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Determination of Mancozeb residues in apple juice samples followed by Gas Chromatography Mass spectrometry method

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ABSTRACT

The present study was performed to validate gas chromatography mass spectrometry analytical method for the determination of mancozeb residues in apple juice samples. The analytical method is based on the extraction procedure by using mineral acids . The extraction solution was sucked into the flask, the dripping funnel was quickly exchanged for the gas inlet. After 2 hours, the adsorption tube containing the isooctane was removed and closed. The solution was transferred into a sample vial and analysed using GC-MS. A Rxi-624Sil MS (30m length x 0.32mm I.D. x 1.8 µm particle size capillary column is used for the separation. The method has linearity over the range 0.03 to 5.0 mg/L. Recovery study was conducted at 0.03 and 0.3 mg/L fortification levels. The average mean recoveries were calculated as 85.28 % at 0.03 mg/kg level and 90.11 % at 0.3 mg/L level. The limit of quantification in juice was established as 0.03 mg/L.

Key words: GC-MS-EI method, Mancozeb, Carbon disulphide and apple juice

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INTRODUCTION

Mancozeb belongs to the dithiocarbamate group of fungicides and to the class of compounds called ethylene bis dithiocarbamates [1]. Mancozeb become first discovered in 1962 as a fungicide still it has an crucial role to play. Globally mancozeb is registered to be used on greater than 70 exceptional plants and gives protection towards greater than four hundred diseases. The direct effect of mancozeb upon middle biochemical procedures inside the fungus outcomes in inhibition of spore germination [2,3]. Mancozeb presentations the traits of a regular multi-web site protactant- only fungicide, the compound remains on the surface of the leaf and does not penetrate through the cuticle where systemic redistribution can arise. that is truely essential due to the fact penetration of a trendy toxophore which include mancozeb into plant cells could probable motive phytotoxicity. Fortuitously, mancozeb has an exceptional file of crop safety over a wide variety of crops and environmental conditions [4,5]. Apples are a prime agricultural crop in world. Due to its high economic value as well as the large number of plant diseases, bugs, and mites that infest apples during the developing season, massive quantities of pesticides are regularly necessary for the protection of this crop. This will result in residues on (or in) the fruit at harvest. The maximum wide-spread apple disease, accounting for plenty of the apple pesticide use worldwide, is apple scab, because of the fungus Venturia inaequalis. This disease may be treated by applications of mancozeb. Now a day's formers are regularly spraying mancozeb fungicide on apple plants. The mancozeb residues may be accumulated in the final harvest of

apple fruits and its products. The usage of these apples and its products by the humans, it will be affecting their health. So that the present study was conducted to determine the mancozeb residues in apple juice samples. The general method of analysis of dithiocarbamates is based on their decomposition to liberate carbon disulfide (CS_2) using 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. Several published methods are available based on this principle. Majority of these methods have practical difficulties while analyzing the active component by titration due to the interference with the dirty components. Sometimes the CS_2 liberation may not be completed or leaked while trapping or the reverse flow may contribute to the negative results forcing the analyst to do multiple sample analysis which is a time consuming process. The method adopted in the present study is based on the decomposition of dithiocarbamates by hot mineral acid to the amines and carbon disulfide (CS_2) followed by the entrapment of released Carbon disulfide in isooctane solvent and then analysing using GC-MS.

MATERIALS AND METHODS

Standards, reagents and samples

The analytical standards of Carbon disulfide (99.9%) and the test item mancozeb (95.2%) were obtained from Sigma Aldrich. The analytical grade solvents i.e., Phosphoric acid, Iso octane and Ethanol were purchased from Rankem, New Delhi. The analytical grade reagents i.e., EDTA, Sodium sulphate and Concentrated Sulfuric acid were purchased from Merck Limited and apple juice was purchased from local fruit juice shop.

Standard stock solution

Carbon disulfide stock solution (500 mg/L) was prepared in Iso octane and stored at -20°C. The stock standard solution was used for up to 3 months. Working standard solution of suitable concentrations was prepared immediately prior to sample preparation by diluting the stock solution using Iso octane.

