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DETERMINATION OF CARBOFURAN AND ITS METABOLITES BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY USING ON-LINE TRACE ENRICHMENT

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SUMMARY

The concentration technique known as on-line trace enrichment was applied to the analysis of over 100 drinking water samples for carbofuran and five of its carbamic and phenolic metabolites. Levels of 1 ppb* could be determined from 5 ml of sample. The proposed method demonstrates shorter total analysis time than previous methods but is readily applicable only to relatively clean samples. A gas chromatographic method used as a confirmatory technique for detecting carbofuran and 3-hydroxycarbofuran is also presented. External standards data, detection limits, relative retention volumes, and sample spike compound recoveries are presented for the trace enrichment method.

INTRODUCTION

Carbofuran (2,2-dimethyl-2,3-dihydrobenzofuranyl-7-N-methylcarbamate) is a broad-spectrum insecticide–nematocide used for the control of insects in such crops as alfafa, peanuts, rice, corn, rapeseed, and potatoes. Although less persistent than organochlorine pesticides, carbofuran (I, Fig. 1) is more toxic (rat oral LD_{50} of 11 mg/kg)[1] and is a cholinesterase inhibitor. Recent findings of carbofuran and Aldicarb in groundwater supplies of Long Island, New York, have raised concern over the compounds' migration and transport characteristics after their application to crops. The carbamic (II, III) and phenolic (IV–VI) metabolites (Fig. 1) are also of interest because of their toxicity, and they may serve as indicators of previous carbofuran contamination. As part of a U.S. Environmental Protection Agency (EPA) project surveying groundwater for selected pesticides, Midwest Research Institute (MRI) sought a method to analyze some 130 samples for carbofuran and its metabolites.

Previous methods of analysis for the determination of carbofuran and its me-

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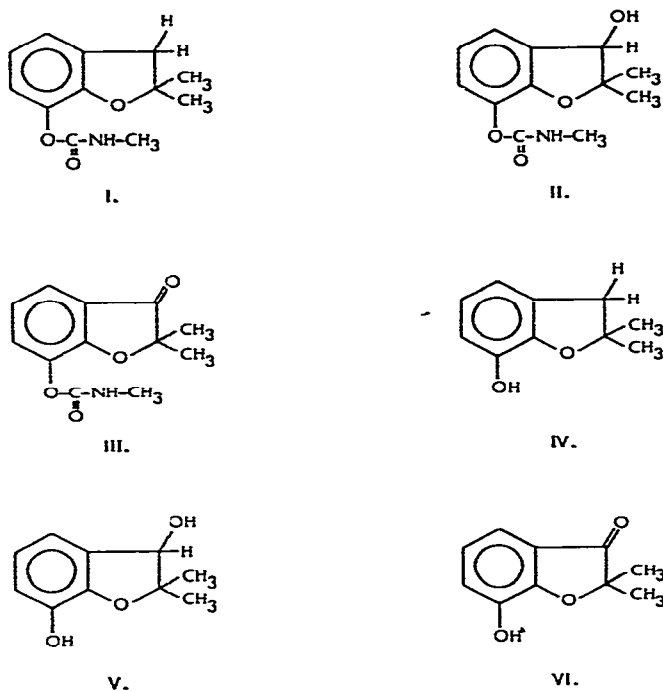* Throughout this article, the American billion (10^9) is meant.

Fig. 1. Structures of carbofuran (I), 3-hydroxycarbofuran (II), 3-ketocarbofuran (III), carbofuran phenol (IV), 3-hydroxycarbofuran-7-phenol (V), and 3-ketocarbofuran-7-phenol (VI).

tabolites have been directed towards various plant and animal tissues. These methods generally have employed extensive cleanup procedures to separate the compounds of interest from interfering lipids and other hydrophobic materials and have frequently employed derivatization procedures to enhance chromatography and sensitivity. Gas chromatographic (GC) methods have used both derivatization procedures[2-5] and direct chromatography with nitrogen specific detection[6-8] to achieve the needed sensitivity. Additional direct GC methods have been applied to water and soil matrices[9-11]. Recent methods have employed high-performance liquid chromatography (HPLC) as a means of separation and detection utilizing both derivatization[12-14] and non-derivatization[15-17] procedures. All of these methods, however, require an extraction step, if not also a cleanup and derivatization step, somewhere in the procedure.

