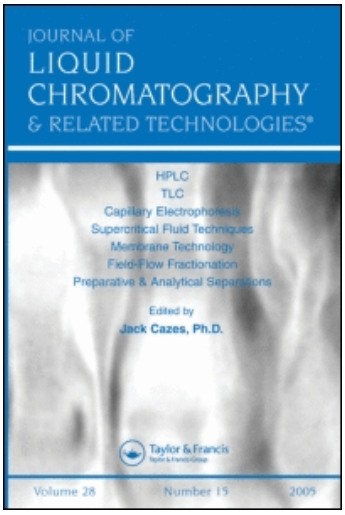

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DETERMINATION OF THIABENDAZOLE IN FRUITS AND VEGETABLES BY CAPILLARY ELECTROPHORESIS

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ABSTRACT

A capillary electrophoresis method was developed to quantify thiabendazole in various fruits and vegetables. Thiabendazole was extracted by adding 20 mL of methylene chloride to a 5 gram sample followed by homogenization and centrifugation. A 5 mL aliquot of the supernatant was removed and evaporated under nitrogen. The residue was reconstituted in 1 mL of HPLC grade water acidified with phosphoric acid (pH 3). Quantitation was performed using UV photodiode detection at 298 nm. Intra-assay and inter-assay reproducibility studies run at concentrations from 0.88 ppm to 20.88 ppm indicated the procedure was reproducible.

A comparison was made between CE and HPLC for thiabendazole. The linear regression was y = 1.0927 x -0.3631 with a correlation coefficient of 0.91. The detection limit is 0.40 ppm.

INTRODUCTION

Thiabendazole (TBZ), a pre- and post-emergence fungicide, (Figure 1) has been used since 1968.[1] It is classified as a benzimidazole and has a solubility in water of 10 g/L, 25°C at pH 2.0.[2] Its high water solubility along with its stability at low pH makes TBZ analysis possible by capillary electrophoresis (CE).

TBZ is used primarily for post harvest treatment of fruits and vegetables to protect from *Fusarium roseum, Collelotrichum musae, Verticullium theobromae, Thielaviopsis paradoxa, Botryodiplodia theobromae, Deightoniella torulosa, and Nigrospora spp.*[3] Control of fungal diseases on fruits and vegetables is imperative to prevent crop loss during storage. Monitoring of pesticide residues on food, especially fruits and vegetables, has become increasingly important with passage of the Food Quality Protection Act of 1996 (FQPA). The tolerance limits set by the EPA for residues of thiabendazole, as of October 26, 1998, are 10 ppm for apples (post-harvest), 10 ppm for citrus fruit (post-harvest), 10 ppm for pears (post-harvest), and 10 ppm for potatoes (pre- and post-harvest).[4] However, these tolerances could change in the future because of the FQPA.

Methods for analyzing TBZ are numerous and include high performance liquid chromatography (HPLC), gas chromatography (GC), and enzyme linked immunosorbent assay (ELISA). Most HPLC methods use fluorescence detection. Bushway et al[5] determined TBZ in fruits, potatoes and their processed products by partitioning TBZ into methylene chloride. This method is very simple and has recoveries ranging from 77 to 135 %. Arenas et al.[6] analyzed TBZ on oranges, grapefruit, tangerines, and lemons. Ethyl acetate was used as the extraction solvent with clean up provided by cation exchange solid phase extraction columns (SPE). Average recoveries were 96 %.

Arenas et al.[7] also developed a method for TBZ in whole green bananas and ripe banana pulp. Hiemstra et al.[8] determined thiabendazole and carbendazim in various crops with SPE clean-up. Samples were extracted with acetone followed by solvent partitioning with dichloromethane-petroleum ether. Diol-bonded SPE cartridges were used for sample cleaning. Fermin et al.[9] uses spectrofluorimetry for TBZ residues in pears.

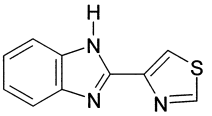

Figure 1. Chemical structure of thiabendazole.

