Column Preconcentration and Spectrophotometric Determination of Ziram and Zineb in Commercial Samples and Foodstuffs Using (1,2′-Pyridylazo)-2-naphthol (PAN)–Naphthalene as Adsorbate

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A procedure has been developed for the determination of zinc(II) bis(dimethyldithiocarbamate) (ziram) and zinc(II) ethylenebis(dithiocarbamate) (zineb) after preconcentration on a column using naphthalene-(1,2′-pyridylazo)-2-naphthol (PAN) as adsorbent. Ziram and zineb are quantitatively retained on the column in the pH range of 9.0−12.5 and at a flow rate of 1−2 mL/min. The solid mass consisting of the Zn−PAN complex along with naphthalene is dissolved from the column with 5 mL of dimethylformamide (DMF). Absorbance of the complex was measured at 550 nm; Beer's law is obeyed over the concentration ranges of 2.0−22.0 μg of ziram and 5.0−19.8 μg of zineb in 10 mL of the final DMF solution. Ten replicate determinations on a sample solution containing 20 μg of ziram and 18 μg of zineb gave a mean absorbance of 0.33 with relative standard deviations of 0.80 and 0.70%, respectively. The interference of various ions has been studied. The method has been employed for the determination of ziram and zineb in commercial samples and in various foodstuffs, and the results were compared with the earlier reported methods.

INTRODUCTION

Ziram (**Figure 1**) is an agricultural dithiocarbamate fungicide used on a wide variety of plant fungi and diseases. It may be applied to the foliage of plants, but it is also used as a soil and/or seed treatment. It is also used as an accelerator in rubber manufacturing, packaging materials, adhesives, and textiles. Another use of the compound is as a bird and rodent repellent. Ziram is often marketed as a wettable powder or as granules.

Zineb (**Figure 1**) is used as a fungicide to prevent crop damage in the field and to protect harvested crops from deterioration in storage or transport. Zineb is used to protect fruit and vegetable crops from a wide range of foliar and other diseases. It is available as wettable powder and dust formulations.

Analytical methods are required for monitoring pesticides in various environmental samples. A number of methods such as chromatography (*1*, *2*), titrimetry (*3*, *4*), voltammetric (*5*), polarography (*6*), headspace gas chromatography (GC) (*7*, *8*), spectrophotometric (*9*), and capillary electrophoresis (*10*) have been reported for the detection and analysis of ziram and zineb. In one such method ziram and zineb are determined on the basis
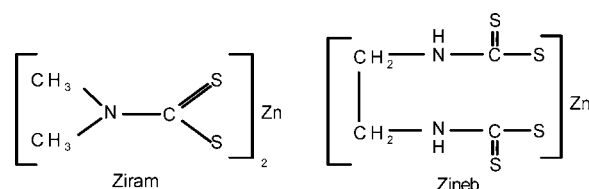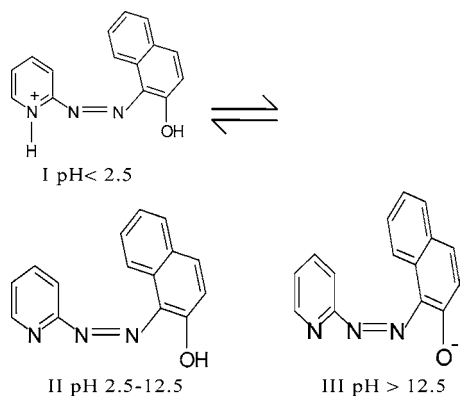

Figure 1. Ziram [zinc(II) bis(dimethyldithiocarbamate)] and zineb [zinc(II) ethylenebis(dithiocarbamate)].

of their decomposition by hot mineral acids to amine and carbon disulfide; liberated carbon disulfide is then absorbed in methanol potassium hydroxide solution, and the potassium methyl xanthate thus formed is titrated iodometrically (*11*). In another approach, carbon disulfide is absorbed in an ethanol solution containing copper(II) and an alkylamine to form copper dialkyldithiocarbamate, which can be determined photometrically (*12*−*14*). Zineb has also been determined by converting it into molybdenum (*9*) and copper (*15*) complexes.

