

Flow-injection Spectrophotometric method for the determination of Ziram (Dithiocarbamate Fungicide)

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Abstract. A flow injection spectrophotometric method for the determination of ziram is described. The method is based on the monitoring of the absorbance at 590 nm of the complex formed between zinc from ziram and arsenazo(III). The method is characterised by a detection limit of 0.35 ppm (signal to noise of 3) and a linear range of 2.30 – 37.40 ppm ziram. The inter and intraday percent relative standard deviation for the determination of 2.34 ppm ziram is 0.16 and 0.15%, respectively. The incorporation of an additional flow line of 1% (w/v) sodium fluoride as masking agent allows the determination of 23.40 ppm ziram in the presence of equal amount of foreign ions such as Mn^{2+} , Cu^{2+} , Mg^{2+} , K^{+} and thiram to be conducted satisfactorily. The method has been applied to the direct analysis of food samples such as cucumbers, cabbages, potatoes and tomatoes spiked with ziram.

Abstrak. Satu kaedah spektrofotometri secara suntikan aliran bagi penentuan ziram diterangkan. Kaedah ini berasaskan pemantauan keserapan kompleks pada 590 nm yang terbentuk diantara zink daripada ziram dan arsenazo(III). Kaedah ini dicirikan dengan had pengesanan 0.35 ppm (isyarat kepada bisingan 3) dan julat linear dari 2.30 - 37.40 ppm ziram. Peratus sisihan piawai relatif bagi antara hari dan intra hari untuk penentuan 2.34 ppm ziram adalah masing-masing 0.16 dan 0.15 %. Penambahan suatu aliran 1 %(w/v) natrium florida sebagai agen penopong membolehkan penentuan 23.40 ppm ziram dalam kehadiran pada paras yang sama ion asing seperti Mn(II), Cu(II), Mg(II), K(I) dan tiram dilaksanakan dengan memuaskan. Kaedah ini telah digunakan untuk penentuan terus sampel makanan seperti timun, kobis, ubi kentang dan tomato yang telah dipakukan dengan ziram.

Keywords : Flow injection analysis, ziram, dithiocarbamate, fungicide.

Introduction

Dithiocarbamates have found wide applications in diverse areas – examples include its use as vulcanization additives and antioxidants in the rubber industry [1]; as antiwear, extreme pressure and antioxidant additives in lubricating oils and greases [2]. Its ability to form complexes with metal ions readily lends itself as a useful analytical reagent for the preconcentration of metals and the treatment of wastewaters [3,4]. Another major application of the dithiocarbamates is in the agricultural sector where it is widely used as fungicides, seed disinfectant and nematocides [1,2]. Being a non-systemic but protectant fungicide [5], residues of undecomposed dithiocarbamates are found in fruits, vegetables, etc., where they are applied prior to fungus infection. Although the acute toxicity of dithiocarbamates in general are relatively low [5,6], dithiocarbamates and its degradation products are of much concern as they are suspected to be carcinogenic, mutagenic, goitrogenic and teratogenic [5-7]. Effective from Jan 1, 1998, according to European Community guidelines, the maximum allowable dithiocarbamate residue for some common dithiocarbamates such as maneb, mancozeb, metiram, propineb and zineb (sum expressed as CS_2) in vegetables and fruits is 0.05 mg kg^{-1} [6]. The possibility of underground water contamination by water-soluble dithiocarbamates such as nabam, metham and ziram is also of concern

as these compounds have been reported to be easily transported in soils [8].

Conventional analytical methods reported for the determination of dithiocarbamates involve treatment with hot mineral acid to form the corresponding amines and carbon disulphide. The latter is normally being determined spectrophotometrically [6,9,10], using iodometric titration [11], and head-space gas chromatography [12,13]. The main drawbacks of these acid decomposition methods is not only in the lengthy steps involved, but also the method itself do not differentiate among the dithiocarbamates. Furthermore, endogeneous compounds also release carbon disulphide when some matrices are heated with acid, contributing to false positive results [12].