The selection of an analytical procedure was constrained by the requirements of (a) 1-ppb sensitivity, (b) an interest in all forms, (c) the limited sample volume of 200–400 ml, and (d) the preference for a single procedure. These criteria eliminated the published GC and HPLC methods in that no single procedure was applicable to the wide range of compounds. A new procedure was developed using on-column trace enrichment[18-21] to meet all the criteria.

EXPERIMENTAL

Reagents

Solvents were purchased as distilled-in-glass grade from Burdick & Jackson

Labs. (Muskegon, MI, U.S.A.) and degassed by vacuum filtration through a membrane filter. Chromatographic grade water was obtained from a Milli-Q-Reagent grade water system (Millipore, Bedford, MA, U.S.A.), which was modified to produce a lower concentration of organic contaminants by installing two charcoal cartridges at the end of the train. Aqueous filters (0.45 μm) were also obtained from Millipore. The sodium sulfate was obtained from Mallinkrodt (St. Louis, MO, U.S.A.). Chromatographic standards were obtained from the EPA Health Effects Research Laboratory (Research Triangle Park, NC, U.S.A.).

Apparatus

A Varian Model 3700 gas chromatograph equipped with a thermionic specific detector (TSD) and a Varian Model CDS-111 integrator was used in this study. The column utilized was a 0.91 m × 2 mm I.D. glass column packed with 3% OV-101 (Supelco, Bellefonte, PA, U.S.A.). After the initial temperature of 150°C was held for 1 min, the temperature was increased at a rate of 10°C/min to 180°C. The injector and detector temperature were 190°C and 200°C, respectively. The detector flow-rates were 3 ml/min for hydrogen and 175 ml/min for air, and the detector bead current was 4.5 A.

The liquid chromatograph consisted of two Waters series 6000A solvent delivery systems controlled by a Model 660 solvent programmer, a single-channel Model 440 detector operated at 280 nm, and a modified U6K injector (Waters Assoc., Milford, MA, U.S.A.). The U6K injector was adapted for this work by replacing the original 2-ml injection loop with a 1 m × 2.64 mm I.D. × 3.18 mm O.D. length of stainless-steel tubing. A μBondapak C_{18} (300 × 3.9 mm I.D.) column obtained from Waters Assoc. was used. The peaks were integrated by a Varian Model CDS-111 integrator. The standard operating procedures selected for trace enrichment reversed-phase elution analysis utilized a flow-rate of 2 ml/min starting at 100% water and stepping up to 50% methanol by setting the profile selector to the 1 position with a program length (delay) of 5 min.

Sample preparation

For GC analysis, a 100-ml aqueous sample was placed in a 250-ml separatory funnel and extracted with three 50-ml portions of dichloromethane for 1 min each time. The extracts were each drained into a 250-ml Kuderna–Danish apparatus through a 10-g column of Na_2SO_4. A Snyder column was attached and the extract concentrated to *ca.* 5 ml on a stream bath. The concentrated extract was transferred to a 7-ml vial and evaporated to dryness under a stream of nitrogen. Acetone (1 ml) was added, and the extract was stored in a refrigerator until analyzed by GC–TSD.

For the trace enrichment method, the general procedure was to measure the sample into a 10-ml volumetric flask and inject 10 μl of a 5-ng/μl solution of carbaryl internal standard into the solution. The spiked sample was transferred to a 10-ml glass/Teflon syringe equipped with a 0.45-μm aqueous filter, and 5 ml were injected into the sample loop of the U6K injector. The sample was then loaded onto the column by switching the inject lever to inject and simultaneously starting the recorder, integrator, and solvent programmer.

