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DETECTION OF CARBOFURAN AND METABOLITES DIRECTLY OR AS THEIR HEPTAFLUOROBUTYRYL DERIVATIVES USING GAS–LIQUID OR HIGH-PRESSURE LIQUID CHROMATOGRAPHY WITH DIFFERENT DETECTORS

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SUMMARY

The gas chromatography of the heptafluorobutyryl derivatives of carbofuran, 3-ketocarbofuran and 3-hydroxycarbofuran is examined with electrolytic conductivity detection (halogen mode). The reaction consists of heating the compounds with heptafluorobutyric anhydride in the presence of trimethylamine catalyst. Although as little as 200 pg of carbofuran, 400 pg of 3-ketocarbofuran and 100 pg of 3-hydroxycarbofuran can be detected by electrolytic conductivity, the minimum detectable quantities by electron capture are about 20-fold less for each derivative. Application to the analysis of carbofuran and 3-ketocarbofuran in corn, potato, turnip and wheat is carried out. This technique is compared to direct gas chromatography in the nitrogen mode and to direct high-pressure liquid chromatography with UV detection at 254 nm using extracts of field-treated turnips.

INTRODUCTION

There are several methods described in the literature for the analysis of carbofuran and its metabolites, 3-hydroxycarbofuran and 3-ketocarbofuran. Cook *et al.*[1] reported on the direct analysis of the first two in corn by microcoulometric gas–liquid chromatography (GLC) which was capable of detecting quantities as low as 0.025 ppm. Williams and Brown[2] reported on the direct analysis of the same two compounds in small fruits by GLC with Coulson electrolytic conductivity detection. They found that 0.2 ppm could be analysed. Several procedures which utilize chemical derivatives have also been reported. Butler and McDonough[3] developed a method for the determination of carbofuran and its two metabolites in tomatoes, potatoes, cucumbers, lettuce and soil. The method involved hydrolysis of the carbamate linkages to form the free phenols which were then treated with trichloroacetyl chloride and determined as the corresponding trichloroacetates by GLC with electron capture detection (ECD). They reported a sensitivity of about 0.04 ppm for 3-hydroxycarbofuran and about 0.01 ppm for carbofuran and 3-ketocarbofuran. Wong and Fisher[4] reported on the

analysis of carbofuran and its metabolites in animal tissue by direct trifluoroacetylation and GLC–ECD. The reaction procedure was based on work described earlier for trifluoroacetylation of methylcarbamate insecticides[5,6]. The derivatives were sensitive enough to be detected in sub-nanogram quantities.

We have found the direct GLC of carbofuran and its metabolites difficult to carry out quantitatively when low (>20 ng) nanogram levels are determined. Thus, the present work describes an investigation into the detection of carbofuran and its two major metabolites in corn, potato, turnip and wheat by GLC with electrolytic conductivity detection (ElCD; halogen mode) of their heptafluorobutyl derivatives. In addition, a comparative study of the direct analysis of these compounds by high-pressure liquid chromatography (HPLC) with a UV detector (254 nm) and GLC–ElCD in the nitrogen mode was made, using as a sample the extracts from carbofuran field-treated turnips.

EXPERIMENTAL

Apparatus

A Microtek MT 220 gas chromatograph was equipped with a Coulson electrolytic conductivity detector in the halogen mode. The column consisted of a 120 cm × 4 mm I.D. glass U-tube packed with 3% OV-1 or 3% OV-17 on Chromosorb W HP (80–100 mesh). Operating conditions were: helium carrier and sweep, 50 ml/min; hydrogen, 50 ml/min; transfer unit, 210°; pyrolysis furnace, 820°; d.c. bridge potential, 30 V. A 0.004 in. diameter stainless-steel wire was inserted into the capillary water entrance to the mixing chamber[7]. This improved detector sensitivity 3-fold.

The HPLC system consisted of a Waters Assoc. Model 6000A pump and a Model 440 UV detector (254 nm) connected to a 1.0-mV recorder. The column was 25 cm × 2.8 mm I.D. stainless steel, slurry-packed with LiChrosorb SI-60 (5 μm). The mobile phase was 5% isopropanol in isooctane. Samples were injected via a modified sample loop injector which allows for syringe injection at ambient pressure[8].

