Development of Quantitative Analysis Method of Carbendazim in Tomatoes by High-Performance Liquid Chromatography with Fluorescence Detection (HPLC-FLD)

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Abstract Carbendazim belongs to the benzimidazole fungicides, which can be used for control lots of fungi pathogens. High-performance liquid chromatography is frequently used for the analysis of carbendazim in all kinds of samples. In most occasions, the developed methods were applied for the simultaneous detection of a huge number of pesticides. Thus, an analytical method via UPLC-FLD was developed, and the sample preparation process was optimized by studying the effect of extraction solvent, approach, time and purification absorbent on the recovery rate of carbendazim. The results showed the optimized method for analysis was ultrasonication-assisted extraction with acetonitrile for 1 min, and subsequent purification by C18. In this occasion, the established analytical method of carbendazim in tomato samples displayed good linearity, accuracy and precision.

Key words Carbendazim; Tomato; High-performance liquid chromatography; Analytical method

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Carbendazim belongs to the benzimidazole fungicides, which can be used for control lots of fungi pathogens through the inhibition of cell division and mitotic microtubule formation[1]. However, its half-life in soil and water lasting for a hundred days, which lead to its residue in soil, vegetables and fruits[2]. Based on the nature of the environment, the half-life of carbendazim varies from 3 d to 12 months[3]. Carbendazim has not only been found in bunches of fruits and vegetables, but also in fish, meat and eggs. Besides, some works have reported its residue in commercial food, such as honey, wines and juices[4]. Thus, it is of great importance to monitor the amount of carbendazim in food.

In order to detect carbendazim, a lot of analytical methods have been established, such as chromatographic methods[5-6], colorimetric method[7-8], fluorescence method[9-10], and Raman method[11-12]. Among them, high-performance liquid chromatography is frequently used for the analysis of carbendazim in all kinds of samples[13]. In most occasions, the developed methods were applied for simultaneous detection of a huge number of pesticides.

In this study, an analytical method for analyzing carbendazim in tomatoes was established. In addition, the effect of sample preparation process on recovery rate was studied to obtain the optimized extraction and purification approach for carbendazim.

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Materials and Methods

Materials and reagents

Standard of carbendazim and sodium chloride (NaCl) was purchased from J&K Scientific (Beijing, China). Acetonitrile and methanol with HPLC-grade were provided from Fisher Scientific (MA, New Jersey, USA). Primary secondary amine (PSA) with particle size of 40 - 60 μm, C18 with particle size of 45 μm, graphitized carbon black (GCB) with particle size of 45 μm and silicon with particle size of 40 - 75 μm were provided from Bonna-Agela Technologies (Tianjin, China). Ultrapure water was obtained from a Milli-Q system by Merck Millipore (Merck KGaA, Darmstadt, Germany). Tomato samples were purchased from local market, which were homogenized and storied in -18 ℃.

Standard solution preparation

The carbendazim standard stock solution (100 mg/L) was provided by precisely weighing 10 mg of carbendazim in 100 ml of volumetric flask, which was subsequently dissolved in methanol. Then, a series of working standard solutions were obtained from dilution of stock solution with methanol. All standard solutions were stored in the dark at -18 ℃ for further use.

UPLC-FLD analysis

The analysis of carbendazim was carried out by an Ultimate 3000™ HPLC system and a FLD-3000 fluorescence detector (Thermo Fisher Scientific, USA) with an Inertsil ODS-3 column (4.6 mm i.d. ×250 mm, 5 μm particle size). The mobile phase was composed with acetonitrile and water (70 : 30, *v/v*), the flow rate was 1 ml/min, and the injection volume was 10 μl. The excitation and emission wavelengths of carbendazim were 285 and 315 nm.

Optimization of sample preparation

Tomato samples were analyzed via QuEChERS procedure for the extraction and purification of carbendazim. In general, 2 g of tomato sample was placed in 30 ml centrifuge tube, which was then added with 10 ml of extraction solvent. After 10 min of sonication, 1 g of NaCl was added, and sonication was performed for 5 min. Then, the tube was centrifuged at 4 000 r/min for 3 min, and 1 ml of supernatant was transferred in another 2 ml centrifuge tube containing 50 mg purification absorbent. The centrifuge tube was capped and vortexed for 3 min, and centrifuged at 5 000 r/min for 1 min. The supernatant was filtered and analyzed with UPLC-FLD. In the sample preparation, the extraction solvent, approaches, time and absorbent types on recovery rate of carbendazim were studied.

Method validation

The stock carbendazim standard solution was diluted to a series of working solutions at various levels (0.5, 1, 5, 10, 15, 25 mg/L) with extracted sample solution, which were determined by UPLC-FLD method. The calibration curves were conducted according to the peak areas of carbendazim and concentration of working solutions. Then, three concentrations of carbendazim (2.5, 5, 25 mg/L) were spiked in the blank tomatoes for evaluating the accuracy and precision of the method, and five replicates were analyzed for every concentration.

Data calculation and analysis

The recovery rate of carbendazim was calculated based on following equation:

$$\text{Recovery rate}\ (\%) = C_1/C_0 \times 100$$

Where C_1 (mg/kg) is the detected concentration of carbendazim in samples, and C_0 (mg/kg) represent the spiked concentration of carbendazim in samples.

