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Research Article

A GC-MS method for the Determination of Mancozeb and Metiram (as CS_2) residues in Aquatic Tox medium

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Abstract: A simple, and sensitive validated GCMS-EI analytical method was developed for the determination of dithiocarbamates (Mancozeb and Metiram) residues in different aquatic tox mediums. The tox mediums were those which provide nutrients and help the growth of different aquatic organisms for their survival and multiplication. The constituent of different mediums includes blended water for fish**,** M4 Medium for Daphnia magna, OECD TG 201 medium for Alga and 20XAAP Medium for lemna. The dithiocarbamates residue involves the reduction of ditioncarbonate moiety under strong acidic conditions in presence of stannous chloride ($SnCl_2$) as reducing agent, evolution of carbon disulfide (CS_2) insitu extraction of the CS_2 into a layer of iso-octane subsequently analysis of CS_2 content by GC–MS in SIM mode. The established optimum conditions provided 5.0 min retention for CS2 and the total time of chromatographic analysis was 7.0 min. The linear graph has the lowest detection 0.01 mg/L. The lowest limit of quantification was 0.03 mg/L. The method was validated at levels of 0.03 and 0.3 mg/L. Recovery spiked samples of mancozeb and Metiram in different mediums were in the range 91-98%, with relative standard deviations 0.78 to 2.2 % (n=5). The proposed method can be applied successfully for the determination of dithiocarbamate residues in different aquatic solutions.
Keywords: Dithiocarbamates, Carbon disulfide, Aquatic Tox medium, GC-MS-EI method.

INTRODUCTION

The ditihocarbamate fungicides used in crop protection services are the complex metal salts which contains manganese (maneb), iron (ferbam), zinc (zineb, mancozeb, propineb). The dithiocarbamates posses the potential for the treatment of broad spectrum of pathogens of mre than 400 species over 70 crops [1]. They were characterized based on their potential activity against different plant pathogens. In combination with modern systemic fungicide, they were also used to manage resistance and to broaden the spectrum of activity. The so called “maneb group” (zineb, maneb, mancozeb, propineb, metiram) fungicides are most frequently detected chemical in several of the export commodities like grapes, and that this group also had the highest frequency in exceeding maximum residue limits (MRLs) [2]. The World Health Organization (WHO) classified few of the dithiocarbamates as being hazardous [3] Which lead to use of an array of different methods for the analysis of their residues in different substrates.

The general methods of analysis are based on the decomposition of dithiocarbamates to liberate carbon disulfide (CS_2) using lead acetate or by hot mineral acid to the amines. The liberated CS_2 is subsequently trapped in a digestion solvent and the active ingredient is determined by Iodometric titrations [4-13]. Several of these published methods are titrations methods and suffers from practical difficulties while analyzing the active by titration due to the interference associated with the dirty samples. Some times the CS_2 liberation may not be complete or it may leak while trapping or the reverse flow may contribute to the negative results forcing the analyst to do multiple sample analysis.

EXPERIMENTAL PROCEDURES

Instrumentation

GC-MS conditions for the determination of Dithiocarbamates

The configuration of GC–MS system used includes a GC-17A (Shimadzu, Kyoto, Japan) gas chromatograph coupled with QP-5050A Mass-Selective Detection (MSD) and GC Solution software, the detector was set in selective ion monitoring mode (SIM) mode. The ions m/z 76 and m/z 78 were used as qualifier ions (Figure 1) and the target ion used for the measurement was the ion at m/z 76. The CS2 peak separation was obtained on a Supelco SPB-1 capillary column (30 m length, 0.32 mm internal diameter, 4.0µm film thickness). The injection system was operated in split mode with a split ratio of 10:1. The injector and the transfer line temperatures were 250ºC and 300ºC, respectively. The oven temperature program was 30ºC, held constant for 4.5 min and ramp at 70ºC /min raised the column temperature up to 120ºC, held

constant for 3.0 min. The carrier gas used was helium (purity 99.999%) at a flow rate of 2.0 ml /min and the sample volume injected onto the column was 1.0 µL. A Shimadzu GCMS solutions Chromatography Software was used for acquisition of data and calculation of peak area.. The carrier gas used was helium (purity 99.999%) at a flow rate of 2.0 ml /min and the sample volume injected onto the column was 1.0 µL. A Shimadzu GCMS solution Chromatography Software was used for acquisition of data and calculation of peak areas. The retention time of CS_2 was 5.0 min and the total time of chromatographic analysis was 7.0 min.

