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Determination of Dithiocarbamate Fungicide Residues in Food by a Spectrophotometric Method Using a Vertical Disulfide Reaction System

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Dithiocarbamates are a class of fungicides extensively used in many crops worldwide. The current residue definition of dithiocarbamates in food for compliance with maximum residue limits, at national and international levels, is total residues arising from the use of any or each dithiocarbamate fungicide, determined as CS₂. The analytical method most frequently used to analyze dithiocarbamate residues in food for monitoring purposes was proposed more than 30 years ago. In this method, total dithiocarbamates are decomposed to CS₂, which is purified and reacted with a cupric reagent. The yellow complex formed is quantified by spectrophotometry. In this paper, a new reaction system for the purification and complexation of CS₂ is proposed. The new system is less fragile than the traditional design, is easier to assemble, and allows for a higher sample throughput, in addition to being of low cost. Recovery of added mancozeb, thiram, or ziram (0.15–8.0 mg/kg) in rice, beans, apple, banana, orange, papaya, tomato, cucumber, and potato ranged from 82 to 120%, with relative standard deviations from 0 to 10% ($n = 3$ or 5). Analysis of apple, tomato, and papaya samples with field-incurred dithiocarbamate residues showed comparable results using both the traditional and the new reaction systems.

Keywords: *Dithiocarbamate fungicides; CS₂ reaction system; spectrophotometry; food analysis*

INTRODUCTION

Dithiocarbamate fungicides are used worldwide on a range of crops, mainly due to their high efficiency in controlling plant fungal diseases and relatively low mammalian acute toxicity. The toxicological significance of the ethylenebis(dithiocarbamate) residues in food stems from the metabolite or degradation product ethylenethiourea, known to be carcinogenic and teratogenic in laboratory animals (*1*).

To guarantee the use of pesticides in the field according to good agricultural practices and to protect consumer health, regulatory agencies establish tolerances, or maximum residue limits (MRL), in treated crops. The methodology needed to analyze residues of dithiocarbamates in food is not always trivial, mainly due to the polymeric character and insolubility of some of these compounds (Figure 1). Reversed-phase HPLC methods have been proposed for the analysis of thiram and other dithiocarbamates in food using UV (*2, 3*) or electrochemical detection (*3, 4*). However, these methods are not selective enough to distinguish among members within the ethylenebis(dithiocarbamate) subclass (nabam, maneb, zineb, and mancozeb) or within the *N,N*-dimethyldithiocarbamate subclass (ziram and ferban).

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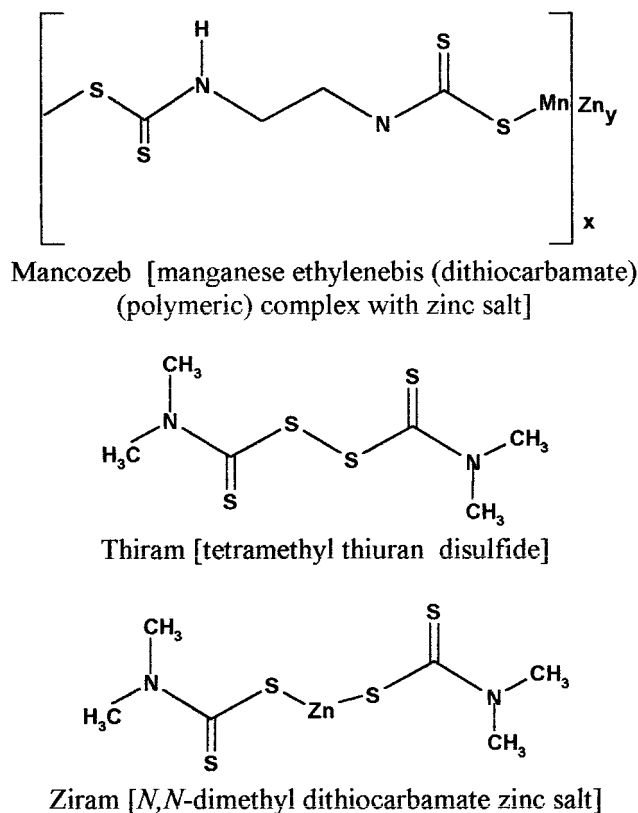


Figure 1. Dithiocarbamate fungicides representative of three subclasses.

