

INDIRECT DETERMINATION OF DITHIOCARBAMATE FUNGICIDES (ZINEB AND FERBAM) IN SOME FOODSTUFFS BY FLAME ATOMIC ABSORPTION SPECTROMETRY

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Abstract

A simple, sensitive and rapid flame atomic absorption spectrometric (FAAS) method for the indirect determination of zinc ethylenebisdithiocarbamate (zineb) and ferric dimethyldithiocarbamate (ferbam) was described. Metals liberated upon decomposition of the fungicides were determined by FAAS and the zineb and ferbam content was calculated by using stoichiometric relationship between the metal and corresponding fungicide. The method was successfully applied to the analysis of water, tomato and wheat grain for the determination of zineb and ferbam. The fungicides were determined with relative standard deviation and relative error less than 5%.

Key words: Atomic absorption spectrometry, Zineb and ferbam, Tomato, Wheat grain, Water

Bazı Gıdalarda Ditiyokarbamat Fungisitlerin (Zineb ve Ferbam) Alevli Atomik Absorpsiyon Spektrometri İle Dolaylı Tayini

Çinko etilenbisditiyokarbamat (zineb) ve demir(III) dimetilditiyokarbamatın (ferbam) dolaylı tayini için basit, duyarlı ve hızlı bir alevli atomik absorpsiyon spektrometrik (FAAS) yöntem açıklandı. Fungisitlerin parçalanmasıyla açığa çıkan metaller FAAS ile tayin edildi ve zineb ve ferbam içeriği, metal ile karşılık gelen fungusit arasındaki stokiometrik ilişki kullanılarak hesaplandı. Yöntem, zineb ve ferbam tayini için su, domates ve buğday danesi analizine başarıyla uygulandı. Fungisitler, %5'ten daha düşük bağıl standart sapma ve bağıl hata ile tayin edildi.

Anahtar kelimeler: Atomik absorpsiyon spektrometri, Zineb ve ferbam, Domates, Buğday, Su

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INTRODUCTION

The water, soil and food contaminants belong to the family of carbamate and organophosphate pesticides, which are ubiquitously used in agriculture and highly persistent in the environment, polluting our water supply and food chains (1). Therefore, many studies have been performed for the accurate determination of pesticide residues.

Flame atomic absorption spectrometric (FAAS) analysis is the most straightforward and widely used method for the determining zinc and iron in various matrices (2,3). In recent years, some organic substances have been determined by FAAS indirectly (4-8). Some analgesic-inflammatory drugs have been determined by AAS by forming a complex with copper (II) amine sulphate (4). Jiang et al. have described an indirect determination of ascorbic acid based on its reducing action to Fe(III) (5). Yebra et al. have described an indirect method for the determination of quinine (6). El Reis et al. have determined captopril in pharmaceutical preparation by AAS (7). Blas et al. have proposed a new procedure for the indirect determination of organophosphorus pesticides by coupling flow injection analysis and atomic absorption spectrometry with hydride generation (8). Cassella et al. have determined zineb by flow injection atomic absorption spectrometry in commercial formulations of pesticide (9). Türker and Sezer have proposed a method for indirect determination of maneb in some foods and drug formulations by flame atomic absorption spectrometry (10). Agarwal et al. have determined ziram and zineb residues in fog-water samples by visible spectrophotometry and by flame atomic absorption spectrometry after separating them with solvent extraction (11).

Dithiocarbamates are applied as pesticides in agriculture and as vulcanization accelerators and anti-oxidants in rubber industry (12). Thiocarbamates and dithiocarbamates are two of the most important families of fungicides (13). The latter family includes compounds such as ferbam, zineb, ziram, nabam and maneb. Ferbam is a broad spectrum fungicide for the control of certain diseases in fruit tree, small fruits and berries etc. Ferbam is foliar protectant against scab, rust, mold and many fungus diseases on fruits, vegetables, melons and ornamentals. Principal uses of ferbam are in the control of apple scab and cedar apple rust, peachleaf curl, tobacco blue mold, and cranberry diseases. Zineb is also used to protect fruit and vegetable crops from a wide range of foliar and other diseases. As dithiocarbamate (DTC) complexes are known to have toxicological and mutational effects (14), it is necessary to separate, identify and determine the DTC complexes by a rapid and accurate method.

