Comparison of Micellar Electrokinetic Capillary Chromatographic Method with High-Performance Liquid Chromatographic Method for the Determination of Imidazolidine-2-thione (Ethylenethiourea) in Formulated Products

Chi-Chu Lo* and Yi-Ming Hsiao

Pesticide Chemistry Department, Taiwan Agricultural Chemicals and Toxic Substances Research Institute, 11 Kuang Ming Road, Wufeng, Taichung Hsien, Taiwan, Republic of China

A micellar electrokinetic capillary chromatographic (MECC) method was developed for routine analysis of the carcinogenic compound imidazolidine-2-thione (ETU) in commercial ethylenebis-(dithiocarbamate) fungicides (EBDC). The MECC method was compared with a previously developed high-performance liquid chromatographic (HPLC) method. Both methods demonstrated good precision, accuracy, linearity, and sensitivity. The relative standard deviations (RSD) for precision ranged from 0.04 to 5.09% for the MECC method and from 0.47−5.22% for the HPLC method. The recoveries for accuracy ranged from 98.3 to 100.6% for the MECC method and from 99.5 to 104.9% for the HPLC method. No matrix interference was observed in either method. The column efficiency of MECC ($N = 12491$) was 15 times higher than that of HPLC ($N = 813$). The instrument detection limit (IDL) and the method detection limit (MDL) of the MECC method were 0.25 and 0.30 μg/mL, which were 25 and 15 times higher than the IDL (0.01 μg/mL) and the MDL (0.02 μg/mL) of the HPLC method, respectively. The most important advantages of the MECC method over the HPLC method are the shorter run time and the reduction in solvent waste. The total run times for analyzing a sample were 11 min for the MECC method and 15 min for the HPLC method, and the volume of waste solvent for the MECC method (49.0 μL/sample) was 153 times less than that for the HPLC method (7.5 mL/sample). This research has proven that the routine ETU analysis in formulated EBDC products by the MECC method is comparable with traditional the HPLC method and the MECC method is better than the HPLC method if the run time and the cost of solvent are considered.

Keywords: *MECC; HPLC; ETU; sensitivity, column efficiency; precision; accuracy; solvent volume*

INTRODUCTION

Capillary zone electrophoresis (CZE) is an efficient separation technique in which charged solutes are differentially transported through open capillaries under the influence of an applied field (Jorgenson and Lukacs, 1981). In our previous work, the CZE technique has successfully separated the antibiotic fungicides blasticidin S (Lo et al., 1995) and kasugamycin (Lo and Hsiao, 1996). However, CZE is not very effective in separating nonionic compounds. An alternative approach to separate neutral compounds was first developed by the addition of surfactant ions to the mobile phase at concentrations above their critical micelle concentration (cmc) (Terabe et al., 1984). Neutral compounds are then separated on the basis of their differential partitioning between an electroosmotically pumped aqueous mobile phase and the hydrophobic interior of the micelles, which are moving at a velocity different from that of the mobile phase due to electrophoretic effects (Sepaniak and Cole, 1987). This technique was then designated micellar electrokinetic capillary chromatography (MECC) (Burton et al., 1986). MECC separations have primarily employed sodium dodecyl sulfate (SDS) and can provide for the effective separation of neutral compounds (Burton et al., 1987); the neutral compounds must have reasonable solubility in the aqueous mobile phase for effective separation (Sepaniak and Cole, 1987).

Imidazolidine-2-thione (ethylenethiourea; ETU) is a carcinogenic metabolite and degradation product of the ethylenebis(dithiocarbamate) fungicides (EBDC), and a specification of a maximum of 0.5% (w/w) ETU content, based on active ingredient, in commercial formulations of EBDC is mandated in Taiwan. Thus, routine surveys of the ETU in EBDC products by the high-performance liquid chromatography (HPLC) method were conducted in Taiwan (Lo and Ho, 1993). However, there are at least two problems associated with the HPLC system. First, the HPLC method required a large amount of solvent, usually 7.5 mL of mobile phase solvent for one sample, and would produce a large amount of toxic solvent wastes if the sample number increased. Second, the performance of the HPLC column would eventually become unstable when routine ETU analyses were conducted. Therefore, a specific method that can reduce the amount of solvent used in analyzing ETU is needed. A high-performance capillary electrophoresis (HPCE) method was characterized by its low solvent loading (Lo et al., 1995); thus, the research of using the HPCE method with the MECC technique was conducted, because ETU is a neutral, water soluble compound. The sensitivity and the reproducibility of the MECC method compared to the HPLC method are reported.

