



# Supercritical fluid chromatographic-tandem mass spectrometry method for monitoring dissipation of thiacloprid in greenhouse vegetables and soil under different application modes

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## ABSTRACT

A rapid, sensitive and effective supercritical fluid chromatographic-tandem mass spectrometry (SFC-MS/MS) method was developed to analyze thiacloprid for the first time. The SFC-MS/MS conditions were optimized with the ultra-performance convergence chromatography (UPC<sup>2</sup>) BEH column (100 mm × 3.0 mm, 1.7 μm particle size) and thiacloprid was eluted at 1.22 min in gradient mode with CO<sub>2</sub>/methanol as mobile phase. The 0.1% formic acid in methanol (v/v) was used as postcolumn compensation solution to improve sensitivity. The ABPR pressure, flow rate of mobile phase and flow rate of compensation pump were set at 1800 psi, 1.8 mL/min, and 0.1 mL/min, respectively. The average recoveries of thiacloprid in soil at four spiking levels (5, 10, 100, 1000 μg/kg) ranged between 78.8% and 107.1% with relative standard deviations (RSDs) lower than 12.2% and the limit of quantitation (LOQ) was 5 μg/kg. The proposed method can distinctly improve the analysis efficiency by 2–12 times and reduce the solvent consumption by 5%–95% compared with reported methods. It was applied to investigate the dissipation rates of thiacloprid in greenhouse vegetables and soil under different application modes. The half-lives of thiacloprid in cucumber and soil were 9.55–20.44 days and 3.74–9.14 days separately under different application modes, 10.60 days in tomato under foliar spraying. The residues in vegetables under root irrigation were all less than that under foliar spraying. The results could offer useful data for risk assessment of thiacloprid in agricultural production.

## 1. Introduction

Tomato (*Lycopersicon esculentum* Mill.) and cucumber (*Cucumis sativus* Linn.) are important vegetable products which widely cultivated in greenhouse worldwide [1,2]. The greenhouse production system is high profitable and productive which has become the mainstream of cultivation mode of tomato and cucumber in facility agriculture. It provides higher planting density and lower light intensity for planting greenhouse vegetable than open fields, however, the condition can cause serious occurrence of pests and diseases because of the high temperature and moisture in greenhouse that lots of pesticides are applied [3,4].

Thiacloprid ((Z)-3-(6-chloro-3-pyridylmethyl)-1,3-thiazolidin-2-

ylidenecyanamide) (Fig. 1) belongs to the second generation neonicotinoid pesticides and is the first chloronicotinyl insecticide which has strong insecticidal activity against sucking and chewing insects such as aphids, whiteflies, weevils, etc. [5]. In addition, recent study showed that thiacloprid could effectively control root-knot nematode (*Meloidogyne* spp.) in cucumber [6]. Thiacloprid is selective agonists of nicotinic acetylcholine receptor (nAChRs) in nervous system of insects and pervasively toxic to insects in minute quantities, also it is systemic insecticide and could protect all parts of the plant. Many neonicotinoids such as imidacloprid, clothianidin, thiamethoxam have been temporarily banned by the European Commission (2013) [7]. Moreover, those three neonicotinoids are persistent and would be accumulated in soil after continuous applications [8]. The cyano-containing neonicotinoid

**Abbreviations:** SFC-MS/MS, supercritical fluid chromatographic-tandem mass spectrometry; UPC<sup>2</sup>, ultra-performance convergence chromatography; HPLC, high-performance liquid chromatography; UPLC, ultrahigh-performance liquid chromatography; ELISA, enzyme-linked immunosorbent assay; MEKC, micellar electrokinetic chromatography; PSA, primary secondary amine; GCB, graphitized carbon black; RCF, relative centrifugal force; ESI, electrospray ionization; MRM, multiple-reaction monitoring; LOQ, limit of quantification; ABPR, auto back pressure regulator; RSD, relative standard deviation; T<sub>1/2</sub>, half-life

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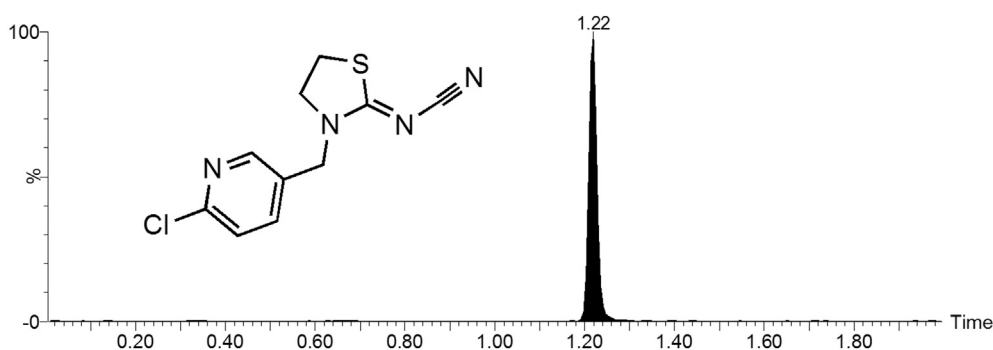


