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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF ARYL N-METHYLCARBAMATE RESIDUES USING POST-COLUMN HYDROLYSIS ELECTROCHEMICAL DETECTION

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SUMMARY

A high-performance liquid chromatographic technique is reported which selectively detects the phenolic moiety of aryl N-methylcarbamates at low nanogram levels. The carbamates are separated on a C-8 column using an acetonitrile–water gradient mobile phase. The eluted carbamates are hydrolyzed in-line by post-column addition of base, which also serves as electrolyte in the electrochemical (coulometric) detection of the resulting phenols. The technique was tested with six carbamates (bufencarb, carbaryl, carbofuran, 3-hydroxycarbofuran, isoprocarb and methiocarb) and four crops (apples, cabbage, grapes and tomatoes). Optimum detector responses and stability for the carbamates were attained at 0.60 V. Detector response to the carbamates in the presence of crop coextractives was 99% of theoretical with a standard deviation of 2.8% at the 0.05- to 0.1-ppm fortification level. The lower limit of quantitation is 0.01 ppm. Other electrochemical detectors were evaluated for suitability for this work.

INTRODUCTION

N-Methylcarbamates are widely used for control of insect pests on agricultural crops. Residues of these insecticides have been found in a variety of crops[1] by a high-performance liquid chromatographic (HPLC) determinative technique[2]. This technique transforms the carbamate moiety of the eluted insecticides through post-column hydrolysis to methylamine with subsequent derivatization to an isoindole product, which is monitored with a fluorescence detector.

Confirmation of the identity and amount of pesticide residue is essential for providing credence to residue findings. The confirmatory technique must selectively detect the pesticide residue, based on a different analytical principle and/or compound functionality from that used in the original analytical determination. In addition to the carbamate moiety, the aryl N-methylcarbamates contain a phenolic moiety, which, if selectively detected, would provide the needed confirmatory data.

Several analytical methods have been reported for detection of the phenolic moiety of aryl N-methylcarbamate insecticides. Johnson[3] reported a method for car-

baryl in which this carbamate is hydrolyzed to 1-naphthol, the phenol reacted with *p*-nitrobenzenediazonium fluoborate and the resulting product measured colorimetrically. Holden[4] reported a procedure in which the phenols of hydrolyzed carbamates are derivatized to dinitrophenol ethers, which are then determined by gas chromatography with electron-capture detection. This technique was unsatisfactory for determination of 3-hydroxycarbofuran, a toxic metabolite of carbofuran. Kissinger *et al.*[5] briefly described a pre-column hydrolysis technique for carbofuran in which the resulting phenol is chromatographed on a reversed-phase column with a slightly acidic mobile phase and detected with an electrochemical detector. Recently, Olek *et al.*[6] applied this pre-column hydrolysis technique to several aryl N-methylcarbamates. They subjected the hydrolyzed phenols to liquid partitioning and solvent evaporation steps prior to determination by HPLC. The phenols are eluted from a reversed-phase column with a methanol–aqueous acetic acid–lithium perchlorate mobile phase and detected with an electrochemical detector operated at +0.9 or +1.0 V using a glassy carbon working electrode and Ag/AgCl reference electrode. Addition of acetic acid to the mobile phase suppresses ionization of the phenols, thereby increasing peak retention and eliminating or reducing peak tailing[7]. Unfortunately, phenolic oxidations at low pH are difficult and require a high applied potential[8] that can result in high background currents and baseline pump noise[9]. Kissinger *et al.*[8] proposed raising the mobile phase pH by post-column addition of base to reduce the oxidation potential and thereby reduce background currents and pump noise.

A confirmatory determinative technique based on the selective detection of the phenolic moiety was desired that would eliminate the separate and manual predeterminative hydrolysis-derivatization steps of current methods to reduce analysis time, and require minimal change in equipment and chemicals from those used in the HPLC post-column fluorometric determinative method[2], which is based on the carbamate moiety of the aryl N-methylcarbamates.

This paper describes an HPLC technique with electrochemical detection (ED) that meets these requirements for determination of the phenolic moiety of aryl N-methylcarbamate insecticide residues in crops. The intact carbamates are separated on a reversed-phase HPLC column using a gradient acetonitrile–water mobile phase. The eluted carbamates are hydrolyzed in-line with dilute sodium hydroxide at 100°C and the resulting phenols are detected with a coulometric electrochemical detector. The sodium hydroxide also serves as the electrolyte.

