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DETERMINATION OF CARBOFURAN RESIDUES IN POTATO, ONION AND TURNIP: COMPARATIVE ANALYSES USING GAS CHROMATOGRAPHY–SELECTED ION MONITORING OR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY*

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SUMMARY

Potato, onion and turnip were analyzed for residues of carbofuran, 3-hydroxycarbofuran and 3-ketocarbofuran to 0.01 ppm by separation and quantitation of heptafluorobutyryl (HFB) derivatives using gas chromatography–chemical ionization mass spectrometry with selected-ion monitor detection at 228 a.m.u. Extracts of acid-digested crops (5 g/ml) required cleanup by liquid–solid chromatography and treatment with 30 μl/ml of heptafluorobutyric anhydride (HFBA) before reproducible derivative formation and interference-free analysis could be achieved. This concentration of HFBA necessitated that excess reagent and by-products be removed before analysis. The effects of this and other modifications on former preparative procedures and recoveries are discussed. Analysis of the cleaned-up extracts by gas chromatography of the HFB derivatives using electron-capture detection was still impossible because of interference. The chromatographic properties of the three carbamates and their respective phenols on adsorption and reversed-phase high-performance liquid chromatography were determined and the applicability of the technique to the analysis of the cleaned-up extracts was investigated and found to be unsatisfactory.

INTRODUCTION

We have previously discussed[1] the difficulty in analyzing for carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranol methylcarbamate; CF) and its metabolites, 3-hydroxycarbofuran (2,3-dihydro-2,2-dimethyl-3,7-benzofurandiol 7-(methylcarbamate); HOCF) and 3-ketocarbofuran (2,2-dimethyl-7-[((methylamino)carbonyl)-oxy]-3(2H)-benzofuranone; COCF) to less than 0.1 ppm in crops, particularly those extracted by the acid digestion procedure of Cook *et al.*[2]. Because these carbamates possess a single nitrogen atom, the specificity of the mass spectrometric (MS)–

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selected-ion monitoring (SIM)* detection makes it possible to take advantage of the fact that they or their heptafluorobutyryl (HFB) derivatives produce, on fragmentation in the mass spectrometer, ion-species different from those produced by the majority of co-extractives. This selectivity, coupled with the prior gas chromatographic (GC) separation of the carbamate–HFB derivatives from one another and from other single-nitrogen containing plant components which may fortuitously produce the same ionic species, has made the required analyses on a number of crops possible with a minimum of sample preparation. Because of the nature and/or amount of the co-extractives in potato, onion and turnip, extracts of these crops at concentrations of 5 g/ml required liquid–solid chromatography clean-up and three-fold higher concentrations of heptafluorobutyric anhydride (HFBA) than used previously before reproducible derivatization and interference-free chromatograms could be achieved. We wish to describe procedures used for these crops and to discuss the effect of these modifications on the operational simplicity and overall recovery of the carbamates by our general method.

The necessity to clean up these extracts before analysis by GC–MS made it appropriate to examine more commonly available and less expensive instrumental techniques for carbofuran analysis. In addition to GC with electron-capture detection (ECD) for HFB derivatives, direct high-performance liquid chromatography (HPLC) appeared to have potential. Adsorption and reversed-phase HPLC of the carbamate insecticides have been investigated[3] and a procedure for the adsorption HPLC analysis of carbofuran and its carbamate metabolites in organic solvent extracts of a variety of crops has been described[4]. The chromatographic behavior of the phenolic metabolites of the three carbamates and their potential for interference were not discussed. Therefore we also describe here the HPLC behavior of the three phenolic metabolites as well as our attempts to analyze potato, onion and turnip extracts by HPLC and GC–ECD.

MATERIALS AND METHODS

Instrumentation and chromatographic conditions

The GC chemical ionization (CI) MS–SIM and GC–ECD instrumentation has been described previously[1]; further to that, pertinent MS ion-source parameters were: electron energy, +100 V; ion-energy, +3 V; repeller, +3 V; lens, −3 V; filament emission, 1 mA; temperature, 130 °C; pressure (methane), 1 torr. The electron multiplier (CDEM) was operated at −1700 V at an analyzer pressure of $5 \cdot 10^{-6}$ torr. The SIM was calibrated daily at 228 a.m.u. using perfluorotributylamine (FC-43) and the unit mass marker was used as a secondary reference.

The Finnigan GC used a 150 cm × 2 mm I.D. glass U-tube column packed and operated as follows: 3% OV-17 on 80–100 mesh Chromosorb W HP at 160 °C for potato and onion and 3% OV-1 on the same support at 150 °C for turnip. The injector was maintained at 200 °C and after each analysis the column was heated rapidly to 250 °C and held there for 5 min while the effluent was diverted from the mass spectrometer. Typical retention times observed were: (a) on OV-17,

* The term "selected-ion monitoring" has been substituted for "mass fragmentography"[1] as a more accurate description of the technique used.

