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High Performance Liquid Chromatographic Method for Determination of Six N-Methylcarbamates in Vegetables and Fruits

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Because of the growing concern about persistence of the pesticides on fresh produce in recent years, the short-life insecticides, such as N-methylcarbamates, have gained popularity with the pesticide chemical industry and farm growers. Subsequently, the task of monitoring these insecticide residues in fresh vegetables and fruits on a daily basis for a regulatory laboratory like ours becomes inevitable.

The idea of using a modern liquid chromatography (HPLC) for testing the thermal labile carbamate residues (Sparacino and Hines 1976) in produce has been considered by the staff of this laboratory for many years. However, the heavy pigment content in crops has made the popular HPLC UV-visible detector almost unusable. Interest was restored by the successful demonstration of the post-column derivatization technique (Krause 1980; Muth and Erro 1980; Krause 1978). In addition, the highly fluorescent derivative formed by the reaction of orthophthaldehyde (OPA) with primary amines from alkali hydrolysis of N-methylcarbamates seemed satisfactory for solving the problem (Moye et al. 1977). However, attention was quickly focused on the lengthy time-consuming sample preparation. In order to adapt the method into our daily routine multi-residue analysis, a simple and fast method had to be developed for the purpose.

MATERIALS AND METHODS

Reagents

(a) Water - High purity distilled water filtered by a Millipore water filtration kit. Degas procedure is not necessary.
(b) Acetonitrile - MCB OmniSolv AX 0142 (Spectrum Chemical Mfg. Corp., Gardena, CA 90248).
(c) Methanol - MCB OmniSolv MX 0488 (Spectrum Chemical Mfg. Corp., Gardena, CA 90248).
(d) Petroleum ether - MCB OmniSolv PX 0424 (Spectrum

Chemical Mfg. Corp., Gardena, CA 90248).

(e) 2-mercaptoethanol - Reagent grade (Fisher Scientific Company, Fair Lawn, NJ 07410).

(f) Sodium hydroxide solution, 5 N and 0.05 N - Weigh 200 g sodium hydroxide (Spectrum Chemical Mfg. Corp., Gardena, CA 90248) into 1 L volumetric flask, and dilute to volume with distilled water to make the 5 N NaOH solution. Pipet 10 ml 5 N NaOH into 1 L volumetric flask, and dilute to volume with distilled water to make the 0.05 N NaOH solution.

(g) Sodium tetraborate solution 0.1 M - Weigh 38.14 g ACS grade sodium tetraborate decahydrate (Fisher Scientific Company, Fair Lawn, NJ 07410) into a 1 L beaker. Pour about 800 ml distilled water and stir until the sodium tetraborate crystals dissolve. Adjust pH to 10.5 with 5 N NaOH. Transfer the solution into 1 L volumetric flask and dilute to volume with distilled water.

(h) OPA solution - Weigh 0.5 g O-phthalaldehyde (Fisher Scientific Company, Fair Lawn, NJ 07410) into a 1 L volumetric flask. Pipet 10 ml methanol and swirl flask until the O-phthalaldehyde dissolve. Add 50 ml sodium tetraborate solution and 1.0 ml 2-mercaptoethanol; then dilute to volume with distilled water.

(i) Carbamates standard solutions -
    (1) Stock solution - 1 mg carbamates/ml methanol.
    (2) Intermediate solution - 5 µg carbamates/ml methanol.
    (3) Working solution - 1 µg carbamates/ml methanol.

General Apparatus

(a) Food chopper - Model 8181-D (Hobart Mfg. Co., Troy, OH 45374).

(b) Homogenizer - Omni-Mixer (DuPont Co., BioMedical Division, Newton, CT 06410).

(c) Water bath - Model WBT-100, equipped with thermostat (Barnstead Still & Sterilizer Co., Boston, MA 02132).

(d) Water filtration kit - Filter holder, teflon-faced glass 47 mm and membrance disc filter, pore size 0.45 µm (Millipore Corp., Bedford, MA 01730).

