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New Chromogenic Reagent for Spectrophotometric Determination of Ethylene Bis-dithiocarbamate Pesticide Residues in Vegetables

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The use of pesticides, mainly ethylene bis-dithiocarbamates (EBDCs) has been reported to be abusive in the North West Region (Cameroon) but due to limited analytical facilities there are very limited data on their concentrations in foods from the local milieu. This study aimed to develop a simple spectrophotometric method to monitor residue levels of EBDCs in vegetables in order to improve food safety measures.
Methodology: The analytical technique was based on the degradation of EBDCs in acidic medium in the presence of tin (II) chloride to liberate carbon disulfide which was absorbed by a new chromogenic reagent (silver nitrate/diethanolamine) and subsequently quantified spectrophotometrically at 410 nm.
Results: A good linearity (R^2=0.999), practical detection limit (0.02 µg/mL), theoretical limit of quantification (20.04 µg/mL) of the method were established. The precision of the method was within the generally accepted interval for pesticides analysis. The analysis of the three selected vegetables revealed EBDC residue levels of 2.96, 6.34 and 7.92 mg/kg in parsley, huckleberry and tomato samples, respectively. These levels exceeded the maximum residue levels recommended by EFSA (2019) and FAO/WHO (2020).
Conclusion: The developed method was simple, rapid, and easy to use for the estimation and control of residue levels of EBDC in vegetables.

Keywords: Carbon disulfide; chromogenic; ethylene bis-dithiocarbamates; spectrophotometry; vegetables.

1. INTRODUCTION

Most economies in Africa are largely agrarian-based with about 66% of wetlands used for agriculture (IWMI, 2006). In Cameroon, agriculture predominantly rural plays a key role in employment, gross domestic product level and poverty reduction (World Bank, 2022). This agriculture yields diverse cash and food crops which contribute significantly to the country's food security (Tata et al., 2013) and reduction of hunger, one of the United Nations sustainable goals.

The Western highlands of Cameroon are made up of wetlands that are favorable for agriculture which is the most practiced activity for about 70% of the population throughout the year (Suh et al., 2024). Crop production is one of the major means of income generation for the population in the North West Region (NWR) of Cameroon. Vegetable production represents about 50% of the regional production estimated at 185,542 tons spanning throughout a surface area of 123,769 hectares (AGRI-STAT, 2012). Vegetables play a significant role in human nutrition, especially as sources of vitamins (C, A, B1, B6, B9, E), minerals, dietary fibers and phytochemicals. Vegetables in the daily diet have been highly associated with improvement of gastrointestinal health, good vision, and reduced risk of heart disease, stroke, chronic diseases such as diabetes and some forms of cancer (Keating et al., 2010).

The ever-increasing demand for vegetables due to their importance in human nutrition and health unfortunately still requires widespread use of pesticides. Residues of pesticides such as dithiocarbamates (DTCs) contaminate crops causing food safety issues with significant health consequences in consumers (animals and humans). The major concern of pesticides in non-target organisms is their toxic effects such as alteration of the reproductive systems and fetal development as well as their capacity to cause cancer and asthma (Gilden et al., 2010). Though maximum residue levels (MRLs) of DTCs have been defined in vegetables, their residue levels in these crops may exceed MRLs due to farmers' malpractices and non-respect of good agricultural practices, habits commonly observed within our communities (Manfo et al., 2012; Nantia et al., 2017a).

Among DTCs/EBDCs used in Cameroon, fungicides especially mancozeb/mancozam, maneb, pencozeb, and zineb are highly prevalent (Nantia et al., 2017a; Sonchieu et al., 2017). mancozam and pencozeb are non-systemic preventive fungicides that belong to the EBDC class. They form complexes with different metal ions and are characterized based on that property. For example when a complex is formed with manganese, it is referred as maneb, zinc (zineb), manganese and zinc (mancozeb or mancozam), and sodium (nabam). EBDCs possess a carbon disulfide (CS_2) moiety in their structure. Acid hydrolysis of EBDCs releases

CS_2 which can be quantified using appropriate methods (Keshavayya et al., 2012; Kavatsurwa et al., 2014; Mozzaquatro et al., 2019).

The United Nations aims to ensure global access to safe food as one of its sustainable development goals. Hence, there is need to screen foods to control toxicant residue levels. Accurate but sophisticated methods to determine EBDC residues as CS_2 (Türker & Sezer, 2005; Keshavayya et al., 2012; Mozzaquatro et al., 2019) exist but they are expensive and need trained personnel to operate while their maintenance is costly. Some chromogenic reagents (Mozzaquatro et al., 2019) have been used to determine EBDC residues in food commodities but they are very expensive and not available in limited resource settings. Hence the determination of EBDC residues in vegetables is still problematic for many developing countries including Cameroon. Therefore a simple sensitive, rapid and accurate spectrophotometric method to determine mancozam and pencozeb in vegetables was proposed in this study. It is based on using a new chromogenic reagent of silver nitrate/diethanolamine that reacts with CS_2 liberated from vegetables leading to the formation of a yellow product (complex) having a maximum absorption at λmax (410nm).