* Address correspondence to this author at Advisor, Pesticide and Toxic Chemical Control, Chinese Agric. Tech. Team, c/o Ministry of Agric. and Water, Riyadh 11195, Saudi Arabia.

S0021-8561(96)00655-3 CCC: $14.00

Table 1. EBDC Products Purchased from Markets for ETU Analysis

formulation, % w/w	active ingredient
A, 65% WP	zinc *N*,*N*′-ethylenebis(dithiocarbamate) 65%
B, 80% WP	manganese *N*,*N*′-ethylenebis(dithiocarbamate) 80%
C, 37% SC	manganese *N*,*N*′-ethylenebis(dithiocarbamate) 37%
D, 80% WP	manganese ethylenebis(dithiocarbamate) complex with zinc salt 80% [Mg^{2+}, 16%; Zn^{2+}, 2%; ethylenebis(dithiocarbamate) 62%]
E, 33% SC	manganese ethylenebis(dithiocarbamate) complex with zinc salt 33%
F, 70% WP	zinc *N*,*N*′-propylenebis(dithiocarbamate) 70%
G, 58% WP	methyl *N*-(2-methoxyacetyl)-*N*-(2,6-xylyl-DL alaninate) 10% manganese ethylenebis(dithiocarbamate) complex with zinc salt 48%
H, 62.25% WP	2-*p*-(chlorophenyl-2-(1*H*-1,2,4-triazol-1-ylmethyl)hexane nitrile 2.25% manganese ethylenebis(dithiocarbamate) complex with zinc salt 60%
I, 64% WP	2-methoxy-*N*-(2-oxo-1,3-oxazolidin-3-yl)-acet-2′,6′-xylidide 8% manganese ethylenebis(dithiocarbamate) complex with zinc salt 56%
J, 72% WP	1-(2-cyano-2-methoxyiminoacetyl)-3-ethylurea 8% manganese ethylenebis(dithiocarbamate) complex with zinc salt 64%
K, 73% WP	methyl(*N*-phenylacetyl-*N*-2,6-xylyl-DL-alaninate 8% manganese ethylenebis(dithiocarbamate) complex with zinc salt 65%
L, 63% WP	copper oxychloride 43% (Cu 25%) manganese ethylenebis(dithiocarbamate) complex with zinc salt 20%
M, 78% WP	basic copper sulfate 70% (Cu 17.5%) zinc *N*,*N*′-ethylenebis(dithiocarbamate) 4% manganese *N*,*N*′-ethylenebis(dithiocarbamate) 4%

MATERIALS AND METHODS

Standard and Samples. Analytical grade ETU (purity 98%) purchased from Aldrich Chemical Co., Inc. (Madison, WI), was used for the preparation of the analytical working standard solutions. Analytical ETU working standard solutions of 0.02, 0.04, 0.06, 0.08, and 0.10 μg/mL in aqueous solvent of 2% acetonitrile (avoid breathing vapors, may cause skin irritation) were prepared for HPLC calibration curves, and analytical ETU working standard solutions of 0.5, 1.0, 1.5, 2.0, and 2.5 μg/mL in aqueous running buffer solution were prepared for MECC calibration curves.

The aqueous running buffer solution for MECC analysis was composed of 100.0 mM sodium dodecyl sulfate (SDS), 10.0 mM sodium tetraborate solution ($Na_2B_4O_7$), and sodium dihydrogenphosphate solution (Na_2HPO_4) to give pH 9.6. The solutions were filtered through a 0.45-μm nylon filter and degassed before use.

