A Rapid, Direct High-Performance Liquid Chromatographic Method for the Simultaneous Quantitative Determination of Carbaryl and α-Naphthol in Aqueous Samples

A direct, on-column enrichment technique for the high-performance liquid chromatographic quantitative analysis of aqueous samples of carbaryl and α-naphthol at the parts-per-billion level is described. A 2-mL volume of aqueous sample, contained in the sample loop of a Waters Associates U6K injector, is flushed onto the head of a 4.0 mm × 30 cm μBondapak C_{18} column, where the solutes are adsorbed and concentrated. Elution with a 60:40 methanol–water mobile phase at a flow rate of 1.0 mL/min provided good separation of carbaryl and α-naphthol from each other and from all unidentified UV-absorbing compounds present in the water. The utility of Sep-PAK C_{18} cartridges (Waters Associates) for storing field-collected aqueous carbaryl samples was demonstrated. Samples can be stored on the cartridges at ambient temperature for 5 days with subsequent recoveries of 98% and 89% for carbaryl and α-naphthol, respectively.

The simultaneous determination of carbaryl (I) and its hydrolysis product, α-naphthol (II), in aqueous, foliar, crop, and insect media has been accomplished by several different techniques. Common to all, however, is the general methodology of sample preparation prior to the final step of derivatization and/or separation–analysis, which usually involves an initial solvent extraction and concentration, followed by solvent partitioning, Florisil column chromatography, collection of the appropriate eluate, concentration, and finally analysis. Utilizing a modification of this cleanup procedure, Johnson and Stansbury (1965) isolated I and II from the benzene extracts of dead bees. Following Florisil chromatography, the eluate was chemically extracted and the concentrations of the separated carbaryl and α-naphthol were determined colorimetrically (*p*-nitrobenzenediazonium fluoroborate) with recoveries of 89% and 86%, respectively. The gas chromatographic (GC) analysis of the compounds of interest in natural water involved similar preliminary cleanup procedures except that Amberlite XAD-8 resin replaced Florisil and, following concentration of the appropriate eluate fraction, derivatization with heptafluorobutyric anhydride preceded GC analysis. Recoveries of 94–102% (carbaryl) and 82–90% (α-naphthol) were reported (Nagasawa et al., 1977). The efficacy of high-performance liquid chromatography (HPLC) for the separation of I and II was first reported by Sparacino and Hines (1976), who, using stock solutions, investigated the normal phase separation performance of 10-μm Si-10, CN-10, and NH_2-10 (Varian) columns with 2-propanol–heptane (preferred) and methylene chloride–heptane mobile phases. Simultaneously, Lawrence (1977) described a similar HPLC screening method (column, 5-μm LiChrosorb Si 60; solvent, 5% 2-propanol in isooctane) for I (II was not determined) in cabbage, corn, wheat, and potato after cleanup (vide supra) of the acetone-blended sample. Estimated recoveries were generally >70% at 0.1–1.0 ppm. To determine the amount of I in forest foliage, soil, sediment, and stream water, Pieper (1979) employed the cleanup procedure described and reversed-phase HPLC (column, 37–50-μm Bondapak C_{18}–Corasil; solvent, 40% acetonitrile in water). Recoveries were 89.5% (grass), 86.5% (geranium), 75% (aspen), 49.8% (Douglas fir), 99.7% (stream water), 106% (soil), and 106.5% (sediment).

As part of a study on the acute and subacute toxicity of carbaryl to beneficial aquatic insects, a rapid quantitative analytical method was required to follow the aqueous hydrolysis of I to II during the 24-h period. The half-life of I is dramatically affected by pH, being 15 min at pH 10 and 10.5 days at pH 7, and the rate of hydrolysis increases 2–3 times with a temperature increase from 20 to 30 °C (Aly and El-Dib, 1971). The simultaneous rapid determination of carbaryl (I) and α-naphthol (II) would permit, for the first time, the direct examination of the environmental effects (temperature, pH, sunlight) on the degradation of I, and implicit in this goal was the elimination of the multimanipulative time-consuming variable-temperature cleanup procedure. Particularly attractive was the possibility of developing the trace enrichment technique first suggested as a qualitative "screening" method for pesticide residues in drinking water by Waters Associates (1976). That the technique could be made quantitative was subsequently demonstrated by Schauwecker et al. (1977) in their study of peptides in urine. Utilizing some of the principles presented, a rapid quantitative HPLC enrichment/analysis procedure was developed for carbaryl (I) and α-naphthol (II) in the parts-per-billion (ppb) range in well water, the particulars of which we wish to report at this time.