Verma et al. (5) describe the use of tetraacetonitrile-copper (I) perchlorate for the spectrophotometric determination of thiram in grain, and Malik (6) proposed the determination in grain of ferbam as its iron(II)-bathophenanthroline-tetraphenylborax complex.

A multiresidue methodology that could discriminate among all of the dithiocarbamate compounds in a single analysis would be advantageous for monitoring and dietary risk assessment purposes. As this methodology is not currently available, the MRLs of dithiocarbamates, at both the national (7–10) and international levels (11), are defined as total residues arising from the use of any or each dithiocarbamate fungicide, determined as CS₂.

Gas chromatographic methods have been described for the determination of the CS₂ formed after acid hydrolysis of dithiocarbamates in food using a head-space technique (12–14) or after the partitioning of the CS₂ into an organic solvent (15, 16). However, the method most frequently used worldwide, mainly because of its low cost and minimal analytical requirements, was proposed by Cullen (17) and modified by Keppel (18). This method is based on the spectrophotometric determination of the cupric complex formed with the CS₂ evolved from the acid decomposition of dithiocarbamates in the presence of stannous chloride as a reducing agent. Recently, a flow injection system for the spectrophotometric quantification of the CS₂ partitioned into hexane was reported (19).

The apparatus for the purification of the CS₂ formed after hydrolysis and formation of the CS₂ cupric complex used in the Cullen and Keppel method has been utilized by laboratories over the years with no significant changes from its original design (7, 8, 20). The objective of this work was to propose a new CS₂ reaction system, less fragile than the traditional design, with the poten-

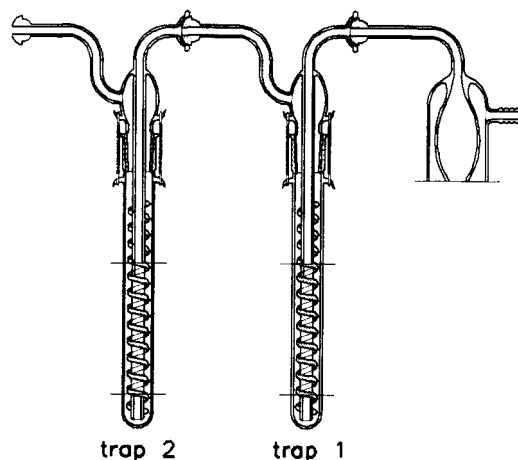


Figure 2. Traditional in-series two-trap CS₂ reaction system.

tial of decreasing analysis times and increasing sample throughput while maintaining good analytical performance and low cost.

MATERIALS AND METHODS

Reagents. All chemicals were of analytical grade, obtained from Vetec or E. Merck. Solutions of copper(II) acetate monohydrate (color reagent) were prepared (0.48 g in 100 g of diethanolamine/1 L of water) and kept at 5 °C protected from light. An acid stannous chloride solution was prepared by dissolving 31.25 g of SnCl₂·2H₂O in 500 mL of concentrated HCl and bringing the volume to 2.5 L. A stock solution of carbon disulfide (0.002 mg/mL) was prepared in ethanol. Nitrogen gas was of commercial grade (White Martins). A 0.543 mg/g stock mixture of mancozeb (74.5% purity and 41.8% CS₂; Dr. Ehrenstorfer), a 0.541 mg/g stock mixture of maneb (95% purity; Ultra Scientific, North Kingstown, RI), and a 0.612 mg/g stock mixture of ziram (98% purity; Ultra Scientific) were prepared in milk sugar lactose (E. Merck). A 0.0150% stock solution of thiram (99.8% purity; Uniroyal Chemical, Middlebury, CT) was prepared in acetone. The traditional and proposed reaction systems were made by Vidrolima Comércio, Importação e Serviços em Vidros Ltda, Brasília, DF, Brazil.

Standard Curve. Known volumes of the CS₂ stock solution were transferred to 25 mL volumetric flasks, 15 mL of the color reagent was added, the volume was completed with ethanol, and the solution was allowed to stand for 15 min. The absorbance was measured in a continuous flow Gilford Stasar III spectrophotometer (Gilford Instruments, Oberlin, OH) at 435 nm. A typical standard curve ranged from 0.38 to 23 μg of CS₂/mL, corresponding to 0.10–6 mg of CS₂/kg in the food sample.

Apparatus. The acid decomposition of the dithiocarbamates occurs in a two-neck round-bottom boiling flask placed in a heating mantle. The flask is connected to a nitrogen inlet tube and to a condenser, which is then connected to the traditional or proposed CS₂ reaction system.