EBDC [*N*,*N*′ethylenebis(dithiocarbamate)] fungicides were purchased from markets in different areas of Taiwan during 1995−1996, and all products had been manufactured within one year before purchase. Sample A was a zineb product [zinc *N*,*N*′-ethylenebis(dithiocarbamate)], samples B and C were maneb products [manganese *N*,*N*′-ethylenebis(dithiocarbamate)], samples D and E were mancozeb products [manganese ethylenebis(dithiocarbamate) complex with zinc salt], sample F was a propineb product [zinc *N*,*N*′-propylenebis(dithiocarbamate)], samples G−L were mancozeb products mixed with metalaxyl [methyl *N*-(2-methoxyacetyl)-*N*-(2,6-xylyl-DL-alaninate), sample G], myclobutanil [2-*p*-(chlorophenyl-2(1*H*-1,2,4-triazol-1-ylmethyl)hexane nitrile), sample H], oxadixyl [2-methoxy-*N*-(2-oxo-1,3-oxazolidin-3-yl)-acet-2′,6′-xylidide, sample I], cymoxanil [1-(2-cyano-2-methoxyiminoacetyl)-3-ethylurea, sample J], benalaxyl [methyl-(*N*-phenylacetyl-*N*-2,6-xylyl-DL-alaninate), sample K], and copper oxychloride (sample L), respectively. Sample M was a product mixed with zineb, maneb, and copper sulfate (Table 1).

Anionic surfactant SDS (purity 99%, GC) from Sigma Chemical Co. (St. Louis, MO) was used as received.

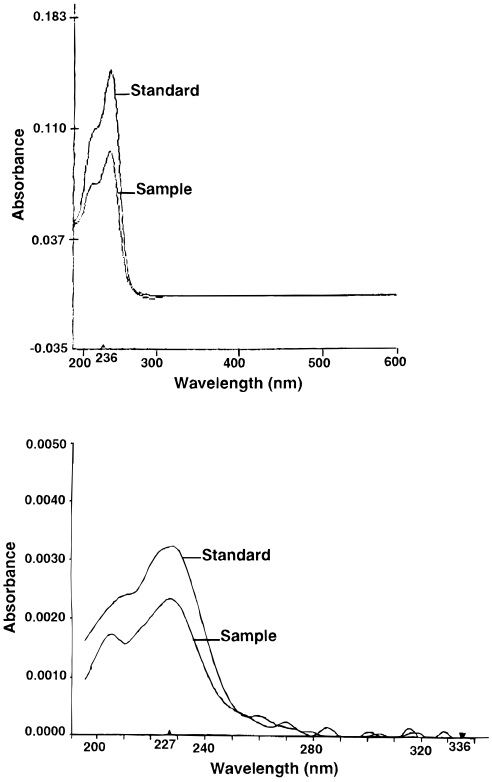

Figure 1. UV spectrum of ETU standard and ETU extracted from formulated samples. Maximum absorption occurred at 236 nm in 2% aqueous acetonitrile solution, and 236 nm was used for ETU determination by the HPLC method (top). Maximum absorption occurred at 227 nm in aqueous running buffer solution, and 225 nm was selected for ETU determination by the MECC method (bottom).

HPLC and MECC Determinations. The HPLC method was conducted on a Beckman HPLC with a Model 126 programmable solvent module, a Model 168 diode array detector operated at 236 nm, and a Model 507 autosampler, and a sample injector valve with a 20-μL sample loop was used to analyze ETU in formulated EBDC products. Separations were achieved on a stainless steel column (250 mm × 3.2 mm i.d.) with Inertsil C_8 (5 μm) operated at room temperature. The mobile phase was water/acetonitrile (98:2, v/v) with a flow rate of 0.5 mL/min, and the volume of waste solvent was estimated about 7.5 mL per sample, because the total run time was set about 15 min.

The MECC method was performed using a Biofocus 3000 automated capillary electrophoretic apparatus. A Biofocus cartridge capillary 148-3040 (50 cm × 50 μm i.d., uncoated) was employed. The column temperature was 20 °C. A regulated dc power supply able to deliver 15 kV was used. The sample was introduced into the capillary vessel using pressure injection mode at 3 psi × s, and the volume of sample introduced into the capillary vessel was calculated to be 4.2 nL (Bio-Rad, 1993; Lo et al., 1995). The elution of a solute was monitored by an on-column UV−vis detector (227 nm) at the negative pole (Figure 1).

Column efficiency is expressed in terms of theoretical plates (*N*) (Jorgenson and Lukacs, 1981; Lo et al., 1995)

$$N = 16(t_r/W)^2 \quad (1)$$

where t_r is the retention time of the peak and *W* is the peak width at a given height (the tangents to the side of the peaks and extrapolatated to the baseline for *W*).

