Published in J. Agric. Food Chem., December, 1994 ,pp. 2817-2823, by the American Chemical Society

Supercritical Fluid Extraction and HPLC Analysis of Benzimidazole Fungicides in Potato, Apple, and Banana

Nadav Aharonson,† Steven J. Lehotay,* and Medina A. Ibrahim

Beltsville Agricultural Research Center, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland 20705

An analytical method employing supercritical fluid extraction (SFE) of the benzimidazole fungicides thiabendazole, benomyl/carbendazim, and thiophanate-methyl was developed and tested with potato, banana, and apple matrices. Ion-pairing HPLC coupled with UV absorbance or fluorescence detection was used for analysis. Sample preparation involved shredding a 100 g sample, mixing a 3 g subsample with 2 g of Hydromatrix, and packing a 10 mL vessel. Optimal SFE conditions were determined by varying the amount, density, and flow rate of CO_2, extraction temperature, type and amount of solvent modifier, and trapping/elution conditions of the extracted pesticides. Optimal conditions for extraction were obtained at 400 atm, 60 °C, 90 g of CO_2, 3% methanol modifier, and 2 mL/min flow rate. The extracts were collected on a 1 mL C_{18} trap at 25 °C and eluted with 10 mL of methanol. Potato, apple, and banana extracts were analyzed by HPLC without additional cleanup. Recoveries were 90 ± 12% for the benzimidazole fungicides in fortified samples. Incurred potato and banana samples were also analyzed.

Keywords: *Supercritical fluid extraction (SFE); residue analysis; benzimidazole fungicides; fruits and vegetables*

INTRODUCTION

Supercritical fluid extraction (SFE) is slowly being recognized as a potential alternative to the "classical" solvent-based extraction and cleanup methods. The application of SFE to pesticide residue analysis has been demonstrated in recent years for a number of pesticides in various sample matrices, such as soil (J. L. Snyder *et al.*, 1993), grain (King *et al.*, 1993a,b; Skopec *et al.*, 1993), and meat (Cross *et al.*, 1993; Maxwell *et al.*, 1992; France and King, 1991). Many of the previous studies have focused on relatively nonpolar pesticides which exhibit higher solubility in CO_2 (J. M. Snyder *et al.*, 1993). Less information is available on SFE for residue analysis of more polar compounds (Cross *et al.*, 1993; Messer and Taylor, 1992), and for the extraction of fruits and vegetables (Howard *et al.*, 1993; Wigfield and Lanouette, 1993; Lehotay and Ibrahim, 1994).

Compared with traditional solvent-based methods of extraction (Luke and Masumoto, 1986), SFE obviates the use of organic solvents, enables automation, generates little waste, and reduces time, space, and glassware required for extraction (Hawthorne, 1990). An additional advantage is that SFE is not an exhaustive extraction, and when coupled with collection on solid-phase sorbents, extraction and cleanup of the sample occur in a single step. In a previous study, Lehotay and Ibrahim (1994) demonstrated that the extraction of various vegetables by SFE was clean enough for direct injection to GC/MS. The selection of the appropriate SFE conditions such as CO_2 density, temperature, modifier, type of solid phase used for trapping the analytes, and elution solvent can be manipulated to overcome most chromatographic interferences. However, not all pesticides can be analyzed by GC methods, and for multiresidue extraction approaches, other detection methods should be considered.

The benzimidazole fungicides thiabendazole (TBZ), benomyl, carbendazim (MBC), and thiophanate-methyl were the pesticides chosen for this study. Benomyl converts to MBC before or during analysis, and usually both are detected as total MBC. Benomyl/MBC and thiophanate-methyl cannot be analyzed by GC methods, and thiabendazole has reduced sensitivity using GC. UV absorbance and fluorescence are the common detection methods for these compounds (Aharonson and Ben-Aziz, 1973; Gilvydis and Walters, 1990), and immunoassay techniques offer an alternative approach (Brandon *et al.*, 1992; Newsome and Collins, 1987). The group of pesticides chosen for this study represents the most commonly used benzimidazole fungicides which are being applied as preharvest and postharvest treatment for many crops (*Farm Chemicals Handbook '92,* 1992). Regulatory tolerance levels are 7.0 and 1.0 μg/g for benomyl/MBC, 10.0 and 0.4 μg/g for TBZ, and 7.0 and 0.2 μg/g for thiophanate-methyl in apple and banana, respectively, and 10.0 μg/g for TBZ in potato (*Code of Federal Regulations*, 1991). Regulatory laboratories detected benomyl/MBC in 10.5% of analyzed apples, and TBZ was found in 26% of bananas, 10% of potatoes, 51% of apples, and 40% of oranges and grapefruit analyzed, albeit below regulatory tolerance levels (Agricultural Marketing Service, 1993).

The goal of this study, which is part of an effort in this Laboratory to develop a multiresidue method employing SFE, was to evaluate SFE for the extraction and cleanup of the benzimidazole fungicides TBZ, benomyl/MBC, and thiophanate-methyl in potatoes, apples, and bananas. A second objective was to evaluate SFE for the extraction of pesticides that are difficult to analyze or cannot be analyzed by GC and require HPLC for quantitation.

* Author to whom correspondence should be addressed (e-mail SLEHOTAY@asrr.arsusda.gov).

