Determination of Carbendazim Residues in Grains by Solid-Phase Extraction and Micellar Electrokinetic Chromatography with Ultraviolet Detection

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Abstract

A new method for the determination of carbendazim residues in grains is described. Samples are extracted with hydrochloric acid, cleaned up by solid-phase extraction, and quantitated by micellar electrokinetic chromatography (MEKC) with on-column ultraviolet detection. Recoveries ranging from 82.6 to 94.6% are obtained for rice and wheat samples spiked with carbendazim at 0.1 and 1.0 mg/kg. The lower detection limits are 0.05 ppm. Measurement of carbendazim via MEKC results in sharp and reproducible peaks that facilitate quantitation. This new approach also shows superiority over currently available methods in terms of procedural simplicity and solvent economy.

Introduction

Carbendazim (methyl benzimidazol-2-ylcarbamate) is a benzimidazolic systemic fungicide with protective and curative action (1). It is frequently used to control a wide spectrum of diseases in crops. Two closely related fungicides, benomyl[methyl 1-(butylcarbamoyl)benzimidazol-2-ylcaxbamate] and thiophanate-methyl[dimethyl 4,4'-(*o*-phenylene)bis(3-thioallophanate)] can be readily degraded into carbendazim, not only when applied to crops but also in contact with water or under moist soil (2,3). Whereas benomyl and thiophanate-methyl may breakdown shortly after application, the produced carbendazim remains for a longer time. Therefore, their total residues are usually determined in the form of carbendazim, and a single maximum residue limit (MRL) has been recommended for this group of fungicides (4).

Determination of carbendazim relies chiefly on chromatographic means, though other methods such as spectrophotometry (5), spectrofluorometry (6,7), and immunoassay (8–10) have been reported. Due to its nonvolatility and thermal lability, carbendazim cannot be analyzed by gas chromatography unless properly derivatized (11,12). High-performance liquid chromatography (HPLC) remains the prevailing method for the measurement of residual carbendazim. Most HPLC analyses are carried out on silica-based reversed-phase columns. However, simple mobile phases (e.g., CH_3OH–H_2O or CH_3CN–H_2O) often cause poor peak shape and irreproducible results (4,13,14). Obvious peak broadening and tailing originates from ionization equilibrium and strong interaction of basic carbendazim with residual silanol groups on the packing surface. Modifying the mobile phase with a buffer (3,15–18), amine (19,20), or ion-pairing reagent (21–23) is a generally adopted measure to improve column efficiency and reproducibility. Performing HPLC under normal phase conditions has been attempted (14,24). A polymer-based reversed-phase column operating with a simple mobile phase was reported by A. Di Muccio et al. (4); good peak symmetry and high column efficiency were achieved.

In most residual analyses of carbendazim, rigorous sample preparation procedures are involved, and few innovative efforts can be found. Samples are typically extracted with a polar organic solvent. Cleanup steps include repeated pH adjustment, solvent partitions, and column adsorption. Not only are a series of laborious steps required, but quite a large amount of organic solvent is consumed, resulting in environmental hazards and waste disposal difficulties.

High-performance capillary electrophoresis (HPCE) has been experiencing rapid development since pioneering studies done by Jorgenson et al. (25) and Terabe et al. (26) in the 1980s. Because of its high column efficiency, this technique not only makes resolution of closely related compounds easy, but also facilitates separation of targeted chemicals from matrix components in real samples. Consequently, the need for extensive sample cleanup could be minimized, rendering method development more straightforward. In recent years, application of this new technique to pesticide residue analysis has begun to gain attention (27–30). Some unique advantages over con-

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ventional chromatographic methods have been noted. Wigfield and coworkers (31) reported residue measurement of two bipyridylium herbicides (paraquat and diquat) in potatoes by capillary zone electrophoresis (CZE). Detection could be done free of interferences from coextractives. Krynitsky et al. (32) described determination of five sulfonylurea herbicides in grains by micellar electrokinetic chromatography (MEKC). The method claimed to be faster and more sensitive than the approved HPLC method.

In this study, a new way to determine residual levels of carbendazim in grains was developed. Samples were extracted in an acidic aqueous medium, purified and concentrated with a C_{18} reversed-phase cartridge, and finally quantitated by MEKC. Through the combination of solid-phase extraction (SPE) and MEKC, sample pretreatment was simplified, and reproducible detection of carbendazim was achieved.

