Journal of Chromatography, 140 (1977) 209–218

CHROM. 10,254

SIMPLIFIED METHOD FOR THE DETERMINATION OF RESIDUES OF CARBOFURAN AND ITS METABOLITES IN CROPS USING GAS–LIQUID CHROMATOGRAPHY–MASS FRAGMENTOGRAPHY

R. A. CHAPMAN and J. R. ROBINSON

Agriculture Canada Research Institute, University Sub Post Office, London, Ontario N6A 5B7 (Canada)

(Received March 10th, 1977)

SUMMARY

The gas–liquid chromatographic behaviour of 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate (carbofuran), its 3-keto-, and 3-hydroxy-derivatives, their respective phenolic hydrolysis products and the heptafluorobutyryl (HFB) derivatives of the carbamates and phenols were studied by examining the column effluent using chemical ionization mass spectrometry. In contrast to the behaviour of the carbamates, their HFB derivatives consistently produced ions having intensities proportional to the quantities injected. The common base-peak ion at 228 a.m.u. was used to quantitate these materials at the 0.02–1 ppm level in field-treated carrots, celery, tomatoes and corn with minimal sample preparation.

INTRODUCTION

The determination of traces of insecticidal carbamates is difficult and development of precise methods for the measurement of residue levels has not kept pace with those techniques available for organochlorine and organophosphorus pesticides. Recent reviews[1,2] of carbamate analysis have surveyed the use of gas–liquid chromatography (GLC) for the analysis of carbofuran (CF) and its metabolites 3-hydroxycarbofuran (HO-CF) and 3-ketocarbofuran (CO-CF) (Fig. 1). The suitability of GLC

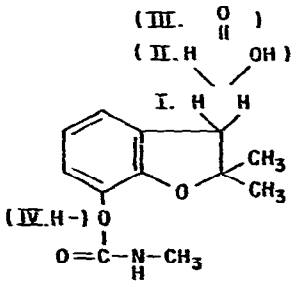

Fig. 1. Chemical structures of carbofuran (I) and its metabolites:3-Hydroxycarbofuran (II), 3-ketocarbofuran (III) and associated phenols (IV).

for the analyses of monomethyl carbamates is a matter of some controversy[3,4]. In general it appears that thermal decomposition is not a major obstacle when the amounts of CF are greater than 1 ng. At this level responses for equivalent amounts of CO-CF and HO-CF are often much smaller. To reduce thermal decomposition and/or to improve sensitivity, derivatives suitable for GLC separation and detectable with electron-capture detectors (ECD) have been prepared. The degree of analytical success attained with these derivatives has been somewhat dependent on the substrate being analyzed. For example, the analysis of the 2,4-dinitrophenyl ethers of the phenols derived from arylcarbamates appears an acceptable method for the analysis of the carbamates in soil. The carbamates are extractable from the substrate under conditions which do not extract the corresponding phenol residues which otherwise would interfere[5–7]. In the case of plant and animal material and water, the analyst is not so fortunate. More elaborate procedures have been devised[8] based on the separation of phenols utilizing their weak acidity compared to the neutral carbamate.

Unfortunately CO-CF and HO-CF are much more susceptible to hydrolysis by the alkaline reagents required to effect the separation than CF is. As a result, accurate analyses of these oxidation products are difficult or impossible to obtain using such methods. A more acceptable alternative, free of this problem, is the derivatization of the intact carbamates. Acylation[9–11] and alkylation[12,13] of the N-methylcarbamates have been reported and acylations have been more fully investigated, particularly with fluorinated carboxylic acid anhydrides which produce acylated carbamates and phenols having strong electron-capturing characteristics. We have successfully combined such procedures with GLC–ECD for the rapid analyses of HFB derivatives of CF and carbofuran phenol (CF-P) in phosphate buffers and biological media. However, interfering responses have largely precluded the use of the ECD for the analyses of these derivatives in plant and animal material except for the work reported by Wong and Fisher[14]. The polar nature of the carbamates hinders their separation from potentially interfering materials prior to derivatization, and separation subsequent to derivatization is rendered impossible by the hydrolytic instability of these derivatives. The use of an electrolytic conductivity detector in the halogen mode for the analysis of the heptafluorobutyryl (HFB) derivatives of a number of methylcarbamates has been reported recently[15]. It lacks the sensitivity attainable with the ECD and remains relatively non-specific since materials which will interfere with the analysis of many of the carbamates are also detected in the crop extracts.