† On sabbatical from Agricultural Research Organization, Volcani Center, Bet Dagan 50250, Israel.

MATERIALS AND METHODS

SFE. A Prepmaster (Suprex, Pittsburgh, PA), equipped with a solvent-modifier pump, automated variable restrictor, and Accutrap solid-sorbent collection system, and a 7680T module SFE (Hewlett-Packard, Avondale, PA), equipped with a 1050 series solvent-modifier pump, automated variable restrictor, and solid-sorbent collection, were used in this study.

HPLC. The HPLC system consisted of two Model 510 pumps and gradient controller (Waters, Milford, MA), ISS-100 autosampler (Perkin-Elmer, Norwark, CT), Model 490 programmable multiwavelength detector, (Waters), Model 1046A programmable fluorescence detector and 3365 Series II Chemstation (Hewlett-Packard). The HPLC column was an Ultrasphere ODS, 5 μm particle size, 4.6 mm i.d. × 250 mm (Beckman, Fullerton, CA). For confirmation of the identity of the extracted fungicides, SFE sample extracts were analyzed with a 5989A mass spectrometer equipped with a 59980B particle beam LC/MS interface and a 1050 Series HPLC (Hewlett-Packard). Conditions consisted of an injection volume of 20 μL, a flow rate of 0.5 mL/min, a methanol mobile phase, and 250−275 °C ion source temperature.

Chemicals. CO_2 used for the Suprex SFE was of SFC/SFE grade supplied with 1800 psi He headspace and dip tube (Air Products, Allentown, PA), and SFC/SFE grade CO_2 (no He headspace) with dip tube (MG Industries, Valley Forge, PA) was used for extractions with the Hewlett-Packard SFE. Hydromatrix (Varian, Harbor City, CA), a diatomaceous earth material described previously for use in SFE (Hopper and King, 1991), was used to absorb water from the sample. All solvents used were of pesticide or HPLC grade (Fisher, Fair Lawn, NJ). The HPLC ion-pairing mobile phase, which was prepared according to the procedure given in Gilvydis and Walters (1990), consisted of 1 g of decanesulfonate sodium salt, 10 mL of triethylamine (Aldrich, Milwaukee, WI), and 7 mL of phosphoric acid (Fisher), diluted to 1000 mL with water purified by the Nanopure system (Barnstead, Boston, MA). The pesticide standards, thiabendazole (TBZ) or 2-(4′-thiazolyl)-benzimidazole, benomyl or methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate, and carbendazim or methyl 2-benzimidazolecarbamate (MBC), and thiophanate-methyl or 4,4′-*o*-phenylenebis[3-thioallophanate] were obtained from the U.S. Environmental Protection Agency (Beltsville, MD) with purity >98%. For comparison, 100 μg/mL pesticide standard solutions in methanol were obtained from Accu-Standard, Inc. (New Haven, CT).

Sample Preparation. Potatoes, apples, and bananas were purchased at a local store to serve as blanks or fortified samples for recovery studies. Incurred potato and banana were provided by the State of Texas Department of Agriculture and by the State of Florida Department of Agriculture. These samples were previously extracted according to the procedure of Luke and Masumoto (1986) and analyzed by GC/electrolytic conductivity detection (ELCD). For SFE, a 100 g portion of the fruit or vegetable was shredded and mixed using a food processor. A 3 g subsample for the Suprex SFE or 2.1 g subsample for the Hewlett-Packard SFE was mixed with a glass rod in a beaker with 2 or 1.4 g of Hydromatrix, respectively. This mixture was packed into a 10 (Suprex) or 7 mL (Hewlett-Packard) extraction vessel. The spiking solution was added to the middle of the vessel and solvent allowed to evaporate for 15 min. Also, spiking was carried out on potato external to the vessel before mixing with Hydromatrix. Spiking levels varied between 0.025 and 1 μg/g per sample, and four replicates were extracted and analyzed for each experiment. For the TBZ-incurred samples, which arrived precut and frozen, the vessels were loaded with the frozen sample mixed with the same ratio of Hydromatrix, and half of the samples were spiked with 0.5 μg/g MBC as an internal standard in the vessel. The 10 mL SFE extract in methanol was concentrated to the desired volume (1−3 mL) under a stream of nitrogen and analyzed by HPLC.

Extraction. Instrumental parameters, which were varied individually to determine optimal extraction conditions, consisted of the following: CO_2 extraction fluid with or without solvent modification; 10 (Suprex) or 7 mL (Hewlett-Packard) extraction vessels; 160−510 atm extraction pressures; temperatures ranging from 45 to 75 °C; CO_2 flow rates of 1.5 to 2.5 mL/min; extraction amounts of 15, 60, 75, 90, 100, and 150 g of CO_2; and collection of analytes on glass beads or C_{18} solid phase at 10 or 25 °C trap temperature. Fixed parameters were 50 °C restrictor temperature and 2 min static extraction prior to the dynamic step, and methanol was used for eluting the analytes from the trap. Ultimately, the chosen extraction conditions for the Suprex instrument were as follows: 400 atm; 60 °C, 2 min static time, 90 g CO_2 dynamic step at 2 mL/min flow rate; C_{18} collection; and 10 mL methanol flush at 1 mL/min and 25 °C. For the Hewlett-Packard SFE, optimal extraction conditions were as follows: 350 atm; 50 °C; 2 min static time; 2.5 mL/min CO_2 flow rate; 50 g of CO_2, and collection on a C_{18} trap at 20 °C. Flushing the analyte from the trap was done at 55 °C and 0.5 mL/min in three 1.6 mL fractions of methanol into 1.8 mL vials. In some experiments, TBZ was dispersed into three vials, whereas MBC always appeared in the first fraction.