For GLC analysis in the nitrogen mode, the nickel wire catalyst was inserted into the quartz tube followed by a 2-cm plug of strontium hydroxide-coated glass wool. The ion-exchange column was replaced with one containing mixed H/OH resin (bottom 2/3) and strong cation-exchange resin (top 1/3). All other conditions were unchanged.

A Hewlett-Packard Model 5713A with ^{63}Ni detector operated at 250° was used for ECD of the derivatives. The column was 4 ft. × 4 mm I.D., 3% OV-17 on Chromosorb W HP (80–100 mesh) at a carrier gas (argon–methane, 95:5) flow-rate of 40 ml/min and a temperature of 160°.

Reagents

Stock solutions of carbofuran (2,3-dihydro-2,2-dimethylbenzofuran-7-yl-N-methylcarbamate), 3-hydroxycarbofuran (2,3-dihydro-2,2-dimethyl-3-hydroxybenzofuran-7-yl-N-methylcarbamate) and 3-ketocarbofuran (2,3-dihydro-2,2-dimethyl-3-ketobenzofuran-7-yl-N-methylcarbamate) were prepared in acetone (1 mg/ml). Working solutions were prepared by dilution with acetone. All organic solvents were glass-distilled residue-free grade. The heptafluorobutyric anhydride (HFBA) was used as received (P.C.R., Gainesville, Fla., U.S.A.). The trimethylamine (TMA) solution was

prepared by adding cooled (0°) ampoules of anhydrous trimethylamine (Eastman) to cool tared benzene to produce a molarity of 1.0. An aliquot of the solution was diluted to 0.025 *M* in a 250-ml volumetric flask. This solution was stable for at least 4 months.

Derivatization

An 80-μl volume of HFBA was added to a 20-ml test tube with a PTFE-lined screw cap containing the insecticide residue. Following this, 2.0 ml of TMA solution were added. The tube was capped, gently shaken and heated for 60 min at 70°. The cap was removed and the contents washed with 3 × 10 ml distilled water to remove excess reagent. An aliquot of the benzene layer was used for GLC. For storage purposes the benzene layer was removed and dried with anhydrous sodium sulfate. The derivatives were stable for about 2 weeks when refrigerated. Although no melting or boiling points were taken, the heptafluorobutyryl (HFB) derivatives appear to have similar physical characteristics as other HFB derivatives.

Sample analysis

Carbofuran an 3-ketocarbofuran were extracted by blending 35 g of spiked (0.1–1.0 ppm) food with 100 ml of acetone in a Sorvall homogenizer for 4 min. The homogenate was suction-filtered through a 150-ml medium-porosity sintered glass funnel. The filtrate was transferred to a 500-ml separatory funnel containing 200 ml of petroleum ether–methylene chloride (1:1). The contents were shaken and the lower (aqueous) layer was drawn off into a 250-ml separatory funnel containing 15 ml saturated sodium chloride and extracted with 2 × 70 ml of methylene chloride. The organic extracts from both funnels were combined and dried over 5 g anhydrous sodium sulfate for at least 10 min. The solution was then filtered through a 150-ml medium-porosity sintered glass funnel into a 1000-ml round-bottom flask. 1.0 ml of 1% OV-17 in hexane was added as a keeper. The contents were reduced just to dryness at 30° by rotary vacuum evaporation. The residue was transferred with hexane to the top of a 12 × 2 cm I.D. 2% deactivated Florisil column. The column was then washed with 50 ml 30% methylene chloride in hexane. This fraction was discarded. The carbofuran and 3-ketocarbofuran were eluted with 100 ml acetone–hexane (3:20). Keeper solution (1.0 ml) was added and the fraction evaporated just to dryness at 30° by rotary vacuum evaporation. The residue was transferred to a 20-ml screw-cap test tube with a small volume of acetone and reduced to dryness at room temperature under a stream of nitrogen. The contents were then treated as described in the derivatization procedure. For direct analysis by GLC in the nitrogen mode or by HPLC, the residue was dissolved in isooctane and not carried through the heptafluorobutyrylation.

The extraction of samples for 3-hydroxycarbofuran was carried out as described by Williams and Brown[2]. However, blank samples were used and the final column eluate was reduced to dryness, spiked at 0.1 ppm with 3-hydroxycarbofuran and derivatized as described above.

