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Note

Simultaneous determination of trace concentrations of benomyl, carbendazim (MBC) and nine other pesticides in water using an automated on-line pre-concentration high-performance liquid chromatographic method

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Methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate (benomyl) is used worldwide as a systemic fungicide for disease control in crops. The analysis of benomyl residues in water is made difficult by the varying instability of the compound in different organic solvents [1–3] and its low solubility in water [4]. Benomyl also decomposes in water, but at a rate slower than that in organic solvent [5,6].

High-performance liquid chromatographic (HPLC) methods are most popular for the analysis of benomyl but most employ the determination of the degradation product methyl 2-benzimidazolecarbamate (carbendazim or MBC) after quantitative conversion of the parent compound [7–9]. These techniques are lacking in that the MBC that is produced from the parent benomyl during the sample preparation procedure cannot be distinguished from MBC that was present in the sample as a natural degradation product of benomyl. This methodology, which is not acceptable in principle, has been widely used in the past, however, for the following two reasons. The main reason is that the determination of intact benomyl residues is exceptionally difficult. Another reason is that MBC is also fungitoxic, and the fungitoxicity of benomyl is, in fact, thought to be due to the presence of MBC [10].

An HPLC method for the simultaneous determination of benomyl and MBC in aqueous media has been described [11]. Benomyl is quantitatively converted by treatment with base to 3-butyl-2,4-dioxo-*s*-triazino[1,2-*a*]benzimidazole (STB), while

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MBC present in the sample matrix is unaffected and is determined as MBC. This technique is suitable for the analysis of benomyl and MBC at the low ppm level.

In this paper, we present an automated method for the simultaneous determination of benomyl, MBC (carbendazim) and nine other presticides at the ppb[a] level. Benomyl is determined as the intact parent compound and any MBC in the sample can be determined exclusively as the natural degradation product.

For reasons discussed above, it would be advantageous to minimize exposure of the sample to any organic solvent during the sample preparation procedure. On-line pre-concentration (or trace enrichment) offers the possibility of isolating intact analytes directly from an aqueous sample matrix by retaining them on a solid sorbent contained in a short pre-column. A subsequent valve switching allows mobile phase to flush analytes from the pre-column to the HPLC analytical column without further sample manipulation. Marvin *et al.* [12] have described an automated on-line pre-concentration method for the determination of pesticide residues in drinking water in conjunction with HPLC and UV detection. With the inclusion of a buffered mobile phase, the technique has been modified to include benomyl, MBC and aminocarb, as well as the eight pesticides included in the original study. These include propoxur, carbofuran, carbaryl, propham, captan, chloropropham, barban and butylate. All of the aforementioned pesticides are of concern in Ontario environmental samples.

MATERIALS AND METHODS

Solvents

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

Preparation of buffer solutions

Solutions of Na_2HPO_4 and KH_2PO_4 were prepared individually at 0.067 *M*. The two resulting solutions were mixed at 3:2 (v/v) and the pH adjusted to 6.8. This solution was diluted to 5% in water.

Pesticides

Solid pesticide standards were obtained from the United States Environmental Protection Agency, Research Triangle Park, NC, U.S.A. Purities of the individual standards ranged from 97.5 to 100%. Benomyl was purchased commercially as Benlate wettable powder (Wilson Labs., Laval, Canada, 50% active ingredient). The pesticides, listed in the order in which they appear in the chromatograms, are (1) MBC, (2) aminocarb, (3) propoxur, (4) carbofuran, (5) carbaryl, (6) propham, (7) captan, (8) chloropropham, (9) barban, (10) benomyl and (11) butylate.

Preparation of stock standard solutions

Solid standards (with the exception of benomyl and MBC) were dissolved in acetonitrile and diluted in acetonitrile. MBC was dissolved in methanol and diluted in

[a] Throughout this article, the American billion (10^9) is meant.

methanol while benomyl was prepared as a suspension in distilled water.

