

METHOD VALIDATION OF DITHIOCARBAMATES RESIDUES ON SOME EGYPTIAN COMMODITIES USING GC-MASS INSTRUMENT

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ABSTRACT

Dithiocarbamates are a non systemic group of fungicides widely used to protect crops from fungal diseases. An applicable GC-Mass method is found to be specified to determine the residues of dithiocarbamates. The current methodology used to determine dithiocarbamates group (Maneb, Mancozeb, Metiram, Propineb, Thiram, Zineb) involves the analysis of carbon disulphide (CS₂) generated after hydrolysis of the compound present in samples. The method performance was tested using recovery tests on different types of matrices (fresh fruits, vegetables and water samples). The average recoveries of CS₂ at different concentration levels of CS₂ varied between 70-120 %. The limit of quantitation is 0.05 ppm. The method showed to be linear up to 0.5 ppm. The reproducibility expressed as relative standard deviation was less than 12%. The measurement uncertainty in terms of expanded uncertainty and expressed as relative standard deviation (at 95 % confidence level and coverage factor of k = 2) is in the range of ± 24 %. A total of 71 samples of selected commodities (Green beans, Green peas, Green broccli, Green onion, Potato leaves, Peanut and Water) were analyzed to assess levels of Dithiocarbamates during the period of January to December 2010 taken from different markets of Egypt.

INTRODUCTION

Ethylenbisdithiocarbamates (EBDCs) are a group of fungicides very effective in controlling fungus diseases such as powdery mildew infesting vegetable crops. Dithiocarbamates pesticides are used all over the world because of their low acute toxicity combined with strong activity and low cost of production. These compounds are non systemic group of pesticides widely used in agriculture as insecticides, herbicides, and fungicides. Dithiocarbamates can result in neuropathology, thyroid toxicity and developmental toxicity to the central nervous system on laboratory animals. The EBDC Mancozeb was considered to be a multipotent carcinogenic agent in a long-term rat study. In Egypt, hundred tons of EBDCs fungicides were released to control plant pathogens which infesting vegetables causing damage especially in the worm humid climate, with increased effect in green house, which necessitates more frequent application of EBDCs (Dogheim *et al*, 2002). Residues of active substances, which were found in the agricultural products from exporting farms in 2010, belonged to Dithiocarbamates group: Maneb, Mancozeb, Metiram, Propineb, Thiram, and Zineb. As a continuation of the previous work on EBDCs, previous validation done by using UV-

Spectrophotometer with LOQ 0.2 ppm (Gadalla *et al*, 1996), the method is improved where fruit and vegetable samples are heated in a two-phase system of Isooctane / stannous (II) chloride in diluted hydrochloric acid. The carbon disulphide which is formed is dissolved in the organic phase (isooctane) and determined by Gas Chromatograph with Mass Selective Detector with LOQ 0.05 ppm. Analysis with GC followed by MSD-detection has better selectivity and specificity than the spectrophotometric method (Cesnik and gregorcic, 2006). This article presents the validation of this method in three matrices: Apple, Tomatoes and Green beans.

MATERIALS AND METHODS

Materials

Carbon Disulphide, extra-pure >99% (Sigma).

Ethylenebisdithiocarbamates (EBDCs) trihydrated, >95% purity.

De-ionized Water, Generated by Millipore water-purification system.

Hydrochloric acid, fuming 35%-37%, Merck or equivalent grade.

Stannous (II) chloride dihydrate solution, $\text{SnCl}_2 \cdot \text{H}_2\text{O}$, >98% (Sigma).

2,2,4-trimethylpentane (Isooctane solvent), 99%, Pestiscan or equivalent grade.

Acetone solvent, Pestiscan or equivalent chromatography grade.

CS₂ Stock Solution with concentration of 630 $\mu\text{g CS}_2/\text{ml}$ is prepared in Isooctane.

CS₂ Intermediate Standard Solutions with the concentration of 6.3 $\mu\text{g CS}_2/\text{ml}$ is prepared from the stock solution. Standard solutions have to be prepared prior to use. Because of the volatility of carbon disulphide and isooctane, lab glassware needs to be quickly and tightly closed.

CS₂ Calibration Standard Solutions with the concentrations: 0.01, 0.05, 0.1 and 0.5 $\mu\text{g CS}_2/\text{mL}$ were prepared in Isooctane to construct the multi-level calibration curve for the GC-MSD system. The standards for calibration are stable for one week when kept in a refrigerator.

Stannous (II) chloride Solution:

20 g stannous (II) chloride dihydrate was dissolved in 500 mL concentrated hydrochloric acid (35%-37%), the solution was decanted in a one-liter volumetric flask and filled to the designated mark with de-ionized water (Milli Q). The solution has to be prepared prior to use.