Analytical standards, Reagents and Solutions

The analytical standard materials of Mancozeb (purity 82.10%), Metiram (purity 85.80%) were obtained from was purchased from sigma Aldrich. The hydrochloric acid, ethylenediaminetetraacetic acid disodium salt (EDTA) used were AR grade, methanol, acetonitrile and Isooctane of HPLC grade were purchased form Merck (Darmstadt, Germany). Stannous chloride (purity 98%) was obtained from Aldrich. Milli-Q water was obtained from Millipore India Ltd, Bangalore, India. Analytical standard solutions of mancozeb and Metiram were made in 0.2 M Disodium EDTA. Purity correction and CS2 conversion factor was incorporated in the preparation of analytical standard solutions. For each pesticide a stock solution of 500 mg/L was prepared, which was serially diluted to produce working standard solutions. The working standard solutions were prepared freshly and used.

Stock solutions of Na_2EDTA (100g/L) and 8N HCl were prepared in Milli-Q water. 3% Stannous chloride solution was prepared in 8N hydrochloric acid.

Test medium

Test medium is a constitute of different macro nutrients, salts and vitamins. This helps in the survival of different organisms during exposure of different compounds.

Blended water

It is a mixture of well water and reverse osmosis water in the ratio of 1:1.7 liters. This provides enough nutrients for the survival of fish during test item exposure.

M4 Medium

It is a combination of Trace elements, Marco nutrients and vitamins .The composition was given in Table 1.

OECD TG 201 medium

This helps in the growth of green alga as it provides the required nutrients and useful salts which helps in their growth and multiplication. The composition was given in Table 2.

20X AAP Medium

This medium with different constitutes of nutrients will help in the growth and survival of alga during test item exposure.The composition details were given in Table 3.

Digestion procedure

To a known volume of homogenized standard/sample taken in 250 ml crimp top vials, 5ml of 10% Na_2 EDTA solution in boiled distilled water was added, swirled the contents to mix, then 15 ml of HCL/$SnCl_2$ [8 N / 3%(w/v)] mixture was added along with 20 ml of iso-octane and immediately capped the crimp top vials. The vials were placed in a hot air oven at 95° C for one hour. After digestion period the Iso-octane layer was collected in GC vials and injected in to GC-MS.

Method validation

The method for the determination of dithiocarbamate residues was validated in terms of method specificity, linearity, assay accuracy, precision, limit of determination and quantification. The results are expressed in term of its CS_2 content.

Method specificity

Specificity was confirmed by injecting the iso-octane trap of medium (Aqua Medium meant for fish toxic study, M4 Medium, OECD TG 201 and 20X AAP Medium).

Linearity

The calibration solution was prepared by digesting analytical standards in stannous chloride solution and trapped the liberated CS2 in iso-octane. The linearity of the method was evaluated by preparing different calibration solutions. A series of calibration solutions were prepared by diluting the stock solution into 10 mL volumetric flasks, and brought to volume with dimethyl sulfoxide. The working calibration solutions were pipetted into separate clean 250 mL capacity crimp top vials giving rise to a series of solutions containing 0.01, 0.10, 1, 5, 10, 50 and 100 µg/mL. All the standard solutions were processed and qualitative analysis was based on the retention time (5.0 min) of carbon disulfide by injecting carbon disulfide solutions made in Iso-octane.