The mass spectrometer appeared a potentially useful alternative to the ECD for this type of analysis. Using specific ion monitoring, sensitivities in the picogram range are attainable for many materials. The carbofuran compounds, and their HFB derivatives, contain only one nitrogen atom and possess odd-number molecular weights; in a chemical ionization mass spectrum their large, protonated molecular ions (and protonated fragments containing the carbamyl nitrogen) will appear at even mass-to-charge (*m/e*) values. Their phenols, having lost the nitrogen atom, will appear at odd *m/e* values together with most of the potentially interfering materials of vegetable origin which, fortuitously, present a very low background at even *m/e* values above 200 a.m.u. This provides the basis for a more specific detection system, with very high sensitivity, for derivatives of intact carbamates.

We wish to report the development of a simple method for the analysis of

carbofuran and its two common oxidation products based on the determination of HFB derivatives by chemical ionization (CI) mass fragmentography in crude crop extracts and to record our observations on the GLC and mass spectrometric (MS) properties of the materials pertinent to this analysis.

MATERIALS AND METHODS

Instrumentation

A Micro-Tek 220 gas chromatograph fitted with a 120 cm × 4 mm I.D. glass column packed with 5% OV-1 on 100–200 Varaport-30 and equipped with a flame ionization detector (FID) and a ^{63}Ni electron-capture detector was used for preliminary studies on the quantitative preparation, the separation and the stability of the HFB derivatives. For experiments involving MS and subsequent residue analysis, a Finnigan 9500 gas chromatograph was fitted with a similar column and coupled directly to a Finnigan 3200 quadrupole mass spectrometer equipped with a CI source and a three-channel Promim* specific ion monitor. The most satisfactory mode of operation for our purpose was to use methane at 5–10 ml/min as the combined carrier–CI reagent gas. This flow-rate provided the optimum CI source pressure (1 Torr) and the column was operated isothermally (145°) to give efficient GLC resolution of the carbamate derivatives of interest. The major portion of the solvent was diverted from the mass spectrometer for up to 3 min following injection. Conventional CI mass spectra of the GLC eluates were recorded with a light-beam oscillograph. Mass chromatograms and fragmentograms were obtained by recording the respective ion integrator or specific ion monitor outputs with a multi-channel paper-chart recorder and peak heights were measured for analysis. All instrument operation and data extraction were performed manually.

Chemicals and crops

Analytical grade CF, CO-CF, HO-CF, CF-P were supplied by Niagara FMC, (Burlington, Ontario Canada). The 3-hydroxycarbofuran phenol (HO-CF-P) and 3-ketocarbofuran phenol (CO-CF-P) were prepared by suitable hydrolysis of the parent carbamate. Standard solutions were prepared at 100 μg/ml in benzene and diluted as required. Reagent-grade chloroform and benzene were purified and glass-distilled in our laboratory and were free of interfering responses. The heptafluorbutyric anhydride (HFBA) (Pierce, Rockford, Ill., U.S.A.) and pyridine (Fisher, Pittsburgh, Pa., U.S.A.) were used as received.

Samples of carrots, tomatoes, celery and corn were from crops grown and treated with CF at conventional levels for insect control at our field station.

Extraction

Crops were extracted by the acid digestion procedure of Cook *et al.*[16] within a day of harvest and the chloroform extracts of the hydrolysates were stored over anhydrous sodium sulfate in a freezer until analyzed. Appropriate aliquots of the chloroform extracts were solvent-exchanged to benzene in preparation for analysis.