Currently the most common analytical method for dithiocarbamates is based on acid hydrolysis with the measurement of the released carbon disulfide by head-space GC analysis (15). The method is nonspecific because it does not distinguish dialkyl dithiocarbamates (such as ziram and ferbam) or thiuram disulfide (such as thiram) from ethylenebisdithiocarbamates. Other methods for the determination of dithiocarbamates rely on the measurement of the metallic portion of the compounds (16, 17). Malik et al. present a modification of usual CS₂ evolution method that converts ferbam to an iron phenantroline complex that is adsorbed on microcrystalline naphthalene; then spectrophotometric measurement of the complex in dimethylformamid (18). Ziram was determined by UV-visible spectrophotometry based on the dissociation of dithiocarbamate complex of zinc with thiocyanate and rhodamine 6G to form colored complex (19). In another study, spectrophotometric determination of ziram, ferbam and zineb with diphenylcarbazone was described (12).

In this study, a new procedure for the indirect determination of dithiocarbamate pesticides zineb (zinc ethylenebis(dithiocarbamate)) and ferbam (ferric dimethyldithiocarbamate) by FAAS is described. This proposed method is not requiring complex and time consuming pretreatment procedures such as extraction. It is also not required to dissolve food sample completely. It is sufficient to decompose the pesticides present generally on the surface of sample. The method was successfully applied to the determination of zineb and ferbam in water, tomato and wheat grain. The results were compared with those obtained by UV-VIS spectrometry.

EXPERIMENTAL

Apparatus

A Philips PU 9285 Model flame atomic absorption spectrometer equipped with deuterium lamp background correction, zinc and iron hollow cathode lamps (HCL) and air-acetylene burner was used for the determination of zinc and iron. The instrumental parameters for zinc and iron were as follows: Wavelength, 213.9 nm and 248.3 nm, lamp current, 7.5 mA and 12 mA, bandpass, 0.5 nm and 0.5 nm, fuel flow rate, 1.2 L min⁻¹ and 0.9 L min⁻¹, respectively. Spectrophotometric measurement was performed by an Unicam UV2-100 Model UV-visible spectrophotometer with a 10 mm quartz cell. The wavelength of maximum absorption used for zineb and ferbam was 284 nm and 293 nm, respectively.

Reagents

Doubly distilled deionized water and analytical reagent grade chemicals were used unless otherwise indicated. Zinc and iron stock solutions (1000 µg mL⁻¹) were prepared by dissolving the appropriate amounts of zinc nitrate hexahydrate and iron powder, respectively. The working solutions were prepared by dilution from the stock solution. Zineb [Riedel de Haén, 69.7 %(m/m)] and ferbam [Riedel de Haén, 93.2 %(m/m)] was used as received. For spectrophotometric analysis, 100 µg mL⁻¹ stock solutions of zineb and ferbam were prepared by dissolving in 0.5 mol L⁻¹ sodium hydroxide and in acetonitrile, respectively. Working solutions of lower concentrations were prepared by appropriate dilutions with their solvents. In order to avoid the influence of the possible degradation of fungicides on the results, the working solutions were freshly prepared every day by diluting the stock solutions to appropriate volumes.

General procedure

Atomic absorption spectrometric method

A known amount of zineb and ferbam as a water suspension was added separately to tomato and wheat grain to prepare spiked samples. For preparing the spiked wheat grain and tomato samples, 6.1 mg of zineb (purity of 69.7 %(m/m)) and 40.0 mg of ferbam (purity of 93.2 %(m/m)) were added to 20 mL of water separately and each obtained suspensions was mixed thoroughly for at least 30 min with about 1 g of accurately weighed wheat grains and 50 g of tomato broken into pieces in a blender. Spiked water samples were prepared by diluting the stock solutions of the fungicides (100 µg mL⁻¹) with water to 4.20 µg mL⁻¹ for zineb and 7.50 µg mL⁻¹ for ferbam. Then, 20 mL of concentrated HCl was added to the each spiked samples and, the mixtures obtained were heated for 10 min in order to decompose the fungicides completely. The mixtures were then

filtered with a filter paper to separate the food residue from the solution. The food residue was washed with two 10 mL portions of 1 mol L⁻¹ HCl to provide the complete extraction of fungicide to the solution. Filtrate and washings were collected together into a 100 mL volumetric flask and diluted to the mark with water. 1 mL of this solution was diluted again to 10 mL to obtain the metal concentration within the working range of the method (0.2 µg mL⁻¹ - 2 µg mL⁻¹ for zinc and 1 µg mL⁻¹ - 8 µg mL⁻¹ for iron) for flame atomic absorption determination. Then, zinc and iron present in these solutions were determined by FAAS using a calibration graph drawn by using the metal (Zn and Fe) standard solutions. Calibration solutions were simulated to the sample solution by adding appropriate amount of diluted HCl to the calibration solutions. The last dilution and FAAS determination procedures were repeated five times. Concentration of zineb and ferbam was calculated by using stoichiometric relationship between metal and corresponding fungicides (1 g of zinc is equivalent to 4.21 g of zineb and 1 g of iron is equivalent to 7.458 g of ferbam).