Preparation of (mancozeb) stock solution

A stock solution of mancozeb was prepared in a volumetric flask by dissolving 5.26 mg of mancozeb (95.2%) in 10 mL of 0.25M EDTA solution, which resulted in a concentration of 500.3 µg/mL, taking into account the purity of mancozeb (95.2%).

Sample preparation

Representative 10.0 mL portions of apple juice fortified with 200 µL of working standard stock solution. The sample was allowed to stand at room temperature for one hour, before it was kept at refrigerator condition, until analysis.

EXTRACTION PROCEDURE

Experimental setup

The test system consisted of a 500 mL round bottom two-neck flask equipped with a reflux condenser and gas inlet tubing, placed in a heating mantle. A row of three adsorption tubes were connected via gas piping to the top of the reflux condenser. The first adsorption tube was filled with 10 mL of sulphuric acid (conc.), the second remained empty and the third was filled with exactly 10 mL of isooctane and immersed in a Dewar vessel containing ethanol and dry ice. The gas piping with pinch cock was connected to a membrane pump which sucked a flow of nitrogen through the apparatus controlled by a rotameter (set to approx. 30 mm ± 5 mm, corresponding to 240 mL/min).

Extraction

10 mL of representative juice sample was transferred into the round bottom two-neck flask to which 50 g of sodium sulphate was added, sample was spiked with the fortified sample solution . The pinch cock of the gas piping was opened and a stream of nitrogen was sucked through the apparatus. Prior to that, 200 mL of the extraction solution (35% H_3PO_4) was brought to the boil in the microwave. The hot but not boiling solution was filled into a dropping funnel which was inserted into the second neck of the round bottom two-neck flask. Because of the low pressure in the apparatus, the extraction solution dripped slowly into the flask with the cock of the dripping funnel partly opened. Care was taken that the gas did not escape through the dripping funnel. Then the heating mantle was switched on. When all of the extraction solution was sucked into the flask, the dripping funnel was quickly exchanged for the gas inlet. After 2 hours, the adsorption tube containing the isooctane was removed and closed. The solution was transferred into a sample vial and analysed using GC-MS. High fortifications were diluted accordingly.

Chromatographic separation parameters

The GC-MS system used, consisted shimadzu Gas Chromatograph GC17AQP5050A equipped with mass spectrometer, Auto injector and interfaced with GCMS solution software, equipped with a Rxi-624Sil MS (30m length x 0.32mm I.D. x 1.8 µm film thickness). Column oven temperature was maintained with program ie., Initial temperature 40°C held for 4 min, ramp @40°C /min to 200°C held for 5 min. The injector temperature is 200 °C, Interface temperature is 220°C, Column flow (Nitrogen) is 2.0 mL/min, Acquisition mode is SIM and the injected sample volume was 1µL with split mode (1:25).

Method validation

Method validation ensures analysis credibility. In this study, the parameters accuracy, precision, linearity and limits of detection (LOD) and quantification (LOQ) were considered[5]. The accuracy of the method was determined by recovery tests, using samples spiked at concentration levels of 0.05 and 0.5 mg/kg. Linearity was determined by different known concentrations (0.03, 0.3, 1.0, 2.0, 3.0 and 5.0 mg/L) were prepared by diluting the stock solution. The 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 control (untreated) sample [6, 7]. The limit of quantification (LOQ, mg/L) was determined as the lowest concentration of a given fungicide giving a response of 10 times the baseline noise.

