Journal of Chromatography, 243 (1982) 307–314
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 14,866

DETERMINATION OF ALDICARB, ALDICARB SULFOXIDE, ALDICARB SULFONE AND CARBOFURAN RESIDUES IN WATER USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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(First received October 7th, 1981; revised manuscript received March 2nd, 1982)

SUMMARY

A method was developed for the residue analysis of aldicarb, its toxic metabolites (aldicarb sulfoxide and aidicarb sulfone), carbofuran and its metabolites (3-hydroxy- and 3-ketocarbofuran) in water. The procedure involved extracting with methylene chloride, initial separation and clean-up with a Sep-Pak™ silica cartridge, followed by high-performance liquid chromatography employing a variable-wavelength ultraviolet detector set at 220, 247 or 280 nm. The method was applied to the analysis of 1-l well water samples with a sensitivity of 1 ppb* and recoveries greater than 83%.

INTRODUCTION

Aldicarb [2-methyl-2-(methylthio)propionaldehyde-O-(methylcarbamoyl) oxime; Temik®] is a soil-applied insecticide, registered in Canada for the control of insects, mites and nematodes in potatoes. The two principal and most toxic oxidation products of aldicarb are the sulfoxide and sulfone. Although these two can undergo further degradation to their respective non-toxic oximes, it is aldicarb sulfoxide which is the most potent cholinesterase inhibitor of the group and is responsible for the high systemic activity and the long-term persistence of insecticidal activity after application of aldicarb[1,2]. Previous methods of residue analysis for crop samples such as potatoes, carrots, cotton plants, sugar beets, have involved gas chromatography (GC) as the end-determinative step. Normally, the residues are extracted, cleaned up and separated from any oxime interference via silica or Florisil® column chromatography and the aldicarb, aldicarb sulfoxide and aldicarb sulfone residues determined collectively or separately, after peracid oxidation, as aldicarb sulfone using a gas chromatograph equipped with a flame photometric detector (sulfur-mode of operation) or an electrolytic conductivity detector (nitrogen-mode of operation)[3–5]. The majority of GC procedures employ a Carbowax 20M column which results in al-

* Throughout this article, the American billion (10^9) is meant.

0021-9673/82/0000–0000/$02.75

dicarb[2], and in some instances aldicarb sulfoxide[4], having a retention time(s) too short to allow sufficient separation from the solvent peak. Therefore, identification and determination has been accomplished, after quantitative conversion by peracetic acid, as aldicarb sulfone. In order to simplify clean-up, eliminate the derivatization step and still quantitatively determine aldicarb and its metabolites individually, a high-performance liquid chromatographic (HPLC) procedure was investigated and found to be applicable to aldicarb down to the 0.05 ppm and for aldicarb sulfoxide and sulfone at the 0.1 ppm level in potato tubers[5].

Carbofuran (2,3-dihydro-2,2-dimethylbenzofuran-7-yl methylcarbamate) is also a soil-applied insecticide registered in Canada for the control of insects, mites and nematodes in potatoes. A number of HPLC procedures have been reported for carbofuran and its two non-conjugated metabolites 3-hydroxy- and 3-ketocarbofuran. Lawrence and Leduc[6] used a LiChrosorb Si 60 column and trimethylpentane–2-propanol as mobile phase for their UV detection, at 254 nm (3-ketocarbofuran) and 280 nm (carbofuran and 3-hydroxycarbofuran), in foods at the 0.02–0.05 ppm level. Nelsen and Cook[7] hydrolyzed carbofuran to its respective 7-phenol prior to reversed-phase HPLC analysis, and Krause[8] separated carbofuran and other carbamates and/or metabolites on a μBondapak C_{18} column, then performed a post-column fluorometric technique. Conversely, Lawrence and Leduc[9] preferred the pre-column Dns derivatization procedure prior to separation on LiChrosorb Si 60 and fluorescence detection. Although Lee and Westcott[10] satisfactorily detected carbofuran and its 3-hydroxy metabolite down to the 0.2 ppm level in rape plants by direct reversed-phase HPLC on a Spherisorb ODS column with an aqueous methanol mobile phase and 280 nm UV detector, Robinson and Chapman[11] found both adsorption and reversed-phase HPLC unsatisfactory for carbofuran and five of its metabolites in potatoes, onions and turnips due to co-eluting interferences.

