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SHORT COMMUNICATIONS

Stabilization of Methyl [1-(Butylcarbamoyl)-1*H*-benzimidazol-2-yl]carbamate (Benomyl) in Hydrochloric Acid Solutions

INTRODUCTION

The systemic fungicide benomyl [methyl [1-(butylcarbamoyl)-1*H*-benzimidazol-2-yl]carbamate] has been successfully used for the control of many plant diseases for many years (Chiba et al., 1987; Delp, 1987; Hall, 1980; Hammerschlag and Sisler, 1973; Köller et al., 1982). According to a recent paper (Liu et al., 1990) the U.S. National Research Council has estimated the use of benomyl at 2 000 000 lbs of active ingredient per year. Because of its widespread use and suspected carcinogenic activity, the determination of benomyl in various crops, such as apples, peaches, oranges, and tomatoes, and water is frequently required.

Due to its instability in water and organic solvents (Chiba and Doornbos, 1974; Chiba, 1977; Chiba and Cherniak, 1978), benomyl was determined by many workers as its stable degradation compound, carbendazim (methyl 1*H*-benzimidazol-2-yl-carbamate, commonly known as MBC). The determination was made by high-performance liquid chromatography (HPLC) after quantitative conversion of the parent benomyl to carbendazim (Austin et al., 1976; Bardalaye and Wheeler, 1985; Gorbach, 1980; Kirkland, 1973; Kirkland et al., 1973; Spittler et al., 1984; Zweig and Gao, 1983). The major problem associated with this method is that carbendazim, which is produced from benomyl as its degradation product during the analysis, cannot be distinguished from the carbendazim that was present in the sample as the natural degradation product of benomyl. Moreover, since carbendazim is also fungitoxic, and its biological activity is different from that of benomyl, analytical determination of benomyl as carbendazim has only limited applications.

Considering the above-mentioned problems, Chiba and Singh (1986) developed an indirect reversed-phase HPLC (RP-HPLC) method for the simultaneous determination of benomyl and carbendazim in water. In this method, benomyl is determined as 3-butyl-2,4-dioxo-*s*-triazino[1,2-*a*]benzimidazole (STB) after its quantitative conversion to STB at pH 13. During the treatment of benomyl (and carbendazim) with alkaline solution (pH 13), carbendazim remained mostly intact and was determined as carbendazim along with STB. Although this method can be used for the accurate determination of benomyl and carbendazim in water and other matrices, such as wettable powders, development of a RP-HPLC method for direct determination of benomyl and stabilization of benomyl samples after sampling remained highly desirable.

In recent papers (Singh et al., 1990; Marvin et al., 1990), we have shown that low concentrations of benomyl in water can be determined by a RP-HPLC method without any observable conversion of benomyl to carbendazim. However, it was not possible to prepare benomyl stock solutions of the desired strength. Stock solutions of benomyl are normally prepared in organic solvents. Due to its instability in organic solvents, benomyl rapidly converts to carbendazim before aqueous solutions can be prepared by dilution with water.

Aqueous solutions of benomyl can be prepared by using an ultrasonic technique (Singh and Chiba, 1985) or with the aid of a surfactant (Chiba and Northover, 1988). These methods are rather impractical for routine HPLC analyses because it is very difficult to prepare standards containing known and desired concentrations of benomyl in aqueous media. In view of the above difficulties, it is quite obvious that direct determination of benomyl in aqueous media, preparation of stable stock standards of benomyl of desired concentrations in aqueous media, and stabilization of benomyl-containing samples after sampling are desirable goals for the determination of benomyl in various types of samples.

In this paper, we report that, contrary to previously published papers (Pyysalo, 1977; Spittler et al., 1984), benomyl can be stabilized in acidic media. Standard aqueous solutions of desired concentrations of benomyl can be prepared in hydrochloric acid (HCl) and are stable over long periods of time.

MATERIALS AND METHODS

Solvents. Acetonitrile was of HPLC grade from Caledon Laboratories, Ltd., Georgetown, ON, Canada. Water was distilled in glass in the laboratory.

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Table I. Observed First-Order Rate Constants (k) for Benomyl Decomposition to Carbendazim at Ambient Temperatures in Various Concentrations of Hydrochloric Acid

HCl, M	k, s^{-1}	$t_{0.5}$, h
0.001	3.6×10^{-5}	5.3
0.01	3.1×10^{-5}	6.0
0.1	1.2×10^{-5}	1.6×10
1.0	7.7×10^{-7}	2.5×10^{2}
2.5	1.3×10^{-7}	1.5×10^{3}
5.0	stable	
10.0	stable	

Chemicals. Benomyl and carbendazim analytical standards were obtained from E. I. du Pont de Nemours and Co., Inc.

