

Determination of Carbendazim in Orange Juice

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Key Words

Food Analysis, Food Safety, HPLC, On-Line SPE, QuEChERS Method

Goal

To develop an efficient and simple high-performance liquid chromatography (HPLC) method combined with on-line solid-phase extraction (SPE) for sensitive determination of carbendazim in orange juice using a Thermo Scientific™ Dionex™ UltiMate™ 3000 x2 Dual Rapid Separation LC (RSLC) system

Introduction

Carbendazim (structure shown in Figure 1) is a fungicide used in many countries to improve crop production; however, its slow degradation rate and high toxicity may be damaging to human health. China regulates the maximum residue limit of carbendazim in foods at 0.5 mg/kg.¹ In the U.S., the Environmental Protection Agency has not approved carbendazim for use as a fungicide on oranges, nor has it established a tolerance, or an exemption from the need for a tolerance, for carbendazim in orange juice. Thus, carbendazim in orange juice is an unlawful pesticide chemical residue under the U.S. Federal Food, Drug, and Cosmetic Act. Therefore, it is necessary to establish efficient analytical methods to detect the presence of the pesticide residue at low concentrations.

Sample preparation is key for the sensitive determination of carbendazim in orange juice, a complex sample matrix. Several methods are reported for the extraction of carbendazim from orange juice using SPE and liquid/liquid extraction.²⁻⁷

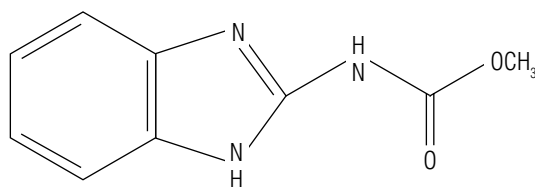


Figure 1. Structure of carbendazim.



Equipment

- UltiMate 3000 x2 Dual RSLC system, including:
 - DGP-3600RS Dual-Gradient RS Pump (P/N 5040.0066) with SRD 3600 Integrated Solvent and Degasser Rack (P/N 5035.9230)
 - WPS-3000TRS Wellplate Sampler, Thermostatted (P/N 5840.0020), with 1000 µL sample loop and 1000 µL syringe
 - TCC-3000RS Thermostatted Column Compartment (P/N 5730.0000)
 - DAD 3000RS Diode Array Detector (without Flow Cell, P/N 5082.0020) and Semi-Micro Flow Cell for DAD-3000 and MWD-3000 Series, SST, 2.5 µL Volume, 7 mm Path Length (P/N 6082.0300)
- Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System (CDS) software version 7.1 or higher

Consumables

- Thermo Scientific™ Target2™ Nylon Syringe Filters, 0.45 µm, 30 mm (Fisher Scientific P/N F2500-1)
- Syringe, 5 µL, Sterile, Luer-Lok (Fisher Scientific P/N 14-823-220)

Reagents and Standards

- Deionized (DI) water, 18.2 MΩ-cm resistivity
- Methanol (CH₃OH), 99.8%, HPLC Grade (Fisher Scientific P/N AC610090040)
- Acetonitrile (CH₃CN) HPLC Grade (Fisher Scientific P/N AC610010040)
- Acetone (CH₃COCH₃), HPLC Grade (Fisher Scientific P/N AC 67-64-1)
- Ammonium Acetate (CH₃COONH₄), Crystalline/HPLC (Fisher Scientific P/N A639-500)
- Acetic Acid (CH₃COOH), Optima™ LC/MS (Fisher Scientific P/N A113-50)
- Magnesium Sulfate (MgSO₄), Anhydrous (Fisher Scientific P/N M65-500)
- Sodium Acetate (CH₃COONa), Anhydrous (Fisher Scientific P/N S210-500)
- Ammonium Hydroxide (NH₄OH), 5% (v/v) (Fisher Scientific P/N 62816)
- Carbendazim Standard (AccuStandard® P/N 10605-21-7)

Preparation of Solutions and Reagents

Ammonium Acetate Mobile Phase, 100 mM

Dissolve 7.71 g of CH₃COONH₄ in 1 L of DI water and mix well.

Working Standard Solutions for Calibration

To prepare a stock standard solution of carbendazim, weigh 1.0 mg of standard and dilute with acetone to 100 mL in a volumetric flask. The concentration of the stock standard solution will be 10 mg/L. To prepare working standard solutions for calibration, add the proper amount of stock standard solution and dilute with DI water.

Sample Preparation

An orange juice sample was purchased from a local supermarket in Shanghai, China.

Sequentially add 3 g of orange juice, 3 mL of 1% methanol in acetonitrile (v/v), 1.2 g of MgSO₄, and 0.3 g of sodium acetate to a 10 mL centrifuge tube. Shake the tube for 1 min, then centrifuge for 3 min at 5000 rpm. Transfer the extract (upper layer) into a 5 mL syringe and filter it through a 0.45 µm syringe filter. Prior to injection, add 2 mL of 0.625% ammonium hydroxide to make basic 1 mL of extracted sample.