Sample collection and storage

Samples were collected from either private or community well sources. Water was taken directly from a household tap after allowing the water to run for 1 min, and placed in a 1-l bottle containing 1 g of sodium thiosulfate. The bottle was sealed with a PTFE-lined cap and maintained at 4°C during shipment and storage. Sampling began October 29th, 1979, and ended December 22nd, 1979.

RESULTS AND DISCUSSION

The trace enrichment method discussed here was originally developed to quantitate and verify the base hydrolysis of carbofuran (I) to carbofuran phenol (IV). Preliminary GC studies indicated that almost no carbofuran or 3-hydroxycarbofuran (II) was recovered after 1 week in tap water at a pH of 9.5. The GC method, however, could not show the concurrent increase in the phenolic hydrolysis products, and it was this fact that prompted the development of the trace enrichment method.

Monitoring the stability of carbofuran in water (spiked at 100 ppb) by the trace enrichment method gave the results shown in Fig. 2. This agrees with previous findings[1], which show the hydrolytic instability of carbofuran and its carbamic metabolites. Base hydrolysis of carbofuran begins immediately in tap water at pH 9.5, and its hydrolysis product, carbofuran phenol, is formed concurrently with mass balance of the two compounds varying between 80 and 113% recovery. At pH 6.5, no formation of the phenolic product from carbofuran was observed over the period of the 1-week study. Similar results might be expected for the hydrolysis of 3-hydroxycarbofuran (II) to 3-hydroxycarbofuran-7-phenol (V) and 3-ketocarbofuran (III) to 3-ketocarbofuran-7-phenol (VI)[1], but results were not quantitated due to lack of standards at the time the stability studies were being carried out.

Since hydrolysis occurs in basic solution and since many of the samples to be analyzed could be expected to be basic, the presence of carbofuran or its major plant metabolite, 3-hydroxycarbofuran, in drinking water would be highly unlikely[22-25]. Because of the limited sample volume (less than 100 ml in some cases), the proposed GC method utilized a very sensitive and specific detector, the TSD. This fact, however, eliminated the method's usefulness for the more probable phenolic metabolites. It was these facts, then, that led to the use of the less selective but equally sensitive HPLC method for the analysis of carbofuran and its metabolites.

The initial selection of the HPLC solvent system for the separation of carbofuran and metabolites was based on earlier work completed at MRI which analyzed for carbofuran in wastewater utilizing a simple dichloromethane extraction method with solvent exchange into acetonitrile. The solvent system used for the earlier work was acetonitrile–water (1:1). When this system was used to chromatograph carbofuran phenol, it was found that the phenol and its precursor (carbofuran) coeluted. Different ratios of acetonitrile to water did not affect resolution, but when the solvent system was changed to methanol–water, resolution was achieved. Initially the methanol–water solvent system included 1% (v/v) acetic acid in the aqueous phase to ensure protonation of the phenolic metabolites. The acetic acid was soon eliminated, however, because it did not affect resolution of these metabolites.