---

* Promim is a registered trademark of The Finnigan Corporation, Sunnyvale, Calif., U.S.A.

Derivatization

Heptafluorobutyrylation was carried out by treating the carbamate or phenol (up to 100 μg), or the crop extract (equivalent to 10 g of crop) in 5–10 ml of benzene with 4 drops of pyridine and 0.1 ml of HFBA at room temperature for 15–16 h (overnight). If the reaction mixture was to be analyzed by GLC–MS without water-washing, it was convenient to dilute 1 ml of the standard at 10 × the desired final concentration, or the extract equivalent to 10 g of crop, to about 8 ml in a 10-ml volumetric. The pyridine and HFBA were then added and the reaction allowed to proceed. Before analysis the samples were made up to volume with benzene. When the reaction mixture was to be washed free of excess reagent, as was required for analysis by FID or ECD in some preliminary experiments, or to reduce background noise on a second or third specific ion-channel on the mass spectrometer, the reaction was carried out with 5 ml of an appropriate concentration of standard or crop extract in a test tube fitted with a PTFE-lined screw cap. After reaction the benzene was shaken with 5 ml of water three times. The water was removed each time after centrifugation using a disposable pipette. The washed benzene was dried with sodium sulfate before analysis. Washed samples were analyzed within 24 h to minimize changes due to hydrolysis of the derivative; unwashed samples were normally used within 5 days of preparation.

Mass spectra, chromatographic behaviour, ion selection and calibration

Mass spectra were determined between 125–650 a.m.u. on the GLC eluate from injection of 50–100 ng of CF, CO-CF and HO-CF and amounts of the CF-HFB, CO-CF-HFB and HO-CF-DIHFB equivalent to these amounts of carbamate. The intensities of the major ions were measured and normalized and pertinent data for the HFB derivatives are given in Table I. Mass fragmentograms were recorded using the equivalent of 1 ng of each of the carbamate HFB derivatives and three channels of Promim, one set at *m/e* 228 and the others at appropriate values. A composite fragmentogram is shown in Fig. 2. The intensities of the ions at 228 a.m.u. were measured for 4–7 injections of various amounts of CF-HFB and HO-CF-DiHFB

TABLE I

METHANE-CHEMICAL IONIZATION MASS SPECTRA OF THE HFB DERIVATIVES OF CARBOFURAN, 3-KETOCARBOFURAN AND 3-HYDROXYCARBOFURAN

Compound injected	*Relevant major ions (m/e)*	*Relative abundance (%)*	*Structural assignment, protonated molecular ion of*
CF-HFB (MW = 417)	418	20	CF-HFB
	361	9	CF-P-HFB
	228	100	Methylamine-HFB
	165	30	CF-P
CO-CF-HFB (MW = 431)	432	27	CO-CF-HFB
	375	14	CO-CF-P-HFB
	228	100	Methylamine-HFB
	179	43	CO-CF-P
HO-CF-DiHFB (MW = 629)	416	37	HO-CF-DiHFB − HFB Acid
	359	16	HO-CF-P-DiHFB − HFB Acid
	228	100	Methylamine-HFB
	163	42	HO-CF-P − H_2O