Analysis. HPLC determination was based on the method described by Gilvydis and Walters (1990). The fluorescence detector was connected in tandem, following the UV detector. The UV detector was set at 285 nm, and for TBZ, the fluorescence detector was set at 305 nm excitation and 345 nm emission. For MBC, the fluorescence detector was set at 282 nm excitation and 307 nm emission. At the latter setting, TBZ and MBC were detected, but the TBZ response was less than optimal. The mobile phase was 55/45 ion-pairing solution/methanol. For optimal quantitation and stability of the fluorescence detector, the ion-pairing solution was recycled for 24 h prior to analysis. Flow rate of the mobile phase was 0.8 mL/min (typical pump pressure of 2600 psi). The same standard solution that was used for spiking the sample was diluted to make calibration standards of 0.25, 0.5, 1, and 2 μg/mL MBC and TBZ in methanol. Once it was determined that the calibration curves were consistent over several weeks using UV detection (relative standard deviation of 3%), the 0.5 and 1 μg/mL standards were analyzed in duplicate with each sequence of samples.

RESULTS AND DISCUSSION

Optimization of SFE conditions for the extraction of MBC and TBZ from fruits and vegetables was evaluated by recovery studies of potatoes fortified at concentrations of 1 μg/g MBC and 0.5 μg/g TBZ. Matrix interferences in HPLC were negligible at these levels when analyzed by UV and fluorescence detection.

CO_2 Density and Extraction Temperature. The objective of this experiment was to determine the effect

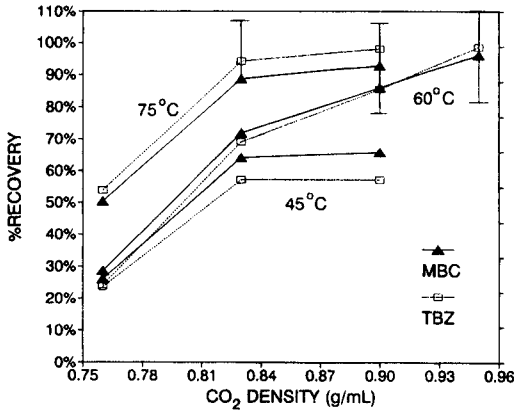

Figure 1. Effect of CO_2 density and extraction temperature on the SFE recoveries of TBZ and MBC from potato fortified at 0.5 and 1.0 μg/g, respectively.

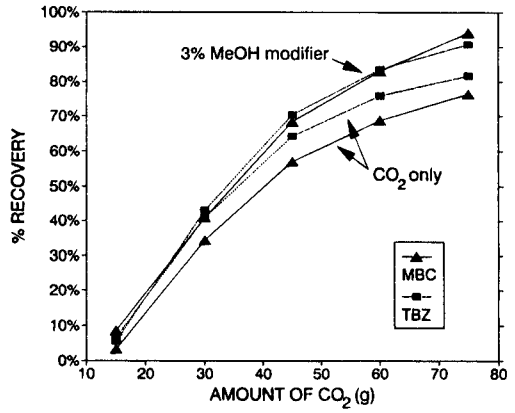

Figure 2. Effect of the amount of CO_2 used in SFE on the recoveries of MBC and TBZ from fortified potato. Extractions were performed with 3% methanol modifier and without modifier.

of CO_2 density and extraction temperature on the extraction efficiency (measured as percent recovery) of MBC and TBZ. Extraction temperatures of 45, 60, and 75 °C were chosen, and pressures corresponding to 0.76, 0.83, 0.90, and 0.95 g/mL were set at the chosen temperatures. Extraction CO_2 volume was 60 g in all cases with the 10 mL extraction vessel. As shown in Figure 1, two trends were observed. An increased density of CO_2 at the same temperature led to increased recoveries, and increased temperature at the same density also increased recoveries. As mentioned previously, matrix interferences were not significant at the 1 μg/g spiking level; however, with the increase in density or temperature at the lower spiking levels, a subsequent increase in the amount of interfering coextractives in the potato became more prominent. For that reason, moderate conditions of 400 atm and 60 °C (0.90 g/mL CO_2) were chosen for further experiments. The error bars shown in Figure 1 for MBC at the higher recoveries were typical for all of the results in this experiment. Standard deviations of more than 15–20% indicated that the conditions were not optimal, and further experiments, as described below, were needed.

