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Rapid on-line precolumn high-performance liquid chromatographic method for the determination of benomyl, carbendazim and aldicarb species in drinking water

CHRIS H. MARVIN[a] and IAN D. BRINDLE
Department of Chemistry, Brock University, St. Catharines, Ontario L2S 3A1 (Canada)
C. DAVID HALL
Ontario Ministry of the Environment, Rexdale, Ontario M9W 5L1 (Canada)
and
MIKIO CHIBA*
Research Station, Agriculture Canada, Vineland Station, Ontario L0R 2E0 (Canada)
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ABSTRACT

A reversed-phase high-performance liquid chromatographic (HPLC) method has been developed for the determination of trace concentrations of benomyl, carbendazim, aldicarb, aldicarb sulphoxide and aldicarb sulphone in drinking water. A 10-ml sample of water is passed through a 3-cm precolumn, packed with 5-μm C_8 sorbent, at a flow-rate of 5 ml/min. The HPLC system is then switched to an acetonitrile–water gradient elution program. The preconcentrated analytes are eluted from, and separated by, the 3-cm C_8 precolumn and determined by UV absorption. The total analytical time is 25 min. The lowest detectable concentrations are in the range of $2.5 \cdot 10^{-9} - 11.0 \cdot 10^{-9}$ g/ml for the five analytes investigated with 10 ml of sample.

INTRODUCTION

The use of solid sorbents for the preconcentration of organic pollutants in water is well documented [1,2]. When any of these solid sorbents is contained in a precolumn, and connected directly to an analytical column in a high-performance liquid chromatographic (HPLC) instrument, a technique known as on-line preconcentration results [3]. This method has received much recognition in the past several years owing to its relative simplicity, good sensitivity, and adaptability to automation [4,5].

Prior to the development of the combined technique, in which a precolumn and an analytical column were connected in series, sample water was passed through an analytical column in order to concentrate analytes at the head of the column packing.

[a] Present address: Department of Chemistry, McMaster University, Hamilton, Ontario L8S 4L8, Canada.

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Subsequent application of mobile phase to the analytical column resulted in the separation and determination of analytes [6,7]. This technique, a precursor to on-line preconcentration, is known as on-column preconcentration. Later analysts found it unwise to pass large volumes of aqueous sample through an analytical column as degradation of the column packing occurred. As a result, separate precolumns were installed to preconcentrate the analytes in the sample water [8].

A wide variety of commercially packed precolumns are now available. Goewie *et al.* [9] have shown that these precolumns can provide large numbers of theoretical plates. This fact presents the possibility of developing a rapid on-column preconcentration technique, employing a short precolumn, for both the preconcentration and separation of analytes.

In this paper, we report the development of an automated on-column preconcentration method for the determination of benomyl, its degradation product carbendazim (MBC), aldicarb, and its degradation products aldicarb sulphoxide and aldicarb sulphone. These analytes are of concern in Ontario environmental samples. Aldicarb (manufactured by Union Carbide under the tradename Temik) is quite toxic as evidenced by its LD_{50} value (for rats) of 0.6 mg/kg. Aldicarb sulphoxide and aldicarb sulphone are relatively polar and difficult to separate owing to their short retention times on reversed-phase material [10]. Residues of intact benomyl are difficult to determine because of its instability in organic solvent [11], and its low solubility in water [12]. Marvin *et al.* [13] have reported an automated on-line preconcentration technique or the determination of benomyl and MBC in water, and Chaput [10] has reported an on-line preconcentration method for the determination of aldicarb species in water.

Also reported in this paper are the factors affecting the use of the short precolumn for both the preconcentration and separation of the aforementioned analytes from water. These factors included: (1) size of packing material used in the precolumn; (2) properties of the solid sorbent phase; (3) sample volume; (4) minimum detectable concentrations; and (5) sample matrix.

EXPERIMENTAL

Solvents

Acetonitrile was of HPLC grade from Fisher Scientific (Fairlawn, NJ, USA), and Caledon Laboratories (Georgetown, Canada). Water used for preparation of standards was distilled in glass in the laboratory.

Pesticides

Solid aldicarb, aldicarb sulphoxide, and aldicarb sulphone standards were obtained from Union Carbide Agriculture Products (Research Triangle Park, NC, USA). Analytical standard of MBC and benomyl was obtained from Du Pont. Benomyl formulation was purchased commercially as Benlate wettable powder (WP) (Wilson Labs., Laval, Canada, 50% active ingredient). Using the method developed by Chiba and Singh [14], the active ingredient in the Benlate WP was determined to be 54.5%, of which 86% was benomyl and 14% was MBC.