The present work extends the utility of the HPLC procedure to the simultaneous determination of aldicarb, its sulfoxide and sulfone metabolites, carbofuran, 3-hydroxycarbofuran and 3-ketocarbofuran in water samples with a method sensitivity of 1 ppb.

EXPERIMENTAL

Reagents

Buffer solution. The buffer solution was obtained by dissolving 16.73 g of K_2HPO_4 and 0.523 g of KH_2PO_4 in 1 l of water (purified by passage through a Milli-Q™ System) to give pH 8.4.

Mobile phase. Mobile phases were (1) Acetonitrile–water (2:3) for aldicarb and carbofuran, (2) acetonitrile–buffer (1:9) acidified to pH 7.6 with phosphoric acid for aldicarb sulfoxide, and (3) acetonitrile–buffer (14:86) acidified to pH 7.6 for aldicarb sulfone.

Standard stock solutions. Standard stock solutions were made by dissolving 10 mg of aldicarb, its sulfone or sulfoxide or carbofuran, separately in 100 ml of acetonitrile. These solutions were diluted to final working solutions containing 1 ng/μl of aldicarb, carbofuran, 3-hydroxycarbofuran, 3-ketocarbofuran, in acetonitrile, aldicarb sulfoxide (in acetonitrile–buffer (1:9)) and aldicarb sulfone (in acetonitrile–buffer (14:86)), respectively.

Solvents and chemicals. HPLC-grade acetonitrile and methanol, glass-distilled chloroform and methylene chloride were purchased from Caledon Labs. (Georgetown, Canada). The sodium sulfate was obtained from J. T. Baker (CA, U.S.A.) and pre-washed with methylene chloride prior to use.

Absorbent. Sep-Pak™ silica (Waters Assoc., Milford, MA, U.S.A.) was used as absorbent.

Apparatus

The high-performance liquid chromatograph consisted of a Spectra-Physics Model 740 pump, a Rheodyne 7121 injection valve, a commercially available 25 cm × 4.2 mm I.D. RP-18 10 μm column (Brownlee Labs., Santa Clara, CA, U.S.A.) coupled to a 7.6 cm × 2 mm I.D. Co-Pell:ODS guard pre-column (25–37 μm; Whatman, Clifton, NJ, U.S.A.) and a Schoeffel Instruments Model 770 Spectroflow multi-wavelength UV detector operated at 220 nm (aldicarb sulfone and sulfoxide), 247 nm (aldicarb and 3-ketocarbofuran) and 280 nm (carbofuran and 3-hydroxycarbofuran).

Extraction procedure

To 1 l of well water in a 2-l erlenmeyer flask were added 200 g of sodium sulfate; the mixture was stirred until dissolution was complete. The solution was transferred to a 2-l separatory funnel and extracted with three 200-ml portions of methylene chloride. Each lower organic layer was drained through 40 g of sodium sulfate into a 1-l round-bottomed flask, and the combined extracts (including a 50 ml methylene chloride wash of the sodium sulfate) were evaporated to 2 ml on a rotary evaporator and transferred to a Sep-Pak silica gel cartridge (which had been previously washed with 10 ml of methylene chloride) surmounted by a 10-ml syringe; the syringe was charged with the 2 ml of solution which was pumped through the cartridge. The round-bottomed flask was washed with 2 ml of chloroform, transferred to the syringe, and then pumped through the silica gel cartridge. This was repeated twice more with 5.5 ml of chloroform, *i.e.*, a total of 15 ml of solvent was passed through the cartridge; this constituted the first fraction containing aldicarb, carbofuran and 3-ketocarbofuran. The cartridge was further eluted with 4 ml of acetonitrile–chloroform (1:1), which was collected (*i.e.*, fraction 2 contained aldicarb sulfone and 3-hydroxycarbofuran). The procedure was repeated using 5 ml of methanol–chloroform (1:1) to give fraction 3, which contained aldicarb sulfoxide. Each fraction was evaporated to dryness in a 10-ml centrifuge tube using nitrogen. The resulting residues were redissolved in the appropriate mobile phase (see above), and 40 μl of each were injected onto the HPLC column with the mobile phase flowing at 1 ml/min.