Assay accuracy and Repeatability

Recovery studies were carried out at 0.01 and 0.1 mg/L fortification levels for Mancozeb, and Metiram (n=5 for each at two fortification levels) by spiking 10 mL (aquatic mediums meant for fish, daphnia, alga and lemma) samples with the appropriate volumes of working standard solutions. After spiking, samples were handled and processed as above described digestion procedure.

Detection and Quantification Limits

The lowest limit of detection (LOD, mg/L) was determined as the lowest concentration giving a

response of 3 times the baseline noise defined from the analysis of three control (untreated) samples. The limit of quantification (LOQ, mg/L) was determined as the lowest concentration of a given pesticide giving a response of 10 times the baseline noise.

Stability of Analytical standard stock solutions

To determine the stability of the analytical standard solutions, the calibration solutions were stored under refrigerated conditions (4-8ºC) for a period of 30 days and aliquots was analyzed.

RESULTS

Specificity

There were no matrix peaks in the chromatograms to interfere with the analysis of CS2 shown in Figure. 2. Furthermore, the retention time of CS_2 was relatively constant at 5.0 ± 0.2 min.

Linearity

Calibration curve at all concentration ranges were better described by quadratic equation with correlation coefficient > 0.99. Good linear correlation coefficients (R^2=0.999) between concentration (x) and peak area (Y) were also found at lower concentration ranges.

Assay accuracy and precision

The recovery data for various mediums are shown in Table 1. Recoveries of mancozeb, and Metiram were > 91%. The method was validated over the fortification level 0.01 – 0.1 mg/L. The relative standard deviation values obtained by this method are summarized in **Table 1**. These numbers were calculated from five (5) replicate analyses of a given sample (mancozeb and Metiram) made by a single analyst on one day. The repeatability of the method is satisfactory (RSDs <5 %).

Table 1: Preparation of M4 medium Nutrients (Daphnia Magna)

Sl.N	Chemical Name	Formula	mg/l
Trace elements			
1	Boric acid	H_3BO_3	57190
2	Manganese chloride	$MnCl_2.4H_2O$	7210
3	Lithium chloride	LiCl	6120
4	Rubidium chloride	RbCl	1420
5	Strontium chloride	$SrCl_2.6H_2O$	3040
6	Sodium bromide	NaBr	320
7	Sodium molybdate	$Na_2MoO_4.2H_2$	1230
8	Cupric chloride	$CuCl_2.2H_2O$	335
9	Zinc chloride	$ZnCl_2$	260
10	Cobalt chloride	$CoCl_2.6H_2O$	200
11	Potassium iodide	KI	65
12	Sodium selinite	Na_2SeO_3	43.8
13	Ammonium vanadate	NH_4VO_3	11.5
14	EDTA*	$Na_2EDTA.2H_2$	5000
15	Ferrous sulphate*	$FeSO_4.7H_2O$	1991
Macro nutrients			
16	Calcium chloride	$CaCl_2.H_2O$	293800
17	Magnesium sulphate	$MgSO_4.7H_2O$	246600
18	Potassium chloride	KCl	58000
19	Sodium hydrogen	$NaHCO_3$	64800
20	Sodium silicate	$Na_2SiO_3.9H_2O$	50000
21	Sodium nitrate	$NaNO_3$	2740
22	Potassium phosphate	KH_2PO_4	1430
23	Potassium phosphate	KH_2PO_4	1840
Vitamin stock solutions			
24	Thiamine hydrochloride	--------	750
25	Cyanocobalamine (B12)	--------	10
26	Biotin	--------	7.5

* Both EDTA and Ferrous sulphate solution were prepared separately, poured together and autoclaved.

