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ORIGINAL ARTICLE

Determination of some benzimidazole fungicides in tomato puree by high performance liquid chromatography with SampliQ polymer SCX solid phase extraction

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KEYWORDS

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Abstract High performance liquid chromatography (HPLC) coupled with solid phase extraction (SPE) was optimized for extraction and quantification of two benzimidazoles fungicides (carbendazim and benomyl) in tomato puree. Results indicate that HPLC using an Agilent ZORBAX Eclipse plus C18 column (4.5 mm × 100 mm, 3.5 μm) and SPE using Agilent SampliQ SCX (55 mg, 3 mL) is an excellent combination for extraction and analysis of these compounds. Recoveries ranged from 90.0 to 95.5 percent with RSDs below 5 percent and limit of detections of 5 μg/kg.

1. Introduction

Due to the high toxicity and slow degradation rate, a lot of organochlorinated pesticides are prohibited and replaced by relatively mild pesticides such as organophosphorous or carbamate pesticides. However, the nature of thermal liability of the carbamate pesticides gives a great limitation in determination of trace level residues. Direct analysis using gas chromatography (GC) is not recommendable since the carbamate pesticides sample can be broken down in the hot column during the analysis (Stan and Klaffenbach, 1991; Sparacino and Hines, 1976; Liu et al., 1990). Methyl (1-butylcarbamoyl)-2-benzimidazole carbamate (benomyl) has been widely used as a systematic fungicide for on variety of food crops and ornament plants (National Research Council Regulating Pesticides in food, 1987) .Besides its carcinogenic activity, it has been known for numerous years that chronic, subchronic, and acute administration of benomyl to rats and mice result in male reproduction damage (WHO Environmental Health Criteria 148, 1993). The acute systematic toxicity of benomyl is very low with a rate LD_{50} of 10 g/kg approximately (Lim and Miller, 1997). In contrast, a single dose of 100 mg/kg is capable of eliciting a testicular lesion (Hess et al., 1991). It has been speculated that benomyls metabolite, carbendazim, cause testicular toxicity by the same mechanism of which they act as fungicides (Marti and Mooser, 1990). Benomyl is rarely soluble in water and rapidly degrades to carbendazim in the environment (Table 1). The degradation rate is dependent on the pH, temperature, and moisture (Davidse and Flash, 1997). High pH

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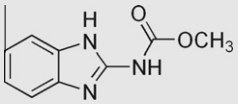
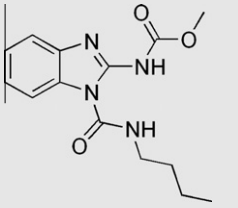
Table 1 Fungicides analyzed in this study.

Name	*p*Ka	Log *P*	Structure
Carbendazim	4.5	1.45	
Benomyl	4.7	1.49	

and temperature accelerate the break down rate of benomyl (Mallat et al., 1977). The benomyl metabolite, carbendazim, is relatively stable in water, and it is known that the compound is intact at least 8 days at pH 8 in water (Cano et al., 1987; USEPA, 1998). Due to these behaviors, the direct measurement of benomyl concentration is not feasible. However, determination of carbendazim concentration, an indirect method, is widely accepted. The concentration of the carbendazim is transformed to benomyl concentration by multiplication of molecular ratio of benomyl to carbendazim (Kawasaki and Murayama, 1999).

Carbendazim has both protective and curative activity against a wide range of fungal diseases, it is toxic to humans, animals and plants and also is very persist in water, wastewater, soil, crops, and food (Kim et al., 2001). A Agilent SampliQ SCX SPE cartridge was used to extract fungicides from tomato puree (Chenhao and Yan, 2003). The purpose of this study to analyze several samples of tomato puree from Jordanian markets and report the concentration of carbendazim and benomyl in those products.

2. Experimental section

2.1. Materials and chemicals

All reagents and solvents were HPLC analytical grade. Fungicide standards were purchased from Sigma–Aldrich Trading Co. (Shanghai, China). Tomato Puree (food grade) was purchased from a local market (city of Aqaba – Jordan).

Phosphate buffer was prepared as 1.42 g of sodium dihydrogen phosphate and 1.4 g of disodium hydrogen phosphate in a 1 L water, which adjusted pH to 4.0.

Stock solution (0.1 mg/mL) was prepared in methanol and kept in the freezer (−20 °C). Working solutions were prepared using the stock solution which were diluted with methanol. The working solutions should be prepared every week and need to be stored at 5 °C.

The SPE cartridges were Agilent SampliQ SCX 3 mL, 60 mg. The analysis was performed on an Agilent 1200 HPLC with variable wavelength detector (VWD). The analytical column was an Agilent ZORBAX Eclipse Plus C18 (4.5 mm × 100 mm, 3.5 μm). Agilent 0.45 mm filter membranes were used to filter sample solutions prior to HPLC analysis.

2.2. HPLC conditions

Column: ZORBAX Eclipse Plus C18 (4.5 mm × 100 mm) 3.5 μm.
Flow rate: 1.0 mL/min.
Injection volume: 20 μL.
Detection wavelength: 285 nm.
Mobile phase: phosphate buffer–acetonitrile (75:25).