Fig. 1. The chemical structure and chromatogram of thiacloprid.

thiacloprid is less toxic to honeybees than nitro-containing neonicotinoids (imidacloprid, clothianidin, thiamethoxam) and many formulations based on thiacloprid are still allowed to be sprayed on flowering crops, however, it has behavioral effects towards honeybees [9–11]. Thus, droplet drift of thiacloprid under foliar spraying may have potential risk to beneficial insects. The maximum residue limits (MRLs) of thiacloprid in tomato and cucumber were all 0.5 mg/kg regulated by European Union.

The analytical methods for thiacloprid on plants and environmental matrices have been established in many studies using high performance liquid chromatography with diode-array detection (HPLC-DAD), liquid chromatography-electrospray mass spectrometry (LC-MS), liquid chromatography-tandem mass spectrometry (LC-MS/MS), liquid chromatography/time-of-flight mass spectrometry (LC/TOF-MS), high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS), ultrahigh-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS), micellar electrokinetic chromatography (MEKC), enzyme-linked immunosorbent assay (ELISA), Time-resolved fluorescence immunoassay (TRFIA), etc. [12–21]. Chromatographic methods for separation and determination of thiacloprid were dominant among all the analytical methods, and immunoassays such as ELISA and TRFIA are useful for preliminary screening analytes which supplement chromatographic methods. Supercritical fluid chromatography-tandem mass spectrometry (SFC-MS/MS) is a complementary and high-performance analytical technique. The supercritical fluid (carbon dioxide, CO<sub>2</sub>) is chosen as the primary mobile phase for SFC-MS/MS, which is non-toxic, non-explosive, non-reactive, easy acquired, low cost and has high miscibility with organic solvents [22,23]. The use of SFC can reduce the analytical time and consumption of organic solvents comparing to LC procedures, which benefits from the low viscosity and high diffusivity of CO<sub>2</sub> [24,25]. SFC is appropriate for analysis of non-polar compounds, however, the addition of organic modifier or co-solvent could increase the solubility of non-polar and polar compounds in the mobile phase and improve the peak shape [25,26]. Furthermore, the sensitivity is improved by adopting post-column polar solvent compensation technology in combination of SFC and mass spectrometry (MS), and large proportion of CO<sub>2</sub> facilitates evaporation during ionization procedure [27]. All these advantages have aroused an increased interest for the application of SFC-MS/MS nowadays. To the best of our knowledge, study of thiacloprid based on SFC-MS/MS has not been reported.

The studies of degradation behavior of pesticide could provide essential data for evaluating its insecticidal effect, ensuring food safety and assessing environmental risk [28]. The degradation rates of thiacloprid in greenhouse tomato leaves, stems and soil after soil application were described in previous study [29]. Moreover, dissipation kinetics of thiacloprid in tomato and soil under foliar spraying were reported [30,31]. The dissipation rate of thiacloprid in cucumber after foliar spraying was investigated according to literature [20]. However, the system research on the field dissipation study of thiacloprid on greenhouse vegetables considering different application modes was

limit. The purpose of this study was to establish a SFC-MS/MS method for monitoring thiacloprid in cucumber, tomato and soil and apply the method to explore the pharmacokinetic degradation of thiacloprid under foliage spraying and root irrigation. The results could provide essential data and guidance for proper use of thiacloprid.

