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HIGH-PRESSURE LIQUID CHROMATOGRAPHIC ANALYSIS OF CARBOFURAN AND TWO NON-CONJUGATED METABOLITES IN CROPS AS FLUORESCENT DANSYL DERIVATIVES

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SUMMARY

Carbofuran, and non-conjugated 3-hydroxycarbofuran and 3-ketocarbofuran were extracted from carrots, corn and potatoes with acetone and partitioned into hexane–methylene chloride. The organic extract was evaporated to a small volume for clean-up on a 2% deactivated Florisil column. All three carbamates were eluted with 15% acetone in hexane. The pesticide residues were hydrolysed to their corresponding phenols with 0.1 *M* sodium carbonate followed by derivatization with dansyl chloride in acetone. The derivatives were extracted and analysed by high-pressure liquid chromatography with fluorescence detection (excitation, 360 nm; emission, >400 nm). Absolute recoveries for all three compounds were between 50 and 65% for spiked samples by the extraction method used. Detection limits approached 0.01 ppm in the foods studied.

INTRODUCTION

High-pressure liquid chromatography (HPLC) has recently been shown to be useful in many cases for the trace analysis of pesticide residues in foods[1–3]. With adequate clean-up many carbamates and ureas can be directly detected in foods by UV absorption at low levels. Carbofuran and its non-conjugated metabolites, 3-hydroxycarbofuran and 3-ketocarbofuran, can be determined in foods such as carrots, corn, potatoes and wheat at 0.1 ppm[4]. While this level might be adequate for compliance purposes, a more sensitive method might be desired in certain instances such as for degradation or tissue distribution studies. We have developed a method for carbofuran and two of its non-conjugated metabolites, which is sensitive enough to detect 5–10-ppb levels in a variety of foods and can easily be used to confirm results of the direct HPLC approach. The technique is based on the formation of a fluorescent derivative of the phenolic hydrolysis products of the carbamates[5,6]. The derivatives then are determined by fluorometry after HPLC separation.

EXPERIMENTAL

Apparatus

The HPLC system consisted of a Waters Assoc. Model 6000A pump and an LDC FluoroMonitor Model 1209 fluorescence detector (with 360 nm (max) excitation and >400 nm emission filters) which was connected to a 1-mV recorder and used at attenuation 4× throughout. The chromatography column (25 cm × 2.2 mm I.D.) was slurry-packed with LiChrosorb Si 60 (5 μm). Samples were injected with a Valco 7000-p.s.i. loop injector. The mobile phase was 3% acetone in 2,2,4-trimethylpentane (TMP) for carbofuran and 3-ketocarbofuran while 15% was used for 3-hydroxycarbofuran.

Reagents

Stock solutions of carbofuran [7-(2,3-dihydro-2,2-dimethyl) benzofuranyl N-methylcarbamate], 3-hydroxycarbofuran [7-(2,3-dihydro-2,2-dimethyl-3-hydroxy) benzofuranyl N-methylcarbamate] and 3-ketocarbofuran [7-(2,3-dihydro-2,2-dimethyl-3-oxo) benzofuranyl N-methylcarbamate] were prepared in 2-propanol (1 mg/ml). Standard working solutions were prepared from this by dilution with TMP. The crops examined were carrots, corn and potatoes.

The derivatization solution was prepared by dissolving dansyl chloride (5-dimethylaminonaphthalene-1-sulfonyl chloride) in acetone (2 mg/ml). Sodium carbonate (0.1 *M*) was used to effect the hydrolysis of the pesticides and to catalyse the dansylation reaction. All organic solvents were distilled in glass, or pesticide grade.

Sample extraction and clean-up

Sample extraction and column chromatographic clean-up were carried out exactly as described earlier[4]. Briefly, 35 g of spiked food were blended with 100 ml of acetone and then suction filtered. The extract was partitioned with 200 ml of hexane–methylene chloride (1:1, v/v) followed by two extractions of the lower aqueous phase with 75 ml methylene chloride. The combined organic extracts were dried and evaporated to a small volume then cleaned up on a 2% deactivated Florisil column. Carbofuran and 3-ketocarbofuran eluted together with 55 ml of 15% acetone in hexane while 3-hydroxycarbofuran eluted later with a further 70 ml of the same solvent.

Derivative preparation

The fractions containing the carbamates were evaporated separately to about 5 ml by rotary evaporation at 30°, quantitatively transferred to individual 20-ml test tubes with PTFE-lined screw-caps, then evaporated just to dryness at room temperature under a stream of nitrogen. A 0.1-ml volume of acetone was added to dissolve the residue, followed by 0.5 ml of 0.1 *M* sodium carbonate.