To prepare a 2 µg/kg spike of orange juice, add 6 µL of 1 mg/L carbendazim standard solution to 3 g of orange juice. For the remainder of the procedure, follow the instructions above for the preparation of the orange juice sample.

Conditions

On-Line SPE

Column:	Thermo Scientific™ Acclaim™ Trinity™ P1, 3 µm Analytical, 3.0 × 100 mm (P/N 071387)
Mobile Phase:	A, Methanol; B, H ₂ O
Gradient:	B, 0–2 min, 100%; 2.1–4.5 min, 50%; 4.6–10 min, 100%
Flow Rate:	0.7 mL/min
Inj. Volume:	1000 µL

Separation

Column:	Thermo Scientific™ Acclaim™ PolarAdvantage II (PA2), 3 µm Analytical, 3.0 × 150 mm (P/N 063705)
Mobile Phase:	A, Acetonitrile; B, 100 mM Ammonium Acetate
Gradient:	B, 0–3 min, 100%; 6.5 min, 50%; 6.6–10 min, 100%
Flow Rate:	1.0 mL/min
Temperature:	30 °C
Detection:	UV absorbance at 285 nm
MSV Time Program:	0 min, 1_2 2.0 min, 6_1 4.5 min, 1_2

Results and Discussion

Salting-Out Extraction of Orange Juice

Although SPE and liquid/liquid extraction are time- and solvent-consuming methods, they typically have been used for the extraction of carbendazim from fruit juice. Therefore, a simple salting-out extraction method that saves both time and solvent was developed for this application. The salting-out extraction method has been reported for the determination of pesticides in food.⁸⁻¹⁰ In these applications acetonitrile containing 1% acetic acid, combined with simultaneous liquid partitioning formed by adding NaCl and MgSO₄, have been the typical protocol. However, experimental results showed that using sodium acetate instead of NaCl improved the recovery of carbendazim from 70 to 87% in the spiked orange juice sample. Therefore, the reagents and solvent used for the salting-out extraction in this application were acetonitrile (containing 1% acetic acid), sodium acetate, and MgSO₄.

Column Selection

The Acclaim Trinity P1 column—based on nanopolymer silica hybrid technology—provides reversed-phase, anion-exchange, and cation-exchange retention mechanisms. This technology ensures separation of the anion-exchange and cation-exchange regions of the phase and is used as the SPE stationary phase for the on-line SPE HPLC analysis of paraquat and diquat in environmental waters.^{11,12} Similar to paraquat and diquat, carbendazim is a basic compound with a similar basic functional group. Therefore, the Acclaim Trinity P1 column was selected for SPE. Additionally, a simple and economical methanol/water mobile phase was used for on-line SPE. This arrangement yielded over 98% on-line SPE efficiency, which was estimated by comparing the peak area of a 5 µg/L carbendazim standard solution obtained with 1000 µL injection volume using on-line SPE to the peak area of a 500 µg/L carbendazim standard solution obtained with 10 µL injection volume without SPE.

The Acclaim PA2 column provides enhanced hydrolytic stability from pH 1.5–10 and is compatible with 100% aqueous mobile phases. In addition to low pH stability, it can be used to analyze basic compounds under the high-pH conditions necessary to reduce peak tailing. In this study the Acclaim PA2 column was chosen to provide good peak shape for carbendazim.

Evaluation of On-Line SPE

Figure 2 shows a typical flow schematic of on-line SPE directly coupled to the HPLC column using one six-port (2p to 6p) valve. The method works as follows: The prepared orange juice sample is directly injected onto the system and delivered to the SPE column (Acclaim Trinity P1 column) for enrichment (1_2 position) using a methanol/water mobile phase delivered by the first pump. The analytical Acclaim PA2 column is simultaneously equilibrated using the second pump of the dual-pump module. After the analyte—carbendazim—is bound to the SPE column and impurities are washed out, the SPE column is switched into the analytical flow path (6_1 position) to elute the bound carbendazim that is then separated on the analytical column using an ammonium acetate buffer (i.e., acetonitrile mobile phase) and detected by the UV detector. This method is easily accomplished using the UltiMate 3000 x2 Dual RSLC system.

Prior to sample analysis, this configuration was evaluated using the standards. The retention times of the 0.5 and 100 µg/L carbendazim standards analyzed under the same conditions were 7.453 min and 7.457 min, respectively. The peak area ratio of the 0.5 and 100 µg/L standards was 1:204, which corresponds closely to the theoretical value of 1:200. Peak symmetries of the 0.5 and 100 µg/L standards were 1.01 and 0.92, respectively. These three sets of data demonstrate that the combination of the Acclaim Trinity P1 and PA2 columns provides sufficient retention, good peak shape, and fast analysis for carbendazim, and—at least with standards—shows that there is sufficient capacity for the analysis.