The pesticides, listed in the order in which they appear in the chromatograms, are (**1**) aldicarb sulphoxide; (**2**) aldicarb sulphone; (**3**) aldicarb; (**4**) MBC; and (**5**) benomyl.

Preparation of stock standard solutions

Solid standards (with the exception of benomyl) were dissolved in methanol and diluted in methanol. Benomyl was prepared as a suspension of Benlate WP in distilled water.

As benomyl decomposes in water at room temperature [15], and the rate of decomposition is temperature dependent [16], benomyl standard solutions should be refrigerated. Benomyl standard suspensions containing more than its solubility in water, should be thoroughly stirred before dilution to ensure an even distribution of particulate matter in any aliquot removed.

The individual stock standard solutions were diluted with water at different concentrations because of their varying sensitivities to ultraviolet (UV) detection.

Water samples

Standard water samples were prepared by diluting the combined standard solutions (prepared as above) to 1000 ml with distilled water from the laboratory unless otherwise noted.

HPLC apparatus

The HPLC system consisted of a Waters Model 600 Powerline solvent delivery system, a Waters WISP Model 710B sample processor, a Waters Model 484 tunable absorbance UV detector, a Fisher Recordall series 5000 strip chart recorder, and an NEC Powermate 2 computer system (NEC Information Systems, Boxborough, MA, USA) incorporating Waters 810 chromatography software (Waters Assoc., Millford, MA, USA).

The factory-packed precolumns were 5-μm Spherisorb C_{18} (80 Å pore size), 5-μm C_8 (80 Å pore size), 7-μm C_{18} (300 Å pore size), and 5-μm CN (80 Å pore size) 3 cm × 4.6 mm I.D. cartridges from Brownlee Labs. (Santa Clara, CA, USA)[a]. The 10-μm C_{18} (300 Å pore size) 3-cm precolumns were laboratory packed using 10-μm Vydac Reverse Phase TP-201 (Separations Group, Hesperia, CA, USA).

The on-line preconcentration apparatus (Fig. 1) incorporated a high-pressure in-line filter with a 0.5-μm frit from Mandel Scientific (Guelph, Canada), and a Rheodyne Model 7000 two-position six-port switching valve, which was equipped with a Rheodyne Model 5701 air actuator controlled by a Rheodyne Model 7163 solenoid valve kit (Rheodyne, Cotati, CA, USA).

HPLC operating conditions

Wavelength, 220 nm; chart speed, 0.5 cm/min; detector sensitivity, 0.200 a.u.f.s. (1 mV = $1 \cdot 10^{-3}$ a.u.); recorder range, 10 mV f.s.; column temperature, ambient.

On-line preconcentration

A 10-ml volume of water sample was passed through the precolumn while the apparatus was in the "load" position unless otherwise noted.

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[a] The stationary phases currently available from Brownlee Labs. may differ from those used in this study.

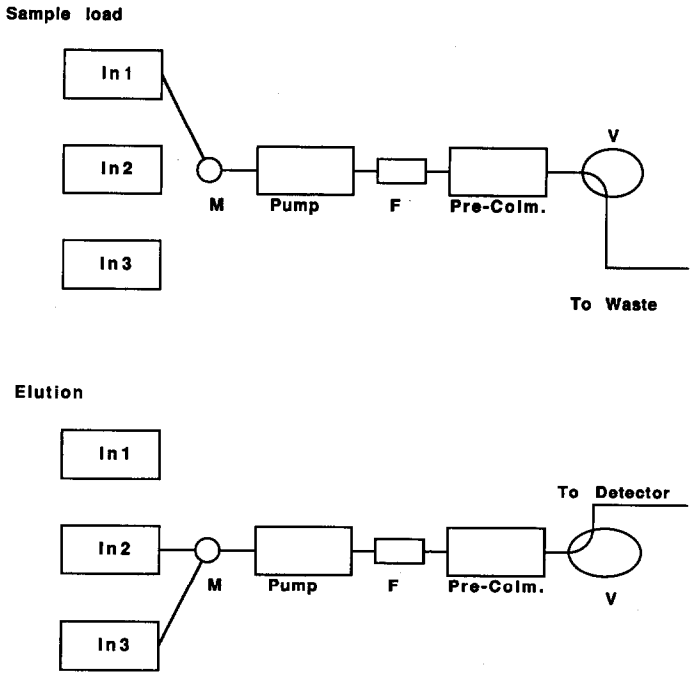

Fig. 1. Schematic diagram of the valve switching system and the directions of liquid flow. V, M, In and F = Valve, mixer, solvent inlets and filter, respectively; Pre-colm. = precolumn. During the sample loading step, In1 dispenses sample water. During the elution steps, In2 dispenses water, and In3 dispenses acetonitrile as part of the mobile phase.