Traditional CS₂ Reaction Systems. A system based on the design of Cullen (17) consists of two traps in series (Figure 2). Trap 1 contains 20 mL of 10% NaOH solution, and trap 2 contains 15 mL of color reagent. A modification of this design was proposed by Thier and Zeumer (21) and includes a third trap containing 10 mL of a 30% Pb(CH₃COO)₂·3H₂O solution, which is connected to the condenser and to the NaOH-containing trap. The purpose of both NaOH and lead acetate solutions is to purify the CS₂ formed, removing volatile interferences, mainly H₂S and SO₂.

Proposed Vertical CS₂ Reaction System. The new system is composed of two segments assembled in a vertical format (Figure 3). The first segment (32 cm total length) contains an antireflux retention valve, a diffuser tube, and a trap containing the 10% NaOH solution (trap 1). The second segment (27

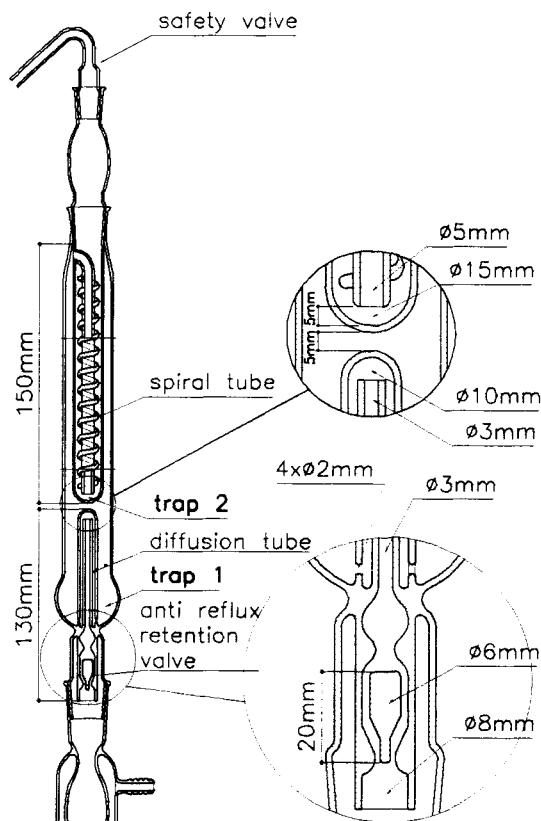


Figure 3. Proposed vertical CS₂ reaction system.

cm total length), which is inserted inside the first segment, contains the reaction tube (trap 2), with the color reagent.

Sample Preparation and Analysis. Dithiocarbamates are nonsystemic fungicides, and their residues are expected to remain on the surface of the crop. Polished rice and dry beans were analyzed without any prior processing. The other commodities (apple, orange, papaya, banana, cucumber, tomato, and potato) were frozen and subsequently cut into pieces. Spiked samples were used to test the traditional and new CS₂ reaction systems. Of each sample, 100 g was transferred to the two-neck round-bottom flask, and a known volume (thiram) or mass (mancozeb, maneb, or ziram) of the dithiocarbamate stock solution or mixture was added. The flask was agitated, 175 mL of stannous chloride acid solution was added, and the condenser and the N₂ inlet were connected. The traditional two-trap (Figure 2), three-trap in series, or new CS₂ reaction system (Figure 3) was connected to the condenser and the N₂ gas and heating mantle turned on. Gas flow (100–180 mL/min) and heat were controlled to produce a gentle and constant gas flow and reflux. After 45 min, the system was turned off, the solution in trap 2 was transferred to a 25 or 50 mL volumetric flask, the volume was completed with ethanol, and the absorbance was measured at 435 nm. For all crops, a control sample with no dithiocarbamate added was analyzed. No dithiocarbamate was found in any control sample tested.

RESULTS AND DISCUSSION

Traditional CS₂ Reaction Systems. The system proposed initially for the spectrophotometric analysis of dithiocarbamates consists of a two-neck round-bottom flask connected to a condenser, which is connected to two traps in series (Figure 2). Air or nitrogen is introduced through the system, and a vacuum line is connected to the last trap (17, 18, 20). Thier and Zeumer (21) modified this design by the addition of a third trap containing lead acetate solution to aid in the removal of interferences. They used air to carry gases through the system. The methodology recommended by the

AOAC International to analyze dithiocarbamates in fungicide formulations utilizes a similar system, containing three traps in series and a vacuum line to pull gas through the system (22). In this methodology, CS₂ is reacted with methanolic KOH in the last trap and xanthate formed is titrated with I₂ solution.