RESULTS AND DISCUSSION

The use of TMA significantly increased the reaction rate of HFBA with the carbamates, without creating interferences, as compared to the direct reaction. A

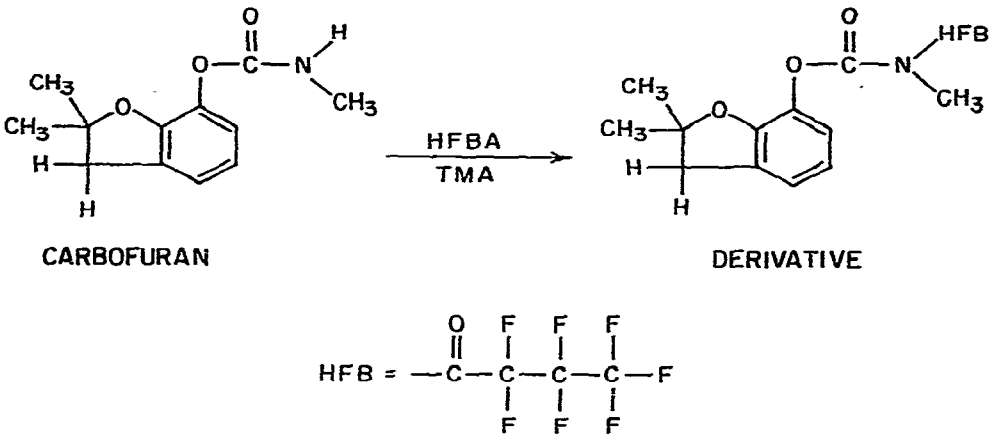

Fig. 1. Reaction scheme for the formation of the HFB derivative of carbofuran.

reaction scheme for the formation of the products is shown in Fig. 1. The reaction went equally well within the range of 5–100 μg of carbamate without changes in reagent quantity. Fig. 2 shows a chromatogram of a mixture of the products of the three compounds studied with ElCD. The 3-hydroxycarbofuran produced a response twice that of carbofuran. This was not surprising since the former has twice the number of fluorine atoms due to the additional attachment of an HFB moiety at the 3-OH position. The 3-ketocarbofuran derivative was less sensitive than carbofuran

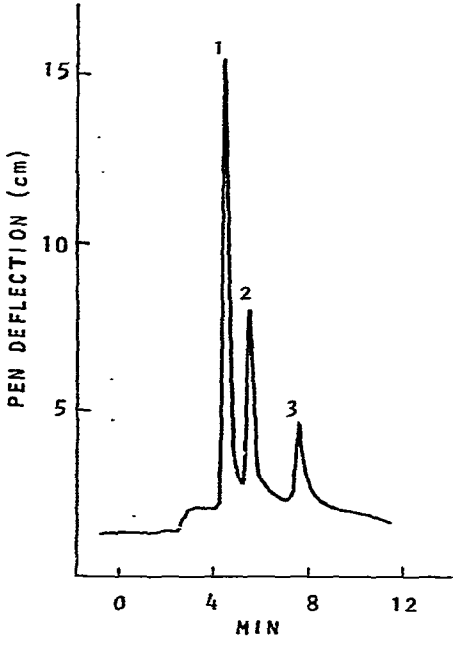

Fig. 2. Gas chromatogram of the HFBA derivatives of (1) 3-hydroxycarbofuran, (2) carbofuran, and (3) 3-ketocarbofuran. Column, 3% OV-17 at 160°; 4 ng of each; attenuation, 16×.

although theoretically, the results should be similar. It is possible that the presence of the keto group makes the derivative less stable to GLC. The quantity of pesticide or metabolite required to produce a 12-cm peak on 4 × attenuation was about 3 ng for carbofuran, 1.5 ng for 3-hydroxycarbofuran and 6.4 ng for 3-ketocarbofuran. Calibration curves showed a greater than linear increase with an increase in nanograms injected when peak heights were used. This is the result of the tailing of the peaks which is an inherent characteristic of the Coulson detector. Peak areas provided a linear range from 2–50 ng for the derivatives.

The ECD results of the derivatives proved to be about 20-fold more sensitive than the corresponding ElCD results. The order of sensitivity was the same with 3-OH > carbofuran > 3-keto. Less than 20 pg of 3-hydroxycarbofuran produced a 2-cm peak at 16 × attenuation. The linear range of detection extended from 0.05 to 2 ng of derivative, by peak height.