Instrumentation. The liquid chromatograph used was a Perkin-Elmer Series 3 equipped with a 50-μL Rheodyne syringe loop-type injector and a Perkin-Elmer LC-55 UV detector.

HPLC Operating Conditions. A Vydac reversed-phase C-18, 5 μm, 15 cm × 4.6 mm (i.d.), column was used. The UV detector was operated at 280 nm. The chart speed was 1 cm min^{-1}, and the detector sensitivity was 0.05 AUFS at a recorder range of 1.0 mVFS. The total system was operated at ambient temperature.

Mobile Phase. A mixture of 45% acetonitrile and 55% water was used isocratically.

Flow Rate. The flow rate was fixed at 1.0 mL min^{-1}.

Procedure. A sample of benomyl was dissolved at 1130 mg L^{-1} in 10 M HCl, and the kinetic rate constant of benomyl decomposition in the solution was determined by measuring the benomyl peak height with time as

$$k = (2.303/\Delta t) \log (C_1/C_2)$$

where C_1 is the benomyl peak height at time t_1, C_2 is the benomyl peak height at t_2, and $\Delta t = t_2 - t_1$. Similarly, benomyl solutions were prepared in various concentrations of HCl (0.001–5 M), and the kinetic rate constants were determined. The k values so calculated at different time intervals compared favorably, confirming first-order rate kinetics. Since the use of the analytical column is recommended between pH 2 and 7, these benomyl solutions were diluted to an HCl concentration of 0.01 M prior to the injection of 50 μL of solution onto the analytical column.

Mass Spectrometry. Analysis of benomyl solution (in 5 M HCl) was carried out on a Kratos Concept IS double-focusing mass spectrometer (Kratos Analytical, Urmston, Manchester, U.K.) using a fast atom bombardment (FAB) source, at room temperature.

RESULTS AND DISCUSSION

In a recent paper (Singh et al., 1990) we observed that the value of the rate constant (k) of benomyl conversion to carbendazim in aqueous solutions remained nearly constant between pH 2 and 7 with mean k at $(3.16 \pm 0.38) \times 10^{-5}$ s^{-1}. However, at pH 1, the value of k decreased sharply to 1×10^{-5} s^{-1}. A similar trend in the k vs pH plots was also observed by Calmon and Sayag (1976), in partially aqueous (50% methanol + 50% water, v/v) solutions. The sharp decrease in the value of k at pH 1 prompted us to investigate the kinetics of benomyl conversion to carbendazim in higher (higher than 0.1 M) HCl concentrations.

The results of the kinetic studies of benomyl conversion to carbendazim in various HCl concentrations are presented in Table I. These results clearly indicate that benomyl is substantially more stable in 1.0 M HCl compared with more dilute acid concentrations. The stability of benomyl increased with an increase in acid concentrations. Benomyl remained stable (within experimental error) in 5 and 10 M HCl solutions for at least 14 days at ambient temperatures. There was no difference in the peak heights of benomyl immediately after, and 14 days after, the preparation of sample solutions in 5 and 10 M HCl.