(e) Sample filtration tube - Bond Elut C18. (Analytichem International, Harbor City, CA 90710).

(f) Centrifuge - Model CS (International Equipment Co., Boston, MA).

HPLC Apparatus and Installation

Detail of installation of HPLC and post-column derivatization is presented in Fig. 1.

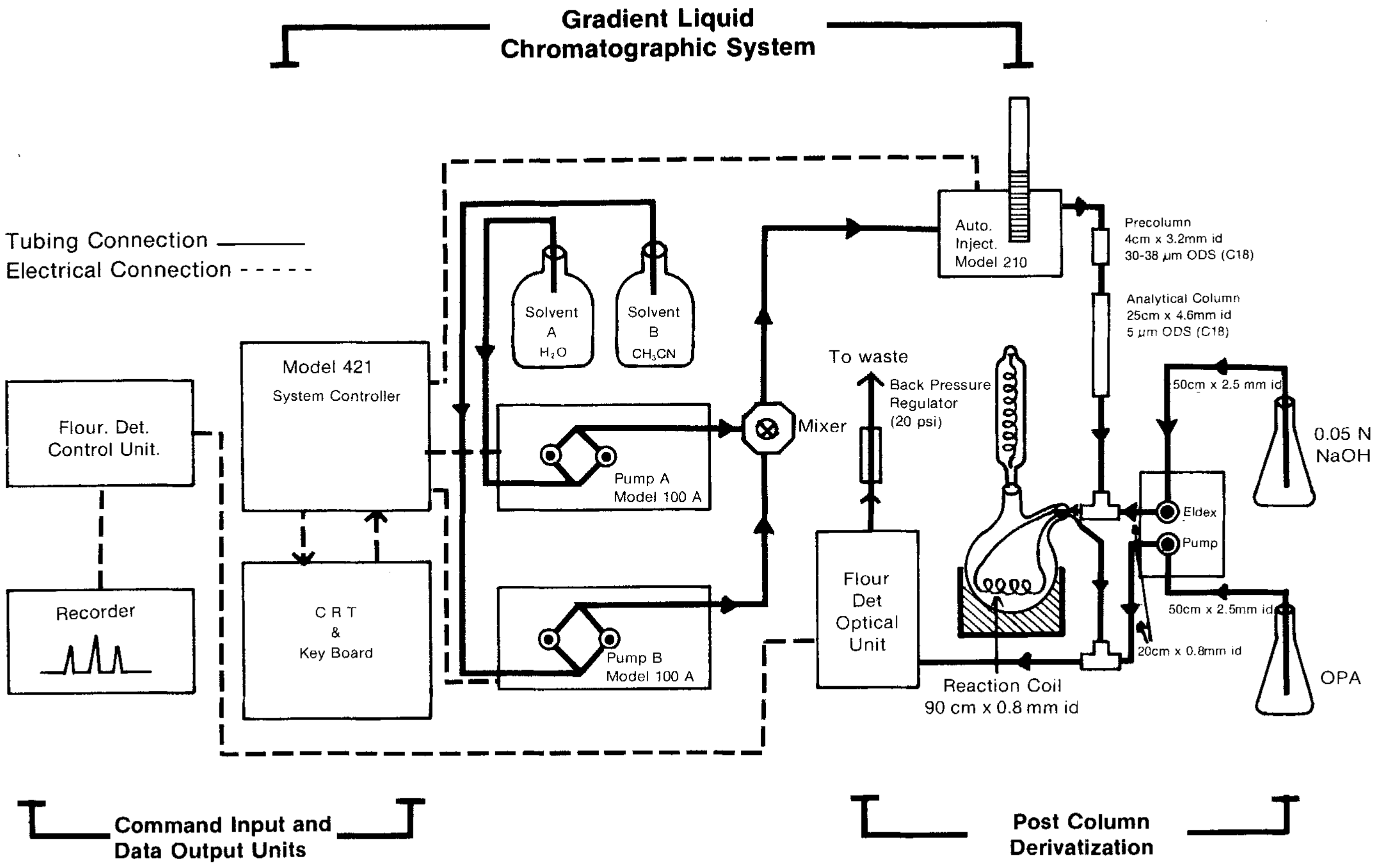

Fig. 1 Detail of installation of HPLC and post column derivatization.