2. MATERIALS AND METHODS

2.1 Plant Material

Experimental vegetable samples (*Solanum nigrum*, *Solanum lycopersicum* and *Petroselinum crispum*) were cultivated on one of the experimental fields of the University of Bamenda by the researchers from April to August 2022. These vegetables were used as control samples. Field vegetables were purchased from a local market in Santa, North West Region of Cameroon. A quantity of 2 kg of each sample was collected and separately packaged in polythene bags. The samples were then placed in separate compartments of a cooler containing ice blocks. Once in the laboratory the samples were manually cut into ~ 0.5 cm pieces immediately before analysis. The selection of vegetables was based on a survey conducted by the researchers on types of vegetables cultivated and commonly consumed by the inhabitants.

2.2 Apparatus and Reagents

A UV-Vis Spectrophotometer UV 752 (D) PEC Medical USA was used for all the measurements. Absorbance was measured with a 10.00 mm quartz cuvette (EN ISO 9001) which matched with the cells of the spectrophotometer. A digital scale and 500 mL Soxhlet equipment were used to weigh reagents and extract carbon disulfide, respectively.

Chemicals used were of analytical grade reagents. Carbon disulfide (>99 % purity) was supplied by Sigma Aldrich (Germany). Other chemicals including silver nitrate, diethanolamine, tin (II) chloride, hydrochloric acid, and ethanol were purchased from SGC LABO (Douala, Cameroon).

2.3 Carbon Disulfide Extraction Equipment

This was constituted of a 500 mL soxhlet apparatus. Each chopped vegetable was separately transferred into 500 mL round bottomed flask of the soxhlet apparatus initially containing 100 mL of hydrolyzing solution (0.1 M $SnCl_2$/HCl). The flask was then heated at 90°C for 45 min. The condensate was collected in the thimble and later transferred into a 50 mL conical flask.

2.4 Preparation of Experimental Solutions

2.4.1 Silver nitrate solution and the chromogenic reagent/solution

A 0.03 M solution of silver nitrate was prepared by dissolving 0.8 g of silver nitrate in 150 mL of distilled water. For the chromogenic reagent a 10 mL/150 mL chromogenic solution was prepared by diluting 10 mL of diethanolamine in 150 mL of 0.03 M silver nitrate solution.

2.4.2 Standard carbon disulfide solution

A standard stock solution was prepared by diluting 0.1 mL of carbon disulfide with 25 mL absolute ethanol to obtain 5.01 µg/mL (Stock 1). Then, working standard solutions were prepared by transferring increasing volumes of stock 1 into separate 50 mL calibrated volumetric flasks and 25 mL of ethanol added to cover the concentration range of 0 to 160.32 µg/mL.

2.4.3 Hydrolyzing reagent

A solution of tin (II) chloride/hydrochloric acid (0.1M) was prepared by dissolving 20 g of stannous (II) chloride dihydrate in 500 mL

concentrated HCl in a 1000 mL volumetric flask and filled to the designated mark with distilled water (Keshavayya et al., 2012). The solution was used immediately.

2.5 Determination of Wavelength (λmax)

The UV-VIS spectrophotometer UV 752 (D) power button was turned on and then allowed to calibrate for 15 min. About 3 mL of the chromogenic solution/reagent blank were transferred in a 10 mm quartz cuvette and the wavelength varied from 380 to 650 nm.

2.6 Optimization of Experimental Conditions Optimization of Experimental Conditions

2.6.1 Determination of the concentration of the chromogenic solution

In order to establish the concentration of the chromogenic solution used in the study, different concentrations of silver nitrate were investigated. Accurately measured amounts of silver nitrate (0.6 to 0.9 g) were separately dissolved in distilled water in 250 mL volumetric flasks. Each amount was weighed in duplicates and dissolved in 100 and 150 mL water, respectively. After the preparation of each solution, 10 mL of diethanolamine were added and the absorbance of the resulting mixture was measured at 410 nm.

2.6.2 Effect of order of addition of reagents

The order of addition of reagents silver nitrate (A), diethanolamine (B), and distilled water (C) was evaluated to ascertain its effect on absorbance. Firstly, the order of addition was A+B+C. Secondly it was B+A+C and thirdly, C+A+B. Lastly, the chromogenic solution (D) and diethanolamine (E) were added and absorbance recorded at 410 nm.