Reproducibility, Calibrations, and Detection Limit

Figure 3 shows an overlay of chromatograms of six consecutive 1000 µL injections of a carbendazim standard (5 µg/L) following on-line SPE under the described chromatographic conditions. The RSDs of retention time and peak area were 0.061 and 0.843, respectively, demonstrating good method reproducibility.

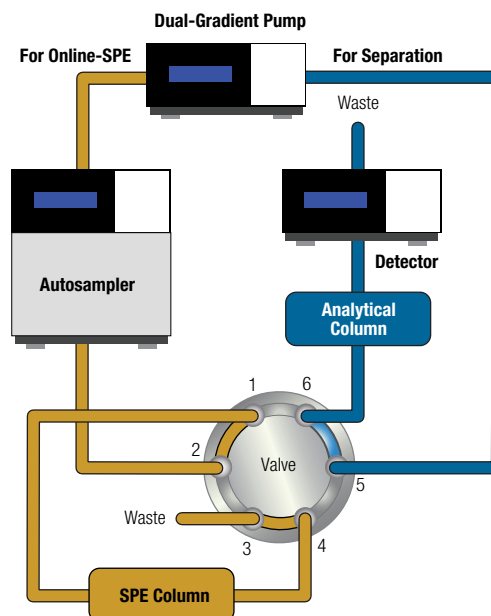


Figure 2. Flow schematic of on-line SPE.

On-Line SPE

Column: Acclaim Trinity P1, 3 µm Analytical, 3.0 × 100 mm
 Mobile Phase: A, Methanol; B, H₂O
 Gradient: B, 0–2 min, 100%; 2.1–4.5 min, 50%;
 4.6–10 min, 100%
 Flow Rate: 0.7 mL/min
 Inj. Volume: 1000 µL onto the on-line SPE cartridge

Separation

Column: Acclaim PA2, 3 µm Analytical, 3.0 × 150 mm
 Mobile Phase: A, Acetonitrile; B, 100 mM Ammonium Acetate
 Gradient: B, 0–3 min, 100%; 6.5 min, 50%; 6.6–10 min, 55%
 Flow Rate: 1.0 mL/min
 Temperature: 30 °C
 Detection: UV absorbance at 285 nm
 MSV Time
 Program: 0 min, 1_2
 2.0 min, 6_1
 4.5 min, 1_2

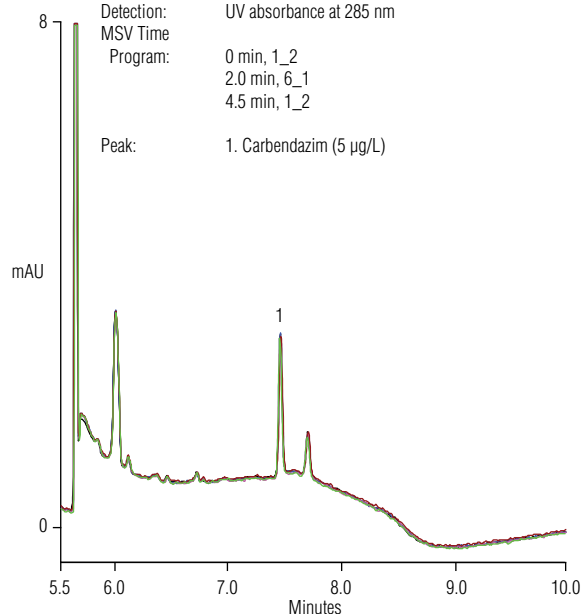


Figure 3. Six consecutive carbendazim standard injections (5 µg/L).

Calibration linearity was investigated by making 1000 μL injections of carbendazim at eight different concentrations: 0.5, 1.0, 2.0, 5.0, 10, 20, 50, and 100 $\mu\text{g/L}$. Linearity was observed from 0.5 to 100 $\mu\text{g/L}$ when plotting the concentration versus the peak area, and the coefficient of determination was ≥ 0.998 , as shown in Figure 4. This calibration curve was used to quantify carbendazim in orange juice.

The method detection limit (MDL) for carbendazim was calculated using the equation:

$$\text{Detection limit} = S t_{(n-1, 1-\alpha=0.99)}$$

The symbol S represents the standard deviation of replicate analyses, n represents the number of replicates, $t_{(n-1, 1-\alpha=0.99)}$ represents the Student's t value for the 99% confidence level with $n - 1$ degrees of freedom. Seven replicate injections of reagent water spiked with 0.5 $\mu\text{g/L}$ of the carbendazim standard were used to determine the MDL of carbendazim, which was 0.2 $\mu\text{g/L}$.