Amount of CO_2. Figure 2 presents the recoveries of MBC and TBZ versus the amount of CO_2 used in the extraction. This experiment was carried out under identical conditions as in the previous study, except conditions were set at 400 atm and 60 °C, and CO_2 extraction volume was varied. The results were obtained by stopping the extraction every 15 g of CO_2 without releasing the pressure and eluting the analytes

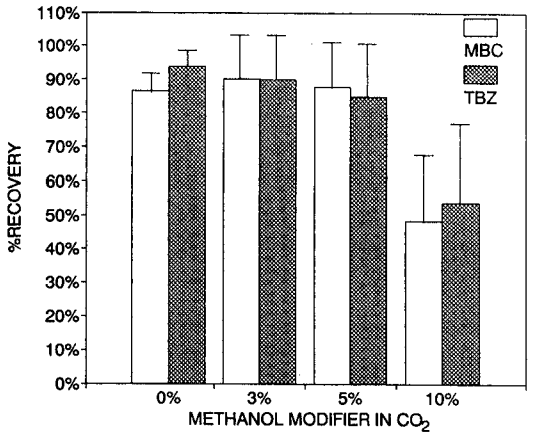

Figure 3. Effect of percent methanol modifier used in SFE on the recoveries of MBC and TBZ from fortified potatoes.

after each step from the C_{18} trap with 10 mL of methanol. The results showed that 75 g of CO_2 was required for 90 ± 12% recoveries of MBC and TBZ. The reproducibility with 75 g of CO_2 was improved when compared to the results shown in Figure 1 (60 g of CO_2 was used in that experiment). Maximum recovery could be achieved with more CO_2 but at the expense of additional time and CO_2 for extraction.

Effect of Modifier. The addition of a solvent modifier to CO_2 is intended to improve the extraction of more polar compounds (Hawthorne, 1990). On the other hand, a modifier may lead to increased co-extractives which may interfere with the analysis. Therefore, this experiment was designed to evaluate the effect and the need for the addition of a modifier to the CO_2. Figure 3 shows that the addition of 3% or 5% methanol as modifier did not significantly improve the extraction efficiency using 100 g of CO_2, and that, for practical purposes, these fungicides could be extracted without modifier. However, as shown in Figure 2, the use of 3% methanol modifier appeared to slightly improve recoveries, but the difference was within the standard deviations of the measurements. A higher concentration of methanol (10%), when 100 g of CO_2 was passed through the 10 mL vessel, resulted in a decreased recovery of MBC and TBZ, probably due to oversaturation of the system with methanol. Acetone and ethyl acetate were also examined as potential modifiers, and the results were similar to those shown for 3% and 5% methanol but methanol gave fewer matrix interferences in HPLC/UV chromatograms.

Effect of Moisture. If nothing was done to remove water from the samples, high moisture content presented difficulties with water carry-over into the restrictor. Hydromatrix, as noted previously (Hopper and King, 1991), was added to absorb the water in the sample. Table 1 summarizes the recoveries of TBZ and MBC from potato that was mixed with different amounts of Hydromatrix. At a ratio of higher than 3 g of Hydromatrix to 1 g of potato (3:1), no MBC and TBZ were extracted because the sample was too dry. At the ratios of 1.5:1 to 0.5:1 the recoveries were essentially the same. A Hydromatrix:water content in the sample of 1:1 was used for further extractions to avoid any chance of clogging the restrictor. Although the use of modifiers in the CO_2 gave small differences in recoveries (Figures 2 and 3), the effect of water in the vessel was dramatic (Table 1). In effect, water in the matrix acted as a modifier of the supercritical CO_2.

Fortified Samples. Table 2 summarizes the recoveries obtained for MBC and TBZ in fortified potatoes,

Table 1. Effect of Moisture Content Governed by the Addition of Hydromatrix on MBC and TBZ Recoveries from Potato

	Hydromatrix:potato ratio[a] (w/w)									
	3:1		1.5:1		1:1		0.67:1		0.5:1[b]	
	MBC	TBZ	MBC	TBZ	MBC	TBZ	MBC	TBZ	MBC	TBZ
% recovery	0	0	84	78	86	78	90	90	91	85
SD	NA[c]	NA	10	10	7	8	12	13	7	9

[a] Higher Hydromatrix:potato ratio corresponds to a drier sample. [b] Restrictor clogging developed at higher moisture content. [c] NA, not applicable.

Table 2. SFE Recoveries of MBC and TBZ from Potato, Apple, and Banana Fortified at Different Concentrations (Analyzed by HPLC/UV Detection)

commodity, spiking level (μg/g)	no. of replicates	% recovery ± SD	
		MBC	TBZ
potato, 1.0	30	91 ± 12	90 ± 13
0.5	20	94 ± 14	91 ± 11
0.1	8	91 ± 10	83 ± 12
0.05	6	81 ± 19	84 ± 16
apple, 1.0	4	81 ± 5	–[a]
0.5	4	–[a]	78 ± 7
banana, 1.0	6	85 ± 6	–[a]
0.5	6	–[a]	78 ± 10

[a] The apple and banana samples were spiked with twice as much MBC as TBZ.