TABLE 2: PREPARATION OF OECD TG 201 MEDIUM (GREEN ALGA)

Sl. No.	Composition	mg/L
1.	$NaHCO_3$ (Sodium Hydrogen Carbonate)	50.0
2.	NH_4Cl (Ammonium Chloride)	15.0
3.	$MgCl_2.6H_2O$ (Magnesium Chloride)	12.0
4.	$CaCl_2.2H_2O$ (Calcium Chloride)	18.0
5.	$MgSO_4.7H_2O$ (Magnesium Sulphate)	15.0
6.	KH_2PO_4 (Potassium Dihydrogen Phosphate)	1.60
7.	$FeCl_3.6H_2O$ (Ferric Chloride)	0.064
8.	$Na_2EDTA.2H_2O$ (E.D.T.A. Disodium Salt)	0.100
9.	H_3BO_3 (Boric Acid)	0.185
10.	$MnCl_2.4H_2O$ (Manganese (II) Chloride)	0.415
11.	$ZnCl_2$ (Zinc Chloride)	0.0030
12.	$CoCl_2.6H_2O$ (Cobaltous Chloride)	0.0015
13.	$Na_2MoO_4.2H_2O$ (Sodium Molybdate)	0.0070
14.	$CuCl_2.2H_2O$ (Copper (II) Chloride)	0.00001

TABLE 3: PREPARATION OF 20X AAP medium (LEMNA GIBBA)

Stock Solution No.	Composition	Concentration in stock solution (g/L)	Concentration in prepared medium (mg/L)
A1	$NaNO_3$ (Sodium Nitrate)	26.0	510
	$MgCl_2.6H_2O$ (Magnesium Chloride)	12.0	240
	$CaCl_2.2H_2O$ (Calcium Chloride)	4.4	90
A2	$MgSO_4.7H_2O$ (Magnesium Sulphate)	15.0	290
A3	$K_2HPO_4.3H_2O$ (Dipotassium Hydrogen Phosphate)	1.4	30
B	H_3BO_3 (Boric Acid)	0.19	3.7
	$MnCl_2.4H_2O$ (Manganese (II) Chloride)	0.42	8.3
	$FeCl_3.6H_2O$ (Ferric Chloride)	0.16	3.2
	$Na_2EDTA.2H_2O$ (E.D.T.A. Disodium Salt)	0.30	6.0
	$ZnCl_2$ (Zinc Chloride)	3.3 mg/L	66 µg/L
	$CoCl_2.6H_2O$ (Cobaltous Chloride)	1.4 mg/L	29 µg/L
	$Na_2MoO_4.2H_2O$ (Sodium Molybdate)	7.3 mg/L	145 µg/L
	$CuCl_2.2H_2O$ (Copper (II) Chloride)	0.012 mg/L	0.24 µg/L
C	$NaHCO_3$ (Sodium Hydrogen Carbonate)	15	300

Table 4: Recoveries of the three dithiocarbamates tested from fortified different mediums

Medium	Fortification level (mg/L)	Mancozeb		Metiram	
		Mean Recovery (%)	% RSD	Mean Recovery (%)	% RSD
Blended water	0.01	98.12	2.36	98.25	2.73
	0.1	97.56	2.02	98.42	0.98
OECD TG 201	0.01	96.58	1.58	96.40	1.64
	0.1	95.87	2.14	95.76	1.21
M4	0.01	96.75	1.45	100.18	2.32
	0.1	97.95	1.69	98.62	1.28
20XAAP	0.01	97.65	1.84	97.45	1.75
	0.1	95.99	2.78	98.24	2.03

Average of five replications

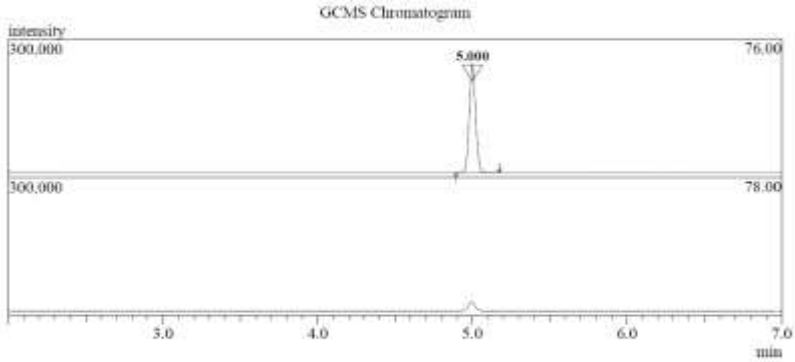

Figure 1: Representative GC-MS scanned Chromatogram at m/z 76 and m/z 78 ions of standard dithiocarbamate