Alternative methods that do not involve acid digestion procedures are preferred; spectrophotometric [2,14-17], electrochemical [18], high performance liquid chromatographic [1,19,20] and more recently capillary electrophoresis [21] and biosensor [5,22] approaches have been reported. The biosensor approach that was based on the aldehyde dehydrogenase inhibition [5], although exhibiting low detection limits (claimed 1.48 ppb maneb), is severely hampered by poor operational stability. The spectrophotometric methods are based on the formation of coloured complexes formed between the

metallic component of the dithiocarbamates with reagents such as rhodamine 6G [2], 1-(2'-pyridylazo)-2-naphthol (PAN) [14] and 4-(2'-pyridylazo) resorcinol (PAR)[15]. All these methods use manual procedures and some require a liquid-liquid extraction step[15]. Flow-injection methodology, with the exception of a fluorimetric flow-injection method for the determination of metham and nabam [8], that deals with the assay of dithiocarbamates have never been reported. This paper describes the development of a flow-injection spectrophotometric method for the determination of ziram, one of the most common dithiocarbamate fungicide [1]. The method is based on the ligand exchange reaction in which zinc from ziram is complexed with the spectrophotometric reagent arsenazo(III). Arsenazo(III) was used as the spectrophotometric reagent as the molar absorption coefficient for Zn-arsenazo(III) complex ($\epsilon = 2.5 \times 10^4/\text{mol}^{-1} \text{ cm}^{-1}$) is almost similar to the Zn-PAR complex ($\epsilon = 8.7 \times 10^4/\text{mol}^{-1} \text{ cm}^{-1}$) [23] and further more the reagent is readily available in our laboratories. As will be shown later, the developed method is suitable for the routine determination of micro quantities of ziram in food samples such as potato, cucumber, tomatoes and cabbages.

Experimental

Chemical and Reagents

Zinc dimethyldithiocarbamate (ziram), 3,6-bis(2'-arsenazophenylazo)-4,5-dihydroxynaphthalene-2,7-

disulphonic acid (arsenazo(III)), sodium fluoride, sodium citrate, sodium thiosulphate, sodium tartrate, manganese(II) chloride, calcium chloride, magnesium chloride, tetrametiltiuram disulphide (thiram) and diethanolamine were purchased from Fluka, Switzerland. Boric acid, potassium chloride and cupric acetate were obtained from May & Baker, England, R & M Marketing, UK and Ajax Chemicals, Australia, respectively. Lead acetate and mercury(II) chloride were purchased from Hopkins & Williams, England. Maneb and dibam were obtained from Chem Service, USA and TCI, Japan, respectively. Solutions of carbon disulphide and hydrochloric acid were obtained from Riedel de Haen, Germany while methanol and sulphuric acid were obtained from J.T. Baker, USA. In almost all instances, analar grade reagents were used.

Apparatus

A blender with stainless steel blades (Toshiba, model TP-1500) was used to blend food samples. The extraction of ziram that was spiked to food samples was performed using a wrist-action flask shaker (Stuart Scientific). Manual absorbance measurements for the conventional determination of ziram were made using a Hitachi U-2000 double beam spectrophotometer with a 1.0 cm corex glass cuvette. A glass calomel electrode with an internal reference electrode that was connected to an Orion Research Expandable Ion Analyzer model EA 940 was used for the measurements of pH of aqueous solutions.

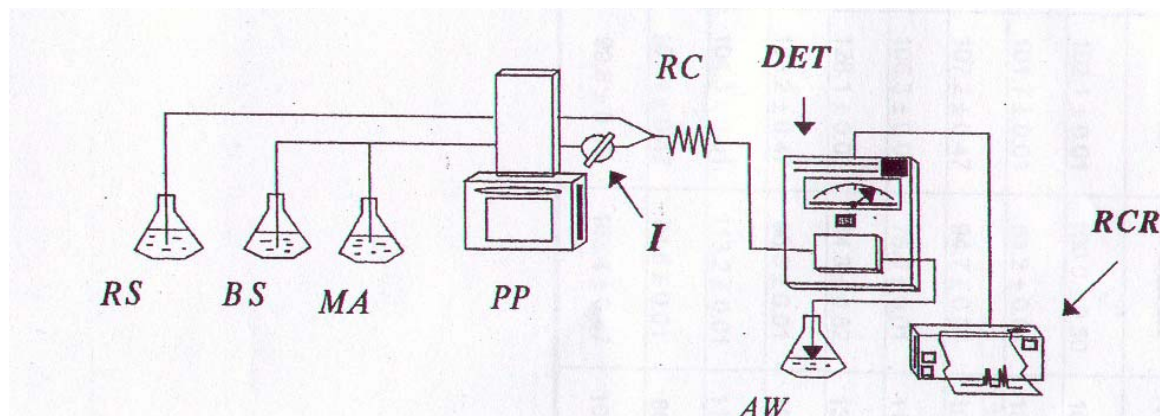


Figure 1: FIA manifold adopted for the determination of ziram. Masking agent (MA) was used only for recoveries and applications on spiked ziram to food samples. RS, reagent stream; BS, buffer stream; PP, peristaltic pump; RC, reaction Coil; DET, detector; AW, aqueous waste; and RCR, recorder.