EXPERIMENTAL

Chemicals

Bufencarb, carbaryl, carbofuran, 3-hydroxycarbofuran, 1-naphthol, methiocarb and the 7-phenol of 3-hydroxycarbofuran were obtained from the Environmental Protection Agency, Pesticides and Industrial Chemicals Repository (Research Triangle Park, NC, U.S.A.). Isoprocarb was obtained from Bayer AG (Leverkusen, F.R.G.). The phenol of methiocarb was obtained from Mobay Chemical (Kansas City, MO, U.S.A.). All carbamates were dissolved and dilutions made in distilled-in-glass grade methanol (Burdick & Jackson Labs., Muskegon, MI, U.S.A.). The HPLC acetonitrile was Burdick & Jackson distilled-in-glass UV grade. HPLC water was produced by a Milli-Q water system (Millipore, Bedford, MA, U.S.A.) consisting

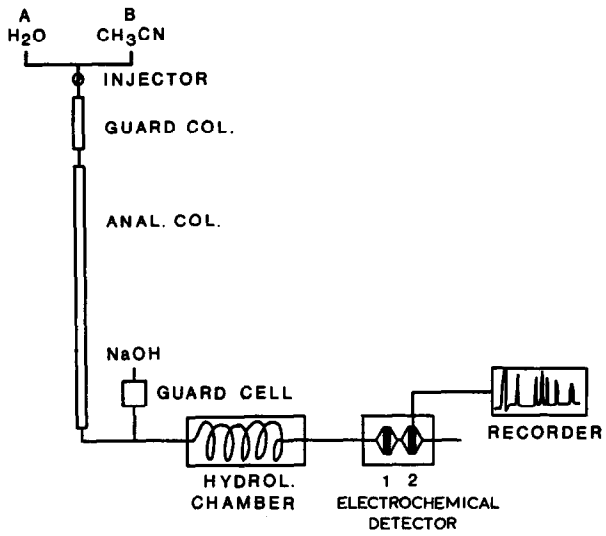

Fig. 1. HPLC–ED determinative system.

of a prefilter, charcoal, ion exchange and Organex cartridge. The 0.1 *M* sodium hydroxide solution was prepared from Fisher certified ACS grade sodium hydroxide pellets (Fisher Scientific, Pittsburgh, PA, U.S.A.) and Milli-Q purified water.

Apparatus

Initial ED data for the phenolic moieties of the carbamates were obtained with a Model CV-27 cyclic voltammograph (Bioanalytical Systems, West Lafayette, IN, U.S.A.). A glassy carbon working electrode, palladium reference electrode and platinum auxiliary electrode were used.

A diagram of the chromatographic determinative system is shown in Fig. 1. The mobile phase and sodium hydroxide solutions were contained in Ultraware HPLC solvent reservoirs (Kontes, Vineland, NJ, U.S.A.) and degassed with helium (99.995%) purified with in-line Hydro-Purge II and Oxy-Purge traps (Alltech/Applied Science Labs., Deerfield, IL, U.S.A.). These solutions were delivered separately with two Model SP8700XR pumps (Spectra-Physics, San Jose, CA, U.S.A.). Injections were made into the column with a Spectra-Physics Model SP8780XR autosampler fitted with a 20-μl loop. The stainless-steel guard column (2 cm × 2 mm I.D.) was packed with 30- to 40-μm Perisorb RP-8 pellicular packing material (Upchurch Scientific, Oak Harbor, WA, U.S.A.). The stainless-steel analytical column (25 cm × 4.6 mm I.D.) was packed with 6-μm spherical Zorbax C-8 packing material (DuPont, Wilmington, DE, U.S.A.). The guard and analytical columns were contained in a Model 2080 HPLC column oven (Varian, Palo Alto, CA, U.S.A.). A stainless-steel column (30 cm × 2.1 mm I.D.) packed with 10-μm spherical PRP-1 packing material (Hamilton, Reno, NV, U.S.A.) was placed between the sodium hydroxide pump and post-column tee to provide 70-bar (1000+ p.s.i.) back pressure for the pump. (The PRP-1 column was packed according to manufacturer's instructions.) The column eluant and sodium hydroxide solution were combined at an Upchurch