Spectrophotometric method

In order to check the accuracy of the atomic absorption method, zineb and ferbam have also been determined spectrophotometrically. UV-visible spectrum was obtained with zineb standard solution (4 µg mL⁻¹) against water with 1 cm quartz cell and maximum absorption wavelength was found as 284 nm. For spectrophotometric determination of zineb in wheat grain, spiked wheat grain sample was prepared by adding 10 mL of zineb solution containing 6.1 mg of zineb (corresponds to 4.252 mg of pure zineb) to 1 g of wheat grain and mixing for 30 min. The spiked water and wheat grain samples were then treated with 30 mL of 0.5 mol L⁻¹ NaOH in order to dissolve the zineb. The mixture was mixed with magnetic stirrer for 30 min to provide complete dissolution of the zineb. Then, the mixture was filtered with a filter paper and the residue was washed with two 10 mL portions of 0.5 mol L⁻¹ NaOH in order to provide the complete separation of zineb. Filtrate was diluted to 100 mL with water. 1 mL of this solution was diluted again to 10 mL with water and analyzed spectrophotometrically for zineb determination. This last dilution and measurement procedure was repeated five times.

UV-visible spectrum was obtained with ferbam standard solution (7.5 µg mL⁻¹) against acetonitrile with 1 cm quartz cell and maximum absorption wavelength was found as 293 nm. For spectrophotometric determination of ferbam in wheat grain, spiked wheat grain sample was prepared by adding 10 mL of ferbam solution containing 8.0 mg of ferbam (corresponds to 7.46 mg of pure zineb) to 1 g of wheat grain and mixing for 30 min. The spiked wheat grain and water samples were then treated with 30 mL acetonitrile in order to dissolve the ferbam. The mixture was mixed with magnetic stirrer for 30 min to provide complete dissolution of the ferbam. Then, the mixture was filtered with a filter paper and the residue was washed with two 10 mL portions of acetonitrile to provide the complete separation of ferbam. Filtrate was diluted to 100 mL with acetonitrile. 1 mL of this solution was diluted again to 10 mL with acetonitrile and analyzed spectrophotometrically for ferbam determination. This last dilution and measurement procedure were repeated five times.

RESULT AND DISCUSSION

Determination of zineb

The general atomic absorption spectrometric and UV-Vis spectrophotometric procedures mentioned above were applied for the determination of zineb in spiked water, wheat grain and tomato samples. A test with untreated wheat grain and tomato samples has also been carried out by adding the same amount of water instead of zineb to the same amount of wheat grain and tomato. The corrected zinc concentration was calculated by subtracting the value obtained with untreated sample from the value obtained with spiked sample. Untreated sample of tomato showed negligible zinc concentration corresponding to negligible concentration of zineb. As shown in Table 1, the accuracy and precision of the determination of zineb in spiked samples was quite satisfactory. The relative standard deviations (RSD) are less than 5% and percent relative errors are about 2% for all of the samples studied. Recovery of zineb from all the spiked samples is above 95%.

For spectrophotometric analyses, absorbances of calibration solutions prepared by diluting the ferbam stock solution were measured at the maximum absorption wavelength (284 nm) and the calibration graph [$A = 0.1004 C - 0.0022$, where, C is the zineb concentration in $\mu\text{g mL}^{-1}$, $r^2 = 0.9999$] was obtained.

The results obtained by flame atomic absorption spectrometric and spectrophotometric methods were also in accordance with each other (Table 1). Spectrophotometric determination of zineb in tomato sample could not be performed, because colored substances liberated from tomato interfere with the absorption peak of zineb.

Table 1. Determination of zineb

Method	Sample	Zineb concentration**		RSD, %	Relative error, %
		Added	Found*		
FAAS	Water	4.20	4.3 ± 0.3	5.6	2.4
	Wheat	4252	4340 ± 90	1.7	2.1
	Tomato	85.04	86 ± 2	1.6	1.2
UV-Vis	Water	4.20	4.26 ± 0.03	0.6	1.2
	Wheat	4252	4200 ± 200	3.8	-1.2

*Mean ± ts/ \sqrt{N} (95% confidence level), N=5.