The HFBA derivatives were found to be about 2–4 times more sensitive to both ECD and ElCD than their equivalent TFA derivatives (prepared by an earlier method[4]). Also the HFBA products were much more stable than the TFA derivatives and could be stored, refrigerated, for up to 2 weeks while the latter products decomposed significantly after 1–2 days. Because of these facts the HFBA products were considered more useful in terms of sensitivity and stability.

The application of the HFBA method to food samples is shown in Fig. 3 for carbofuran and 3-ketocarbofuran. It can be seen that both can be readily detected at the 0.1-ppm level. Minimum detectable levels of these two were about 0.005–0.02 ppm for carbofuran and 0.01–0.05 ppm for 3-ketocarbofuran depending upon the background interference from the crops studied. Turnip provided the worst interferences of the foods studied. It is possible that the relatively high sulfur content of this crop caused the extraneous GLC peaks since sulfur can produce a detector response in the halogen mode due to the formation of H_2S. Recoveries with the described extraction procedure ranged from 85 to 100% at the spiked levels studied.

The detection of the 3-hydroxycarbofuran derivative in the presence of turnip extract cleaned up by the Williams and Brown[2] method is illustrated in Fig. 4. Peak 1 represents the detector response to 0.4 ng in the equivalent of 4 mg of sample (0.1 ppm). Recovery studies were not carried out at this time as the extraction method has been shown earlier to recover > 80% of the residues[1–3].

Fig. 5 shows a chromatogram of the three compounds by direct HPLC at 254 nm. The HPLC sensitivity was poor for carbofuran and 3-hydroxycarbofuran but very good for 3-ketocarbofuran. It was possible to detect less than 0.02 ppm of the latter in turnip.

The direct GLC results (Coulson nitrogen mode) showed poor sensitivity compared to the derivatives. This necessitated injection of a larger quantity of sample for analysis. Also the direct GLC was not as reproducible as the derivatives and often much conditioning was required before reliable results could be obtained.

A comparison of the HFBA method to the other two was carried out for carbofuran and 3-ketocarbofuran in extracts obtained from field-treated turnips. In this experiment the method used in cleaning up the turnip extract precluded any residue of 3-hydroxycarbofuran that might be present. The extraction and clean-up were essentially that described above with the exception that 5% Florisil was used for column clean-up and the pesticide and metabolite eluted completely with methyl-

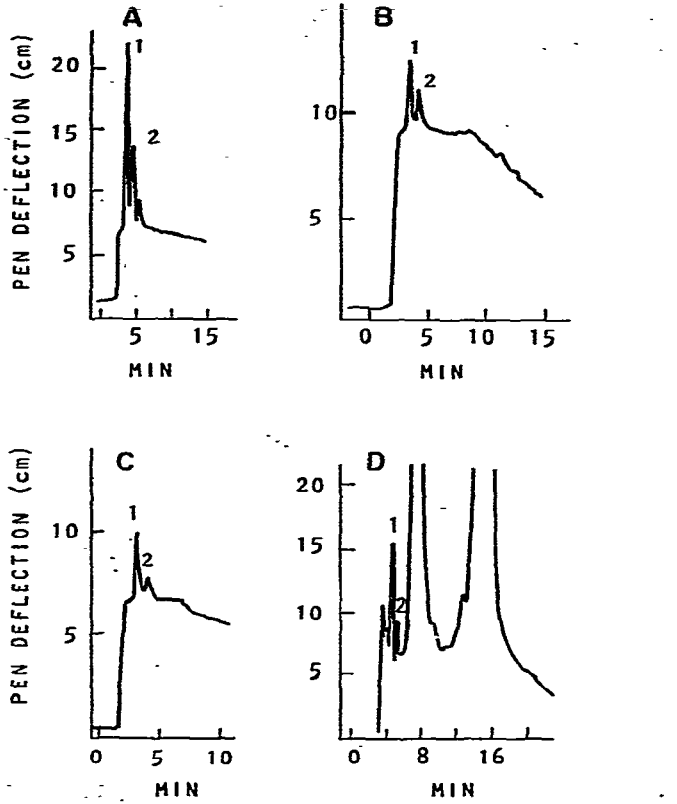

Fig. 3. Sample analysis of carbofuran (1) and 3-ketocarbofuran (2) at 0.1 ppm level. A: potato; 30 mg sample injected; attenuation, 4×. B: wheat, 14 mg sample injected; attenuation, 8×. C: corn, 20-mg sample injected; attenuation, 8×. D: turnip, 40 mg sample injected, attenuation, 8×. Column; 3% OV-1 at 160°; flow-rate, 40 ml/min.

