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DETERMINATION OF BENOMYL BY HIGH PERFORMANCE LIQUID
CHROMATOGRAPHY/MASS SPECTROMETRY/SELECTED ION MONITORING

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ABSTRACT

Methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate (benomyl) was analyzed by reversed-phase high performance liquid chromatography/mass spectrometry/selected ion monitoring (HPLC/MS/SIM) using 2-aminobenzimidazole (2-AB) as internal standard. Benomyl was quantitatively converted to methyl 2-benzimidazolecarbamate (carbendazim) by acid hydrolysis and the latter was recovered by partitioning into ethyl acetate. The minimum detectable level in apples, peaches and tomatoes was 0.025 ppm. Recoveries of benomyl fortified at 0.1 ppm were in the range 85-110% in all three commodities. Twenty-five samples each of tomatoes, peaches and apples were analyzed. Four apple samples contained levels of 0.15-0.59 ppm benomyl, while eighteen of the peach samples analyzed had levels of 0.25-3.48 ppm. Trace levels of benomyl were also found in three tomato samples and one apple sample.

Benomyl (methyl 1-(butylcarbomoyl)-2-benzimidazolecarbamate) is a fungicide which is widely used for the control of a variety of plant diseases. Unfortunately, benomyl causes hepatocellular carcinomas or combined hepatocellular neoplasms in both male and female mice at all doses tested, with 500 ppm being the lowest dose tested. Its degradation product and metabolite, methyl 2-benzimidazolecarbamate (carbendazim), was also carcinogenic at the same site. Cancer risk from benomyl has been calculated as 6.8×10^{-5} and 7.2×10^{-6} based on tolerance levels and average expected residue levels, respectively (Wiles, 1987). Benomyl has proven to be a difficult analytical target because it decomposes readily in many common organic solvents, as well as in water (Calmon and Sayag, 1976). Because of this instability, residues of benomyl in crops are determined by acid hydrolysis of benomyl to the stable compound, carbendazim, which is then analyzed by HPLC on strong cation-exchange or reversed phase (Bardalaye & Wheeler, 1985) columns.

We have adapted these methods to the determination of benomyl in peaches, apples and tomatoes by reversed phase HPLC/MS.

MATERIALS AND METHODS

Chemicals

Benomyl and carbendazim reference standards were donated by the EPA (Research Triangle Park, NC). 2-Aminobenzimidazole (2-AB) was purchased from Alfa Products, (Danvers, MA). Acetonitrile, ammonium acetate, ethyl acetate, and hexane were high purity HPLC grade (Fisher Scientific, Springfield, NJ). ACS grade anhydrous sodium sulfate, hydrochloric acid, and sodium hydroxide were also purchased from Fischer Scientific.

Samples

Twenty five samples of tomatoes (thirteen New Jersey grown, seven Mexican, one Israeli, and four from Florida), apples (thirteen New Jersey, nine New York, one

Pennsylvania, and one California) and peaches (thirteen New Jersey, seven South Carolina and five Georgia) were collected by the New Jersey Department of Health from New Jersey farms and supermarket distribution centers.

Instrumentation

A Kratos Spectraflow 400 Liquid Chromatograph (Kratos, Ramsey, NJ) interfaced to a Vestec Model 201 Thermospray LC/MS (Vestec Corp., Houston, TX) and controlled by a Teknivent Vector/One Data System (Teknivent Corp., St. Louis, MO) on a Compac Deskpro 286 Personal Computer was used. The HPLC column was a 25 cm x 4.6 mm i.d. Whatman Partisil 5 ODS-3 (Whatman, Clifton, NJ) with a phase particle size of 5 μ m. The mobile phase was 85% acetonitrile/15% 0.1 M ammonium acetate buffer at a flow rate of 1 mL/min. The UV detector was set at 280 nm. The mass spectrometer was operated in the positive ion filament mode, and selected ion monitoring of m/z 192 (carbendazim) and m/z 134 (2-AB) was performed. Scan time was 0.5 second, and sweep width was 0.1 amu. The vaporizer tip temperature was held at 190°C.