RESULTS AND DISCUSSION

Aldicarb and its metabolites exhibit strong UV absorbance characteristics at the shorter wavelengths, *i.e.*, 193–199 nm, in addition to secondary maxima at 245–248 nm for aldicarb and aldicarb sulfoxide[1,2]. Previous HPLC work on potato extracts[5] showed that aldicarb sulfoxide and sulfone were conveniently detected at 220 nm since they were chromatographed with very similar solvent systems. However, 247 nm was chosen for aldicarb since many interferences were encountered below 210 nm in both water and potato extracts.

With acetonitrile–water (2:3) as the mobile phase, aldicarb displayed a retention time of 5.9 min when monitored at 247 nm. No interferences were observed in control extracts (Fig. 1). Similar chromatograms were obtained for aldicarb sulfone, isolated from the silica gel cartridge fraction 2, and aldicarb sulfoxide (fraction 3). They appeared at 7.6 and 6.9 min, respectively, with acetonitrile–buffer (14:86) (sulfone) and acetonitrile–buffer (1:9) (sulfoxide) as mobile phases flowing at 1 ml/min. Therefore, initially three injections were required for quantitative analysis. With the described procedure, recoveries were within the range 83.6–102% for levels of 1–5 ppb for all three compounds (Table I). During the course of method development, it was decided to include carbofuran, another important insecticide used on potatoes, in the analytical scheme since it is currently registered in Canada for the control of soil and foliar-feeding insects. Like aldicarb it eluted in fraction 1 from the silica gel

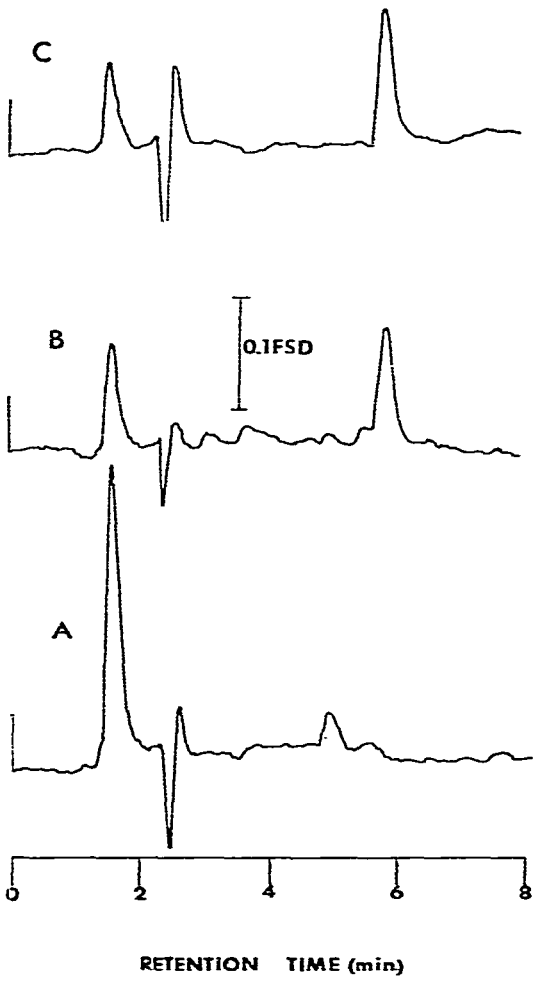

Fig. 1. Typical HPLC chromatogram for aldicarb at 247 nm. (A) Water control (fraction 1); (B) 2 ppb recovery of aldicarb (85%); (C) 40 ng of standard. Mobile phase, acetonitrile–water (2:3) at 1 ml/min.