Apparatus and Equipment

Stainless steel cutters.

Water bath, Jeoitech BS-21, temperature controlled, supplied with shaker.

SCHOTT DURAN[®] lab bottles with plastic coating to minimize liquid loss.

SCHOTT DURAN[®] lab screw caps.

PTFE/Silicone seal (protection against contents escaping).

Graduated Cylinder: 250, 500, and 1000 ml.

Volumetric Flasks, Grade A: Hirschmann Labogerate or Brand, 10, 25, 50, 100, and 1000 ml.

Automatic Pipettes, suitable for handling volumes of 10 μl to 100 μl , and 100 μl to 1000 μl .

Glass Pipettes, Grade A: Hirschmann Labogerate or Brand, 0.5, 1, 2, 5, and 10 ml.

Analytical Balance, Mettler Toledo, AG204: 0.1mg to 210g range.

Precision Balance, Mettler Toledo, GG4002-S: Delta range, 0.5g to 4100g.

Water Purification System, Millipore TKA-LAB-UPW.

GC-MSD System; Agilent gas chromatograph system 5890 Plus II series equipped with 5972 mass selective detector.

Procedure

Sampling

Residues of dithiocarbamates decompose rapidly especially in chopped samples. Therefore, test samples for dithiocarbamate determination should be analyzed immediately upon their arrival to the lab and analyzed after cutting to preclude decomposition of the chemical.

Test sample preparation is taking place by cutting sample into parts to ensure that the analytical sample and analytical portion is homogeneous. The degree of homogeneity achieved must also allow residues to be quantitatively digested and extracted (Gadalla *et al*,1996).

Digestion and Extraction Steps

50 g of the sample is placed in a laboratory bottle where 40 ml Isooctane is added and a 100 ml solution of freshly prepared stannous (II) chloride is added using a graduated cylinder. Care must be taken to avoid any loss of CS₂ from the apparatus. The sealed bottle is placed in a water bath at 80°C and heated for 90 minutes. The solution is mixed approximately every 15 minutes and replace immediately into water bath. The bottle is taken out of the bath after 90 minutes, invert quickly many times, and then keep in deep freezer for extra one hour, let to be cooled and the upper phase (isooctane top layer) is transferred into a labeled GC vial using a Pasteur pipette for analysis.

Determination

Chromatographic Conditions of GC-Mass System

Splitless Inlet, Agilent 30 m × 0.25 mm × 0.25 μm HP-5MS column, Helium carrier gas, 1.3 ml/min constant flow mode, oven temperature 70 °C (2 min), 20 °C/min to 270 °C (6 min) program, injection volume 1.0 μL, source, quad, transfer 230 °C, 150 °C and 280 °C respectively. Inject 1μl of the sample extracts into the GC-Mass instrument. Parameters to determine the identity of the Dithiocarbamates analyte present in the sample extract includes: i) The retention time (RT), ii) Monitoring of ions m/z = 76 & 78, and iii) the relative abundance of the recorded masses (2 ions; m/z = 78 around 10% abundance of m/z = 76). The parameters obtained for CS₂ analyte to be identified in the sample extract are compared with those obtained in the calibration solution(s).

RESULTS AND DISCUSSION

Recovery Tests

Recovery results for the different levels are shown in Tables 1.

Table 1: Recovery Tests Results

Compound	Product	Spiking level (mg/kg)	N	Average Recovery (%)	CV (%)
EBDC (as CS ₂)	Apple	0.05	5	84	13.7
	Green Beans		6	92	12.1
	Apple	0.1	6	110	5.9
	Green Beans		5	98	9.3
	Apple	0.5	6	88	14.2
	Green Beans		6	104	6.2

Limit of Detection (LD)

The limit of detection in green beans samples was theoretically estimated and found to be 0.015 mg/kg as shown in the following table;

#	Compound	n	Green Beans		
			Expected (mg CS ₂ /kg)	S (mg/kg)	LD (3*S) (mg/kg)
1	EBDC (Expressed as CS ₂)	6	0.05	0.005	0.015

S=Standard deviation.

Limit of Quantification (LOQ)

The lowest practical limit of quantification was estimated by repeated measurements for spiked samples of apple and green beans at the expected lowest level 0.05 mg CS₂/kg standard. The average recovery percentages were 84% and 92% for apple and green beans respectively. The coefficient of variation measured as relative standard deviation was less than 14%.

Compound	Product	LOQ (mg/kg)	N	Average Recovery (%)	CV (%)
EBDC (as CS ₂)	Apple	0.05	5	84	13.7
	Green Beans		6	92	12.1

CV=The coefficient of variation.