Sample Preparation

Benomyl was extracted and hydrolyzed by a procedure based on the method of Bardalaye and Wheeler (1985) with some modification. Fifteen pounds of the cored or pitted crop were chopped and mixed. One hundred gram samples were extracted with 150 mL of 1:1 methanol/ethyl acetate using a Waring blender for 1 minute at low speed. The cake was washed with an additional 150 mL portion of the extracting solvent after filtration under reduced pressure through glass-fiber filter paper (Fisher Scientific). The filtrate was transferred to a 1 L evaporation flask containing 2.5 mL of 1 M hydrochloric acid. This solution was concentrated to ca. 50 mL by rotary evaporation at 40°C. The concentrate was extracted with 10 mL hexane and the hexane layer was discarded. The acidic solution was adjusted to pH 7.5-8.0 with 1 M sodium hydroxide. Carbendazim was recovered from this basic aqueous phase by extracting with three

100-mL portions of ethyl acetate. The combined ethyl extracts were dried by passing through anhydrous sodium sulfate supported by glass wool in a large funnel. The sodium sulfate was washed with an additional 50 mL of ethyl acetate. The dried ethyl acetate extract was then concentrated to 3-5 mL by rotary evaporation at 35°C and transferred to a graduated centrifuged tube to which were also added the flask rinsings. The solution was then evaporated to near dryness under a gentle stream of nitrogen. Ten μ L of 3 mg/mL of 2-AB in chloroform was added as internal standard and the volume was adjusted to 1.0 mL with 1:1 methanol/acetonitrile. The solution was filtered through a 0.45 μ m HPLC syringe filter and 20 μ L of the filtrate was analyzed by HPLC/MS/SIM.

Preparation of Calibration Curve

A stock solution of carbendazim was prepared by dissolving 10 mg of carbendazim reference standard in methanol in a 100 mL volumetric flask. A series of standard solutions were prepared by adding various volumes of the stock solution into graduated tubes, adding 20 μ L of 2-AB internal standard stock solution (3 mg/mL in chloroform), and diluting to the 2-mL mark with 1:1 methanol/acetonitrile. These standard solutions were made to contain 1, 2, 5, 10, 30, and 50 ng/ μ L of carbendazim and 30 ng/ μ L of 2-AB internal standard and were analyzed by HPLC/MS/SIM with duplicate injections for each concentration level. We monitored m/z 192 (carbendazim) and m/z 134 (2-AB) and the peak area ratios of m/z 192 over m/z 134 were plotted against the amounts of carbendazim in the standard solutions. A linear calibration curve with a regression coefficient of 0.992 was obtained. In the recovery studies and sample analyses, benomyl was calculated by multiplying the amount of carbendazim with the molecular weight factor, 290/191 (the molecular weight of benomyl is 290).