(a) Gradient liquid chromatographic system - Model 324 comprising a 421 Microprocessor System Controller, two Model 100A pumps, a stirred mixing chamber, a Model 210 sample autoinjector with 20 µl injection loop. (Beckman Instruments, Inc., Berkeley, CA 94710).

(b) Precolumn - 4 cm x 3.2 mm I.D. column containing octadecyl (C18) groups chemically bonded to 30-38 µm glass beads. (Whatman, Inc., Clifton, NJ 07014).

(c) Analytical column - 25 cm x 4.6 mm I.D. column containing 5µm ultrasphere ODS packing. (Beckman Instruments, Inc., Berkeley, CA 97410).

(d) Post column derivatization system or sodium hydroxide and OPA solution delivery system - Contains a Model AA-72-S-8 Eldex dual piston pump (Eldex Laboratories, Inc., Menlo Park, CA 94025), a 500 ml NaOH reservoir, a 500 ml OPA reservoir and a 90 cm x 0.8 I.D. teflon reaction tubing in a 500 ml 2-neck pyrex boiling flask (Corning Glass Works, Corning, NY 14830) with a coil type 24/40 pyrex condenser (Corning Glass Works, Corning, NY 14830) connected to one neck and a heating mantle (Briscoe Mfg. Co., Columbus, OH) beneath the flask.

Connect NaOH solution to one piston inlet of Eldex pump with a 50 cm x 2.5 mm I.D. teflon tubing. At the piston outlet, connect a 20 cm x 0.8 mm I.D. tubing to the tee 0.8 mm I.D. for joining the outlet of analytical column and the inlet of reaction tubing through the vacant neck into the boiling flask. Use the same size of tubings as NaOH solution for connecting OPA reservoir to the Eldex pump, and then, join the reaction tubing outlet and detector tubing inlet at the second tee. Put about 300 ml of distilled water into the 2-neck boiling flask to cover the reaction tubing.

(e) Fluorescence detector - Gilson Spectral Glo Fluorometer (Gilson Medical Electronics, Inc., Middleton, WI 53562) contains two units:

 (1) Optical unit with a 15 µl flow cell, excitation 360 nm and emission 455 nm filters. Suggest setting this unit close to the reaction tubing outlet to reduce the dead volume.

 (2) Attenuation control unit. Suggest placing the unit near the recorder; therefore, the sensitivity range can be selected based on peak response on the recorder chart.

(f) Back pressure regulator - Connect a regulator, 4.23 mm flanged tubing seat, at the end of waste line. (Applied Science, State College, PA 16801).

(g) Recorder - Ten-inch strip chart recorder. (Beckman Instruments, Inc., Fullerton, CA 92634).

HPLC Chromatographic Condition

Set the flow rate of the mobile phase at 1.5 ml/min. Inject the sample (20 µl) and begin linear gradient from 5% acetonitrile and 95% water to 70% acetonitrile and 30% water in 12 min. Restore the initial condition in 1 min. and retain the initial condition for the next 3 min. Set the flow rate of 0.05 N NaOH and OPA solution to 0.8 ml/min., individually. Heat the water in the boiling flask to 95°±5° C. Adjust the fluorescence detector range to 200 for 20 ng carbofuran to respose 20% full scale on a recorder chart. Set the recorder chart speed to 5 mm/min.

Note: This linear gradient program is short and only takes 16 min. for testing each sample. In order to synchronize the system, it is necessary to load a dummy vial at the first injection position in a series of samples. A vial of standards is suggested after the six sample vials.

