Determination of Benzimidazole Fungicides in Apple Juice by SampliQ Polymer SCX Solid-Phase Extraction with High-Performance Liquid Chromatography

Application Note

Food Safety

Authors

Chenhao Zhai and Yun Zou
Agilent Technologies Co., Ltd.
412 Ying Lun Road
Waigaoqiao Free Trade Zone
Shanghai 200131
China

Carol Ball
Agilent Technologies, Inc.
200 Regency Forest Drive
Cary, NC 27518
USA

Abstract

Solid-phase extraction (SPE) coupled with high-performance liquid chromatography (HPLC) was optimized for extraction and quantification of two fungicides (carbendazim and thiabendazole) in apple juice. Results indicate that SPE using Agilent SampliQ SCX (60 mg, 3 mL) and HPLC using an Agilent ZORBAX Eclipse Plus C18 column (4.6 mm × 100 mm, 3.5 µm) is an excellent combination for extraction and analysis of these compounds. Recoveries ranged from 92.1 to 99.4 percent with RSDs below 5 percent and limits of detection of 4 µg/kg.

Introduction

Because of the indiscriminate use of pesticides for different applications, important environmental problems are emerging that are a risk to plant, animal, and human health. Fungicides are one group of these pesticides that are used primarily to control spoilage of crops as a result of fungal attack. Fungicides in general represent approximately 20 to 25 percent of all pesticides used. Benzimidazole fungicides are systemic pesticides, widely used in agriculture for pre- and post-harvest treatment for control of a wide range of pathogens. These substances are applied directly to the soil or sprayed over crop fields and hence are released to the environment. They readily penetrate plants through the roots and leaves and can directly enter natural waters by drainage from agricultural land. Most of these compounds persist in the environment after application; some even remain for years. Two of the main compounds in the benzimidazole family are carbendazim and thiabendazole (Table 1). 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 persistent in water, wastewater, soil, crops, and food. Thiabendazole is used to control fruit and vegetable diseases such as mold, rot, and blight, and is used as a veterinary drug to treat worms.

Agilent SampliQ SCX SPE cartridge was used to extract fungicides from apple juice. This application note describes the implementation and optimization of the method described in SN/T 1753-2006 and the results of validation.

Experimental

Materials and Chemicals

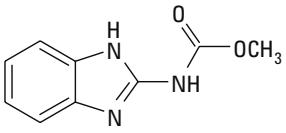
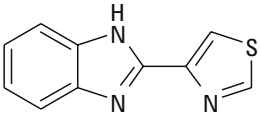
All reagents and solvents were HPLC or analytical grade. Fungicide standards were purchased from Sigma-Aldrich Trading Co. (Shanghai, China). Apple juice (food grade) was purchased from a local market.

Phosphate buffer: 1.38 g sodium dihydrogen phosphate and 1.41 g disodium hydrogen phosphate in 1,000 mL water, adjust pH to 3.0

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 diluted with methanol. The working solutions should be prepared every week and need to be stored at 4 °C.

The SPE cartridges were Agilent SampliQ SCX 3 mL, 60 mg (p/n 5982-3236). The analysis was performed on an Agilent 1200 HPLC with variable wavelength detector (VWD). The analytical column was an Agilent ZORBAX Eclipse Plus C18 3.5 µm 100 mm × 2.1 mm id (p/n 959793-902). Agilent 0.45-µm filter membranes (p/n 5185-5836) were used to filter sample solutions prior to HPLC analysis.

Table 1. Fungicides Used in this Study

No.	Name	pKa	Log P	Structure
1	Carbendazim CAS # 10605-21-7	4.48	1.45	
2	Thiabendazole CAS # 148-78-8	4.7	2.39	

HPLC Conditions

Column:	ZORBAX Eclipse Plus C18 100 mm × 4.6 mm 3.5 µm (p/n 959793-902)
Flow rate:	1.0 mL/min
Injection volume:	20 µL
Detection wavelength:	288 nm
Moblie phase:	Phosphate buffer-Acetonitrile (73:27)

Sample Preparation

Weigh 10 g apple juice, dilute to 100 mL with water, and mix with a glass rod for 1 minute. Transfer the diluted sample to a 250-mL Erlenmeyer flask and adjust pH to 10 with 2 mol/L NaOH solution. Divide the sample between two or three 50-mL polypropylene centrifuge tubes and centrifuge for 10 minutes at 4,000 rpm. Recombine the supernatants into a glass beaker.

SPE Purification

The procedure used for the SPE extraction is shown in Figure 1. The Agilent SampliQ SCX cartridges were conditioned with 3 mL of methanol, followed by 3 mL 0.15 mol/L NH_4OH solution with gravity flow (about 1 mL/min).

