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Short Communication

Determination of carbendazim in blueberries by reversed-phase high-performance liquid chromatography

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ABSTRACT

A fluorescent reversed-phase high-performance liquid chromatographic method was developed for the analysis of carbendazim in blueberries. Recoveries of fortified blueberries at 27 and 810 ng/g were more than adequate with good precision. Forty commercial blueberry samples were analyzed and the amount of carbendazim ranged from none detected (detection limit of 15 ng/g) to 155 ng/g. Confirmation of carbendazim in the blueberry samples was made by enzyme immunoassay and UV photodiode array.

INTRODUCTION

Carbendazim (methyl benzimidazole-2-yl carbamate) and benomyl (methyl 1-(butylcarbamoyl) benzimidazole-2-yl carbamate), systemic benzimidazole fungicides, are used as either preharvest or postharvest treatment on fruit and vegetables to prevent Botrytis and rotting during refrigeration [1]. Since benomyl metabolizes quite rapidly to carbendazim in fruits and vegetables [2,3], it is usually quantified as carbendazim. Tolerances range from 0.2 to 35 ppm. There has been some long standing concern as to the safety of carbendazim [4,5]. Because of possible health effects [4,5], widespread use [6] and insufficient residue data [6], there is a need to monitor carbendazim in food commodities.

Previous methods for carbendazim analysis in foods have focused on spectrophotometric and chromatographic procedures [1,5,7,8]. However, high-performance liquid chromatography (HPLC) has become the method of choice for benzimidazole fungicides [5,7,8]. Of these HPLC procedures none included the analysis of carbendazim in blueberries.

This paper describes a reversed-phase fluorescence HPLC method for the analysis of carbendazim in blueberries (fresh, frozen, highbush and lowbush) that is extemely sensitive.

EXPERIMENTAL

Materials and chemicals

Solvents were HPLC grade (VWR, Boston, MA, USA) except for the methanol used for the extraction which was ACS grade (Fisher Scientific, Fairlawn, NJ, USA). Carbendazim was obtained from the US Environmental Protection Agency (Research Triangle Park, NC, USA), Acid almunia was from Sigma (St. Louis, MO, USA) and the basic alumina from Fisher Scientific.

Blueberries were obtained from local stores and processors. Enviroguard carbendazim enzyme immunoassay kits were purchased from Millipore Corporation (Bedford, MA, USA).

Apparatus

The HPLC system consisted of a Waters Assoc.

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(Milford, MA, USA) 510 pump, a Valco pneumatic injector (VICI Instruments, Houston, TX, USA), a Waters 470 Fluorescence detector, a Hewlett-Packard (Avondale, PA, USA) 1040A photodiode array detector and a Hewlett-Packard 3396A integrator.

Chromatography

An Ultracarb 30 ODS column (stainless steel, 15 cm × 4.6 mm I.D.) (Phenomenex, Torrance, CA, USA) was employed for the separation along with a mobile phase comprised of acetonitrile–methanol–water–monoethanolamine (135:30:235:0.05) at a flow-rate of 1.0 ml/min. When UV detection was used 25 μl of sample was injected at a wavelength of 286 nm and 0.04 a.u.f.s. As for fluorescence, the excitation was set at 286 nm and the emission at 310 nm. A 5-μl volume was injected into the fluorescence system because of its greater sensitivity. Linearity for the fluorescence detector was from 0.25 ng to 500 ng injected while the UV was from 1.25 ng to 2500 ng injected.

Extraction procedure

The extraction procedure was a modification of the method of Gilvydis and Walters [8]. A 50-g amount of blueberries was blended with 100 ml of methanol for 5 min. (If one wants to make sure that all benomyl is converted to carbendazim, then 10 ml of 1 *M* hydrochloric acid may be added to the methanol before blending). The extract was vacuum filtered through No. 42 filter paper and the filter cake rinsed with an additional 50 ml of methanol before transferring the filtrate to a 500 ml sepatory funnel containing 100 ml of 1% NaCl. A pH adjustment was made by adding 40 ml of 2.0 *M* NH_4Cl (pH 9.5) to the separatory funnel. This mixture was partitioned twice with 100 ml aliquots of dichloromethane. The combined dichloromethane fractions were dried over Na_2SO_4 and evaporated to dryness using a rotary evaporator at 40°C. A 2-ml volume of acetonitrile–methanol (50:50) was used to dissolve the residue followed by 2 ml of water. A 1-ml aliquot of this dissolved residue was passed through an alumina column made with a Pasteur pipette that contained 5 mm of acid alumina and 5 mm of basic alumina. A 5- or 25-μl aliquot was injected into the HPLC system depending upon the system employed.