Method Linearity

Method linearity was tested by performing recovery tests at different concentration levels and different commodities. The method showed to be linear from the LOQ up to 0.5 mg CS₂/kg. The recovery is within the range 70-120%.

Accuracy

Accuracy expresses the closeness of a result to a true value. Accuracy is expressed in terms of two components: "Trueness" and "Precision".

Trueness

The trueness of a method is an expression of how close the mean of a set of results (produced by the method) is to the true value.

Method Bias

To check trueness of the method, spiked samples are used at different levels on different samples. The data for bias are described in the Table 2.

Table (2): Method bias

Green Beans						
Compound	Level(1)		Level(2)		Level(3)	
	Mean Rec.%	Bias	Mean Rec.%	Bias	Mean Rec.%	Bias
EBDC (Expressed as CS ₂)	92%	-9%	98%	-3%	104%	4%
Apple						
Compound	L1		L2		L3	
	Mean Rec.%	Bias	Mean Rec.%	Bias	Mean Rec.%	Bias
EBDC (Expressed as CS ₂)	84%	-16%	110%	10%	88%	-12%

Certified Reference Material

The method trueness was tested by using Certified Reference Material provided by TestQual (EUCRL-FV, Spain) (round 22). Test sample matrix was potato with assigned value 0.299 mg/kg with 25 RSD%. Table 3 shows accepted z-score.

Table (3): CRM Result

#	Compound	Assigned (mg CS ₂ /kg)	Found (mg CS ₂ /kg)	S	z-score	Remarks
1	EBDC (Expressed as CS ₂)	0.299	0.25	0.075	-0.7	Satisfactory

Precision

Precision is a measure of how close results are to one another. The two most common precision measures are (repeatability) and (reproducibility).

Repeatability

The relative standard deviation (CV %) for results was 5.9 %. The CV% for repeatability should be lower than 20%.

Reproducibility

Reproducibility tests were done by fortifying 15 samples (apple, green beans, and pomegranate) at level of EBDC standard that is equivalent to 0.1 mg CS₂/kg and were analysed at different times by different analysts. As shown in Table 4, the relative standard deviation (CV %) was 11.79%.

Table (4): Reproducibility Test Results

#	Compound	n	Expected (mg CS ₂ /kg)	Mean Recovery (%)	CV (%)
1	EBDC (Expressed as CS ₂)	15	0.1	101	12

Uncertainty of Measurements:

Accumulated data from different quality control procedures were used for estimation of measurement uncertainty for Dithiocarbamate method.

Relative standard uncertainty due to precision (U_{precision}) comes from spike samples was found to be 11.79 %. The bias of the analytical procedure was investigated from recovery data using spiked samples (SANCO/10684/2009). The mean recovery was 101 % was observed with standard deviation s = 11.9 and n= 15. The standard uncertainty was

calculated as the standard deviation of the mean standard uncertainty= 3.1 %, Relative Standard Uncertainty (U_{Bias}) = 3%. Combined uncertainty was found to be 11.8 %. Expanded uncertainty is obtained by multiplying the combined uncertainty by a coverage factor k, for confidence level of 95% k is 2. The expanded uncertainty (at 95 % confidence level) was found to be less than 24 %.

Summary of levels of Dithiocarbamates During The Period of January to December 2010

Some 71 samples comprising 8 different fresh fruit, vegetables and water were purchased from the Egyptian Market between January and December 2010 and analysed for the residues of Dithiocarbamates. Of the 71 samples, Dithiocarbamates residues were either absent or complied with the Maximum Residue Limit (MRL) in 71.4% of samples. Dithiocarbamates were detected in 7 samples (9.9%), and were suspected of exceeding the MRL in 2.8% of samples (in Green Broccoli). Summary of results are shown in the following table.

Commodity	Total No. of samples analysed	Contaminated samples of each type		Free samples of each type		Less than LOQ		MRL (mg/kg) (CS ₂)	Violative samples	
		No.	%	No.	%	No.	%		No.	%
Green Beans	53	5	9.4	35	62.5	13	23.2	1.0	-	-
Green Peas	3	-	-	3	100	-	-	0.2	-	-
Green Onion	1	-	-	1	100	-	-	1.0	-	-
Green Broccoli	2	2	100	-	-	-	-	1.0	2	100
Potato Leaves	1	-	-	1	100	-	-	0.3	-	-
Peanut	3	-	-	3	100	-	-	0.1	-	-
Water	8	-	-	8	100	-	-	0.1	-	-
Total	71	7	9.9	51	71.4	13	23.2%		2	2.8

Conclusions

According to the validation, the proposed method is suitable for the determination of dithiocarbamates residue content in many sample types. The method is linear in the range from 0.05 mg/kg to 5.0 mg/kg. The limit of detection (0.015 mg/kg) and the limit of quantitation (0.05 mg/kg) ensure that the method is suitable for the determination of residues regarding the prescribed MRLs. Inter-laboratory comparisons and recoveries have proved that the method is precise and accurate.

