Use of High-Performance Liquid Chromatographic and Atomic Absorption Methods To Distinguish Propineb, Zineb, Maneb, and Mancozeb Fungicides

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A high-performance liquid chromatographic (HPLC) method with ultraviolet detection at 272 nm is capable of distinguishing between the propineb and ethylenebis(dithiocarbamate) (EBDC) fungicides, which could not be indentified by the traditional CS_2 method. By combination of an HPLC and an atomic absorption method, the EBDC fungicides zineb, maneb, and mancozeb could be further distinguished by comparing the total Zn and Mn content in formulated products

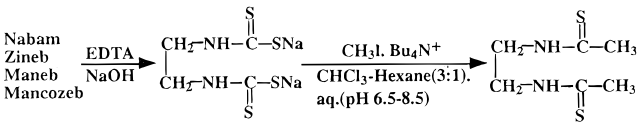
Keywords: *HPLC; atomic absorption; selective analysis; propineb; zineb; maneb; mancozeb*

INTRODUCTION

Traditionally, fungicide dithiocarbamates are analyzed with a colorimetric method based on the amount of carbon disulfide formed by acid hydrolysis (Keppel, 1969). However, this method is not selective, because all *N*,*N*′-ethylenebis(dithiocarbamate) (EBDC) fungicides and propineb [zinc *N*,*N*′-propylenebis(dithiocarbamate)] released carbon disulfide upon heating with acid. Furthermore, this method is not very accurate. Therefore, it is important to develop a method that can distinguish the active ingredienta zineb [zinc *N*,*N*′-ethylenebis(dithiocarbamate)], maneb [manganese *N*,*N*′-ethylenebis(dithiocarbamate)], mancozeb [manganese ethylenebis(dithiocarbamate) complex with zinc salt], and propineb from each other.

Stevenson (1972) developed a color spot test method and Afsar and Demirate (1987) developed a modified color spot test method to distinguish between maneb, zineb, mancozeb, and a selected mixture. These methods have the same limitations, the color depends on the type and concentration of metal present in the sample, the determinations are based on color differences that are difficult to describe accurately, and no propineb sample is tested.

Gustafsson and Thompson (1981) developed a high-pressure liquid chromatographic (HPLC) method to determine the thiram salts of *N*,*N*′-alkylenebis(dithiocarbamic acid), and *N*,*N*-dimethyldithiocarbamic acid by methylating these fungicides with methyl iodide:



This method showed some selectivity among some dithiocarbamates fungicides. For examples, the methyl derivative of metham-Na (sodium *N*-methyldithiocarbamate) eluted first from the HPLC column, followed by the methyl derivatives of diram (ammonium *N*,*N*-dimethyldithiocarbamate), ziram (zinc *N*,*N*-dimethyldithiocarbamate), and ferbam (ferric *N*,*N*-dimethyldithiocarbamate); the methyl derivative of thiram (tetramethylthiuram disulfide); and the methyl derivative of nabam [disodium *N*,*N*′-ethylenebis(dithiocarbamate)], zineb, maneb, and mancozeb. The methyl derivative of propineb was the last compound to be eluted out from the HPLC column. Therefore, distinguishing between zineb and propineb was possible because the methyl derivative of zineb eluted out earlier than the methyl derivative of propineb. However, distinguishing the EBDC fungicides (zineb, maneb, and mancozeb) by HPLC was impossible because they form the same derivative [i.e., dimethyl *N*,*N*′-ethylenebis(dithiocarbamate)] on methylation.

Table 1. Contents of Zn and Mn in the Standard and Wettable Powder (WP) of Zineb, Maneb, and Mancozeb

sample	Zn (%)	Mn (%)
standard		
zineb	23.7	ND[b]
maneb	0.9	18.8
mancozeb	2.5	22.0
formulation product		
zineb		
1,[a] 650 g kg^{-1} WP	17.9	trace
2, 650 g kg^{-1} WP	13.8	ND
3, 650 g kg^{-1} WP	14.4	ND
4, 700 g kg^{-1} WP	17.7	ND
5, 720 g kg^{-1} WP	16.9	ND
maneb		
6, 800 g kg^{-1} WP	0.2	14.3
7, 800 g kg^{-1} WP	0.4	14.6
8, 800 g kg^{-1} WP	ND	14.6
9, 800 g kg^{-1} WP	0.6	14.2
10, 800 g kg^{-1} WP	0.1	14.7
mancozeb		
11, 800 g kg^{-1} WP	2.4	14.3
12, 800 g kg^{-1} WP	2.6	13.9

[a] Different manufacturers arranged in numerical order. [b] Not detected.