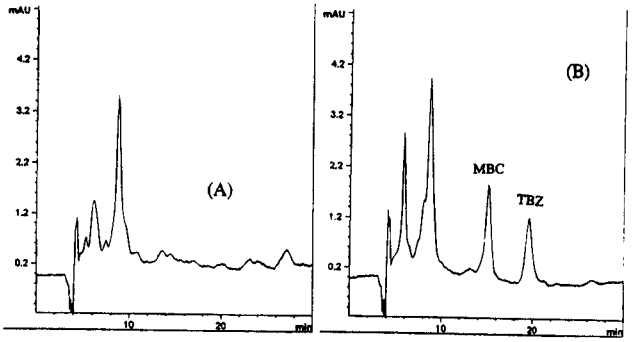

Figure 4. HPLC/UV chromatograms of untreated SFE potato extracts: (A) blank potato; (B) potato fortified with 0.5 μg/g MBC and 0.25 μg/g TBZ.

apples, and bananas at different spiking levels (1.0, 0.5, 0.1, and 0.05 μg/g). Extractions carried out at 400 atm, 60 °C, and 90 g of CO_2 resulted in recoveries of 91 ± 12% for MBC, at the 1 μg/g level, and 90 ± 13% for TBZ. These results were calculated from 30 extractions in 8 separate experiments at the same conditions. Fewer extractions were done at lower spiking levels and with apple and banana. At lower concentration, matrix interferences in UV detection affected quantitation (as seen in the chromatograms in Figure 4). There was no need for additional cleanup of the extract obtained from SFE. The analytes were collected on either glass beads or C_{18} at 10 °C or 20 °C, respectively, eluted with 10 mL methanol, and concentrated to the desired volume (1–3 mL), and 30 μL was injected for HPLC. No serious matrix interferences were observed with analysis by UV detection at 285 nm at concentrations above 0.1 μg/mL MBC and 0.05 μg/mL TBZ. Figure 4 shows a typical HPLC chromatogram of an untreated control and fortified potatoes at 0.5 μg/g MBC and 0.25 μg/g of TBZ. Similar chromatograms were obtained from SFE extracts collected on either glass beads (80–100 mesh) or C_{18}. Under those conditions, the C_{18} trap did not contribute much to the cleanup of the extracted sample. With glass beads, some losses of analytes were observed and the trap was cooled to 10 °C. Several other potential sorbents and solvents are available which could be used in the trap for additional cleanup at the lower levels, but this aspect has not yet been fully investigated.

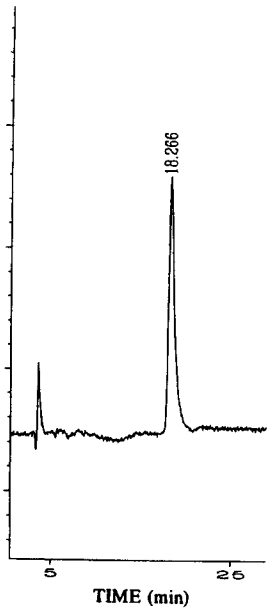

Figure 5. HPLC/fluorescence chromatogram of untreated SFE extract of potato fortified with 0.1 μg/g TBZ.

On the basis of the results presented in Figure 4, the limits of detection (LODs) in potato for MBC and TBZ by HPLC/UV detection were calculated as 0.041 and 0.024 μg/g, respectively. Calculation of LODs was made by taking three fifths of the peak-to-peak noise at the retention time of a blank potato chromatogram and dividing by the slope of a calibration curve using peak heights of the standards.

HPLC/Fluorescence Analysis. Determination of sample extracts by HPLC coupled to a fluorescence detector enabled detection of TBZ at much lower concentrations without the need for any additional cleanup. Figure 5 shows a chromatogram of TBZ at 0.1 μg/g concentration in a potato extract analyzed at 305 nm excitation and 345 nm emission. LOD was 0.0012 μg/g TBZ in potato using the fluorescence detector at these conditions. The only problem encountered was related to the effect of the ion-pairing mobile phase on the fluorescence signal of TBZ. This problem was solved by recycling the mobile phase for 24 h prior to the analysis. Determination of MBC with the fluorescence detector at 282 nm excitation and 307 nm emission did not improve the sensitivity of MBC versus the UV detection. However, fewer interfering peaks occurred with fluorescence. At these wavelengths, TBZ could be detected together with MBC but at reduced sensitivity than obtained at 305 nm excitation and 345 nm emission. The use of the two detectors in tandem was very helpful in overcoming matrix interferences at the 0.05–0.1 μg/g level of MBC. Higher MBC response by

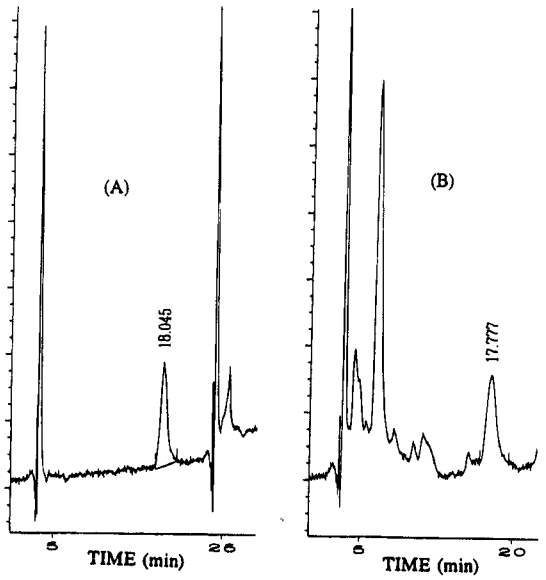

Figure 6. HPLC/UV chromatograms of (A) 1.0 μg/mL thiophanate-methyl standard and (B) untreated SFE extract of potato fortified with 1.0 μg/g thiophanate-methyl. The values above the peaks refer to retention time. Peak area for the standard is 1707 and for the sample 1590 (93% recovery).

