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METHOD FOR THE DETERMINATION OF MANCOZEB RESIDUES IN TEST MEDIA FOR ECOTOX STUDIES

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Abstract

A GC Analytical Method for the determination of mancozeb residues in test media samples has been developed and Validated. The mancozeb samples were digested and estimated by Gas Chromatograph (GC) using Flame Photometric Detector (FPD). The Method Validation consists of: Specificity, Linearity, Limit of Detection (LOD) Limit of Quantitation (LOQ), Precision (% RSD) and Accuracy (% Recovery). The Linearity ranged from 0.002 to 0.102 mg/L. The LOD for tap water, reconstituted water and algal media samples was 0.002 mg/L and the corresponding LOQ was 0.006 mg/L. The precision for tap water, reconstituted water and algal media at LOQ level was 5.50%, 6.00% and 5.36%, respectively and the corresponding values at 10 x LOQ level was 3.50%, 2.29% and 3.85%, respectively. The accuracy for tap water, reconstituted water and algal media at LOQ level was 85.66%, 83.67% and 93.84%, respectively and the corresponding values at 10 x LOQ level was 89.03%, 87.30% and 90.92%, respectively. The detailed Analytical data will be presented.

Mancozeb

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Common Name	:	**Mancozeb**
IUPAC Name	:	**Manganese ethylenebis(dithiocarbamate) (polymeric) complex with zinc salt**
CAS Name	:	**[[1,2-ethanediylbis[carbamodithioato]](2-)] manganese mixture with [[1,2-ethanediylbis[carbamodithioato]](2-)]zinc**
CAS RN	:	**[8018-01-7]**
Composition	:	**The ISO definition is a complex of zinc and maneb containing 20% mangenese and 2.55% of zinc**
Form	:	**Greenish yellow powder**
Melting Point	:	**Decomposes at 192 to 204 °C**
Solubility	:	**Water - 6.2 ppm (pH 7.5 at 25 °C)** **Organic Solvent - Insoluble**

Mancozeb

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Stability : **Stable under normal dry condition**

Hydrolysis (25°C)

- **pH 5, DT_{50} : 20 Days**
- **pH 7, DT_{50} : 17 Hours**
- **pH 9, DT_{50} : 34 Hours**

Application : **Fungicide**

Mode of Action : **Fungicide with protective action**

Uses : **Control fungal disease : Blight, leaf spot, rust, downy mildew, scab, etc. in field crops, fruits, nuts, vegetables and ornamentals etc.**

Analysis

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☆ Product

Analysis by decomposition with acid and measurement of the carbon disulfide liberated, either by GC or by a titrimetric method.

- **CIPAC 1980, 1A, 1288.**
- **Identification: Colorimetric (CIPAC 1994, F, 320)**
  U.V. Absorbance (ibid., 411).

☆ Residue

Determined by reaction with acid to form carbon disulfide which is measured by standard method.

- **Analyst 1981: 106, 782**
- **Pestic. Anal. Man., 1979, II;**
- **Manu. Pestic. Residue Anal., 1987, I, S21;**
- **Anal. Methods Residues Pestic., 1988, Part II).**

Structure

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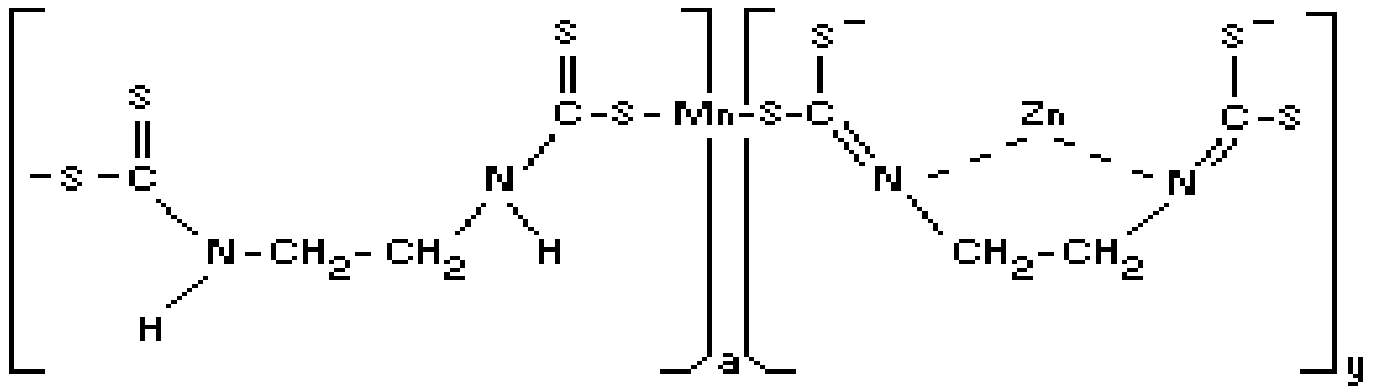
Empirical Formula

$$\left[-SCSNHCH_2CH_2NHCSSMn-\right]_x (Zn)_y$$

Chemical Structure



Method Development

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The mancozeb residues in test media (tap water, reconstituted water and algal media) is determined by digesting the test media and the evolved CS_2 gas estimated by capillary gas liquid chromatograph (GLC) using Flame Photometric Detector (FPD) in Sulfur mode.