However, all of these methods suffer from the following disadvantages:

1. Methods other than gas chromatography are indirect and time-consuming, and their sensitivity is low.

2. GC methods are sensitive but suffer from a lack of the selectivity because all dithiocarbamate pesticides evolve carbon disulfide on acid hydrolysis. Moreover, the dithiocarbamate

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Figure 2. Structure of PAN [(1,2′-pyridylazo)-2-naphthol] at various pH values.

Figure 3. Absorbance curves of PAN (●) and its zinc complex (■). Conditions: ziram = 20 μg or zineb = 18 μg, pH 9.2, PAN (0.01%) = 0.5 mL, Triton X-100 = 2 mL.

decompositions are strongly dependent on temperature and on individual dithiocarbamtes. The evolution of CS_2 requires >2 h for the decomposition of dithiocarbamte.

3. High-performance liquid chromatography (HPLC) methods are expensive as these involve the use of toxic and expensive solvents.

Here, we present a relatively simple, rapid, sensitive, and selective spectrophotometric method for the determination of ziram and zineb after its preconcentration onto (1,2′-pyridylazo)-2-naphthol (PAN)−naphthalene adsorbate in the column, and this can be determined subsequently using spectrophotometry at 550 nm. Earlier we have used PAN for the determination of maneb in various commercial and synthetic samples (*16*). Ziram and zineb react with PAN (**Figure 2**), forming colored complexes.

$$Zn-Dtc + PAN \rightleftharpoons Zn-PAN + Dtc$$
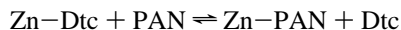
PAN acts as a tridentate ligand complexing with zinc through the ortho-hydroxyl group, the azonitrogen nearest the phenolic ring, and the hetrocyclic nitrogen atom, giving two five-membered chelate rings.

MATERIALS AND METHODS

Apparatus and Reagents. A Spectronic-20 spectrophotometer (Bausch and Lomb) and an ECIL digital pH-meter (Hyderabad, India) were used during the investigation. A 0.01% PAN (Merck, GR) solution in methanol was prepared by dissolving 10 mg in solvent and diluting to 100 mL. Ziram and zineb were obtained from Wilson Laboratories (Mumbai, India); their purity was checked by elemental analysis and by melting point. Separate 0.1% stock solutions of ziram in 0.1 M NaOH and zineb in 1.0 M NaOH were prepared by dissolving 100 mg of solute, respectively, in 100 mL of base solution. Boric acid buffer (pH 9.2) was prepared by mixing boric acid solution (0.2 M) with an equal volume of KCl (0.2 M) solution, and the pH was adjusted to 9.2 with 0.2 M NaOH. A 0.001 M solution of alkali metal salt for anions (bromide, chloride, fluoride, acetate, etc.) and a 1×10^{-4} M solution of different metal salts for cations [Pb(II), V(V), Th(IV), Sb(VI), Ni(II), Co(II), Mo(VI), Fe(II), Fe(III), Hg(II), Cd(II), Cu(II), and Mn(II)] were used to study the interference of these ions as they are easily available. Ten percent solutions of the surfactant sodium lauryl sulfate (SLS) (Merck) (w/v), Triton X-100 (TX-100) (SDS) (v/v), cetyl pyridinium bromide (CPB) (BDH) (w/v), and cetyl trimethylammonium bromide (CTAB) (Loba) (w/v) were prepared in distilled water for the solubilization of the complexes.