EXPERIMENTAL SECTION

All analyses were performed on a Waters Model ALC/GPC 204 liquid chromatograph equipped with a Model 440 UV detector absorbing at 280 nm, a Model 660 solvent programmer, a Model 6000A pump, a Model M-45 pump, and a U6K universal injector with a standard 2-mL sample loop. A 4.0 mm × 30 cm μBondapak C_{18} column was employed, with HPLC-grade methanol and acetonitrile (Omni-Solve, MCB Reagents, filtered prior to use) and HPLC water (prepared by passing filtered, deionized water through a Bondapak C_{18}/Porasil B water cleanup column, Waters Associates).

The 2-mL sample loop of the U6K injector was completely filled with a filtered aqueous solution of carbaryl (I) and α-naphthol (II) by injecting an excess of the test solution. With the injector in the "inject" position, the mobile phase flushed the aqueous sample onto the column, thus adsorbing and concentrating the solutes at the head of the column. In this way an "enrichment" of the solutes was achieved, resulting in a sampling procedure capable of handling concentrations as low as 5 ppb. Aqueous sample blanks were also run with each mobile phase tested.

To optimize the analytical conditions, we determined the α values (separation factors) and the capacity factors (*k's*) for I and II as well as the UV-absorbing interfering substances found in the water. Mobile phases consisting of methanol–water and acetonitrile–water in ratios of 50:50,

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Table I. Elution Parameters for Test Solutes as a Function of Mobile-Phase Composition

mobile phase		k' [a] W	k' [a] carbaryl (I)	k' [a] α-naphthol (II)	α value [b] $\alpha_{I/W}$	α value [b] $\alpha_{II/I}$	R [c] $R_{I/W}$	R [c] $R_{II/I}$
CH_3OH-H_2O:	50:50	8.3	10.2	12.2	1.23	1.20	3.1	3.3
	60:40	5.5	5.9	7.0	1.08	1.19	1.5	3.2
	70:30	4.2	4.3	4.9	1.02	1.13	1.4	2.5
CH_3CN-H_2O:	50:50	5.8	6.1	6.6	1.05	1.08	1.1	2.0
	60:40 [d]	4.5-4.8	4.5	4.8	~1.0	~1.0	~1.0	~1.0
	70:30 [d]	3.6-5.0	3.7	3.9	~1.0	~1.0	~1.0	~1.0

[a] Calculated from $k' = (t - t_0)/t_0$, where $t = t_r$ of peak of interest and $t_0 = 1.6$ min (see Figure 1). [b] Calculated from $\alpha = k'_B/k'_A$. [c] Calculated from $R = \Delta k'/(w_A + w_B)$ where $\Delta k'$ is the difference in k' values of indicated peaks and w = the respective base widths. [d] Peaks coeluted.

60:40, and 70:30 were evaluated in this study at a flow rate of 1.0 mL/min, and the UV detector setting of 0.02 AUFS.

The method was quantitatively validated by running calibration curves using quaduplicate aqueous solutions of 5.0, 10.0, 15.0, and 20.0 ppb of I and II, which were injected 3 times. Detector response (Y) was measured in terms of peak height and peak area (peak height times width at half peak height).

Since the hydrolysis rate for I increases rapidly above pH 6.0 (Aly and El-Dib, 1971), and the pH of the well water used in the tests was 8.14, the effects of acidification and refrigeration on sample deterioration during workup and analysis were investigated. A test solution of I in well water was prepared, and one aliquot was immediately refrigerated, another acidified with acetic acid to approximately pH 3.5, and a third was held at 25 °C. After 24 h, these samples were analyzed and the percent recovery of carbaryl was determined. The utility of Sep-PAK C_{18} cartridges as possible "storage" containers for field-collected samples was determined as follows: Each cartridge was flushed with 5 mL of HPLC-grade acetonitrile, followed by 5 mL of purified water. A 2-mL aliquot of a 1.0-ppm solution of either I or II was adsorbed onto each of six cartridges, and the cartridges were stored in their aluminum foil lined packets at 25 °C. Triplicate cartridges were analyzed after periods of 24 h and 5 days by flushing the cartridge with 5 mL of HPLC-grade methanol, and the percent recovery of both compounds was determined by the method described herein, using an injection volume of 25 μL of methanol sample solution rather than the 2-mL volume used with aqueous samples.

