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Simultaneous Determination of Benomyl and Morestan Residues in Waters by Synchronous Solid-Phase Spectrofluorimetry

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In this paper a new, sensitive, and simple method for simultaneous determination of pesticides morestan and benomyl at trace levels in waters is reported. Both chemicals, showing native fluorescence in solution at neutral medium, were fixed on C-18 silica gel at pH 1, giving a fluorescent system. The benomyl–morestan–silica gel system, after dry, was packed in a 1-mm silica cell and its synchronous fluorescence spectra were recorded at $\Delta\lambda$=80 nm for determination of benomyl and $\Delta\lambda$=25 nm for determination of morestan. Measurements of fluorescence were performed at λ_1= 289 nm and λ_2= 367 nm for benomyl and morestan analysis, respectively. The applicable concentration ranges were from 0.5 to 15.0 ng·ml^{-1} for benomyl and from 0.6 to 15.0 ng·ml^{-1} for morestan, with relative standard deviations of 1.2 and 1.5% for benomyl and morestan, respectively, being 0.15 and 0.18 ng·ml^{-1} its respective detection limits. The method was applied to the simultaneous determination of residues of both pesticides in water of different provenances.

KEY WORDS: Benomyl and morestan determination; solid-phase spectrofluorimetry; pesticides residues; water analysis.

INTRODUCTION

Methyl[1-(butylcarbamoyl)benzimidazol-2-yl] carbamate (benomyl) and [6-methyl-2,3-quinoxalinediyl cyclic *S,S*-dithiocarbonate] (morestan) are used in commercial formulations as fungicide and insecticide, respectively, in agriculture [1,2]. Generally, both compounds are not mixed in commercial formulations, but as a consequence of their use in ornamental fruit and crops, they could be present in the residual waters coming from agricultural manipulations.

Solubility of the morestan only enables its direct determination at trace levels because it is practically insoluble in water [2]. Benomyl is more soluble than morestan, but its instability in water and in organic solvents [3] is the cause of its determination as methyl-2-benzimidazole carbamate, commonly named carbendazime (MBC), after quantitative conversion of the benomyl to MBC [4–6].

As the biological activity of both MBC and benomyl is different, the determination of benomyl as MBC has only limited applications. Moreover, the aqueous solutions of benomyl are stable in acidic mediums [7].

Several methods have been reported for the determination of morestan or benomyl, as well as for the simultaneous determination of these chemicals with other pesticides.

Morestan has been determined by thin-layer chromatography (TLC) and measurement of the fluorescence produced [8,9]. A mixture of residues of morestan and other pesticides in water has been studied using gas chromatography (GC) and different detectors for deter-

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mination of the components [10,11]. Polarography with a previous step of separation by TLC is another technique also used for determination of morestan at trace level in waters [12].

For determination of benomyl previous quantitative conversion to MBC, several methods have been reported. We would like to mention here the use of HPLC [4–6] and the method proposed by Marvin *et al.* [13] by HPLC using a previous automated on-line preconcentration.

In previous papers the authors have reported solid-phase spectrofluorimetry as an appropriated technique for the analysis of waters containing residues of organic compounds such as pesticides [14,15] or polycyclic aromatic hydrocarbons [16].

Following this research line, we now propose the application of synchronous fluorescence in the solid phase for resolution of mixtures of the above-mentioned pesticides.

We have not found a method for simultaneous determination of benomyl and morestan in our search, and for this reason we propose here a new method for its simultaneous determination in water without conversion to MBC. It is simple and sensitive, and only conventional instrumentation is required.

EXPERIMENTAL

Instrumentation

Fluorescence measurements were performed using a Perkin–Elmer LS-5 fluorometer equipped and checked as described in the previous paper [14]. A Canon BJ-300 printer for graphical representations, a Crison 501 digital pH-meter with a combined glass-satured calomel electrode, and an Agitaser 2000 rotating agitator were also used. As container for the analyte–silica gel system beads, a STARNA standard rectangular silica cell (Type 1,Q,1,6) with a 1-mm path length was used for the fluorescence measurements.