HOCF–DiHFB 2.6 min; CF–HFB 3.8 min; COCF–HFB 5.5 min; (b) on OV-1, CF–HFB 5.5 min; HOCF–DiHFB and COCF–HFB 7.5 min.

The HPLC system consisted of a Constametric IIG pump, a Spectromonitor III variable-wavelength ultraviolet detector operated at 280 nm (Laboratory Data Control, Riviera Beach, FL, U.S.A.) and a Rheodyne Model 710 valve loop injector (Rheodyne, Berkeley, CA, U.S.A.) with a 20-μl loop. For adsorption chromatography a 25 cm × 4.6 mm I.D. stainless-steel column of 10 μm Spherisorb was used with hexane–isopropanol (95:5) flowing at 1 ml/min. Retention times were CF phenol, 7 min; CF, 15 min; HOCF and COCF, 27 min; HOCF phenol, 29 min. COCF phenol was not eluted under these conditions but did elute in 6 min (twice the retention times of the other five components) with hexane–isopropanol (50:50). Reversed-phase chromatography was carried out on a similar-sized column of 5 μm Spherisorb ODS using acetonitrile–water (25:75) at the same flow-rate. Retention times were HOCF phenol, 5 min; HOCF, 8 min; COCF phenol, 11 min; COCF, 17 min; CF phenol, 19 min; CF, 24 min. Peak heights ranged from 100 mm for HOCF to 15 mm for CF (0.02 AU = 255 mm) for 200 ng. Control crop extracts in acetonitrile were subjected to a preliminary examination on reversed-phase using acetonitrile–water (50:50). Under these conditions the retention times for HOCF (and its phenol) and CF (and its phenol) were 4 and 6 min, respetively. Peak heights of *ca.* 30 mm (0.005 AU = 255 mm) were observed for 20 ng of carbamate.

Chemicals and crops

Chemical sources were as reported previously[1]. In addition, the GC column packing for the Finnigan 9500 were from Chromatographic Specialties (Brockville, Canada), the Florisil (60–100 mesh, normally activated by the manufacturer, 2.5% moisture at use) was from Floridin (Pittsburgh, PA, U.S.A.), the silica gel (100–200 mesh, Davidson grade 923, 1.5% moisture at use), sodium lauryl sulfate and sodium chloride were from Fisher (Toronto, Canada). The antifoamer (Antifoam A compound) was from Dow-Corning (Toronto, Canada). The FC-43 was supplied by Finnigan (Sunnyvale, CA, U.S.A.). The acetonitrile, hexane and isopropanol used for HPLC were HPLC grade from Fisher (Toronto, Canada). Methane gas was "Linde", UHP grade from Union Carbide (Toronto, Canada). Sample vials were from Varian Canada (Georgetown, Canada).

Standards for HPLC and for the fortification of aqueous solutions were prepared at 100 μg/ml in HPLC grade acetonitrile and diluted as required.

Samples of potato, onion and turnip were from crops grown and treated in the field with formulated carbofuran at conventional levels along with appropriate untreated control crops.

Extraction

Crops for residue analysis were extracted within 24 h of harvest. Some fortification and derivatization studies were done on refrigerator-stored materials.

The extraction method used was basically that of Cook *et al.*[2] with some further modifications. A 100-g sample of vegetable was mixed with 250 ml of water in a blender at 20,000 rpm for 2 min. The suspension was quantitatively transferred to a 1-l boiling flask by rinsing the blender jar thoroughly several times with portions of a total of 250 ml of 0.5 *N* aqueous HCl which were added to the flask.

The suspension was refluxed for 1 h. Foaming can be a problem during the initial heating and was reduced by insulating the upper portion of the flask to minimize heat loss. Old potatoes were particularly troublesome and required the use of a few drops of antifoaming agent. After cooling slightly the condenser was rinsed with a small portion of water and the aqueous suspension was filtered with suction using a Buchner funnel and Whatman No. 1 paper. Before the residue in the funnel reached dryness the boiling flask was rinsed with 250 ml of 0.5 *N* aqueous HCl at room temperature and the rinse was added to the plant residue in the funnel. The filtrate (*ca.* 800 ml) was quantitatively transferred to a 2-l separatory funnel with small water rinses. The solution was cooled to room temperature (usually under running water), treated with *ca.* 0.25 g of sodium lauryl sulfate and saturated with salt (*ca.* 500 g). The salt-saturated filtrate was extracted four times with 100 ml of chloroform by shaking vigorously for 2 min. The chloroform extracts were passed through anhydrous sodium sulfate suspended over glass wool in a funnel and collected in a 500-ml boiling flask. On completing the extraction, the sodium sulfate was rinsed with *ca.* 20 ml of chloroform. The chloroform extract was evaporated to less than 100 ml on a rotary evaporator under vacuum, made up to 100 ml and transferred to a bottle for storage at *ca.* —20 °C. The effect of acid-digestion was examined by fortifying 500 ml of 0.25 *N* aqueous HCl with 1 μg of each of the carbamates in acetonitrile and processing the solutions through 1-h reflux, transfer to the separatory funnel with the 250-ml acid rinse and extraction. The recovery of 1 μg of the materials from a salt-saturated mixture of 250 ml water and 500 ml of 0.5 *N* aqueous HCl was also examined to estimate the efficiency of the extraction step alone. Data are included in Table I.