RESULTS AND DISCUSSION

The k' values of carbaryl (I), α-naphthol (II), and the last eluting compound of those indigenous UV-absorbing well water compounds (designated "W") obtained by using various ratios of the mobile phases MeOH–H_2O and CH_3CN-H_2O are shown in Table I. With all the mobile phases tested, the unidentified organic UV-absorbing compounds present in the water eluted first, followed by I and II. Although some of the water–organic compounds were not retained on the column and were eluted with the solvent front, others were sufficiently retained to coelute with I and II in those solvents having higher concentrations of the stronger solvent CH_3CN (Ku and Freeman, 1977) such as 60:40 and 70:30 CH_3CN-H_2O. With 50:50 CH_3CN-H_2O, only marginal peak separation was achieved between W and carbaryl (I), as indicated by $\alpha_{I/W} = 1.05$. For carbaryl (I) and α-naphthol (II), the $\alpha_{II/I}$ value was 1.08 with the same solvent composition, and the resolution value (R) between the peaks of I and W of 1.1 proved to be unacceptable in as much as base-line separation between the two peaks was required. It is interesting to note that such a solvent system provided excellent resolution of carbofuran and its metabolite, 3-hydroxycarbofuran, on the same column (Sparacino and Hines, 1976) although the effect of this solvent combination on I and II was not reported.

When the weaker solvent MeOH was used as the organic modifier in the mobile phase, the 70:30 MeOH–H_2O ratio provided only marginal resolution of I and W ($\alpha_{I/W} = 1.02$; $R_{I/W} = 1.4$). Although the 50:50 solvent ratio resulted in excellent peak resolution ($R_{I/W} = 3.1$ and $R_{II/I} = 3.3$), the total time for analysis was greater than 20 min/sample, and it was felt that a decrease in analysis time could be realized without sacrificing resolution by increasing the organic solvent concentration. For a mobile composition of 60:40 MeOH–H_2O, k' values for the peaks of interest fell within the desired range of 1 to 10, with the values $\alpha_{I/W} = 1.08$ and $\alpha_{II/I} = 1.19$ while $R_{I/W} = 1.5$ and $R_{II/I} = 3.2$, indicating good peak separation of W and I and I and II. Equally important, the analysis time was decreased to approximately 13 min. Figure 1 shows typical chromatograms of a water blank and a test solution with 60:40 CH_3OH-H_2O, the negative deflection resulting from the large (2-mL) sample injection volume of water.

The calibration curves were computed by relating the response variables, peak height and peak area (y), and the known concentration of compound (x) by using the regression equation $y = Ax + B$. Correlation coefficients (r^2) of >0.99 were obtained for the regression equation, relating concentrations of carbaryl and α-naphthol to the mean peak height and mean peak area over the concentration range tested. The statistical data obtained are given in Table II. For comparison of the relative precision of the two methods for predicting the unknown weight of the compound as a function of detector response, the regression was inverted (Williams, 1959) and the number of determinations necessary to obtain the true weight ± 10% at least 95% of the time (m value) calculated. Peak area proved to be much more precise, requiring only two determinations as opposed to five or six with peak height. This analytical method has been successfully used to follow the hydrolysis of ppb concentrations of I to II during 24-h toxicity tests for a group of aquatic insects (McClelland, et al., 1981). The replicability of the method described in the actual toxicity studies was in agreement with the m values calculated for the standard solutions described above.