When the final step gradient system to methanol–water (1:1) was being chosen, it was shown that up to 50 ml of water could be passed through the analytical C_{18}
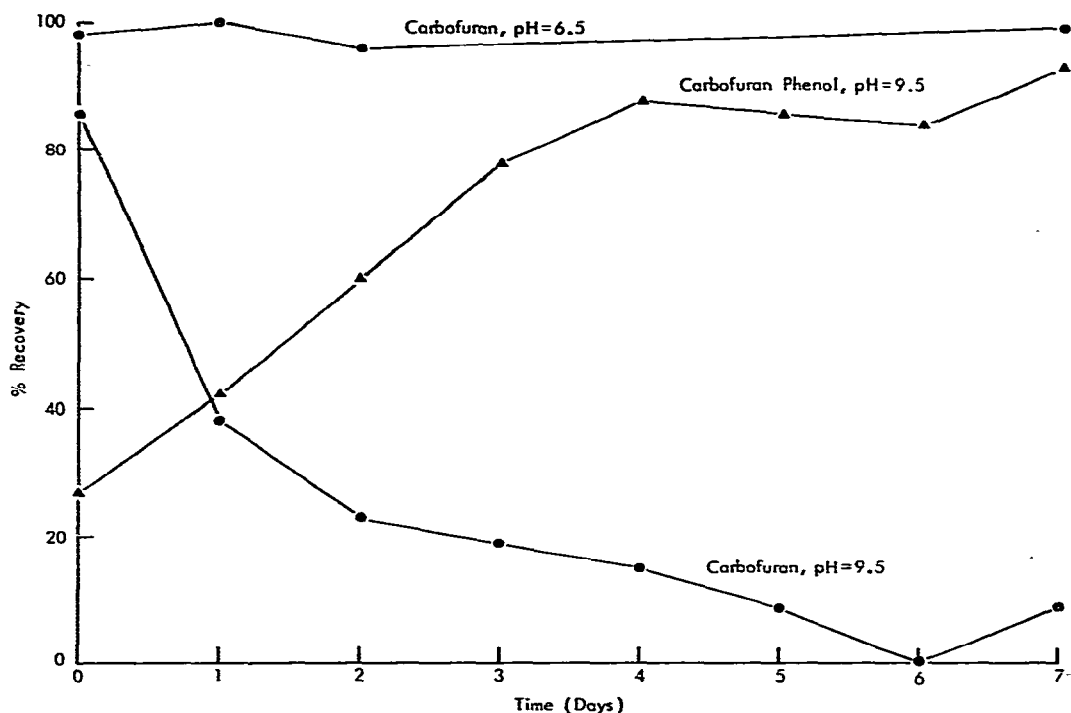
Fig. 2. Carbofuran stability in tap water at pH 9.5 and 6.5.

column, after a 10-μl injection of the compounds of interest in methanol, without altering the shape or resolution of the peaks. This fact assured us of being able to inject sizeable amounts (1–5 ml) of drinking water directly onto the analytical column. The Waters U6K injector was modified to accept a 5-ml sample volume by removing the standard 2-ml sample loop and replacing it with *ca.* 6-ml volume loop for the sample. The use of the step gradient to methanol–water (1:1) after 5 min of pumping 100 % water at 2 ml/min allowed the 5-ml sample to be concentrated on the head of the column and gave an additional 2.5 min for any non-retained polar constituents to be eluted from the column before the step to methanol–water.

Detection limits based on 5 ml of sample as well as retention time windows relative to Carbaryl are given in Table I. The detection limits amounts for these compounds produced a signal which was twice the noise level at 0.005 a.u.f.s. For an individual sample, the detection limits could be higher due to interfering peaks. This was especially true for the first four metabolites, but virtually no peaks were seen to interfere with carbofuran or carbofuran phenol.

Retention time windows were used to identify carbofuran and its metabolites since the absolute retention times of the compounds and the internal standard varied slightly with the differences of sample matrices. The earlier eluting metabolites, which were most affected by changes in polarity and system anomalies, were assigned larger retention time windows than the later eluting compounds because of their greater variability.

All samples were screened for carbofuran and its metabolites (except 3-keto-

TABLE I

RELATIVE RETENTION TIMES AND DETECTION LIMITS FOR CARBOFURAN AND ME-
TABOLITES

Compound	Detection limit (ppb)*	Relative retention time window**
3-Hydroxycarbofuran-7-phenol	1	0.67 ± 0.01
3-Hydroxycarbofuran	1	0.71 ± 0.01
3-Ketocarbofuran	5	0.80 ± 0.005
3-Ketocarbofuran-7-phenol	5	0.81 ± 0.005
Carbofuran	1	0.91 ± 0.005
Carbofuran phenol	1	0.95 ± 0.005
Carbaryl (internal standard)	—	1.00

* Determined at 280 nm in a 5-ml sample.
** Determined on a C_{18} column using a step gradient from 100% water to 50% methanol after 5 min.
Flow-rate 2 ml/min.

carbofuran) by comparison with external standards. Standard curves were generated
daily from three to four mixed standards by using linear regression. A compilation of
the average areas for each compound and the relative standard deviations for each
compound at a given level over the period of analysis (*ca.* 5 weeks) are given in Table
II. Also given in Table II are correlation coefficients for standard curves constructed
from the average areas for each compound.