Elution

The following gradient program was run after switching the valves of the "elute" position from the "load" position:

Elapsed time (min)	Flow-rate (ml/min)	Acetonitrile (%)	Water (%)	Curve
Initial	1.0	10	90	
2.5	1.0	10	90	
10.0	1.0	60	40	6
10.5	1.5	60	40	11
15.0	1.5	70	30	6
20.0	1.0	10	90	6

Curve 6 denotes a linear change and curve 11 denotes an immediate change to the described conditions.

RESULTS AND DISCUSSION

The on-line preconcentration valving apparatus is shown in Fig. 1. This apparatus is a modification of the one described in previous papers by Marvin and co-

workers [5,13], but is substantially simpler as only unidirectional elution is required. A simple valving apparatus employing only one valve is sufficient.

A 5-μm C_8, a 5-μm C_{18}, a 7-μm C_{18}, and a 5-μm CN sorbent were investigated for both their retention properties and their abilities to provide adequate separation of the analytes. There was little difference in the degree of retention of the analytes on the 5-μm C_8 and C_{18} precolumns, but the C_8 packing demonstrated superior resolution of MBC and aldicarb. The use of a 5-μm CN or a 7-μm C_{18} resulted in very poor seperation of aldicarb sulphoxide and aldicarb sulphone. A 10-μm C_{18} stationary phase was also investigated, but dit not provide enough theoretical plates to result in adequate separation of the aldicarb sulphoxide and aldicarb sulphone.

Table I shows the pesticides used in the study, their retention times, a comparison of peak areas between those obtained from a preconcentration sample and those obtained by a straight injection of a concentrated standard containing an equal amount of the pesticides, the sample pesticide concentrations, and the minimum detectable concentrations for a 10-ml sample. The minimum detectable concentrations (MDC values) were calculated using a 5:1 signal-to-baseline-noise ratio. The peak-area standard deviations for five replicate samples averaged approximately 3% for each of the analytes.

A sample volume of 7.5 ml was found to result in the best resolution of aldicarb sulphoxide and aldicarb sulphone. Volumes greater than 10 ml result not only in the loss of aldicarb sulphoxide and aldicarb sulphone due to breakthrough, but also gave poorer separation of the two early-eluting analytes. These results are in agreement with those of Chaput [10]. Chaput calculated a 50% loss in theoretical plate number for sample volumes of 20 ml compared with volumes of 10 ml. This theoretical plate loss is due to broadening of the weakly retained analyte peaks as the volume of sample passed through the precolumn increases. This results in little or no separation of the aldicarb sulphoxide and aldicarb sulphone when the maximum sample volume of 10 ml is exceeded.

On the basis of these observations, we judged that a sample volume of 10 ml,

TABLE I

SELECTED PESTICIDES, THEIR RETENTION TIMES (t_R) AVERAGE PERCENTAGE PEAK AREAS ± S.D. AS COMPARED WITH THOSE OBTAINED FROM A STRAIGHT INJECTION OF EQUAL AMOUNTS OF THE PESTICIDES, SAMPLE CONCENTRATIONS AND MDC VALUES FOR A 10-ml SAMPLE AT 220 nm

The data were obtained from five replicate 10-ml samples using the described method and a 5-μm C_8 precolumn. The MDC values were calculated using a 5:1 signal-to-baseline-noise ratio.

No.[a]	Pesticide	t_R (min)	Percentage peak area ± S.D. (%)	Sample concentrations (10^{-9} g/ml)	MDC (10^{-9} g/ml)
1	Aldicarb sulphoxide	4.1	92±2	138	7.0
2	Aldicarb sulphone	4.7	102±3	117	9.0
3	Aldicarb	9.2	104±1	112	11.0
4	MBC	10.3	95±4	47	2.5
5	Benomyl	12.7	94±5	68	9.0

[a] The pesticides are numbered to correspond with those in the figures.

loaded at 5 ml/min onto a 5-μm C_8 precolumn, to be the best combination of experimental conditions. Fig. 2 shows a chromatogram resulting from a distilled water sample containing the five compounds of interest. The sample was analyzed under the aforementioned conditions. The total analytical time is 25 min for one sample. It is interesting to note that MBC can be determined without the use of a buffered mobile phase, which had been necessary in the past to produce a sharp MBC peak for accurate quantitation [13].