Preparation and Use of the Column. Twenty grams of naphthalene was dissolved in 40 mL of warm acetone (35 °C) with stirring. The solution was transferred to a beaker containing 1500 mL of distilled water, and 100 mL of 0.01% PAN solution and 50 mL of TX-100 were added. The resulting solution was stirred continuously for 2 h and allowed to stand for another 2 h. The supernatant solution was
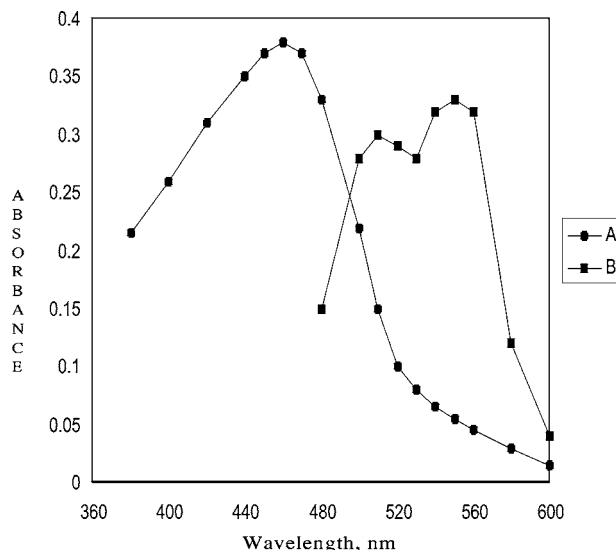
decanted. The naphthalene−PAN adsorbent obtained in the slurry form was washed twice with distilled water and packed to a depth of 1.5−2.0 cm in a glass column (7 mm i.d. × 150 mm) fitted with a quartz wool plug. The packed column was conditioned to pH 9.2 with 3.0 mL of borate buffer with the flow rate of 1 mL/min. For analysis, sample solution was passed through the column at 1−2 mL/min. The column material was dissolved in 5 mL of DMF, and the absorbance of the solution was measured at 550 nm against a reagent blank prepared similarly.

RESULTS AND DISCUSSION

Absorption Spectra. The absorption spectrum of the Zn−PAN complex (in DMF) recovered from the column was recorded against a reagent blank. The complex shows an absorption maximum at 550 nm (**Figure 3**).

Preparation of the Calibration Curve. To a known volume of the sample solution containing 1−20 μg of ziram or 1−18 μg of zineb were added borate buffer and distilled water to make 10 mL of solution. The rest of the procedure is the same as discussed above in the preparation and use of the column.

Beer's Law and Sensitivity. Under the conditions described above, a calibration graph drawn for the determination of ziram or zineb was linear over the concentration ranges of 2−22 and 5−19.8 μg, respectively, per 10 mL of the final solution. Ten replicate determinations on these sample solutions of 20 μg of ziram and 18 μg of zineb gave a mean absorbance of 0.33 with relative standard deviations (RSD) of 0.80 and 0.70%, respectively. The molar absorptivity of the Zn−PAN complex formed from ziram or zineb ($A = \epsilon lc$, where A is absorbance measured, ϵ is the molar absorptivity, l is the path length, and c is concentration) was calculated to be 5.06×10^4 L mol^{-1} cm^{-1}, and Sandell's sensitivity values (*17*), which is the number of micrograms determined per milliliter for an absorbance of 0.001, were found to be 0.0063 and 0.0055 μg cm^{-2} for ziram and zineb, respectively.

Effect of Standing Time. The absorbance of the solution was measured at different intervals of time, and it was observed that the absorbance of the solution remained constant for >24 h.

Effect of Aqueous Phase Volume. It was observed that the adsorption was quantitative and complete when the aqueous
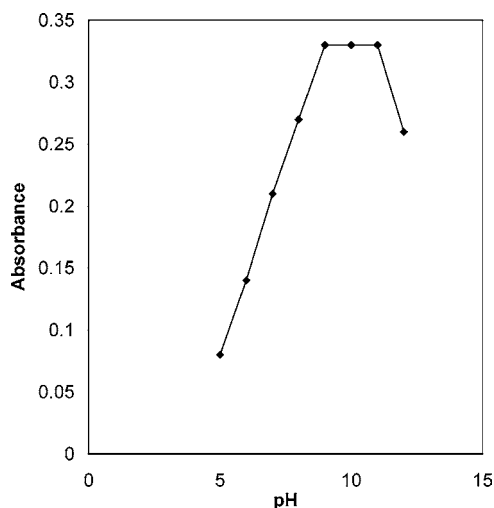
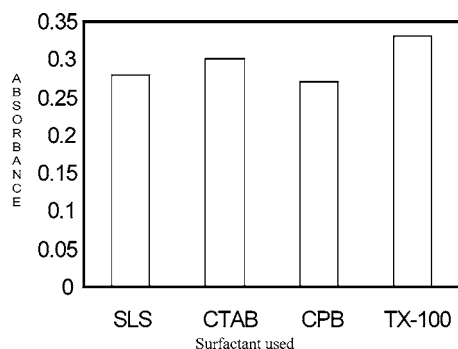**Figure 4.** Effect of pH on the absorbance of the Zn–PAN complex.