Guidelines

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- **SANCO guideline (SANCO/3029/99 rev. 4)**
- **U.S. EPA OPPTS 860.1340 (August, 1996).**

Method Validation

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The GC method for the estimation of digested mancozeb samples was validated. The validation consist of :

(i) Specificity

(ii) Linearity

(iii) Limit of Detection (LOD)

(iv) Limit of Quantitation (LOQ)

(v) Precision (% RSD)

(vi) Accuracy (% Recovery)

Sample Digestion Procedure

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A volume of 5.0 mL test media was transferred into a reagent bottle of 160 mL capacity. A volume of 2.5 mL 10% EDTA and 5.0 mL 3% $SnCl_2$ in 8N HCl reagents was transferred into the same bottle. The bottle was crimped and heated in the water bath at 80°C for 1 hr. The evolved CS_2 trapped in the vial was directly injected onto GC using FPD with a gas tight syringe.

Instrumental Parameters

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Instrument	:	**Gas Chromatograph (Perkin Elmer, Clarus 500)**
Column	:	**DB- 5 (30m x 0.25 mm; 0.25µm film thickness)**
Detector	:	**Flame Photometric Detector (FPD), Sulfur mode**
Column Temp.	:	**40°C (5 min hold)**
Injector Temp.	:	**200°C**
Detector Temp.	:	**250°C**
Carrier gas	:	**Nitrogen**
Carrier Flow	:	**1.0 mL/minute**
Hydrogen Gas Flow	:	**75 mL/minute**
Air Gas Flow	:	**105 mL/minute**
Injection volume	:	**500 µL**

Specificity

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The specificity of the method was studied by injecting the gas onto gas chromatograph (GC) evolved after the digestion of

- **Reagents blank**
- **Mancozeb reference standard working solution**
- **Test substance working solution**
- **Untreated Control test media**
  - **i) Tap Water**
  - **ii) Reconstituted water**
  - **iii) Algal media**

Since there was no interference between components with each other or with any of their impurities and as well as the reagents blank. The method was considered to be specific for the reference compounds.

Linearity

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The linearity of analytical method was established by injecting the CS_2 evolved after the digestion of the five different concentrations of digested mancozeb standard solutions samples viz., 0.002, 0.006, 0.010, 0.051 and 0.102 mg/L of onto Gas Chromatograph in duplicate. The mean peak areas of CS_2 evolved after the digestion of mancozeb samples were plotted against concentration (mg/L).

The calculation for the followings was also performed.

i) Intercept (a)

ii) Slope (b)

iii) Correlation of coefficient (r).

The coefficient of correlation (r) was 0.999.

Linearity

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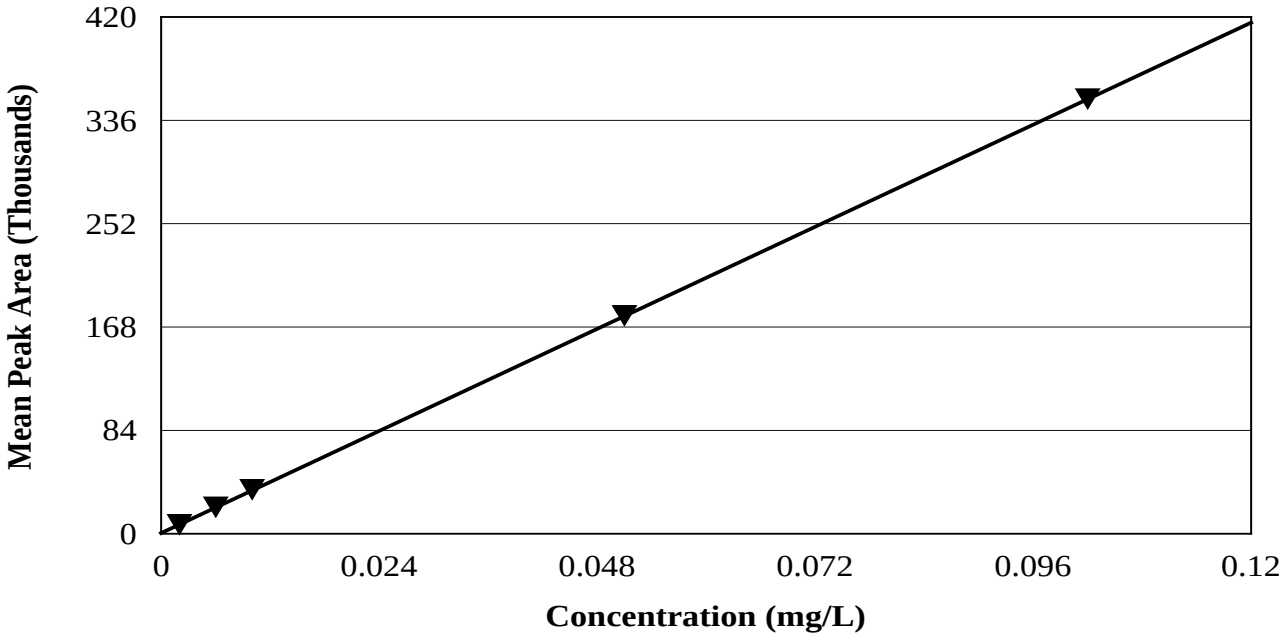
Concentration (mg/L)	Replication	Peak Area Count	Mean Peak Area Count	% Variation
0.002	R1	7050	7045.50	0.13
	R2	7041		
0.006	R1	21346	21237.00	1.02
	R2	21128		
0.010	R1	35767	35541.50	1.26
	R2	35316		
0.051	R1	179037	176929.50	0.68
	R2	177822		
0.102	R1	352828	353082.00	0.14
	R2	353336		