2.6.3 Stability of the chromogenic solution

The stability of the chromogenic solution was studied over a period of time (2 h). Briefly, 2 mL of the chromogenic solution were transferred in a 10 mm quartz cuvette and absorbance measured at 410 nm. A new aliquot was obtained from the flask and placed in the spectrophotometer for determination of absorbance after every 5 min.

2.6.4 Stability of the complex

Briefly, 10 mL of the chromogenic solution were transferred in a 50 mL conical flask. Then 0.1 mL stock 1 was added and after homogenization the absorbance was measured at 410 nm at 5 min interval.

2.6.5 Quantity of sample, volume of hydrolyzing reagent, time and temperature of heating

The amount of sample, time and temperature of heating, and the volume of the hydrolyzing reagent depend on each other. Hence, the parameters were not investigated individually. This experiment was carried out using blank sample from the field. A scale was used to accurately measure 10, 15 and 20 g of parsley sample. The sample was transferred separately in a 500 mL round-bottomed flask of the soxhlet equipment, and 0.1 mL of stock 2 was added. Thereafter 50 mL of the hydrolyzing solution were added and the flask was placed on a heating system (50 - 90 ^{o}C) for 1h. The volumes of the condensate were collected after heating and optical densities were measured at 410 nm.

2.7 Method Validation

2.7.1 Calibration curve, linearity and range

In order to quantitatively analyze EBDC fungicides (mancozam and pencozeb) in the form of carbon disulfide it was necessary to establish a calibration curve. The latter was presented as a variation of absorbance which was recorded in triplicate against the concentration of carbon disulfide to prove linearity. Calibration parameters were generated from a stock solution of concentration 5.01 µg/mL. Aliquots of 20.04 - 160.32 µg/mL were transferred in separate 50 mL volumetric flasks containing 10 mL of the chromogenic solution. Then the reading was performed at 410 nm using a UV-Vis Spectrophotometer UV 752 (D).

2.7.2 Limit of detection

The limit of detection (LOD) was established practically and theoretically. Practically it was determined by the successive gradual dilution of the minimum concentration of the standard solution that was used in the preparation of the calibration curve (Mohammed & Ahamed, 2013). In this case, 0.1 mL of stock 2 was pipetted into a calibrated 50 mL conical flask containing 10

mL of the chromogenic solution. Then 2 mL ethanol solution were added sequentially and after every addition absorbance was measured at 410 nm after 5 min. Theoretically LOD was calculated according to the International Conference on Harmonization guidelines as LOD =3.3 x σ/S, where σ is the standard deviation of y-intercept of regression lines (standard deviation of response) and S is the slope of calibration curve (Miller & Miller, 2005).

2.7.3 Limit of quantification

The limit of quantification (LOQ) was determined according to the ECDGHFS guideline which states that the limit of quantification of a method is defined as the lowest level of fortification that met the validation criteria (recovery between 70 and 120% and relative standard deviation (RSD) lower than 20%) (ECDGHFS, 2017). The lowest level of fortification was taken from the analytical range which was used to plot the graph of absorbance against the concentration of carbon disulfide. The lowest level of fortification that produced satisfactory results for the recovery of carbon disulfide was 20.04 µg/mL. In order to realize the recovery of carbon disulfide, 0.1 mL corresponding to 20.04 µg/mL of carbon disulfide was diligently mixed with 20 g of blended parsley and 100 mL of tin (II) chloride/hydrochloric acid were added, this in a 500 mL round-bottomed flask of soxhlet equipment. The flask was placed on a heating system and then heated at 90 ^{o}C for 45 min. Absorbance was measured at 410 nm. The percent recovery was calculated as amount of carbon disulfide recovered (g) divided by the amount spiked multiplied by one hundred.

2.7.4 Precision

In other to evaluate the precision of the proposed method, stock 2 was used. Briefly, 0.1 mL of stock 2 was transferred in a 50 mL conical flask containing 10 mL of the chromogenic solution. The absorbance was measured three times during the same day (intra-day precision) and three consecutive days (inter-day precision) (Sameer et al., 2011; Nantia et al., 2017a; Nantia et al., 2017b).

2.7.5 Reproducibility

The reproducibility of the method, also called the day to-day precision or intermediate precision, or the inter-day precision was assessed by performing analyses of stock 2 by two different analysts. To validate the reproducibility of the method, 1.0 mL of the stock 2 was pipetted into a 50 mL conical flask containing 10 mL of the chromogenic solution. The absorbance was measured at 410 nm in triplicate by each analyst.