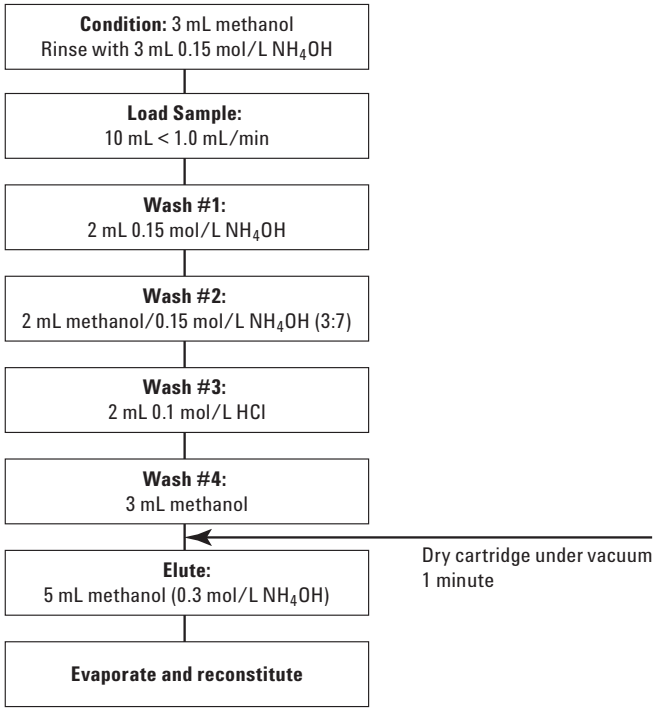


Figure 1. Fungicides in apple juice SPE procedure.

Load 10 mL supernatant liquid to SampliQ SCX cartridges at a speed about 1 mL/min. After the sample effuses completely, wash the cartridge with 2 mL of 0.15 mol/L NH_4OH, 2 mL of a solution of methanol and 0.15 mol/L NH_4OH (3:7), 2 mL of 0.1 mol/L HCl and 3 mL methanol. All three wash steps were under gravity flow. Discard all of the effluents. Dry the cartridge under negative pressure below 2.0 kPa for 1 minute. Finally, elute the cartridge with 5 mL of 0.5 mol/L NH_4OH in methanol, under gravity flow. Collect the eluent and dry it under nitrogen. Dissolve the resulting residue and bring it to a constant volume of 1 mL using the mobile phase. Then filter the residue through a 0.45-µm filter membrane and analyze.

Results and Discussion

Linearity, 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 the injection concentration at which the signal-to-noise ratio was between 2 and 3. The linear range was between 25 and 500 µg/kg. The linearity and LOD are shown in Table 2.

Table 2. Linearity and LODs of Fungicides

Compound	Regression equation	Correlation coefficient	LOD (µg/kg)
Carbendazim	Y = 75.781× – 0.4018	0.9999	4
Thiabenzole	Y = 108.07× – 0.6984	0.9999	4

Recovery and Reproducibility

Recoveries were calculated for spiked fungicide standards in apple juice at 25, 50, 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 in Figure 2 and Figure 3. The recovery and reproducibility data are shown in Table 3.

