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Note

Reversed-phase high-performance liquid chromatographic separation of carbofuran and its five metabolites

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Use of high-performance liquid chromatography (HPLC) for the determination of carbofuran residues has been described. Most reports include methods for the determination of 3-hydroxycarbofuran metabolites in plants[1–5]. Frei *et al.*[6] separated Dns derivatives of N-methylcarbamate pesticides, including carbofuran, in water and soil using a β,β'-oxydipropionitrile column. Moye and St. John[7] reported the use of a C_{18} column in the determination of carbofuran and other N-methylcarbamates. Sparacino and Hines[8] reported the separation of 25 carbamate pesticides, including 3-hydroxycarbofuran and carbofuran, using HPLC with silica, CN and NH_2 columns. Lawrence[9] reported the determination of carbamate pesticide residues, including carbofuran, in crops after separation on a silica column. Lawrence and Leduc[10] developed an analytical procedure for residues of carbofuran, 3-hydroxycarbofuran and 3-ketocarbofuran that used a silica column. We needed a method to separate carbofuran and its metabolites 3-hydroxycarbofuran, 3-hydroxy-7-phenol, 3-ketocarbofuran, 3-keto-7-phenol and 7-phenol, using HPLC. No report could be found on the use of HPLC for the separation of carbofuran and these five carbofuran metabolites.

Because of the variations in retention times and irreversible adsorption often observed with silica HPLC columns, the use of a reversed-phase column was explored. The HPLC separation of carbofuran and its five non-conjugated metabolites using a C_{18} reversed-phase column is described here.

EXPERIMENTAL

Apparatus

A liquid chromatograph (Model 1084B; Hewlett-Packard, Avondale, PA, U.S.A.) equipped with a variable-wavelength UV detector (operated at 220 nm) and a 24 cm × 4.6 mm I.D. Zorbax ODS analytical column with a 5 cm × 4.6 mm I.D. Permaphase ODS guard column (DuPont, Wilmington, DE, U.S.A.) was used.

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Reagents

Methanol and acetonitrile were liquid chromatographic quality (Burdick & Jackson, Muskegon, MI, U.S.A.). Water was filtered through a Milli-Q water purification system (Millipore, Bedford, MA, U.S.A.). The following compounds (see Fig. 1 for structures), used as reference standards, were obtained from FMC (Middleport, NY, U.S.A.): carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate, I); 7-phenol (2,3-dihydro-2,2-dimethyl-7-hydroxybenzofuran, II); 3-OH-carbofuran (2,3-dihydro-2,2-dimethyl-3-hydroxybenzofuranyl-7-methylcarbamate, III); 3-OH-7-phenol (2,3-dihydro-2,2-dimethyl-3,7-dihydroxybenzofuran, IV); 3-CO-carbofuran (2,3-dihydro-2,2-dimethyl-3-ketobenzofuranyl-7-methylcarbamate, V) and 3-CO-7-phenol (2,3-dihydro-2,2-dimethyl-3-keto-7-hydroxybenzofuran, VI). Standards were prepared in methanol or acetonitrile and were stored at −20°C when not in use.

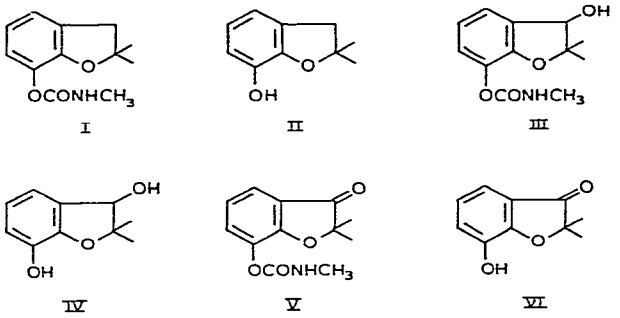

Fig. 1. Carbofuran and its five metabolites: I = carbofuran; II = 7-phenol; III = 3-OH-carbofuran; IV = 3-OH-7-phenol; V = 3-CO-carbofuran; VI = 3-CO-7-phenol.

High-performance liquid chromatography

Chromatographic parameters: column oven temperature 40°C; UV detector: sample beam wavelength 220 nm, reference beam wavelength 430 nm; mobile phase: System A (isocratic) acetonitrile–water (25:75), 2 ml/min; System B (gradient) methanol in water 40, 44, 62, 100 and 100% for retention times of 0, 8, 15, 17 and 30 min, 1.5 ml/min. Following gradient elution, the mobile phase was returned to starting conditions (methanol–water, 40:60) and the system was equilibrated for 10 min before any subsequent injections.