RESULTS AND DISCUSSION

Specificity

Aliquots of carbon disulfide standard, control sample solution and solvent (iso-octane) were assayed to check the specificity. There were no matrix peaks in the chromatograms to interfere with the analysis of residues shown in (**Figure. 1 and Figure. 2).** Furthermore, the retention time of carbon disulphide was 2.6 min (Approximately) and the mass fragment selected for evaluation was m/z 76. Shown in (**Figure. 3).**

Figure.1. Representative Chromatogram at juice control

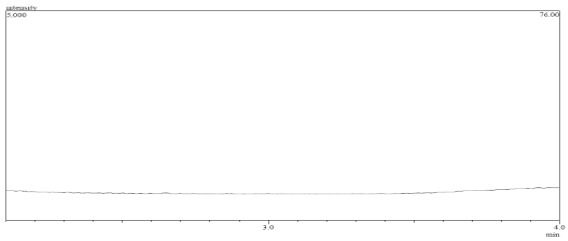


Figure.2. Representative Chromatogram at fortification level of 0.3 mg/L

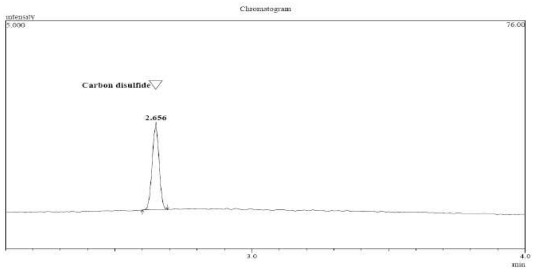
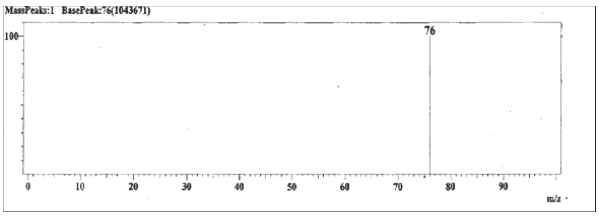


Figure.3. Representative mass spectrum of carbon disulphide



Linearity

Different known concentrations of standards (0.03, 0.3, 1.0, 2.0, 3.0 and 5.0 mg/L) were prepared in isooctane by diluting the stock solution into 10 mL different volumetric flasks. These solutions were injected into a GC-MS and calibration plot was constructed for the peak area recorded Vs the concentration. The peak areas obtained from different concentrations of standards were used to calculate linear regression equation. This was Y=8835.66X + 86.32 with correlation coefficients of 0.9999. The data are presented in **Table 1** and the curve was presented in **Figure. 4.**

Table 1. Linearity data for carbon disulfide

Concentration in mg/L	Area in µv*sec
0.03	289
0.3	2678
1	8745
2	17901
3	27026
5	43987
Slope	8835.66
Intercept	86.32
Correlation coefficient	0.9999

Fig. 4. Representative Calibration curve of carbon disulphide

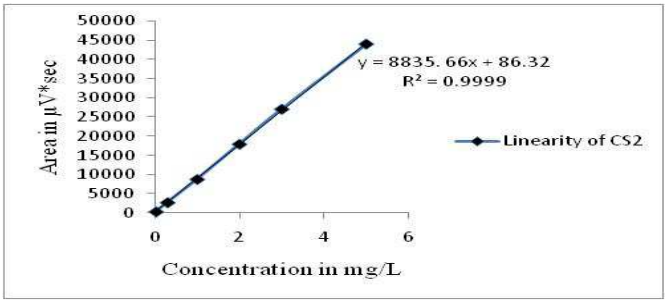


Table 2.. Recoveries of the carbon disulphide from fortified apple juice sample control sample (n=6)

Fortification Concentration in mg/L	Replication	Recovey (%)
0.03	R1	84.23
	R2	83.29
	R3	85.37
	R4	87.18
	R5	86.12
	R6	85.47
	Mean	**85.28**
	STDEV	**1.37**
	RSD in %	**1.61**
0.3	R1	88.78
	R2	88.21
	R3	89.36
	R4	92.22
	R5	91.58
	R6	90.49
	Mean	**90.11**
	STDEV	**0.75**
	RSD in %	**0.81**

Accuracy and Precision

Assay accuracy of the method was checked at two concentration levels LOQ (0.03 mg/L), 10 x LOQ (0.3 mg/L) and 2 control samples. Five determinations were made at each concentration level to check repeatability along with two control samples for comparison.