Figure 5. Effect of surfactant on the absorbance of the Zn–PAN complex.

Table 1. Determination of Ziram or Zineb in Synthetic Mixtures

percentage composition of individual compounds in the synthetic mixture	amount of ziram or zineb (μg)		recovery[a] (%, RSD)
	taken	found	
ziram, 20 thiram, 15 NaDDC,[b]10 ferbam, 10	20.00	20.10	100.5 ± 1.2
zineb, 20 ferbam, 10 vapam, 10 NaDDC,[b] 15	10.00	10.0	100 ± 1.5
ziram, 15 thiram, 10 vapam, 12 dibam, 10	12.00	12.01	100.08 ± 1.4
zineb, 15 NaDDC,[b] 10 ferbam,[c] 10 vapam, 10	10.00	10.12	101.2 ± 1.66

[a] Mean of five replicate determinations ± standard deviation. [b] Sodium dimethyldithiocarbamate. [c] Ferbam was masked with 1.0 mL of 5% sodium fluoride solution.

phase volume in the preconcentration step was <1500 mL; when the volume >1500 mL, there was a decrease in absorbance.

Effect of Flow Rate. The flow rate was varied from 0.5 to 8 mL/min. It was found that the flow rate did not affect adsorption in this range. A flow rate of 1−2 mL/min was used in all experiments.

Effect of pH. The effect of pH on the retention of the Zn−PAN complex was studied by varying the pH of the solution with 1 M NaOH and 0.1 M HCl solutions. As shown in **Figure 4** the maximum absorbance was observed in the pH range of 9−12.5. The effect of pH shown in **Figure 4** clearly explains that at lower pH the complex formation is not complete, as it is dependent on the OH⁻ ion concentration in the solution to cause the ionization of the phenolic OH⁻ of the naphthol (**Figure 2**). Hence, buffer of optimum pH 9.2 was selected for the present study.

Choice of Solvent. The complex is soluble in dimethylformamide, acetonitrile, dimethyl sulfoxide, and chloroform, but it was insoluble in benzene and carbon tetrachloride. There was little difference in the absorbance in these solvents; thus, DMF, being cheap and readily available, was selected for the present study.

Choice of Surfactant. The complex was soluble in DMF, etc., but to increase the sensitivity, the surfactant was used. The effects of various surfactants such as SLS, TX-100, CPB, and CTAB were studied on absorbance. All of these surfactants readily solubilize the Zn−PAN complex, as the results shown in **Figure 5**, and TX-100 was preferred as it shows maximum absorbance in comparison to other surfactants used.

Interferences. To evaluate the interference of diverse ions, solutions containing 20 μg of ziram or 18 μg of zineb and various amounts of different alkali metal salts or metal ions

were prepared and then analyzed. The following ions did not interfere in the determination 20 μg of ziram or 17.5 μg of zineb: acetate (15 mg), bromide (12 mg), chloride (3 mg), fluoride (2 mg), citrate (15 mg), metabisulfite (7 mg), thiocyanate (0.2 mg), tartrate (0.2 mg), and EDTA (0.07 mg). Of the metal ions examined, Pb(II) (0.3 mg), V(V) (0.03 mg), Th(IV) (0.35 mg), Sb(VI) (0.15 mg), Ni(II) (0.31 mg), Co(II) (0.001 mg), and Mo(VI) (0.14 mg) did not interfere. Fe(II) and Fe(III) were masked with 1.0 mL of 5% sodium fluoride solution; Hg(II), Cd(II), and Cu(II) were masked with 2.0 mL of 5% sodium citrate solution; and Mn(II) was successfully masked with 1.5 mL of 1% potassium bromide solution. The masking ion forms the stronger complex as compared to the PAN complex; therefore, these were used as masking agents. It was also observed that there is no effect of the masking agent on the absorbance at these levels.