Orange Juice Analysis

Figure 5 shows an overlay of chromatograms of a carbendazim standard (5 $\mu\text{g/L}$), an orange juice sample, and a blank. This analysis shows that carbendazim was not detected in the orange juice sample. To judge method accuracy, three injections of an orange juice sample were spiked with 2.0 $\mu\text{g/kg}$ of carbendazim standard and the average recovery value was 87%.

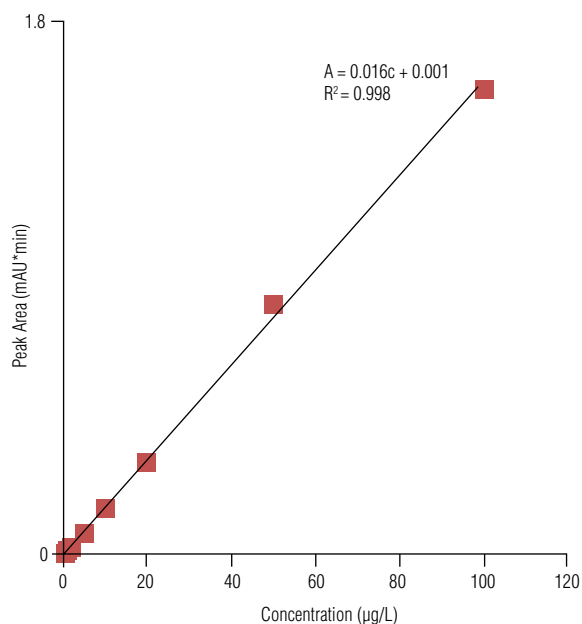


Figure 4. Calibration curve for carbendazim.

Conclusion

This work describes a reproducible and accurate on-line SPE HPLC with UV absorbance detection method for determining carbendazim in orange juice. This application was performed using an UltiMate 3000 x2 Dual RSLC system controlled by Chromeleon CDS software version 7.1. A salting-out extraction combined with on-line SPE simplified the extraction and improved HPLC detection sensitivity. The measured MDL of carbendazim in orange juice was 0.2 $\mu\text{g/L}$.

On-Line SPE

Column: Acclaim Trinity P1, 3 μm Analytical, 3.0 \times 100 mm
 Mobile Phase: A, Methanol; B, H_2O
 Gradient: B, 0–2 min, 100%; 2.1–4.5 min, 50%; 6.6–10 min, 100%
 Flow Rate: 0.7 mL/min
 Inj. Volume: 1000 μL onto the on-line SPE cartridge

Separation

Column: Acclaim PA2, 3 μm Analytical, 3.0 \times 150 mm
 Mobile Phase: A, Acetonitrile; B, 100 mM Ammonium Acetate
 Gradient: B, 0–3 min, 100%; 6.5 min, 50%; 6.6–10 min, 100%
 Flow Rate: 1.0 mL/min
 Temperature: 30 $^\circ\text{C}$
 Detection: UV absorbance at 285 nm
 MSV Time
 Program: 0 min, 1_2
 2.0 min, 6_1
 4.5 min, 1_2

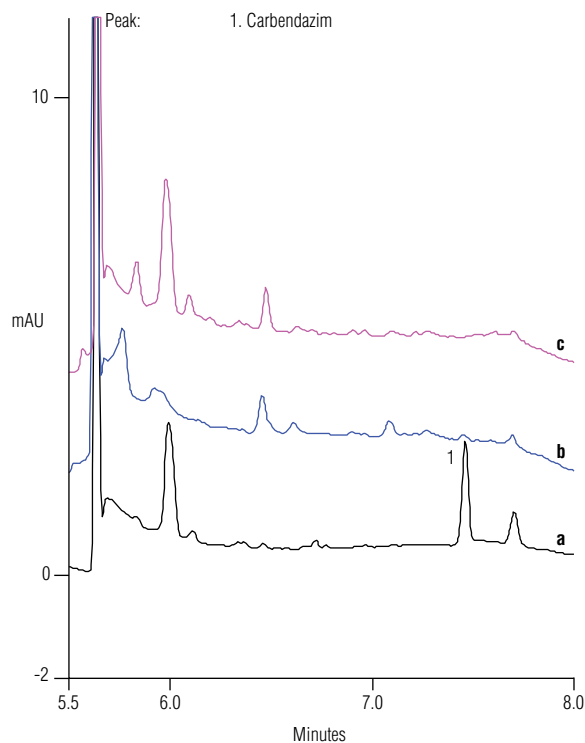


Figure 5. Samples of (a) carbendazim standard (5 $\mu\text{g/L}$), (b) orange juice, and (c) blank.

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