Preliminary atomic absorption analyses of commercial EBDC fungicides collected from market showed that the Zn content was high in zineb products (Zn > 13.8%), low in mancozeb products (Zn < 2.6%), and in trace amounts in maneb products (Zn < 0.6%). The Mn content was high in both maneb products (Mn > 14.2%) and mancozeb products (Mn > 13.9%), but low or in trace amounts in zineb products (Zn%, trace or not detected; Table 1). These results indicate that the

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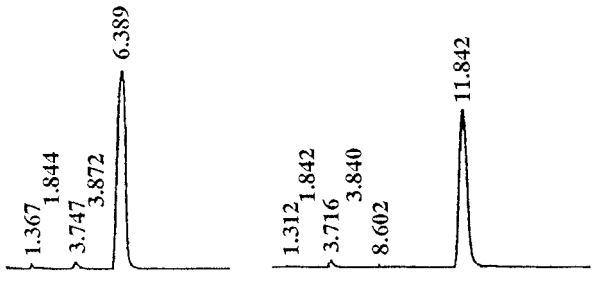


Figure 1. HPLC chromatograms of ETU (left; retention time, 6.389 min) and PTU (right; retention time, 11.842 min).

atomic absorption method can be used to distinguish the EBDC products by comparing the total Zn and Mn contents.

Thus, a two-phase determination procedure is used to identify the active ingredient of commercial propineb products. First, the HPLC method developed by Gustafsson and Thompson (1981) is applied to investigate the active ingredient in propineb fungicide and determine whether it is a real propineb product or it is a falsely claimed EBDC product. Second, the atomic absorption method is used to determine whether the EBDC product is a zineb, a maneb, or a mancozeb product by analyzing the total Zn and Mn contents.

MATERIALS AND METHODS

Apparatus. A Varian atomic absorption (AA) spectr-30 AA was used for the determination of total Zn and Mn contents in the samples. The light sources were single-element hollow-cathode lamps, and the operating parameters were as given by the manufacturer. The wavelengths and slit widths for Zn and Mn were 213.9 and 1.0 and 279.5 and 0.2, respectively. The atomization mode was air/acetylene flame. All the analyses were conducted in triplicate. Mass measurements by direct-insert method were recorded on a Hewlett-Packard 5970B mass selective detector (EI, 70 eV).

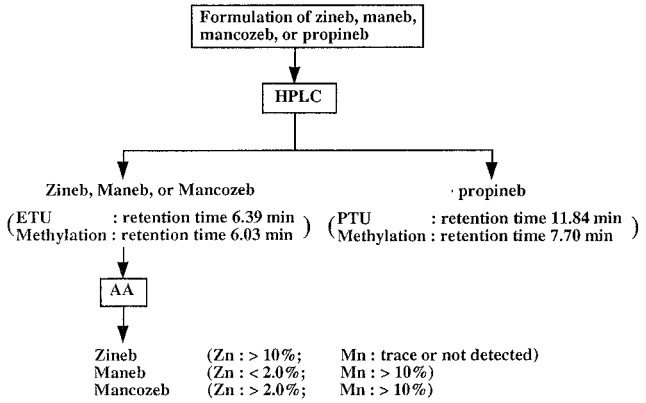
ETU and PTU Determination. Analytical grade imidazolidine-2-thione (ethylenethiourea, ETU) was purchased from Aldrich Chemical Company, Inc., Milwaukee, WI (purity 98%). Analytical grade 4-methylimidazolidine-2-thione (PTU) was provided by Bayer AG (99.5% purity). Analytical ETU and PTU standard solutions of 1.0, 5.0, 10.0, and 50.0 mg/L in methanol were prepared for HPLC calibration curves.

The propineb formulation (2.5 g) was weighed directly into a 100-mL flask, methanol (40 mL) was added, and the flask was shaken vigorously for 5 min. The solution was filtered through a 0.45-μm Millipore filter into a 50-mL volumetric flask, made to volume with methanol, and 10 μL of the clear filtrate was immediately injected into the HPLC. The PTU and ETU contents were calculated by comparison with the calibration curves from a series of standard PTU or ETU solutions. Each analysis was done in triplicate.