Limited data were obtained for 3-ketocarbofuran-7-phenol because of its rela-
tively low sensitivity and its tendency to coelute with a compound present in the
methanol as the column began to lose resolution. Although the relative standard
deviations for the compound responses were large, especially at the lower levels, the
correlation coefficients for the standard curves show good agreement. 3-Ketocarbo-

TABLE II

EXTERNAL STANDARD SUMMARY

Statistics	Standard levels (ppb)	Compounds				
		3-Hydroxy-carbofuran-7-phenol	3-Hydroxy-carbofuran	3-Keto-carbofuran-7-phenol	Carbofuran	Carbofuran phenol
Number of	1	15	15	2	21	21
data points	5	25	24	10	26	25
	25	20	16	5	19	21
Average area	1	29.4	14.9	3.0	9.8	9.2
	5	88.5	50.0	22.7	39.0	41.2
	25	313.9	251.4	119.4	229.4	246.4
Relative	1	40.5	44.0	—*	29.8	29.4
standard	5	25.6	29.0	27.3	22.6	21.5
deviation	25	12.1	16.4	20.8	17.5	8.7
Correlation		0.9991	0.9998	0.9999	0.9994	0.9995

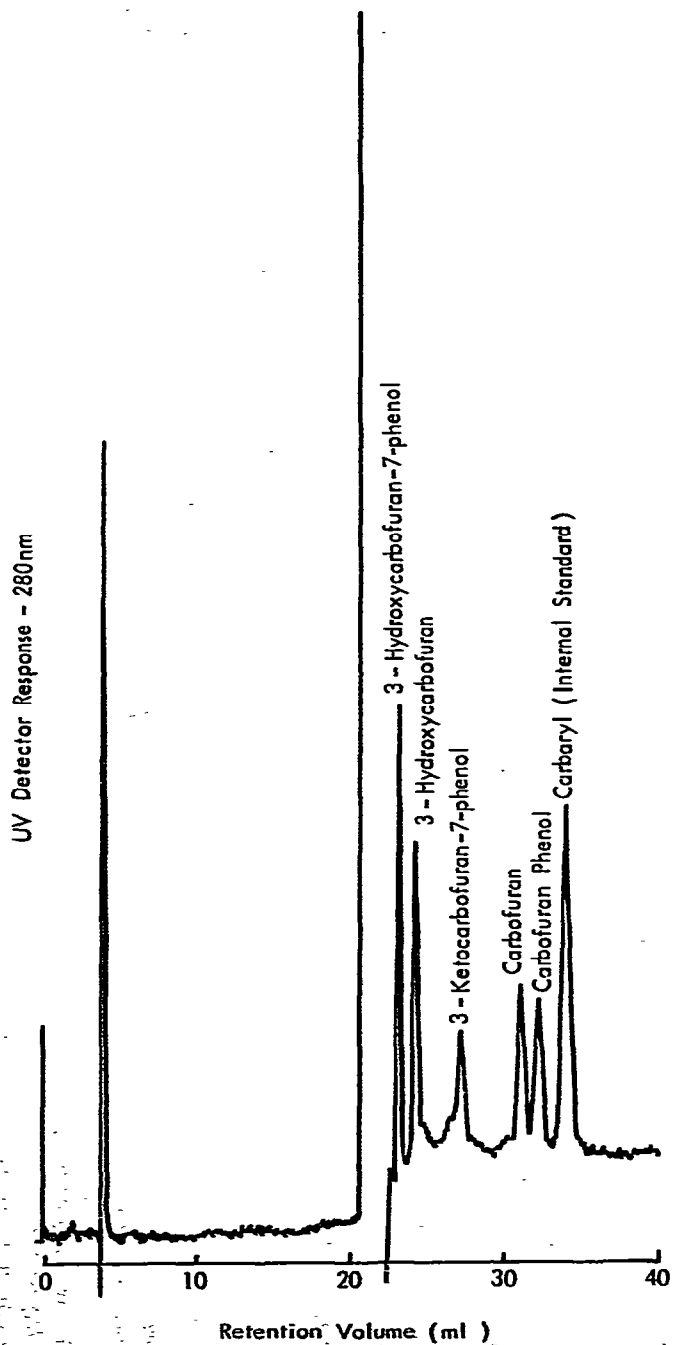* Insufficient data to calculate relative standard deviation.