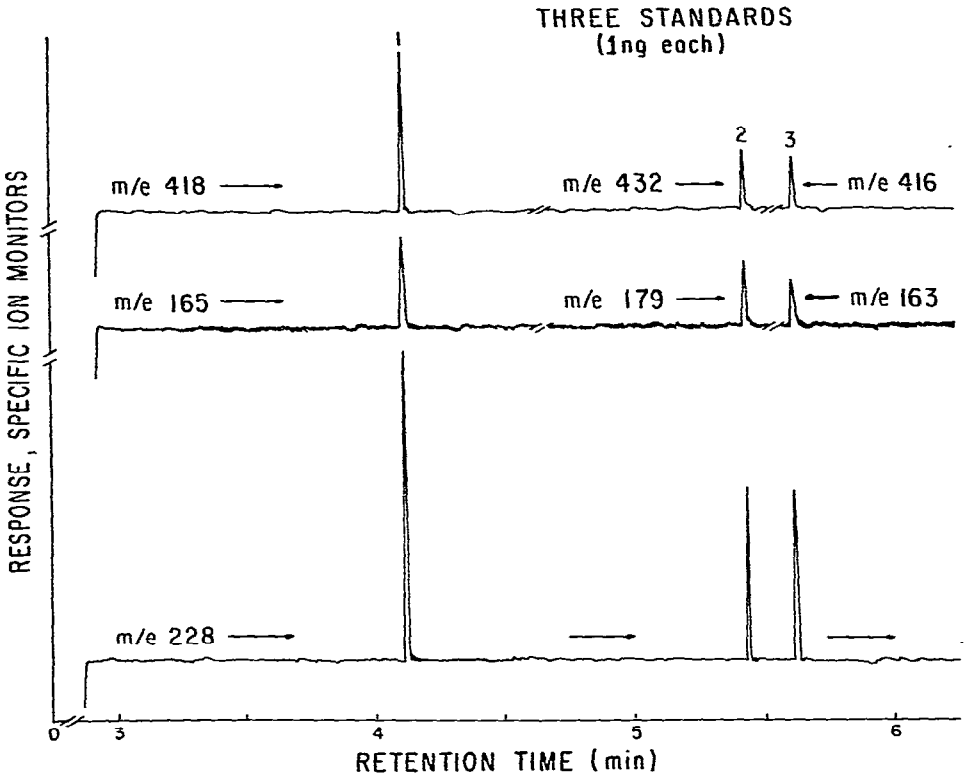

Fig. 2. Reconstituted mass fragmentogram of the HFB derivatives of carbofuran and metabolites. 1 = CF-HFB, 2 = CO-CF-HFB, 3 = HO-CF-DiHFB.

equivalent to between 10 and 1000 pg of carbamate and the results are summarized in Fig. 3. The respective coefficients of variation (CV% = 100 × standard deviation/mean), for the responses for the HFB derivatives equivalent to 10, 25, 50, 100, 250, 500 and 1000 pg of CF and HO-CF were 16.7, 8.6; 8.7, 9.7; 6.1, 5.9; 3.3, 1.6; 1.5, 0.9; 3.0, 2.8 and 0.6, 1.5. The 228-a.m.u. ion from CO-CF-HFB behaved similarly but was not as rigorously determined because preliminary analysis of the crops indicated significant amounts of this metabolite were not present. Constant (100 pg) amounts of un-derivatized CF, CO-CF and HO-CF were also injected and, at this level, only phenolic fragment ions at 165, 179 and 163 a.m.u. were observed at the carbamate retention times. The much shorter retention times for similar amounts of directly injected CF-P, CO-CF-P, HO-CF-P, and their HFB derivatives were also observed by not activating the solvent-divert valve.

Residue analysis

Samples of derivatized crop extracts (usually 1 μl and unwashed) at a concentration equivalent to 1 g/ml were injected into the chromatograph and the intensities of the ions at 228 a.m.u. coincident with the retention times of CF-HFB, CO-CF-HFB and HO-CF-DIHFB were measured. From this preliminary determination an estimate of the concentration of these components, if present, was made by comparison with the calibration responses for derivatized standards. Samples of the