TABLE I

RECOVERY OR REACTION OF CARBAMATES AT VARIOUS STEPS IN PROCEDURE

Carbamate concentrations equivalent to 0.01 ppm in crop.

Step	*Substrate*	*Crop*	*Recovered or reacted (%)*		
			CF	*HOCF*	*COCF*
1 Extraction	Aqueous acid	—	100	98	102
2 A Column chromatography*	Standards in C_5H_6	—	102	94	100
B Column chromatography**	Crop extract	Potato	98	104	87
		Onion	97	104	99
		Turnip	98	70	97
3 Derivatization	Column-chromatographed crop extract	Potato	91	102	81
		Onion	93	97	96
		Turnip	92	102	109
4 Acid digestion and extraction	Aqueous acid	—	81	96	93
5 Total procedure	Blended crop	Potato	83	101	95
		Onion	82	97	103
		Turnip	83	67	90

* Values for Florisil. Silica gel gave similar results.

** Potato and onion on Florisil, turnip on Florisil followed by silica gel (see text).

Liquid–solid chromatography

Chromatography columns containing (bottom to top) 2 g sodium sulfate, 15 g Florisil and 5 g sodium sulfate were prepared in 1.5-cm-I.D. glass tubes having a coarse glass frit at the bottom and a ground joint at the top to permit attachment of a solvent reservoir. A 50-ml aliquot of the chloroform extract of crop was evaporated to *ca.* 3 ml on a rotary evaporator under vacuum and solvent-exchanged to benzene by repeating the evaporation three times with 15 ml of benzene. The crop residue was suspended in *ca.* 5 ml of benzene. The prepared column was wet completely with benzene (*ca.* 20 ml) and the extract was transferred to the column. The flask containing the extract was thoroughly rinsed four or five times with benzene (*ca.* 25 ml total) and each rinse was added to the column only after the previous portion had passed into the column and in such a way as to transfer most of the undissolved residue to the column. The sides of the column were rinsed down with a small portion of benzene and it was eluted with the remainder of a total of 100 ml of benzene used for transfer, rinsing and elution. The benzene fraction was discarded. Slow elution may occur and can usually be rectified by gently disturbing the surface of the Florisil immediately below the sodium sulfate layer with a suitable glass rod. The column was then eluted with a total of 200 ml of benzene–acetone (90:10) using the first 25 ml of this solvent to rinse the flask originally containing the extract before adding it to the column. The total eluate was evaporated to less than 10 ml on a rotary evaporator under vacuum and made up to 10 ml with benzene.

In addition, the Florisil eluate of turnip was solvent-exchanged to benzene to remove all the acetone and applied to a second column containing 15 g of silica gel in place of Florisil. This column was eluted with 100 ml of benzene–acetone (90:10) which removed both CF and COCF followed by 100 ml of benzene–acetone (80:20) which contained the HOCF. Each fraction was made up in 10 ml of benzene as above.

Recoveries from the column were determined for 0.5 μg of each carbamate added both directly to the column in benzene (Table I, step 2A) as well as to control crop extracts immediately prior to their being placed on the column (step 2B). Preliminary work on the column chromatography using 100 μg of materials was monitored by reversed-phase HPLC. At this level more than 50% of the three phenols were eluted from Florisil with the 200 ml of benzene–acetone (90:10) required to elute the parent carbamates.

Derivatization

The procedure was essentially the room-temperature pyridine-catalyzed heptafluorobutyrylation described previously[1]. Duplicate 1-ml samples of the column-chromatographed crop extracts (concentration equivalent to 5 g/ml) were placed in 2-ml screw-cap vials and treated with 8 μl of pyridine and 30 μl of HFBA. The vials were capped with PTFE-faced silicone rubber septa, shaken vigorously and allowed to stand at room temperature for at least 15 h. The efficiency of derivatization was checked by comparing the response from solutions of 0.05 μg/ml of the carbamates in a solution of column-chromatographed control crop extract equivalent to a crop concentration of 5 g/ml with the same concentrations of standard carbamate in pure benzene (Table I, step 3). In preliminary experiments it was determined that 30 μl of HFBA was sufficient to derivatize completely the carbamates present in these extracts.