Table 3. Reproducibility of SFE Recoveries of MBC and TBZ in Fortified Potato during the Same Day and between Different Days[a]

commodity, set	% recovery ± SD	
	MBC	TBZ
potato, set 1	97.8 ± 9.7	88.1 ± 9.4
set 2	88.7 ± 1.2	85.5 ± 6.4
set 3	82.7 ± 3.5	72.3 ± 4.2
set 4	87.8 ± 3.3	82.8 ± 5.8

[a] Spiking level was 1 ppm, and each set consisted of four replicates extracted on the same day. Analysis was by HPLC/UV detection.

fluorescence could be obtained without the use of the ion-pairing reagent. When using just a methanol/water mobile phase, the peak of MBC was similar to the peak obtained with ion-pairing HPLC, but TBZ gave a much broader peak. Therefore, if the detection limit of MBC must be lowered, a methanol/water mobile phase with fluorescence detection could be used.

Extraction and Analysis of Thiophanate-Methyl. The recovery of thiophanate-methyl from potato using the SFE procedure on the HP SFE at 1 μg/g spiking level was higher than 90% (Figure 6). The LOD of thiophanate-methyl was calculated as 0.3 μg/g in the sample using HPLC/UV detection at 285 nm. It was demonstrated that this fungicide did not decompose during SFE at 300 atm and 50 °C. This fact was confirmed by injection of the extract on HPLC/MS (Figure 7).

Day-to-Day Fluctuations. From a large number of experiments using the Suprex SFE, it became clear that fluctuation of the results was lower within a set of extractions performed on the same or adjacent days, whereas larger deviations occurred over the course of several days. Table 3 presents an example of typical results of four sets of data. The standard deviations were on average 5% within the same set and typically 12% between sets. The HPLC/UV gave less than 3% RSD for the standards analyzed over a period of weeks. Therefore, the fluctuations in SFE recoveries were not due to HPLC quantitation error but a result of SFE considerations. The definitive reason for the problem is not clear, but possible instrumental factors include

Table 4. Comparison of the Analytical Results for TBZ in the Incurred Potato and Banana Samples Using the SFE Method and Traditional Solvent-Based Method

sample	solvent-based extraction and GC/ELCD TBZ concn (μg/g)	SFE and HPLC results	
		TBZ concn (μg/g)	i.s.[a] % recovery
banana 1	0.33	0.29 ± 0.05	80 ± 16
banana 2	0.17	0.13 ± 0.02	73 ± 29
potato 1	0.26	0.61 ± 0.11	87.9
potato 2	0.17	0.49 ± 0.04	85.1
potato 3	0.13	0.33 ± 0.05	92.8

[a] MBC was added to the samples at 0.5 μg/g to serve as a matrix internal standard.

flow rate stability, level and purity of the CO_2 in the cylinder, trapping efficiency, and analyte rinsing efficiency. Other factors may include sample packing and how the CO_2 dispersed in the vessel.

HPLC/MS. Several samples that were extracted by SFE were also analyzed by HPLC/MS for confirmation of the identity of MBC and TBZ chromatographic peaks. Ion-pairing solution was not used in this analysis because the salts were not suitable for the mass spectrometer.

Results from the Hewlett-Packard SFE. The method development presented in this study was carried out with the Suprex instrument. When the Hewlett-Packard SFE became available during the latter stages of this research, experiments at extraction conditions as close as possible to those used with the Suprex were applied with the Hewlett-Packard SFE. The pressure limit was 350 atm, and to remain at the 0.9 g/mL CO_2 density, the extraction temperature was set to 50 °C at maximum pressure (350 atm). No modifier was used with the Hewlett-Packard instrument. The recoveries of fortified potato and banana samples were generally equal to the results with the Suprex extractor. The same conclusion was made in a previous study (King *et al.*, 1993b).

Incurred Samples. After a method had been developed and tested with three types of fortified commodities, verification of the results with incurred samples, which had been previously analyzed using the Luke and Masumoto (1986) procedure and GC/ELCD analysis, was performed. Potato and banana samples were provided by state laboratories and analyzed for the presence of TBZ using the SFE and HPLC procedure. Table 4 lists the results of the SFE approach versus the results obtained by the state laboratories using the traditional approach. MBC was added to some of the samples in the SFE vessels as an internal matrix standard to help account for possible extraction fluctuations. For banana, the SFE method gave 12% and 23% lower values for the 0.33 and 0.17 ppm samples, respectively. The recoveries for bananas on average were 80%, which accounts for these differences. If the concentrations determined by SFE were corrected for the known recoveries, then the results fall within the variability of the method (12% RSD).