2.7.6 Accuracy and specificity

The accuracy (trueness) and specificity of the proposed method were assessed by performing triplicate recovery experiments at one concentration level (20.04 µg/mL). The experiment was performed on a control sample. A 0.1 mL of carbon disulfide working standard corresponding to 20.04 µg/mL was pipetted into a 500 mL round-bottomed flask of the soxhlet equipment containing 20 g of blended control parsley and the flask was manually homogenized. Then 100 mL of the hydrolyzing mixture were added and after homogenization the mixture was heated at 90 ^{o}C for 45 min. The condensate in the thimble was collected and transferred to a 50 mL conical flask containing 10 mL of the chromogenic solution and the yellow complex formed was homogenized and the absorbance measured at 410 nm after 5 min.

2.7.7 Recovery analysis

The recovery analysis was performed on one control sample (parsley). In this case the control sample was blended and 20 g of sample was introduced in a 500 mL flask. Then 0.1 mL solution of carbon disulfide stock 2 was added, the mixture homogenized and 100 mL tin (II) chloride/ hydrochloric acid solution were added. The mixture was heated at 90^{o}C for 45 min. Thereafter the condensate was collected and transferred in a 50 mL conical flask containing 10 mL chromogenic solution. After homogenization the absorbance of the solution was measured three times at 410 nm after 5 min.

2.7.8 Application of the method

The developed method was applied to determine EBDC residues as carbon disulfide in some common vegetables (huckleberry, tomatoes, parsley) through chopped extraction. In summary, 20 g of each sample was separately transferred to a 500 mL flask containing 100 mL of the acid digestion solution and placed in a heating system. After heating for 45 min at 90^{o}C the content of the flask was then transferred to a 50 mL conical flask containing 10 mL of the complexing solution. Thereafter the flask was homogenized and the yellow complex was

measured in a spectrophotometer at 410 nm after 5 min.

3. RESULTS AND DISCUSSION

3.1 Absorption Characteristics

The proposed method involved the reaction of silver nitrate/diethanolamine in solution with carbon disulfide hydrolyzed by hydrochloric acid in the presence of tin (II) chloride to give a yellow colored product that was measured against a blank scanned from 380-650 nm for absorbance versus wavelength. The maximum absorbance was recorded at 410 nm. However the absorbance decreased as the wavelength increased starting from 410 nm (Fig. 1). Therefore, λmax of 410 nm was used in all subsequent experiments. Previous studies obtained close λmax when characterizing chromogenic compounds for different analyses (Mozzaquatro et al., 2019; Kanakashree et al., 2024).

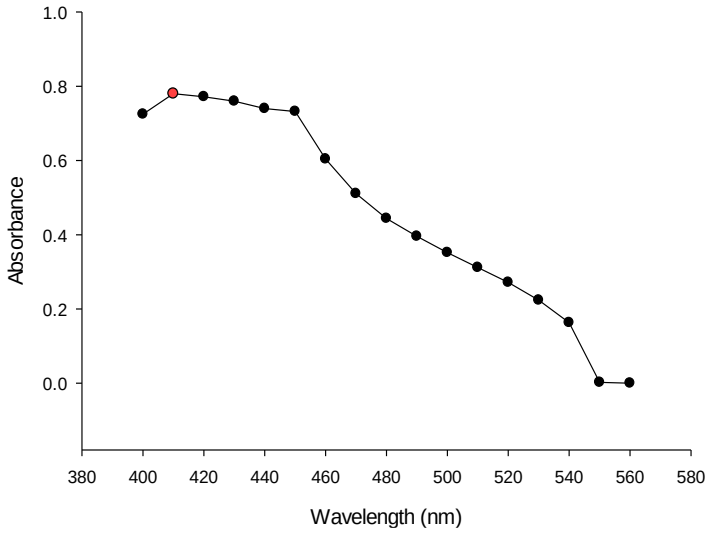

Fig. 1. Absorption spectrum of 410 nm

Table 1. Effect of the concentration of chromogenic compound

Concentration of chromogenic compound (M)	Time interval (min)	Absorbance
	0	0.000
0.023	5	0.000
	10	0.000
	15	0.000
	20	0.000
	0	0.345
	5	0.341
	10	0.346
0.027	15	0.456
	20	0.462
	0	0.613
	5	0.631
0.031	10	0.634
	15	0.635
	20	0.652
	0	0.401
	5	0.347
0.035	10	0.288
	15	0.253
	20	0.231

Table 2. Effect of order of addition of individual reagents and solutions on absorbance

Reagents	A+B+C			B+A+C			C+A+B		
Absorbance (nm)	0.337	0.257	0.216	0.343	0.295	0.151	0.105	0.056	0.054
Mean ± SD	0.270±0.062			0.260±0.100			0.071±0.029		
Solutions	**D+E**				**E+D**				
Absorbance (nm)	0.442	0.442		0.441	0.441		0.442	0.442	
Mean ± SD	0.441±0.001				0.441±0.001				

3.2 Optimization of Experimental Conditions

Various parameters affected the intensity of the colored complex. Therefore, the effects of those parameters were studied and the reaction conditions were optimized.