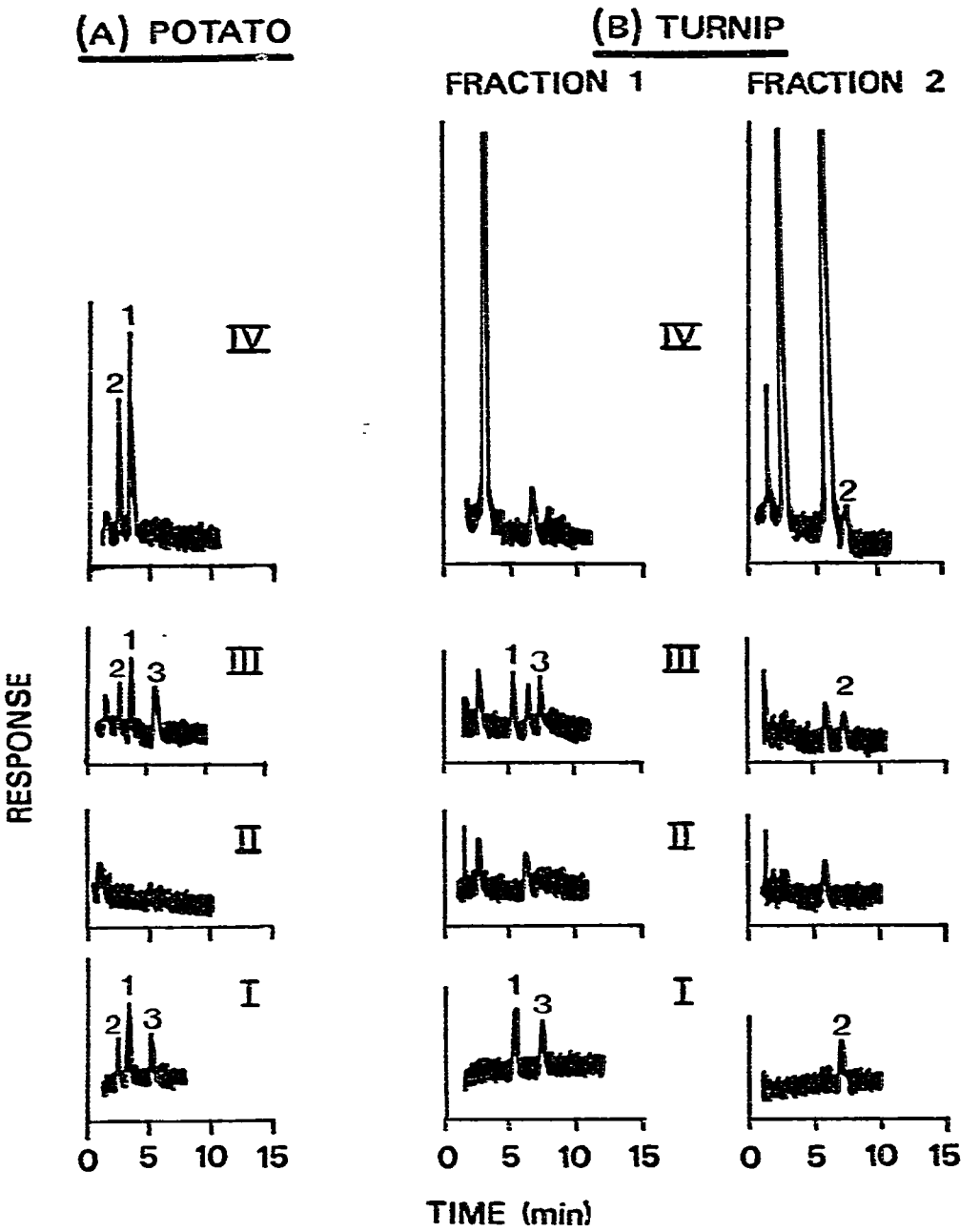

Fig. 1. Selected-ion chromatograms (mass 228) of CF-HFB (1), HOCF–DiHFB (2) and COCF–HFB (3) and unknown crop components on OV-17 (A) and OV-1 (B). I, Standards, 50 pg. II, Control crops after cleanup, 5 g/ml, 1 μl injected. III, Same as II but fortified with carbamates at 0.01 ppm at blending. IV, Field-treated crops, 5 g/ml, 1 μl injected.