2.3. Sample preparation

A 10 g tomato puree was weighted, diluted to 100 mL with water, and stirred with a glass rod for 2 min. Then, the diluted sample was transferred to a 250 mL Erlenmeyer flask and the pH was adjusted to 9.5 using 2.0 M of NaOH solution. The sample was then divided to two or three 50 mL polypropylene centrifuge tubes and centrifuged for 15 min at 4000 rpm. The supernatants were transferred into a glass beaker.

2.4. SPE purification

The Agilent SampliQ SCX cartridges were conditioned with 3 mL of methanol, followed by 3 mL of a 0.15 M of NH_4OH solution with gravity flow (about 1 mL/min).

A 10 mL of the supernatant liquid was loaded to the SampliQ SCX cartridges at a speed about 1 mL/min. After the sample effuses completely, the cartridges were washed with 2 mL of 0.15 M of NH_4OH, 2 mL of the methanol solution, 0.15 M of NH_4OH (3:7), 2 mL of 0.1 M of HCL , and 3 mL of methanol. All three wash steps were under the gravity flow. All effluents were discarded. The cartridges were dried under negative pressure below 2.0 KPa for 1 min. Finally, the cartridges were eluted with 5 mL of 0.5 M NH_4OH in methanol under nitrogen. The dissolved resulting residue was brought to a constant volume of 1 mL using the mobile phase. Then the residue was filtered through a 0.45 μm filter membrane and analyzed.

3. Results and discussion

3.1. Linearity and limits of detection

Stock solutions were diluted to different concentrations and analyzed by HPLC. Linear regressions were calculated for the tetracyclines using the areas and the solution concentrations. The limit of detection (LOD) was calculated using the signal-to-noise ratio 3. The linear range was between 25 and 500 μg/kg. The linearity and LOD are given in Table 2.

3.2. Recovery and reproducibility

Recoveries were calculated for spiked fungicide standards in tomato puree at 50, 75, and 100 μg/kg levels. The analysis was performed in replicates of six at each level. The chromatograms of the blank and spiked standard (100 μg/kg) are shown

Table 2 Linearity and LODs of fungicides.

Compound	Regression equation	Correlation coefficient	LOD (μg/kg)
Carbendazim	$y = 75.45x - 0.3525$	0.89	3.55
Benomyl	$y = 101.18x - 0.5462$	0.89	3.55

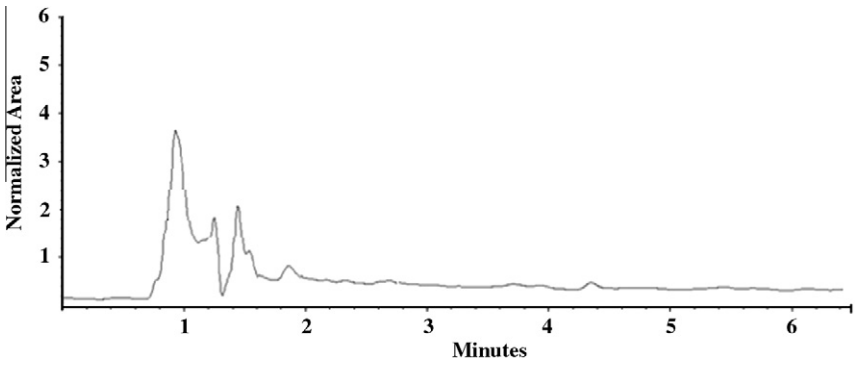


Figure 1 Chromatogram of blank.

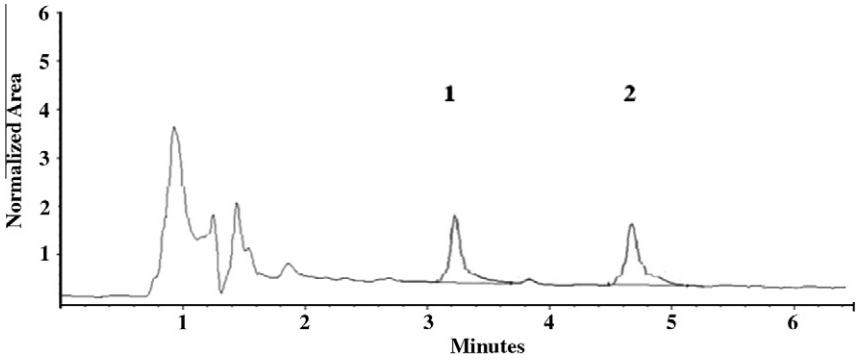


Figure 2 Chromatogram of tomato puree sample spiked at 100 μg/kg (carbendazim is labeled 1 and benomyl is labeled 2 on the chromatogram).

Table 3 Recoveries and RSDs of fungicides in tomato puree by SPE.

Compound	Spiked level (μg/kg)	% Recovery	% RSD ($n = 6$)
Carbendazim	50	86.8	2.95
	75	87.5	2.28
	100	85.6	2.30
Benomyl	50	87.3	1.35
	70	83.5	3.85
	100	83.4	2.68

in Figs. 1 and 2. The recovery and reproducibility data are given in Table 3.

4. Conclusion

The Agilent SampliQ SCX provides a simple and effective single cartridge SPE method for the purification and enrichment of fungicides in tomato puree. The recovery and reproducibility results based on solution standards are acceptable for fungicide residue determination in tomato puree under the Jordanian regulation. The impurities from tomato puree were minimal and did not interfere with any of the fungicides analyzed.

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