## 2. Material and methods

### 2.1. Chemicals and reagents

Thiacloprid standard (98.5% purity) was purchased from China Standard Material Center (Beijing, China). The thiacloprid commercial product applied in the working area was 22% thiacloprid and spirotetramat suspension concentrate (containing 11% thiacloprid) which was obtained from Bayer corporation (Leverkusen, Germany), the commercial name of the used formulation was Wente. Chromatographic grade methanol and acetonitrile were acquired from Sigma-Aldrich (Steinheim, Germany). Chromatographic grade formic acid was purchased from Thermo Fisher Scientific (Waltham, MA, USA) respectively. Analytical grade sodium chloride (NaCl), anhydrous magnesium sulfate (MgSO<sub>4</sub>) and acetonitrile were purchased from Beihua Finechemicals (Beijing, China). Ultra-pure water was prepared from a Milli-Q system (Bedford, MA, USA). CO<sub>2</sub> (99.999% purity), N<sub>2</sub> (99.95% purity) and Ar (99.999% purity) were obtained from Haike Yuanchang Gas (Beijing, China). Sorbents including Primary secondary amine (PSA, 50 μm) and graphitized carbon black (GCB, 120–400 mesh) were purchased from Bonna-Agela Technologies (Tianjin, China).

The standard stock solutions (100 mg/L) of thiacloprid were prepared in pure acetonitrile. The standard working solutions and matrix-matched standard solutions (5, 10, 50, 100, 500 and 1000 μg/L) required for construction of calibration curves were prepared from standard stock solutions by serial dilution with pure acetonitrile and blank matrix extraction, respectively. The blank matrix including vegetables and soil were collected from the control plot. All solutions were stored in the refrigerator in the dark at 4 °C.

### 2.2. Field trial and sample collection

The field experiments of tomato and cucumber were carried out under solar greenhouse conditions according to the guidelines for pesticide residue trials (NY/T 788–2004), which was issued by the Ministry of Agriculture of the People's Republic of China [32]. Tomato and cucumber seeds were purchased from Botong Nongyi seeds (Beijing, China) and were cultivated in trial plots (Langfang, China, 116.8°E, 34.1°N) of the Institute of Plant Protection, Chinese Academy of Agricultural Sciences. The temperature and moisture were 22 ± 5 °C and 70–90% in the solar greenhouse during experiment, respectively. The physicochemical properties of soil were as follows: organic matter (1.2%), pH (7.8), soil texture (15.1% sand, 45.1% silt, 39.8% clay). The trial plots had no history of thiacloprid application or other compounds with similar structure to thiacloprid. Four 30-m<sup>2</sup>-sized trial plots (three

replicates and one control) were selected for each experimental set and a buffer zone was designed between the trial plots.

In fruiting period, the thiacloprid commercial product was applied at the dose of 216 g of active ingredient per hectare (1.5 times of the recommended dosage according to NY/T 788–2004 [32]) by foliage spraying and root irrigation. Approximately 1000 g of representative samples (tomato, cucumber and soil) from each trial plot were collected at day 0 (2 h after application), and 1, 3, 5, 7, 10, 14, 21, 28 and 35 days after treatment. The soil samples were taken from the depth of the soil surrounding the roots, the fruit samples were chopped and homogenized. All samples were stored in dark at  $-20^{\circ}\text{C}$  until analysis.

### 2.3. Instrumental parameters

Supercritical fluid chromatography system for thiacloprid analysis was performed on a Waters UPC<sup>2</sup> system (Waters, Milford, MA, USA), which consists of an UPC<sup>2</sup> binary solvent manager, an UPC<sup>2</sup> sample manager, an UPC<sup>2</sup> convergence manager, the Waters 515 compensation pump and an UPC<sup>2</sup> column manager equipped with a UPC<sup>2</sup> BEH column (100 mm  $\times$  3.0 mm, 1.7  $\mu\text{m}$  particle size) (Waters, Milford, MA, USA). The mobile phase was composed of CO<sub>2</sub> (solvent A) and methanol (solvent B) which was pumped at a flow rate of 1.8 mL/min. The gradient elution program was as follows: 0.0 min (95% A), 0.2 min (76% A), 1.5 min (76% A), 1.6 min (95% A), 2.0 min (95% A). The injection volume was 1  $\mu\text{L}$  and the temperatures of the column heater and sample manager were kept at  $40^{\circ}\text{C}$  and  $5^{\circ}\text{C}$  separately.

The Xevo-triple quadrupole (Xevo-TQD) mass spectrometer (Waters, Milford, MA, USA) was used for qualitative and quantitative analysis of thiacloprid which is equipped with an electrospray ionization (ESI) source and performed in the positive ionization mode. The nebulizer gas (99.95% nitrogen) and the collision gas (99.99% argon) were set at a pressure of  $2 \times 10^{-3}$  mbar in the T-Wave cell. The source parameters were optimized and performed as follows: the capillary voltage was 3.5 kV, the source and desolvation temperatures were set as  $150^{\circ}\text{C}$  and  $500^{\circ}\text{C}$ , respectively. The cone gas and desolvation gas flows were held at 50 L/h and 1000 L/h separately.