GC–MS analysis

Distilled water (0.5 ml) was added to the vial containing the derivatized sample, the re-capped vial was shaken vigorously and the phases allowed to separate. Standards, normally derivatized with 10 μl/ml of HFBA were washed once, but for crop extracts the aqueous phase was discarded and the washing repeated twice more. The benzene phase was immediately subjected to GC–MS–SIM analysis. Typical SIM-generated chromatograms are illustrated in Fig. 1. Samples were usually derivatized in duplicate and at least three 1-μl aliquots from each vial were analyzed against suitable concentrations of external standards with conventional statistical precautions observed throughout. Samples obtained and analyzed as described contained no interferences at the retention times of the three carbamate–HFB derivatives. Total-method recoveries were determined at the 0.01 ppm level by the addition of 1 μg of each of the carbamates in a total of 1 ml of acetonitrile during the initial blending of control crops with water. Data are included in Table I.

RESULTS AND DISCUSSION

Gas chromatography: columns and conditions

In our initial work, the separation of the HFB derivatives of COCF and HOCF on OV-1 was adequate for analysis. Subsequent columns prepared with this liquid phase were not as efficient and these two derivatives were not separated using the initial conditions. Columns packed with 3% OV-17 gave good resolution of the three derivatives at 160 °C and are now used routinely. Use of OV-1 may be advantageous in some cases, *e.g.* turnip (see below), but our column now requires prior separation of HOCF and COCF.

With continued use of the GC–MS method for carbofuran analysis, we have become aware of a slight but cumulative decrease in sensitivity and increase in background noise with each sample analyzed even for water-washed samples. Heating the column at 250 °C for 5 min between isothermal analyses while diverting the effluent from the MS solved this problem and reduced the MS ionizer maintenance by 75%.

Mass spectrometry: SIM calibration

The SIM cannot be tuned conveniently on the sample itself because of the very low levels being measured. Permanent background peaks at *m/e* 227 and 229, previously used for tuning, were found to have considerable mass defect; the 228 ion-fragment used for quantitating the N-methylcarbamate–HFB derivatives more closely resembles the fully-fluorinated ions provided by FC-43 in this respect. Thus, use of the latter compound for direct calibration of the SIM has provided better sensitivity.

Extraction

The method described incorporates the results of a study of the extraction of carbofuran and its metabolites[5]. Salt-saturation of the aqueous phase permits essentially quantitative extraction (see Table I, step 1) of all three carbamates at a level equivalent to 0.01 ppm with 400 ml of chloroform. This represents a sizable saving in organic solvent from the original procedure[2]. Recoveries of HOCF and COCF after 1 h acid digestion in the absence of crop material were high but only about 80% of the carbofuran could be recovered (see Table I, step 4). We are currently investigating this loss. Extraction efficiency is not adversely affected by the presence

of crop material as recoveries from the total procedure were not appreciably different from those observed in the other steps (see Table I, step 5).

Derivatization, clean-up and derivative stability

In our initial work we had successfully derivatized concentrations of 0.01 to 10 μg/ml of the three carbamates in crop extracts at concentrations equivalent to 1 g/ml with 10 μl/ml of HFBA providing analysis to *ca.* 0.02 ppm. Because of the limit in sensitivity of the detection system, increasing the analytical sensitivity for both detectability and precision must involve higher extract concentrations. Reaction of crop extracts at concentrations equivalent to 5 g/ml showed that 10 μl/ml of HFBA was insufficient to ensure derivatization of 0.01 μg/ml of the carbamates in the presence of this five-fold increase in extractives. The carbamates were completely derivatized at an HFBA concentration of 30 μl/ml but the interference from derivatized extractives was too great to permit analysis even after water-washing. Carbamate standards reacted with 30 μl/ml of HFBA were also not analyzable until the excess HFBA and associated by-products were removed by water-washing. Clearly the GC–MS analysis of crude crop extracts was limited to concentrations equivalent to *ca.* 1 g/ml and concentrations of 5 g/ml would require prior removal of a portion of the extractives but without loss of carbamates.

The clean-up of CF and its metabolites on Florisil has been used for organic solvent extracts of crops in preparation for HPLC analysis[4]. Initial column chromatography of the carbamates at the 100 μg level on our Florisil, monitored by HPLC, indicated quantitative recovery with 200 ml of benzene–acetone (90:10). This recovery was confirmed for standards at the 0.5-μg level using GC–MS–SIM (Table I, step 2A). Column-chromatographed control onion and potato extracts (concentration equivalent to 5 g/ml) were interference-free when analyzed by GC–MS–SIM on OV-17 after derivatization with 30 μl/ml of HFBA and three water washes (Fig. 1, AII). Similarly-treated turnip extract showed a greatly improved background but carbamate derivative interferences were still observed on OV-17 or OV-1. Chromatography of standards on silica gel, monitored by HPLC showed that CF and COCF could be easily separated from HOCF by eluting with benzene–acetone (90:10 and 80:20, respectively). The quantitative recovery at the 100-μg level was again confirmed at the 0.5-μg level by GC–MS–SIM. The two fractions from the chromatography of the Florisil eluate of turnip extract on silica gel (concentration equivalent to 5 g/ml) were interference-free when analyzed by GC–MS–SIM on OV-1 following derivatization and water-washing (see Fig. 1, BII). The separation of HOCF from CF and COCF on Florisil as reported by Lawrence and Leduc[4] and the effectiveness of a single chromatography of crude turnip extract on silica gel were not examined as the number of analyses required did not justify the work involved.