Linearity



Intercept with y-axis (a) = 535.43
Slope of the line (b) = 3457066.44
Correlation co-efficient or 'r' value = 0.999

Equation : Y = bX + a
Y =3457066.44 + 535.43

Limit of Detection (LOD)

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The limit of detection (LOD) was determined by injecting the CS_2 evolved after the digestion of different concentrations of standard solutions of mancozeb viz., 0.010, 0.006 and 0.002 mg/L onto GC in duplicate. The lowest detectable concentration was determined by calculating the signal to noise ratio (S/N) of 3.0 ± 0.5:1 for mancozeb. The lowest detectable concentration (LOD) of mancozeb by the method was 0.002 mg/L.

Limit of Quantitation (LOQ)

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The limit of quantitation (LOQ) was determined by injecting the the CS_2 evolved after the digestion of different concentration of test media fortified with mancozeb viz. 0.010, 0.006 and 0.002 mg/L onto GC in duplicate.

The lowest detectable concentration was determined by calculating the signal to noise ratio (S/N) ≥ 10:1 . The LOQ for all the media was 0.006 mg/L with signal to noise ratio of 10.18:1, 10.24:1 and 10.45:1 for tap water, reconstituted water and algal media, respectively.

Precision (% RSD)

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Precision of the analytical method was determined by analysing the CS_2 evolved after the digestion of test media samples fortified at different concentrations i.e, LOQ and 10 x LOQ levels. The precision (% RSD) data for mancozeb fortified in tap water, reconstituted water and algal media is provided below:

Precision (% RSD) of Mancozeb in Tap Water, Reconstituted Water and Algal Media

Parameter	Tap Water		Reconstituted Water		Algal Media	
	LOQ Level	10 x LOQ Level	LOQ Level	10 x LOQ Level	LOQ Level	10 x LOQ level
Precision (% RSD)	5.50	3.50	6.00	2.29	5.36	3.85

Accuracy (% Recovery)

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Accuracy (% recovery) of the analytical method was determined by analysing the CS_2 evolved after the digestion of test media samples fortified at different concentrations i.e, LOQ and 10 x LOQ levels as per SANCO guideline. The accuracy (% recovery) data for mancozeb fortified in tap water, reconstituted water and algal media is provided below:

Accuracy (% Recovery) of Mancozeb in Tap Water, Reconstituted Water and Algal Media

Parameter	Tap Water		Reconstituted Water		Algal Media	
	LOQ Level	10 x LOQ level	LOQ Level	10 x LOQ Level	LOQ Level	10 x LOQ level
Accuracy (% Recovery)	85.66	89.03	83.67	87.30	93.84	90.92

Typical Chromatograms

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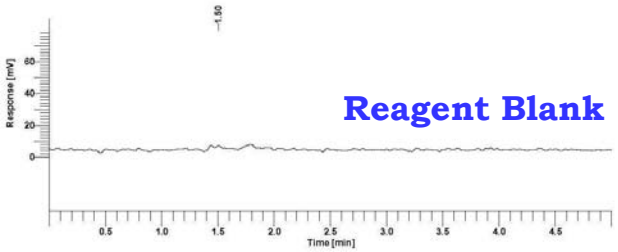
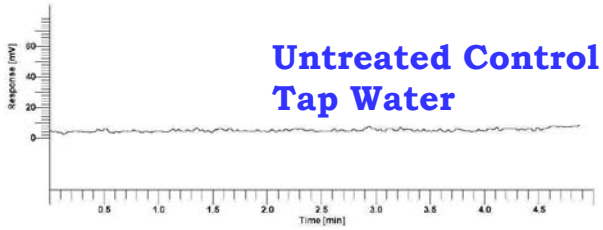
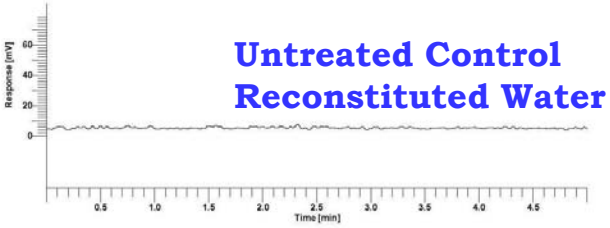