0.5-mm I.D. stainless-steel tee. The hydrolysis coil was a 3 m × 0.48 mm I.D. stainless-steel tube contained in a modified Model 5360 Barber-Colman gas chromatograph oven fitted with a Model 700-115 proportional temperature controller (RFL Industries, Boonton, NJ, U.S.A.). The effluent from the hydrolysis coil was passed through a Model 5010 dual analytical ED cell (ESA, Bedford, MA, U.S.A.) with potential applied by an ESA Model 5100A Coulochem electrochemical controller. All chromatograms were recorded on a Spectra-Physics Model 4200 computing integrator. An ESA 5020 guard cell was in-line between the PRP-1 column and tee. ESA filters containing 0.2-μm porous graphite filter elements were placed in-line after each pump and immediately before the analytical ED cell.

HPLC operating parameters

The column oven and hydrolysis chamber were operated at 35 and 100°C, respectively. The mobile phase flow-rate was adjusted to 1.50 ± 0.02 ml/min with acetonitrile–water (50:50). The flow-rate of the 0.1 *M* aqueous sodium hydroxide solution was adjusted to 0.50 ± 0.02 ml/min. (Note: flow-rates were determined by timing the collection of a measured volume of eluant flowing from the detector.) The system was equilibrated at acetonitrile–water (20:80) for 10 min prior to injection of the prepared sample or standard. Immediately after injection, a 25-min linear gradient to acetonitrile–water (70:30) was begun.

The ED guard cell was set at +0.70 V. Detectors 1 and 2 of the analytical cell were set at +0.20 and +0.60 V, respectively, or as indicated. The multiplier gain switch was set to X1, and the gain thumbpot switch was adjusted so that 20 ng of carbofuran produced 50% full scale response on the computing integrator set at an attenuation of 8. The time constant was set at 0.4 s. The analytical cell was flushed with 6 *M* nitric acid at the end of each day as a preventive maintainance measure to remove any materials absorbed on the electrodes.

Samples

Crop samples were prepared for the HPLC–ED determination by the reported HPLC post-column fluorometric method[2]. Briefly, the sample is extracted with methanol and crop coextractives are removed by liquid–liquid partitioning and a Nuchar-silanized Celite adsorbent mixture. The prepared samples were fortified with carbamates just prior to injection in studies to determine the effect of crop coextractives on the detector's response to the phenolic moiety of the hydrolyzed carbamates.

RESULTS AND DISCUSSION

ED oxidation parameters

Cyclic voltammetric data were collected to determine the effect of phenolic structure, solution pH and electrolyte concentration on the oxidation potential. A solvent mixture of acetonitrile–water (1:1) was used to simulate the mobile phase used in the HPLC–fluorometric method[2] and sodium hydroxide, or other compounds as indicated, was added to serve as the electrolyte.

The phenols of the aryl N-methylcarbamates produced well-defined oxidation waves in basic media, as shown in Fig. 2. The oxidation peak potential was lowest (0.56 V) for 1-naphthol (metabolite of carbaryl), and highest (0.72 V) for the phenol

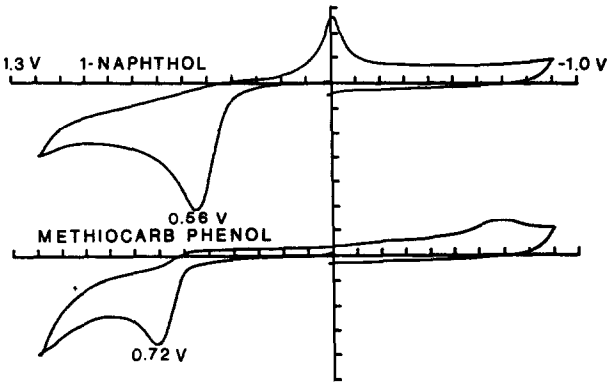

Fig. 2. Cyclic voltammograms of the phenols of two carbamates; analyte (1 m*M*) in 0.1 *M* sodium hydroxide in acetonitrile–water (1:1); glassy carbon working electrode and palladium reference electrode. Scan rate was 200 mV/s and sensitivity was 0.2 mA/V.

of methiocarb. Also, peak potentials were lowest for the phenols at a high pH and the oxidation potential of water was well separated from that of the phenols. The data from this work are in agreement with those of Kissinger *et al.*[8] The oxime moieties of the oxime N-methylcarbamates were not electrochemically active with the parameters used.