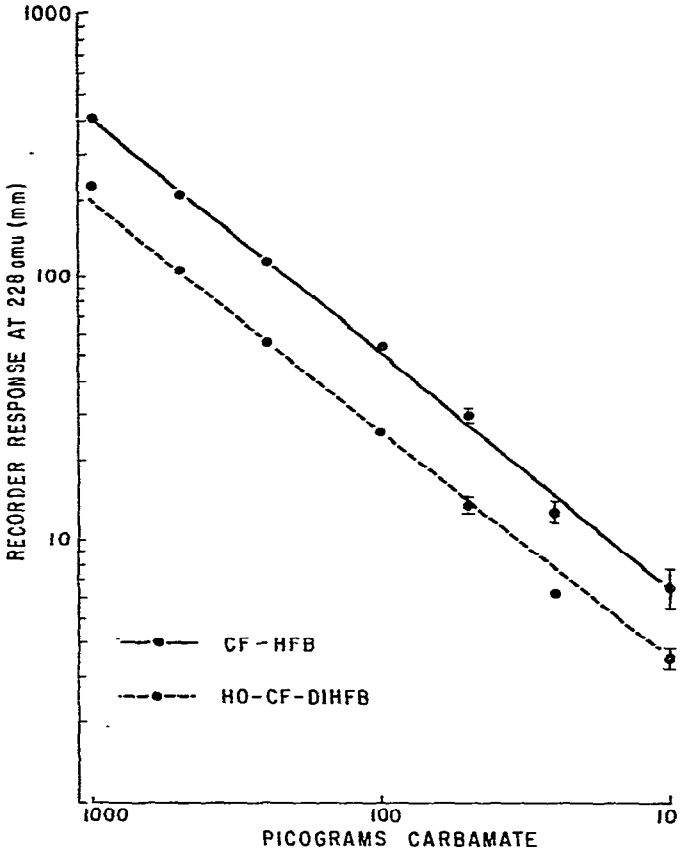

Fig. 3. Response characteristics of the *m/e* 228 ion produced by methane-CI of various quantities of CF-HFB and HO-CF-DiHFB eluted from GLC.

control extract were then fortified with the component(s) of interest at slightly above and below the estimated concentration(s) and derivatized as usual. The unknown was then re-analysed by comparison of the 228-a.m.u. intensity with that of the component(s) in the fortified control.

RESULTS AND DISCUSSION

Extraction

The acid digestion procedure of Cook *et al.*[16] was chosen as it appears to be the best available for CF and its degradation products in crops[17]. It is generally known that considerable quantities of HO-CF can be conjugated in plants[18–20] and although the toxicological significance of HO-CF in this form is not known it seems best to determine the total carbamate present rather than only the portion soluble in organic solvents. In future it may prove worthwhile to distinguish between the two forms. Although the acid digestion procedure is commonly accepted, it should be noted that in the original report the fortification recoveries of CF and HO-CF varied

from 57 to 84% and 51 to 101%, respectively. It is not clear whether the variation arises in the extraction or in the subsequent analysis. We plan to examine this aspect more carefully in future work.

Derivatization

The development of procedures for the perfluoroacylation of N-methylcarbamates has been thoroughly reviewed by Dorough and Thorstenson[2]. In our hands the procedure of Seiber[11] failed to convert CO-CF and HO-CF quantitatively to their respective mono- and di-HFB derivatives. Increasing the reaction time or the amount of acid anhydride did not improve the conversion markedly. For the procedure adopted, based on the work of Shafik *et al.*[21], the minimum time for completion of the reaction was not examined as the time allowed fitted conveniently into our work schedule and provided quantitative conversion. The amounts of reagent used were shown to be sufficient to derivatize completely at least 100 μg of each carbamate in the presence of the extractives from 10 g of each crop studied. The derivatives are relatively stable in the reaction mixture and some samples have been stored in capped hypo vials in the dark at room temperature for 2–3 weeks without showing significant changes in the concentrations of the carbamate derivatives. Changes in the concentration of the derivatives in water-washed samples were usually detectable after 2–3 days under the same conditions.