Capillary conditioning between runs was conducted by rinsing with 0.5 M NaOH (1 min), H_2O (1 min), and running buffer (1 min) at 100 psi, and the volume of waste solvent was calculated to be 49 μL per sample, because only rinsing solvent

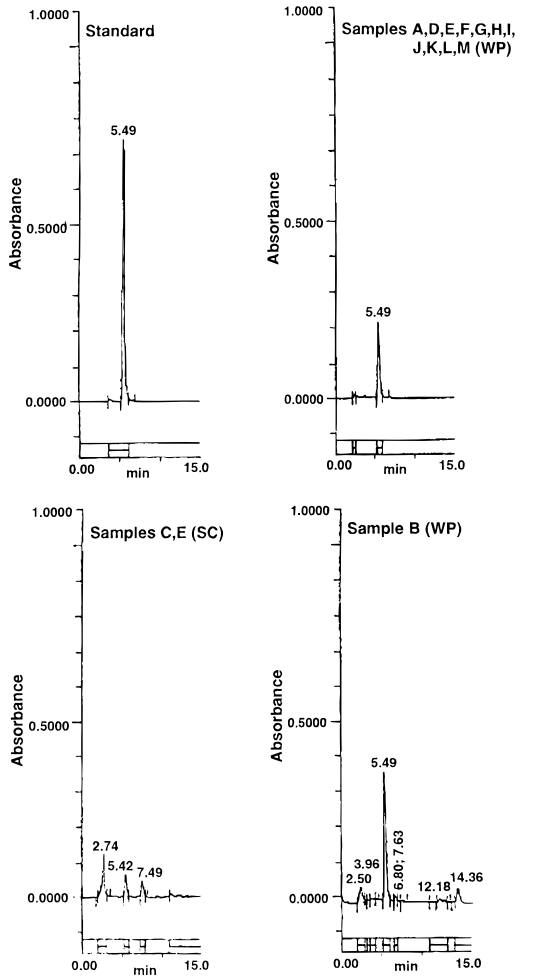

Figure 2. Typical HPLC elution profiles of ETU standard (25 μg/mL) and ETU extracted from formulated products. The retention time of ETU ranged from 5.42 to 5.49 min. Chromatograms of samples A and D−M were similar, and chromatograms of samples C and E were similar. Sample F was a zineb product that was falsely claimed to be a propineb product.

and running buffer were treated as waste solvent in the MECC method. The volume of waste solvent from the MECC method was 153 times less than that from the HPLC method.

The linearity, detection limit, precision (expressed as RSD), and accuracy (expressed as recovery) were used to compare the selectivity, sensitivity, and reliabilities of the HPLC and the MECC methods.

ETU Extraction. A proper amount of sample (0.1 g) was weighed into a 15 mL centrifuge tube. Exactly 6 mL of solvent (2% acetonitrile) was added. The mixture was mixed with a mixer (Thermolyne 37600 Mixer) for 1 min. The extract was centrifuged at 1006*g* for 10 min (Sigma 320). The supernatant was transferred to a 25 mL volumetric flask. The extraction was repeated twice, the supernatants were combined and made up with 2% acetonitrile solution to 25 mL, and a proper aliquot was injected into an autosampler vial through a 0.45 μm nylon syringe filter (Lida Manufacturing Corp.).

Recovery. The recoveries of ETU from formulated products were determined by pipetting a 0.2 mL aliquot of ETU standard solution (1.0 mg/mL of 2% acetonitrile) to a 100 mg portion of each of the formulated products (0.2% w/w). Another 100 mg portion of each of the formulated samples served as a blank. The spiked and unspiked formulated samples were then mixed separately for 1 min and were extracted for ETU analysis.

The percent recoveries were calculated as the difference between the amount of ETU found in the spiked and in the nonspiked samples, expressed as a percentage of the amount of ETU added.