Multiple reaction monitoring (MRM) was applied to the detection of thiacloprid with a dwell time of 163 ms per ion pair and the specific MS/MS parameters were as follows: a  $m/z$  253.0 was chosen as the precursor ion, the cone voltage was set to 24 V. The  $m/z$  126.0 and  $m/z$  90.0 were selected as quantitative ion and qualitative ion, and the corresponding collision energy were optimized as 20 V and 30 V separately. Masslynx NT v.4.1 (Waters Corp.) software was used to analyze the obtained data.

### 2.4. Sample preparation

The extraction and purification procedures were performed based on QuEChERS methodology according to the method described in previous literature [12]. The frozen samples were thawed at room temperature, then a portion of 10 g homogenized samples were weighed into 50 mL PTFE centrifuge tubes with screw caps. Next, ultra-pure water (5 mL) and 0.2% (v/v) formic acid acetonitrile (10 mL) were added for soil, 10 mL acetonitrile were added into fruit sample tubes. The samples were shaken vigorously for 10 min at oscillation frequency of 1350 per minute (CK-2000 high-throughput grinder, TH Morgan, Beijing, China). Then, 1 g NaCl and 4 g anhydrous MgSO<sub>4</sub> were added to the tubes followed by an additional shaking for 5 min and centrifuged for 5 min at 2588  $\times$ g relative centrifugal force (RCF). Subsequently, 1.5 mL acetonitrile supernatant was transferred into a 2 mL micro-centrifuge tube containing sorbents (50 mg PSA + 150 mg anhydrous MgSO<sub>4</sub> for soil, 50 mg PSA + 10 mg GCB + 150 mg anhydrous MgSO<sub>4</sub> for tomato and cucumber). Afterwards, the samples were vortexed for 1 min and centrifuged for 5 min at 2400  $\times$ g RCF. Finally, the resulting supernatant was filtered into an autosampler vial by a 0.22- $\mu\text{m}$  nylon syringe filter for SFC-MS/MS injection.

### 2.5. Method validation

The method was validated according to European Union SANTE/11813/2017 regulatory guidelines [33], the evaluated parameters including selectivity, linearity, matrix effect, limit of quantitation (LOQ), accuracy, precision and stability. The blank samples (cucumber, tomato, soil) were analyzed to verify that no interference peak existed around the retention time of thiacloprid. The linearity was evaluated by analyzing the standard working solutions and matrix-matched standard solutions in triplicate ranging from 5 to 1000  $\mu\text{g/L}$ . And several parameters including slope, intercept and correlation coefficients ( $R^2$ ) were calculated. Matrix effect was determined by following equation: matrix effect (%) = [(slope of calibration curves in matrix – slope of calibration curve in solvent)/slope of slope of calibration curve in solvent]  $\times$  100%. The LOQs was defined as the minimum concentrations which provide satisfactory recoveries (70%–120%) with relative standard deviations (RSDs)  $< 20\%$ .

Recovery experiments were performed to evaluate the accuracy and precision of the method. Five replicates of spiked blank soil samples (10 g for each) at four spiked levels (5, 10, 100, 1000  $\mu\text{g/kg}$ ) were prepared in three days. The samples were vortexed for 30 s and left for 30 min to ensure that thiacloprid can penetrate into the soil uniformly. The extraction and purification procedures were carried out which were described above. The stability of the standard working solutions and matrix-matched standard solutions was tested monthly by injecting a newly prepared working solution. All the samples used in stability experiment was stored at  $-20^{\circ}\text{C}$ . The concentration of stock solution was compared with newly prepared solution through Student's paired  $t$ -test at 95% probability.

### 2.6. Data analysis

The degradation kinetics of thiacloprid in tomato, cucumber and soil were estimated according to the first-order kinetic equation, the degradation rate constants ( $K$ ) and half-life ( $T_{1/2}$ ) were calculated using following equations [34]:

$$C = C_0 e^{-Kt} \quad (1)$$

$$T_{1/2} = \ln 2/K \quad (2)$$

where  $C_0$  and  $C$  indicate the concentrations of the thiacloprid at time 0 and time  $t$ , respectively.  $K$  is the degradation rate constant. The concentrations in different matrix were calculated by external matrix-matched standard regression equations. The retention factor ( $k$ ) of thiacloprid was calculated by the following equation [34]:

$$k = (t - t_0)/t_0 \quad (3)$$

where  $t$  is the retention time of thiacloprid and  $t_0$  is void time ( $t_0 = 0.31$  min), which is determined using 1,3,5-tri-*tert*-butylbenzene under the chromatographic condition described above.

