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Determination of Copper-Based Fungicides by Flame Atomic Absorption Spectrometry Using Digestion Procedure with Sulfuric and Nitric Acid

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SUMMARY

Copper-based fungicides can be effectively digested by treatment with a mixture of concentrated sulfuric and nitric acid in exactly 15 minutes for the rapid determination via copper using flame atomic absorption spectrometry (AAS). Under optimum conditions, the results of copper fungicide analysis were consistent to those obtained by the AOAC's recommended method. Recovery values ranged from 98.63 to 103.40%. Relative standard deviation values are lower than 2%. The proposed digestion procedure applied for determination of copper ranged from 100 to 594 g Cu kg^{-1} in different commercial fungicide products.

Keywords: Copper fungicides; Digestion procedure (H_2SO_4 + HNO_3); Copper determination; Flame atomic absorption spectrometry (AAS)

INTRODUCTION

Copper-based fungicides belong to a pesticide class that has been used to control fungal diseases in pome and stone fruit orchards, vineyards and vegetable crops for more than a hundred years (Merry et al., 1983). They can be applied alone or in combination with other fungicides for seed treatment, foliar application or soil drenching (Gharieb et al., 2004). Some registered fungicide products containing copper compounds as their active ingredients, such as Blue Vitriol, Bordeaux Mixture, Cupravit, Cuproxat, and Funguran, are currently used extensively (Tomlin, 2006).

The toxicity of copper fungicides is due to copper's ability to precipitate proteins and cause coagulation of the cytoplasm (Hughes and Pole, 1989). Also, excess of copper has inhibitory effect on the growth of fungal strains, which can lead to reduced auxin, gibberelin and cytokinin levels (El-Mehalawy, 1999). Taking into account these important facts, it is evident that copper content should be controlled to ensure final product quality.

A few spectrophotometric methods are currently available for copper determination in copper-based fungicides. In one such method, copper is determined by spectrophotometric bathocuproine method

(Rangaswamy et al., 1970). Bathocuproine disulfonate forms an orange coloured chelate complex with copper. Extraction of the coloured compound is relatively slow and complexation occurs in acid medium. In another approach, copper in environmental products, pharmaceuticals and standard alloy samples is determined by the analytical reagent benzildithiosemicarbazone (Reddy et al., 2003). The molar absorptivity value of the complex reveals that the reagent is more sensitive in copper determination than earlier reagents had been.

Copper is also determined spectrometrically after digestion of fungicide samples with nitric acid to elemental copper, which can be easily measured at 324.7 nm by atomic absorption in oxidizing air-acetylene flame, and this is the most widely used method (Rasero, 1981).

In this work, rapid spectrometric method with sample preparation using a mixture of sulfuric and nitric acid is proposed. The digestion procedure with two mineral acids allows a highly convenient determination of copper in different fungicide products by atomic absorption spectrometry (AAS).

MATERIAL AND METHODS

Reagents and samples

All the solutions were prepared from analytical grade reagents. Copper working solution was prepared from 1000 mg L^{-1} copper standard solution (Solutions, Plus Inc, Varian) by transfering 10 mL and diluting to 100 mL with deionized water. More dilute copper solutions were made by pipetting appropriate volumes (1.0, 2.0, 3.0, 4.0 and 5.0 mL) of the standard solution with deionized water (up to 100 mL volumetric flasks) in order to obtain final concentrations of: 1.0, 2.0, 3.0, 4.0 and 5.0 mg Cu L^{-1}, respectively.

Several commercial copper fungicide products were used in the analysis: Blue Jet 50 DF ("Mumbai", India), Copper Oxychloride, Bordeaux Mixture, Blue Bordo WDG, Cuprozine 35 WP and Funguran OH ("Galenika", Serbia). The declared copper content in these products ranged from 100 g Cu kg^{-1} (Bordeaux Mixture) to 594 g Cu kg^{-1} (Blue Bordo WDG). The exact copper content was determined by flame atomic absorption spectrometry.

