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High Performance Liquid Chromatography Method for Residues Analysis of Thidiazuron in Apple and Soil

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Abstract A rapid, sensitive and reliable analytical method for thidiazuron residues in apple and soil was established. The residual levels of the pesticide in apple and soil were determined by high performance liquid chromatography (HPLC) with UV detector. Samples of apple and soil were extracted with acetonitrile–water solutions, and then cleaned up by Florisil or C_{18} cartridges. The results showed good linearity ($r^2 = 1.000$) over the concentration range of 0.01–5.0 mg/L. Limits of quantification (LOQ) of the method were 0.01 mg/kg for both soil and apple. Recovery from the apple and soil samples were 83.36%–84.08% and 85.27%–89.83%, respectively, and the corresponding relative standard deviations (RSDs) of the recovery data were 0.155%–0.524% and 0.475%–4.79% for the three fortified levels (0.01, 0.1, 0.5 mg/kg). The analyte in the samples were further confirmed by electrospray ionization tandem mass spectrometry (ESI–MS/MS). It was demonstrated that the proposed method was simple and efficient, and particularly suitable for detecting thidiazuron residues in apple and soil.

Keywords Thidiazuron · Residues determination · Solid-phase extraction · High performance liquid chromatography with UV detector

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Thidiazuron [1-phenyl-3-(1,2,3-thidiazol-5-yl)urea] (Fig. 1), is a plant growth regulator developed by Schering AG (now Aventis Crop Science). It has been used to stimulate high rate of axillary shoot proliferation on many woody plant species (Fiola et al.1990; Malik and Saxena 1992). According to Capelle et al. (Capelle et al. 1983), thidiazuron directly promotes growth due to its own biological activities in a fashion similar to that of an N-substituted cytokinin or it may induce the synthesis and accumulation of an endogenous cytokinin. In China, thidiazuron is being investigated for promoting growth of apple.

To the best of our knowledge, no analytical methods are published in literatures to determine thidiazuron residues in apple. Although thidiazuron residues have been reported in some environmental materials, such as water (Potter et al. 2000), soil (Guo et al. 2010), cotton seed (Cai et al. 2009; Guo et al. 2005), melon (Wang et al. 2010). Therefore, the development of a readily applicable method for residual analysis of thidiazuron in apple is necessary. In this paper, a simple HPLC–UV method combined with solid phase extraction was established to detect the residues of thidiazuron in apple and soil. The residues of thidiazuron in the samples were further confirmed by electrospray ionization tandem mass spectrometry (ESI–MS/MS).

Materials and Methods

Thidiazuron standard (99.5% purity) was provided by Jiangsu Huifeng Agrochemical Co., Ltd. (Jiangsu, China). All the reagents were analytical-grade, except that methanol for HPLC analysis was chromatographic-grade, and all of them were purchased from Dikma Limited (China). SPE columns (Florisil, 500 mg, 3 mL; C_{18}, 500 mg, 3 mL) were purchased from Agela Technologies (Beijing, China).

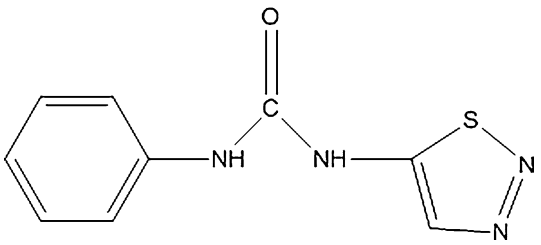

Fig. 1 Chemical structure of thidiazuron

High Performance Liquid Chromatography (Shimadzu LC-20AT) equipped with an analytical column (250 mm × 4.6 mm I.D., 5 μm C_{18}) attached to a UV detector. The chromatographic conditions used for the analysis of thidiazuron residues were as follows: the mobile phase was methanol: water (60/40, *v/v*) with a total flow of 0.9 mL/min. The injection volume was 20 μL for all samples. Detection was performed at 290 nm. Under these conditions, the retention time of thidiazuron was about 11.87 min. All measurements were carried out at room temperature.

ESI–MS/MS analysis was performed in negative mode (ESI+). The source conditions were typically as follows: capillary voltage 3 kV, source temperature 120°C and desolvation temperature 350°C. The cone and desolvation gas flows were 50 and 500 L/h, respectively. The acquisition was performed in full-scan mode in the ranges *m/z* 50–300.