Experimental

Reagents and materials

Carbendazim standard was purchased from Chem Service (West Chester, PA). A 100-µg/mL stock solution was prepared by dissolving the standard in HPLC-grade methanol. Working standards were prepared by diluting the stock solution with an appropriate CE buffer.

Sodium dihydrogen phosphate dihydrate, sodium carbonate monohydrate, and sodium dodecyl sulphate (SDS) were obtained from Fluka (Buchs, Switzerland). Sodium hydroxide and concentrated hydrochloric acid were supplied by BDH (Poole, UK). Purified water was prepared with a Milli-Q system (Millipore, Bedford, MA). SPE cartridges containing 500 mg C_{18} bonded sorbent were obtained from Whatman (Clifton, NJ)

Instruments

A laboratory-built CE system was used to carry out experiments. The system included an LS30-2R4-3/3.5 high-voltage power supply (High Voltage Technology, New York, NY), a modified MicroUVIS20 detector (Carlo Erba, Milan, Italy) with wavelength set at 210 nm, and an LCI-100 integrator (Perkin-Elmer, Norwalk, CT). Separation was performed on a fused-silica capillary (50-µm i.d.; 375-µm o.d.; total length, 55 cm; effective length, 45 cm) from Polymicro Technologies (Phoenix, AZ). The detection window was made by burning off a small section of outer coating. The column was pretreated by first flushing with 1M NaOH for 20 min followed by 10 min with 0.1M NaOH, 5 min with Millipore water, and finally 20 min with running buffer. All CE buffers and sample solutions were filtered through 0.45-µm nylon filter devices. Samples were injected into the capillary by gravity with an injection time of 10 s and an injection height of 10 cm. All experiments were carried out under ambient temperature.

Sample preparation

Finely ground grains (20 g) were weighed into a 250-mL conical flask. 50 mL 1M hydrochloric acid was added, and the mixture was stirred with a magnetic stirrer for 30 min. The extract was vacuum-filtered through a Whatman filtration assembly. The filter cake was mixed again with 50 mL 1M HCl, stirred for another 30 min, and filtered. The combined flitrate was neutralized by slowly adding sodium carbonate powder until release of carbon dioxide ceased. The resulting solution was centrifuged for 5 min at 7000 rpm to remove sediments. The supernatant (approximately 95 mL) was then ready for subsequent SPE.

SPE cleanup

The Whatman ODS-2 cartridge was connected to a vacuum manifold. The cartridge was preconditioned by passing 5 mL methanol and then 10 mL Millipore water through it. The sample solution was loaded into a separating funnel that was connected to the SPE cartridge. A vacuum was applied to keep the column flow rate at 3 mL/min. When all the solution had passed through the cartridge, 20 mL of Millipore water was used to rinse the cartridge. The washing was discarded. Finally, 2 mL of methanol was employed to desorb the cartridge. The eluate was collected in a small glass vial. This methanol solution was evaporated to near dryness by introducing a gentle stream of nitrogen, and then 1 mL of low-ionic-strength buffer was added. The solution was sonicated prior to CE quantitation.

Sample spiking trials

After a 20-g sample of finely milled grain (rice or wheat) was placed in a conical flask, an appropriate volume of carbendazim stock standard (100 µg/mL in methanol) was added. This spiked sample was blended and allowed to air dry. Sample extraction, cleanup, and CE quantitation were done as usual. Recovery was obtained by comparing the amounts that were found with the amounts that were added (based on the external standard method).

Results and Discussion

Migration behavior of carbendazim

Carbendazim is a weak base. Therefore it can undergo protonation to form the corresponding cation. Initially, CZE was attempted to analyze this compound. With 20mM HAc–NaAc

Table I. Effect of Surfactant Concentration on Peak Height and Column Efficiency for Carbendazim*

SDS (mM)	Average peak height (mm)	Migration time (min)	Plate number†
20	14.2 ± 0.4	4.38 ± 0.03	4.5×10^4
40	26.0 ± 0.5	6.19 ± 0.04	1.9×10^5
60	33.8 ± 0.7	7.22 ± 0.05	2.6×10^5
80	36.0 ± 1.0	8.02 ± 0.05	3.2×10^5

* Buffer: 40mM sodium phosphate at pH 7.0; voltage: +18 kV; injected sample: 10 ppm carbendazim dissolved in running buffer with a corresponding concentration of SDS. Data are from three replicates.