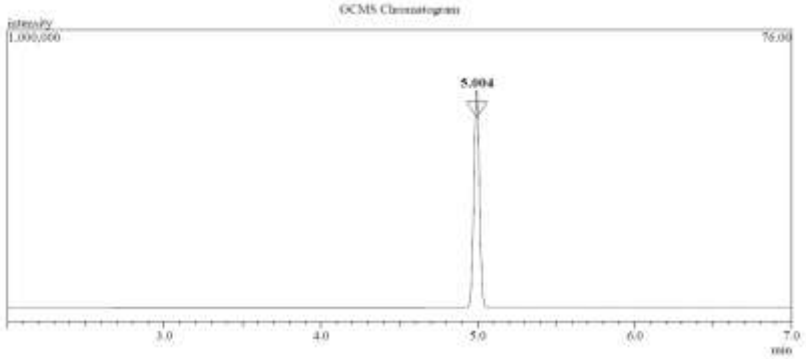

Figure 2: Representative GC-MS Chromatogram of standard dithiocarbamate tested from fortified medium

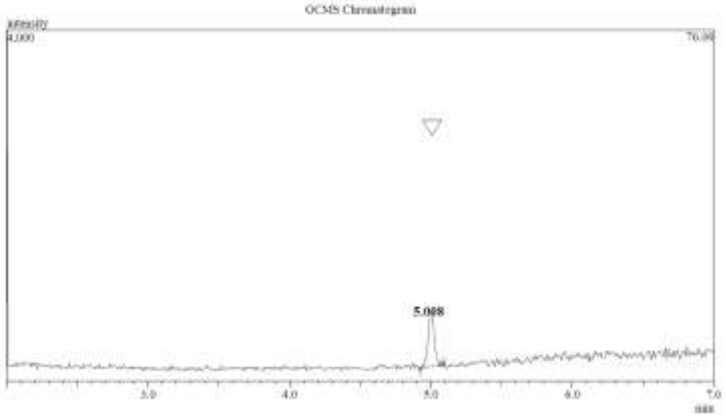

Figure 3: Representative GC-MS Chromatogram at fortification level of 0.01 mg/L

Detection and Quantification Limits

The limit of quantification was determined to be 0.01 mg/L. this quantization limit was defined as the lowest fortification level evaluated at which acceptable average recoveries were 91-98%, RSD <3%. This quantification limit also reflects the fortification level at which an analyte peak is consistently generated at a level approximately 10 times the baseline noise in the chromatogram.The limit of detection was determined to be 0.003 mg/L at a level of approximately three times the back ground of control injection around the retention time of the peak of interest. The LOD and LOQ values were 0.003 mg/L and 0.01 mg/L.

Stability of analytical standard stock solutions

It is well-known that dithiocarbamates are not stable in solution (30,31), From the storage stability test (4-8ºC) it was concluded that mancozeb decomposed at a rate of 4% per day, and only 20-25% of the initial concentration was recovered after 1 week of storage. On other hand, solution of Metiram was stable, 84% and 87% of the initial concentrations were recovered respectively after one week of storage. Nevertheless, it is recommended that for all tested compounds fresh stock and working standard solutions are made for the daily update of calibration curves and recovery studies.

Conclusion

Appropriate analytical methodology for the determination of dithiocarbamate(Mancozeb and Metiram) residues in aquatic tox medium (meant for fish, Daphnia, Algae and lemna) has been established and validated. This method indirectly measures the residual dithiocarbamates as concentration of Carbon disulphide. This was found to be satisfactory in terms of linearity of response, system precision, assay accuracy and quantification.

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