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Determination of Thiamethoxam Residues in Tomato by High Performance Liquid Chromatography with UV Detection

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A simple and novel analysis method to detect thiamethoxam residues in tomato was developed using solid phase extraction coupled with high performance liquid chromatography with ultraviolet detection. The pesticide residues present in matrix were extracted with acetonitrile and purified by Pesticarb/NH_2 solid phase extraction cartridge. Mean recoveries for the analyte at the levels of 0.01, 0.05 and 0.10 mg kg^1 ranged from 83 to 85 % with relative standard deviations less than 7.5 % and limit of quantification was 0.01 mg kg^1. This method has been applied to analyze thiamethoxam in real samples of tomato. The half-life of thiamethoxam in tomato was 4.6 days and the residues in tomato were below the maximum residue limit of European union (0.1 mg kg^{-1}) after one day.

Key Words: Thiamethoxam, Tomato, Residue analysis, Solid phase extraction.

INTRODUCTION

Thiamethoxam, 3-(2-chloro-thiazol-5-ylmethyl)-5-methyl-[1,3,5]oxadiazinan-4-ylidene-*N*-nitro-amine (Fig. 1), is a new neonicotinoid insecticides with contact, stomach and systemic activity against sucking and chewing insects[1]. It binds at the postsynaptic nicotinic acetylcholine receptor and blocks the electron transfer between nerve cells. Thiamethoxam is registered for use in various crops and vegetables because of the broad-spectrum activity, therefore there is possibility that thiamethoxam residues may remain in the food of human. The maximum residue limits of thiamethoxam in tomato were 0.1 mg kg^{-1} in European Union, 0.2 mg kg^{-1} in Korea, 0.25 mg kg^{-1} in America and 0.5 mg kg^{-1} in Japan. There has been no maximum residue limit and safe interval of thiamethoxam in Chinese legislation yet.

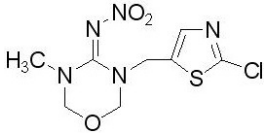

Fig. 1. Chemical structure of thiamethoxam

Numerous methods have been developed for determination of thiamethoxam residues in crops, vegetables, soils and environmental water by HPLC coupled with UV detector, diode-array detector and mass spectrometry[2-6]. Enzyme-linked immunosorbent assay have been applied to analyze thiamethoxam residues in fruits and honey samples[7-10]. Evaluation of the inhibition esterases activity on Apis mellifera has been used as bioindicators for insecticide thiamethoxam residues[11]. Solid-phase extraction, solid matrix partition, pressurized fluid extraction and microwave-assisted extraction were carried out for thiamethoxam residues determination[12-15]. The residue dynamics of thiamethoxam in tomato and soil were also examined. However, the pretreatment method was liquid-liquid extraction, which was time consuming, labor intensive and wasteful of large amounts of organic solvents[16].

The purpose of this study is to develop a simple, sensitive and selective method for quantification of thiamethoxam residues in tomato. Based on the residue analysis method, degradation behaviour and final residue of thiamethoxam in tomato were investigated at the same time. It would help Chinese government to establish maximum residue limit of thiamethoxam and make environmental safety assessment in tomato.

EXPERIMENTAL

Thiamethoxam standard (99.7 %) and its formulation (WG, 25 %) were provided by Syngenta China Co. Ltd. (Shanghai, China); All the reagents were of analytical-grade, except that methanol for HPLC analysis was chromatographic grade and all of them were purchased from Dikma Co. Ltd. (Beijing, China); Pesticarb/NH_2 (250 mg/250 mg) solid phase extraction cartridge were obtained from Agela Technologies (Beijing, China).

Field trials: Experiments were conducted in 2009 in a tomato garden located at Daxing County, Beijing, China. Each experimental plot was 30 m^2 and separated with isolation belt. One control plot and three replicate plots for each treatment were set.

In the dissipation field test, the formulation of thiamethoxam (WG, 25 %) was sprayed onto the tomato field by a portable motor prayer for one time at the high level of 84.38 g active ingredient per hectare, which was diluted with water. Tomato samples were collected at random on 0 (after 2 h of spray), 1, 2, 3, 5, 7, 10, 14, 21, 28 days after the treatments and stored in a deep freezer (-20 ºC) before further analysis.

In the final residue field test, the formulation of thiamethoxam (WG, 25 %) was sprayed for two and three times at the designed two dosages: 56.25 g (low level) and 84.38 g (high level) active ingredient per hectare in the same way. The interval of spray was 7 days. Tomato samples were collected at random at the different time intervals and stored in a deep freezer (-20 ºC) before further analysis.