Mass spectra, chromatographic behaviour, ion selection and calibration

The mass spectra of a variety of N-methylcarbamates produced by electron impact (EI) and CI of samples introduced via the probe inlet have been reported[22,23]. Under these conditions the corresponding phenol, or protonated phenol in the case of methane-CI, is observed as the base peak. Samples of CF, CO-CF and HO-CF introduced at sub-nanogram levels into our methane-CI system *via* the GLC behaved similarly; the respective protonated molecular ions at 222, 236 and 238 a.m.u. were not seen but ions were observed at 165, 179 and 163 a.m.u. corresponding respectively to $[CF\text{-}PH]^+$, $[CO\text{-}CF\text{-}PH]^+$ and $[HO\text{-}CF\text{-}PH-18]^+$. Free phenols, injected for comparison, had much shorter retention times than the carbamates so there is no question that the signals observed were due to the fragmentation of a portion of the intact carbamate that had survived passage through the GLC column. The intensities of these ions varied erratically when constant amounts were injected and this, coupled with the fact that they are at odd *m/e* in CI–MS and would undoubtedly suffer from interference from crop extractives led us to abandon further thought of utilizing a combination of GLC of underivatized carbamates and methane CI–MS for analysis. Attempts to produce protonated molecular ions having even *m/e* values by use of a "softer" CI reagent were fully successful with isobutane but this led to a more rapid fouling of the ion source and necessitated too frequent cleaning for routine use. The stability and linearity of the intensities of these $[MH]^+$ ions from the carbamates were not examined.

Mumma and Khalifa[24] have reported that EI fragmentation of the trifluoroacetyl (TFA) derivatives of carbaryl and its metabolites produced the TFA derivative of the corresponding phenols as the major fragment and the TFA derivative of methylamine as a minor fragment. In our system, the methane-CI fragmentation of CF-HFB, CO-CF-HFB and HO-CF-DiHFB fortuitously produced the even *m/e* ion

at 228 a.m.u. corresponding to the protonated HFB derivative of methylamine (*i.e.*, protonated N-methyl heptafluorobutyramide) as the base peak for all three compounds, as well as providing additional ions (see Table I and Fig. 2) which can be used for further identification when sample size and level of interference permit. The composite mass fragmentogram shown in Fig. 2 illustrates the use of these ions for the identification of the compounds eluted. The *m/e* 228 ion response shown was recorded at the same sensitivity range for the equivalent of 1 ng of each carbamate while the other responses shown were recorded at various ranges. The intensity of the 228-a.m.u. ion was directly proportional to the amounts of CF-HFB and HO-CF-DiHFB injected over the range equivalent to 10–1000 pg of carbamate demonstrating its suitability as a basis of analysis (see Fig. 3). Predictably the larger coefficients of variation occurred at the lower levels. The data indicated that the standard error for estimating CF and HO-CF is about $\pm 10\%$ at the 25-pg level. Although the methane-CI fragmentation produces the same base-peak ion for each HFB derivative, the compounds are all sufficiently well separated by GLC (see Figs. 2 and 4), to permit accurate analysis based on the 228-a.m.u. ion signal. The method should be applicable to any N-methylcarbamate-HFB derivative. For our purposes HFBA was preferred over trifluoroacetic anhydride and pentafluoropropionic anhydride as the