Results and Discussion

Effect of extraction solvent

Methanol and acetonitrile were the most common extraction solvent in pesticide residue analysis[14-16]. However, it is reported that the mixing solvent could help the extraction of analytes in samples. Hence, the effect of methanol, acetonitrile, ethanol, and mixing solvent (methanol and acetonitrile) on the recovery rate of carbendazim were studied. The results are shown in Fig. 1. It could be found that acetonitrile was the optimum solvent in the extraction because all the carbendazim could be isolated from the tomato.

Effect of extraction approach

The extraction approach is important in sample preparation, which can greatly influence the extraction efficiency[17]. In order to choose an effective extraction approach, the effects of ultrasonication, vertexing and shaking with oscillator on recovery rate were studied. As shown in Fig. 2, the recovery rate by three approaches were all exceed 90%, while ultrasonication is more convenient as it can realize batched sample process. Hence, ultrasonication was chosen as the extraction approach for the analysis of carbendazim in tomato samples.

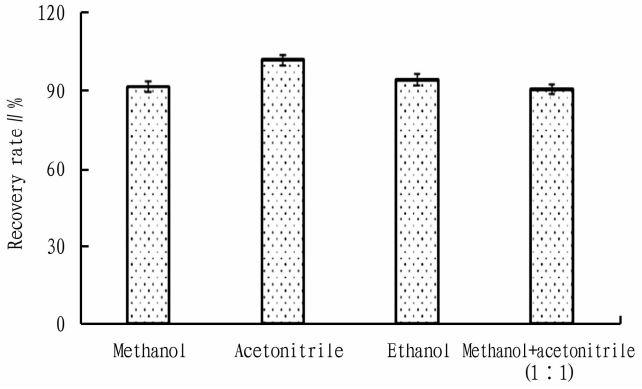

Fig. 1 Effect of extraction solvent on the recovery rate of carbendazim in tomato samples

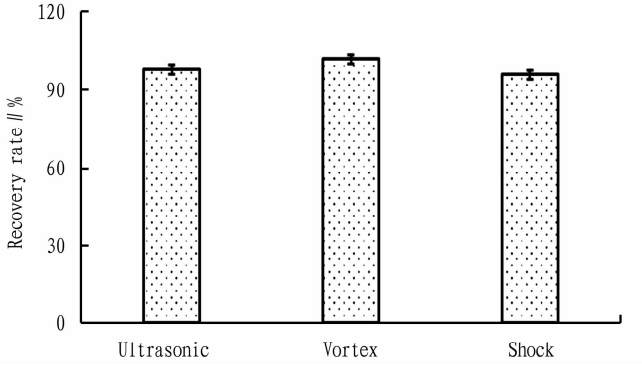

Fig. 2 Effect of extraction approach on the recovery rate of carbendazim in tomato samples

Effect of extraction time

The effect of extraction time was studied. The results are shown in Fig. 3. Generally, the extraction had a positive relation with the extraction efficiency of analytes, while the recovery rate basically remained constant with the extraction time varying from 1 to 10 min. The reason might be carbendazim was easily transformed from tomato samples to solvent, which quickly established the distribution equilibrium.

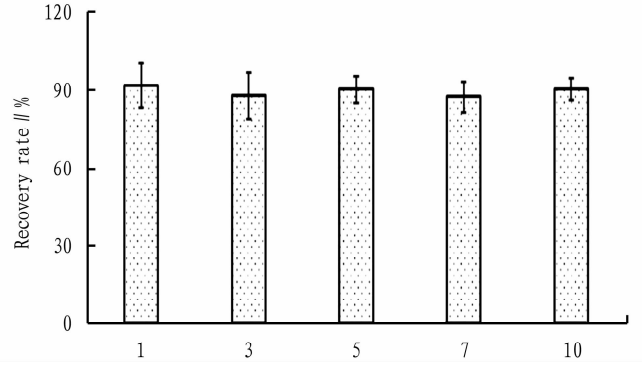

Fig. 3 Effect of extraction time on the recovery rate of carbendazim in tomato samples

Effect of adsorbent types

The purification adsorbent was of huge influence on the recovery rate of carbendazim[18]. In this experiment, C18, PSA, silica and GCB were tested as the adsorbent in the sample preparation. As shown in Fig. 4, the recovery rates with the silica and C18 were in the range of 70% – 120%, which met the standard for the recovery rates. The typical chromatography of analytical samples using silica and C18 are shown in Fig. 5. It could be found that the purification efficiency of C18 was better than that of silica.