SAS 9.4 (SAS Institute, Beijing, China) was used for data analysis, and statistically significant difference was observed if the  $P$  value was  $< 0.05$  by comparing three corresponding replicates. The data of the replicates were attained by calculating the average value of the three replicate injections.

## 3. Results and discussion

### 3.1. Optimization of the SFC-MS/MS conditions

Methanol was chosen as the co-solvent considering it has the higher elutropic strength and better ionization for MS detection than other alcohols and better chromatographic performance compared to acetonitrile in SFC separation [35]. The higher flow rates resulted in shorter retention time, the flow rate was set at 1.8 mL/min considering the allowable pressure of column. To achieve shorter retention time and

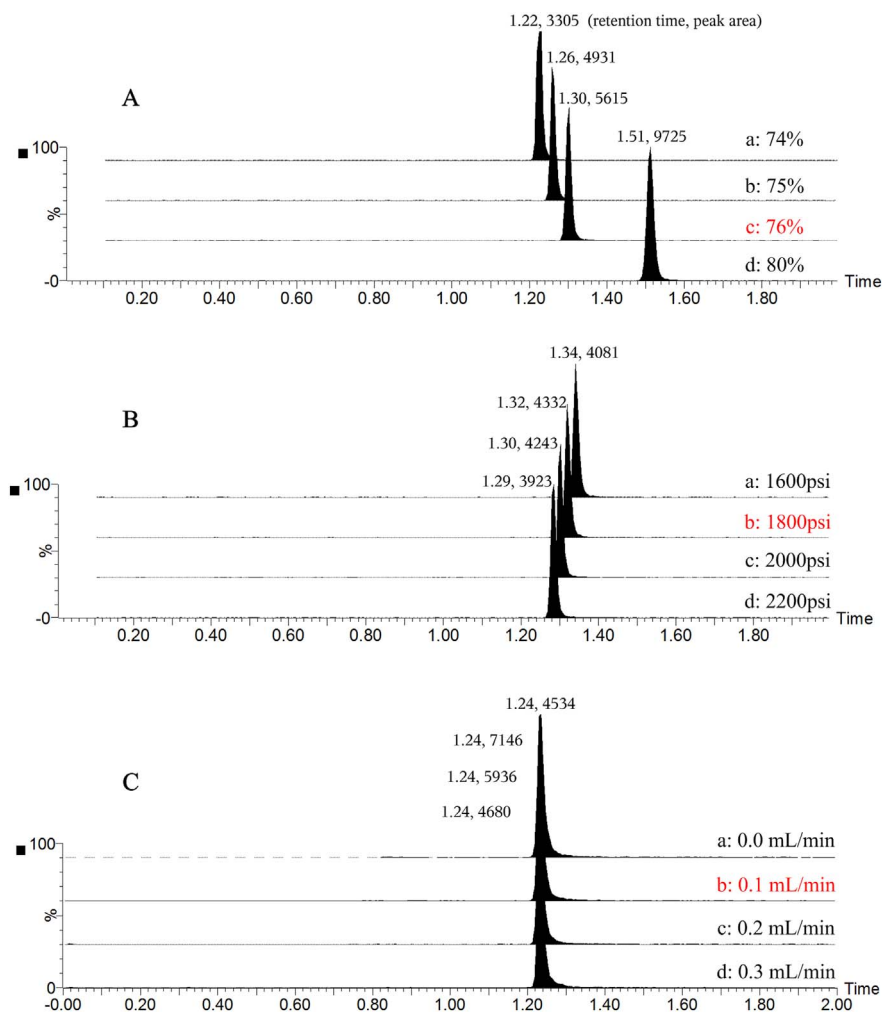


Fig. 2. Comparison of effects of different SFC-MS/MS parameters (A. the proportion of co-solvent at 0.2–1.5 min in the gradient elution program; B, ABPR pressure; C, flow rate of 1515 compensation pump), the concentration of thiocloprid for optimization was 100  $\mu\text{g}/\text{kg}$ .

Table 1

Recovery and relative standard derivation (RSD) of thiocloprid in soil at different spiked levels.