Numerous GC methods have also been developed to determine TBZ residues. Oishi et al.[10] determined TBZ residues in citrus and apple juices. Tanaka et al.[11] determines TBZ as its methyl derivative in orange, lemon, grapefruit, and banana.

Thiabendazole can also be measured by ELISA and numerous papers have been published by this method. ELISA is unique in that it uses no complex or costly instrumentation and can be tested quickly. Bushway et al.[12-14] determined TBZ residues in potatoes, fruit juices, and bulk juice concentrates.

Even though HPLC, GC and ELISA techniques are more sensitive, a detection limit of 0.40 ppm by CE is adequate since the tolerance limits set by the EPA is 10 ppm.

Capillary electrophoresis (CE) is an analytical technique that is useful for the separation of anions, cations, and neutral molecules. Separation is based on the differential movement of charged species by attraction or repulsion in an electric field. Cations migrate towards the cathode while anions migrate towards the anode. Neutral molecules move toward the cathode at the rate of the bulk solution. When using uncoated columns with a pH greater than 5 the movement of bulk solution is faster than the velocity of the anions, which allows all molecules to move toward the cathode where detection occurs.

CE is beginning to be employed in method development for pesticide residues. This paper describes a capillary electrophoresis procedure to quantitate thiabendazole residues in grapefruit, oranges, lemons, pears, apples, and potatoes.

EXPERIMENTAL

Materials

All chemicals used were analytical grade. Sodium phosphate, monobasic, was purchased from Sigma (St. Louis, MO, USA). Methylene chloride (HPLC grade) was bought from Fisher (Fair Lawn, NJ, USA). Phosphoric acid was from Fisher Scientific (Fair Lawn, NJ, USA). Thiabendazole (TBZ), 2-(thiazol-4-yl)benzimibazole (99%), was purchased from (Riedel-de Haën, Germany).

Standard Preparation

A stock standard was prepared by dissolving 25 mg of TBZ into a 25 mL volumetric flask and bringing to volume with ethyl acetate. Working standards were then made from this stock in order to complete the fortification and reproducibility studies.

Sample Extraction

Organic produce was obtained from a local store in Bangor, Maine to conduct spiking and reproducibility studies. The produce tested included potatoes, grapefruits, oranges, lemons, apples, and pears. A sample was placed in a food processor and blended to create a homogenous mixture. A 5 gram sub-sample was placed into a 50 mL conical centrifuge tube. Methylene chloride, 20 mL, was added to the sample and mixed with a polytron (Brinkman Instruments, Westbury, NY) for approximately two minutes. The extract was centrifuged for 10 minutes at 5000 x g. A 5 mL aliquot of the bottom layer (methylene chloride) was placed into a 20-mL glass vial. The sample was evaporated under nitrogen and reconstituted in one milliliter of acidified water (pH 3.0 using phosphoric acid). All samples were filtered with a 0.2 μm filter before adding a 100 μL aliquot to the injection vial.

CE Analysis

Quantitation of thiabendazole was performed on a Hewlett-Packard (Avondale, PA, USA) 3^D CE capillary electrophoretic system equipped with a photodiode array detector with an extended light path capillary. At the beginning of each day the capillary was flushed with new running buffer (20 mM sodium phosphate monobasic pH 7.0) for 10 minutes. It is important to make the buffer up daily and filter prior to use. When the capillary was not being used it was stored in running buffer. Prior to injection the capillary was flushed for two minutes with fresh running buffer.

The wavelength monitored was 298 nm. The capillary column had an i.d. of 75 μm with a bubble factor of 2.7 and a total length of 48.5 cm and effective length of 40 cm.

Rinsing, sample introduction, and separation were all controlled by a HP Vectra XM2 with CHEMSTATION software. Sample introduction into the system was performed hydrodynamically for 5 seconds at 50 mbar. The system was run under normal polarity with a current of 35 μA. Capillary temperature was maintained at 20° C and the sample carousel at 25.8°C. Corrected peak areas were used for all quantitations.

