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Short Communication

Determination and confirmation of benomyl and carbendazim in water using high-performance liquid chromatography and diode array detection

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ABSTRACT

This paper describes a direct determination of benomyl and carbendazim in a single analytical run and their confirmation by absorption spectra using diode array detection. A simple technique has been developed for preparation of benomyl calibration standard. Benomyl was dissolved in chloroform prior to a high dilution in water for HPLC.

Detection limits, precision, and accuracy of data are presented. The method is simple, rapid, accurate, and precise.

INTRODUCTION

Benomyl [methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate] is used as a systemic fungicide and miticide in agricultural crops. It is marketed with the trademark of Benlate which contains 50% benomyl. It is stable in the dry state but splits into butyl isocyanate to form carbendazim (methyl 2-benzimidazolecarbamate) or MBC in acid solution and organic solvents. Both benomyl and MBC are considered to be fungitoxic.

Most of the analytical methods used for determining benomyl involve conversion of benomyl to carbendazim [1–4] or formation of its derivative [5]. Direct analysis of benomyl as parent molecule has been difficult because of its instability in solvents [6,7] and poor solubility in water [8].

EXPERIMENTAL

Apparatus

Analytical procedures were performed using a Hewlett-Packard 1090 liquid chromatograph equipped with a Hewlett-Packard diode array detector, PV5 solvent delivery system, autoinjector and a Hewlett-Packard 79994A "Chem Station" for data acquisition. A Brownlee cyano column (25 cm × 4.6 mm × 5 μm) was obtained from Rainin (Emeryville, CA, USA). A filter (0.45 μm particle size) and a guard column of the same composition were used on-line before the column.

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Reagents

Benomyl (99.9%) was purchased from Ultra Scientific (North Kingston, RI, USA). Carbendazim (99.0%) was obtained from the Pesticides and Industrial Chemical Repository, United States Environmental Protection Agency Repository. Chloroform and ammonium acetate were obtained from Aldrich (Milwaukee, WI, USA).

Procedure

Carbendazim stock standard solution was prepared by weighing 10 mg of pure carbendazim and dissolving in 10 ml methanol. This stock standard, when stored at 4°C and protected from light, is stable for six months. Aqueous standards ranging from 5 μg/l to 2 mg/l were prepared by spiking 10 ml of water with different volumes of stock standard. A calibration curve of concentration versus area counts was prepared.

Standard solution of benomyl (1000 or 100 mg/l) was prepared by dissolving 10 mg in 10 or 100 ml chloroform. Immediately 20 μl of the 1000 mg/l standard solution were spiked into 10 ml reagent water and shaken vigorously for 20 s to dissolve the chloroform solution and then 200 μl of this aqueous calibration standard (2 mg benomyl/l) were injected immediately into the HPLC system for analysis. Similarly, each aqueous standard ranging in concentration from 5 μg/l to 2 mg/l was prepared from neat benomyl powder and analyzed immediately so that contact of benomyl with chloroform and water before injection into the HPLC system was achieved in 1–2 min. Only 1 to 20 μl chloroform for aqueous standard preparation was used because chloroform has limited solubility (0.5% V/V) in water. The standard solution of benomyl (100 mg/l) mentioned above was used for the preparation aqueous calibration standards at concentration levels of <200 μg/l. The benomyl calibration curve was established after adjusting the benomyl loss by conversion to carbendazim, if any, as follows:

benomyl concentration that gives response (mg/l) = benomyl concentration prepared (mg/l) − [carbendazim concentration (mg/l) × 1.52]

Carbendazim concentration refers to the concentraton of carbendazim peak which appeared in the benomyl standard chromatogram as calculated from the carbendazim calibration curve prepared above.

The standard solutions, spiked reagent water, drinking water, and wastewater were analyzed using 200 μl injections with methanol–0.05 *M* ammonium acetate (35:65, v/v) as the mobile phase at 1 ml/min and 25°C temperature. The diode array detector was scanned from 210 to 400 nm wavelength to get the absorption spectra and the 286 nm wavelength was used to monitor the quantitation. Molar absorptivities for benomyl and carbendazim, dissolved in chloroform and methanol respectively, were determined at 286 nm wavelength. The detection limit was established by using the lowest concentration of the analyte that gives a 2:1 signal-to-noise ratio.