Determination of Ziram and Zineb in Synthetic Mixtures. Aliquots of the samples were prepared by mixing solutions of ziram and zineb with other dithiocarbamate solutions, which were then analyzed by the general procedure. The study was conducted to mark the fact that by using this method ziram and zineb can be determined even in the presence of other dithiocarbamates. The results of the determinations are given in **Table 1**.

Dithiocarbamates containing no transition metal ion, for example, dibam (sodium dimethyldithiocarbamate), vapam (sodium monomethyl dithiocarbamate), nabam [sodium ethylenebis(dithiocarbamate)], sodium *N*-methylanilinecarbodithioate, and potassium morpholine-4-carbodithioate, did not interfere in the determination of ziram or zineb. Ferbam (ferric dimethyldithiocarbamate) was masked with 1 mL of 5% sodium fluoride solution, and maneb [manganese ethylenebis(dithiocarbamate)] was masked 1.5 mL of 1% potassium bromide solution. However, ziram and zineb interfere in their individual analyses.

Determination of Ziram and Zineb in Crops (Rice/ Potatoes). The method was applied to the determination of ziram and zineb in crops. A known amount of ziram in 0.1 M sodium hydroxide and zineb in dimethylsulfoxide was crushed with 20 g of the crop with a pestle and mortar and shaken mechanically with 100 mL of DMSO for 1 h. The mixture was filtered, and the residue in the funnel was washed with DMSO (3 × 10 mL). The extracts were combined and evaporated to

Table 2. Determination of Ziram and Zineb in Grains and Potatoes

sample analyzed	crop	ziram/zineb added (μg)	ziram/zineb found[a] (μg)			
			present method (RSD)	recovery (%)	diphenylcarbazone method (RSD)	recovery (%)
ziram	grain	10.00	9.80 ± 2.4	98.0	9.75 ± 2.6	97.5
		20.00	19.90 ± 2.2	99.5	19.80 ± 2.3	99.0
	potato	20.00	19.80 ± 2.14	99.0	19.70 ± 2.15	98.5
		40.00	39.00 ± 2.12	97.5	38.90 ± 2.13	97.3
zineb	grain	10.00	9.90 ± 2.12	99.0	9.85 ± 2.13	98.5
		20.00	19.50 ± 2.10	97.5	19.25 ± 2.12	96.3
	potato	20.00	19.60 ± 2.14	98.0	19.50 ± 2.16	97.5
		30.00	29.50 ± 2.13	98.3	29.40 ± 2.14	98

[a] Each result is the mean of five experiments.

Table 3. Determination of Ziram and Zineb in Commercial Samples

sample	ziram/zineb taken (μg)	ziram/zineb found[a] (μg)			
		present method	recovery	diphenylcarbazone method	recovery
dithan Z-78	10.00	9.99 ± 2.39	99.9	9.98 ± 2.52	99.8
	12.00	12.10 ± 2.25	100.8	12.09 ± 2.43	100.8
	15.00	15.15 ± 2.12	101.0	15.14 ± 2.32	100.9
	20.00	20.00 ± 2.11	100.0	20.00 ± 2.21	100.0
ziram 27% SC	10.0	10.01 ± 2.42	100.1	10.00 ± 2.64	100.0
	15.00	15.00 ± 2.32	100.0	14.95 ± 2.31	99.7
	18.00	18.00 ± 2.22	100.0	17.85 ± 2.25	99.2
	20.00	20.02 ± 2.12	100.1	20.00 ± 2.12	100.0

[a] Each result is the mean of five experiments.