As benomyl decomposes at room temperature [13], benomyl standard solutions should be refigerated. Benomyl standard suspensions containing benomyl, at greater concentrations than its solubility in water, must be thoroughly stirred before dilution to ensure an even distribution of particulate matter in any aliquot removed.

The individual stock standard solutions were combined at different concentrations because of their varying sensitivities to UV detection. The combined standard solution thus prepared was diluted with water to make standard water samples as below.

Water samples

Standard water samples were prepared by diluting 1 ml of the combined standard solution (prepared as above) to 1000 ml with distilled water from the laboratory unless otherwise noted.

HPLC apparatus

The HPLC system consisted of a Waters (Milford, MA, U.S.A.) 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 (Boxborough, MA, U.S.A.) Powermate 2 computer system incorporating Waters 810 chromatography software.

Pre-columns were 5-μm Spherisorb C_{18} and C_8 3 cm × 4.6 mm I.D. cartridges from Brownlee Labs. (Santa Clara, CA, U.S.A.). Analytical columns were a Supelcosil LC-8 5-μm 25 cm × 4.6 mm I.D. (Supelco, Bellefonte, PA, U.S.A.), and a Phenomenex Spherisorb C_{18} 5-μm 15 cm × 4.6 mm I.D. (Phenomenex, Torrance, CA, U.S.A.).

The on-line pre-concentration apparatus (Fig. 1) in corporated two high-pressure in-line filters with 0.5-μm frits from Mandel Scientific (Guelph, Ontario, Canada), and three Rheodyne Model 7000 2 position 6-port switching valves, one of which was equipped with a Rheodyne Model 5701 air actuator controlled by a Rheodyne Model 7163 solenoid valve kit (Rheodyne, Cotati, CA, U.S.A.).

Unidirectional elution from a C_{18} pre-column onto a C_{18} analytical column was used as a standard procedure.

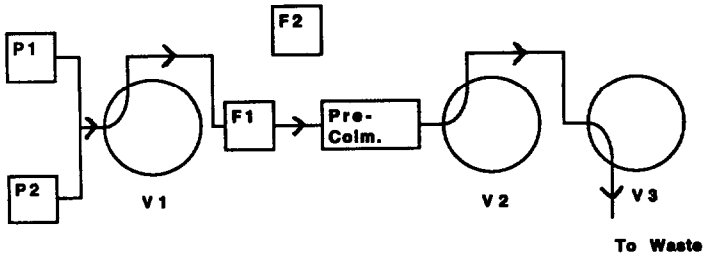
HPLC operating conditions

Wavelength, 220 nm; flow-rate, 1.5 ml/min; chart speed, 0.5 cm/min; detector sensitivity, 0.075 A.U.F.S. (1 mV = $1 \cdot 10^{-3}$ A.U.); recorder range, 10 mV F.S.; column temperature, ambient.

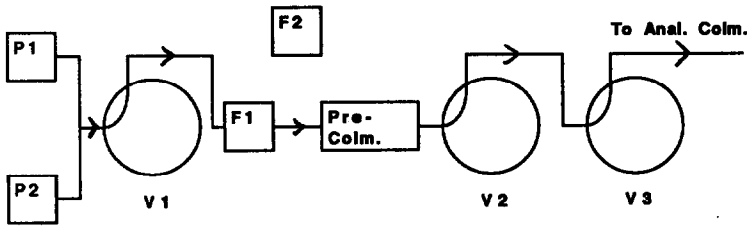
On-line pre-concentration

A 100-ml volume of water sample was passed through the pre-column while the apparatus was in the "load" position unless otherwise noted.