The HPLC method was basically that of Van Damme et al. (1981) and was conducted on a Shimadzu LC-9A pump, equipped with a UV spectrophotometer (SPD-6AV) and a 250 $\times$ 4 mm i.d. stainless steel column packed with Lichrospher 60 RP-select B (5 μm; Merck). The operating conditions were as follows: column temperature, ambient; mobile phase, water + tetrahydrofuran (99.95 + 0.05, by volume); flow rate, 0.8 mL min^{-1}; wavelength, 233 nm (Figure 1).

Methylation and Determination. Standards of zineb (purity 80%), maneb (purity 97%), mancozeb (purity 83%), and propineb (purity 75%) were purchased from RDH (Riedel-de Haën, Germany). Methylation was basically according to the method of Gustafsson and Thompson (1981). Standard zineb of 0.01 g was added to a 100-mL beaker with 50 mL of EDTA (0.25 M) and stirred for 5 min. The EDTA extracts were filtered through a Whatman GF/B glass microfiber filter. The extraction beaker and the filter were rinsed with 20 mL of water. The pH of the solution was adjusted to 6.5−8.5 by addition of 8 mL of HCl solution (2 M) and 5 mL of aqueous tetrabutylammonium hydrogen sulfate solution (0.41 M). The mixture was shaken in a separatory funnel for 5 min at room temperature with 30 mL of 0.05 M methyl iodide (iodomethane, cancer suspect agent) in chloroform (highly toxic cancer suspect agent):hexane (3 :1, v/v). The organic phase was collected and the aqueous layer was rinsed with another 10 mL of the methyl iodide solution. The organic phases were combined, and 5.0 mL of 1,2-propanediol in chloroform (20%, v/v) was added and concentrated by rotary evaporator at 30 °C. The residue was diluted with 5.0 mL of methanol, and 10 μL was injected onto the HPLC column by syringe loaded with a PVDF syringe filter (0.45 μm) and analyzed by HPLC.

Scheme 1. Procedure To Distinguish Zineb, Maneb, Mancozeb, and Propineb Fungicides



The same methylation derivation procedures were followed for the standards of mancozeb, maneb, propineb, and the commercial propineb formulations.

A Shimadzu liquid chromatograph with a UV spectrophotometric detector operated at 272 nm was used to analyze the methyl derivatives of zineb and propineb and their formulations. Separations were achieved with a stainless steel column (125 $\times$ 4 mm i.d.) with Lichrospher 60 RP-select B (5 μm) preceded by a guard column of similar packing (4 $\times$ 4 mm i.d.) at room temperature. The mobile phase was water:acetonitrile (3:2, v/v) with a flow rate of 1.0 mL/min (Figure 2).

Distinguishing Zineb, Maneb, Mancozeb and Propineb. The active ingredient content of the propineb fungicides were determined by the retention time of the methyl derivative of propineb by an HPLC method. The active ingredient contents of zineb, maneb, and mancozeb were determined by an atomic absorption method. If the Zn content was high in products (Zn > 10%), the sample was considered to be a zineb product; if the Mn content was high (Mn > 10%) and the Zn content was <2% in product, the sample was considered to be a maneb product. If the Zn content was >2% and the Mn content was >10% in product, the sample was considered to be a mancozeb product (Scheme 1).

RESULTS AND DISCUSSION

ETU and PTU Identification. Routine HPLC analyses of the ETU content of EBDC fungicides showed that one of the commercial propineb 70% wettable powder (WP) samples (sample A) was contaminated with 5.27% ETU (Table 2); ETU should be a degradation product of EBDC fungicides, and PTU should be a degradation product of propineb (WHO, 1988; Figure 3). This erratic result was further analyzed by mass spectrometry. The suspect propineb product (sample A) was extracted and recrystallized with methanol and analyzed. The mass spectrum (Figure 4) confirmed that the recrystallized compound was ETU not PTU, because its molecular weight was the same as that of ETU (MW = 102.0) and not the same as that of PTU (MW = 116.0).