Standard solution (400 mg/L) of thidiazuron was prepared in methanol. Working calibration solutions of 0.01, 0.05, 0.1, 0.5, 1.0, 2.0, 5.0 mg/L were made by serial dilutions. Working solutions between 0.01 and 5.0 mg/L were suitable for recovery assay and dissipation study. All these solutions were protected against light with brown containers and were stored in a refrigerator at 4°C.

Apple sample: 20 g sample of apple homogenized was extracted with 110 mL acetonitrile–water (10/1, *v/v*) by shaking thoroughly in a 250 mL conical flask for 1 h on a mechanical horizontal shaker. The extracts were filtered through a filter paper and washed with another 30 mL acetonitrile–water (10/1, *v/v*), and then the supernatant was combined and transferred quantitatively to a 250 mL separator funnel. 5 g NaCl was added to the separator funnel, and shaken for 3 min. The acetonitrile layers were combined, dehydrated with anhydrous sodium sulfate, and evaporated to near dryness with a vacuum rotary evaporator at 45°C. Then the extract was made to dryness under a gentle nitrogen stream, 5 mL of acetone-petroleum ether (3/7, *v/v*) was added to dissolve the sample before purification by Florisil cartridge. The Florisil cartridge was conditioned with 4 mL acetone-petroleum ether (3/7, *v/v*). 3 mL extract solution was loaded onto the cartridge and the eluate was discarded. The analyte was eluted with 6 mL acetone-petroleum ether (4/6, *v/v*) and the eluate was concentrated to near dryness at 40°C by a vacuum rotary evaporator and completely dried under a nitrogen purge. The resulting residue was re-dissolved in 2 mL methanol for HPLC–UV analysis.

Soil Sample: 20 g sample of soil was was extracted with 90 mL acetonitrile–water (8/1, *v/v*) by shaking thoroughly in a 250 mL conical flask for 1 h on a mechanical horizontal shaker. The extracts were filtered through a filter paper and washed with another 30 mL acetonitrile–water (10/1, *v/v*), and then the supernatant was combined and transferred quantitatively to a 250 mL separator funnel. 5 g NaCl was added to the separator funnel, and shaken for 3 min. The acetonitrile layers were combined, dehydrated with anhydrous sodium sulfate, and evaporated to near dryness with a vacuum rotary evaporator at 45°C. Then the extract was made to dryness under a gentle nitrogen stream, 3 mL of methanol–water (3/7, *v/v*) was added to dissolve the sample before purification by C_{18} cartridge. The C_{18} cartridge was conditioned with 2 mL methanol, followed by 4 mL methanol–water (3/7, *v/v*). 2 mL extract solution was loaded onto the cartridge and the eluate was discarded. The cartridge was washed with 2 mL methanol–water (3/7, *v/v*), and the eluate was discarded. The analyte was eluted with 2 mL methanol–water (7/3, *v/v*) and the eluate was concentrated for HPLC–UV analysis.

Results and Discussion

Quantification was accomplished by using standard curves constructed by plotting corresponding analyte concentrations against peak areas under proposed chromatographic conditions. A good linearity was achieved from 0.01 to 5.0 mg/L with $R^2 = 1.000$. The standard curve equation was $y = 47{,}711.103x + 617.302$, where $y =$ peak area, $x =$ thidiazuron concentration.

The fortified recovery experiment was set at three concentration levels (0.01, 0.1, 0.5 mg/kg), and with three repetitions at each level. Blank analyses were performed in order to check interference from the matrix. As shown in Table 1, the fortified recoveries of thidiazuron from soil and apple samples were 85.27%–89.83% and 83.36%–84.08%, respectively; and the relative standard deviations (RSDs) of the recovery data were 0.475%–4.59% and 0.155%–0.524%. All the recoveries and RSD values were within the permissible range. The representative chromatograms for apple and soil spiked with 0.1 mg/kg of thidiazuron and blank were shown in Fig. 2. According to the Fig. 2b, d, there have shown no peak in blank samples at the retention time. So that it was hardly any signal interference from the matrix. Confirmation tests by ESI–