Sample preparation: A 20 g aliquot of chopped and homogenized tomato sample was extracted with acetonitrile (50 mL) by shaking thoroughly in a 250 mL-flask for 0.5 h on a mechanical horizontal shaker and ultrasonic extraction for 0.5 h. The mixtures were filtered through a 12 cm-funnel and the solid residues were washed with another 20 mL of acetonitrile. The combined filtrate and 2 g of sodium chloride were transferred to a 250 mL-separatory funnel and then shaken for 0.5 min. After 2 min, the lower aqueous layer was discarded, the organic portions was filtered through anhydrous sodium sulfate and evaporated to dryness at 45 ºC on the rotary evaporator. The residue was dissolved in 2mL for further cleanup.

Purification procedure was performed on a Pesticarb/NH_2 solid phase extraction column. The Pesticarb/NH_2 column was preconditioned with 3 mL of acetonitrile, followed by 3 mL of toluene. 2.5 mL of residue was directly loaded onto the column and the effluent was discarded. The column was rinsed with 3 mL of toluene and the rinsing was also discarded. The analyte was eluted with 5 mL of toluene-acetonitrile(70/30, v/v). The collected eluate was evaporated to dryness at 45 ºC by rotary evaporator. The residue was dissolved in 1 mL of methanol-water (25/75, v/v) and then filtered through a 0.45 µm filter before HPLC analysis.

HPLC analysis: An Agilent 1100 series HPLC system (Agilent Technology) equipped with an UV detector and Agilent Chemstation software was used. The baseline separation of this compound by an C_{18} column (250 mm × 4.6 mm I.D., 5µm, kromasil 100 A, Dikma) was obtained with a mobile phase of methanol/water (25/75, v/v/v) with a flow rate of 1.0 mL/min. Wavelength for UV detection was 254 nm and temperature for chromatographic separation was 20 ºC. Under these conditions the retention time of thiamethoxam was about 10.2 min.

Standard preparation and calibration curve: The standard solutions of thiamethoxam (0.05, 0.5, 1.0, 5.0 and 10.0 mg L^{-1}) for linearity were prepared by diluting the stock standard solution. Calibration curves were generated by plotting peak area *versus* its concentration. All solutions were stored in a refrigerator at 4 ºC.

Recovery assay: Samples of untreated tomato were fortified with thiamethoxam standard solutions to reach concentration of 0.01, 0.05 and 0.10 mg kg^{-1} and then they were processed according to the above procedure. Five replicates for each concentration were conducted.

RESULTS AND DISCUSSION

Method validation: The linear calibration curve was obtained over the concentration range of 0.05-10.0 µg g^{-1} with a RSD of 0.999 (y = 69.48x - 1.13, R^2 = 0.999). The average recoveries of thiamethoxam in tomato were 83-85 % and the RSDs of the recovery data were 2.7-7.5 % (Table-1). All recoveries and RSDs were within the permissible range. The representative chromatograms for blank and blank tomato spiked with thiamethoxam (Fig. 2) showed that there was no impurity interference from the matrix. The limit of detection for thiamethoxam was 1.3×10^{-10} g which was set at a signal-to-noise (S/N) ratio of 3:1. The limit of quantification was 0.01 mg kg^{-1} in tomato in this method based on S/N ratio with 10:1 as the minimum.

TABLE-1
RECOVERIES OF THIAMETHOXAM IN TOMATO (n=5)

Spiked levels (mg kg^{-1})	Average recoveries (%)	RSD (%)
0.01	85	7.5
0.05	84	2.7
0.1	83	3.1

