical column is protected from particulate matter, irreversably absorbed organics, etc., by the utilization of a 4-cm guard column packed with a reverse phase C_{18} type pellicular support.

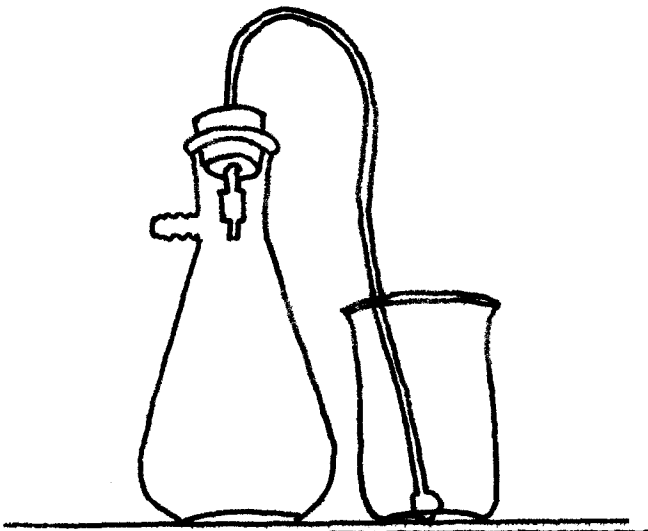

Fig. 1. Concentration apparatus: The beaker is filled with sample and vacuum draws it through the Sep-pack. Note the Sep-pack is inserted in the bottom of the stopper which is then placed in the vacuum flask.

Determination. With the chromatographic parameters set as follows (see Fig. 2)

Column	C_{18} Ultrasphere, 15 cm
Mobil phase	20 - 55% CH_3CN/H_2O
Program rate	20 min
Flow rate	1.6 mL/min
Wave length	340 nm excitation, 455 nm emission
Attenuation	1 x 50
Column temperature	50°C

and the post column reaction parameters set up as optimized by KRAUSE (1978), namely

0.5 mL/min of .05 N NaOH

0.5 mL/min of Reaction Solution

16 s hydrolysis time at 100°C with NaOH

1.3 s reaction time with Reaction Solution

and considering that a full scale response could easily be obtained for 10-15 ng of carbaryl at higher levels of attenuation it was therefore decided that a practical level of 50-100 ng full scale response should be chosen as it would exhibit a very stable base line. 1.0 ppm full scale response would therefore dictate an injection of 0.05 to 0.1 g. With a 10 µL injection loop and a 50 g sample a 5-10 mL final sample volume would be needed.

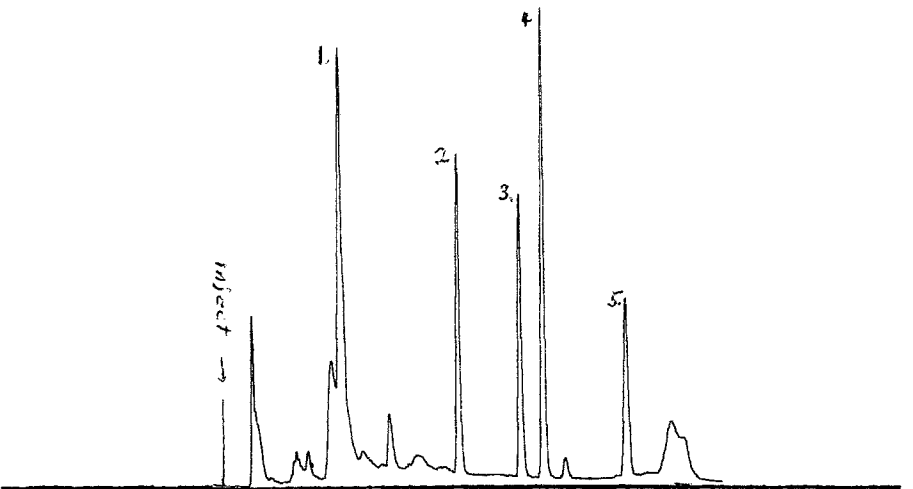

Fig. 2. HPLC - fluorescence detector chromatogram of pear sample fortified at 1.0 ppm. Compounds (50 ng each) are 1-methomyl, 2-aldicarb, 3-carbofuran, 4-carbaryl, 5-methiocarb.