Instrumentation

A flame atomic absorption spectrometer SpectrAA Varian 220 was used for all measurements in oxidizing air-acetylene flame. The absorption signal of copper at a most sensitive analytical wavelength was measured using a Varian hollow cathode lamp for copper, operated at 7 mA (Table 1). A pneumatic nebulizer with a glass impact bead was used. Spectral slit width was 0.7 nm and conventional burner for air-acetylene flame was placed in position at 0.7 cm.

Table 1. Instrument parameters used for copper determination by AAS
Tabela 1. Instrumentalni parametri u toku određivanja bakra metodom AAS

Instrument parameters Instrumentalni parametri	Cu
Wavelength (nm) Talasna dužina	324.7
Flame type Tip plamena	Air-acetylene, oxidizing Vazduh-acetilen, oksidujući
Lamp current (mA) Struja lampe	7.0
Slit width (nm) Širina otvora plamenika	0.7
Working range (mg L^{-1}) Radni opseg standarda	0-5
Nebulization rate (mL min^{-1}) Brzina raspršivanja	5.0

Sample preparation

Approximately 0.5 g of each test sample was weighed and placed in a 250 mL volumetric flask. Concentrated sulfuric acid was added and heated for 15 minutes. Then nitric acid was added dropwise into the solution. The solution was mixed vigorously and finally diluted to 250 mL with deionized water. After cooling, solution was filtered and then appropriate solution volume was pipetted into 100 mL of deionized water.

To determine copper content in fungicide products, the obtained solutions were nebulized by digestion with sulfuric and nitric acid, and atomic absorption signals of copper were measured, based on standard calibration curve. In order to compare the results obtained by digestion with both sulfuric and nitric acid, six fungicide products were simultaneously analysed using an officially recommended method with nitric acid as solution for sample digestion (Rasero, 1981).

Analysis of results

A two-sided F-test (P = 0.05) was used to test whether the recommended AOAC method and the proposed method involving a different digestion procedure differed in term of precision. Interferences by the reagents used and other metals in the samples analysed were also investigated.

RESULTS AND DISCUSSION

Treating copper fungicide samples with a mixture of concentrated sulfuric and nitric acid is an effective way of completely digesting them. Under appropriate conditions, the presence of acids used does not alter the atomic absorption signal of copper. The proposed procedure enables effective digestion of fungicide samples within 15 minutes. The recovery values were all very close to 100%, i.e. 98.63-103.40%, and their interval is narrower than the digestion results obtained by the proposed official method, which ranged from 91.35 to 104.12%.

Effect of sample amount and digestion time

In order to investigate the effects of different sample amounts and different digestion duration times, several amounts of the Blue Bordo WDG product were digested in various time intervals. A relatively large sample amount (about 1.0 or 0.75 g) was left to be digested over a prolonged period of time, and hence produced incorrect decreased results since the actual amount of copper was less than estimated.

During the study of digestion time nessecesary for releasing copper from the complex fungicide matrix, it was clarified that this factor had influence on the expected amount of copper (Table 3). The results are best when digestion time is 15 minutes. Shorter time periods enable complete digestion of organic matter, while digestion periods longer than 15 minutes result in higher concentrations of copper (Table 3).

Generally, the best results were obtained when the initial weight of samples analyzed was about 0.5 g. Also, digestion of the mixture consisting of both sulfuric and nitric acids was most effective when lasting 15 minutes.

The results obtained are consistent with the official spectrometric method of analysis using nitric acid only for sample preparation. The calculated F value (F = 1.50) is less than the critical level, so there is no significant difference between the two methods at the 5% probability level.

Interferences

The influence of other metals that are usually present in fungicide products on the atomic absorption signal of copper was studied. Copper is highly tolerant of other metals present in the sample. Thus, lead, cadmium and arsenic (present in very low concentrations), have no effect on copper content determination by atomic absorption.