Fortification Studies

To ascertain the percent recovery, grapefruit, oranges, lemons, and potatoes were spiked at levels of 0.88, 1.76, 5.28, 8.80, 14.08, and 19.36 ppm. The apple and pear samples were spiked at 0.93, 1.86, 5.10, 9.28, 13.92, and 20.88 ppm. At each concentration level five separate samples were extracted and analyzed.

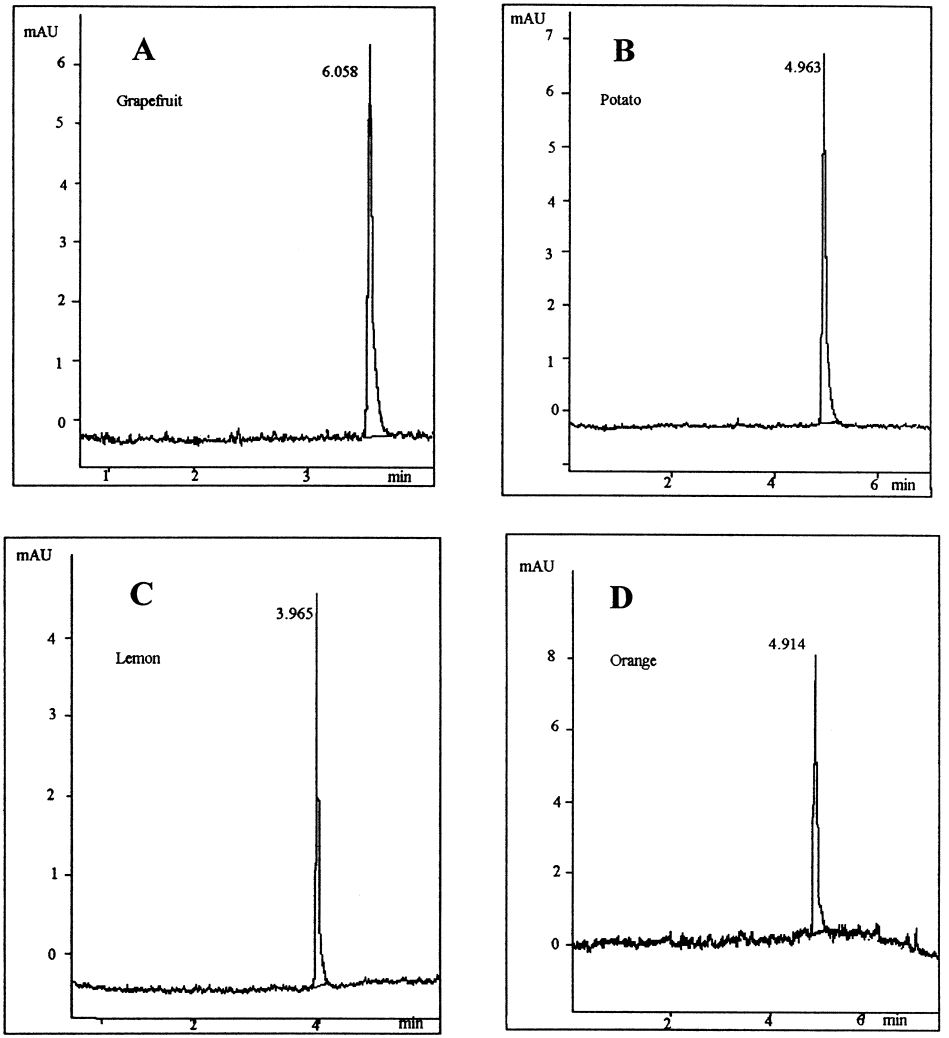

Figure 2. Electropherograms of (A) Grapefruit (B) Potato, (C) Lemon, (D) Orange, at 1.76 ppm.