Reagents

Benomyl stock solution (Lab. Prof. Riedel-de Haën; 100 $mg \cdot l^{-1}$) was prepared by exact weighing of the reagent and dissolution in 5 *M* hydrochloric acid aqueous solution. Working solutions were prepared by adequate dilution with the same 5 *M* HCl aqueous solution.

Morestan stock solution (Lab. Dr. Ehrenstorfer; 100 $mg \cdot L^{-1}$) was prepared by exact weighing of the reagent and dissolution in ethanol (98%, v/v), making further dilutions with deionized water.

C-18 silica gel was used as solid support without pretreatment.

All solvent and reagents were of analytical reagent grade unless stated otherwise.

Procedure

Samples of water, acidified with 5 *M* HCl, were filtered through a filter glass (0.45-μm pore size) and collected in a glass container carefully cleaned with HCl. These samples were stored at 4°C until analysis, taking the usual precautions to avoid contaminations.

Likewise, measurement of relative fluorescence intensity (RFI) of the gel beads containing the analytes packed in a 1-mm silica cell was the diffuse transmitted fluorescence (DTF). This DTF was emitted from the unexcited surface of the cell, where the optimum angle between the cell plane and the excitation beam was 45° in all cases [14].

Basic Procedure

A 500-ml water sample containing from 0.5 to 15.0 $ng \cdot ml^{-1}$ of benomyl and from 0.6 to 15.0 $ng \cdot ml^{-1}$ of morestan was acidified with HCl to pH 1. The sample was placed in a 1-L glass bottle and 250 mg of C-18 silica gel was added. After mechanically shaking the sample for 15 min the gel beads were collected and dried by filtration under suction and packed in a 1-mm silica cell. A blank sample containing all reagents except morestan and benomyl was prepared and treated in the same way. Synchronous spectra of the sample and the blank were recorded at $\Delta\lambda_1 = 80$ nm and $\Delta\lambda_2 = 25$ nm, the optima wavelength being 289 nm for determination of benomyl and 367 nm for determination of morestan. In all instances and RFI measurements were performed when the temperature in the cell compartment was 20.0 ± 0.5°C. Standard curves were constructed in the same way using solutions of benomyl and morestan of known concentration.

RESULTS AND DISCUSSION

Benomyl in solution remains stable only in acidic medium [7], but when it is fixed in C-18 silica gel it shows the synchronous fluorescence spectrum shown in Fig. 1. Morestan, fixed in C-18 silica gel in the same way as described above for benomyl, showed the synchronous fluorescence spectrum shown in Fig. 1. These synchronous spectra were recorded at $\Delta\lambda = 80$ nm for benomyl

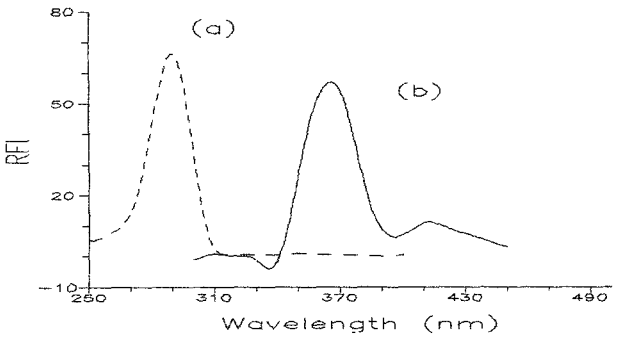

Fig. 1. Synchronous spectra of benomyl (a) and morestan (b) recorded to $\Delta\lambda = 80$ nm and $\Delta\lambda = 25$ nm, respectively.

Table I. Optimum Experimental Variable Values

Variable	Value
pH	1.0
Stirring time	15 min
Sample volume	500 ml
Temperature of measurements	20.0 ± 0.5°C
Reactant addition order	Not affected
Stirring speed	60 rpm
Amount of gel	250 mg

and $\Delta\lambda$=25 nm for morestan. The fixation pH of both pesticides in the silica gel was 1.0 in all instances.

Optimization of Variables

Temperature

The effect of temperature on the fixation process of the analytes on the gel and, hence, on the fluorescence emission was studied. The fixation process was independent of the temperature, in the range 5–40°C measuring the RFI at 20.0±0.5°C. On the other hand, RFI decreases when the temperature of the system increases, the effect being totally reversible.