Table 2. Content of copper in fungicide samples analysed
Tabela 2. Sadržaj bakra u analiziranim uzorcima fungicida

Sample Uzorak	Content of Cu declared Deklarisani sadržaj bakra (g Cu kg^{-1})	Content of Cu found – Nađeni sadržaj bakra (g Cu kg^{-1})			
		Digestion with HNO_3 Digestija HNO_3	RSD values* Vrednosti RSD (%)	Digestion with H_2SO_4 : HNO_3 mixture Digestija smešom H_2SO_4 : HNO_3	RSD values* Vrednosti RSD (%)
Blue Jet 50 DF	500.00	514.24	0.35	507.62	1.14
Copper Oxychloride	570.00	593.50	1.78	589.40	0.55
Blue Bordo	200.00	201.51	0.52	200.47	0.93
Funguran-OH	500.00	501.10	1.18	504.08	0.93
Cuprozin 35 WP	510.00	521.72	0.65	512.36	1.73
Bordeaux Mixture	100.00	91.35	0.71	98.63	0.60

* Relative standard deviation values were obtained for six replicates
* Vrednosti relativne standardne devijacije dobijene na osnovu šest ponavljanja

Table 3. Cu content after different Blue Bordo WDG sample treatments
Tabela 3. Sadržaj Cu nakon različite pripreme uzorka Blue Bordo WDG

Approximate sample initial weight Približna početna masa uzorka (g)	Mineral acid Neorganska kiselina (5 mL)	Digestion time Vreme digestije (min)	Cu content Sadržaj Cu ($g\ kg^{-1}$)	RSD values* Vrednosti RSD (%)
1.01	$H_2SO_4 : HNO_3$	20	197.48	1.84
0.75	$H_2SO_4 : HNO_3$	20	198.50	0.71
0.50	$H_2SO_4 : HNO_3$	10	199.71	0.42
0.50	$H_2SO_4 : HNO_3$	15	200.47	0.93
0.50	$H_2SO_4 : HNO_3$	20	201.16	1.58
0.50	$H_2SO_4 : HNO_3$	25	201.18	0.73
0.50	HNO_3	15	201.51	0.52
0.50	H_2SO_4	15	202.60	0.94

* Relative standard deviation values were obtained for six replicates
* Vrednosti relativne standardne devijacije dobijene na osnovu šest ponavljanja

Treatment of copper-based fungicides with sulfuric and nitric acids for 15 minutes enables a precise atomic absorption determination of copper, compared to the official AOAC method using nitric acid only.

Under the working conditions used, the reagents employed to digest the samples did not alter the spectrometric signal of copper. Interferences of other metals present in typical concentrations in the fungicide products were not observed. As a result, copper can be determined by running calibration curves from aqueous solutions.

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Određivanje bakarnih fungicida metodom plamene atomske apsorpcione spektrometrije primenom digestije sumpornom i azotnom kiselinom

REZIME

Fungicidi na bazi bakra se uspešno mogu određivati plamenom atomskom apsorpcionom spektrometrijom praćenjem apsorpcije bakra, nakon digestije uzoraka koncentrovanom sumpornom i azotnom kiselinom u toku 15 minuta. Pod optimalnim uslovima, rezultati analize su u saglasnosti sa onima dobijenim zvaničnom AOAC metodom, s tim što su vrednosti prinosa u užem intervalu (98.63 do 103.40%) u odnosu na odgovarajuće vrednosti dobijene AOAC metodom. Vrednosti relativne standardne devijacije su manje od 2%. Predloženi postupak digestije se može primeniti za određivanje uzoraka bakarnih fungicida koji sadrže od 100 do 594 g Cu kg^{-1}.

Ključne reči: Fungicidi na bazi bakra; vlažna digestija (H_2SO_4 + HNO_3); određivanje bakra; plamena atomska apsorpciona spektrometrija (AAS)