In those experiments designed to determine the effect of refrigeration and acidification, recovery of carbaryl from the untreated standard solution was only 27% after 24 h at 25 °C. Immediate refrigeration of the sample at 4 °C retarded sample deterioration to the extent that 94% of the carbaryl was recovered after 24 h. Recovery of carbaryl from the acidified (pH 3.5) sample after 24 h at 25 °C was still 97%, and acidification was judged to be adequate to

Table II. Statistical Relationships[a] of Concentration of Carbaryl and α-Naphthol and Peak Height and Area

method	compound	concn, (x), ppb	detector response (y) mean	SD	r^2	m	slope (A)	intercept (B)
concn vs. peak height[b]	carbaryl	5.0	24.6	1.7				
		10.0	48.2	1.0	0.992	4.04	5.16	−2.04
		15.0	75.2	1.3				
		20.0	101.6	4.9				
	α-naphthol	5.0	18.1	1.6				
		10.0	38.8	0.9	0.991	5.23	4.44	−4.92
		15.0	61.0	1.4				
		20.0	84.8	4.7				
concn vs. peak area[c]	carbaryl	5.0	73.9	4.4				
		10.0	146.2	1.6	0.997	1.25	15.12	−2.79
		15.0	225.1	4.0				
		20.0	299.7	7.6				
	α-naphthol	5.0	55.2	3.8				
		10.0	136.7	1.0	0.996	1.92	16.71	−30.62
		15.0	212.8	4.8				
		20.0	308.4	6.6				

[a] General equation is $y = Ax + B$; r^2 = square of correlation coefficient; m = number of determinations required for true concentration ± 10%, 95% of the time. [b] Peak height measured in millimeters. [c] Peak area = peak height times width at half-height.

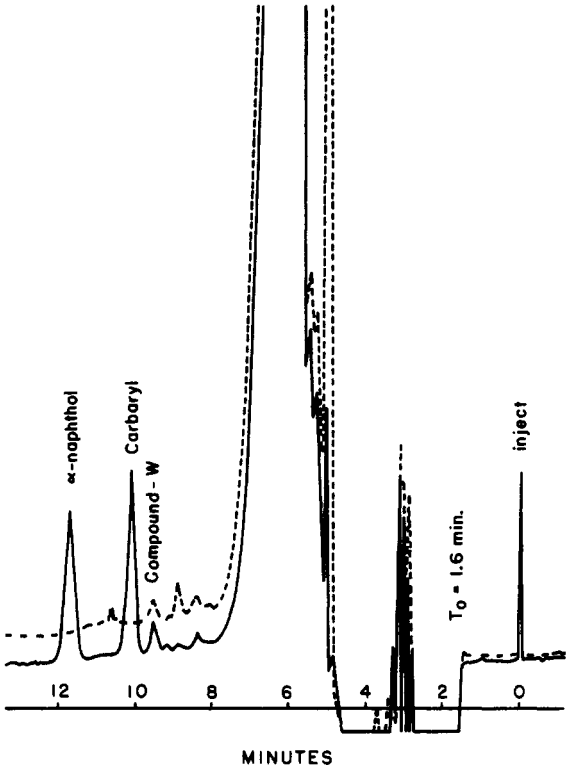

Figure 1. Liquid chromatograms of a 10-ppb aqueous solution of carbaryl (I) and α-naphthol (II) (—) and a water blank (- - -) on a μBondapak C_{18} column: mobile phase 60:40 methanol–water; flow rate 1.0 mL/min; sensitivity 0.02 AUFS. The negative deflection results from the large (2-mL) aqueous sample volume.

prevent sample deterioration in the laboratory toxicity studies where samples could be analyzed rapidly.

The results of the Sep-PAK "storage" studies were suprising. Recoveries of I and II from the cartridges were 100% and 97%, respectively, after 24 h of room temperature storage. Even more impressive was the fact that after 5 days of storage, 98% of carbaryl (I) and 89% of α-naphthol (II) were recovered unchanged. These cartridges would appear to offer a convenient method of storing aqueous samples collected on an extended field trip where analysis will be delayed and where facilities for refrigeration or storage of bulky aqueous samples are limited.

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Alice S. Jones*[1]
Louis A. Jones[2]
Felton L. Hastings[1]

[1]U.S. Department of Agriculture Forest Service
Southeastern Forest Experiment Station
Forestry Sciences Laboratory
Research Triangle Park, North Carolina 27709
[2]Department of Chemistry
North Carolina State University
Raleigh, North Carolina 27650

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