The decrease in the RFI (in solid phase) was 1.4% at 10°C, 4.1% at 20°C, 7.6% at 40°C, and 10.0% at 60°C for morestan and 2.7% at 10°C, 11.1% at 20°C, 25.9% at 40°C, and 38.9% at 60°C for benomyl. This effect can be explained as due to internal conversion processes as the temperature increases, facilitating nonradiative deactivation of the excited singlet state. All RFI measurements reported here were made at 20.0±0.5°C.

Table II. Statistical Analysis of the Determination of Benomyl (0.5–15.0 ng · ml^{-1}) and Morestan (0.6–15.0 ng · ml^{-1}) in Mixtures

Pesticide determined	Concentration of coexisting compound	Slope	Intercept	Corr. coeff.
Benomyl	Morestan			
	—	6.82	0.05	0.999
	4.4	6.80	0.08	0.999
	8.8	6.79	−0.10	0.999
Morestan	Benomyl			
	—	5.18	0.03	0.999
	4.4	5.09	−0.17	0.998
	8.8	5.08	0.09	0.999

Table III. Analytical Parameters (Sample Volume, 500 ml)

	Pesticide	
Parameter	Benomyl	Morestan
Intercept	0.05	0.03
Slope	6.8	5.2
LDR[a]	0.5–15.0	0.6–15.0
Correlation coefficient	0.999	0.999
Detection limits[b]	0.15	0.18
Quantification limits[b]	0.50	0.62
RSD %	1.2	1.5

[a]Linear dynamic range (ng · ml^{-1}).
[b]Expressed as ng · ml^{-1}.

Table IV. Effect of Foreign Species on the Determination of 5.0 ng · ml^{-1} of Benomyl and Morestan

Foreign species	Tolerance level (ng · ml^{-1})
Chlorate, carbonate, sulfate, nitrate, acetate, iodide, bromide, chloride, fluoride, Ca^{2+}, Mg^{2+}, Al^{3+}, Fe^{3+}, and alkaline ions	> 20,000
Carbaryl	310
o-Phenylphenol	88
Thiabendazole	500
Carbendazime	5000
Dichlone	100
Lindane	800

pH Dependence

To check the influence of pH on the RFI measurements of morestan and benomyl on the process of fixation of both compounds in the gel, different values of pH were tested using hydrochloric acid and sodium hydroxide solutions.

Table V. Recoveries of Benomyl and Morestan in Water Samples[a]

Sample		Benomyl Taken	Benomyl Found[b]	Benomyl Rec (%)		Morestan Taken	Morestan Found	Morestan Rec (%)
Genil River (Granada)	(a)	2.22	2.19	98.6	(d)	2.22	2.17	94.6
		4.44	4.37	98.4		2.22	2.15	96.8
		8.88	8.95	100.8		2.22	2.12	95.5
	(b)	2.22	2.16	97.3	(e)	4.44	4.35	97.9
		4.44	4.34	97.7		4.44	4.45	100.2
		8.88	8.84	99.5		4.44	4.32	97.3
	(c)	2.2	2.14	96.4	(f)	8.88	8.77	98.7
		4.44	4.45	100.2		8.88	8.70	97.9
		8.88	8.79	98.9		8.88	8.52	95.9
Quentar Dam (Granada)	(a)	2.22	2.20	99.1	(d)	2.22	2.22	100.0
		4.44	4.46	100.5		2.22	2.23	100.4
		8.88	8.89	100.1		2.22	2.20	99.1
	(b)	2.22	2.21	99.5	(e)	4.44	4.42	99.5
		4.44	4.46	100.5		4.44	4.43	99.8
		8.88	8.90	100.2		4.44	4.43	99.8
	(c)	2.22	2.23	100.5	(f)	8.88	8.82	99.3
		4.44	4.46	100.5		8.88	8.80	99.1
		8.88	8.79	98.9		8.88	8.80	99.1

[a](a) In the absence of morestan; (b) in the presence of 4.4 ng · ml^{-2} of morestan; (c) in the presence of 8.8 ng · ml^{-1} of morestan; (d) in the absence of benomyl; (e) in the presence of 4.4 ng · ml^{-1} of benomyl; (f) in the presence of 8.8 ng · ml^{-1} of benomyl.
[b]These data represent the average of three determinations expressed as ng · ml^{-1}.