Fig. 3. Mixed 5-ppb standard. Column, μBondapak C₁₈; solvent program, step gradient to 50% methanol in water after 5 min; flow-rate 2 ml/min.

TABLE III

SAMPLE SPIKE RECOVERIES

Sample code	Compounds									
	3-Hydroxy-carbofuran-7-phenol		3-Hydroxy-carbofuran		3-Keto-carbofuran-7-phenol		Carbofuran		Carbofuran phenol	
	Spiking level*	% R**	Spiking level	% R	Spiking level	% R	Spiking level	% R	Spiking level	% R
01-069-0500C***	5	94	5	77	5	105	5	90	5	90
01-069-206C	5	85	5	93	5	82	5	93	5	111
01-109-1701C	5	49	5	82	10	obs	5	62	5	73
04-019-301C	5	114	5	67	10	obs	5	85	5	82
04-021-0355-01	1	obs†††	1	297	2	obs	1	107	1	100
04-027-102C	5	64	5	74	5	118	5	96	5	102
06-029-0180C	5	76	5	93	5	105	5	79	5	81
06-039-201C†	10	obs	10	60	20	obs	10	67	10	90
06-039-203C	1	71	1	105	2	obs	1	53	1	74
06-039-203C	2	82	2	92	4	obs	2	168	2	150
06-039-203C	5	obs	5	48	10	obs	5	76	5	105
06-039-203C	10	121	10	60	20	obs	10	65	10	99
06-099-1395C	5	90	5	152	5	90	5	94	5	95
06-099-305C	5	86	5	80	5	111	5	123	5	79
13-321-201C	5	97	5	96	5	110	5	103	5	101
13-321-201C††	5	99	5	105	5	100	5	97	5	119
13-321-2740C	5	94	5	84	10	75	10	85	5	88
26-049-306C	5	68	5	74	10	105	5	63	5	63
27-119-102-001	5	46	5	87	10	84	5	82	5	105
28-133-0345C	5	88	5	77	10	obs	5	obs	5	obs
28-133-103C	5	90	5	82	10	obs	5	obs	5	obs
36-051-301C	5	137	5	74	10	obs	5	72	5	63
45-089-304C	5	obs	5	74	10	obs	5	77	5	91
48-279-106C	5	obs	5	97	10	obs	5	77	5	91
Average recovery		87		93		99		87		93
Relative standard deviation		26		52		14		28		21

* Spiking levels are given in ppb.

** Percentage recovery.

*** C indicates this sample was a composite.

† Sample has pH of 9.6; was spiked and stored 24 h before analysis.

†† Sample spiked after filtration in this instance.

††† Recovery obscured by interfering peak.

furan was not screened in the samples because of its hydrolytic instability and because it virtually coelutes with its hydrolysis product. Since it hydrolyzes *ca.* 165 times more rapidly than carbofuran[1], it was more likely to be found as 3-ketocarbofuran-7-phenol.

The trace enrichment method was applied to the analysis of 132 well-water samples or sample composites. The results of the analyses of all well-water samples were negative (below the detection limits). Those samples suspected of containing carbofuran or any of its metabolites were subjected to either spiking at 5 ppb and reanalysis by trace enrichment or extraction and GC–TSD confirmation.

Interpretation of the sample chromatograms was facilitated by spiking one sample out of a group of samples which showed the same pattern of peaks. The addition of the compounds of interest to a sample such as this eliminated any doubt as to the identity of a given peak in the unspiked samples and also gave method recovery values for those compounds in that sample matrix. Table III gives percent recoveries and spiking levels for 20 samples.

