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Determination of Mancozeb analysis method using LC/MS/MS

Eun-seong Park, Jong-gon Son, Eun-ho Son, Jung-hwa Jin, Nam-suk Kim, Su-myeong Hong
National Institute of Agricultural Sciences, RDA, Wanju, Korea

ABSTRACT

This study was conducted on the development of analysis method of Mancozeb belonging to dithiocarbamate group which is effective in the control of anthracnose and rot disease of the fruits and vegetables. The present CS_2 analysis method takes long time to analyze and present HPLC analysis has solubility problem of the fungicides and low recovery rates. The objectives of this study was to solve these problems by making the derivative of dithiocarbamate fungicides and detecting this derivatives by HPLC. This improved method was confirmed with LC/MS/MS analysis.

Mancozeb is a macromolecule containing metal ions that are sparingly soluble in water and not soluble in organic solvents. Therefore, it was completely dissolved in a chelating reagent, 0.25M-EDTA disodium salt, and then derivatized with a $CHCl_3$: C_6H_{14} (3:1) solution of 0.05M-CH3I for the accurate analysis.

The new HPLC analysis used the mobile phase (acetonitrile : distilled water, 50/50 v/v) and the column (C18, 250 mm × 4.6 mm, inner diameter 5 μm). As the results of LC/MS/MS analysis for dithiocarbamate, the detection limit of EBDC was 0.0025 mg/L and the quantitative limit was 0.005 mg/L. The standard curve for this was Y=7.17656e6X+3113.28681 and r^2 was equal to 0.9957.

MATERIALS & METHODS

Table 1. Molecular structure and physicochemical properties of Mancozeb

Common name	Mancozeb		
CAS No	8018-01-7		
Chemical name	Manganese ethylenebis(dithiocarbamate)(polymeric) complex with zinc salt		
Solubility	Water : 2-20 mg/L, Organic solvents : practically insoluble in organic solvents		
Molecular formula	$[C_4H_6MnN_2S_4]_XZn_Y$	Vapor pressure	1.33×10^{-5} Pa
Molecular Weight	271.2 g/mol	Density	1.9938 g/ml (20℃)
Appearance	Light yellow powder	log Pow	1.33

- Reagent : Mancozeb Standard product, Methyl iodide, Acetonitrile(HPLC grade)
  Disodium dihydrogen ethylenediaminetetraacetate dihydrate(EDTA)
  Tetrabuthylammonium hydrogen sulfate(TBAH), Distilled water

Solution preparation

An aliquot 25.5 mg (±0.1 mg) of standard was shaken in 20 mL of 0.25M EDTA-disodium salt solution(pH 9.5-10.5) for 10 min in a closed 50 mL tube.
4 mL of the extracts was added to 16 mL of 0.25M EDTA-disodium salt solution and the mixture was shaken for 5min

↓

3 mL of 0.41M TBAH and 20 mL of 0.05M-CH_3I in $CHCl_3$:C_6H_{14}(3:1) were added into 3 mL of the extracts. Then the pH was adjusted to 7.2-8.0

↓

The mixture was shaken lightly about 1 min. And then was methylated for 5 min. The lower layer was dehydrated with Na_2SO_4.

↓

3 mL of the solvent of CH_3I were added with 0.2 mL of 20% 1, 2-propanediol in acetonitrile and evaporated at 30℃ in a rotatory evaporator. The residue was diluted with 3 mL of acetonitrile and was analyzed by HPLC and LC/MS/MS

Instrument Analysis

- HPLC Analysis : Injection volumn : 10 μl, 272 nm, Flow rate : 0.7 mL/min , Column Temperature : 35℃
- LC/MS/MS Analysis : ExionLC™, AB SCIEX, USA