For morestan, the dependence of the fluorescence developed on the pH values presents maximum RFI at pH values from 1.0 to 8.0. However, as the benomyl solution remains stable only at pH values lower than 1.5, pH = 1.0 was selected as the working pH. Hydrochloric acid, 5 *M*, was used for the fixation of pH.

Other Experimental Conditions

As the use of a large amount of the gel lowered RFI measurements, only the amount required to fill the cell and facilitate handling (250 mg) was used in all instances.

The order of addition of reagents did not affect the results obtained.

To check the stirring times necessary for maximum RFI development, different samples prepared and treated in the same way were equilibrated with equal amounts of the gel during 5, 10, 15, 20, and 25 min. The results obtained show that the developed RFI is independent of time from 10 min.

Effect of Sample Volume on Sensitivity

In previous papers [14–16] we have mentioned that one of the main advantages of solid-phase spectrofluorimetric methods is the potential increase in sensitivity with an increase in the sample volume taken for analysis. This effect can be assessed by measuring the RFI of gel equilibrated with different volumes of solution containing the same concentration of pesticides and proportional amounts of the other reagents. The nature of the experimental data suggest a linear dependence of RFI vs sample volume in the range 100–500 ml. For sample volumes higher than 500 ml the increase in RFI is not significant. The results obtained are summarized in Table I.

Analytical Parameters

To test the mutual independence of the analytical signals for benomyl and morestan, i.e., to show that the signal produced by every one is independent of the concentrations of the other, three calibration graphs were obtained from RFI measurements for standards containing between 0.5 and 15.0 ng·ml^{-1} of benomyl, in the absence of morestan and in the presence of 4.4 and 8.8 ng·ml^{-1} of morestan, respectively. Following the same procedure, three calibration graphs were prepared for standards containing between 0.6 and 15.0 ng·ml^{-1} of morestan, in the absence of benomyl and in the presence of 4.4 and 8.8 ng·ml^{-1} of benomyl, respectively.

The results obtained are summarized in Table II, from which the independence of the analytical signals of both pesticides can be deduced because the slope of the corresponding calibration graphs remained constant in all cases. Also, the values of the correlation coefficients and the low values for the intercepts indicate a good linearity for all calibration graphs obtained.

The reproducibility of the proposed method was checked with a series of 10 samples having a concentration of 5.0 ng·ml^{-1} of morestan and 5.0 ng·ml^{-1} of benomyl. The relative standard deviations (RSD) (P=0.05, n=10) for different sample volumes were studied. The results obtained are summarized in Table III. This table also lists the results obtained in estimating the precision (RSD) of the packing operation calculated from 10 measurements, the detection limits (k=3) [17], and the quantification limits (k=10) [18].

Effect of Foreign Species

The study of the influence of foreign species potentially present in the determination of benomyl and morestan in water was undertaken. A 20 mg·ml^{-1} level of each ionic species was tested first, and if interference occurred, the ratio was reduced progressively until disappearance. Organic species were tested at different concentration levels depending on their respective solubilities in water. The tolerance level was defined as the amount of foreign species inducing error not exceeding ±5% in the determination of the analyte (Table IV).

Applications of the Method

To check the accuracy of the proposed method, a recovery study was carried out on various types of waters. These samples were analyzed after adequate additions of benomyl and morestan to see eventual effects on water coming from agricultural manipulations. Water samples were taken from the Genil River and Quentar Dam (Granada, Spain). The volume used was 500 ml in all cases, the results obtained being summarized in Table V. The measurements included in this table were performed for solutions containing different concentrations of one pesticide in the absence or in the presence of constant amounts (4.4 and 8.8 ng·ml^{-1}) of the other pesticide.

Conclusion

This paper provides a practical application of synchronous spectrofluorimetry in combination with solid-phase spectrofluorimetry to multicomponent pesticide analysis at trace levels.

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