Compound	Matrix	Spiked level ( $\mu\text{g}/\text{kg}$ )	Intraday (n = 5)						Interday (n = 15)	
			Day 1		Day 2		Day 3			
			Average recoveries (%)	RSD <sup>a</sup> (%)	Average recoveries (%)	RSD <sup>a</sup> (%)	Average recoveries (%)	RSD <sup>a</sup> (%)	RSD <sup>b</sup> (%)	
Thiocloprid	Soil	5	96.7	7.7	106.5	6.0	107.1	7.3	8.1	
		10	104.6	9.0	96.5	7.5	99.6	8.8	8.6	
		100	83.4	6.0	78.8	6.4	100.3	5.4	12.2	
		1000	104.1	4.8	98.2	7.6	92.1	9.7	8.6	

<sup>a</sup> Intraday RSD (n = 5).

<sup>b</sup> Interday RSD (n = 15).

better resolution on the UPC<sup>2</sup> BEH column, the proportion of co-solvent and auto back pressure regulator (ABPR) pressure were investigated (Fig. 2), which are important parameters affecting the chromatographic separation by influencing the density and solubility of mobile phase [36]. The retention time was decreased when the modifier proportion of mobile phase increased from constant 10% (0.2–1.5 min) to constant 26% (0.2–1.5 min) in the gradient elution program in one injection. However, when the constant proportion of co-solvent content changing from constant 20% to constant 26% at 0.2–1.5 min in one injection, the MS signal intensity was in decline. We chose 24% Methanol in mobile phase at 0.2–1.5 min to get relatively satisfied MS signal intensity and retention time of thiocloprid. The ABPR pressure varied from 1600 psi

to 2200 psi in 200 psi increments. The retention time was slightly decreased when ABPR pressure increased due to the enhanced density of mobile phase, because the impact of ABPR is more limited in presence of high proportion of methanol which is less compressible fluid. Furthermore, the flow rate of post-column compensation pump solvent was also evaluated from 0.0 mL/min to 0.3 mL/min. The 0.1% formic acid in methanol (v/v) was used as compensation solution, which was used to improve the MS signal response of thiocloprid to some extent. The retention time was not influenced by use of post-column compensation and the highest signal intensity was achieved at 0.1 mL/min (Fig. 2). This phenomenon was caused by the diluting effect on thiocloprid exceeding the ionization enhancement effect of compensation solvent at

**Table 2**  
Comparison of matrix-matched calibration and solvent calibration of thiacloprid (5–1000 µg/kg).

Matrix	Regression equation	R <sup>2</sup>	Slope ratio <sup>a</sup>	Matrix effect (%)	LOQs (µg/kg)	MRL (µg/kg)
Acetonitrile	y = 12.85x + 59.09	0.9992	–	–	–	–
Soil	y = 14.38x + 133.84	0.9968	1.12	+11.87	5	–
Tomato	y = 9.30x + 85.84	0.9999	0.72	–27.67	10	500 (EU)
Cucumber	y = 9.39x + 28.60	0.9968	0.73	–26.93	10	500 (EU)

<sup>a</sup> Slope ratio = matrix/ACN.

high flow rates [37]. The ABPR pressure, flow rate, flow rate of 515 compensation pump were set at 1800 psi, 1.8 mL/min, 0.1 mL/min separately, which were applied to obtain shorter retention time, better peak shape and satisfied signal intensity by comprehensive consideration. The analysis time of thiacloprid was < 1.30 min by using SFC-MS/MS and the retention factor (k) was 2.94 under the selected conditions. The retention time of thiacloprid was in loss ranging from 1.22 min to 1.21 min during the consecutive injections, over 200 mL of methanol was pumped approximately 12 h period, the variation of the retention time may be caused by silyl ether formation on particle surface of the column which could alter the hydrophilicity of the particle surface [38].

### 3.2. Comparison between SFC-MS/MS and reported analytical methods

In order to compare the analytical performance between the established SFC-MS/MS methods and previous chromatographic analysis methods, publications of typical chromatographic methods of thiacloprid were chosen and several parameters are listed in Table 3. The retention time was 1.22 min by using SFC-MS/MS which was improved obviously in comparison with published methods. The running analysis time for per injection was 2 min in our study which reduced by at least half of that reported previously. Organic solvent consumption for per injection for SFC-MS/MS was 0.676 mL, which was lower than the consumption based on other chromatographic methods basically. The assessment of sensitivity is performed on the concentration of thiacloprid which generate a signal-to-noise ratio of 10. SFC-MS/MS provided comparatively high sensitivity (0.52 µg/kg) compared to previous methodologies, which varied from 0.02 µg/kg to 67 µg/kg. In conclusion, SFC-MS/MS technique shows obvious advantages for thiacloprid analysis which could greatly reduce the analysis time of thiacloprid, decrease the consumption of organic solvent, and offer satisfactory sensitivity.