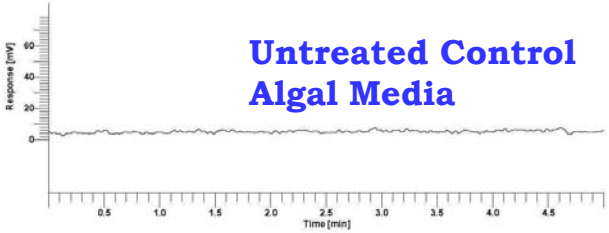
Reagent Blank



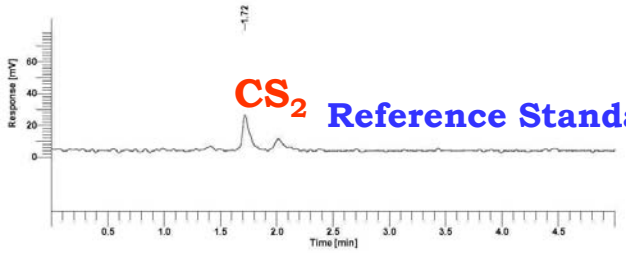
Untreated Control
Tap Water



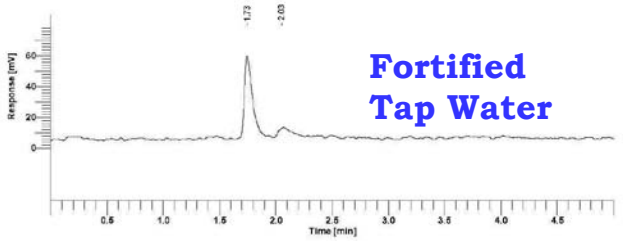
Untreated Control
Reconstituted Water



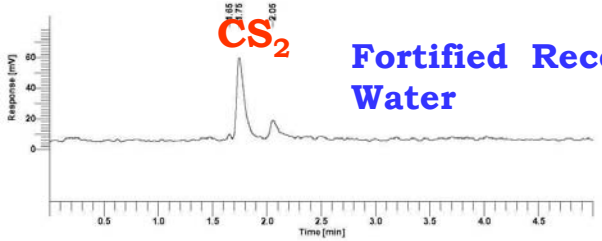
Untreated Control
Algal Media



CS_2 Reference Standard

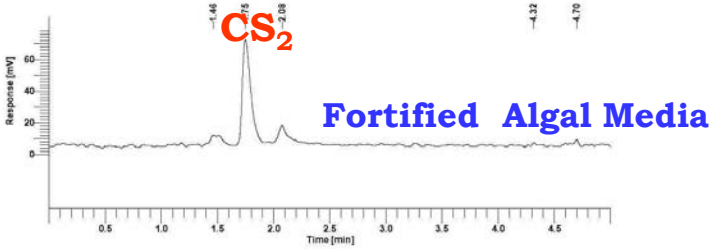


Fortified
Tap Water



CS_2

Fortified Reconstituted
Water



CS_2

Fortified Algal Media

Summary

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Parameters		Results		
Test Media		Tap Water	Reconstituted Water	Algal Medium
Specificity (Non-analyte Interference)		No interference	No interference	No interference
Linear Dynamic Range (LDR)	Concentration Range (mg/L) Intercept (a) Slope of the line (b) Correlation Coefficient (r)	0.002 to 0.102 228.59 3375839.02 0.999	0.002 to 0.102 535.43 3457066.44 0.999	0.002 to 0.102 410.89 3434956.50 0.999
Limit of Detection (LOD) [mg/L]		0.002	0.002	0.002
Limit of Quantitation (LOQ) [mg/L]		0.006	0.006	0.006
Precision (% RSD)	Fortification Level			
	LOQ	5.50	6.00	5.36
	% RSD [Horwitz equation]	23.15	23.15	23.15
	10 x LOQ	3.50	2.29	3.85
	% RSD [Horwitz equation]	16.37	16.37	16.37
Accuracy (Recovery %)	LOQ	85.66	83.67	93.84
	10 x LOQ	89.03	87.30	90.92

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Thank You