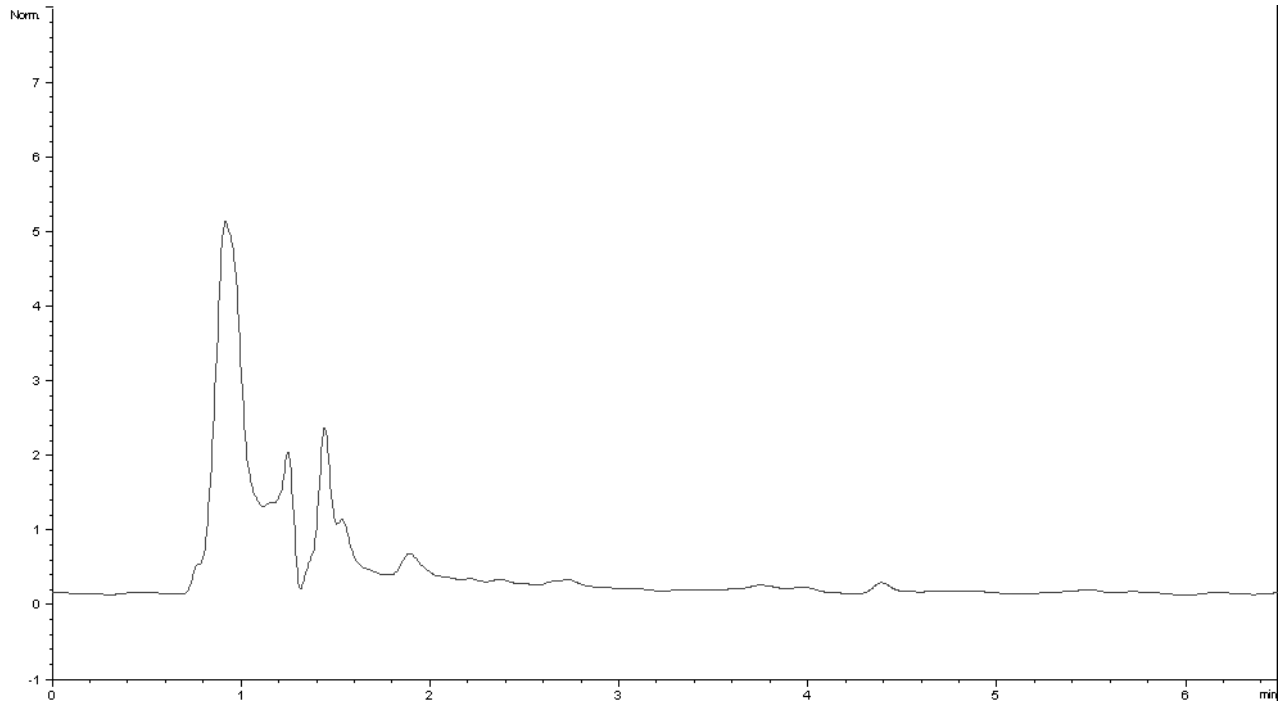


Figure 2. Chromatogram of apple juice blank.

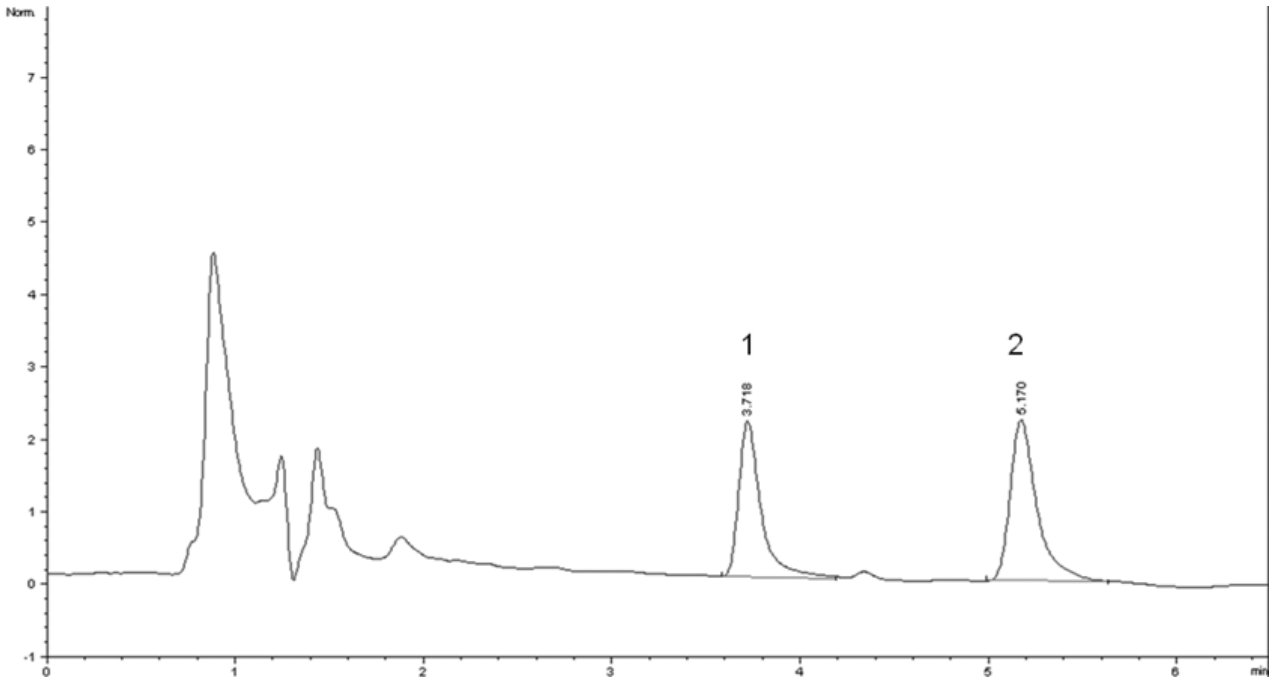


Figure 3. Chromatogram of apple juice sample spiked at 100 µg/kg (1 – Carbendazim, 2 – Thiabenzole).

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Table 3. Recoveries and RSDs of Fungicides in Apple Juice by SPE

Compound	Spiked level (µg/kg)	Recovery (%)	% RSD (n = 6)
Carbendazim	25	98.6	3.99
	50	99.4	3.24
	100	95.9	3.27
Thiabenzole	25	99.0	2.38
	50	92.1	4.90
	100	93.0	3.79

Conclusions

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

References

SN/T 1753-2006, Determination of thiabenzole and carbendazim residues in concentrated fruit juice for import and export – High Performance Liquid Chromatographic method.

For More Information

For additional information on Agilent SampliQ SPE products visit: www.agilent.com/chem/sampliq

For additional information on Agilent HPLC columns visit: www.agilent.com/chem/LCcolumns

interchim
211 Avenue J.F. Kennedy - BP 1140
03103 Montluçon cedex - France
Tél 33 (0)4 70 03 88 55 - Fax 33 (0)4 70 03 82 60
e-mail interchim@interchim.com - www.interchim.com