Table 4. Comparison of Molar Absorptivity with Earlier Methods

fungicide	molar absorptivity (L mol^{-1} cm^{-1})	method	remark	ref
ziram	0.15 × 10^4	molybdenum	require acidic medium (4 M H$_2$SO$_4$) and	*9*
zineb	0.62 × 10^4		equilibrium time of 5 min	
ziram/zineb	8.3 × 10^4	diphenylcarbazone	maneb, zineb, ziram interference can be avoided using masking agents	*18*
ziram	1.59 × 10^4	selenite	other dithiocarbamates interfere	*19*
ziram/zineb	6.9 × 10^4	phenylfluorone + CPB[a]	selective and sensitive	*20, 21*
ziram/zineb	5.06 × 10^4	PAN	column concentration factor is 100 times	present work

[a] Cetylpyridinium bromide.

2.0 mL on a water bath (70−90 °C), and the remaining solvent was removed in a current of dry air at room temperature. The residue was dissolved in NaOH and determined by the procedure described. Untreated samples were taken as reference, and the results indicated good recoveries in all cases. The results of the determinations are given in **Table 2**. The results are in comparison to the diphenylcarbazone method (*18*).

Determination of Ziram and Zineb in Commercial Samples. The method was applied for the determination of ziram and zineb in commercial samples ziram 27% SC and dithane Z-78. The formulated product sample solution was prepared as discussed above and determined according to the general procedure. The results obtained by the present method are compared with those obtained by using the diphenylcarbazone method (*18*). The results of the determinations are given in **Table 3**.

Interferences. To evaluate the interference of diverse ions, solutions containing 20 μg of ziram or 18 μg of zineb and various amounts of different alkali metal salts or metal ions were prepared and then analyzed. The following ions did not

interfere in the determination of 20 μg of ziram or 17.5 μg of zineb: acetate (15 mg), bromide (12 mg), chloride (3 mg), fluoride (2 mg), citrate (15 mg), metabisulfite (7 mg), thiocyanate (0.2 mg), tartrate (0.2 mg), and EDTA (0.07 mg). Of the metal ions examined, Pb(II) (0.3 mg), V(V) (0.03 mg), Th(IV) (0.35 mg), Sb(VI) (0.15 mg), Ni(II) (0.31 mg), Co(II) (0.001 mg), and Mo(IV) (0.14 mg) did not interfere. Fe(II) and Fe(III) were masked with 1.0 mL of 5% sodium fluoride solution; Hg(II), Cd(II), and Cu(II) were masked with 2.0 mL of 5% sodium citrate solution; and Mn(II) was successfully masked with 1.5 mL of 1% potassium bromide solution. The masking ion forms the stronger complex as compared to the PAN complex; therefore, these were used as masking agents. It was also observed that there is no effect of the masking agent on the absorbance at these levels.

Comparison of Sensitivity. The present method is more sensitive than the carbon disulfide evolution methods (*2*, *4*, *11*, *13*, *14*, *19*). By this method it is possible to estimate 0.2 μg/mL of ziram and 0.5 μg/mL of zineb; equivalent to 0.0996 and 0.276 μg/mL of evolved carbon disulfide can be determined,

which is superior to reported methods. The sensitivity of the present method is comparable to that of spectrophotometric methods (*18*). The limit of detection (LOD) from crops is 0.05 ppm for ziram and 0.125 ppm for zineb. As summarized in **Table 4**, the wide applicability and simplicity of this method make it an excellent choice among available methods.

ABBREVIATIONS USED

PAN, (1,2′-pyridylazo)-2-naphthol; Dtc, dithiocarbamate; SLS, sodium lauryl sulfate; TX-100, Triton X-100;CPB, cetyl pyridinium bromide (CPB); CTAB, cetyl trimethylammonium bromide; DMSO, dimethyl sulfoxide; EDTA, ethylenediamine-tetraacetate.

LITERATURE CITED

(1) Gustafsson, K. H.; Falhgren, C. H. Determination of dithiocarbamate fungicides in vegetable food stuffs by high performance liquid chromatography. *J. Agric. Food Chem.* **1983**, *31*, 461−463.