** $\mu\text{g mL}^{-1}$ for water and $\mu\text{g g}^{-1}$ for wheat grain and tomato.

Determination of ferbam

The general atomic absorption spectrometric and UV-Vis spectrophotometric procedures mentioned above were applied for the determination of ferbam in spiked water, wheat grain and tomato samples. A test with untreated wheat grain and tomato samples has also been carried out by adding the same amount of water instead of ferbam to the same amount of wheat grain and tomato. The corrected iron concentration was calculated by subtracting the value obtained with

untreated samples from the value obtained with spiked samples. Untreated sample of tomato showed negligible recovery of iron corresponding to negligible recovery of ferbam. As can be seen in Table 2, the accuracy and precision of the determination of ferbam in spiked samples was quite satisfactory. The relative standard deviations are about 2% and percent relative errors are less than 5%. Recovery of ferbam from all the spiked samples is above 95%. The ferbam concentrations found by spectrophotometrically are also in accordance with the results obtained with FAAS.

Table 2. Determination of ferbam

Method	Sample	Ferbam concentration**		RSD, %	Relative error, %
		Added	Found*		
FAAS	Water	37.28	37.0 ± 0.6	1.3	-0.8
	Wheat	37280	35700 ± 100	0.3	-4.2
	Tomato	746	744 ± 1	0.1	-0.3
UV-Vis	Water	7.50	7.2 ± 0.2	1.7	-4.0
	Wheat	7460	7300 ± 200	2.2	-2.1

*Mean ± ts/√N (95% confidence level), N=5.

**µg mL⁻¹ for water and µg g⁻¹ for wheat grain and tomato.

For the spectrophotometric determination of ferbam, the absorbances of calibration solutions prepared by diluting the ferbam stock solution were measured at the maximum absorption wavelength (293 nm) and the calibration graph [$A = 0.087 C + 0.0307$, where C is the ferbam concentration in µg mL⁻¹, $r^2 = 0.9976$] was obtained. Spectrophotometric determination of ferbam could not be performed in tomato sample, because colored substances liberated from tomato interfere with the absorption peak of ferbam.

Sensitivity, selectivity, precision and accuracy of the method

The sensitivity of the present method is almost the same or less than those of the methods based on the determination of liberated CS₂ and UV-visible spectrophotometry (2). The sensitivity of the present method depends on the sensitivity of metal determination in acidic aqueous medium. Under the optimum conditions for zinc and iron determination in acidic medium, the calibration graph was linear over the concentration range 0.2 µg mL⁻¹ - 2 µg mL⁻¹ for zinc and 1 µg mL⁻¹ - 8 µg mL⁻¹ for iron. These working ranges correspond to about 1 µg mL⁻¹ - 8 µg mL⁻¹ for zineb and 7.5 µg mL⁻¹ - 60 µg mL⁻¹ for ferbam.

The present method is relatively selective for the determination of zineb and ferbam in the presence of other dithiocarbamates. Since it is based on the determination of metal portion of the fungicide, other dithiocarbamates do not interfere with this method. The main disadvantage of the method is the interference of other zinc and iron compounds present in pesticide formulation and in foods naturally.

The precision and accuracy of the method are also satisfactory. As can be seen in Tables 1 and 2, relative standard deviations are less than 6% and the recoveries are above 95% for all samples. Moreover, the results obtained by FAAS are agreed with those obtained by UV-Vis spectrophotometry.

CONCLUSION

The proposed indirect atomic absorption spectrometric method is rapid, simple, precise and accurate. The method described here is advantageous over others studies, since no previous sample treatment is needed, thus minimizing errors due to solvent extraction or hot acid decomposition procedures. No fungicide standards are needed for the calibration. Any solution of the metal salt may be used as calibration solution. The sample pretreatment procedure is also very simple. No extraction and separation are not needed except filtration. Zineb and ferbam were quantitatively recovered from the samples with a high precision and accuracy. In addition, the selectivity of this method is relatively high. It possesses some distinct advantages over determination of dithiocarbamates by gas chromatography involves formation of CS₂, extraction of CS₂ and determination of CS₂. All dithiocarbamates evolve CS₂ on acid digestion results poor selectivity. The method also avoids the use of hazardous chemicals such as pyridine etc. used in certain methods, thus provides environmentally friendly methodology. The main disadvantage of the method is not differentiating among various forms of the zinc and iron. Other zinc and iron sources present in foods or pesticide formulation interfere with the determination. This interference can be eliminated by analyzing the identical sample not containing pesticide and by correcting the results.

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