Reproducibility Studies

To determine within-day reproducibility grapefruit, oranges, lemons, and potatoes were spiked at levels of 0.88, 1.76, 5.28, 8.80, 14.08, and 19.36 ppm. While the apple and pear samples were spiked at 0.93, 1.86, 5.10, 9.28, 13.92,

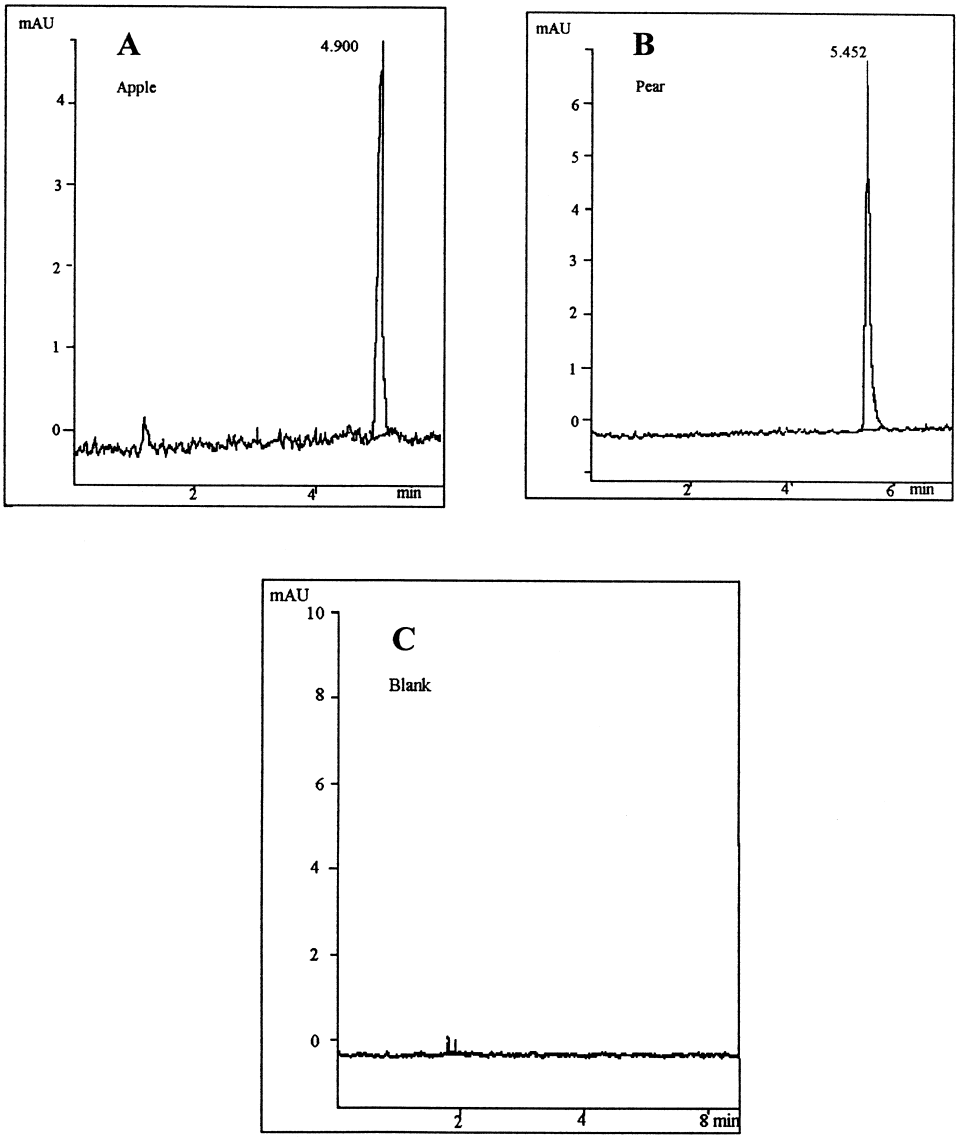

Figure 3. Electropherogram of (A) Apple; (B) Pear at 1.86 ppm. Panel C depicts a blank potato sample. All blank samples had a similar baseline.

and 20.88 ppm. Samples were injected a total of ten times in one day. Between-day reproducibility was determined by preparing samples spiked at each level and analyzed on five separate days.