For potatoes, the state laboratory gave results without correction for their recovery which, for TBZ, was known by the laboratory to be lower than 65%. On the basis of SFE studies employing both UV and fluorescence detectors, the concentrations in the potatoes were consistently 2.5 times higher than the values reported. The state laboratory used a gas chromatographic method with flame photometric detection in the sulfur mode (a nonlinear method of quantitation) for the analysis of

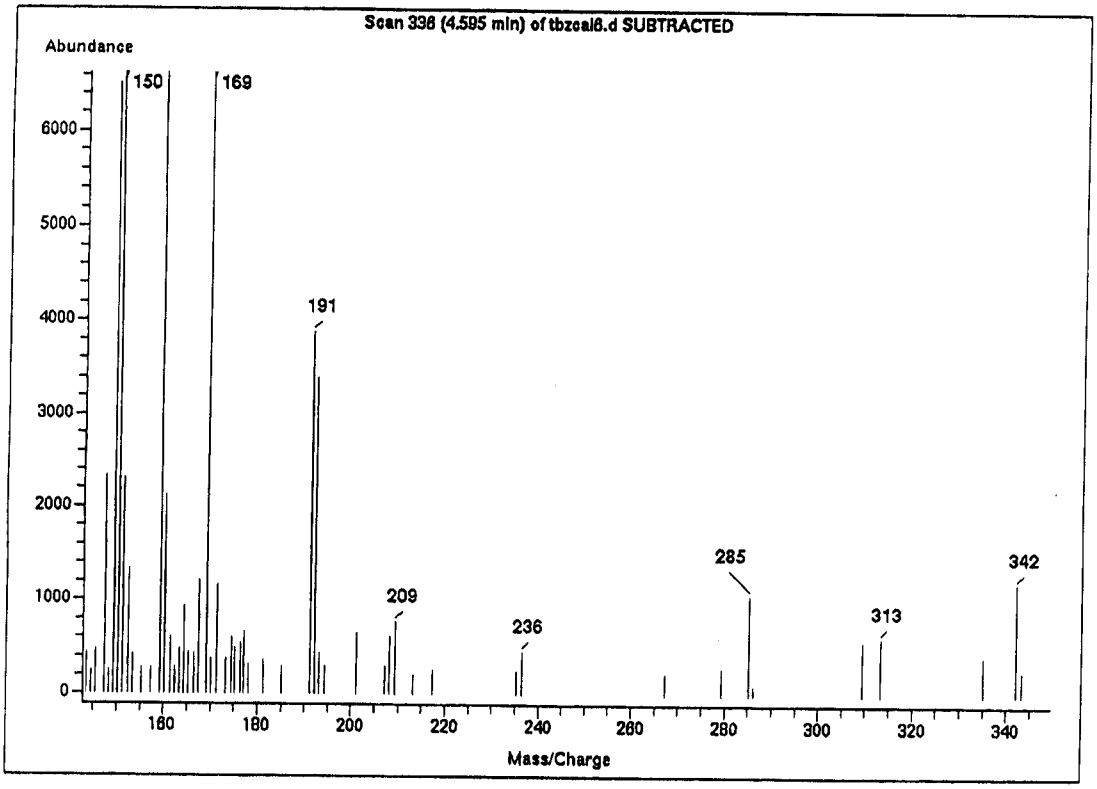

Figure 7. Mass spectrum of thiophanate-methyl from HPLC/MS analysis of an SFE extract of fortified potato.

TBZ. HPLC unquestionably allows better quantitation for the benzimidazole fungicides than GC methods due to better detection sensitivity, linearity, and chromatographic peak shape. It should be noted that, even though the potato sample amounts were only 3 g for the Suprex and 2.1 g for the Hewlett-Packard instruments, the %RSDs (8–18%) of the results from different subsamples were similar to the reproducibility of fortified studies. This indicated that the 2–3 g sample sizes used in SFE were satisfactory.

Conclusions. The results of this study demonstrated that SFE can be used for routine analysis of benzimidazole fungicides with good recoveries. The advantages of SFE versus many solvent-based extraction techniques include increased selectivity, more automation, shorter time of extraction, generation of less waste, and use of less laboratory space and glassware. Additionally, impurities from concentrated extraction solvents are avoided, and extracts are typically cleaner than with solvent-based methods. This issue is of particular importance when HPLC/UV is used for residue analysis. For example, the procedure of Luke and Masumoto (1986) requires 875 mL of organic solvents, at least two cleanup steps, and several hours of manual labor for each 100 g sample.

Difficulties in maintaining optimal SFE instrument conditions over an extended period of time were the major source of fluctuations in recoveries. Results were directly related to how well the instruments were functioning, and currently, optimal performance requires experience and careful attention. This problem is expected to diminish as manufacturers improve current instrument designs. To help account for possible fluctuations, an appropriate internal matrix standard should be used to ensure recoveries for unknown samples. Since this study was part of a larger effort to develop a multiresidue method with SFE, the demonstration that these fungicides could be extracted by SFE and analyzed by HPLC was a significant step in the development of a complete procedure.

ACKNOWLEDGMENT

We express our gratitude to Emy Pfeil for help with the HPLC instrumentation, to the Florida and Texas Departments of Agriculture for analyzing and providing incurred samples, and to Hewlett-Packard for loaning the 7680T SFE instrument.

LITERATURE CITED

Agricultural Marketing Service. *Pesticide Data Program January–June 1992, Report*; U.S. Department of Agriculture: Washington, DC, 1993.

Aharonson, N.; Ben-Aziz, A. An analytical procedure for residue determination of benomyl, MBC, and thiabendazole in various crops. *J. Assoc. Off. Anal. Chem.* **1973**, *56*, 1330–1334.