The effect of base concentration on the phenol oxidation peak potential was determined by using 1-naphthol and the 7-phenol of 3-hydroxycarbofuran as representative phenols. The purpose was to establish the base concentration at which slight changes in concentration would have minimal effect on peak potential shift and hence detector response. The phenolic peak potential decreased 7 mV/0.01 *M* as the molarity of the sodium hydroxide, after mixing with the column eluent, increased from 0.0125 to 0.0625 *M*. However, from 0.00625 to 0.0125 *M* the peak potential change was much greater, 80 mV/0.01 *M*. To optimize system stability, subsequent HPLC–ED studies were conducted at a base molarity of 0.025 *M* when the sodium hydroxide was mixed with the column eluent.

HPLC–ED characteristics

Five representative carbamate insecticides (bufencarb, carbaryl, carbofuran, isoprocarb and methiocarb) and a carbamate metabolite (3-hydroxycarbofuran) were used to investigate the suitability of commercial HPLC–ED detectors, to develop HPLC parameters compatible with the ED detector, to produce hydrodynamic voltammograms to aid in establishing appropriate detector potentials and to establish the detector linear response range.

Two amperometric cell designs, a constant potential coulometric cell and a thin-layer cell, were investigated for their compatibility with the HPLC post-column hydrolysis system. The thin-layer cell (Model 17, Bioanalytical Systems) with dual glassy carbon working electrodes leaked when the needed 4-bar (60 p.s.i.) back pressure was applied to prevent boiling of the mobile phase in the hydrolysis chamber. Attempts to correct this problem were unsuccessful. The coulometric cell (Model 5010, ESA) with dual porous graphite working electrodes did not leak, and because

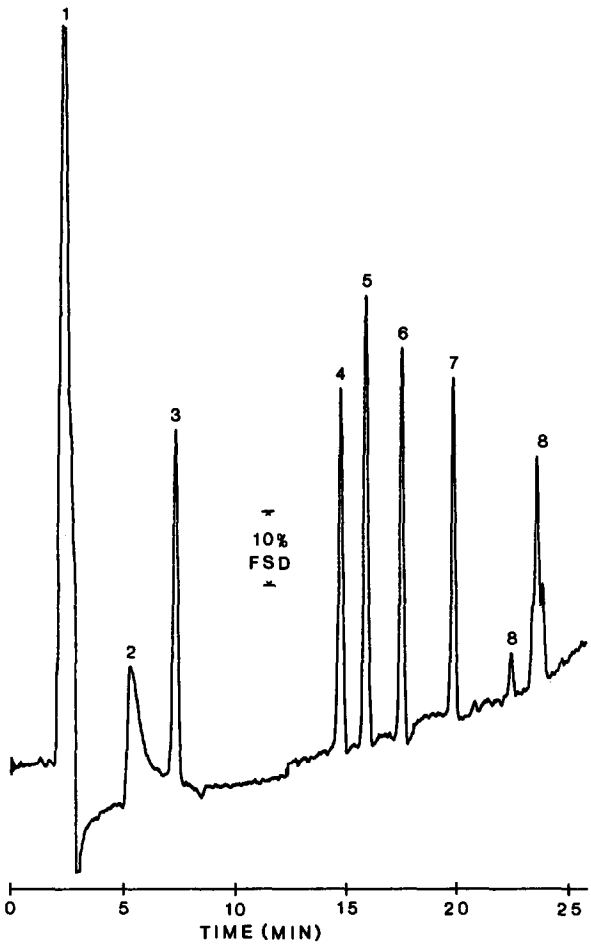

Fig. 3. HPLC separation of six carbamates. 1 = methanol; 2 = oxygen; 3 = 3-hydroxycarbofuran; 4 = carbofuran; 5 = carbaryl; 6 = isoprocarb; 7 = methiocarb; 8 = bufencarb (contains several isomers). Each carbamate represents 20 ng of compound except bufencarb, which represents 40 ng. Chromatographic and ED parameters are described in the text.