Table 1 The recoveries of thidiazuron in apple and soil

Sample type	Amount added (mg/kg)	Recovery (%)				RSD (%)
		1	2	3	Average	
Soil	0.5	89.34	90.03	90.12	89.93	0.475
	0.1	93.13	88.86	84.96	88.98	4.59
	0.01	84.03	86.36	85.43	85.27	1.38
Apple	0.5	84.37	83.57	84.29	84.08	0.524
	0.1	83.67	83.34	83.06	83.36	0.366
	0.01	83.76	83.63	83.89	83.76	0.155

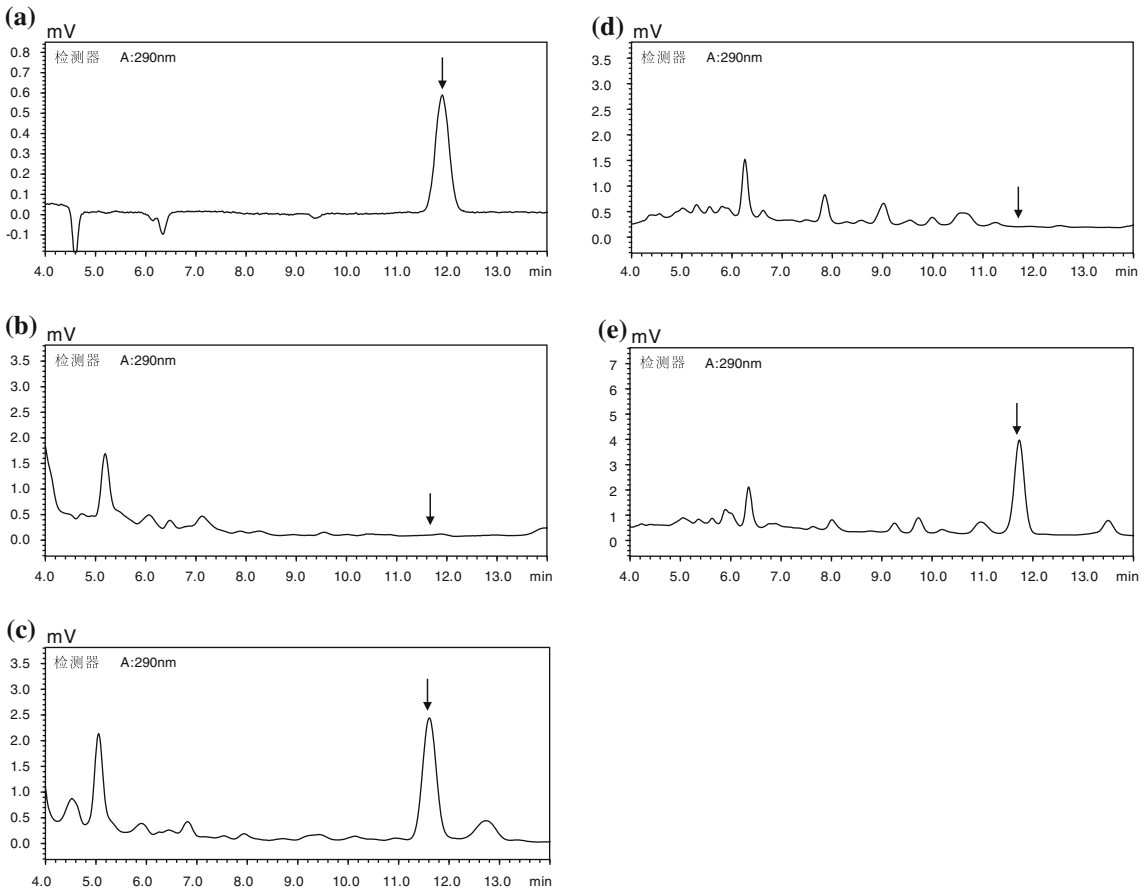

Fig. 2 Chromatograms obtained from the target pesticide: **a** thidiazuron standard solution, **b** blank apple sample, **c** apple spiked with thidiazuron at 0.1 mg/kg, **d** blank soil sample, **e** soil spiked with thidiazuron at 0.1 mg/kg

MS/MS were used to determine whether or not peaks detected at the retention time of the analyte were in the samples. Each isomer of thidiazuron was identified by its retention time and the specific molecule ion peak ($[M+H]^+$at *m/z* 221, $[M–C_7H_4NO]^+$at *m/z* 102 and $[M–C_8H_7N_2O]^+$at *m/z* 73) in MS–MS according to the proposed conditions (Fig. 3).