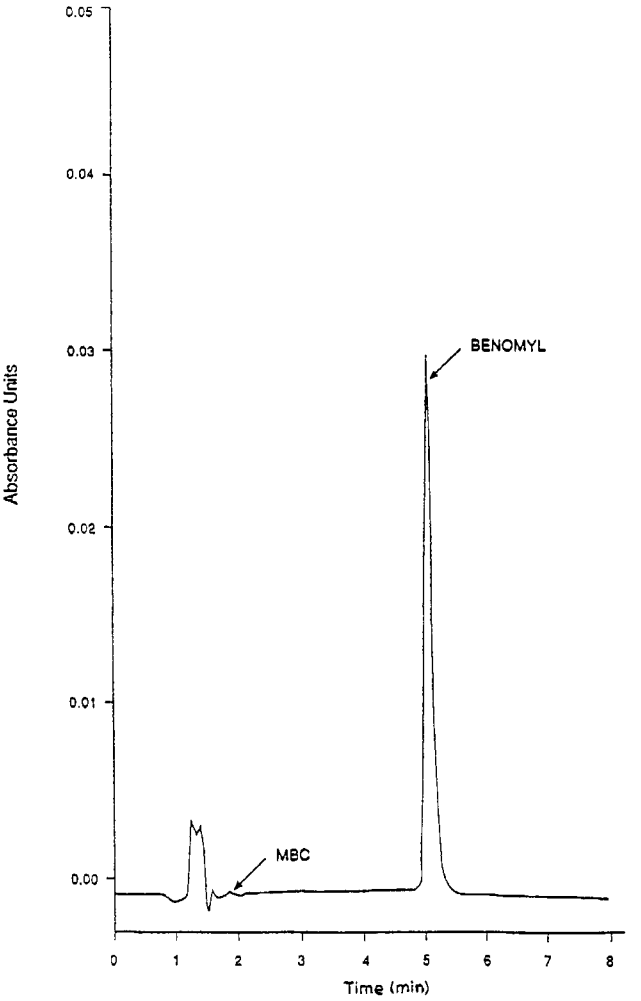

Figure 1. Reversed-phase HPLC chromatogram of a benomyl solution (1.13 μg mL^{-1}) in 0.01 M HCl. The solution was prepared just before the HPLC determination by diluting a 1130 μg mL^{-1} benomyl stock solution in 10 M HCl kept at room temperature for 14 days.

The stabilization of benomyl in relatively higher concentrations of HCl may be explained on the basis of its protonation at the nitrogen of benzimidazole group in the presence of acid. This was first suggested by Calmon and Sayag (1976) during their work on the kinetics of benomyl conversion to carbendazim, in water–methanol (50% v/v) solutions of pH 0–7. We believe that in high concentrations of HCl benomyl is easily protonated as benomyl-$H^+ + Cl^-$. This may be explained on the basis of very high solubility of benomyl in concentrated acidic solutions. Benomyl, which is difficult to dissolve in weakly acidic or neutral solutions (the solubility of benomyl is 4 mg/L or less in aqueous solutions of pH 3–7) (Singh and Chiba, 1985), can be dissolved in very large concentrations (we have dissolved up to 10 000 mg/L) in 5 M or higher concentrations of HCl, almost instantaneously. In contrast, the low solubility of benomyl in neutral and weakly acidic solutions (pH 2–7) can be explained by the lack of protonation. Since stabilization of benomyl is related to its protonation at the nitrogen of benzimidazole group, nonprotonated benomyl will slowly hydrolyze to carbendazim in aqueous solutions of pH 2–7.

Figure 1 shows the HPLC chromatogram of a sample of benomyl prepared in 10 M HCl and kept at room temperature for 14 days. As revealed in Figure 1, a small peak corresponding to carbendazim (approximately 0.7% of the peak height of benomyl) was always present in the chromatogram, even when using the analytical standard grade

benomyl. The presence of a constant level of a trace of carbendazim in the chromatogram for the maximum study period of 14 days suggests that carbendazim is present as a trace impurity in the analytical standard of benomyl.

The presence of benomyl in a 5 M HCl solution was also confirmed by mass spectrometry using a FAB source. A very strong molecular ion of benomyl at mass 291 has indicated the presence of benomyl in the acid solution. The signal counts for benomyl-H^+ molecular ion at m/e 291, in the FAB mass spectra of a 100 mg/L benomyl solution in 5 M HCl, remained virtually the same at 0 and 14 days after the preparation of the solution. This confirmed the stabilization of benomyl in 5 M HCl solution for 14 days.

The results discussed above also suggest that benomyl can be determined quantitatively using the HPLC conditions reported in this paper. The results for the determination of benomyl in a wettable power sample compared well with those obtained using the method developed by Chiba and Singh (1986).

The above results also indicate that stable reference standard aqueous solutions of benomyl can be prepared. Benomyl in water can be stabilized by strongly acidifying the solutions. The acidified benomyl solutions will keep for at least 14 days at room temperature, and probably for longer periods, by storing them at lower temperatures (for example, at 4 °C in a refrigerator). Other alkyl isocyanate homologues of benomyl (Chiba and Northover, 1988; Northover and Chiba, 1989) could also be stabilized in a similar fashion.

CONCLUSIONS

In conclusion, in this paper we report that, for the first time, the preparation of stable stock solutions of benomyl [methyl [1-(butylcarbamoyl)-1*H*-benzimidazol-2-yl]carbamate] in an aqueous medium is possible. The stabilization of benomyl in acidic solutions was confirmed by RP-HPLC. Mass spectrometry, using a FAB source, also confirmed the stabilization of benomyl in a 5 M hydrochloric acid solution. On the basis of these investigations, known amounts of benomyl can be easily dissolved and stabilized in 5 M or higher concentrations of hydrochloric acid. This opens up a real possibility for the preparation of reference standards of benomyl in aqueous (acidic) media. The stability of benomyl in 5–10 M hydrochloric acid also suggests that water samples containing benomyl can be stabilized upon acidification. This stabilization of benomyl-containing aqueous samples was not possible in the past.

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