3.2.1 Concentration of chromogenic compound

The effect of different concentrations (0.02 – 0.03 M) of the chromogenic compound on the absorbance after preparing separately in 100 and 150 mL of distilled water was studied. Absorbance was recorded at 410 nm against time that ranged from 0 to 20 min. The 100 mL solution was not stable. The 150 mL solution was stable for 1 h 25 min. A 0.023 M silver nitrate solution did not affect the response of the detector. The mean values of absorbance of 0.027 M and 0.035 M, respectively did not increase with time. The absorbance of 0.035 M silver nitrate solution decreased with time. Only 0.031 M of silver nitrate in 150 mL of distilled water had absorbance values (nm) that increased with time. A 0.031 M silver nitrate solution (Table 1) was suitable for the study.

3.2.2 Effect of order of addition of individual reagents and solutions

Firstly, 0.8 g of silver nitrate (A) pellet was measured and transferred in a clean and dry calibrated 250 mL volumetric flask. Then, 10 mL diethanolamine (B) and 150 mL of distilled water (C) were added, respectively. The measurements obtained indicated that the order of addition of the chemicals (A+B+C, or B+A+C, or C+A+B) involved in the preparation of the chromogenic solution has an effect on the sensitivity of the detector (lowering effect) and intensity of the yellow complex product formed as shown by the response of the detector. The disparity in absorbance (Table 2) could be due to the formation of different complex ions with varying colors. This order of addition (A+B+C) was not recommended.

When silver nitrate solution (D) was prepared separately and diethanolamine (E) added, the order of addition did not have an effect on the absorbance (Table 2). This could be due to the fact that the diethanolamine did not form any new complex ion with the silver ion which resulted in no significant change in the absorbance of the solution at 410 nm. Thus, the recommended addition, any order (D+E or E+D) was used throughout this work.

3.2.3 Stability of the chromogenic solution

The stability of the chromogenic solution was studied. The results of the experiment indicated that the absorbance values were 0.000 from 0 to 80 min. There was an increase in absorbance from 85 – 100 min then constant from 105- 110 min (Fig. 2). The chromogenic solution was stable for at least 1 h 20 min. The solution was stable during the first 85 min probably because there was no significant change in its properties. This could be attributed to the formation of a colorless complex between silver nitrate and diethanolamine. The increase in absorbance after 85 min can be attributed to the decomposition of the complex as the concentration of the products and reactants change over time. However, the reaction reaches equilibrium, and the rate of complex decomposition and the formation of the product become equal. It was recommended that the chromogenic solution be used within 80 min of the preparation.

3.2.4 Stability of the complex (colored product)

The yellow product obtained by the proposed method was studied at different time intervals in

order to ascertain its stability. The results indicated that the absorbance values of the colored product were not constant within the time interval of 0-120 min. A maximum absorption was recorded after 5 min. Thereafter the absorbance decreased with time. The formation of the yellow complex began immediately when the reactants were mixed leading to an increase in absorbance as more complex was formed. The peak concentration of the complex was around 5 min (Fig. 3) when the reaction likely reached its optimum absorbance. The complex started to decompose after reaching the maximum absorption. This phenomenon led to a decrease in absorbance over time. Similar trend of the stability of the complex was observed in the study of Kanakashree et al. (2024). Therefore it was recommended that absorbance should be measured after 5 min.