TABLE I

PERCENT RECOVERIES OF CARBOFURAN, ALDICARB AND ITS METABOLITES FROM FORTIFIED WELL-WATER SAMPLES

Compound	*Added (ppb)*	*Recovery (%)*	*Average*	*S.D.*
Aldicarb sulfoxide	1	96.3, 100.0	98.15	2.6
	2	88.5, 84.6, 80.6	84.56	3.9
	5	80.7, 90.4, 79.8	83.63	5.9
Aldicarb sulfone	1	93.3, 92.3, 100.0	95.20	4.2
	2	93.8, 100, 100.0	100.00	4.2
	5	107, 106, 93.0	102.00	7.8
Aldicarb	1	86.8, 97.4, 106	96.70	9.6
	2	86.9, 93.7, 83.3	87.97	5.3
	5	91.8, 103.0, 90.7	95.20	6.8
Carbofuran	2	94.1, 88.7, 95.8	92.87	3.7
3-Hydroxycarbofuran	1	102	–	–
	2	95.6	–	–
3-Ketocarbofuran	1	95.2	–	–
	2	97.4	–	–

cartridge. However, carbofuran displays UV maxima at 200, 218 and 280 nm[12], and since the sensitivities at 220 and 280 nm were found to be very similar under the elution conditions used for aldicarb, 280 nm was chosen as the monitoring wavelength. Since carbofuran appeared at 8.74 min, using the acetonitrile–water (2:3) mobile phase, aldicarb and carbofuran could be detected together in the same injection by the simple expedient of changing the monitoring wavelength from 247 nm to 280 nm after the elution of aldicarb (Fig. 2). At the 2-ppb level, carbofuran recoveries were in the range 88.7–95% (Table I).

In recovery studies with the silica gel cartridge, 3-ketocarbofuran eluted in fraction 1 with aldicarb and carbofuran, while 3-hydroxycarbofuran appeared in fraction 2 along with aldicarb sulfone. UV monitoring was performed at 280 nm for 3-hydroxycarbofuran and 247 nm for the 3-keto metabolite to ensure maximum sensitivity, and their elution times were 3.66 and 5.63 min, respectively, using the acetonitrile–water (2:3) mobile phase (Fig. 3). Unfortunately, aldicarb and 3-ketocarbofuran co-eluted under these conditions. Attempts to effect a baseline separation of these two compounds included changes in the mobile phase, use of a buffer, change of columns (*e.g.*, RP-18, 5 μm Spheri-5, and an amino-cyano column) all of which failed to give adequate resolution. However, separation was achieved with a Hamilton PRP-1 reversed-phase resin (a styrene–divinylbenzene copolymer) with acetonitrile–water (3:7) at a flow-rate of 1.5 ml/min. Under these conditions, aldicarb appeared at 6.46 min and 3-ketocarbofuran at 7.99 min. Recoveries from spiked water samples were 95.2% for both 3-hydroxy- and 3-keto-carbofuran at the 1–2-ppb level (Table I).

With the above HPLC procedure, aldicarb residues were determined in wells located near potato fields treated at label rates for control of the Colorado potato