### 3.3. Method validation

The linearity of standard working solutions and matrix-matched standard solutions was evaluated with satisfactory R<sup>2</sup> > 0.9968 ranging from 5 µg/L to 1000 µg/L. The regression equation and relevant parameters were list in Table 2. The matrix effects derive from the presence of matrix component which influence the ionization of the target component, resulting in the signal suppression or enhancement. Different levels of signal suppression or enhancement were observed for thiacloprid in different matrices with the slope ratios of the matrix to the solvent ranging from 0.72–1.12 (Table 2). It was considered that mild signal influence was noticed when absolute value of matrix effect was < 20% and medium signal influence was found when absolute value of matrix effect was between 20%–50% [39]. The mild signal enhancement was observed for thiacloprid in soil with value of matrix effect of 11.87%. Medium signal suppression was found in tomato (matrix effect = –27.67%) and cucumber (matrix effect = –26.93%).

**Table 3**  
Comparison between published chromatographic analysis methods and SFC-MS/MS method on thiacloprid.

Analytical instrument	Retention time (min)	Mobile phase	Gradient range (% organic)	Running analysis time for per injection (min)	Flow rate (mL/min)	Organic solvent consumption for per injection (mL)	Sensitivity <sup>a</sup> (µg/kg)	Authors
LC-MS	14.48	Water with 0.1% acetic acid	5–60	18	1	5.400	20–50	Paola Fidente et al. [21]
LC/TOF-MS	> 12	Water with 0.1% formic acid/acetonitrile	15–100	30	0.6	13.350	1–3	Imma Ferrer et al. [15]
LC-MS/MS	13.19	Water with 0.1% formic acid/acetonitrile with 0.1% formic acid	5–60	20	0.2	0.882	2.5	Zhiming Xiao et al. [16]
LC-MS/MS	5.71	Water with 0.1% formic acid/acetonitrile	20–100	22	0.1	> 1.400	0.36	Mariilena E. Dasenaki et al. [17]
LC-MS/MS	10.40	Water with 0.1% formic acid/acetonitrile	5–80	15	0.35	1.479	2	Han Sol Lee et al. [18]
HPLC-DAD	13.8	50 mM KH <sub>2</sub> PO <sub>4</sub> solution/methanol	5–50	21	0.8	3.540	67	Eiki Watanabe et al. [19]
HPLC-MS/MS	9.52	Water with 0.1% formic acid/acetonitrile with 0.1% formic acid	5–60	20	0.5	1.963	2.35	Maha F. et al. [20]
UPLC-MS/MS	2.05	Water/methanol	10–90	5	0.3	0.726	0.71–1.03	Ying Zhang et al. [12]
UPLC-MS/MS	2.36	Water with 0.1% formic acid/acetonitrile	20–90	5	0.4	0.714	0.0191	Zhihong Shi et al. [47]
UPLC-Orbitrap-MS	9.48	Ammonium formate (4 mM) with 0.1% formic acid/ammonium formate (4 mM) in methanol with 0.1% formic acid	5–100	14	0.25	1.624	–	Marina López-García et al. [48]
SFC-MS/MS	1.22	CO <sub>2</sub> /methanol	5–24	2	1.8	0.676	0.52	In our study

<sup>a</sup> Sensitivity refers to the concentration of thiacloprid which gives a signal-to-noise ratio of 10.