REFERENCES

- Wolfgang Schwack, and Steven Nyanzi Analysis of dithiocarbamate fungicides. Second-derivative UV-spectroscopic determination of CS₂, COS, and thiram, tetramethyl thiuram disulfide (TMTD),
DFG-S-15 Method, Deutsche Forschungsgemeinschaft, Determination of Dithiocarbamates as CS₂.
SANCO/10684(2009), DG-SANCO: Method Validation and Quality Control Procedures for Pesticide Residues Analysis in Food and Feed, Document No..

- Anonymous (2004), Dithiocarbamates residues in Brazilian food and the potential risk for consumers, *Food and Chemical Toxicology*, 42, 1877–1883.
- G. E. Keppel, (1971), *Journal of the Association of Official Analytical Chemists*, 54, 528–532.
- Maximum recommended limit according to Regulation (EC) No 396/2005 and its amended Commission Regulation (EC) No 839/2008.
- Ashok Kumar Malik, *Methods of analysis of dithiocarbamate pesticides: a review*, Werner Faubel Forschungszentrum Karlsruhe, Institut für Instrumentelle Analytik, PO Box 3640, D-76021, Karlsruhe, Germany.
- S. M. Dogheim *et al.*, (2002), *Food Additives and Contaminants*, 19, 1015–1027.
- SFS-EN 12396-1, determination of dithiocarbamate and thiuram disulfide residues.
- US Office of Pesticide Programs (OPP)-(2001), *The Determination of Whether Dithiocarbamate Pesticides Share a Common Mechanism of Toxicity*, December 19.
- Česnik, Ana Gregorčič, *Acta Chim. Slov.* (2006), Validation of the Method for the Determination of Dithiocarbamates and Thiuram Disulphide on Apple, Lettuce, Potato, Strawberry and Tomato Matrix, Helena Baša, 53, 100–104.
- Gadalla *et al* (1996), *Monitoring of dithiocarbamate fungicides in some vegetables*, *Egypt. J. Agric. Res.*, 74 (4).

طريقة لتقدير متبقيات مركبات الدايتيوكاربامات فى بعض المحاصيل المصرية باستخدام GC-Mass .GC- جهاز مطياف الكتلة
عبيد أحمد الجوهري، مصطفى محمد نبيل و أحمد حسن
المعمل المركزى لتحليل متبقيات المبيدات و العناصر الثقيلة فى الأغذية – الدقى – الجيزة.

تعتبر مركبات الدايتيوكاربامات من المبيدات الفطرية الغير نظامية والأكثر استخداما لحماية المحاصيل من الأمراض الفطرية. وقد طبق استخدام جهاز مطياف الكتلة GC-Mass فى تقدير متبقيات الدايتيوكاربامات. وتستخدم الطرق الحالية لتقدير مجموعة الدايتيوكاربامات (مانب – مانكوزب – ميتيرام – بروبينب – ثيرام – زينيب) عن طريق تحليل (ثانى كبريتيد الكربون) الناتج بعد عملية التحلل المائى لهذه المركبات الموجود فى عينات التحليل. و قد أجرت طرق الكفاءة المختبرة باستخدام تجارب الاسترجاع على انواع مختلفة من الأنسجة فى عينات (فواكه طازجة – خضروات – ومياة). تختلف النسبة المتوسطة لاسترجاع ثانى كبريتيد الكربون بين ٧٠-١٢٠ % . ويكون الحد الأدنى للتحليل الذى يمكن تقديره ٠.٠٥ جزء فى المليون. وقد أظهرت الطريقة أنها خطية حتى تركيز ٠.٥ جزء فى المليون. وتراوح الانحراف الفياسى المقدر CV% ما بين ٥.٩ و ١٢%. وقد تم تحليل ٧١ عينة من المحاصيل المختارة (الفاصوليا الخضراء- البسلة الخضراء- البروكلى الأخضر – البصل الأخضر – ورق البطاطس- الفول السودانى والمياة) لتقدير مستويات الدايتيوكاربامات خلال الفترة من يناير حتى ديسمبر ٢٠١٠ من مختلف السواق فى مصر .

قام بتحكيم البحث

كلية الزراعة – جامعة المنصورة
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