Preparation of Sample

Finely chop the vegetable or fruit in a Hobart food chopper. Weigh 50 g sample into a one-pint Mason jar. Accurately add 100 ml acetonitrile to the jar and blend 2 min. with an Omni-Mixer. Let the solids settle for a few minutes. Carefully pour the aqueous phase through sharkskin filter paper; collect filtrate in a 4 oz. glass bottle. Add about 15 g NaCl into the bottle. Cork and shake the bottle vigorously for a minute. Place the bottle in a centrifuge and set the control at about 1,500 rpm for 2 min. Pipet 20 ml top acetonitrile layer into a 50 ml beaker and evaporate nearly to dryness on a 100° C water bath with the aid of a gently air stream. The final evaporation to dryness is made at ambient temperature and with a gentle air stream.

For crops which do not need a clean-up procedure, pipet 5 ml methanol into the beaker and rotate gently to dissolve the solids. Filter the aliquot through Bond Elut (C18) into a 10 ml volumetric flask by gravity. Transfer about 1.5 ml sample into 2 ml vial. Load the vial into automatic sampler's magazine and start the HPLC determination. A set of six samples is recommended for the process.

Clean-up Procedure

For crops needing clean-up procedure including citrus, celery heart, mint, cilantro, parsley and parsnip, weigh about 10 g of activated florisil, which was preheated at 130° C, into a 55 cm x 2 cm I.D. glass column with gentle vebration by a vibrator. Add about 2 cm of anhydrous granular Na_2SO_4 on top of the florisil column. Pre-elute the column with 30 ml of 10% methanol in petroleum ether. Discard. Adjust the flow rate to about 4 ml/min. Place about 3 ml of methanol into beaker with dried extract and pour the dissolved sample onto the column. Using a few drops of methanol, rinse the sample beaker, then add to the column. Elute the column with 100 ml of 10% methanol in petroleum ether. Collect the eluate in a 150 ml beaker as the first fraction. Before the column top is dry, immediately pour 100 ml of 30% methanol in petroleum ether onto the column. Collect the eluate as the second fraction. Concentrate both fractions on a 100° C water bath with gentle air stream to near dryness. The remaining solvent is evaporated at ambient temperature by a gentle air stream. Pipet 5 ml methanol into the beaker and rotate gently to dissolve the solids. Transfer about 1.5 ml sample into 2 ml vial. Load the vial into automatic sampler's magazine and start the HPLC determination. One analyst can manage six columns at the same time. The clear-up procedure takes about 100 min.

RESULTS AND DISCUSSION

In the past three years, we have noticed that approximately 90% of vegetables and fruits contain simple-polarity composition of fluorescence detectable coextractives. The other 10%, citrus, celery heart, mint, cilantro, parsley, parsnip and celeriac, has a much more complicated or broad-polarity composition of fluorescence detectable coextractives. This observation has inspired us to treat the two groups of crops differently.

For the first group, taking advantage of the simple-polarity-composition coextractives for their polar characteristic nature, we programmed a very polar mobile phase (5% acetonitrile and 95% water) on a reversed phase analytical column (ODS, C18) at the beginning of a linear gradient run. As a result, we were able to move the interfering coextractives through the analytical column sooner than the six carbamates which are of interest to us. Most polar coextractives appear in the chromatogram between 1.5 and 4.o min.; they are well ahead of the first carbamate, oxamyl; its retention time is 7.52 min. The separation of the carbamates from the early emerged polar coextractives is demonstrated in Fig. 2. The recovery results for this group are shown in Table 1. The range of the recovery data is from 76% to 99% when crops were fortified with 0.5 ppm of the six carbamates. Each value in the table is an average of three individual