The tubes were capped and incubated in a sand bath at 45° for 30 min at which time hydrolysis to the phenols was complete. The caps then were removed and 2.0 ml of acetone were added followed by 0.5 ml of dansyl chloride solution. The tubes were returned to the sand bath and heated at 45° for another 30 min to complete the derivatization. After this time, the caps were removed and the acetone evaporated from the mixture (at 45°) with a stream of nitrogen. Following this, 1 ml of hexane

was added and the mixture shaken for 30 sec. A 25-μl aliquot of the organic phase was injected into the liquid chromatograph for analysis of 3-hydroxycarbofuran and 3-ketocarbofuran. For carbofuran analysis the hexane was washed 3 times with 10 ml of 0.1 *M* HCl prior to analysis in order to remove an interfering peak.

Additional clean-up for corn

The extraction procedure as described above frequently was found to be inadequate for corn, thus an additional clean-up was carried out which made use of the weakly basic nature of the dansyl derivatives.

The hexane extract from above was washed 3 times with 5 ml 2 *M* HCl. The hexane layer was discarded. The three aqueous washings containing the dansyl derivatives were combined and neutralized with solid sodium carbonate being cautious to keep the effervescence to a minimum. After this, 5 ml of hexane were added and the mixture vigorously shaken for 1 min. When the phases had separated, the hexane was transferred to a 15-ml centrifuge tube and the aqueous phase extracted a second time with 1 ml of hexane. The combined hexane solutions were evaporated to 1 ml for HPLC analysis.

RESULTS AND DISCUSSION

Reaction

The hydrolysis and dansylation reactions proceeded very smoothly for the three carbamates. The 0.1 *M* Na_2CO_3 first hydrolysed the compounds to methylamine and a phenolic moiety. The subsequent addition of dansyl chloride converted these two products to fluorescent dansyl derivatives which were easily separable by HPLC. The phenolic moieties were chosen for the analysis since they were characteristic of each compound. Details of the reaction may be found elsewhere[5].

In the present work an interfering peak from the dansylation reaction appeared at a retention time equal to the dansyl-carbofuran derivative. While this posed no problem at levels near 1.0 ppm, it was significant at 0.1–0.01 ppm. Washing the hexane solution with 0.1 *M* HCl removed the peak without affecting the carbofuran derivative. About 5% of the 3-ketocarbofuran derivative was lost with this treatment. Both the dansyl derivatives of 3-hydroxycarbofuran (phenol) and methylamine were completely removed with the acid treatment but could be recovered upon neutralization and reextraction if desired. However, since 3-hydroxycarbofuran eluted from the Florisil in a different fraction from carbofuran, the washing in this case was not required.

The reactions proved to be reproducible from one day to the next although fresh standards were always carried through the reaction procedure along with samples. The derivatives were very stable and could be refrigerated for several weeks with little decomposition evident. This is an attractive feature of the technique for residue analysis since samples usually cannot be extracted and analysed in the same day.

Chromatography and detection

HPLC of dansyl carbamates has been carried out earlier using liquid–liquid partition or adsorption chromatography on pellicular supports such as Zipax or Corasil I[6]. We have found that adsorption chromatography on microparticulate

silica gel (LiChrosorb Si 60, 5 μm) provided complete separation of the three carbamates with a sensitivity (signal-to-noise ratio) 5–10-fold better than that reported earlier[6]. The detection limit at a 5:1 signal-to-noise ratio was about 0.5 ng for carbofuran. Attempts at reducing the background noise by the addition of an electrical RC circuit to the output terminals, or by cooling the phototube, were insuccessful. An attempt was made to increase the detector sensitivity by using a packed-cell approach described earlier for aflatoxins[7]. This resulted in a 3–5-fold increase in absolute sensitivity but the baseline became very erratic resulting in a net reduction of the signal-to-noise ratio. The packed-cell approach has excellent potential if noise can be reduced by better packing methods.

Fig. 1 shows a chromatographic separation of a reaction mixture of the three carbamate standards. The methylamine peak represents the total methylamine generated from the three compounds. For practical purposes 3% acetone in TMP was used as mobile phase for carbofuran and 3-ketocarbofuran analysis while 15% acetone in TMP was used for the 3-hydroxy derivative.