(2) Brandsteterova, E.; Lehotay, J.; Liska, O.; Garaj, J. High performance liquid chromatographic determination of dimethyldithiocarbamate residues in agricultural products. *J. Chromatogr.* **1986**, *354*, 375−381.

(3) Verma, B. C.; Sidhu, H. S.; Sood, R. K. Determination of thiuram disulfide and analysis of thiuram disulfide−dithiocarbamate mixtures. *Talanta* **1982**, *29*, 703−705.

(4) Clyde, D. D. Determination of xanthates, dithiocarbamates and some fungicides by titration with electrogenerated iodine in anhydrous acetonitrile. *J. Assoc. Off. Anal. Chem.* **1983**, *66*, 646−650.

(5) Ulkhovich, N. A.; Medyantseva, E. D.; Frolova, V. F.; Romanova, O. N. Application of catalytic currents of hydrogen for complexes of metals with sulphur-containing ligands in extraction voltametry. *Zh. Anal. Khim.* **1983**, *38*, 1963−1968.

(6) Halls, D. J.; Townshend, A.; Zumen, P. The polarographic determination of trace dithiocarbamates and their heavy metal complexes. *Analyst* **1968**, *93*, 219−223.

(7) McLeod, H. A.; McCully, K. A. Headspace gas procedure for screening food samples for dithiocarbamate pesticides residues. *J. Assoc. Off. Anal. Chem.* **1969**, *52*, 1226−1230.

(8) Ministry of Agriculture, Fisheries and Foods. Panel on determination of dithiocarbamates residues of pesticides and veterinary products in foodstuffs by a headspace method. *Analyst* **1981**, *106*, 782−787.

(9) Rao, A. L. J.; Verma, N. Spectrophotometer determination of zinc bis(ethylenedithiocarbamate) (zineb). *Talanta* **1989**, *36*, 1041−1043.

(10) Malik, A. K.; Faubel, W. Capillary electrophoretic determination ziram and zineb. *Talanta* **2000**, *52*, 341−346.

(11) Clarke, D. G.; Baum, H.; Stanley, E. L.; Hester, W. E. Determination of dithiocarbamates. *Anal. Chem.* **1951**, *23*, 1842−1846.

(12) Simpson, B. W. Determination of dithiocarbamates in carbon based formulations of maneb and zineb. *Pestic. Sci.* **1971**, *2*, 127−128.

(13) Lowen, W. K. Determination of dithiocarbamates residues on food crops. *Anal. Chem.* **1951**, *23*, 1846−1850.

(14) Cullen, T. E. Spectrophotometric determination of dithiocarbamate residue on food crops. *Anal. Chem.* **1964**, *36*, 221−224.

(15) Rangaswamy, J. R.; Poornima, P.; Majumder, S. K. S. K. Colorimetric method for estimation zineb. *J. Assoc. Off. Anal. Chem.* **1971**, *54*, 1120−1121.

(16) Rao, A. L. J.; Malik, A. K.; Kapoor, J. Extraction spectrophotometric determination of maneb with 1-(2-pyridylazo)naphthol (PAN). *Talanta* **1993**, *40*, 201−203.

(17) Sandell, E. B. *Colorimetric Determination of Traces of Metals*, 3rd ed.; Interscience Publishers: New York, 1959; p 82.

(18) Malik, A. K.; Rao, A. L. J. Spectrophotometric determination of some dithiocarbamates. *Talanta* **1990**, *37*, 1205−1207.

(19) Malik, A. K.; Rao, A. L. J. Spectrophotometric determination of ziram and zineb using diphenylcarbazone. *Talanta* **1991**, *8*, 941−944.

(20) Malik, A. K.; Kapoor, J.; Rao, A. L. J Simple and sensitive spectrophotometric determination of ziram, zineb and ferbam using phenylfluorone. *J. Environ. Monit.* **2000**, *2*, 367−371.

(21) Malik, A. K.; Rao, A. L. J. Spectrophotometric determination of ziram, ferbam and zineb using diphenylcarbazone. *Talanta* **1991**, *8*, 941−944.