Linearity Studies

From a stock standard of thiabendazole various working standards were prepared for linearity testing. The concentrations ranged from 0.5 ppm to 25.0 ppm. Thiabendazole was found to be linear when comparing response to corrected peak area.

RESULTS AND DISCUSSION

The Use of Acidified Water to Reconstitute Samples

In any CE method it is preferable to reconstitute samples in water or diluted running buffer to promote sample stacking. When water was used for reconstitution the current at times fluctuated; it was found that when using acidified water (pH 3.0) currents remained stable. Thiabendazole is very soluble in water at pH 2.0. Acidified water will also charge the TBZ molecule. TBZ migrates from the column in less than seven minutes. Other peaks migrate after TBZ creating a clean window for TBZ. However, the migration times did tend to vary. This was most likely due to protein build up along the capillary walls. This was especially observed when potato samples were analyzed. Since there were no other peaks present the migration time fluctuations did not pose a problem. It was found that if a 1.0 minute rinse with 0.1 M NaOH was used prior to flushing the capillary with buffer the migration time fluctuation was minimal. TBZ then migrates in 3.0 minutes decreasing the analysis time a small amount. Figure 2 shows a grapefruit, potato, lemon, and orange sample spiked at 1.76 ppm. Figure 3 shows an apple and pear sample spiked at 1.86 ppm. Figure 3 also shows a blank sample for a potato; the blank looked the same for all the produce tested.

Quantitation

Corrected peak areas were used for all quantitations since they compensate for differences in solute velocities. In CE, solutes of low mobility remain in the detection window for a longer time than solutes with a higher mobility, and will therefore have increased peak areas. To correct for peak areas one divides the integrated peak area by the migration time.

Table 1

Fortification Studies of Thiabendazole by Capillary Electrophoresis

Sample[a]	Spiking Level (ppm)	Recovery (% CV)
Grapefruit	0.88	96 (6.7)
Grapefruit	1.76	91 (14.1)
Grapefruit	5.28	92 (4.8)
Grapefruit	8.80	89 (4.8)
Grapefruit	14.08	91 (3.9)
Grapefruit	19.36	96 (7.3)
Orange	0.88	83 (11.1)
Orange	1.76	90 (8.8)
Orange	5.28	89 (15.2)
Orange	8.80	100 (10.7)
Orange	14.08	97 (13.0)
Orange	19.36	100 (8.5)
Lemon	0.88	53 (12.3)
Lemon	1.76	51 (10.7)
Lemon	5.28	61 (8.8)
Lemon	8.80	56 (15.6)
Lemon	14.08	64 (15.0)
Lemon	19.36	61 (16.4)
Potato	0.88	86 (21.2)
Potato	1.76	98 (17.2)
Potato	5.28	94 (4.6)
Potato	8.80	95 (8.9)
Potato	14.08	93 (10.5)
Potato	19.36	90 (9.5)
Apple	0.93	66 (11.4)
Apple	1.86	66 (5.8)
Apple	5.10	66 (8.0)
Apple	9.28	75 (9.6)
Apple	13.92	73 (14.6)
Apple	20.88	65 (9.7)
Pear	0.93	73 (7.9)
Pear	1.86	83 (11.5)
Pear	5.10	78 (4.7)
Pear	9.28	80 (3.5)
Pear	13.92	71 (14.8)
Pear	20.88	80 (6.1)

[a] Each spiking level was extracted five separate times for each fruit or vegetable.