Figure 1. A flow sheet of quantitative analysis process of mancozeb

RESULTS

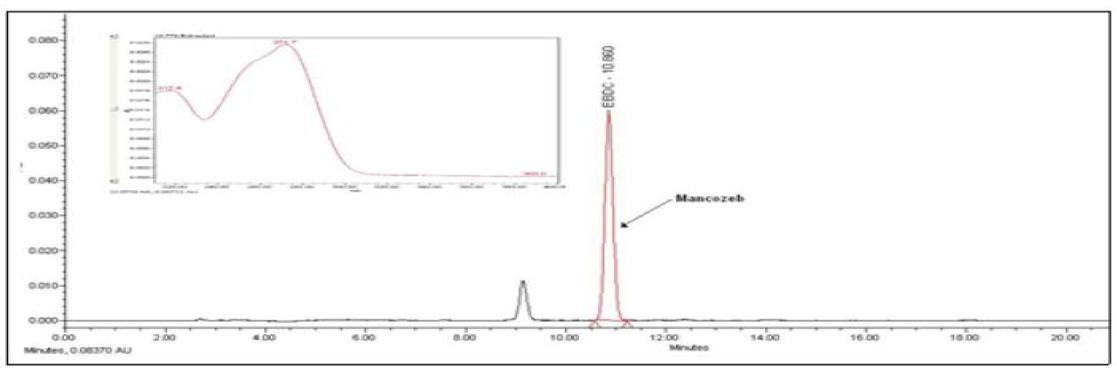

Figure 2. Chromatogram and spectra of 100 ng of Mancozeb

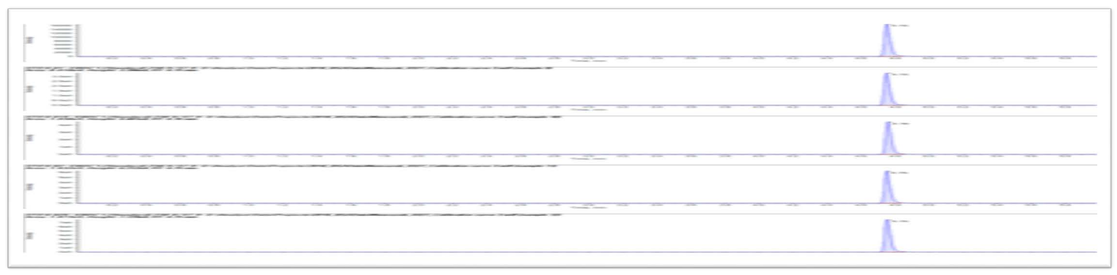

Figure 3. MRM chromatogram by Mancozeb standard concentration

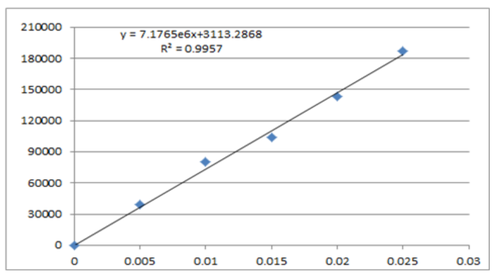

Figure 4. Calibration curve of Mancozeb

Mancozeb (mg/L)	Area	Height	Retention Time
0.005	3.94E+04	1.69E+04	4.75
0.01	8.02E+04	3.40E+04	4.75
0.015	1.04E+05	4.48E+04	4.76
0.02	1.43E+05	6.31E+04	4.75
0.025	1.87E+05	7.73E+04	4.75

Table 2. Instrumental analysis conditions for LC/MS/MS

Instrument	ExionLC™, AB SCIEX, USA
Column	Kinetex® 2.6 um Polar C18 100 Å , 2.1 mm I.D. × 150 mm L
Injection volume	5 ul
Mobile Phase	MPA - MeOH(2mM ammonium formate 0.1% formic acid) MPB - H2O(2mM ammonium formate 0.1% formic acid)

Time(min)	A (%)	B (%)	Flow rate(mL/min)
0	10	90	0.4
3	90	10	0.4
3.1	10	90	0.4
6	10	90	0.4

Column Temp	35 ˚C
Retention time	mancozeb 4.75min

Instrument	QTRAP 5500 system, AB SCIEX, USA					
Precursor ion(m/z)	Ion transition					
	Quantitation ion(m/z)	Dwell time	DP	CE	CXP	EP
238.9	57.9	50	-40	-28	-4	-10

DISCUSSIONS

- Since Mancozeb is poorly soluble in water and solvents, EDTA, a chelating agent, is used to form an EBDC-disodium salt to make an aqueous solution. The EBDC- Bu_4N^+ reacts with CH_3I to form methylated after the formation of EBDC- Bu_4N^+ in the solvent layer by substituting Na^+ ion and $Bu4N^+$ ion for the phase transfer catalyst TBAH in the EBDC-disodium salt aqueous solution
- As a result of verifying the methylated EBDC using LC/MS/MS, the detection limit was 0.0025ppm, the quantitation limit was 0.005ppm, and the linearity (0.0252, 0.02, 0.015, 0.01, 0.005 ppm) was good as the correlation coefficient was 0.9957

REFERENCE

- Gustafsson, K. H., and Tompson, R. A. 1981 High Pressure Liquid Chromatographic Determination of Fungicidal Dithiocarbamates. *J. Agric. Food Chem.* 29:729-732.