Carbofuran recoveries at all levels tested were 87%, with a relative standard deviation of 28% (Table III). In general, recoveries for all metabolites were better than 85%. Sample 06-039-203 composite (see Table III) was spiked at four different levels in an attempt to observe any patterns in recoveries at different spiking levels. No trends were observed and recoveries at the 1-ppb level were good except for 3-ketocarbofuran-7-phenol and carbofuran, both of which were partially or totally obscured by interfering peaks. Sample 13-321-201 composite was spiked at the 5-ppb level both before and after filtration to see if any losses were incurred by filtration; no difference in recoveries was observed. Sample 06-039-201 composite (pH 9.6) was spiked at 10 ppb and allowed to stand for 24 h before analysis. The low recoveries for carbofuran (67%) and 3-hydroxycarbofuran (60%) in this situation can probably be attributed to the base hydrolysis of those compounds to the corresponding phenols although an increase in the phenol recoveries was not observed.

Figs. 3–5 show chromatograms of a 5-ppb mixed standard, a typical sample, and the same sample spiked at 5 ppb. Only five samples were suspected to contain carbofuran or 3-hydroxycarbofuran. These samples were subjected to the GC method, but none were found to contain either carbamate residue. The detection limits were 1 ppb for carbofuran and 5 ppb for 3-hydroxycarbofuran by this method. Poor chromatography for 3-hydroxycarbofuran contributed to its high detection limit.