The two-trap and three-trap in series systems were tested for the analysis of dithiocarbamates in tomato. The traps contain a spiral (Figure 2) to give a homogeneous contact between the gases and the reagents and to guarantee the efficiency of the reactions, namely, the removal of volatile interferences and the complexation of CS₂ with the cupric reagent. The performance of the two-trap system was satisfactory. Recovery of added mancozeb, ziram, or thiram (0.15–4.0 mg of CS₂/kg) ranged from 81 to 105%, and relative standard deviations of triplicate analyses were from 0 to 6%. When the three-trap system was used, recovery of added mancozeb, maneb, or thiram (0.8–5.0 mg of CS₂/kg) ranged from 45 to 75%, and relative standard deviations of triplicate analyses were from 0 to 21%. The low performance of the three-trap system is probably due to the higher number of connections, which can increase the chance of gas leaking. The good performance of the two-trap system shows that the NaOH solution is sufficient to purify the CS₂ prior to complexation and an additional trap with lead acetate solution is not necessary.

The two-trap system was used for two years in the laboratory for monitoring dithiocarbamates in tomato collected in the field and at the market of the Federal District area. During this routine work, however, this system showed some drawbacks. The assembling and disassembling procedure is time-consuming and has to be done very carefully to prevent damage. In addition, the seal of the spherical joints that connect the traps (Figure 2) had to be checked before each analysis to guarantee a leak-free system.

Proposed Vertical CS₂ Reaction System. To solve some of the problems present in the CS₂ reaction system based on the traditional design, we developed a new reaction system (Figure 3). The system is very compact, relatively robust, easy to assemble and clean, and practically free of losses at the connections. The same sample preparation, reaction times, and volumes and concentrations of the reagents were used with both the traditional two-trap and the new reaction systems.

The system is composed of two segments connected vertically to the condenser (Figure 3), which is connected to the two-neck round-bottom hydrolysis flask. From the condenser, the N₂ gas carrying the CS₂ formed by the acid hydrolysis of the dithiocarbamates passes through the antireflux retention valve and the diffuser tube, reaching the NaOH solution (trap 1). After leaving trap 1, the gases enter the tube covered by a spiral in the second segment through an upper entrance and reach the color reagent in trap 2. The spiral is intended to guarantee a homogeneous contact between the gases and the color reagent and an efficient complexation reaction.

The most critical component of the new system is the diffusion tube. The gas flow leaving the diffusion tube reaches trap 1 through four small holes in the base of the tube that contains the diffuser tube. This creates a smoother gas flow through the NaOH solution and increases the extraction efficiency of the interferences formed in the hydrolysis flask. When the diffusion tube

Table 1. Recovery of Added Dithiocarbamate Fungicides in Food Using the Proposed Vertical CS₂ Reaction System

food	compound	amount added (mg of CS ₂ /kg)	av % recovery ^a	RSD ^b (%)
apple	mancozeb	0.15	102	9.9
		1.0	82	7.8
		3.0	98	5.1
	thiram	1.0	110	0
	ziram	1.0	90	5.1
papaya	mancozeb	0.15	120	2.8
		1.0	106	4.9
		3.0	106	3.0
	thiram	0.15	103	2.8
		1.0	91	1.5
	3.0	106	3.0	
orange	mancozeb	0.15	86	4.5
		1.0	95	7.3
banana	mancozeb	0.15	105	3.8
		1.0	93	5.1
		2.0	106	8.2
	thiram	1.0	110	0
	ziram	1.0	87	4.0
dry beans	mancozeb	0.15	102	9.9
		1.0	96	1.0
	thiram	1.0	110	0
	ziram	1.0	90	3.9
polished rice	mancozeb	0.15	120	3.3
		1.0	101	7.5
		8.0	95	6.6
	thiram	1.0	91	2.8
	ziram	0.15	108	1.5
		1.0	110	0
		3.0	106	7.4
potato	mancozeb	0.15	98	10
		1.0	86	4.0
	thiram	1.0	107	2.0
	ziram	1.0	90	2.3
tomato	mancozeb	0.15	107	0
		1.0	105	2.0
		4.0	103	1.4
cucumber	mancozeb	0.15	104	7.2
		1.5	102	3.9
	thiram	0.15	100	0
		1.5	93	0
	ziram	0.15	100	0
	1.5	91	3.8	

^a $n = 3$ in all cases except for thiram in papaya and ziram in rice at 0.15 and 3.0 mg/kg, where $n = 5$. ^b Relative standard deviation.

was removed from the system, large gas bubbles were formed, carrying basic vapor from trap 1 to the color reagent solution in trap 2, changing its pH. The efficiency of the complexation reaction in trap 2 decreased as the pH increased, yielding low recoveries (<60%).

