Degradation Study of Benomyl and Carbendazim in Water by Liquid Chromatography and Multivariate Curve Resolution Methods

E. Mallat, D. Barceló, R. Tauler

1Department of Environmental Chemistry, CID-CSIC, c/ Jordi Girona Salgado 18-26, 08034 Barcelona, Spain
2Department of Analytical Chemistry, University of Barcelona, Av. Diagonal 647, 08028 Barcelona, Spain

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Introduction
Methyl (1-butylcarbamoyl)-2-benzimidazolecarbamate (benomyl) is widely used as a systemic fungicide for crop diseases. Due to its extensive use and carcinogenic activity the determination of benomyl and its main degradation compound 2-benzimidazolecarbamate (carbendazim) have been the object of several studies in environmental water, soil extracts and crops [1].

On account of its instability in organic solvents and its low solubility in water, several approaches were made to decide the degradation path of benomyl in different media. Most methods reported in the literature determine benomyl, after its conversion to a stable degradation compound, by chromatographic methods.

The most common approach is based on the conversion of benomyl to carbendazim, although it does not allow distinction between carbendazim resulting from the degradation of benomyl or not [2]. The conversion of benomyl to other degradation products; 3-butyl-2,4-dioxo-s-triazino[1,2-a]benzimidazole (STB), 1-(2-benzimidazolyl)-3-n-butylurea (BBU) and to 2-aminobenzimidazole (2-AB) were also reported.

Several authors studied the conversion of benomyl to carbendazim in aqueous and in partially aqueous solutions [3, 4] as well as in common organic solvents [5].

Calmon and Sayag [3, 4] determined the pseudo first-order rate constants of benomyl decomposition to carbendazim, and they studied the dependence of pH on degradation rate in aqueous buffers-methanol (50:50, v/v) media. The results showed no dependence of pH on degradation constants between pH 2–7, similar to the observations of Singh et al. [6] in pure aqueous solutions. Kinetic studies of conversion of benomyl to BBU and STB were studied [1]. Conversion of benomyl to STB in aqueous solution at pH 13 is very fast and this compound leads to the formation of BBU in very strong alkaline media. The degradation of benomyl to 2-AB was observed only in strong alkaline media.

Chiba and Cherniak [5] studied the degradation of benomyl in common organic solvents and they reported

Dedicated to Professor W. Haerdi on the occasion of his 70th birthday.
Degradation studies of some pesticides can be performed by UV spectrophotometry and multivariate curve resolution methods [8]. Spectra of the compound recorded at preselected time intervals evolve as the degradation reaction takes place, with noticeable changes in the absorption bands. However, the information provided by UV absorption is unresolved and no selective wavelengths are expected. To solve this problem, mathematical resolution of the species spectra and concentration profiles by means of factor analysis and multivariate curve resolution methods can be applied.

In view of the scarcity of studies in the literature regarding degradation of benomyl and carbendazim by spectrophotometry using chemometrics analysis and by LC-MS, it was decided to apply these powerful techniques in the present work.

Our aims were as follows: (a) to study the degradation of benomyl and carbendazim in different organic solvents and aqueous solutions at different pH by LC, spectrophotometry and multivariate curve resolution; (b) to study the influence of temperature, pH and sunlight on the degradation of carbendazim and identify its photodegradation products; and (c) to identify the degradation compounds of benomyl and carbendazim by LC-MS techniques.

**Experimental**

**Analytical Equipment**

**Chemicals**

Analytical grade benomyl and carbendazim were from Promochem (Wesel, Germany). The LC-grade water and acetonitrile were from Merck (Darmstadt, Germany) and were passed through a 0.45 μm filter before use.

Potassium dihydrogenphosphate, sodium hydroxide, sodium tetraborate, hydrochloric acid and acetic acid were from Merck (Darmstadt, Germany).

**Chromatographic Conditions**

The eluent was delivered by a Model 250 binary high-pressure pump from Perkin Elmer (Norwalk, CT, USA) coupled to a LC-95 UV-VIS spectrometric detector from Perkin Elmer. Samples were injected via a Rheodyne 20 μL loop (Cotati, CA, USA). A C-18 column (250 × 4.6 mm i.d.) 5 μm packing, from Shandon (USA) was used. Gradient elution was carried out with acetonitrile-water, from 30 to 80 % acetonitrile in 20 min, from these conditions to 100 % in 5 min, and back to initial conditions in 10 min. Flow rate was set at 1 mL min⁻¹.

The on-line SPE comprised a MUST column switching device from Spark Holland (AS Emmen, The Netherlands). Precolumn consisted of a stainless steel membrane disk holder, 10 × 2 mm containing C-18. Sample preconcentration was carried out with an SSI model 300 LC pump from Scientific Systems Inc. (State College, PA, USA).

The precolumn was first conditioned with 10 mL acetonitrile and 10 mL water, then 50 mL of the spiked water sample was percolated through the precolumn, and after valve switching components were desorbed by the mobile phase and separated on the analytical column. Desorption of the pesticides from the disk holder was in the back-flush mode to avoid dead volumes and chromatographic tailing.

**Spectrophotometric Conditions**

Spectrophotometric measurements were performed in a lambda-19 UV-VIS Spectrophotometer from Perkin Elmer (Norwalk, CT, USA). Spectra were registered from 200 to 320 nm, with a resolution of 1 nm, every 15 min during 12 hours. Temperature was set with a temperature control device, based on the Peltier effect, at 25 °C.

**LC-APCI-MS Detection**

A VG Platform from Fisons Instruments (Manchester, UK) equipped with an APCI interface was used. The eluent was delivered by a gradient system from Waters 616 pumps coupled to a Waters Model 600 S controller (Waters, Milford, MA, USA). Samples were analysed on-line, and chromatograms recorded under SIM conditions using positive ion mode. The typical fragments of benomyl, carbendazim and its metabolites were selected at m/z values of: 134, 160, 173, 192, 233, 259 and 291.

A cyano column (250 × 4.6 mm i.d.) 5 μm packing from Shandon (USA) was used. Preconcentration cartridges were filled with Lichrolut EN, dp 22 μm, to achieve good recoveries for photodegradation compounds of carbendazim which are more polar than the parent compound. Elution was carried out with acetonitrile and HPLC water both acidified with 0.5 % acetic acid under the same elution conditions.

**Stability Experiments**

A study of the stability of benomyl and carbendazim was performed in media, such as: methanol, acetonitrile and HPLC water acidified with hydrochloric acid (pH 3). Sample concentrations were 2 mg L⁻¹. The study was carried out over 11 days and samples were stored at 4 °C in dark bottles to minimize degradation by sunlight. Samples were injected direct into the HPLC system.

**Spectrophotometric Experiments**

Samples were prepared by dissolving benomyl in aqueous solutions adjusted to pH 3, 4.5, 6, 7.5 and 9. To ensure complete solubilization of benomyl 10 mL methanol were added to a total volume of 100 mL. The first