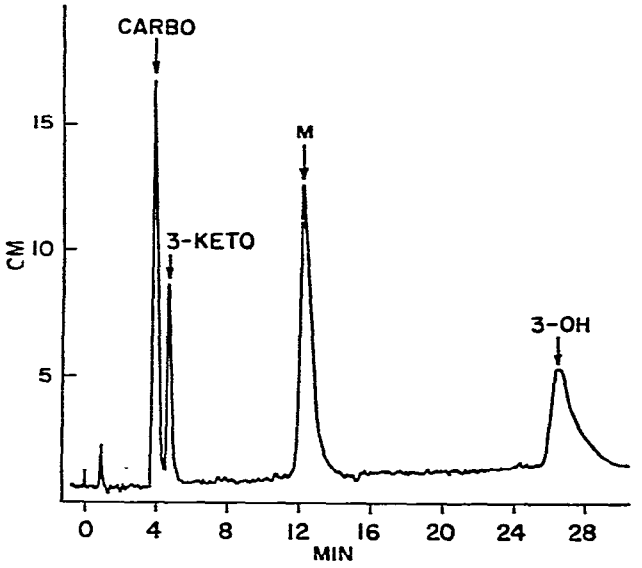

Fig. 1. Chromatogram of a reaction mixture of the three carbamates studied. 12.5 ng each of carbofuran and 3-ketocarbofuran; 25 ng 3-hydroxycarbofuran. Methylamine (M) peak represents the total amount generated from the three carbamates (50 ng carbamate). Solvent, 7% acetone in TMP; flow-rate 1.0 ml/min; attenuation, 4×.

Sample extraction and clean-up

The extraction and clean-up method was the same as that employed earlier[4] which was designed to fit into a multi-pesticide residue procedure. However, for quantitative analysis at levels below 0.1 ppm for the carbamates studied, some minor problems were encountered. Average recoveries of standards (0.5 μg each) from the 2% deactivated Florisil column were 75%, 82% and 81%, respectively, for carbofuran, 3-ketocarbofuran and 3-hydroxycarbofuran. This was the largest source of

losses of any step in the complete procedure. Although the results reported herein are for 2% deactivated Florisil, it was later found that further deactivation of the Florisil to 5% water content improved recovery to about 90% for all three compounds. The absolute recoveries were not affected by food type. Average recoveries through the method (3–12 values each) at 0.1 ppm were 54, 65 and 61%, respectively, for carbofuran, 3-ketocarbofuran and 3-hydroxycarbofuran while at 0.01 ppm they were 50, 55 and 61%, respectively. Carbofuran itself always exhibited the lowest values. The absolute recovery for standards passed through the method were 69, 75 and 80%, respectively, for the same three carbamates at levels equivalent to 0.1 ppm. This mainly reflects the observed losses on the Florisil as mentioned above. The lower levels obtained for the food samples are attributed to additional small losses because of extraction efficiency of the acetone, hydrolysis and dansylation (usually *ca.* 90% conversion to products, compared to a pure standard) and the HCl partitions when required.

Chromatographic analysis

Fig. 2 shows the results obtained for spiked (0.1 ppm) samples of potatoes, carrots and corn. Both carbofuran and 3-ketocarbofuran were easily detected at this level in all samples. The corn results are after treatment with 2 *M* HCl. This treatment resulted in very clean chromatograms and makes detection of low ppb levels feasible. Fig. 3 compares results obtained at 0.01 ppm for the three foods. The corn results are clearly superior to the other two foods in ease of interpretation. The 2 *M* HCl treatment sufficiently cleaned up the extract to permit injection of 500 mg or more of equivalent sample. This approach has definite potential for detection of 1-ppb or lower levels of these carbamates in foods. Analyses of the fraction containing 3-hydroxycarbofuran are shown in Fig. 4. Here, both the phenolic and methylamine

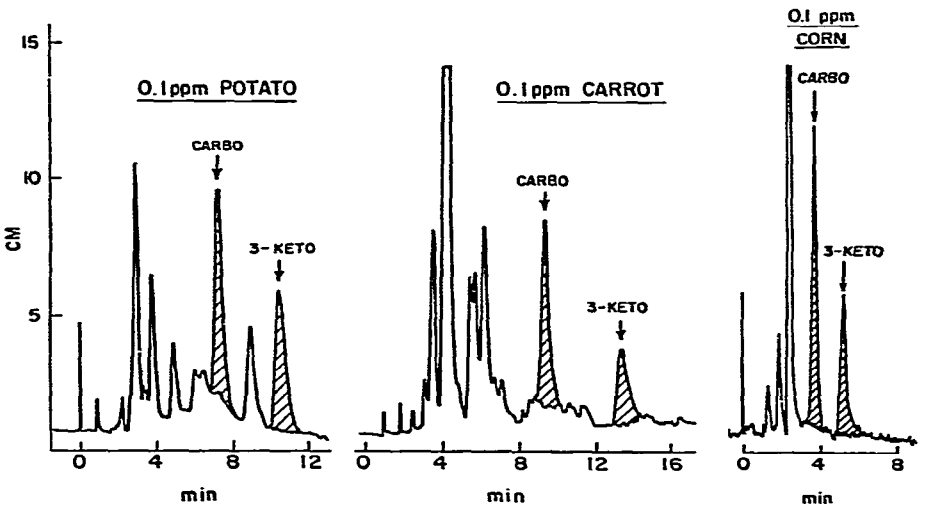

Fig. 2. Analysis of spiked (0.1 ppm) food samples. Potato, 125 mg injected; flow-rate, 1 ml/min; carrot, 125 mg injected; flow-rate, 0.8 ml/min; corn, 125 mg injected; flow-rate, 1.8 m /min. Attenuation, 4×; solvent, 3% acetone in TMP.