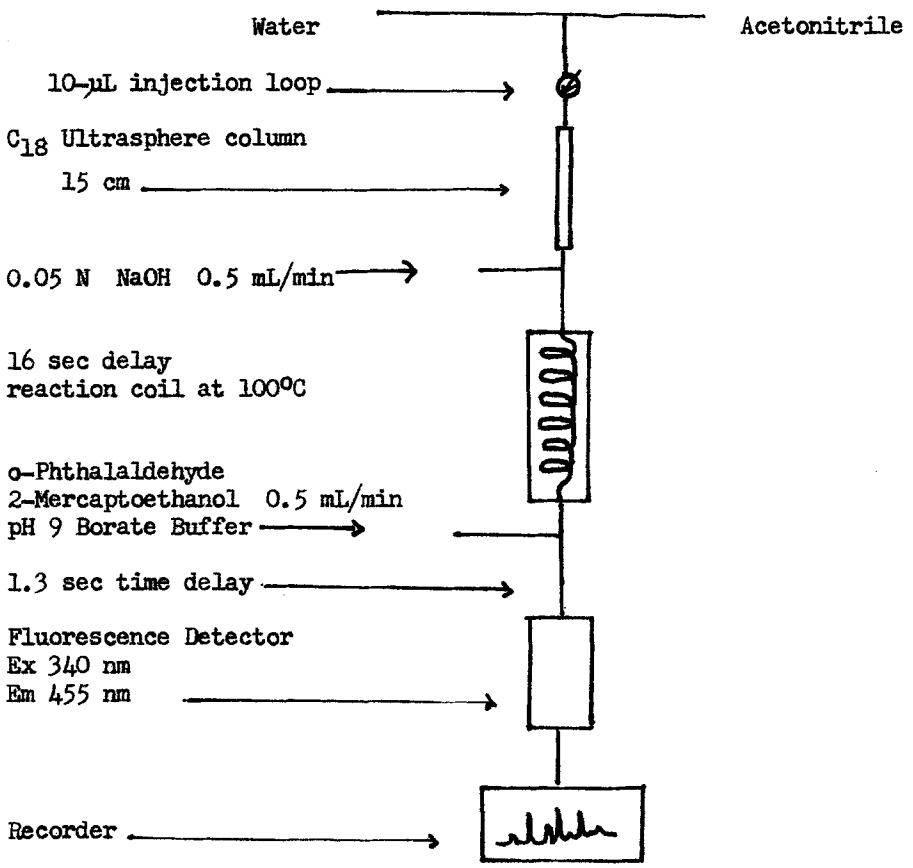

Fig. 3. HPLC - fluorometric post column reactor flow chart.

RESULTS AND DISCUSSION

The method was evaluated by determining the recovery of the carbamates carbofuran, carbaryl, and methiocarb at a level of 5.0 ppm. After weighing 50 g of crop material 250 µL methanol containing 250 µg of each pesticide was added to the sample prior to the solvents, filter cell or blending, etc. Fifteen separate crops were analyzed for both background and the fortified carbamates. The results are listed in Table 1 for all crops. Recoveries averaged 93% for carbofuran, 90% for carbaryl and 85% for methiocarb. The extraction efficiencies were not verified by alternate procedures. One would expect that carbamates on crops as "weathered" residues may react somewhat differently. Other relatively nonpolar N-methyl carbamates showed promise such as propoxur and mexacarbate. An investigation at the 1.0 ppm level was carried out for potatoes, lettuce, cole crops, tomatoes, beets and greenbeans. Average % recoveries were as follows: carbofuran 82, carbaryl 85, and methiocarb 78.

It was observed that recoveries of methomyl and aldicarb were poor, probably because these two compounds are relatively polar. It has been suggested that this characteristic prevents absorption of methomyl and aldicarb on reversed phase Sep-pack absorbant. A normal phase such as a silica Sep-pack could be used as the absorbant with a nonpolar mobil phase such as dichloromethane but one would then expect low recoveries of carbaryl and methiocarb under these conditions as they are relatively nonpolar compounds.

Interferences were quite minimal indicating an easily achieved sensitivity of around 0.01 ppm. Artichokes contain considerable proteinaceous matter which has a tendency to coat the Sep-pack decreasing its adsorptive capacity. This may be overcome by the use of the hydroxylapatite column. Highly pigmented samples such as beets, red cabbage, berries, etc., generally pose few problems as the color is not retained by the Sep-pack. These highly pigmented samples showed some improvement with the use of Hydroxylapatite. The method required about one hour sample preparation time then another 30 min instrument time. The hydroxylapatite cleanup would be in addition to this. It is conceivable that one person, processing samples in batches of at least five, and excluding the hydroxylapatite cleanup step could process ten samples per day.

Methomyl, carbofuran, propoxur, carbaryl, and methiocarb were recovered quantitively from water which had been fortified with these carbamates and passed over the hydroxylapatite column. The data in Table 1 was acquired using the hydroxylapatite cleanup step.

TABLE 1. Carbamate Recoveries of 5.0 ppm

Crop	Carbofuran	Carbaryl	Methiocarb
Pears	103	99	107
Beets	105	95	74
Celery	99	96	103
Lettuce - Romaine	101	96	89
Lettuce - Head	100	82	92
Tomatoes	105	99	104
Onions	90	78	64
Strawberries	76	91	84
Red Cabbage	81	90	90
Parsley	89	67	41
Artichokes	88	70	52
Apples	102	108	107
Potatoes	101	103	92
Mushrooms	95	94	81
Peppers	62	85	94
Average	93	90	85

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