† Plate number was calculated according to the formula: $N = 5.54 \times (\text{migration time/peak width at half-height})^2$.

buffer at pH 4–6, carbendazim could be detected as a broad, tailing peak ahead of electroosmotic flow (electropherogram not shown). Hence this mode was deemed unsuitable for residual analysis. On the contrary, when analysis of carbendazim was performed using MEKC mode (phosphate buffer with SDS surfactant), a symmetric peak with much better efficiency could be obtained. The detection was highly reproducible. From the data in Table I, it can be seen that variation in migration time was less than 1%, and deviation of peak height was within 3%. Following the column flushing procedure as described, the day-to-day variation in migration time was better than 3%, and the deviation of peak response was less than 5%. We believe two factors can account for the excellent column efficiency and reproducibility. First, because MEKC is conducted in a well-buffered system, ionization of carbendazim could be suppressed (thus peak broadening was minimized). Second, unlike in silica-based HPLC, in which a strong interaction exists between the basic carbendazim and residual silanol groups on the stationary phase, SDS micelles (pseudeophase) in MEKC have no such interaction mechanism with the solute, and peak tailing is avoided.

Optimization of MEKC parameters

As in many previous CE separations, on-column ultraviolet (UV) detection was used to detect the peaks. An inherent drawback of this detection method is its low concentration sensitivity because the optical path length is limited to the inner diameter of the capillary. This loss of detection sensitivity can be partially compensated through careful optimization of parameters. From our investigation, two factors (SDS concentration in the running buffer and the sample injection medium) were found to have significant impacts on column efficiency and detection sensitivity. In the following discussion, peak height rather than peak area is used to evaluate detection sensitivity because it better reflects the changes in column efficiency (33).

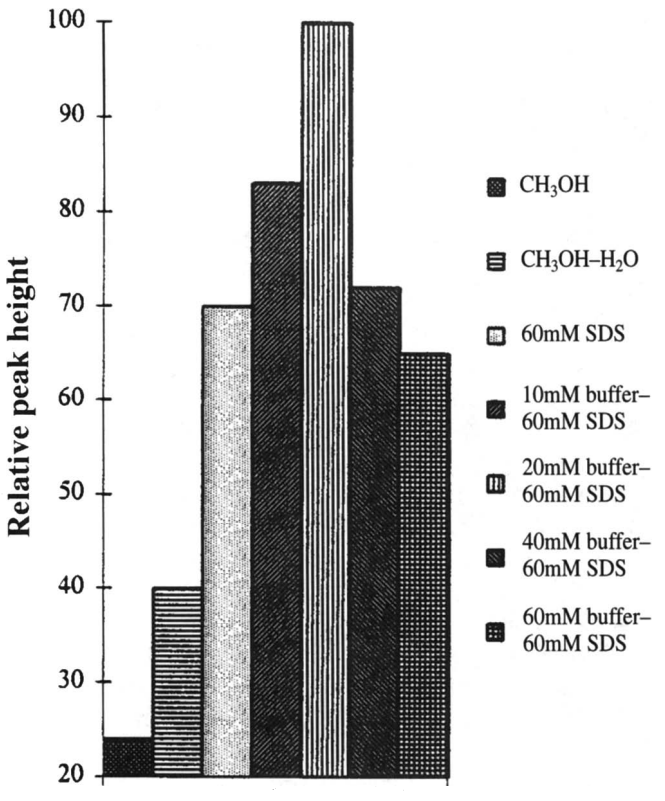

Figure 1. Relative peak height of carbendazim with various injection matrices. Running buffer: 40mM phosphate with 60mM SDS (pH = 7.0); applied voltage: 18 kV; injected sample: 10 ppm carbendazim in corresponding medium.

Table I illustrates the effect of SDS concentration on the peak height and column efficiency of carbendazim. When SDS concentration in the running buffer increased from 20 to 80mM, peak height response from the same amount of carbendazim increased about 150%, whereas column efficiency increased sixfold. The increases in efficiency and detection sensitivity with increasing surfactant concentration have been observed elsewhere (33–35). It has been attributed to the reduction of resistance to mass transfer due to shortening of intermicelle distance (34) or mitigation of longitudinal diffusion because of increased capacity factor and lowering of the diffusion coefficient (33). Though it is possible to achieve higher efficiency by further increasing micellar concentration, higher SDS concentration caused worsening of the signal-to-noise ratio due to heat dissipation.