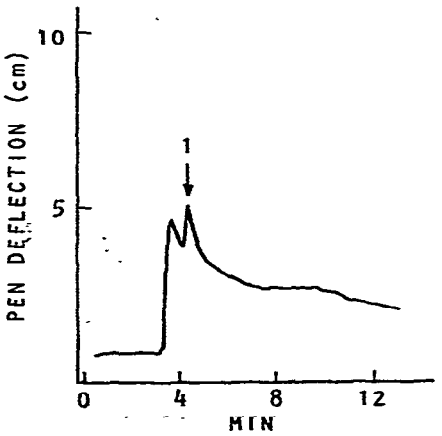

Fig. 4. Chromatogram of the HFBA derivative of 3-hydroxycarbofuran in turnip at 0.1 ppm. Column, 3% OV-1 at 160°; 4.0 mg sample injected; attenuation, 16×; 3-min vent period.

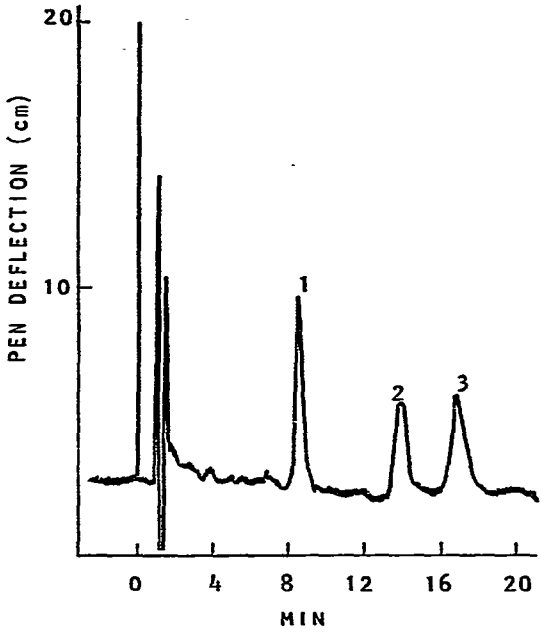

Fig. 5. HPLC chromatogram of (1) carbofuran (80 ng), (2) 3-ketocarbofuran (6.5 ng) and (3) 3-hydroxycarbofuran (90 ng). Flow-rate, 1.0 ml/min; 0.001 a.u.f.s.

ene chloride–acetonitrile–hexane (50:4:46). An aliquot of final extract was analyzed by each method. Table I compares the results. The HPLC and HFBA results were obtained 3–4 weeks after the results of the direct methods which could explain the lower levels obtained with these two, since pesticide degradation during this time was possible, although the samples were refrigerated between analyses. It can be seen that all methods could detect the high levels of carbofuran in the samples. However, 3-ketocarbofuran was not detected by direct GLC at the levels found by the other two techniques due to poor sensitivity.

TABLE I

COMPARISON OF CARBOFURAN AND 3-KETOCARBOFURAN ANALYSES IN TURNIP

Carbo = carbofuran, 3-keto = 3-ketocarbofuran; N.D. = not determined.

Sample	*Quantity found (ppm)*				
	Coulson (N-mode)	*HPLC*		*HFBA-Coulson (halogen)*	
	Carbo	*Carbo*	*3-Keto*	*Carbo*	*3-Keto*
1	1.9	1.2	0.17	2.7	N.D.
2	1.8	1.3	0.1	1.4	0.1
3	2.2	N.D.	N.D.	1.7	0.3
4	2.4	1.7	0.4	1.9	0.5
5	0.4	0.1	0.1	0.1	N.D.
6	7.2	N.D.	N.D.	4.8	0.7

Although carbofuran and its major metabolites can pass directly through the GLC after conditioning, many other methylcarbamates cannot do so reproducibly. Thus, derivative formation such as the one described herein is more generally applicable for the routine screening of this class of compounds. The reaction of HFBA with a number of carbamates for analysis by GLC–ElCD has recently appeared in the literature[9]. The direct HPLC analysis of carbamate insecticides at 254 nm is limited to those which absorb strongly in that region. This is useful for carbamates such as carbaryl, matacil, Zectran and a few others[10].

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