FIA Set-up

The FIA used is as shown in Figure 1. Solutions were propelled by a multi-channel peristaltic pump (Gilson Minipuls 3) through PTFE tubings (0.8 mm i.d.). Samples were injected into a Rheodyne type 500

Teflon rotary injection valve. A Spectronic Mini-20 Spectrophotometer (Milton Roy), equipped with a Uvonic ultramicroflow cell of 20 μ l and 1.0 mm pathlength was used as detector while an x-y recorder (Kipps & Zonen) was used to record the FIA output.

Determination of Spiked Ziram in Food Samples

About 1.00 g of the food sample was weighed, chopped into small pieces and blended. Ziram standard solution was spiked, followed by the addition 100 ml of deionised water. The slurry was thoroughly mixed and left to stand for about half an hour, after which it was filtered using a Whatman 41 filter paper. The filtrate was collected and transferred to an Erlenmeyer flask. 100 ml chloroform was slowly poured to the flask and extraction carried out, aided with a wrist-action flask shaker for 30 minutes at 400 agitations per minute. The aqueous layer was separated from the organic layer and was further extracted three more times, each time using 10 ml of chloroform. The pooled chloroform extracts were evaporated to dryness and reconstituted with borate buffer. This sample was analysed using the FIA procedure and the method of Cullen's [10]. The extraction steps were completed in about 50 minutes. A blank extract for each food sample was also prepared using the same procedure.

Conventional Spectrometric Determination of Ziram

The method of Cullen [10], a modification of the original method of Clarke's [11] was used as comparison to the proposed FIA method. Briefly, in this procedure, to the same food sample that was used for the FIA method, 50 ml 5 M near boiling sulphuric acid was added and gently heated for 45 minutes. The evolved CS_2 was first directed into a trap containing 10% w/v lead acetate to remove interfering gases and finally into a second trap that contains the spectrometric reagents (prepared by dissolving 0.004 g cupric acetate monohydrate and 25 g diethanolamine in a 250 ml volumetric flask with ethanol). After allowing the contents to stand for about 15 minutes, the absorbance of the sample versus the spectrometric reagent was measured manually at 435 nm.

Results and Discussion

The effect of key FIA operating parameters such as

pH and flow-rate of the borate buffer used as carrier solution, injection volume, length of reaction coil and concentration of spectrophotometric reagent used was studied. The following conditions were chosen as a compromise between sensitivity and speed of analysis, and were used for the remaining of the studies:

Flow rate:	1.50 ml min ⁻¹
pH of carrier solution:	8.00
Injection volume:	125 μ l
Length of reaction coil:	900 mm
Concentration of arsenazo(III):	40 ppm

A series of standard solutions of ziram were injected into the manifold under the optimised conditions to test the linearity of the calibration graph. A linear range with correlation coefficient of 0.9982 over a concentration of 2.30 - 37.40 ppm ziram was obtained. The detection limit (signal/noise of 3) was about 0.35 ppm. The sensitivity of the proposed FIA method is at least comparable [6] to the spectrophotometric methods reported. The reproducibility of the method, as suggested by the % within day relative standard deviation of 0.15 % for the repeated injections of 2.34 ppm ziram standards was satisfactory. The day-to-day % relative standard deviation for the determination of the same concentration of ziram, performed over a period of three consecutive days was 0.16 %. Under the optimised conditions, the sample throughput was about 50 samples per hour.

The effect of foreign ions such as the heavy metals, alkali, alkaline earth metal ions; other dithiocarbamates such as maneb and thiram on the method developed was studied by preparing binary mixtures containing 23.40 ppm ziram and the same concentration of foreign species and determined using the FIA method. It was found that all the species tested caused serious interference to the determination (Table 1). To overcome this problem, the effects of a few common masking agents such as fluoride, tartarate, thiosulphate and citrate were studied, by incorporating these reagents to the manifold as shown in Figure 1.