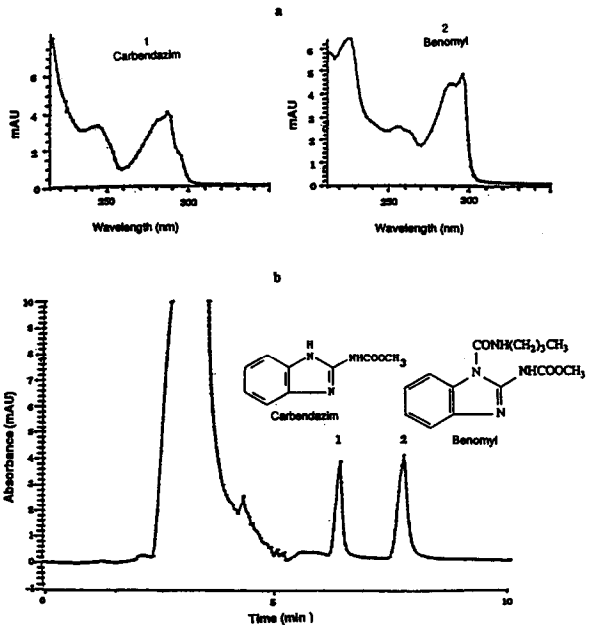

Fig. 1. (a) Diode array spectra for carbendazim and benomyl corresponding to chromatographic peaks 1 and 2, respectively, in (b) UV absorption chromatogram at 286 nm showing carbendazim (1) and benomyl (2) in wastewater at 100 μg/l each using Brownlee cyano column (25 cm × 4.6 mm × 5 μm particle size), solvent methanol–0.05 *M* ammonium acetate (35:65, v/v), 1 ml/min flow-rate, 25°C.

TABLE I

RECOVERY OF BENOMYL AND CARBENDAZIM FROM SPIKED DRINKING WATER AND WASTE WATER

Analyte	Concentration (μg/l)	Number of replicates	Average recovery (%)	Relative standard deviation (%)
Tap drinking water				
Benomyl	5.0	7	109	9.0
	50	7	106	10
Carbendazim	5.0	7	115	10
	50	7	119	14
Waste Water				
Benomyl	50	7	97	9.5
	100	7	93	11
Carbendazim	50	7	107	9.2
	100	7	103	14

RESULTS AND DISCUSSION

Separation and quantitation

Fig. 1 illustrates the separation of benomyl and carbendazim in wastewater. The separation was achieved in 10 min. The high molar absorptivities of benomyl (1.9×10^4) and carbendazim (1.3×10^4) at 286 nm wavelength facilitated an otherwise sensitive assay. The limits of detection for benomyl and carbendazim were 1 μg/l each. Benomyl standard solutions in all solvents, including water, always show some degree of conversion to carbendazim; a correction therefore has to be made. When each calibration aqueous standard at different concentration level was analyzed, only $3.5 \pm 1\%$ ($n = 5$) conversion of benomyl to carbendazim had occurred. Chloroform was used as a solvent because it dissolves benomyl quickly and yet does not accelerate its decomposition [9]. Instrumental response was demonstrated to be sufficiently linear over a range of 1 ng to 4 μg for both benomyl and carbendazim with a correlation coefficient of > 0.99.

Method evaluation

In Table 1 the recovery and precision data for the wastewater samples compared favourably with that from the drinking water samples. This shows that the matrix effect in these cases was minimal. Confirmation of peak identity with a high degree of confidence is possible by comparing the retention times and UV spectra of benomyl and carbendazim (Fig. 1a). The minimum amounts of these analytes required by diode array detection to provide a UV spectra of sufficient quality to permit identification were determined to be 2 ng for carbendazim and 10 ng for benomyl, respectively.

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