These results confirm that some EBDC fungicides contain propineb products, which are used as substi-

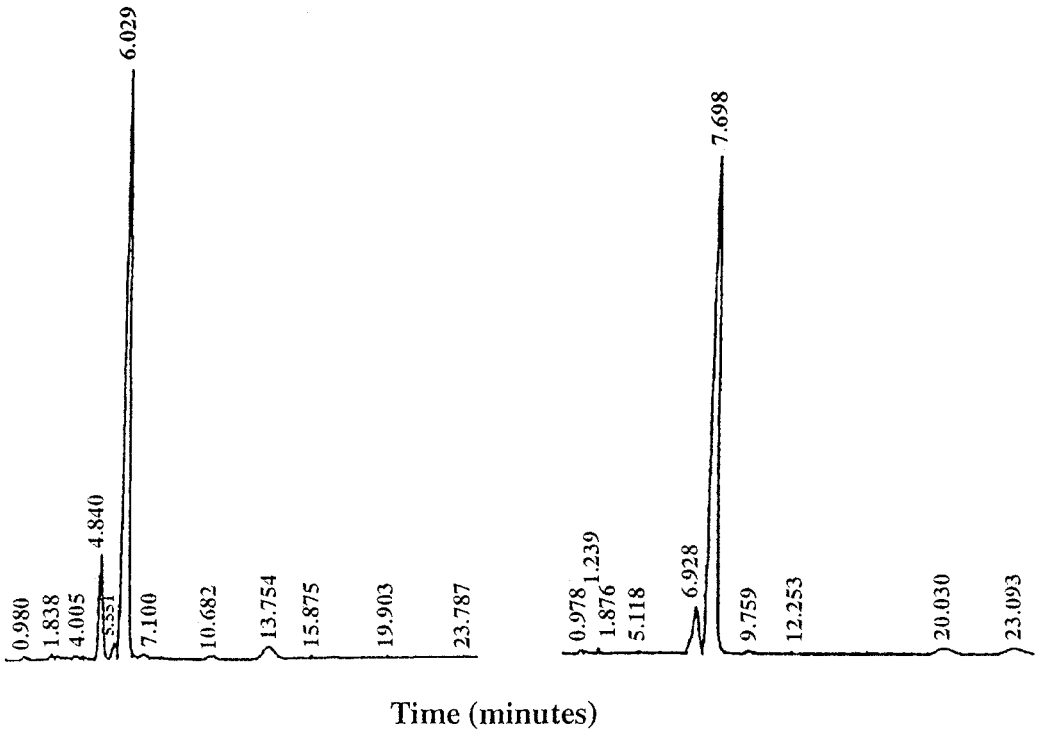
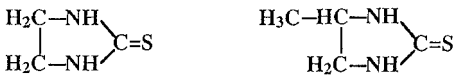


Figure 2. HPLC chromatograms of dimethyl *N,N′*-ethylenebis(dithiocarbamate) (left; retention time, 6.03 min) and dimethyl *N,N′*-propylenebis(dithiocarbamate) (right; retention time, 7.70 min).

Table 2. Analysis of Active Ingredient and Zn and Mn Content in Propineb 70% Wettable Powder (WP) Formulations

formulation[a]	ETU (%)	PTU (%)	active ingredient (HPLC method)	Zn (%)	Mn (%)	active ingredient (confirmed)
A	5.27	ND	EBDC[b]	14.38	ND[c]	zineb
B	0.16	ND	BEDC	21.46	0.04	zineb
C	1.70	ND	EBDC	13.63	ND	zineb
D	ND	0.12	propineb	16.81	ND	propineb
E	0.73	ND	EBDC	18.21	ND	zineb
F	1.88	ND	EBDC	12.47	ND	zineb
G	1.14	ND	EBDC	14.11	0.02	zineb
H	1.35	ND	EBDC	16.34	0.02	zineb
I	1.14	ND	EBDC	15.84	ND	zineb
J	0.53	ND	EBDC	18.19	ND	zineb
K	0.38	ND	EBDC	15.05	ND	zineb
L	1.28	ND	EBDC	16.58	ND	zineb

[a] Different manufacturers arranged in alphabetical order; all claimed to be propineb 70% WP. [b] Ethylenebis(dithiocarbamate) fungicides; such as, zineb, maneb, and mancozeb. [c] Not detected.