Other analysts studying on-line preconcentration [5,10,17,18] have observed that the chromatograms resulting from the analysis of municipal tap waters or natural groundwaters are characterized by large peaks resulting from early-eluting impurities. This was of concern as aldicarb sulphoxide and aldicarb sulphone are eluted on the downslope of these impurities. It was found that this problem could be partially overcome by using UV detection at wavelengths greater than 220 nm. The impurities are much less sensitive to UV detection at wavelengths greater than 230 nm. With the exception of aldicarb sulphone, the other four analytes could be determined at longer wavelengths with substantially less interference from sample matrix impurities. Fig. 3 compares two chromatograms resulting from the analysis of a municipal tap water sample containing 25 ppb (10^{-9} g/ml) of MBC and 100 ppb of benomyl at 220 and 280 nm. The compounds are well separated as sharp peaks in both chromatograms, but the background is substantially better in Fig. 3b (produced at 280 nm) compared to Fig. 3a (produced at 220 nm). Relative peak heights of MBC and benomyl at 280 nm are 60.3 and 94.3%, respectively, of those at 220 nm. Similarly, at 254 nm peak

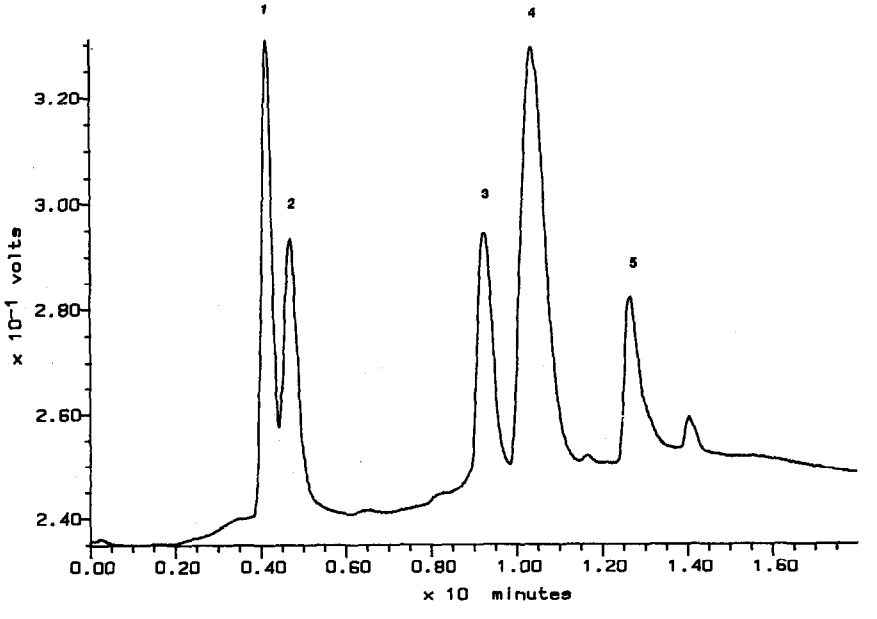

Fig. 2. Chromatogram resulting from the analysis of a 10-ml sample containing the five analytes. The analysis was performed on a 5-μm C_8 precolumn using the described method. The concentrations of the individual pesticides are those listed in Table I. For peak identification, see text.