The limit of detection (LOD) for thidiazuron was estimated to be 0.075 ng, based on signal to noise ratio 3/1. The limit of quantification (LOQ) of thidiazuron in apple and soil were both 0.01 mg/kg in this method. There has been no MRL (Maximum Residue Limit) of thidiazuron in apple legislated by Chinese legislation or FAO/WHO yet, while the MRL of thidiazuron in grape has been set at 0.2 mg/kg by Korea (Zhuang 2010). According to that, the quantification limit of 0.01 mg/kg was suitable for residue analysis of thidiazuron in samples.

Due to the particular physical and chemical properties of thidiazuron, it is not easy to find a suitable extraction solvent to effectively extract thidiazuron from the matrix components. Usually acetonitrile and acetone were used to extract thidiazuron from different samples. In this study,

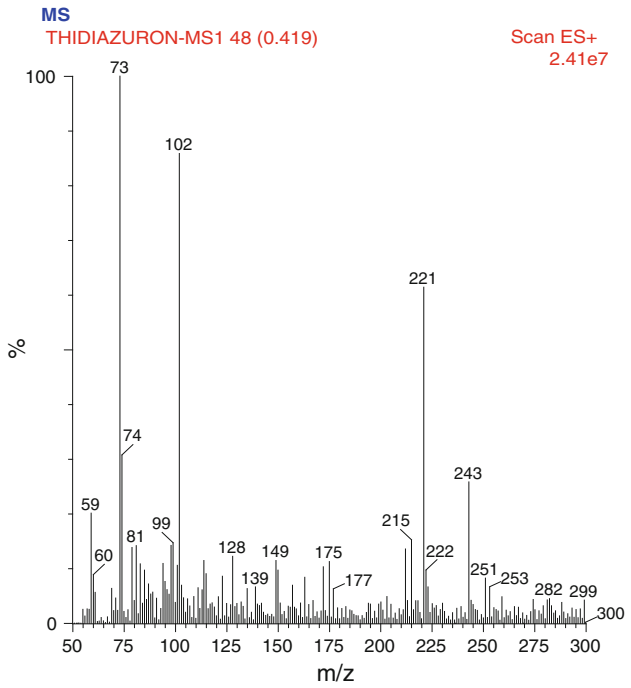

Fig. 3 Mass spectra of thidiazuron in the sample

the fortified recoveries were blow 63.00% when extracted with acetone–water solutions. Acetonitrile–water solutions in different ratios were also investigated. In ratios 5/1 (*v/v*) and 6/1 (*v/v*), the fortified recoveries were both blow 70.45%, but in 8/1 (*v/v*) the recoveries were above 89.78%. For the apple sample, acetonitrile–water (10/1, *v/v*) can make the recoveries was above 87.54%. The results showed that the extraction method was reliable for the residue analysis of thidiazuron in apple and soil samples.

In order to improve the selectivity and reproducibility of the method, cleanup procedures were implemented after the extraction process. Solid-phase extraction (SPE) was used in this method. It is a simple preparation technique based on the separation of liquid chromatography, where the solubility and functional group interactions of sample, solvent and sorbent are optimized to effect the retention and elution (Tekel and Hatrfk 1996). For the apple sample, different sorbents were tested for SPE, such as Florisil and PSA. Poor recoveries were achieved (<51.07%) using PSA cartridges, but best results were obtained using Florisil. The pesticide was not retained by Florisil when eluted with acetone/petroleum ether (4/6, *v/v*) while many interfering matrixes were adsorbed onto the solid phase. It indicated that Florisil provide sufficient cleanup for the crude extract of apple. As for soil cleanup, C_{18} was found to eliminate most interfering peaks and allowed good recoveries at low fortification levels.

A rapid and simple HPLC method was developed and validated for the determination of thidiazuron residues in apple and soil. The proposed method involves optimization of the extraction process, SPE for purification, and HPLC–UV analysis. It showed satisfactory validation parameters in terms of linearity, accuracy, precision and selectivity. And the residues were further confirmed by ESI–MS/MS. As a result of this study the procedure could be utilized for regular monitoring of thidiazuron residues in apple and soil.

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