Table 2

Reproducibility of Thiabendazole by Capillary Electrophoresis

Sample	Spiking Level (ppm)	Intra-Assay[a] (% CV)	Inter-Assay[b] (% CV)
Grapefruit	0.88	3.4	9.6
Grapefruit	1.76	15.4	7.6
Grapefruit	5.28	1.7	12.3
Grapefruit	8.80	2.0	5.6
Grapefruit	14.08	2.7	8.1
Grapefruit	19.36	1.3	6.6
Orange	0.88	5.6	8.9
Orange	1.76	6.1	11.2
Orange	5.28	4.3	4.1
Orange	8.80	2.3	4.7
Orange	14.08	0.8	8.3
Orange	19.36	1.5	7.5
Lemon	0.88	4.5	9.0
Lemon	1.76	2.1	15.2
Lemon	5.28	1.8	7.4
Lemon	8.80	1.6	8.3
Lemon	14.08	0.8	7.1
Lemon	19.36	1.0	12.5
Potato	0.88	4.9	6.6
Potato	1.76	1.9	4.4
Potato	5.28	3.5	5.4
Potato	8.80	2.4	3.8
Potato	14.08	1.8	3.1
Potato	19.36	1.3	3.3
Apple	0.93	4.9	13.5
Apple	1.86	2.4	5.7
Apple	5.10	1.3	7.5
Apple	9.28	1.3	12.6
Apple	13.92	1.1	5.9
Apple	20.88	1.4	5.9
Pear	0.93	2.2	14.3
Pear	1.86	1.6	8.9
Pear	5.10	1.8	8.5
Pear	9.28	0.05	6.5
Pear	13.92	0.06	9.3
Pear	20.88	1.1	8.0

[a] Intra-assay6 % CVs based on ten determinations in one day. [b] Inter-assay % CVs based on determinatins performed on five different days.

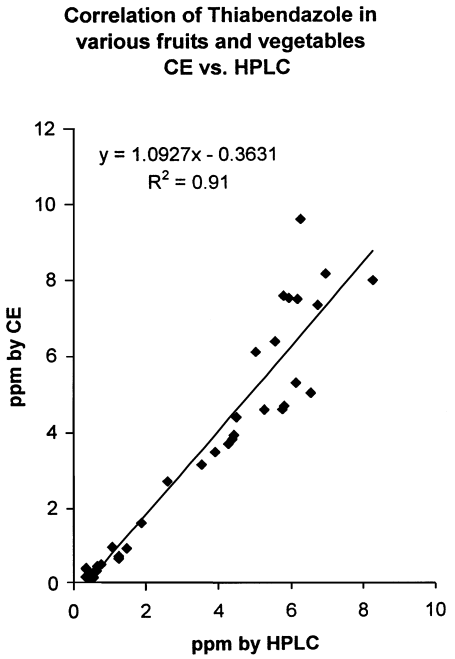

Figure 4. Correlation of all six fruits and vegetables analyzed by high performance liquid chromatography and capillary electrophoresis.

Fortification Studies

Table 1 summarizes the results of fortifying grapefruit, oranges, lemons, and potatoes at 0.88, 1.76, 5.28, 8.80, 14.08, and 19.36 ppm. Apples and pears were fortified at 0.93, 1.86, 5.10, 9.28, 13.92, and 20.88 ppm. Percent recoveries ranged from 89 to 96 % for grapefruit, 83 to 100 for oranges, 51 to 64 for lemons, 86 to 98 for potatoes, 65 to 73 for apples, and 71 to 83 for pears. All percent recoveries are adequate but the lemons had the lowest. Recoveries for lemon and apples are low compared to the other four but they are adequate since they are above 50%.

Reproducibility Studies

Table 2 shows results for within-day and between-day reproducibility. For intra-assay results the % CVs ranged from 0.05 to 15.4 % for samples spiked at each concentration level. The majority of CVs were below 5 %. Inter-assay % CVs ranged from 3.1 to 15.2% and tended to be higher than within-day assays.

Correlation Studies

The newly developed CE method was compared to an established HPLC procedure[5] Thirty-seven samples of produce, comprised of a mixture of all six kinds, were analyzed by both techniques. The 37 samples ranged in TBZ concentration from 0.8 to 9.0 ppm and the linear regression analysis yielded the equation y = 1.0927 x -0.3631 with a pearsons correlation coefficient of 0.91 (Figure 4). Linear regression results indicate that the CE method had a slight low bias.

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