SAMPLE LOAD



UNIDIRECTIONAL ELUTION



BACKFLUSH ELUTION

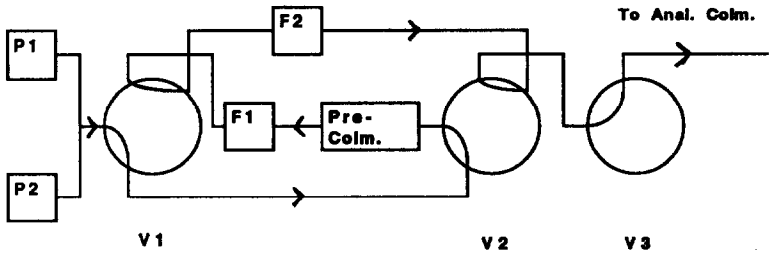


Fig. 1. Schematic of the valve-switching system. V, P, and F denote valves, pumps and filters, respectively. During the sample loading step, P1 dispenses sample. During the elution steps, P1 dispenses water, and P2 acetonitrile as part of the mobile phase. Pre-Colm. = Pre-column; Anal. Colm. = analytical column.

Elution

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

Elapsed time (min)	*Composition of mobile phase: acetonitrile–buffer–water*
Inital	30:70:0
5	30:70:0
15	60:30:10
25	60:30:10
30	30:50:20
35	30:70:0

Changes in the percentage of organic solvent in the mobile phase throughout the gradient program occurred linearly. The final 10 min of the gradient program serve to return the system to the initial conditions to enable another analysis run.

The inclusion of water in the mobile phase, resulting in a ternary gradient system, was essential in order to maintain a flat baseline profile throughout the gradient program. Decreasing the buffer strength in the aqueous phase during increases in the percentage of acetonitrile, results in an optimum baseline profile. If water was not included in the mobile phase, thereby resulting in a binary gradient system, a marked disturbance in the baseline (a huge bump) was unavoidable. Most analyte peaks appeared on the up and down slopes, as well as at the top of the raised baseline. The baseline disturbance can, however, be negated through the use of gradient correction (baseline subtraction).

RESULTS AND DISCUSSION

Fig. 2 shows a chromatogram resulting from the analysis of a distilled water sample containing the eleven pesticides of concern in the study using unidirectional

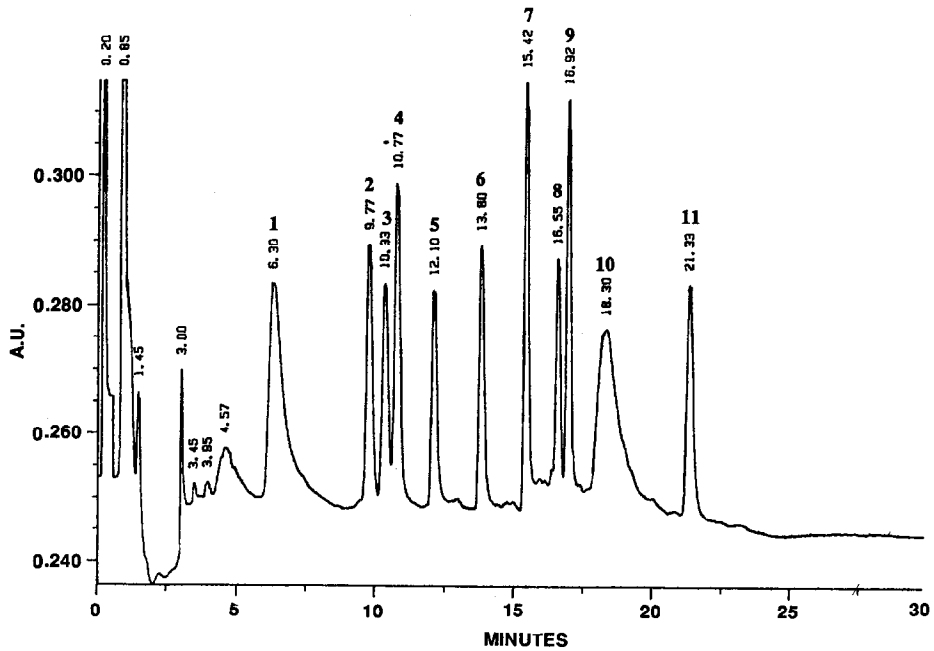


Fig. 2. Chromatogram resulting from the analysis of a 100-ml sample by the described method. The concentrations of the individual pesticides are the same as those listed in Table I.

elution and a C_{18} stationary phase in both the pre-column and the analytical column. Table I lists the pesticides, their retention times, sample concentrations, and minimum detectable concentrations. The minimum detectable concentrations were calculated using a 3:1 signal-to-noise ratio with the exception of benomyl and MBC. The minimum detectable concentrations for benomyl and MBC were calculated using a 6:1 signal-to-noise ratio due to the broadness of the peak profiles. It was essentially impossible to obtain a chromatogram of benomyl without the presence of MBC. Even freshly prepared benomyl analytical standards, prepared in water, were observed to contain a trace amount of MBC.