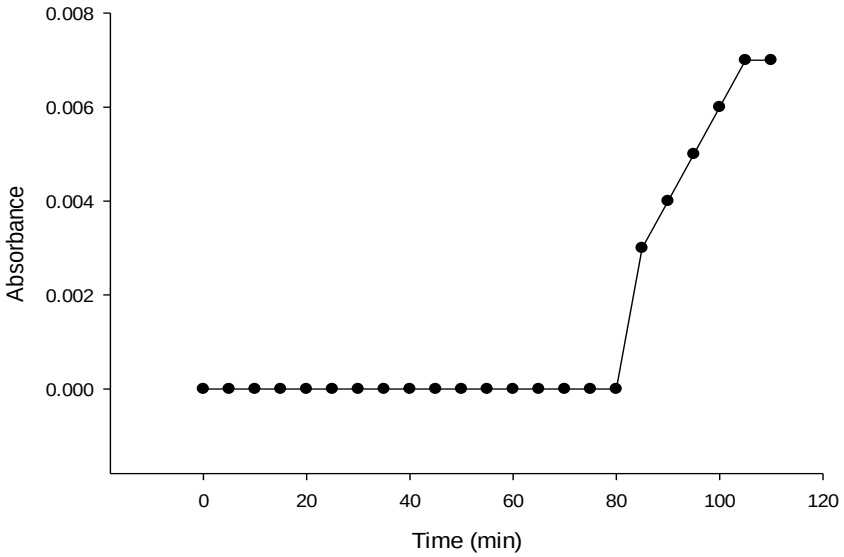

Fig. 2. Stability of the chromogenic solution

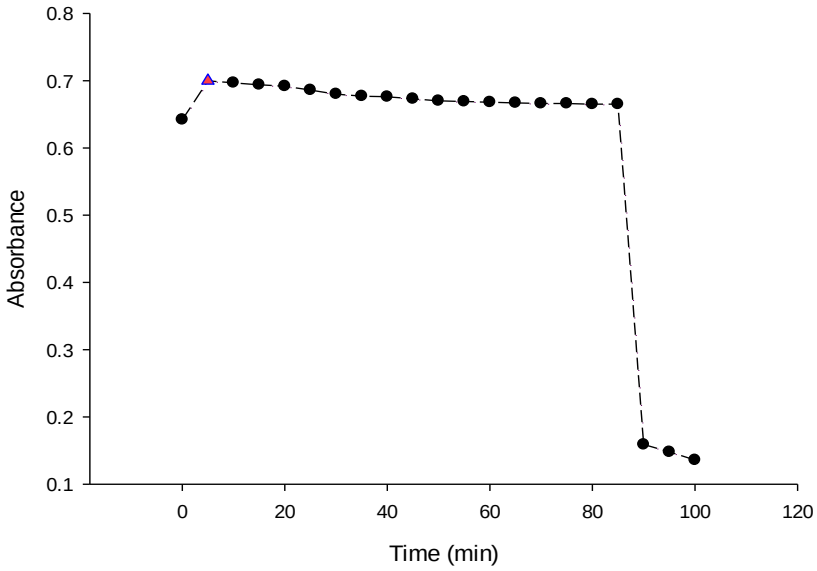

Fig. 3. Stability of the complex (colored product)

Table 3. Effect of the amount of sample, volume of hydrolyzing reagent, heating time and temperature on the absorption

Sample (g)	Time (min)	Concentration of CS_2 (µg/mL)	Temperature (°C)	$SnCl_2$/HCl (mL)	Condensate (mL)	Absorbance (nm)
	45		50	50	0	0.000
	45		60	100	0	0.000
	45		70	100	0	0.000
10	60		60	100	0	0.000
	60		70	100	0	0.000
	60	20.04	90	100	0	0.000
15	45		90	100	8	0.000
	45		50	100	0	0.000
	45		60	100	0	0.000
20	45		70	100	8	0.000
	45		80	100	9	0.000
	45		90	100	17	0.056

3.2.5 Quantity of sample, volume of hydrolyzing reagent, time and temperature of heating

The quantity of sample, the volume of hydrolyzing reagent, time and temperature of heating were studied and results presented in Table 3. No condensate was formed with a 10 g sample after heating in a 50 mL tin (II) chloride/hydrochloric acid solution at 50 °C for 45 min. At lower temperature and smaller volume of tin (II) chloride/hydrochloric acid solution, the reaction proceeded at a slower rate due to reduced kinetic energy of the molecules involved in the reaction. Hence no condensate was formed. Aside, a black ring was formed in the heating flask when 50 ml of hydrolyzing reagent was used hence no absorbance was obtained. After 10 g of sample was used while time and volume of hydrolyzing reagent were varied from 45 – 60 min and 50 – 100 mL respectively and heating from 60 – 90 °C the results showed no condensate. When 15 g of sample were heated for 45 min at 90°C in 100 mL tin (II) chloride/hydrochloric acid solution there was an increase in the volume of the condensate. When the latter was reacted with a chromogenic solution, a white colored product was formed. Hence no absorbance was obtained since a yellow complex was expected instead of white. With 20 g of sample a high absorbance was recorded when the sample was heated at 90 °C for 45 min in 100 mL tin (II) chloride/hydrochloric acid solution. The increase in the quantity of sample, volume of tin (II) chloride/hydrochloric acid solution and temperature resulted in elevated kinetic energy of the molecules involved in the reaction. This led to an increase in the volume of the condensate formed. Hence, 20 g of sample, 100 mL hydrolyzing reagent, heating time and temperature of 45 min and 90 °C, respectively were used throughout the study.

3.2.6 Reaction mechanism

Ethylene bis-dithiocarbamates are derivatives of dithiocarbamic acid. Upon heating in tin (II) chloride/hydrochloric acid solution, the hydrochloric acid provided the proton (H^+) which initiated the process of hydrolysis by protonating the nitrogen to release carbon disulfide. Tin (II) chloride acted as a reducing agent which stabilized the transition state during the hydrolysis process. The sulfur atoms in the released carbon disulfide formed bonds with silver in the silver-diethanolamine complex leading to changes in the electronic structure of the complex. Alterations in the d-orbital electron transition of silver ions due to the coordination of sulfur to silver resulted in the formation of a yellow coloration complex probably due to particular arrangement and interactions among silver, sulfur and carbon disulfide. The complex formed was measured at 410 nm. The proposed reaction is illustrated in Fig. 4.