The N₂ flow rate was also found to be critical and should not be >180 mL/min; otherwise, it could also carry NaOH from trap 1 to trap 2. Higher flow rates can decrease the volume of the alcoholic color reagent solution in trap 2, also affecting the performance of the system. The antireflux retention valve helps to regulate the gas flow, in addition to preventing any reflux in case of a sudden decrease in pressure.

The efficiency and reproducibility of the new system (Figure 3) were tested with different food crops spiked with dithiocarbamates (Figure 1) at levels from 0.15 to 8.0 mg of CS₂/kg. These concentrations are within the range of MRLs for dithiocarbamates in various crops in Brazil (10) and in the Codex Alimentarius (11). The

Table 2. Analysis of Samples with Field-Incurred Dithiocarbamate Residues Using the Traditional In-Series and the Proposed Vertical Reaction Systems^a

crop	traditional		proposed		
	mg of CS ₂ /kg	RSD (%)	mg of CS ₂ /kg	RSD (%)	RSD ^b (%)
apple	1.2	4.7	0.98	12	14
	0.96	13	0.87	10	6.9
	0.29	11	0.34	6.7	11
tomato	0.45	6.9	0.47	4.3	3.1
	0.49	10	0.43	7.3	9.2
papaya skin	0.46	13	0.48	7.1	2.7

^a Each residue value is the average of three replicate samples. ^b Relative standard deviation of residues in both systems.

foods chosen represent the different crop classes that are normally treated with dithiocarbamates: cereal grains (rice), legume vegetables (beans), fruits with edible and inedible peels (apple, banana, orange, and papaya), fruiting vegetables (tomato and cucumber), and root vegetables (potato). The results are shown in Table 1. For all of the compounds and foods tested, the recoveries fell within the range of 82–120%, with relative standard deviations ($n = 3$ or 5) ranging from 0 to 10%.

The vertical CS₂ reaction system described in this paper has been applied successfully in our laboratory for monitoring dithiocarbamates in food for compliance with Brazilian MRLs and for dietary exposure assessment. The system represents a considerable improvement from the traditional in-series two-trap system in three main aspects: (a) reduced space requirements in a laboratory; (b) reduced time needed for assembling/deassembling, resulting in a higher sample throughput; and (c) lowered repair costs. In a laboratory bench space of 1.5 × 0.5 m, where there were two in-series two-trap systems, we now have five new systems. The two-trap traditional system has at least four segments, which have to be handled carefully to avoid damage, demanding ~10 min for assembling, including checking for gas leaks. The vertical system has only two segments, which are much less fragile, requiring <2 min to assemble, and does not need to be checked for gas leaks. The robustness of the new system also reduced considerably its maintenance due to damaged parts, decreasing the costs per analysis.

From over the 100 food samples of rice, beans, tomato, potato, banana, papaya, and orange collected at local market and analyzed in the laboratory using the vertical system, dithiocarbamates were detected in only tomato, apple, and papaya skin. Analysis of samples with field-incurred dithiocarbamate residues was performed using both the traditional in-series and the proposed vertical systems (Table 2). The two systems yield comparable results, with the relative standard deviations (RSD) of the values falling within the same range of each system RSD.

Conclusion. Although chromatographic methods have been applied successfully in the analysis of dithiocarbamates as CS₂ in food, the spectrophotometric method proposed by Cullen (17) and Keppel (18) is still widely used. The method can achieve the sensitivity required for compliance with national and international MRLs, and it is especially suitable for monitoring laboratories in developing countries, due to its low costs and minimal laboratory and analytical expertise requirements. We have proposed a new CS₂ reaction system for use with the spectrophotometric method. The

new system is less fragile than the old system, is easier to assemble, and allows for higher sample throughput. This new reaction system can potentially be applied for the analysis of dithiocarbamates in fungicide formulations to replace the apparatus recommended by the methodology of the AOAC International (22).

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