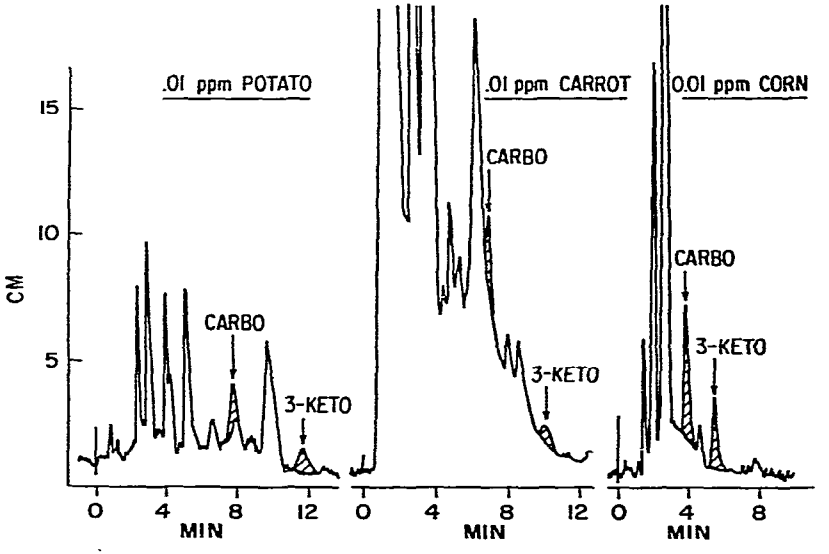

Fig. 3. Analysis of spiked (0.01 ppm) samples. Potato, 125 mg injected, 1.0 ml/min; carrot, 125 injected, 0.8 ml/min; corn, 500 mg injected, 1.8 ml/min. Attenuation, 4×; solvent, 3% acetone in TMP.

products from hydrolysis of the metabolite are detected. The peak height ratios for the two peaks were always similar to that shown in the chromatograms. No problem was encountered in the detection of this compound down to 0.01 ppm in the foods studied.

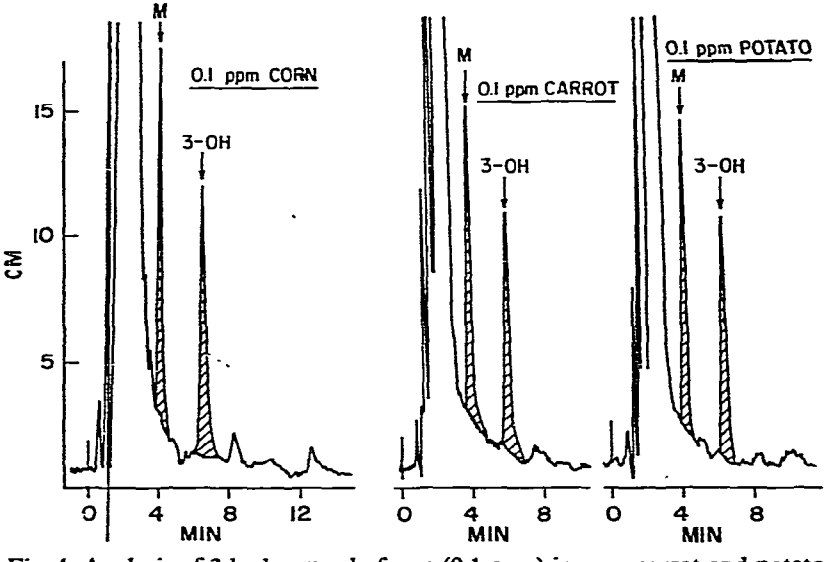

Fig. 4. Analysis of 3-hydroxycarbofuran (0.1 ppm) in corn carrot and potato, 125 mg each injected. Attenuation, 4×; solvent, 15% acetone in TMP; flow-rate, 1.0 ml/min. M = methylamine derivative.

CONCLUSION

The described method shows the potential which fluorescence derivatization has for the sensitive analysis of carbofuran and metabolites in selected foods. The approach makes use of clean-up techniques already well used by residue chemists for gas–liquid chromatographic analysis of pesticides.

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