Choosing a suitable medium for sample injection is another factor worthy of consideration. The influence of different sample media on the peak height response of carbendazim is shown in Figure 1. When carbendazim was dissolved in solvents such as methanol and methanol–water mixtures, the resulting peak height was very low. This phenomenon may be explained in the following manner. When a sample containing a high content of organic solvent is introduced into the capillary, the organic solvent in the sample plug diffuses toward the surrounding buffer solution, causing a breakdown of neighboring micelles. This means that micelles will disappear along quite a long segment of capillary. Consequently, it takes longer for the solute to be incorporated into micelles. This results in a significant dilution of the sample band and loss of detection sensitivity. It was found that dissolving carbendazim in a low-ionic-strength buffer containing SDS was a favorable choice. Peak height reached its maximum when the sample was dissolved in 20mM phosphate buffer with 60mM SDS. A possible explanation is that, under such conditions, carbendazim was largely incorporated into the SDS micelle and behaved as a charged molecule. Thus some sample stacking effect might exist that leads to solute zone sharpening and higher sensitivity.

Other MEKC parameters such as ionic strength, buffer pH, and voltage were selected by balancing the system reproducibility, signal noise level, and analysis time. The final optimized conditions were as follows: buffer, 40mM phosphate with 60mM SDS (pH = 7.0); voltage, 18 kV. The sample for injection was dissolved in 20mM sodium phosphate with 60mM SDS. Under these conditions, the lowest detection limit for carbendazim was 1.0 ppm. By injecting a series of carbendazim standards, the concentration of which ranged from 1.0 to 20 ppm, a linear line was established as: $y = 3948x + 68.9$ (square of the correlation coefficient [r^2] = 0.998), where y is the peak area count, and x is the carbandazim concentration in ppm. Further expansion of the linear range is difficult due to the limited sol-