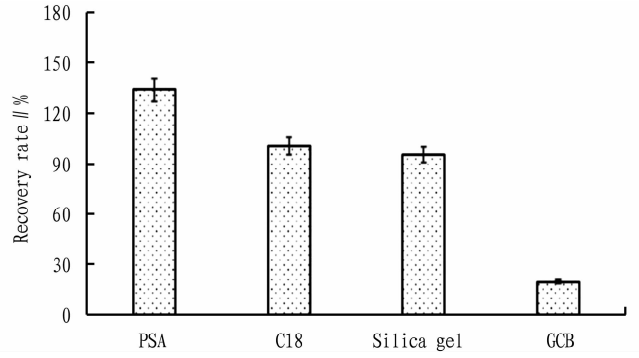

Fig. 4 Effect of adsorbent types on the recovery rate of carbendazim in tomato samples

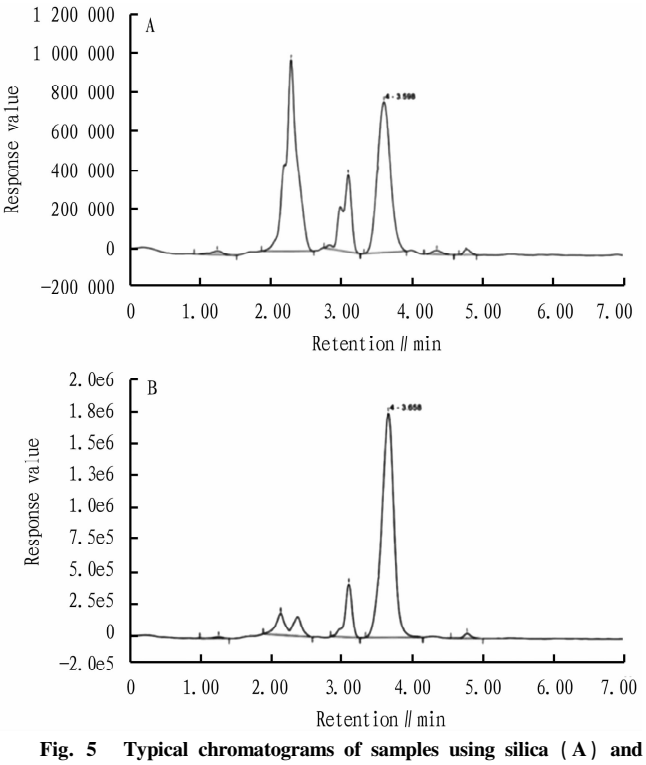

Fig. 5 Typical chromatograms of samples using silica (A) and C18 (B)

Method validation

The calibration curve, recovery rate and precision of carbendazim in tea infusion and leaves are shown in Fig. 6 and Table 1. As shown in Fig. 6, the calibration curve of carbendazim in tomato samples was $y = 155\ 023x + 176\ 969$ with the linear correlation coefficient (R^2) ranging from 0.993 7, which indicated the relation between peak area and carbendazim concentration displayed good linearity. Then, the recoveries of analytes in tomato spiked with three concentrations of carbendazim ranged from 88.7 to 93.3%. In addition, the *RSD* of five replicates for every group were all less than 7%. Hence, the data of recoveries and *RSD* elucidated that the analysis method was of great accuracy and precision.

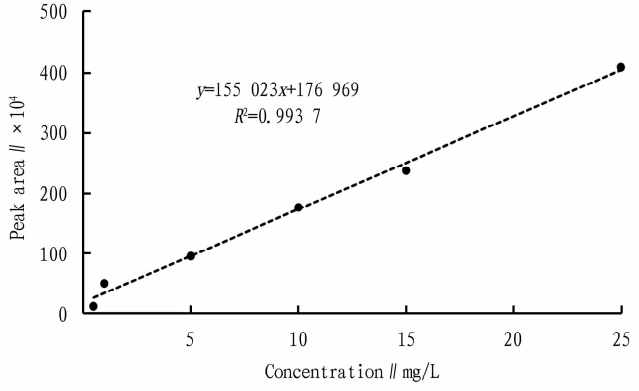

Fig. 6 Calibration curve of carbendazim in sample solution

Table 1 Recovery rate and precision of carbendazim in spiked tomato samples

Analyze	Spiked concentration // mg/kg	Recovery rate // %	*RSD* // %
Carbendazim	2.5	93.3	4.5
	5.0	91.4	2.6
	25.0	88.7	3.5

Conclusions

Carbendazim, as a commonly used systemic fungicide, was applied frequently in vegetables and fruits. In order to detect carbendazim in tomato samples, the analytical method via UPLC-FLD was developed, and the sample preparation process was optimized by studying the effects of extraction solvent, approach, time and purification absorbent on the recovery rate of carbendazim. The results showed the optimized method for analysis was ultrasonication-assisted extraction with acetonitrile for 1 min, and subsequent purification by C18. In this occasion, the established analytical method of carbendazim in tomato samples displayed good linearity, accuracy and precision.

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detected in this study had no impact on health.

To sum up, the problem of pesticide residue of clothianidin in Chinese chives is serious. Through the assessment of dietary intake risk, the pesticide residue was within the safe range. However, with the increasing demand for Chinese chives, farmers have increased the use of clothianidin and related pesticides in pursuit of yield and pest control, which has increased the pesticide residues to a certain extent, which is likely to pose unacceptable risks to human health. Therefore, it is suggested that relevant departments formulate the maximum residue limit standard of clothianidin in Chinese chives, enhance the supervision and management of pesticide use, and strengthen the detection and evaluation of pesticide residues.

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