Derivatization efficiency was checked in these cleaned-up extracts (concentration equivalent to 5 g/ml) by fortifying control extracts after chromatography at 0.05 μg/ml of all three carbamates and reacting with 10–30 μl/ml of HFBA. Good conversion to the HFB derivatives was observed for only the highest concentration of HFBA (Table I, step 3). Column recoveries in the presence of crop extracts were also checked by fortifying crude control extracts immediately prior to chromatography. Except for HOCF in turnip, the presence of crop extractives did not adversely affect the recovery (Table I, step 2B).

Because of the known hydrolytic instability of HFB derivatives of these carbamates, we had hoped that the GC–MS–SIM would be capable of analyzing derivatized samples without water-washing out the excess reagent and by-products and thereby eliminate any variability from the source. In fact most samples treated with HFBA at 10 μl/ml could be analyzed for the carbamates without water-washing[1]. As indicated above 0.05 μg/ml of carbamate standards treated with HFBA at concentrations of 30 μl/ml were not readily analyzable without water-washing and the analysis of unwashed crop extracts was impossible even after column chromatography. With water-washing a requirement for the increased amounts of HFBA required for increased crop extract concentrations, the rate of hydrolytic degradation of the derivatives is of major importance. We previously recommended the water-washed samples be analyzed within 24 h, but at carbamate concentrations of 0.05 μg/ml degradation is detectable in 2–3 h for samples in water-saturated benzene (obtained from water-washing the derivatization reaction mixture). This instability necessitates the derivatization of multiple samples to permit replicate analysis of water-washed mixtures over periods greater than 3 h. Derivatized samples kept at room temperature and dry are usable for at least two weeks.

Residue analysis by GC–MS–SIM

The recovery of the carbamates at the 0.01-ppm level added to the blended crop and subjected to acid-digestion, extraction, column chromatography and derivatization are adequate for residue analysis (see Table I). The lower values for CF clearly reflect the same loss on acid digestion as was encountered in the absence of plant material while the poor recovery of HOCF from turnip reflects the unexplained loss on silica gel chromatography in the presence of the extractives. Other anomalies in the recovery data (*e.g.* of COCF in potato) are smaller or appear to be random and attributable to normal experimental variation. SIM-generated chromatograms for the fortified control crops are shown in Fig. 1, III. The chromatograms shown in Fig. 1, IV are typical of field-treated crops with 0.03 ppm of CF and HOCF present in potato and a trace ($<$ 0.01 ppm) of HOCF present in turnips. The variations in intensity of the non-interfering components in turnip shown (compare Fig. 1, BII and IV) represent the lower and upper limits we have observed. The method readily allows analysis down to 0.01 ppm and detection of 1–2 ppb* in samples representing 5 mg of crop (1-μl injections of crop extracts at concentration equivalent to 5 g/ml).

Attempted analysis by GC–ECD and HPLC

The analyses of HFBA-derivatized crude crop extracts by GC–MS–SIM described previously was impossible by GC–ECD detection because of massive interference. Because there was a possibility that the cleaned-up extracts of onion, potato and turnip described here might be analyzable by this simpler technique, the HFBA-derivatized extracts were examined by GC–ECD after water-washing. Analysis was still impossible because of interference. A typical example is shown in Fig. 2.

The physical properties of these carbamates make HPLC with UV detection seem an attractive technique for direct analysis. The separation of CF and HOCF on both adsorption and reversed-phase HPLC has been described by Sparacino and Hines[3] and the adsorption mode has been used by Lawrence and Leduc[4] for the

* The American billion (10^9) is meant here.