of its design, produces 20-bar (300 p.s.i.) back pressure, thus eliminating the need for an auxiliary backpressure device. Additionally, the ESA Model 5011 cell, which contains a smaller diameter graphite electrode and is not coulometric, was also investigated. This cell was found to plug more readily than the 5010 cell because of its smaller diameter. All subsequent HPLC–ED work was undertaken with the Model 5010 cell.

The six aryl N-methylcarbamates, which vary in polarity, were successfully separated by using the C-8 column and the gradient mobile phase previously reported for the aryl and oxime carbamates[2]. Because the oxime carbamates are not detected by the electrochemical detector, the HPLC solvent parameters were reduced to a 25-min, 20–70% acetonitrile in water gradient. Fig. 3 shows the separation and sharp

symmetrical peaks attained for the six carbamates with this gradient. Thus, only a slight change needs to be made to switch from the fluorescence to the ED system. In addition, by not adding acids, bases and/or salts to the mobile phase, column deterioration is minimized and original column performance maintained. The wide use of the HPLC solvents, acetonitrile and water, in ED analyses shows that they are compatible with electrochemical detectors. However, electrochemical detectors are very sensitive to changes in flow-rate and solution composition (solvent, electroactive impurities and electrolyte concentration)[10]. Short-term and long-term flow-rate stability were attained with a solvent delivery system containing dual pistons arranged in series and a low-pressure solenoid-controlled metering chamber for control of mobile phase composition. The deleterious effects of electroactive impurities (high and changing background currents) were minimized by use of high-purity solvents, thorough removal of oxygen from the mobile phase solvents and sodium hydroxide solution and by coulometric oxidation of oxidizable impurities in the base solution with an ED guard cell. Variation in short-term and long-term electrolyte concentration was minimized by the post-column constant flow-rate addition of sodium hydroxide solution with the previously described delivery system. The "optimized" system resulted in a low coulometric background current ranging from 1.7 to 2.3 μA through the gradient (20% baseline change) and less than 1% full scale short-term baseline noise as shown by the chromatogram in Fig. 3. With less sophisticated delivery systems, pump noise was 3%, and without attention to removal of oxygen and coulometric oxidation of impurities, background currents were in excess of 20 μA and baseline change through a gradient run was in excess of 50%.

Hydrodynamic voltammograms were produced for the six aryl N-methylcarbamates to obtain an understanding of the ED characteristics of the compounds with the HPLC–ED system. Voltammograms for carbofuran and methiocarb are shown in Fig. 4. The coulometric cell uses a palladium reference electrode; if a Ag/AgCl reference electrode had been used, the voltammograms would have been shifted to a more positive voltage by approximately 200 mV. Carbaryl, carbofuran, 3-hydroxycarbofuran and isoprocarb produced similar hydrodynamic voltammograms; oxidation began to occur between 0.25 and 0.40 V with a maximum response observed

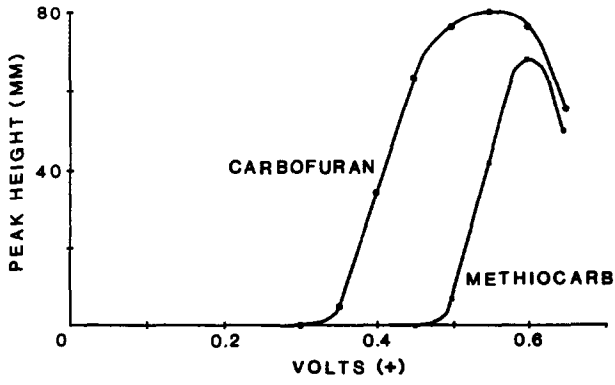

Fig. 4. Hydrodynamic voltammograms of phenols of two carbamates. Amounts of 20 ng of each carbamate injected. Chromatographic and ED parameters are described in the text.

near 0.55 V. Methiocarb and bufencarb produced similar hydrodynamic voltammograms with oxidation beginning at 0.45 V. In all cases response begins to decrease after 0.60 V, as evident in Fig. 4. This decrease appears to be associated with the pronounced increase in background current from 1.4 μA at 0.60 V to 5.1 μA at 0.65 V. The working and reference electrodes and base concentration, which can affect peak potential, were reasonably stable with only a ± 0.05-V change occurring over a period of 2 years.

