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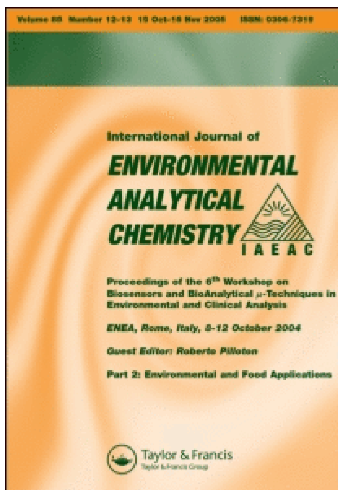

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Thin -layer Chromatographic Separation and Identification of Carbofuran and Two Carbamate Metabolites and their Dinitrophenyl Ethers

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Thin-layer Chromatographic Separation and Identification of Carbofuran and Two Carbamate Metabolites and their Dinitrophenyl Ethers†

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Residues of carbofuran and its 2 carbamate metabolites in fortified fresh water, a sandy loam soil, and plant tissues were extracted with HCl, partitioned into methylene chloride and cleaned up on deactivated Florisil. Aliquots of extracts were chromatographed on silica gel G and GF plates and carbofuran, 3-hydroxycarbofuran, and 3-ketocarbofuran were hydrolysed into their 7-phenols with KOH spray. Spots were visualized with either p-nitrobenzenediazonium fluoroborate or 2,6-dibromobenzoquinone-4-chloroimine. The characteristic color of spots before and after spraying with KOH or with either of the chromogenic reagents in ordinary light and under UV light helped in identification and differentiation of the compounds. As low as 0.05 ppm in water and 0.2 ppm in soil or strawberry, lettuce, carrots, peas and potatoes could be detected. 3-Hydroxycarbofuran in the remaining extracts was converted into 3-ethoxycarbofuran and the compounds were converted into their dinitrophenols and chromatographed on silica gel GF. Good separation of compounds was obtained but the limit of detection was 0.1 ppm in water and 0.3 ppm in

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soil and plant tissues. The procedures were applicable to field-treated rutabaga samples and useful in identity confirmation.

KEY WORDS: Carbofurane, carbamate metabolites, dinitrophenyl ethers, TLC, water, soil, plant tissue.

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INTRODUCTION

Carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl N-methylcarbamate) is a broad-spectrum insecticide-nematocide[1] and its two carbamate metabolites 3-hydroxycarbofuran (2,3-dihydro-2,2-dimethyl-3-hydroxy-7-benzofuranyl N-methylcarbamate) and 3-ketocarbofuran (2,3-dihydro-2,2-dimethyl-3-oxo-7-benzofuranyl N-methylcarbamate) are also toxic.

Thin-layer chromatography has been used in several studies relating to the identification and confirmation of carbofuran and its metabolites.[2-7] However, in each of these studies a significant interference was shown to exist between carbofuran and 3-ketocarbofuran, even though the latter fluoresced blue under UV light. One method which did effect complete separation of these compounds by TLC was a hydrolysis to the respective phenol.[4]

In the present study, the objective was to investigate a silica gel G and GF TLC technique for the identification and detection of carbofuran and its two carbamate metabolites in fortified water, soil, and plant tissues. It was found that identification and differentiation of these compounds could be achieved by evaluating their color response in visible and UV light before and after spraying with p-nitrobenzenediazonium fluoroborate or 2,6-dibromobenzoquinone-4-chloroimine chromogenic reagents.

EXPERIMENTAL

Apparatus and reagents

Precoated TLC glass plates 20 × 20 cm, silica gel G (without fluorescent indicator) and GF (with fluorescent indicator), 250 microns, from Analtech, Inc., Newark, Delaware. Carbamates hydrolysing agent, 1.5 N KOH in methanol. Chromogenic reagents (Eastman

Organic Chemicals, N.Y.), were p-nitrobenzenediazonium fluoroborate, 0.1% (w/v) in methanol, and 2,6-dibromobenzoquinone-4-chloroimine, 1% (w/v) in ethanol, prepared freshly and refrigerated, Carbofuran, 3-hydroxycarbofuran, and 3-ketocarbofuran analytical grade standards (FMC Corporation, Middleport, N.Y.); stock solutions (10 $\mu g/\mu l$) were prepared in benzene and a series of standards were obtained by successive dilution with hexane. All solvents were pesticide grade.

Sample preparation

Samples of fresh water (2 l), a sandy loam soil screened through 2 mm-sieve (100 g), and strawberry, lettuce, carrots, peas and potatoes (100 g) fortified with carbofuran, 3-hydroxycarbofuran, and 3-ketocarbofuran at levels of 0.05, 0.1 and 2.0 ppm were extracted in dilute HCl and partitioned in methylene chloride.[8] Water was acidified to about 0.25 N with concentrated HCl. Soils were refluxed in HCl 0.25 N using a water-cooled condenser for 1 hr and filtered hot through two fluted filter papers, the top paper being Reeve-Angel 802 and the bottom being Whatman 2 V. Plant tissues were chopped, blended with HCl 0.25 N for 3 min in a Servall Omni-Mixer then refluxed in the acid for 1 hr. The hydrolysate was filtered hot through sea sand (washed and ignited, Fisher Scientific Co.) in a coarse fritted Büchner funnel. Extracts were kept at $-5°C$ for about 2 hr then partitioned into methylene chloride. Soil and plant tissue extracts were cleaned up on deactivated Florisil (2.5%) using ethyl acetate:hexane (3:7, v/v) for elution.[9] Organic solvents were removed in a Kuderna–Danish evaporator using a steam bath and residues were redissolved in hexane (1 ml) in a 2 ml vial.