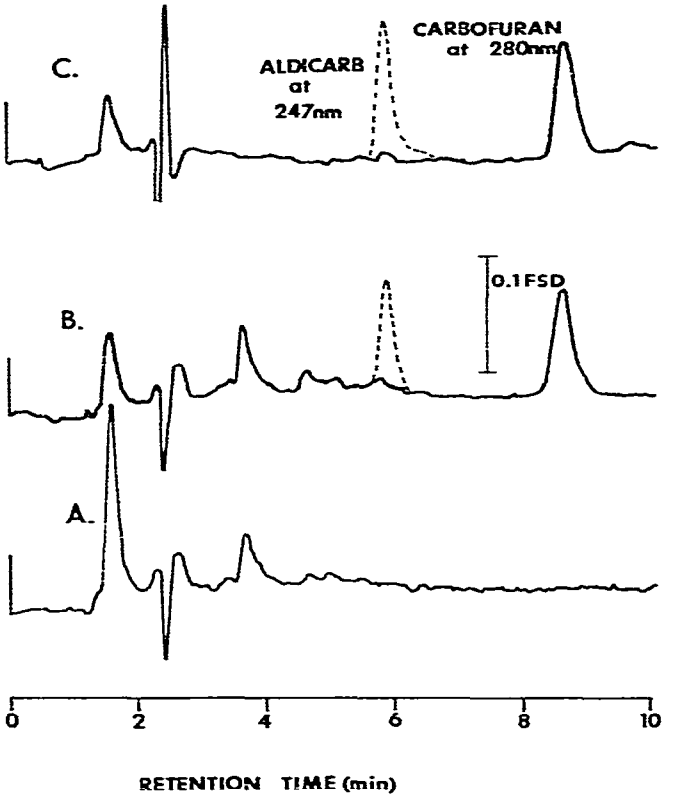

Fig. 2. Typical HPLC chromatogram for carbofuran. (A) Water control (fraction 1); (B) 2 ppb recovery of carbofuran (96%); (C) 40 ng of standard(s). Conditions, see Fig. 1.

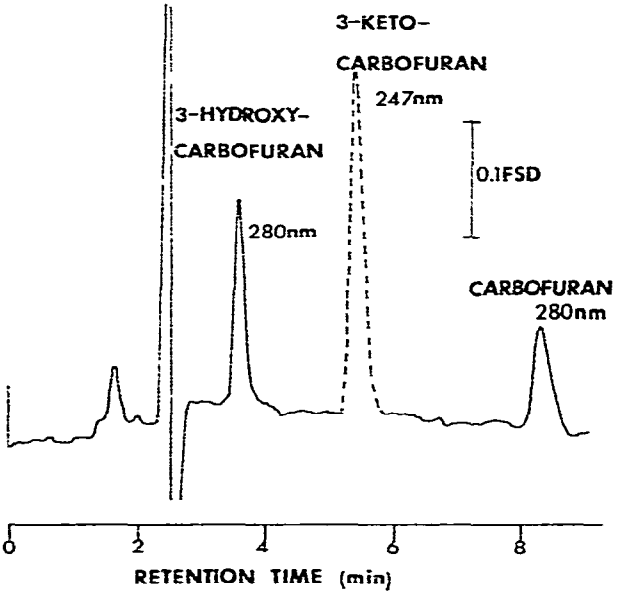

Fig. 3. Separation characteristics of carbofuran, 3-hydroxycarbofuran and 3-ketocarbofuran (40 ng each). Conditions, see Fig. 1.

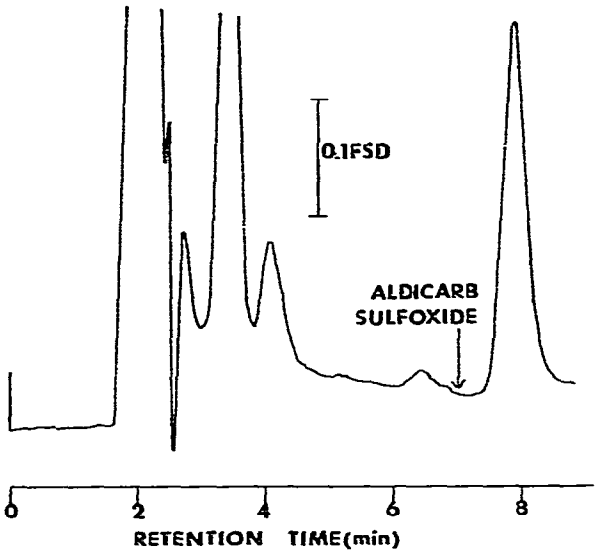

Fig. 4. Water sample extract showing the elution of an interference peak. Mobile phases, 8% acetonitrile–buffer (2:23).