ETU (MW=102) PTU (MW=116)

Figure 3. Structures and molecular weights (MW) of imidazolidine-2-thione (ETU) and 4-methylimidazolidine-2-thione (PTU).

tuted for ETU to avoid the routine ETU analysis required to meet the specification in Taiwan of a maximum of 0.5% ETU content, based on active ingredient, in commercial formulations of EBDCs because ETU is a carcinogen (Graham et al., 1973). There is no limitation on PTU, which is a degradation product of propineb.

Propineb Determination. A general survey of the active ingredient and PTU in propineb products was then conducted. Every propineb formulation of different manufacturers sold in Taiwan was collected from market and the active ingredients and degradation products

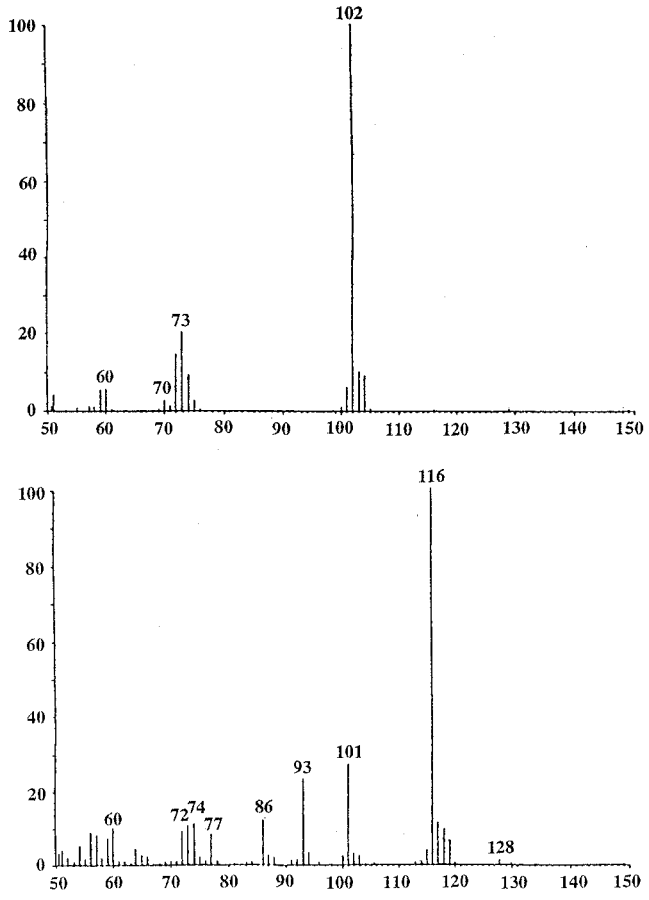


Figure 4. Mass spectra of ETU (MW = 102, above) and PTU (MW = 116, below).

were analyzed by two HPLC methods (233 nm for PTU; 272 nm for methyl derivative of propineb). The data from HPLC analyses showed two facts; first, most of the propineb products were contaminated with ETU, and only one propineb product (sample D) was contaminated

with PTU (Table 2); second, the HPLC retention time of the methylated derivative of sample D was the same as that of the methylated derivative of standard propineb (Figure 2), and the HPLC retention time of other samples after methylation were identical with the retention times of EBDC fungicides (Figure 2). These results proved that Sample D was a real propineb, and other samples were falsely claimed EBDC products.

Zineb, Maneb, and Mancozeb Determination. The falsely claimed EBDC products were then further analyzed by the atomic absorption method, and the results showed that there was no sample of maneb or mancozeb, because there was no Mn detected (or in trace) in these falsely claimed EBDC products (Table 2). All the falsely claimed EBDC products had a Zn content in the range 12.47−21.46%, suggesting that they were zineb products (Scheme 1 and Table 2).

Conclusions. The HPLC methods used here can be applied to distinguish whether commercial propineb products are real propineb products or falsely claimed EBDC products by comparing the retention times of PTU and the methyl ester of propineb. When the commercial product is identified as an EBDC product, then the atomic absorption (AA) method can be applied to determine whether the EBDC product is a zineb product, a maneb product, or a mancozeb product by comparing the total Zn and Mn contents in commercial products. By combination of these two-phase methods (HPLC and AA), the 12 commercial propineb products were identified as 11 zineb products, and one propineb product.

Further research on the use of the HPLC method to quantify the content of active ingredient in formulated EBDCs and propineb fungicides is warranted, because the traditional CS_2 method is not accurate.

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