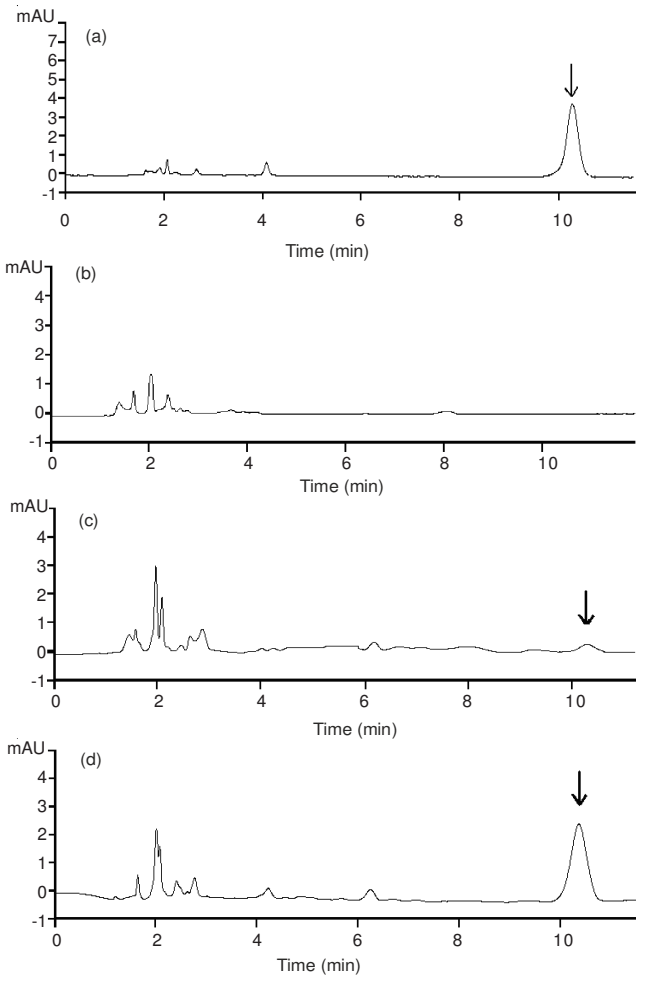

Fig. 2. Representative HPLC chromatograms of thiamethoxam. (A) thiamethoxam standard; (B) untreated tomato sample; (C) untreated tomato sample spiked with thiamethoxam (0.01 mg kg^{-1}); (D) real tomato sample

Dissipation of thiamethoxam in tomato: The dissipation of thiamethoxam residues in tomato followed first-order kinetics (y = 0.094e - 0.153x, R^2 = 0.963) and the half-life was 4.6 days (Fig. 3). The data during the dissipation was showed in Table-2. The average initial deposit of thiamethoxam was 0.079 mg kg^{-1}, which reached to a level of 0.011 mg kg^{-1} after 14 days and the residue was below limit detection after 21 days. The study showed that the dissipation of thiamethoxam in tomato under the field condition is fast.

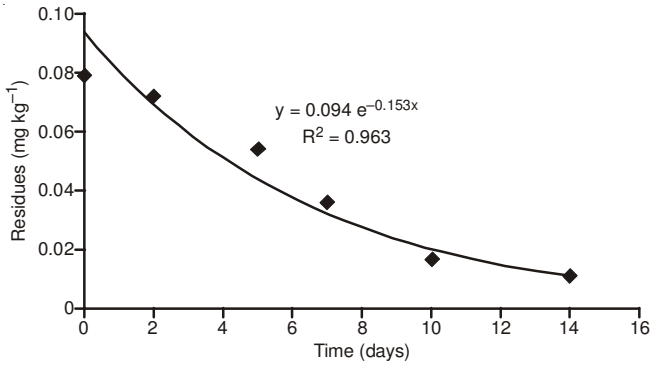

Fig. 3. Digestion dynamic curve of thiamethoxam in tomato

TABLE-2
RESIDUES OF THIAMETHOXAM IN TOMATO

1 (d)	Average residues (mg kg^{-1})	RSD (%)	Dissipation (%)
0	0.079	5.4	–
2	0.072	1.9	8.9
5	0.054	4.5	31.6
7	0.036	7.0	54.4
10	0.017	9.0	78.5
14	0.011	9.1	86.1
21	BLD[1]	–	> 90

[1]BLD: < 0.01 mg kg^{-1}

Final Residues of thiamethoxam in tomato: The final residues of thiamethoxam in tomato were listed in Table-3. The residues were between 0.02 to 0.09 mg kg^{-1} in tomato on the first two days. The maximum residue limits of thiamethoxam in tomato was 0.1 mg/kg (European Union) and there was not any more in Chinese legislation or food and agriculture organization/world health organization yet. According to maximum residue limit set by European Union, applying thiamethoxam in tomato was safety at harvest time and the safe interval was one day. This work would be helpful for the Chinese government to establish maximum residue limits of thiamethoxam in tomato and provide guidance on safe and proper use of the pesticide.

TABLE-3
FINAL RESIDUES OF THIAMETHOXAM IN TOMATO

Dosage [g(a.i.)·ha^{-1}]	Spraying times	Sampling time (d)	Final residue (mg kg^{-1})	RSD (%)
56.25	2	1	0.02	1.6
		2	0.02	9.5
	3	1	0.07	3.1
		2	0.05	8.0
84.38	2	1	0.08	2.0
		2	0.07	5.2
	3	1	0.09	6.8
		2	0.08	8.7

Conclusion

A residue analytical method has been developed and validated for determination of thiamethoxam in tomato. This method was characterized by recovery (between 83 and 85 %), RSDs (below 7.5 %), limit of detection (1.3×10^{-10} g) and limitof quantification (0.01 mg kg^{-1}). The results showed that the half-life of thiamethoxam in tomato was 4.6 days. The final residues of thiamethoxam in tomato were below the maximum residue limits (0.1 mg kg^{-1}, fixed by European Union) after one day, so it could be considered as safty for human beings and environment and the safe interval was one day. This proposed analytical procedure was fast, easy to perform and could be utilized for regular monitoring of thiamethoxam residues in tomato field.

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