TABLE I

SELECTED PESTICIDES, THEIR RETENTION TIMES, SAMPLE CONCENTRATIONS AND MINIMUM DETECTABLE CONCENTRATIONS FOR A 100-ml SAMPLE

The pesticides are numbered to coincide with those in the figures.

Compound	Retention time (min)	Sample concentration (μg/l)	Minimum detectable concentration (ng/l)
(1) MBC (carbendazim)	6.30	2.5	100
(2) Aminocarb	9.77	4.0	65
(3) Propoxur	10.33	4.0	65
(4) Carbofuran	10.77	4.5	70
(5) Carbaryl	12.10	0.5	10
(6) Propham	13.80	2.5	50
(7) Captan	15.42	20.0	460
(8) Cl-Propham	16.55	2.0	30
(9) Barban	16.92	3.0	40
(10) Benomyl	18.30	8.0	500
(11) Butylate	21.33	5.0	150

A buffered mobile phase is necessary for the gradient elution program. Otherwise, the peak profile of MBC is unacceptably broad for quantitation. A ternary gradient of acetonitrile, buffer, and water, was found to produce an optimum gradient profile allowing for accurate quantitation of the analytes. A binary gradient of acetonitrile and buffer can be used if the option of gradient correction is available to the analyst. Even with the buffered mobile phase, peak profiles for benomyl and MBC were much broader than the other compounds. These peak profiles were not improved even when other experimental conditions were investigated. Experiments with a more polar pre-column stationary phase (C_8), backflush and unidirectional elution, and a different analytical column stationary phase (C_8), failed to improve peak profiles for MBC and benomyl. The use of a C_8 analytical column improves separation of the three earlier eluting pesticides (aminocarb, propoxur, and carbofuran) but the peak profile for benomyl is poor and co-elutes with butylate. Unidirectional elution from a C_{18} pre-column onto a C_{18} analytical column was found to be the best combination of experimental conditions.

As shown in Fig. 2, peak widths of MBC and benomyl are substantially broader

than those of the other compounds when using unidirectional elution. As concentrations and volumes of water sample (and accordingly, sample loading time) are increased, peak widths increased. In contrast, peak widths of MBC and benomyl are substantially better if streight injections of 100-μl 8.0-ppm benomyl standard suspensions are made directly onto the analytical column without passing through the pre-column. It appears that the band broadening, apparent in the pre-concentration chromatograms, is a results of a large volume of sample loading.

Results obtained with backflush elution were substantially different from those obtained with unidirectional elution. As shown in Fig. 3, four peaks were observed with a sample that contained only MBC and benomyl. It is clear that both the MBC and the benomyl displayed two peaks each. Of the two benomyl peaks, the first peak represents benomyl eluted from particulate matter retained on the inlet side of the pre-column [either on the 0.50-μm filter (F1) or at the head of the pre-column in Fig. 1]. The second peak represents benomyl which is present as solute in water and adsorbed on the pre-column. This presents the possibility of a method for the quantitation of benomyl and MBC both as solute and in the solid state in water samples. It is interesting to note that the peak shape of the first benomyl peak is substantially sharper than the second. Similar results were observed with MBC.

In the above example, benomyl was analysed at 0.136 ppm (Fig. 3). MBC was present in the sample as the degradation product of benomyl. The solubilities of MBC and benomyl are reported to be approximately 3 ppm in water [4]; thus the concentrations of benomyl and MBC were well within the solubilities of the analytes in water.