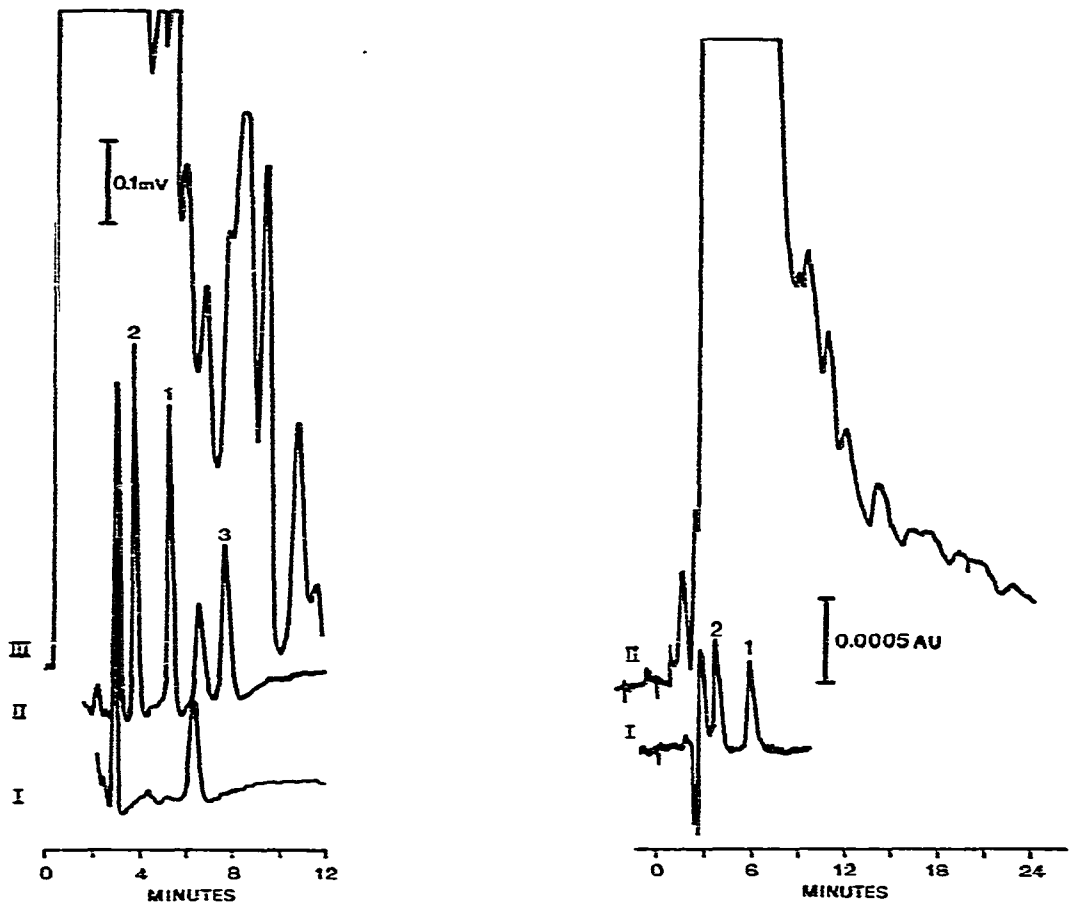

Fig. 2. Electron-capture generated chromatograms of CF–HFB (1), HOCF–DiHFB (2), COCF–HFB (3) and unknown components on OV-17 (att. $1.6 \cdot 10^{-9}$ A/mV). I, Derivatization reaction blank. II, Standards, 100 pg. III, Control potato after cleanup, 5 g/ml, 2 μl injected.

Fig. 3. Reversed-phase HPLC of CF(1), HOCF (2) and unknown crop components (acetonitrile–water (50:50), UV detector 280 nm, att. 0.005 AUFS). I, Standards 20 ng. II, Control turnip after cleanup, fraction 1, 5 g/ml, 20 μl injected.

analyses of CF, HOCF and COCF in the organic solvent extracts of seven crops to 0.1 ppm in favorable cases. Neither paper discusses the chromatographic properties of the corresponding phenols or their potential for interference in the analysis of the carbamates, although the former describes the separation of carbaryl and its corresponding phenol, α-naphthol, in the adsorption mode and their data for reversed phase imply that these components would be more difficult to separate in that mode. Preliminary work has shown that the Florisil chromatography used for clean-up would not separate the phenols and the carbamates. Since the GC–MS–SIM of HFB derivatives was used to avoid interference from the phenolic metabolites, as may be experienced in the analyses of carbamates as derivatives of the corresponding phenols, we believe that any alternative method should also have this advantage.

The chromatographic behaviour of CF, HOCF, COCF and the corresponding phenols was examined on both adsorption and reversed-phase HPLC. On reversed

phase all six components were well separated using acetonitrile–water (25:75), but the retention time of CF (24 min) was too long to produce useful sensitivity for residue analysis. Increasing the proportion of acetonitrile in the solvent reduced the retention of all components and likewise the separation between the carbamates and phenols. At acetonitrile–water (50:50) HOCF eluted in 4 min and CF in 6 min but they were not separated from their respective phenols. At maximum sensitivity (0.005 AUFS) *ca.* 5 ng of CF could be detected (Fig. 3). Column-chromatographed control crop extracts, solvent-exchanged from acetone–benzene to acetonitrile under conditions that were known to remove all benzene and acetone, were examined and found to be non-analyzable because of interference. The chromatogram of turnip shown in Fig. 3 contained the least interference of the three crops. Analysis on reversed-phase was not examined further.