The effects of these masking agents on the determination of 23.40 ppm ziram mixtures in the presence of the foreign species is shown in Table 1, where it was found that the use of 1 % w/v sodium fluoride gave the best overall percent recoveries. The use of citrate, tartarate and thiosulphate, however, were all not satisfactory. The use of sodium fluoride as masking agent for the FIA determination of ziram in food samples such as potatoes, cabbages, tomatoes and cucumbers was next studied. Prior determination using the conventional method [10] indicated that

these samples to be free from dithiocarbamates. The samples were spiked with ziram standard solutions over the concentration range 2.34-46.80 ppm, and after undergoing the pretreatment steps outlined under section 1.4, were analysed using the FIA method. It was found that for all these food samples, ziram concentrations of < 9.36 ppm gave false positive values greater than 100%, indicating interference

from unidentified endogeneous components in these samples (Table 2). However, good recoveries were obtained when the concentration of ziram is > 19.72 ppm for all the food samples studied (Table 2). The sensitivity of the method is sufficient to be used for the determination of ziram in food samples such as cabbages where the maximum residue limit is 20.1

Table 1: Effect of Masking Agents on the Determination of 23.40 ppm Ziram in the Presence of Equal Amount of Interferent (expressed as % recoveries)

Masking Agent Interferent	None	Sodium Fluoride	Sodium Tartarate	Sodium Thiosulphate	Sodium Citrate
Mn ²⁺	162.0 ± 0.50	102.1 ± 0.01	100.0 ± 0.50	101.1 ± 0.01	140.6 ± 0.82
Cu ²⁺	200.0 ± 0.50	101.7 ± 0.01	63.2 ± 0.01	133.3 ± 0.01	176.4 ± 0.47
Hg ²⁺	135.8 ± 0.50	107.2 ± 0.47	94.7 ± 0.01	104.8 ± 0.01	104.1 ± 0.94
Zn ²⁺	181.9 ± 0.50	106.3 ± 0.01	76.0 ± 0.01	110.5 ± 0.50	138.4 ± 0.47
Ca ²⁺	165.6 ± 0.01	126.1 ± 0.01	64.8 ± 0.82	134.6 ± 0.01	194.9 ± 0.01
Mg ²⁺	168.7 ± 0.50	103.2 ± 0.47	96.5 ± 0.01	100.0 ± 0.01	130.3 ± 0.47
K ⁺	111.6 ± 0.01	104.3 ± 0.01	113.2 ± 0.01	118.9 ± 0.01	114.6 ± 47
Maneb	175.4 ± 0.01	107.4 ± 0.47	84.5 ± 0.01	86.1 ± 0.01	91.7 ± 0.50
Thiram	117.5 ± 0.47	99.8 ± 0.43	103.4 ± 0.47	107.3 ± 0.47	96.8 ± 0.50

Table 2: Results for the Determination of Ziram When Spiked To Food Samples (expressed as % recoveries)

Sample Spiked Ziram, ppm	Potato	Cabbage	Tomato	Cucumber
2.34	>> 100	>> 100	>> 100	>> 100
4.68	>> 100	>> 100	108.9 ± 0.01	>> 100
9.36	>> 100	>> 100	110.0 ± 0.01	146.0 ± 0.55
19.72	96.4 ± 0.04	95.8 ± 0.01	90.8 ± 0.01	100.0 ± 0.01
23.40	97.4 ± 0.80	103.7 ± 0.01	94.0 ± 0.01	95.6 ± 0.01
28.08	98.0 ± 0.04	100.9 ± 0.53	90.0 ± 0.01	94.6 ± 0.01
32.76	94.1 ± 0.01	99.7 ± 0.01	91.4 ± 0.01	98.6 ± 0.01
46.80	98.5 ± 1.67	106.5 ± 0.01	99.5 ± 0.45	100.0 ± 0.01

ppm [24]. The method, however, will not be sensitive enough for the analysis of ziram in potatoes, tomatoes and cucumbers as the maximum residue limits are 0.4, 12.1 and 2.0 ppm ziram, respectively [24]. It is envisaged that the sensitivity as well as the selectivity of the method can be further improved by the incorporation of a pretreatment column to the manifold. The use of such columns have been instrumental in the development of an automated enrichment of chemical species prior to the analytical determinations [25 – 28].

Conclusion

A rapid, fairly sensitive and selective method for the determination of ziram is proposed. The use of 1% w/v sodium flouride as masking agent enables the

determination of 23.40 ppm ziram in the presence of the same concentration of foreign species such as Mn²⁺, Cu²⁺, Mg²⁺, K⁺ and thiram to be conducted satisfactorily. When applied to the determination of ziram in real samples such as cabbages, tomatoes, cucumbers and potatoes that were spiked with ziram standards, it was found that the method can be used only when the ziram concentration is at least 19.72 ppm, as ziram concentrations of ≤ 9.36 ppm caused positive interference. Interference from metal ions and other unidentified endogenous components can be reduced further by the on line incorporation a pretreatment column [25 - 28]. The principle of measurement can similarly be extended to other dithiocarbamates such as nabam, ferbam, maneb and zineb that can undergo ligand exchange reactions, provided that suitable spectrophotometric reagents is

used for the complexation of metals from the respective dithiocarbamate.

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