Fig. 1. Chromatogram of an extracted unfortified blueberry sample. Column, Ultracarb 30 ODS; flow-rate, 1 ml/min; wavelength 286 nm excitation and 310 nm emission; eluent, acetonitrile–methanol–water–monoethanolamine (135:30:235:0.05). Peak: 1 = carbendazim.

RESULTS AND DISCUSSION

A typical HPLC chromatogram of an unfortified blueberry extract is shown in Fig. 1. Chromatographic time is rapid with a retention time of 3.5 min and a complete clearance time from other compounds of 8 min. The resolution of the carbendazim peak was 90% from other components present in the blueberry samples. As for peak confirmation two techniques were used to ascertain that the peak at 3.5 min was carbendazim and that there were no co-eluting substances.

First, UV spectra from 190 nm to 350 nm were taken for each carbendazim peak at the up slope, pinnacle and down slope using a photodiode detector. All three spectra for each blueberry sample were the same and agreed with the carbendazim standard. The sensitivity needed for carbendazim confirmation by photodiode array was at least 6 ng carbendazim injected.

The second confirmation technique employed was an enzyme immunoassay for carbendazim. All blueberry samples were analyzed by enzyme immunoassay, including the 20 samples that were shown to contain no detectable residues by HPLC and the agreement between the fluorescent HPLC and immunoassay was excellent. A correlation coefficient of 0.98 was obtained which indicates that the carbendazim peak from the HPLC procedure was free from interfering substances. Furthermore, these results suggest that the immunoassay is a good confirmation technique for carbendazim. The sensitivity needed for carbendazim confirmation by enzyme immunoassay was a sample containing at least 15 ng/g carbendazim.

A recovery study was performed using organic blueberries fortified at 27 and 810 ng/g carbendazim. The blueberries were spiked at the two concentrations on each of six days. Results are shown in Table I. Average recovery for the 27-ng/g fortification was 94% with a coefficient of variation (C.V.) of 14% while the recovery for the 810-ng/g spike was 76% with a C.V. of 3.8%. These recoveries are good and the day to day reproducibility is excellent.

Forty blueberry samples were analyzed using the fluorescent HPLC method. The results are given in Table II. Of these samples, 20 contained measurable levels of carbendazim while the other 20 had no detectable amounts of carbendazim at a detection limit of 15 ng/g. This detection limit was based on a 5-μl injection into the fluorescent system and yielded a signal five times higher than the organic blueberry extract.

TABLE I

RECOVERY OF CARBENDAZIM FROM FORTIFIED ORGANIC BLUEBERRIES

Each spiked sample was extracted on different days.

Sample	Recovery (27 ng/g) (%)	Recovery (810 ng/g) (%)
1	89	76
2	86	73
3	76	74
4	100	76
5	103	77
6	112	81
Mean	94	76
C.V. (%)	14	3.8

TABLE II

CARBENDAZIM LEVELS IN COMMERCIAL BLUEBERRY SAMPLES

Sample	Carbendazim (ng/g)	Sample	Carbendazim (ng/g)
1	59	12	33
2	55	13	23
3	25	14	40
4	54	15	39
5	33	16	102
6	37	17	155
7	37	18	96
8	58	19	78
9	41	20	23
10	59	21–40	ND[a]
11	58		

[a] ND = none detected at a detection limit of 15 ng/g.

Approximately 9 samples per day can be run through the entire procedure. The limiting step is the partitioning with dichloromethane (since it takes much time to wait for the layers to partition after shaking) but this step is really necessary to clean up the sample from interfering substances so that a detection limit of 15 ng/g can be achieved. Since the carbendazim tolerance for blueberries is 7 ppm, it seems ridiculous to obtain such a low detection limit. However, there is an increased interest to know what the actual amounts of pesticides are in food so that the toxicologists and epidemiologists will have accurate data to design and do their studies. Presently, in the USA scientists dealing with the toxicity of pesticides in food assume that each pesticide used on food is present at its tolerance level and as can be seen with blueberries this is not the case. Thus, wrong assumptions can lead to incorrect conclusions.

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