TLC before derivative formation

Twenty-five μl of the concentrated hexane extracts and appropriate standards of carbofuran, 3-hydroxycarbofuran, 3-ketocarbofuran and their mixture were streaked in a 10 mm band on precoated TLC plates. For each developing solvent system, two TLC plates from both types G and GF were prepared. The plates were developed to 10 cm in glass tanks saturated with the developing solvents, then air-dried and viewed under UV light. The plates were then sprayed with

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1.5 N methanolic KOH, re-air dried and reviewed under UV light. One set of plates was oversprayed with the chromogenic reagent p-nitrobenzenediazonium fluoroborate and the other with the chromogenic reagent 2,6-dibromobenzoquinone-4-chloroimine. After drying at room temperature the characteristic color of spots and the background were observed in ordinary light and under UV light. The chromoplates were photocopied for permanent records.

Formation of dinitrophenyl ether derivatives and their identification and detection by TLC

The remaining concentrated extracts in the 2 ml vial above were refluxed with absolute ethanol-concentrated HCl for 30 *min to* ethoxylate the 3-hydroxycarbofuran.[9] The resulting mixtures were extracted with methylene chloride, washed with 0.1 N NaOH in a separatory funnel and carbofuran and its two carbamate metabolites were then converted to their dinitrophenyl ethers using 1-flouro-2,4-dinitrobenzene (1% in acetone) in phosphate buffer pH 11.[9] The derivatized mixtures were isolated in hexane, dried over anhydrous sodium sulfate, and concentrated to 0.2 ml. The aforementioned procedure was also followed using authentic standards (10 μg) each of carbofuran, 3-hydroxycarbofuran, 3-ketocarbofuran, and a mixture of them. Aliquots (20–50 μl) of each derivative solution or mixture were spotted on silica gel GF plates with flourescent indicator and developed in the solvent systems to a 10 cm scored line. When dried, the sheets were viewed under short UV light and the R_f values calculated.

RESULTS AND DISCUSSION

Carbofuran, 3-hydroxycarbofuran, and 3-ketocarbofuran were hydrolysed to their respective 7-phenols on TLC plates when sprayed with KOH solution. These phenols appeared as red–violet spots when oversprayed with p-nitrobenzenediazonium fluoroborate or as blue spots when oversprayed with 2,6-dibromobenzoquinone-4-chloroimine chromogenic reagents, respectively. The background remained unchanged and the lower limit of detection was 0.05 ppm in water and 0.2 ppm in soil and plant tissues. In the permanent photocopies of the chromatograms spots appeared black against a

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white background. Viewing the plates under UV light before or after spraying with KOH solution or either of the two chromogenic reagents helped in the identification of carbofuran and its two metabolites. With Analtech silica gel G plates and under long or short UV-light, carbofuran and its 3-hydroxy metabolite spots were not visible, wheras 3-keto metabolite spots flouresced blue–violet. The background in all cases was non-fluorescent. With plates of fluorescent indicator (GF) and under the long UV-light, the same response of spots as above was observed. However, if the plate was placed under the UV light before the application of any spray solution, carbofuran and hydroxycarbofuran appeared as dark spots whereas ketocarbofuran appeared as a blue–violet fluorescent spot and the background appeared fluorescent green. This is useful in differentiating between carbofuran and 3-ketocarbofuran whose spots were always close to each other in any of the solvent systems (Table I). The fluorescence of 3-ketocarbofuran disappeared upon spraying with KOH solution or overspraying with either of the chromogenic reagents.

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TABLE I

R_f values of carbofuran, 3-hydroxycarbofuran and 3-ketocarbofuran on silica gel TLC plates.

	Solvent system and R_f value	
Compound	Methylene chloride:acetonitrile (9:1, v/v)	Hexane:ethyl acetate (1:9, v/v)
Carbofuran	0.45	0.44
3-Hydroxycarbofuran	0.10	0.20
3-Ketocarbouran	0.52	0.37

Table II shows the R_f values of the 2,4-dinitrophenyl ether derivatives of carbofuran, 3-ethoxy-(originally 3-hydroxy-) carbofuran, and 3-ketocarbofuran in two solvent systems. Spots were small, well defined and appeared dark against a fluorescent background when the plates were viewed under the short UV light. All solvents gave good separation so that the ketocarbofuran derivative did not overlap with that of carbofuran. These are advantages over the chromatography of the underivatized compounds (Table I).

TABLE II

R_f values of 2,4-dinitrophenyl ethers of carbofuran, 3-ethoxycarbofuran and 3-ketocarbofuran on silica gel (GF) TLC plates.

	Solvent systems and R_f value	
Dinitrophenyl ethers	Methylene chloride	Toluene
Carbofuran	0.59	0.34
3-Ethoxycarbofuran	0.36	0.14
3-Ketocarbofuran	0.29	0.08

However, the limit of detection is higher; 0.1 ppm in water and 0.3 ppm in soil and plant.

To check the applicability of the above-described method the procedure was applied to extracts from rutabaga roots field-treated with carbofuran and analysed by gas chromatography.[10] The TLC result confirmed the presence of carbofuran, 3-hydroxycarbofuran and 3-ketocarbofuran in the tubers at harvest.

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