Linearity response curves were obtained by monitoring the potential from 0.2 to 0.6 V (detector 1, 0.20 V, and detector 2, 0.60 V). The gain multiplier was set at X1, and the gain control was set at 27 for the entire linearity study. The current output from the cell was linear from 2 to 1000 ng for carbaryl. (Note: the ESA instrument enables direct light-emitting diode readout of current prior to conversion to millivolts.) However, the output measured after conversion of current to voltage was linear only from 2 to 80 ng. Above 80 ng the response plateaued, because the gain amplifier was overloaded. The other five carbamates produced similar linearity response data. Relative retention times, linear response range data (computing integrator) and nanograms required for 50% FSD for the six carbamates are shown in Table I. The linear response range is approximately 3–60 ng for the carbamates except for bufencarb, for which the range is 12–180 ng. The lower response for bufencarb was anticipated because bufencarb contains several isomers (see Fig. 3) and only the major peak was measured in determining the linear range and 50% FSD response.

System stability

The system's stability under typical laboratory conditions was determined with apples, cabbage, grapes and tomatoes. These crops were selected based on the frequency of reported aryl N-methylcarbamate residue findings[1]. The individual fresh raw crop was chopped; portions were immediately extracted and coextractives removed according to the published method[2]. Volumes of 20 μl of aliquots representing 400 mg of crop were injected into the HPLC column.

TABLE I

CARBAMATE RETENTION, RESPONSE AND LINEAR RESPONSE RANGE DATA

Carbamate	*Relative retention time**	*Response (ng) for 50% FSD***	*Detection limit (ng)****	*Linear range (ng)*
3-Hydroxycarbofuran	0.50	25	0.4	2–70
Carbofuran	1.00	20	0.3	2–60
Carbaryl	1.08	16	0.25	2–50
Isoprocarb	1.19	19	0.3	4–60
Methiocarb	1.34	21	0.35	4–60
Bufencarb	1.60	70	0.65	12–180

* Relative to carbofuran having a retention time of 14 min.

** Instrument sensitivity adjusted so that 20 ng of carbofuran produced a 50% FSD detector response.

*** Signal-to-noise ratio = 3 based on method detector sensitivity parameters.

The effect of applied potential (0.54 *versus* 0.60 V) on the stability of detector response to the six carbamates after injection of crop controls was determined by the injection sequence: mixed carbamate standard; five crop controls (single crop, five chromatographic runs); mixed carbamate standard. Peak heights of the carbamates from the second standard injection were compared to those from the first standard injection. Detector response stability for carbaryl, carbofuran, 3-hydroxycarbofuran and isoprocarb was excellent at both 0.54 and 0.60 V; mean responses for the carbamates after five crop control chromatographic runs were 100.6 and 99.9%, at the respective voltages, of the mean response values determined before crop controls were injected. The standard deviations were 3.1 and 4.1 at 0.54 and 0.60 V, respectively. Each of these carbamates is completely oxidized near 0.55 V.

The data for bufencarb and methiocarb, which do not reach maximum response until near 0.60 V, show the effect of working at or below the maximum response voltage. The mean recoveries for bufencarb were 85.4 and 89.8% at 0.54 and 0.60 V, respectively, showing that detector response for bufencarb decreased after injection of crop controls. At the low potential the standard deviation was much higher (25.5%) than at the high potential (8.0%). Comparative response calculations for methiocarb were 101.2 and 94.9%, respectively, at 0.54 and 0.60 V. The corresponding standard deviations were 19.3 and 0.5%. Although results were similar at both voltages for bufencarb and methiocarb, the system stability (standard deviation) is superior at 0.60 V. These findings are consistent with the hydrodynamic voltammogram data.