beetle. A total of nineteen water samples were taken from wells associated with the potato growing areas in the Canadian Maritimes (*i.e.*, ten from New Brunswick and nine from Prince Edward Island). Sites were chosen where the potato fields had been treated with 10% granular formulations of Temik (technical grade aldicarb) at rates of 11–22.4 kg/ha (*i.e.*, equivalent to 0.97–2.0 lb. of active ingredient per acre). The location of the wells varied from 15 to 300 m adjacent to the potato fields, and 1-l water samples were drawn which were acidified with 10 ml of 0.2 *M* phosphoric acid prior to transport to the laboratory. No detectable residues of aldicarb, its sulfone, sulfoxide, or carbofuran were observed in any of the well water samples above the 1-ppb detection limit. In some samples, a peak appeared which interfered with the detection and quantitation of aldicarb sulfoxide. However, resolution was achieved by a slight alteration in the composition of the mobile phase. When the acetonitrile

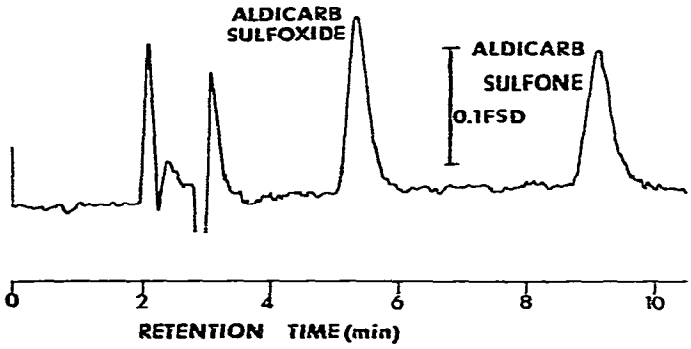

Fig. 5. HPLC chromatogram showing the separation characteristics of aldicarb sulfoxide and sulfone (40 ng each). Mobile phase, 12% acetonitrile–buffer (3:22).

concentration was decreased from 10% to 8%, aldicarb sulfoxide appeared *ca.* 1 min ahead of the interference peak (Fig. 4). However, with a 12% acetonitrile–buffer system, both aldicarb sulfoxide and sulfone could be determined in the same injection (Fig. 5). This also necessitated collecting fractions 2 and 3 in the same receiver prior to the evaporation step. Therefore, the analysis of aldicarb, its two metabolites, and carbofuran can be quantitatively determined in a two-injection procedure.

ACKNOWLEDGEMENT

The authors thank the Agriculture Canada staff of the Plant Products and Quarantine Directorate in the Maritime Region for the collection of the water samples.

REFERENCES

1 N. R. Andrawes, W. P. Bagley and R. A. Herrett, *J. Agr. Food Chem.*, 19 (1971) 732–737.
2 J. C. Maitlen, L. M. McDonough and M. Beroza, *J. Agr. Food Chem.*, 16 (1968) 549–553.
3 J. H. Smelt, N. W. H. Houx, Th. M. Lexmond and H. M. Nollen, *Agr. Environ.*, 3 (1977) 337–347.
4 M. Galoux, J.-C. Van Damme, A. Bernes and J. Potvin, *J. Chromatogr.*, 177 (1979) 245–253.
5 W. P. Cochrane and M. Lanouette, *J. Ass. Offic. Anal. Chem.*, 64 (1981) 724–728.
6 J. F. Lawrence and R. Leduc, *J. Agr. Food Chem.*, 25 (1977) 1362–1364.
7 T. R. Nelsen and R. F. Cook, *J. Agr. Food Chem.*, 27 (1979) 1186–1188.
8 R. T. Krause, *J. Chromatogr. Sci.*, 16 (1978) 281–288.
9 J. F. Lawrence and R. Leduc, *J. Chromatogr.*, 152 (1978) 507–513.
10 Y. W. Lee and N. D. Westcott, *J. Agr. Food Chem.*, 28 (1980) 719–722.
11 J. R. Robinson and R. A. Chapman, *J. Chromatogr.*, 193 (1980) 213–224.
12 C. M. Sparacino and J. W. Hines, *J. Chromatogr. Sci.*, 14 (1976) 549–556.