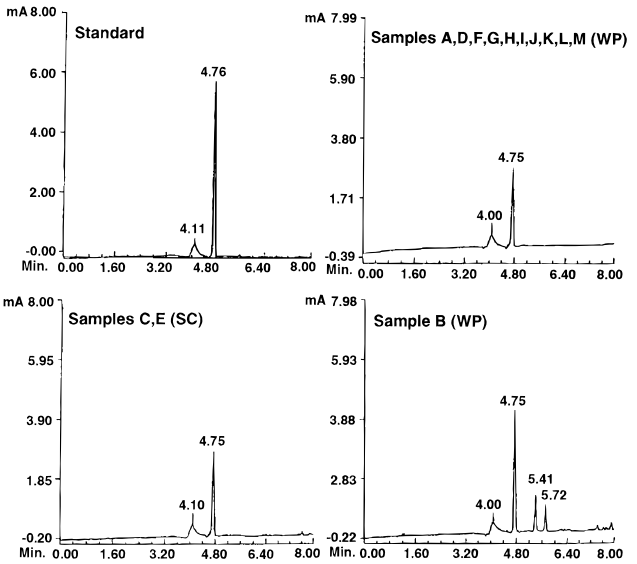

Figure 3. Typical MECC electropherograms of ETU standard (25 μg/mL) and ETU extracted from formulated products. The retention time of ETU ranged from 4.76 to 4.75 min, and the first peak at 4.10 min was a running buffer peak (100 mM SDS). Chromatograms of samples A and D−M were similar, and chromatograms of samples C and E were similar. Sample F was a zineb product that was falsely claimed to be a propineb product.

Limit of Detection. The instrument limit of detection (IDL) was determined by injecting a low concentration of working standard solution to produce a signal that was about 3 times the signal-to-noise ratio (U.S. EPA, 1984). The concentration of working standard solution that corresponds to 5.0 times the IDL is used to determine the method detection limit (MDL). Repeated MECC or HPLC analyses (seven times) produced data for the standard deviation (SD); 3 SD was used as the MDL. Relative standard deviation (RSD) was used to compare the precision of the HPLC and MECC methods. Three replications were conducted in all analyses.

RESULTS AND DISCUSSION

Chromatography of ETU Standard. The UV spectra of the ETU standard in aqueous running buffer solution detected by MECC or in 2% aqueous acetonitrile solution detected by HPLC are shown in Figure 1. Maximum absorption was found at 227 nm for the MECC method, whereas maximum absorption was found at 236 nm for the HPLC method. The shifting of maximum UV absorption was due to the solvent effect. The retention times of the ETU of standard solution or ETU extracted from the formulated products were consistent by the HPLC method (Figure 2). The same consistency was also observed in the ETU electropherograms of the MECC method (Figure 3). The first peak at 4.0 min was a running buffer peak, and this peak could be eliminated by reducing the concentration of SDS in borate−phosphate buffer from 100 to 50 mM (Figure 4).

The analysis of the ETU standard solution from 1.0 to 30.0 μg/mL by the HPLC method showed a good correlation between the concentration (X) and peak area (Y), and the coefficient of determination (r^2) averaged 0.9995. The region of 1.0, 5.0, 10.0, 15.0, 20.0, 25.0, and 30.0 μg/mL was used to calculate the ETU concentration in the formulated products analyzed by the HPLC method. A good linear correlation ($r^2 = 0.997$) between the concentration (X) and peak area (Y) was also found in the MECC method at the concentration of 1.0−30.0 μg/mL. The region of 1.0, 5.0, 10.0, 15.0, 20.0, 25.0, and

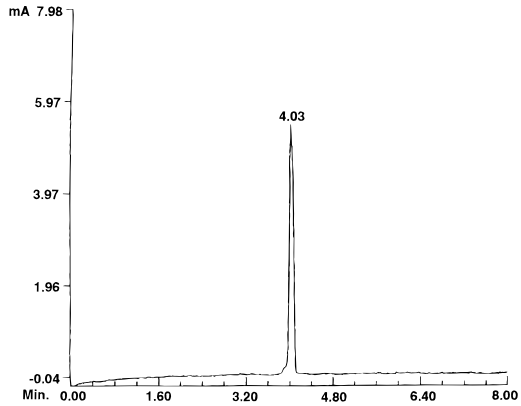

Figure 4. Running buffer peak in the electropherograms of ETU analyses could be eliminated by reducing the SDS concentration from 100 to 50 mM. ETU concentration was 25 μg/mL.

Table 2. Column Efficiency and Detection Limit of the HPLC and MECC Methods

method	column efficiency (N)[a]	IDL (μg/mL)	MDL (μg/mL)
HPLC	813	0.01	0.02
MECC	12491	0.25	0.30

[a] Separation efficiency calculated by $N = 16(t_r/W)^2$.

30.0 μg/mL was used to calculate the ETU concentration in the formulated products analyzed by the MECC method.

Column Efficiency. The column efficiency expressed in terms of theoretical plates (N) was calculated using eq 1 to be 813 for the HPLC column and 12491 for the MECC column. The retention time (t_r) and the peak width (W) of ETU determined by the HPLC method were 5.49 and 0.77 min, and the retention time and the peak width of ETU determined by the MECC method were 4.75 and 0.17 min, respectively. The separation efficiency of the MECC method was better than that of the HPLC method, and the column efficiency of MECC was 15 times greater than that of HPLC.