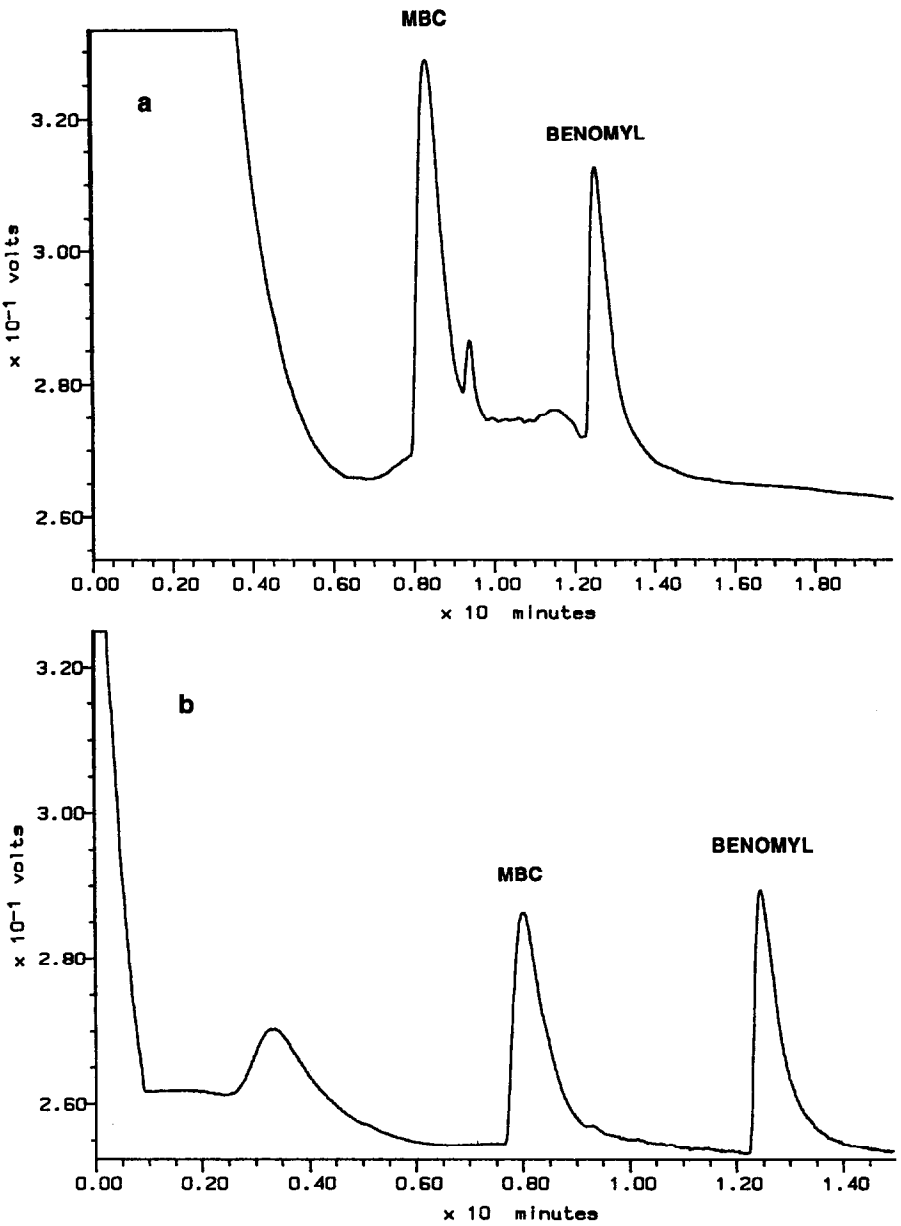

Fig. 3. A comparison of samples containing residues of MBC and benomyl only, at two different UV wavelengths: (a) 220; (b) 280 nm. The samples were analyzed using the described method.

heights of aldicarb sulphoxide and aldicarb were 96.0 and 78.0%, respectively, of those at 220 nm.

Additional experiments with sample water containing only two of the less polar analytes, MBC and benomyl, have shown that sample sizes of the order of 100 ml can

be analyzed using the described method. Even with these larger sample sizes, separation of the analytes was good and the peak profiles were sharp enough for accurate quantitation. The larger sample sizes result in significantly lower MDC values, although the period of time required for analysis becomes longer.

CONCLUSIONS

Our results indicate that a 3-cm precolumn packed with 5-μm C_8 is best. Precolumns packed with 5-μm C_{18}, 10-μm C_{18}, 5-μm CN and 7-μm C_{18} precolumns were less effective. Under the described conditions, the analytes can be detected at the 10 ppb level using 10 ml of sample water. The total analysis time is 25 min.

The on-line preconcentration method described in this paper using a 3-cm precolumn is applicable as a simple, rapid, inexpensive, qualitative and quantitative procedure for all five analytes of interest. Even difficult separations, such as that of aldicarb sulphoxide and aldicarb sulphone, can be accomplished effectively. The method is sensitive and will provide reliable and reproducible results for practical applications. The method also offers the possibility of complete automation for the analysis of many samples.

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