RESULTS AND DISCUSSION

Figure 1 shows the chromatogram obtained from the reversed-phase HPLC of carbendazim and the added internal standard 2-aminobenzimidazole (2-AB). Figure 2 depicts selected ion monitoring chromatograms obtained simultaneously by monitoring the $M + 1$ ions of carbendazim (m/z 192) and 2-AB (m/z 134). The selected ion monitoring chromatogram for peaches spiked with 0.1 ppm benomyl is shown in Figure 3. Similar chromatograms (not shown) were obtained from apples and tomatoes spiked with 0.1 ppm benomyl. UV detection of this sample using our HPLC conditions results in the unusable chromatogram shown in Figure 4. Sensitivity declines sharply below 0.1 ppm, but reasonable chromatograms are still observed at 0.05 ppm (Figure 5). The limit of detection was estimated at about 0.025 ppm. Tomatoes spiked with 1.0 ppm benomyl (the lowest tolerance level for any food) gave recoveries of 108.8, 102.9, 91.6, 90.3 and 75.7% (average = 93.9%, coefficient of variation = 13.6%). At the 0.1 ppm level recoveries of benomyl were 105.0, 100.3 and 85.6% (average = 97.0%, coefficient of variation = 10.4%) in apples, 110.3, 94.4 and 84.9% (average = 96.5%, coefficient of variation = 13.3%) in peaches and 96.4, 92.4 and 92.3% (average 93.7%, coefficient of variation = 2.5%) in tomatoes. Three of the tomato samples (Israel, Mexico and Florida) contained benomyl at trace (ca 0.025 ppm) levels. Two samples of apples grown in New York contained 0.56 and 0.59 ppm benomyl while another sample of New York grown apples contained benomyl at trace levels. Two of the New Jersey grown apple samples contained benomyl at 0.15 and 0.16 ppm. Benomyl was most frequently found in peaches (18 positives out of 25 samples). Seven of the New Jersey grown peaches had benomyl residues of 3.48, 0.66, 0.31, 0.29, 0.28, 0.28 and 0.25 ppm, while five contained no detectable residues. Six South Carolina grown peaches had benomyl residues of 2.08, 1.97, 1.79, 1.02, 0.67, and 0.42 ppm and one sample had no detectable residue. All of the Georgia peaches were positive (2.17, 2.04, 0.93, 0.88 and 0.62 ppm).

The method we have described for the analysis of benomyl is fast, accurate and sensitive while giving a higher degree of confirmation of identity than methods based on UV detection alone. Reversed-phase HPLC allows for elution of carbendazim and 2-AB in less than five minutes as compared to retention times of approximately eighteen minutes (Kirkland et al., 1973; Spittler et al., 1984) for HPLC methods using a strong cation exchange column. The specificity of the mass spectrometer detection system allows us to chromatograph rapidly because none of the peach, apple or tomato co-extracts give $(M + H)$ or $(M + NH_4)^+$ ions at m/z 192 and 134. Thermospray HPLC/MS gives chemical ionization type mass spectra. Fragmentation to numerous ions, such as occurs in electron impact mass spectrometry, is minimized.

2-Aminobenzimidazole was chosen as an internal standard because it has a structure similar to benomyl and carbendazim, and because it has a retention time close to that of carbendazim. Although 2-AB is a suspected decomposition product of benomyl and/or carbendazim, this decomposition occurs only at $pH > 13$ (Chiba & Singh, 1986) and there has never been evidence for the presence of 2-AB residues in plant matrices (Spittler et al., 1984; Bardalaye and Wheeler, 1985). To further assure ourselves that 2-AB cannot arise from our methods, we analyzed apples spiked with 4 ppm benomyl and carried it through our procedure without adding 2-AB just before analysis. No 2-AB was detected.

Benomyl and its principal environmental degradation product, carbendazim are known animal carcinogens and are suspect human carcinogens. None of the samples we analyzed contained residue levels above tolerance (peaches, 15 ppm; apples, 7 ppm; tomatoes, 5 ppm). However, these tolerances were set to reflect acute, not chronic toxicity effects. We hope that the methodology we have developed will be used for a wider study of benomyl residues in food in order to obtain better risk data.

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FIGURES

Figure 1. Chromatogram of carbendazim (A) and 2-AB (B), both at 30 ng/ μ L with 20 μ L injection: integrator attenuation 2^7 and detector range 0.02.

Figure 2. The SIM chromatogram of a standard solution of carbendazim (upper trace) and 2-AB (lower trace) both at 30 ng/ μ L with 20 μ L injection.

Figure 3. The SIM chromatogram obtained from a peach sample spiked with a 0.1 ppm benomyl before extraction and to which internal standard was added to the concentrated extract.

Figure 4. The HPLC chromatogram of a peach sample spiked with 0.1 ppm benomyl before extraction using a UV detector set at 280 nm (carbendazim and 2-AB are completely masked by co-eluting peach extracts).

Figure 5. The SIM chromatograms (monitored at m/z 192) for peaches (A), apples (B) and tomatoes (C), each spiked with 0.05 ppm benomyl before extraction.

TIME (MIN)