Brandon, D. L.; Binder, R. G.; Bates, A. H.; Montague, W. C., Jr. Monoclonal antibody-based ELISA for thiabendazole in liver. *J. Agric. Food Chem.* **1992**, *40*, 1722–1726.

Chester, T. L.; Pinkston, J. D.; Raynie, D. E. Supercritical fluid chromatography and extraction. *Anal. Chem.* **1992**, *64*, 153R–169R.

Code of Federal Regulations, Parts 150–189; Title 40; U.S. GPO: Washington, DC, 1991.

Cross, R. F.; Ezzell, J. L.; Richter, B. E. The supercritical fluid extraction of polar drugs (sulphonamides) from inert matrices and meat animal products. *J. Chromatogr. Sci.* **1993**, *31*, 162–169.

Farm Chemicals Handbook '92; Meister Publishing: Willoughby, OH, 1992.

France, J. E.; King, J. W. Supercritical fluid extraction/enzyme assay: a novel technique to screen for pesticide residues in meat products. *J. Assoc. Off. Anal. Chem.* **1991**, *74*, 1013–1016.

Gilvydis, D. M.; Walters, S. M. Ion-pairing liquid chromatographic determination of benzimidazole fungicides in foods. *J. Assoc. Off. Anal. Chem.* **1990**, *73*, 753–761.

Hawthorne, S. B. Analytical-scale supercritical fluid extraction. *Anal. Chem.* **1990**, *62*, 633A–642A.

Hopper, M. L.; King, J. W. Enhanced supercritical fluid carbon dioxide extraction of pesticides from foods using pelletized diatomaceous earth. *J. Assoc. Off. Anal. Chem.* **1991**, *74*, 661–666.

Howard, A. L.; Braue, C.; Taylor, L. T. Feasibility of thiocarbamate pesticide analysis in apples by supercritical fluid extraction and high-performance liquid chromatography. *J. Chromatogr. Sci.* **1993**, *31*, 323–329.

King, J. W.; Hopper, M. L.; Luchtenfeld, R. G.; Taylor, S. L.; Orton, W. L. Optimization of experimental conditions for the SC-CO_2 extraction of pesticide residues from grains. *J. AOAC Int.* **1993a**, *76*, 857–864.

King, J. W.; Snyder, J. M.; Taylor, S. L.; Johnson, J. H. Translation and optimization of supercritical fluid extraction methods to commercial instrumentation. *J. Chromatogr. Sci.* **1993b**, *31*, 1–5.

Lehotay, S. J.; Ibrahim, M. A. Supercritical fluid extraction and gas chromatography/ion-trap mass spectrometry analysis of pentachloronitrobenzene pesticides in vegetables. *J. AOAC Int.* **1994**, in press.

Luke, M. A.; Masumoto, H. T. Pesticide Residue Analysis of Foods. In *Analytical Methods for Pesticides and Plant Growth Regulators*; Zweig, G., Sherma, J., Eds.; Academic Press: Orlando, FL, 1986; Vol. 15, pp 161–200.

Maxwell, R. J.; Parks, O. W.; Piotrowski, E. G. Improved SFE recovery of trace analytes from liver using an integral micrometering valve–SPE column holder. *J. High Resolut. Chromatogr.* **1992**, 807–811.

Maxwell, R. J.; Pensabene, J. W.; Fiddler, W. Multiresidue recovery a PPB levels of 10 nitrosamines from frankfurters by supercritical fluid extraction. *J. Chromatogr. Sci.* **1993**, 212–215.

Messer, D. C.; Taylor, L. T. Development of analytical SFE of a polar drug from an animal food matrix. *J. High Resolut. Chromatogr.* **1992**, *15*, 238–241.

Newsome, W. H.; Collins, P. G. Enzyme-linked immunosorbent assay of benomyl and thiabendazole in some foods. *J. Assoc. Off. Anal. Chem.* **1987**, *70*, 1025–1027.

Skopec, Z. V.; Clark, R.; Harvey, P. M. A.; Wells, R. J. Analysis of organophosphorus pesticides in rice by supercritical fluid extraction and quantitation using an atomic emission detector. *J. Chromatogr. Sci.* **1993**, *31*, 445–449.

Snyder, J. M.; King, J. W.; Rowe, L. D.; Woerner, J. A. Supercritical fluid extraction of poultry tissues containing incurred pesticide residues. *J. AOAC Int.* **1993**, *76*, 888–892

Snyder, J. L.; Grob, R. L.; McNally, M. E.; Oostdyk, T. S. The effect of instrumental parameters and soil matrix on the recovery of organochlorine and organophosphate pesticides from soils using supercritical fluid extraction. *J. Chromatogr. Sci.* **1993**, *31*, 183–191.

Wigfield, Y. Y.; Lanouette, M. Supercritical fluid extraction of the fortified residues of fluazifop-P-butyl (fusilade II) and its major metabolite, fluazifop-P, in onions. *J. Agric. Food Chem.* **1993**, *41*, 84–88.

Received for review May 9, 1994. Accepted August 29, 1994.[⊗]

[⊗] Abstract published in *Advance ACS Abstracts,* October 15, 1994.