CONCLUSIONS

On-line trace enrichment for the analysis of carbofuran and metabolites in drinking water without the use of a precolumn is an effective means of analysis. The advantages of this method lie in its simplicity and its sensitivity. Instrument modification requires no special valves or fabricated parts and cou'd be easily done in any laboratory with a similar instrument. Since there is virtually no sample preparation other than spiking the sample with the internal standard and filtering, the total analysis time for each sample is essentially the HPLC run time, or *ca.* 30 min (including re-equilibration of the HPLC system). A detection limit of 1 ppb (in 5 ml of sample) is easily achievable for carbofuran and most of its metabolites without benefit of solvent
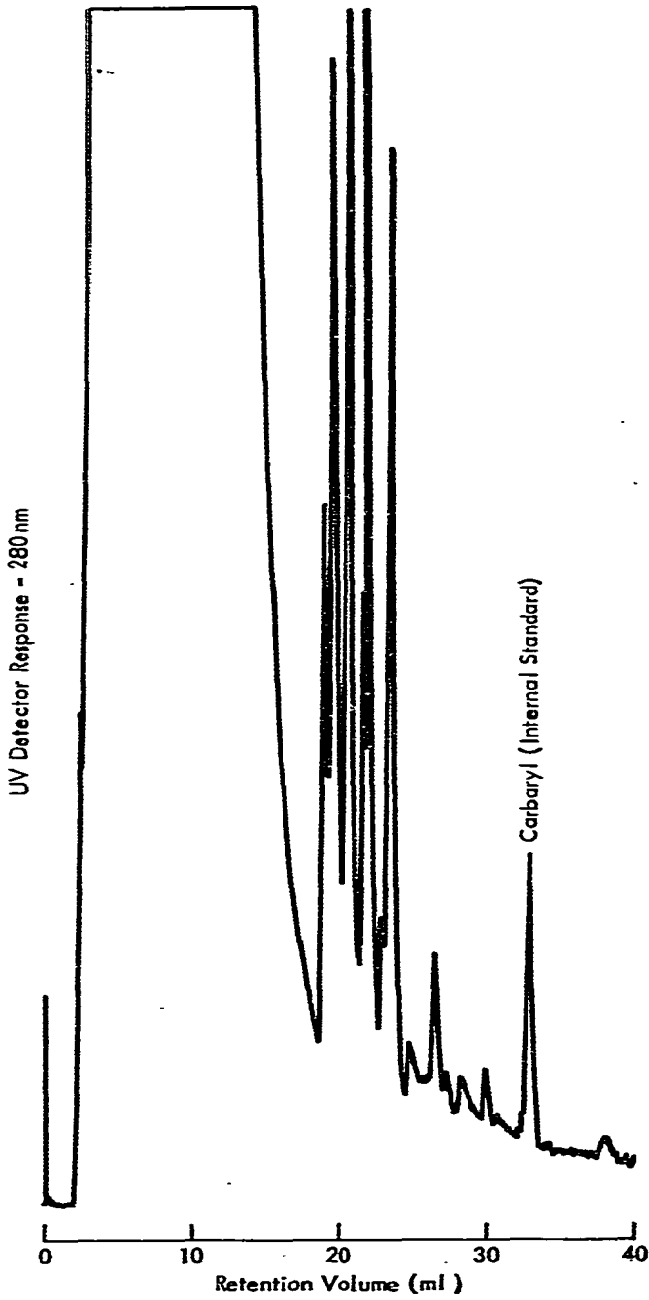
Fig. 4. Sample 06-029-0180 composite; taken from Kern County, CA, U.S.A.
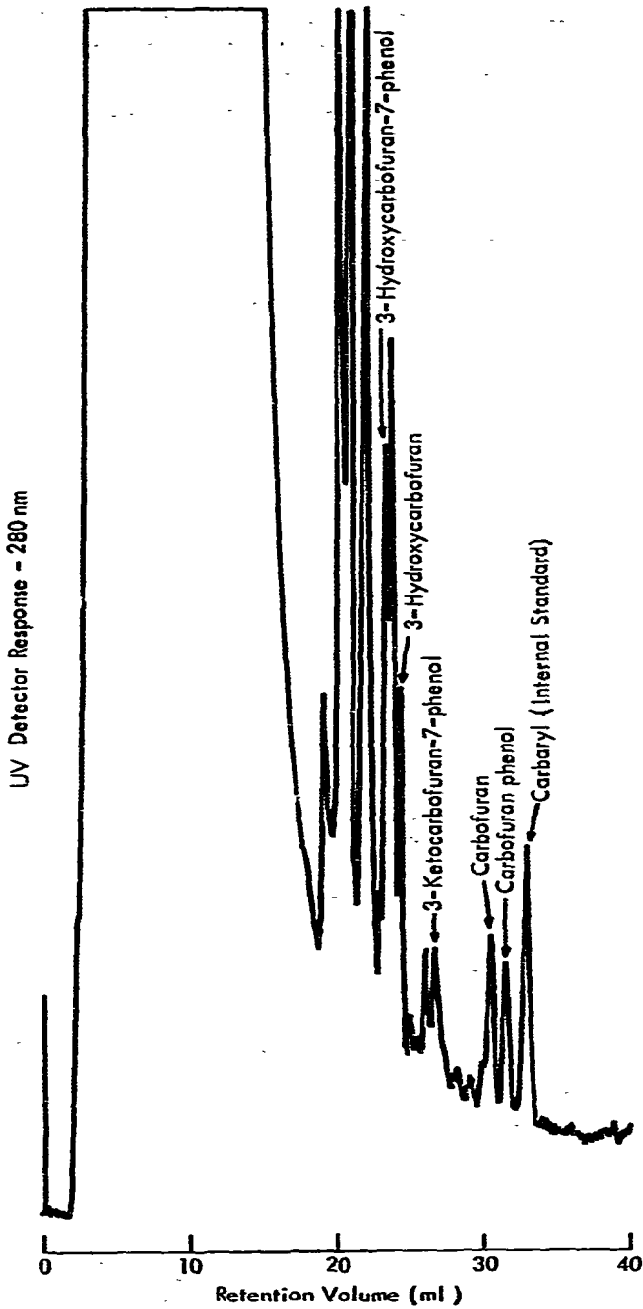
Fig. 5. Sample 06-029-0180 composite spiked at 5 ppb with carbofuran and metabolites.

extraction or derivatization procedures. If the volume of sample concentrated on the head of the column is increased, and this is highly possible, then even lower detection limits can be attained. The basic concept should be applicable, with some modifications, to other compounds with similar polarities and hydrophobic properties.

ACKNOWLEDGEMENT

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