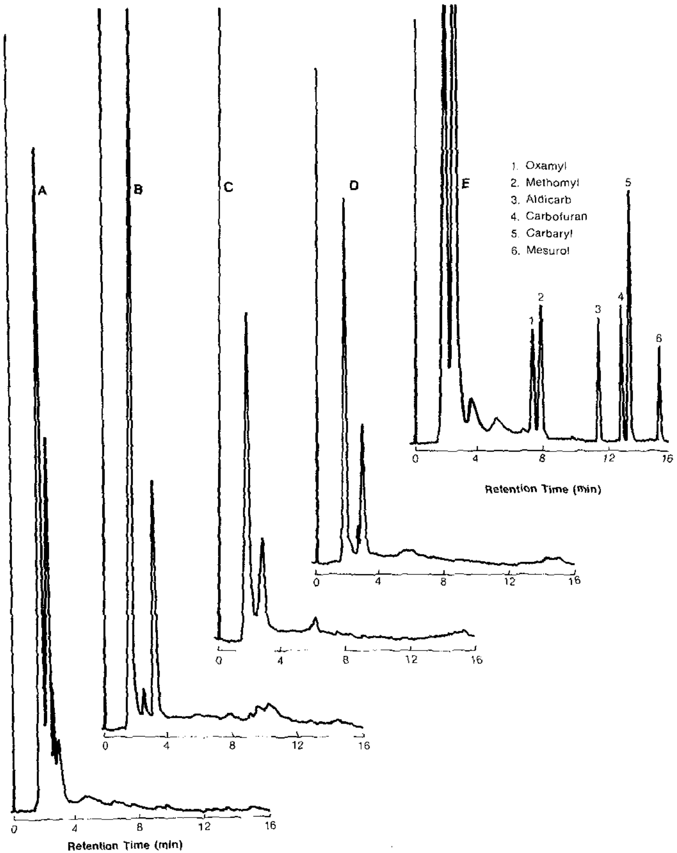

Fig. 2 The separation of the six *N*-Methylcarbamates from the *early* emerged polar coextractives: A, Romaine lettuce; B, Red *cabbage*; C, Pear; D, Cucumber; E, Spinach with fortified carbamates.

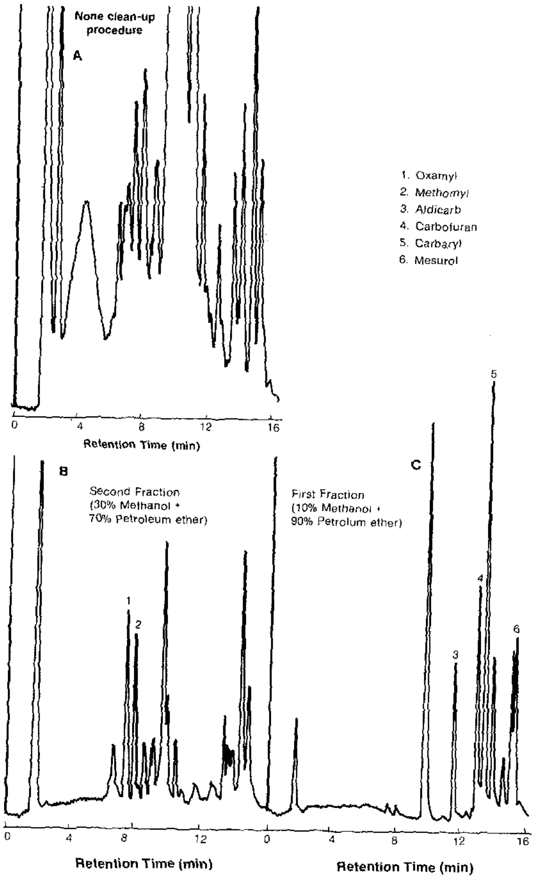

Fig. 3 The comparison of A, a grapefruit sample without clean-up procedure, to B and C, the fortified grapefruit sample with the clean-up procedure.

tests. The best part of the method for this group of crops is its simplicity in sample preparation and short chromatographic time (16 min.). An estimation of 20 samples can be accomplished by two analysts in nine hours. In this case, one hour of lunch time is added to an eight hour working day, because the HPLC equipped with an automation system can be operated without attendance.