3.3 Method Validation

3.3.1 Calibration curve and quantification

After defining all the parameters in the previous parts, a series of sequential dilutions were prepared from the standard solution and a calibration curve with the concentrations of carbon disulfide (0.00-160.32 µg/mL) was obtained.

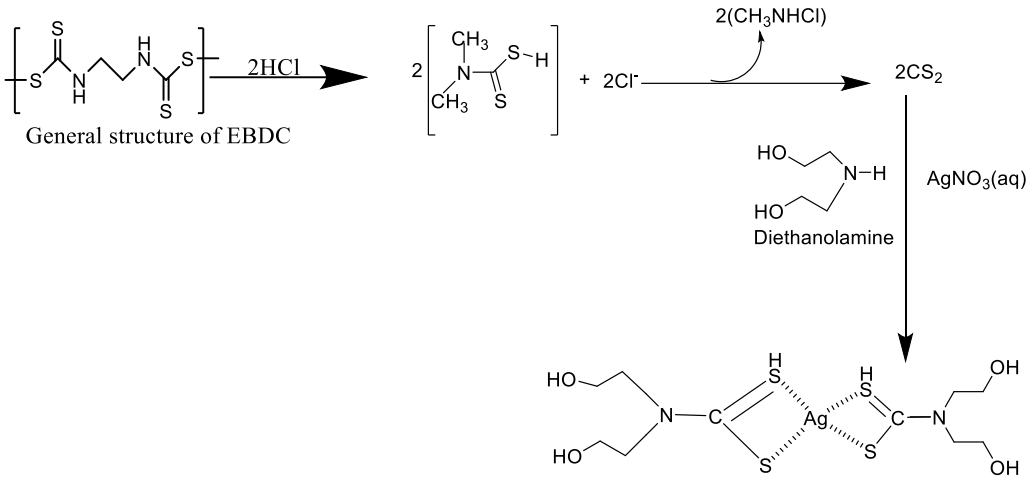

Fig. 4. Proposed acid hydrolysis and complexation reactions for EBDCs forming a yellow complex

Table 4. Calibration parameters

Parameter		Value
Wavelength (nm)		410
Beer's law linear range (µg/mL)		0.00 – 160.32
Molar absorptivity ($L.Mol^{-1}.cm^{-1}$)		7.3×10^{-6}
Sandell's sensitivity ($\mu g.cm^{-2}$)		5.2×10^{-2}
LOD (µg/mL)	Practical determination	0.02
	Theoretical determination	0.03
LOQ (µg/mL)		20.04
Straight line equation		Abs=0.0072x
Slop		0.0072
Intercept		0.0
Correlation coefficient (r)		0.999

Table 5. Accuracy, specificity and recovery of CS_2

Spiked (g)	Absorbance			Mean ± SD	RSD	%RSD	%Recovery
	1.220	1.221	1.222	1.221±0.001			
20.04×10^{-6}	1.221	1.221	1.222	1.221±0.0005	0.164	0.055	84.62
	1.222	1.221	1.222	1.221±0.0005			

Table 6. Intra-day and inter-day precision of the method

Time frame	Intra-day precision		Inter-day precision/reproducibility			
	Mean (nm)	%RSD	Day/Analyst		Mean (nm)	%RSD
Morning	0.296	0.168	Day 1	1st Analyst	0.275	0.181
				2nd Analyst	0.269	0.185
Noon	0.289	0.173	Day 2	1st Analyst	0.286	0.174
				2nd Analyst	0.286	0.174
Evening	0.287	0.174	Day 3	1st analyst	0.267	0.187
				2nd analyst	0.266	0.187

Table 7. EBDC residue in terms of CS_2 (mg/kg) in vegetable samples

S/N	Sample	EBDC residue level	EPA MRLs	EFSA MRLs	FAO/WHO MRLs
1	Tomato	7.916 (mg/kg)	2.5 (mg/kg)	0.03 (mg/kg)	/
2	Huckleberry	6.338 (mg/kg)	Not available	Not available	Not available
3	Parsley	2.962 (mg/kg)	/	/	0.023 (mg/kg)

The limit of detection (LOD) was determined through two methods (practically and theoretically). It was determined practically by the successive gradual dilution of the minimum concentration of the standard solution that was used in the preparation of the calibration graph. Practical and theoretical limits of detection were 0.02 and 0.03 µg/mL, respectively. The limit of quantification (20.04 µg/mL) was established from the lowest concentration of the standard solution that was used to spike vegetables in the recovery analysis which met the validation criteria (recovery between 70 and 120% and RSD lower than 20%) (Kavatsurwa et al., 2014). The limit of detection obtained in this study was similar or lower than that obtained by previously reported spectrophotometric techniques for the determination of ethylene bis-dithiocarbamates in fruits and vegetables (Kavatsurwa et al., 2014; Ahoudi et al., 2018; Mozzaquatro et al., 2019). The Beer's law plot and analytical parameters are given in Table 4.