Sensitivity of the Method. The IDL, defined as 3 times the baseline noise, were estimated at 0.01 μg/mL for the HPLC method and 0.25 μg/mL for the MECC method. The MDL were calculated to be 0.02 μg/mL for the HPLC method and 0.30 μg/mL for the MECC method (Table 2), and the lower detection limit of the HPLC method compared with that of the MECC method was in agreement with previous reports. The MDL of blasticidin S for the MECC method was 0.2 μg/mL (Lo et al., 1995), whereas the MDL for the HPLC method was 0.05 μg/mL (Lo et al., 1996).

ETU Determination. The official specification of a maximum of ETU content, based on active ingredient, in commercial formulations of EBDC is 0.5% (w/w) in Taiwan. Both the HPLC analysis and the MECC analysis showed that the ETU content in all samples was within the official tolerance level (Table 3). However, both the HPLC method and the MECC method indicated that sample F was a falsely claimed propineb product, because sample F was contaminated with ETU, and the degradation compound from authentic propineb product should be PTU (4-methylimidazolidine-2-thione; propylenethiourea); sample F was therefore identified to be a zineb product (Lo et al., 1996).

Precision of the Method. The precision of the analytical method as measured by RSD values in the determination of ETU in commercial formulated products ranged from 0.47 to 5.22% for the HPLC method and from 0.04 to 5.09% for the MECC method (Table 4). All of the RSD values were <10%, indicating that both the HPLC method and the MECC method were excellent (McFarren et al., 1970).

Table 3. Determination of ETU in Formulated Products by the HPLC and MECC Methods

formulation, % w/w	HPLC (%, RSD)	MECC (%, RSD)
A 65% WP	0.069, 2.76	0.069, 050
B 80% WP	0.350, 2.79	0.333, 2.55
C, 37% SC	0.062, 1.69	0.062, 0.25
D, 80% WP	0.075, 4.40	0.076, 0.20
E, 33% SC	0.065, 0.97	0.068, 5.43
F, 70% WP	0.221, 3.04	0.232, 7.04
G, 58% WP	0.119, 1.81	0.122, 0.84
H, 62.25% WP	0.239, 1.68	0.238, 1.15
I, 64% WP	0.207, 1.47	0.206, 2.40
J, 72% WP	0.085, 0.94	0.084, 5.46
K, 73% WP	0.079, 3.53	0.084, 6.44
L, 63% WP	0.037, 0.97	0.038, 0.54
M, 78% WP	0.030, 3.84	0.030, 2.69

Table 4. Recovery of ETU Fortified at 0.2% (w/w) in Formulated Products by the HPLC and MECC Methods

formulation, %, w/w	HPLC (%, RSD)	MECC (%, RSD)
A, 65% WP	100.8, 2.47	99.9, 0.07
B, 80% WP	96.9, 2.73	98.3, 2.91
C, 37% SC	100.3, 0.47	100.1, 0.40
D 80% WP	104.9, 1.31	100.4, 0.07
E, 33% SC	100.4, 2.25	100.2, 3.56
F, 70% WP	103.4, 1.44	101.2, 5.09
G, 58% WP	99.0, 5.22	98.5, 0.21
H, 62.25% WP	98.9, 1.20	99.4, 1.03
I, 64% WP	101.0, 1.47	100.3, 1.72
J, 72% WP	100.8, 3.94	99.9, 2.03
K, 73% WP	101.5, 3.17	98.3, 1.40
L, 63% WP	99.5, 1.23	98.5, 0.44
M, 78% WP	101.8, 4.61	100.6, 1.01
av	100.7, 2.02 (SD) 2.01 (RSD)	99.7, 0.97 (SD) 0.97 (RSD)

Accuracy of the Method. The accuracy of the analytical method was validated by recovery. Commercial samples were fortified with ETU standard, and the recovery of added ETU was analyzed. It was found that the recoveries of ETU ranged from 99.5 to 104.9% for the HPLC method and from 98.3 to 100.6% for the MECC method (Table 4). The average values of recoveries were 100.7% for the HPLC method and 99.7% for the MECC method (Table 4); therefore, there is no major difference between the commercial samples and the analytical methods. The high recoveries of both the HPLC and MECC methods indicated that both methods were accurate.