RESULTS AND DISCUSSION

Two solvent systems were investigated. The first was an acetonitrile–water isocratic elution (System A). Fig. 2 shows the resolution of carbofuran and its five metabolites using this system. The corresponding hydrolyzed compound eluted before its parent compound (II before I, IV before III and VI before V). Resolution of carbofuran and its metabolites was not completely satisfactory for our needs using this system because the resolution of I and II varied, especially when comparing resolution before and after flushing the column with 100% acetonitrile. Long equilibration periods were often required to return the column to optimum resolution following the acetonitrile flush. Reduction of the time required for the separation was

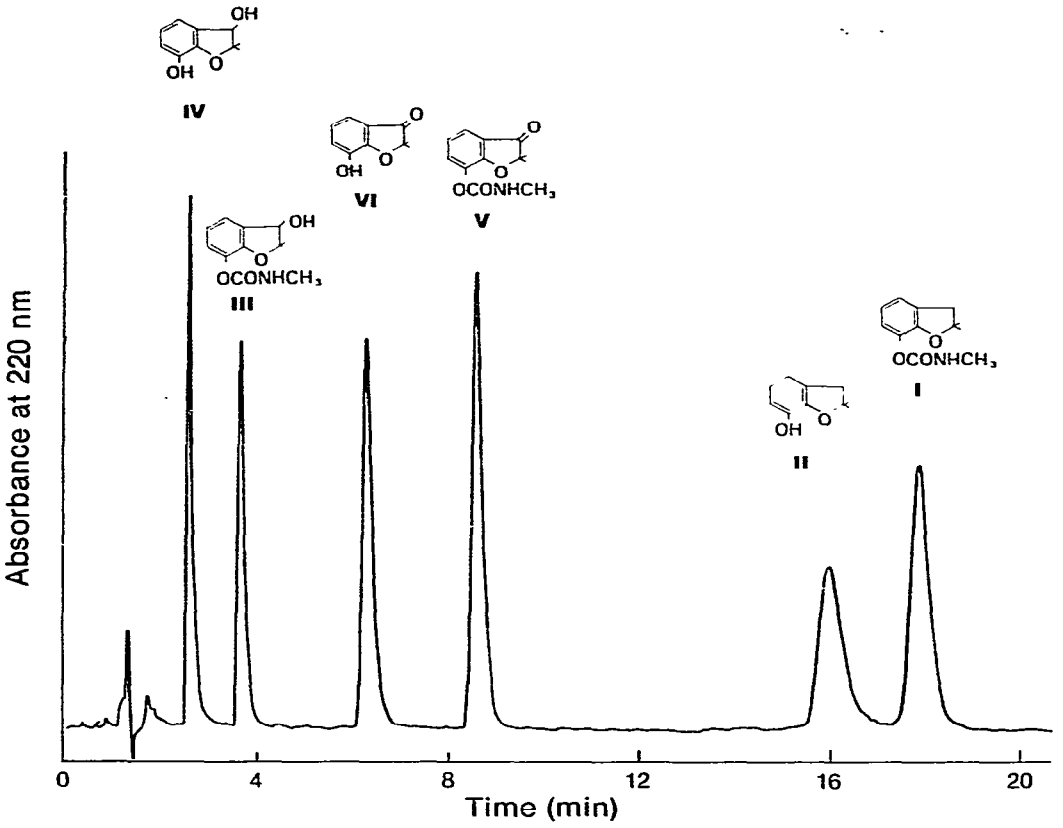

Fig. 2. Chromatogram of carbofuran and its five metabolites. Packing: Zorbax ODS. Column: 25 cm × 4.6 mm I.D. Mobile phase: acetonitrile–water (25:75), 2 ml/min (System A); recorder sensitivity, 0.058 absorbance units full scale; injection volume, 10 μl. Concentration of standards (μg/ml): I, 44.4; II, 48.0; III, 20.0; IV, 40.0; V, 31.6; VI, 12.6.

also desirable. For these reasons, a separation involving gradient elution was also investigated.