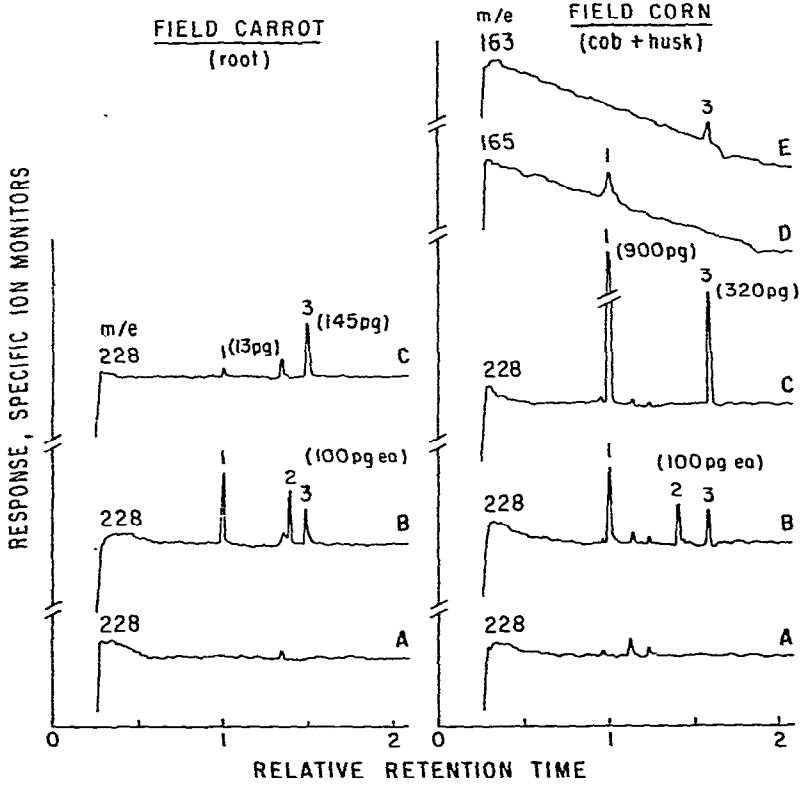

Fig. 4. Reconstituted fragmentograms of HFBA-derivatized carrot and corn extracts used for analysis. A, Extract from control samples; B, extract from crop controls fortified with 0.1 ppm (1) CF, (2) CO-CF and (3) HO-CF; C, D and E, extract from field-treated crop.

derivatizing agent because the increase in molecular weight shifts the selected ion to high a.m.u. values where potential interference is lessened.

Residue analysis

Using the 228-a.m.u. ion for analysis, no interference was observed at the retention times of CF-HFB and HO-CF-DiHFB in samples of carrot, celery, corn (kernels, leaves and stalks, cobs and husks) and tomato extracts that had been prepared for analysis as described, *i.e.* evaporation to a level equivalent to 1 g/ml and reaction with HFBA. Fig. 4 shows typical results on carrot (root) and corn (cob and husk). Each fragmentogram was produced from the equivalent of 1 mg of crop. The residue levels of CF and HO-CF in the corn were sufficiently high to permit confirmation of the components with a second channel of Promim but for the low levels in the carrot the signals on these channels could not be seen above background noise unless the derivatized extract was water-washed to reduce interference. In some cases a small interference was observed for CO-CF-HFB which would limit the minimum level of detectability for this component below 0.05 p.p.m.; no levels above this were observed in any of the extracts. Derivatized carrot, celery and corn extracts contained material(s) which eluted between 25 and 30 min and produced a 228-a.m.u. ion. This of course, does not interfere with the analysis but does lengthen the analysis time considerably. Samples were analyzed by comparison with fortified controls rather than pure standards to eliminate any possibility of the extractives interfering with the formation of the derivative(s) and invalidating the results.

The injection of the equivalent of 1–2 mg of crude crop may offend the classical residue analyst. In our work, we have not observed a deterioration in column performance due to repeated analyses of crop samples. The time saved and the accuracy of the results obtained using this method will rapidly make up for the slight inconvenience and cost of replacing the GLC column when it is required. In using samples which have not been washed free of the large excess of derivatizing reagent a column length of 90–120 cm was found optimum. Shorter columns began to suffer from lack of resolution of CO-CF-HFB and HO-CF-DiHFB while longer columns required considerable time to elute residual HFBA which appeared to alter the response characteristics of the system even when no interference was observed at *m/e* 228. The ion source of a mass spectrometer is subject to fouling with organic materials. The continual presence of relatively large amounts of methane in a CI source accentuates this problem and cleaning was required at 4–5 week intervals to maintain the signal-to-noise levels required.