Conclusion. Both the MECC and HPLC methods offered good precision, accuracy, linearity, and sensitivity. No matrix effect was observed in either method. However, the MDL of the MECC method was higher than the MDL of the HPLC method, but the MECC method provided 15 times higher separation efficiency than the HPLC method. The most important advantages of the MECC method over the HPLC method are the shorter run time and the reduction of toxic solvent waste. First, the solvent waste from the HPLC method was considered to be a toxic waste because it contained acetonitrile, whereas the solvent waste from the MECC method was considered to be a nontoxic waste. Second, the run time can be set to the time required to separate the compounds of interest only, i.e., 5−8 min/sample for

MECC analysis, and all compounds in solution could then be removed from the column by a high-pressure wash for 3 min. Thus, the total run time could be reduced to 8−11 min/sample for the MECC method, compared to 15 min/sample needed for the HPLC method. Lower run cost is another advantage of the MECC method, because the MECC method used much less solvent than the HPLC method did.

This study has proved that routine ETU analysis in formulated EBDC products by the MECC method is comparable with HPLC method and that the MECC method is even better than the HPLC method if the run time and the cost of solvent are considered.

ABBREVIATIONS USED

MECC, micellar electrokinetic capillary chromatography; ETU, imidazolidine-2-thione; PTU, propylenethiourea (4-methylimidazolidine-2-thione); EBDC, ethylenebis(dithiocarbamate); CZE, capillary zone electrophoresis; WP, wettable powder; SC, suspension concentrate.

LITERATURE CITED

Bio-Rad. US/EG Bulletin 1818, Sample stacking to increase detectability in capillary electrophoresis; Bio-Rad: Richmond, CA, 1993.

Burton, D. E.; Sepaniak, M. J.; Maskarinec, M. P. Analysis of B_6 vitamers by micellar electrokinetic capillary chromatography with laser-excited fluorescence detection. *J. Chromatogr. Sci.* **1986**, *24*, 347−351.

Burton, D. E.; Sepaniak, M. J.; Maskarinec, M. P. Evaluation of the use of various surfactants in micellar electrokinetic capillary chromatography. *J. Chromatogr. Sci.* **1987**, *25*, 514−518.

Jorgenson, J. W.; Lukacs, K. D. Zone electrophoresis in open-tubular glass capillaries. *Anal. Chem.* **1981**, *53*, 1298−1302.

Lo, C. C.; Ho, M. H. Determination of imidazolidine-2-thione (ethylenethiourea) in ethylenebisdithiocarbamate formulations. *Pestic. Sci.* **1993**, *37*, 247−251.

Lo, C. C.; Hsiao, Y. M. High-performance capillary electrophoretic method for the determination of antibiotic fungicide kasugamycin in formulated products. *J. Agric. Food Chem.* **1996**, *44*, 2231−2234.

Lo, C. C.; Lee, Y. J.; Chang, C. J. High-performance capillary electrophoretic method for the determination of blasticidin S in formulated products. *J. Agric. Food Chem.* **1995**, *43*, 2892−2895.

Lo, C. C.; Ho, M. H.; Hung, M. D. Use of high-performance liquid chromatographic and atomic absorption methods to distinguish propineb, zineb, maneb, and mancozeb fungicides. *J. Agric. Food Chem.* **1996**, *44*, 2720−2723.

McFarren, E. F.; Lishka, R. J.; Parker, J. H. Criterion for judging acceptability of analytical methods. *Anal. Chem.* **1970**, *42*, 358−365.

Sepaniak, M. J.; Cole, R. O. Column efficiency in micellar electrokinetic capillary chromatography. *Anal. Chem.* **1987**, *59*, 472−476.

Terabe, S.; Otsuka, K.; Ichikawa, K.; Tsuchiya, A.; Ando, T. Electrokinetic separations with micellar solutions and open-tubular capillaries. *Anal. Chem.* **1984**, *56*, 111−113.

U.S. EPA. *Fed. Regist.* **1984**, *49* (No. 209, Oct 26), Part 136.

Received for review September 3, 1996. Revised manuscript received May 5, 1997. Accepted May 15, 1997.[⊗] This study was supported by a grant from the Chinese National Science Council (NSC 86-2113-M-225-001).

JF960655C

[⊗] Abstract published in *Advance ACS Abstracts,* July 1, 1997.