On adsorption HPLC using hexane–isopropanol (95:5) the phenols were also separated from the carbamates. The lack of separation of HOCF and COCF is not a great problem as they can be separated by suitable column chromatography or distinguished by detection at different wavelengths. The HOCF phenol elutes close to these components but the peak is so skewed under these conditions that the amounts likely to be encountered in residue analysis would probably be undetectable. Column-chromatographed control crops analyzed in the adsorption mode showed interferences for carbofuran or HOCF except for the second fraction from turnip. Both onion and potato contained late-eluting components which would severely restrict the number of analyses that could be done unless gradient elution were used. Turnip extracts were free of the late eluters, perhaps a result of the additional clean-up on silica gel. The elution times of the carbamates under these conditions were not the most suitable for residue analysis. Typical chromatograms are shown in Fig. 4. The conditions required to produce a minimum retention time, so as to maximize sensitivity, and still provide for separation of the carbamates from the phenols were not examined as such conditions would clearly have the same effect as was observed for reversed-phase. In general, the degree of clean-up which will permit analyses to 0.01 ppm by GC–MS–SIM is not sufficient for analyses by either GC–ECD or HPLC.

CONCLUSIONS

Prior liquid–solid chromatography and HFBA concentrations of 30 μl/ml are required for the complete derivatization of carbofuran and its carbamate metabolites in crop extracts at concentrations of 5 g/ml. The column chromatography is also effective in removing extractives which interfere with the GC–MS–SIM analyses when converted to HFB derivatives. The required higher concentration of HFBA necessitates that excess reagent and by-products be removed by water-washing before analysis. The HFB derivatives at the 0.05-μg/ml level are sufficiently stable in water-saturated benzene at room temperature to permit use for analysis up to 3 h after water-washing and the derivatization of replicate samples is necessary if repeat analyses extending over greater periods are required. The use of FC-43 to tune the SIM and the elution of sensitivity-depleting impurities from the GC column at 250 °C between analyses improved the performance of the GC–MS–SIM system. Using these procedures, routine interference-free analyses of 0.01 ppm of carbofuran and its carbamate metabolites in crops at extract concentrations of only 5 g/ml are possible. Less capital-intensive techniques such as GC–ECD and HPLC were not generally useful at the level of cleanup achieved. Care should be used in the choice of

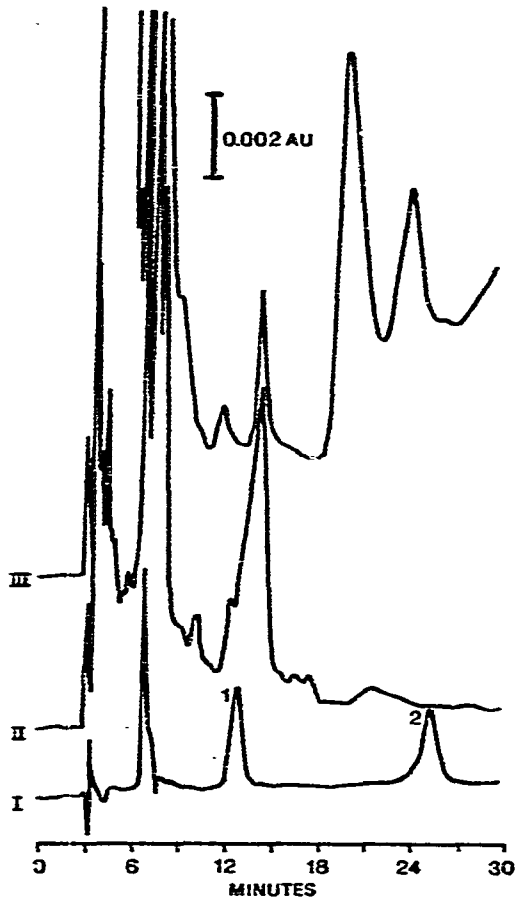

Fig. 4. Adsorption HPLC of CF(1), HOCF(2) and unknown crop components (hexane–isopropanol (95:5), UV detector 280 nm, att. 0.02 AUFS). I, Standards, 200 ng. II, Control turnip after cleanup, fraction 1, 5 g/ml, 20 μl injected. III, Control onion after cleanup, 5 g/ml, 20 μl injected.

conditions for the analyses of carbofuran and its carbamate metabolites by HPLC, because of potential interference from the corresponding phenols.

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