Slight changes in the oxidation potential caused by crop coextractives or solution pH do not affect detector response to carbaryl, carbofuran, 3-hydroxycarbofuran and isoprocarb at 0.54 and 0.60 V. However, for bufencarb and methiocarb, any slight change in oxidation potential at 0.54 V can cause a large change in response, because these compounds are only partially oxidized at 0.54 V. Thus, detector stability is best at 0.60 V, and this voltage was used in subsequent tests.

The effect of crop coextractives on detector response was further studied at 0.60 V by fortifying purified extracts of the crop controls with a mixture of six carbamates. Fortification levels were at 0.05 ppm except for bufencarb, which was at 0.1 ppm. The overall mean recoveries based on standards injected before and after sample injection were 98.9 and 99.2%, respectively. Overall standard deviations were 2.88 and 2.81%. These results further show that detector response to the six carbamates was not affected by the presence of crop coextractives remaining from any of the four crops studied.

Chromatograms of a reagent blank, tomato control and fortified tomato sample are shown in Fig. 5. The reagent control chromatogram contains two peaks. The first peak was found to be due to oxygen from the non-degassed 20-μl injection. This was verified in that after degassing the control with nitrogen, the peak was not observed in the chromatogram. Subsequent aeration of the control with oxygen and sample reinjection resulted in a very large peak having the same retention as the original peak. The oxygen or oxygen-related phenomena were unknown until now. The second peak has been attributed to an apparent trace impurity in the toluene used in the method[2]. No other method reagent produced the second peak. These peaks are separated from the six carbamate peaks.

The tomato control chromatogram contains a peak near the 3-hydroxycar-

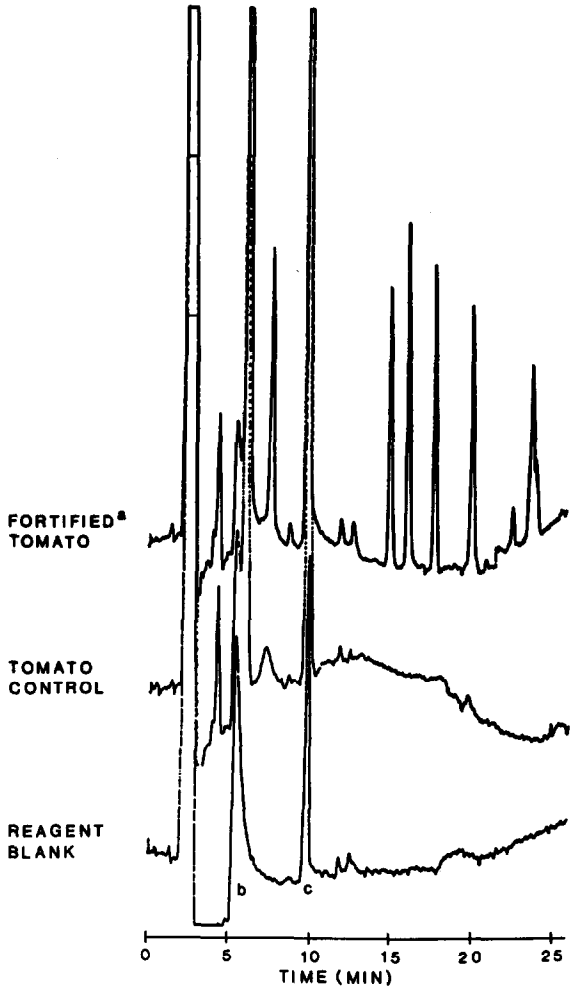

Fig. 5. HPLC chromatograms obtained by using established method[2]. a, Fortification levels at 0.05 ppm for five carbamates and 0.10 ppm for bufencarb; b, oxygen peak from 20-μl injection; c, peak from toluene used in the method.

bofuran peak that caused an 8% erroneously high recovery at the 0.05-ppm fortification. The data were corrected for this interference. Apple and grape control chromatograms contained a peak of similar size near the carbaryl peak. At a tenfold dilution of the crop controls, no crop interferences were observed. Phenolic carbamate metabolites are not recovered through the method, and thus do not interfere.

CONCLUSION

It has been demonstrated that this HPLC–ED technique will provide the qualitative and quantitative confirmatory data needed to substantiate original aryl N-methylcarbamate residue findings, or it can serve as a primary determinative technique.

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