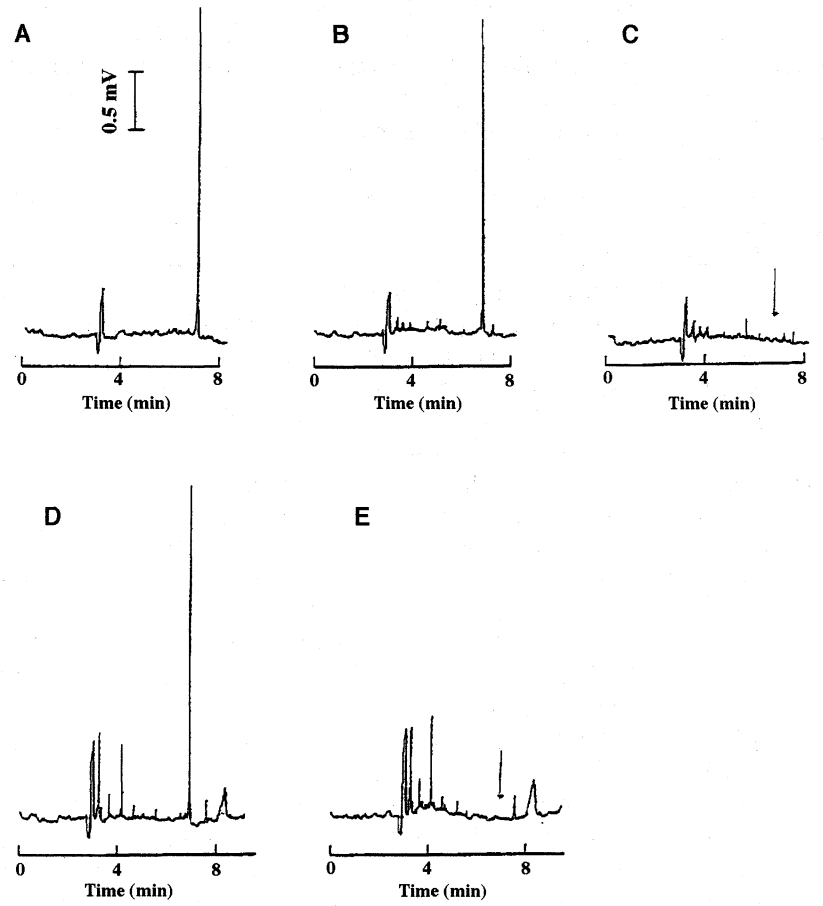

Figure 2. Chromatograms of carbendazim standard and spiked and controlled samples. Capillary: 55 cm x 50 µm fused silica; running buffer: 60mM SDS in 40mM phosphate (pH 7.0); injection media: 60mM SDS in 20mM phosphate; applied voltage: 18 kV; current: 52 µA. (A) Carbendazim standard, (B) spiked rice, (C) controlled rice, (D) spiked wheat, and (E) controlled wheat. Rice and wheat samples were spiked at 1.0 ppm. Detection was performed after 20-fold concentration by SPE. Arrows indicate expected migration time for carbendazim.

Table II. Recoveries of Carbendazim from Fortified Grain Commodities*

	0.1 ppm level		1.0 ppm level	
Grain	Average recovery (%)	Coefficient of variation	Average recovery (%)	Coefficient of variation
Rice	90.1	7.5	93.1	4.4
Wheat	82.6	8.6	94.6	5.6

* Three replicates, each level.

ubility of carbendazim in water. It should be noted that even under optimized conditions, the detection sensitivity was still inadequate for the determination of low-level carbendazim residues. Therefore, sample enrichment was necessary.

Sample extraction and cleanup

Carbendazim forms a water-soluble salt at low pH, thus offering the possibility of extraction of this fungicide in an acidic medium. According to our experiments, when 1M hydrochloric acid was used to soak grain samples, carbendazim was effectively extracted. Simply neutralizing the extract resulted in an aqueous solution that could be conveniently loaded onto a reversed-phase SPE cartridge. Sample enrichment and purification were achieved simultaneously.

By gradually loading a dilute carbendazim standard solution onto the ODS-2 SPE cartridge and monitoring the eluted fractions, it was concluded that the breakthrough volume for carbendazim was over 200 mL. This indicated that the cartridge could provide adequate preconcentration, which could facilitate subsequent CE detection.

Several factors such as organic solvent content and ionic strength had impacts on CE performance for carbendazim. Efforts had to be made during sample cleanup to control these factors. After loading the sample solution onto the cartridge, a washing step was necessary to remove possible coextracted substances and salt components. The elimination of salts helped to guarantee a uniform ionic strength in the concentrated solution. After SPE cleanup, the concentrated sample was transferred to a methanolic medium, which had to be evaporated. The resulting residues were finally dissolved in optimized buffer. By applying SPE, a 20-fold concentration of the sample was obtained. When 1.0 µg carbendazim standard was fortified to a 20-g (0.05 ppm) grain sample following the same sample treatment and MEKC analysis procedure, carbendazim was still detectable with a signal-to-noise ratio greater than 5; this indicates that the detection limits were comparable to most HPLC methods.

It is worth noting that some advantages have been gained by introducing this new sample extraction and cleanup method. First, consumption of organic solvent was greatly reduced. Whereas most methods require at least several hundred milliliters of solvent in steps such as sample extraction, solvent partitioning, and column elution, this method involved only a few milliliters of methanol for the purposes of SPE cartridge preconditioning and desorption. Second, the cleanup procedures are significantly simplified. In traditional methods, repetitive solvent partitions and evaporations are needed. With SPE, these laborious steps were eliminated, and emulsification problems were prevented.

Method validation

Figure 2 presents chromatograms of the carbendazim standards, spiked samples, and controlled samples of rice and wheat (1 ppm spiking level with 20-fold concentration after SPE).

From these chromatograms, it can be seen that most sample matrix components elute closely following the electroosmotic flow. This met our expectation because only highly polar components would be extracted with dilute acid. Wheat samples seemed to contain more complicated coextractives, but this did not cause any serious interference problems.

Table II lists the results of standard addition of carbendazim in rice and wheat samples at 0.1 and 1 ppm levels. The maximum residual limits for carbendazim in cereals vary among different countries, but they are typically in the range of 0.1–1.0 ppm. The satisfactory recoveries show that this new method is suitable for detecting carbendazim residues in grain samples.

Conclusion

In this study, determination of carbendazim residues in grains by HPCE with SPE was demonstrated. With optimization of sample preparation and CE parameters, detection limits comparable to common HPLC methods were obtained. The new method was highly reproducible, required very little organic solvent, and was procedurally simplified as compared with conventional HPLC approaches.

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