For the second group of crops, it is impossible to obtain any result from a HPLC determination without first removing the coextractives; therefore, a clean-up method must be used in the sample preparation. After many attempts, including gel permeation (Ault et al. 1979), Bond Elut, various columns (Official Methods of Analysis 1980) and partitioning methods, we found that the florisil column remove a substantial amount of interfering coextractives and still give us good recovery results. To avoid the overlapping interferences from remaining coextractives, the six carbamates were collected into two solvent fractions. The first fraction is a mixture of 10% methanol in petroleum ether for eluting the less polar carbamates like aldicarb, carbofuran, carbaryl and mesurol. The second fraction is a mixture of 30% methanol in petroleum ether for eluting polar carbamates like oxamyl and methomyl. Then the two fractions are concentrated and detected by a HPLC separately. The benefit of this method is easily recognized by comparing a grapefruit sample without clean-up procedure and the fortified grapefruit sample with the clean-up procedure in Fig. 3. Obviously, the coextractives of uncleaned grapefruit sample have dominated the entire chromatographic spectrum completely; however, in the case of clean-up sample, the six carbamates were isolated from the remaining coextractives. The recovery results for which the crops need florisil column clean-up procedure are presented in Table 2. The recovery range is from 72% to 107%, when crops were fortified with 0.5 ppm of the six carbamates. Like the first group, each value in the table is an average of three replicates.

During our preliminary evaluation, we felt that the polarity composition of the coextractives in each crop of this second group could be similar. We now have a different opinion. We found that we can obtain reasonable recovery results in celery heart, mint and citrus, including grapefruit, lemon and orange; however, additional research studies are needed to remove the coextractives which interfere with oxamyl and mesurol in crops such as cilantro, parsley and parsnip. Apparently, these crops have more complicated polarity-coextractive compositions than our current clean-up procedure can handle. Fortunately, these crops have

Table 1, Recovery (%) results of the crops without clean-up procedure

Crop	Oxamyl	Methomyl	Aldicarb	Carbofuran	Carbaryl	Mesurol
Apple	95	95	76	92	93	88
Cucumber	88	82	83	89	86	81
Green onion	88	94	92	96	94	90
Head lettuce	86	96	83	85	86	86
Mushroom	84	83	91	97	94	87
Pear	94	92	89	96	92	89
Red cabbage	95	92	83	83	83	82
Romaine lettuce	88	87	77	85	87	83
Spinach	99	94	86	91	96	93
Zucchini	88	86	87	89	88	86

Table 2, Recovery (%) results of the crops with clean-up procedure

Crop	Oxamyl	Methomyl	Aldicarb	Carbofuran	Carbaryl	Mesurol
Citrus:						
Grapefruit	98	80	91	89	96	98
Lemon	77	93	107	92	91	93
Orange	93	84	78	83	85	84
Others:						
Celery heart	80	94	96	93	93	100
Mint	72	93	86	91	96	90
Cilantro	[a]	93	98	94	95	93
Parsley[b]	65[a]	95	95	97	97	103[a]
Parsnip[b]	70[a]	77	87	93	91	106[a]

(a) Interference due to coextractives.
(b) Started from 30% acetonitrile instead of 5% in HPLC linear gradient profile.

such a low consumption profile, they only constitute approximately 2% of the samples received by this laboratory annually, according to the California Department of Food and Agriculture Sampling Guide for Pesticide Residue Enforcement. The alternative of separating the oxamyl and mesurol from the interferences is to slow down the flow rate and prolong the chromatogram time; consequently, one will run into the problem of lengthy analysis time and fewer numbers of samples completed in a day. This is not acceptable for a laboratory like ours averaging 13 samples per day.

By using the clean-up procedure, an estimation of six samples can be accomplished by two analysts in nine hours. The increase of time for sample preparation and HPLC chromatogram of two fractions, 10 and 30% individually, has dropped the analytical rate or efficiency down dramatically. However, if a combination of four clean-up samples and six non-clean-up samples may be arranged, a total of ten samples can be accomplished by two analysts in nine hours without much pressure.

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