Assay accuracy samples at LOQ level fortifications were prepared by fortifying 0.1 mL of carbon disulphide fortification solution (0.3 mg/L) into round bottom flask containing 10 mL of juice sample. Samples at LOQ x 10 level fortifications were prepared by fortifying 1.0 mL of carbon disulphide fortification (3.0 mg/L) solution [8].

The recovery data and relative standard deviation values obtained by this method are summarized in **Table 2**.

These numbers were calculated from four (6) replicate analyses of given sample made by a single analyst on one day. The repeatability of method satisfactory (RSDs<5 %).

Detection and Quantification Limits

The limit of quantification was determined to be 0.03 mg/L. The quantitation limit was defined as the lowest fortification level evaluated at which acceptable average recoveries (85-90%, RSD<5%) were achieved. This quantitation limit also reflects the fortification level at which an analyte peak is consistently generated at approximately 10 times the baseline noise in the chromatogram. The limit of quantification was determined to be 0.03 mg/L at a level of approximately three times the back ground of control injection around the retention time of the peak of interest.

Storage Stability

A storage stability study was conducted at -20 ± 1°C with juice samples spiked with 3.0 mg/L of mancozeb Samples were stored for a period of 30 days at this temperature [9,10]. Analysed for the content of mancozeb before storing and at the end of storage period. The percentage dissipation observed for the above storage period was only less than 5% for mancozeb showing no significant loss of residues on storage. The results are presented in **Table 3.**

Table 3. Storage stability Details (n=6)

Fortification Concentration in mg/L	Storage Period in Days	Recovery in %
3.0	0	95.12
		96.77
		95.19
		94.79
		94.55
		95.09
	Average	**95.25**
	STDEV	**0.78**
	RSD in %	**0.82**
	30	93.46
		92.87
		91.89
		93.25
		92.31
		91.45
	Average	**92.54**
	STDEV	**0.79**
	RSD in %	**0.85**

Calculations

The detector signals were registered and integrated using the data systems. The peak area was taken into account to determine the CS2 amount in the specimens. The calibration curves were calculated from the area of the calibration solutions

$y = a + bx$ ------- (1).

where

y: peak area [integration units µ]
x: amount of analyte [ng]
a: ordinate intercept [µ]
b: slope [µ /ng]

The amount of CS2 in the specimen was calculated using the transformed equation (1):

$$x = \frac{y - a}{b}$$ -------- (2).

The concentration of CS_2 in the specimen was calculated from 'x' using equation (2):

$$C_R = \frac{x \cdot V_E \cdot A_1 \cdot F}{V_i \cdot A_2 \cdot W}$$ ---------- (3).

Where

X : amount of analyte [ng]
C_R : analysed concentration of analyte in the specimen [mg/kg]
V_E : final volume (Mancozeb: 10 mL)
A_1 : total extract (Mancozeb: 1 mL)
V_i : injection volume (Mancozeb: 2 µL)
A_2 : aliquot (Mancozeb: 1 mL)
W : specimen weight (Mancozeb: 25 g)
F : conversion factor CS2 → Mancozeb (1.75)

The recovery data was calculated according to equation (4):

$$R = \frac{C_R \cdot 100}{C_F}$$ ---------- (4).

Where
R : recovery (%)
C_R : analysed concentration of analyte in the fortified specimen (mg/kg)
C_F : nominal concentration of analyte in the fortified specimen (mg/kg)

CONCLUSION

The present method is suitable for the determination of mancozeb in different commodities. The method is very specific for the quantification of individual molecules and produces better accurate results when compared with the methods published in the literature. Satisfactory validation parameters such as linearity, recovery, precision and very low limits were obtained and according to the SANCO guidelines [11]. Therefore, the proposed analytical procedure could be useful for regular monitoring authority, scientific researchers and residue analytical labs.

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