Fig. 3 shows a typical chromatogram of carbofuran and its metabolites using a methanol–water gradient elution (System B). The resolution of carbofuran and its five metabolites was satisfactory for our purposes. The order of elution of I and II is reversed compared with that of the isocratic elution using acetonitrile–water. This is not the case for the order of elution of the remaining four metabolites (III, IV, V and VI). Adequate resolution was maintained for consecutive runs using this elution system.

Some problems with the stability of V were encountered. Metcalf *et al.*[1] reported that V is easily hydrolyzed to its phenol (VI) upon standing at 37.5°C in methanol–phosphate buffer solution at pH 9.5. No significant hydrolysis of V to VI was observed when the mixed standards in methanol or acetonitrile were kept at 4°C overnight. Neither was hydrolysis of V observed when these solutions were stored for 1 week at −20°C. No degradation of the other carbofuran metabolites was observed when methanol and acetonitrile solutions were stored at 4°C for a month.

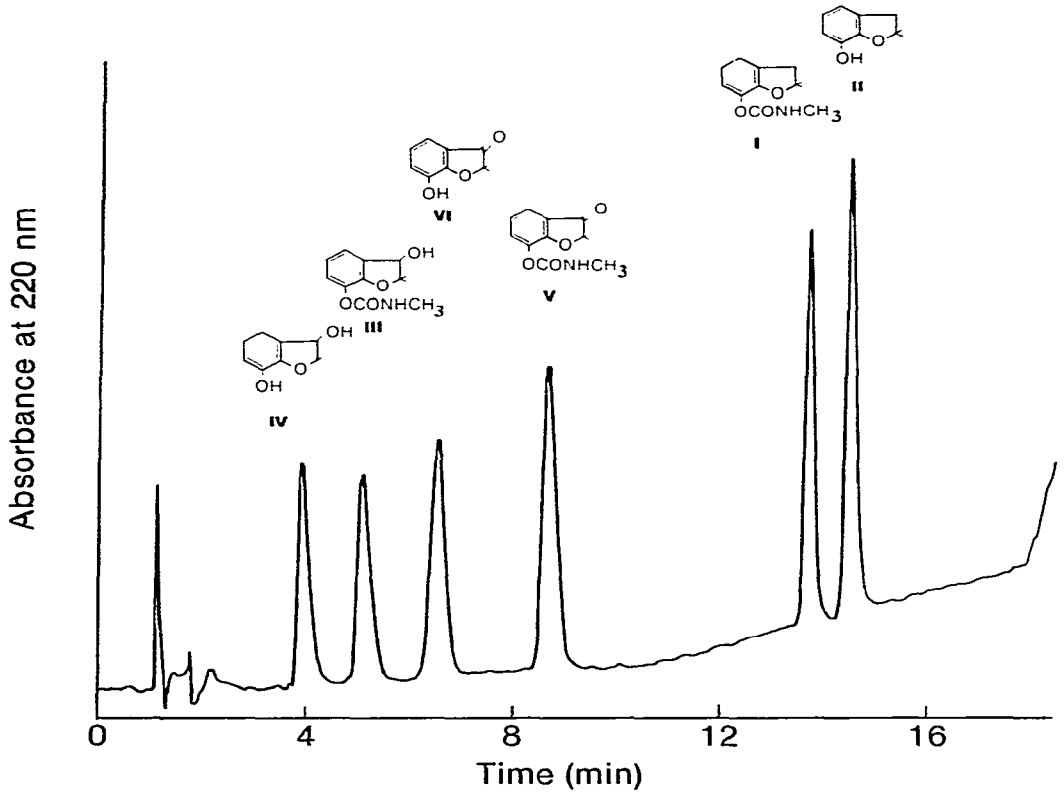

Fig. 3. Chromatogram of carbofuran and its five metabolites. Packing: Zorbax ODS. Column: 25 cm × 4.6 mm I.D. Mobile phase: as described in Experimental, 1.5 ml/min (System B); recorder sensitivity, 0.116 absorbance units full scale; injection volume, 10 μl. Concentration of standards (μg/ml). I, 57.8; II, 61.3; III, 41.8; IV, 36.4; V, 37.3; VI, 28.9.

In our application, these separations were made before collection of fractions and subsequent quantitation of [^{14}C]carbofuran residues in extracts of root crops. We believe that this separation, with UV determination at 220 nm, may be applicable for the quantitative determination of carbofuran residues in some samples without cleanup of the extracts. However, in most cases cleanup of the sample extracts before HPLC determination may be necessary. Data on the extraction study for which these separations were used will be presented in a future publication.

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