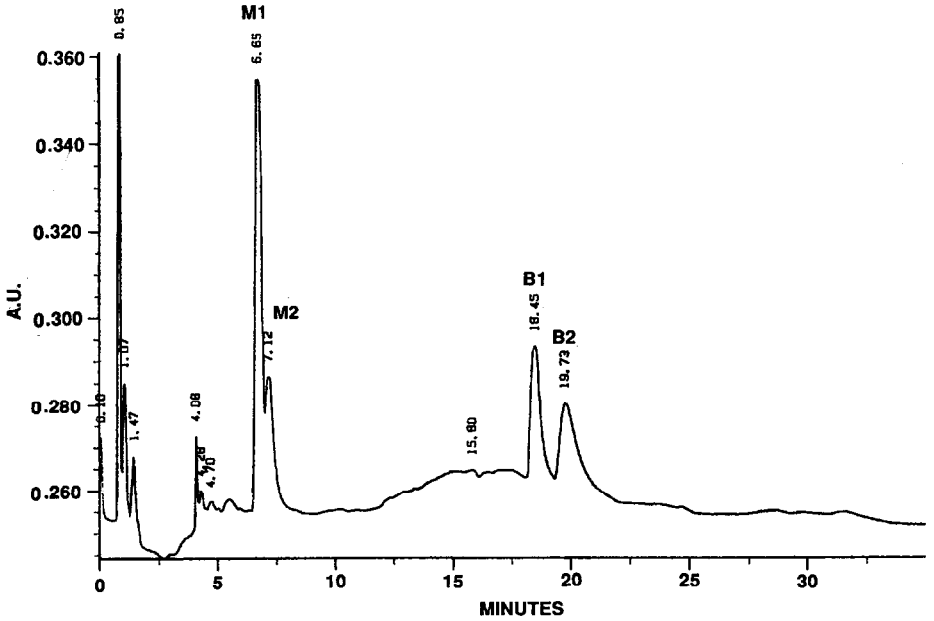


Fig. 3. Chromatogram resulting from the analysis of a 10-ml 0.136-ppm benomyl suspension. Peaks (M1, B1) correspond to MBC and benomyl eluted from particulate matter. Peaks (M2, B2) correspond to MBC and benomyl present as solutes. The sample was analysed by the described method, except that backflush elution was used.

The sample analysis still yielded four peaks, indicating that the analytes were present in the particulate phase, as well as being present as solutes. This may be because the sample was prepared by a 100:1 dilution of a 13.6-ppm stock solution of benomyl and analysed immediately. It appears, therefore, that a period of time is needed for the benomyl to fully dissolve in the water matrix. Further evidence to support this was the observation of a substantial decrease in the area of the first peak, and an increase in the area of the second peak, when the same sample was analysed 45 min later. The sum of the areas of the first and second benomyl peaks were equal for both sample runs. Unidirectional elution yielded a large single peak with a peak area equal to that of the sum of the two peaks obtained from backflush elution.

These findings reveal that consideration must be given to the basic handling procedures of water samples regarding the need for filtration. Depending on the pore size of the filters, regardless of whether they are used on-line or not, analytical results may be substantially different. This is important when analysing samples for compounds which have very low solubilities in water. Benomyl is one of the compounds which merits this consideration, as it is used at the 250–1000-ppm range in agriculture.

The peak profiles of MBC and benomyl can be much improved by elution with a higher percentage of organic solvent in the mobile phase but this results in poor resolution of the other analytes. If benomyl and MBC are the only compounds to be analysed, both compounds can be eluted as sharp peaks by using a gradient program in which the mobile phase contains a higher initial percentage of acetonitrile. Accordingly, the minimum detectable concentrations can be lowered substantially.

CONCLUSION

The method presented in this paper is accurate, sensitive and reproducible. Benomyl in its intact form and MBC, the degradation compound, can be quantitated simultaneously at the low ppb level. Both MBC and benomyl which are present as particulate matter in water can also be quantitated separately if desired.

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