3.3.2 Accuracy, specificity and recovery analysis of the proposed method

Accuracy and specificity were determined based on the recovery of known amounts of carbon disulfide reference standard added to a control sample (parsley) at a selected concentration, 20.04 µg/mL (20.04 x 10^{-6} g**)** within the calibration range (0.0 – 160.32 µg/mL). The accuracy and specificity were assessed as the percentage of CS_2 recovered and also expressed as relative standard deviation of replicate experiments between the measurements. The recovery and percent relative standard deviation were 84.62 % and 0.054 %, respectively (Table 5). The recovery indicated that most of the analyte added to the sample was detected while the method produced consistent results under the same conditions. The results were quite satisfactory as the percent recovery was between 70 and 120, intervals established by European Union (EU, 2015).

Recovery analysis was carried out on a single pesticide free vegetable. Exactly 20 g of parsley were spiked with 20.044x10^{-6} g reference CS_2 and transferred in a 500 mL flask containing the hydrolyzing solution. Then the flask was heated at 90^{o}C for 45 min. As presented in Table 5, the percentage of CS_2 recovered was 84.62%. The good solubility of CS_2 in tin (II) chloride/hydrochloric acid solution allowed for efficient extraction of CS_2 from parsley while the high temperature of 90^{o}C accelerated the reaction rate, leading to a faster extraction and higher recovery. A higher sample size contributed to a good percentage recovery while 45 min allowed for an adequate amount of time for the extraction process to take place. This result indicated that the recovery was within the general accepted interval (70 – 120 %), and similar recovery was noted in earlier studies (Nantia et al., 2017a; Nantia et al., 2017b).

3.3.3 Precision

The precision of the proposed method was determined as intra-day and inter-day precision. Intra-day precision was evaluated in terms of repeatability and analyzed in triplicate on the same day in the morning (6 am), afternoon (12 noon) and evening (6 pm) by same analyst. As presented in Table 6, the percent relative standard deviation (%RSD) for intra-day precision was 0.168, 0.173 and 0.174 in the morning, noon and evening, respectively. %RSD values for the analysts were identical (0.187) in the evening but slightly lower at noon (0.174). These results demonstrate that the precision of the method was within the generally accepted interval for pesticide analysis (EU, 2015).

3.4 Analysis of Vegetable Samples

The developed method was applied to determine mancozam and pencozeb residues levels in three selected vegetables including huckleberry, tomatoes, and parsley. The results presented in Table 7, showed that the vegetables were contaminated by pesticides or related residues. Tomato (*Solanum lycopersicum*) had the highest concentration level (7.92 mg/kg) of mancozam and pencozeb residues. The concentration level of mancozam and pencozeb residues in huckleberry (*Solanum nigrum*) was 6.34 mg/kg while parsley (*Petroselinum crispum*) had the lowest level (2.96 mg/kg). For the tomatoes the Environmental Protection Agency (EPA) and the Food Safety Authority in the USA recommend 2.5 mg/kg (or ppm) (EPA, 2009) and 4 ppm of CS_2 respectively as MRLs (FSA, 2005). The European Union Commission accepts 0.2 mg/kg for parsley (European Commission, 2016). These concentrations surpass the MRLs established by the European Commission and the Environmental protection agency. To the best of our knowledge the MRLs for huckleberry do not exist. The alarming results for the contaminated vegetables may be due to agricultural malpractices such as misuse of pesticides, frequency of application and non-adherence to a minimum of two weeks for the

sales of vegetables after pesticide application. The obtained results suggest possible indirect exposure of the population to the carbamate pesticides through contaminated vegetables.

4. CONCLUSION

The spectrophotometric method developed for determination of EBDCs in vegetables was satisfactory with LOD of 0.02 and 0.03 µg/mL. A new chromogenic reagent (silver nitrate/diethanolamine) was successfully used to quantify EBDCs in vegetables. All the vegetables were contaminated with pesticide residue levels above the European Commission, the EPA and Food Safety Authority of the United States of America. Although EBDC residues have been determined by different techniques, the method described here is viable, low turnaround time (5 min), simple, convenient, requires minimum sample quantity and does not require special working conditions unlike other methods. Moreover the method can be used for routine monitoring of EBDC residues in different food samples, thereby contributing to improve food safety and improving the health of the population.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that no generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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