CONCLUSION

A simple yet sensitive method has been developed for the analysis of carbofuran, 3-ketocarbofuran and 3-hydroxycarbofuran in carrots, celery, corn and tomatoes based on the GLC separation of the heptafluorobutyryl derivatives of the insecticides prepared in crude extracts followed by their chemical ionization to a common ion and the measurement of its intensity with a quadrupole mass spectrometer. Sensitivity is at least 0.05 ppm for all three materials.

ACKNOWLEDGEMENTS

The authors acknowledge the assistance of G. A. McFadden and H. J. Svec for maintaining and treating the crops, of H. S. Simmons and L. L. E. Westendorf for the extraction and derivatization of samples and thank N. L. Jerry for the preparation of the figures.

REFERENCES

1 E. D. Magallona, *Residue Rev.*, 56 (1975) 1.
2 H. W. Dorough and J. H. Thorstenson, *J. Chromatogr. Sci.*, 13 (1975) 212.
3 E. J. Lorah and D. D. Hemphill, *J. Ass. Offic. Anal. Chem.*, 57 (1974) 570.
4 A. Strother, *J. Gas Chromatogr.*, 6 (1968) 110.
5 J. H. Caro, H. P. Freeman, D. E. Glatfelty, B. C. Turner and W. M. Edwards, *J. Agr. Food Chem.*, 21 (1973) 1010.
6 J. H. Caro, D E. Glatfelty, H. P. Freeman and A. W. Taylor, *J. Ass. Offic. Anal. Chem.*, 56 (1973) 1319.
7 L. W. Getzin, *Environ. Entomol.*, 2 (1973) 461.
8 R. F. Cook, *Determination of Carbofuran and 3-Hydroxycarbofuran Residues in or on Rutabagas*, Research Report of Niagara Chemical Division, FMC Corp., Research and Development Dept., Middleport, N.Y., 1973.
9 L. J. Sullivan, J. M. Eldridge and J. B. Knaak, *J. Agr. Food Chem.*, 15 (1967) 927.
10 J. B. Knaak, D. M. Munger, J. F. McCarthy and L. D. Satter, *J. Agr. Food Chem.*, 18 (1970) 832.
11 J. N. Seiber, *J. Agr. Food Chem.*, 20 (1972) 443.
12 R. Greenhalgh and J. Kovacicova, *J. Agr. Food Chem.*, 23 (1975) 325.
13 J. F. Lawrence and G. F. Laver, *J. Agr. Food Chem.*, 23 (1975) 1106.
14 L. Wong and F. W. Fisher, *J. Agr. Food Chem.*, 23 (1975) 315.
15 J. F. Lawrence, *J. Chromatogr.*, 123 (1976) 287.
16 R. F. Cook, R. P. Stanovick and C. C. Cassil, *J. Agr. Food Chem.*, 17 (1969) 277.
17 C. H. van Middelem and A. J. Peplow, *J. Agr. Food Chem.*, 21 (1973) 100.
18 J. B. Knaak, D. M. Munger and J. F. McCarthy, *J. Agr. Food Chem.*, 18 (1970) 827.
19 R. L. Metcalf, T. R. Fukuto, C. Collins, K. Borck, S. A. El-Aziz, R. Munoz and C. C. Cassil. *J. Agr. Food Chem.*, 16 (1968) 300.
20 R. J. Ashworth and T. J. Sheets, *J. Agr. Food Chem.*, 20 (1972) 407.
21 M. T. Shafik, D. Bradway and P. F. Mongan, *Pesticide Paper No. 40, ACS Meeting, Boston, 1972.*
22 J. N. Damico and W. R. Benson, *J. Ass. Offic. Anal. Chem.*, 48 (1965) 344.
23 R. L. Holmstead and J. E. Casida, *J. Ass. Offic. Anal. Chem.*, 58 (1975) 541.
24 R. O. Mumma and S. Khalifa, *J. Agr. Food Chem.*, 20 (1972) 1090.