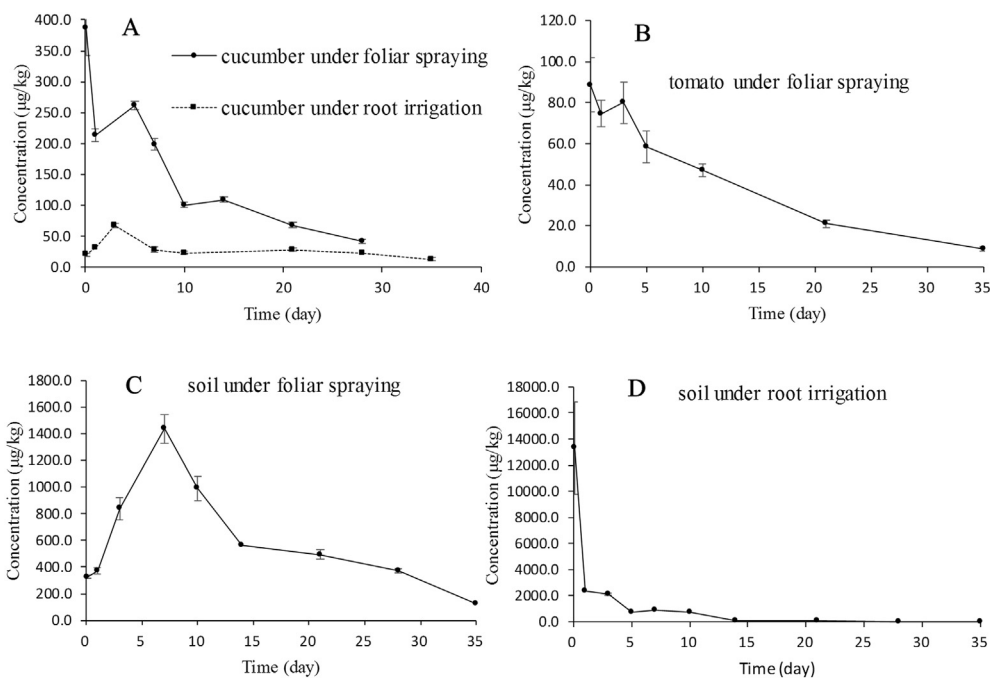


Fig. 3. Concentration versus time plots of thiacloprid in cucumber, tomato and soil samples under different application modes (A. cucumber under foliar spraying and root irrigation; B. tomato under foliar spraying; C. soil under foliar spraying; D. soil under root irrigation).

Table 4

Degradation equations of thiacloprid in cucumber, tomato and soil under different application modes.

Matrix	Application modes	Degradation equation	R <sup>2</sup>	T <sub>1/2</sub> (days) <sup>a</sup>	p <sup>b</sup>
Cucumber	Foliar spraying	C <sub>t</sub> = 301.770e <sup>-0.0726t</sup>	0.9121	9.55 ± 0.78	0.0439 <sup>c</sup>
	Root irrigation	C <sub>t</sub> = 48.280e <sup>-0.0339t</sup>	0.6466	20.44 ± 1.78	
Tomato	Foliar spraying	C <sub>t</sub> = 86.791e <sup>-0.0654t</sup>	0.9931	10.60 ± 0.66	–
	Root irrigation	–	–	–	–
Soil	Foliar spraying	C <sub>t</sub> = 2188.202e <sup>-0.0758t</sup>	0.9213	9.14 ± 0.12	0.0076 <sup>c</sup>
	Root irrigation	C <sub>t</sub> = 3377.633e <sup>-0.1853t</sup>	0.8795	3.74 ± 0.03	

<sup>a</sup> Values refer to the means ± STDEVs (n = 3).

<sup>b</sup> P values from degradation half-lives (T<sub>1/2</sub>) between different application modes using Student's paired t-test at 95% probability.

<sup>c</sup> Statistical significant difference with P < 0.05.

External matrix-matched standards were used to minimize the error in quantitative analysis of all samples.

The method validation for thiacloprid analysis on cucumber and tomato at three spiked levels (10, 50, 500 µg/kg) regarding accuracy and precision were reported in previous study and the limits of quantitation (LOQs) were 10 µg/kg for cucumber and tomato according to European Union SANTE/11813/2017 regulatory guidelines [12,33]. The recoveries and RSDs of thiacloprid in soil were evaluated to validate the accuracy and precision of the method. As shown in Table 1, satisfactory mean recoveries of thiacloprid were in range from 78.8% to 107.1% (n = 15) at four spiked levels (5, 10, 100, 1000 µg/kg) in soil. The intraday RSD (n = 5) and interday RSD (n = 15) were 4.8–9.7% and 8.1–12.2% separately. The LOQ for thiacloprid was 5 µg/kg in soil. The results of recovery experiment proved that the analysis method could achieve satisfactory recovery, precision and sensitivity for analysis of thiacloprid in cucumber, tomato and soil.

### 3.4. Dissipation of thiacloprid in vegetables under different application modes

Under solar greenhouse conditions, the concentration of thiacloprid in cucumber was increased until the third day under root irrigation. The residues in cucumber and tomato reached the highest level at 2 h under foliar spraying. Afterwards, the concentration of thiacloprid decreased

gradually under different application modes (Fig. 3). The pharmacokinetic degradation kinetics for thiacloprid in vegetables were generally in accordance with the first-order kinetics (R<sup>2</sup> = 0.9121 for foliar spraying in cucumber, R<sup>2</sup> = 0.9931 for foliar spraying in tomato, R<sup>2</sup> = 0.6466 for root irrigation), the fitting equations were listed in Table 4. The half-lives (T<sub>1/2</sub>) of thiacloprid in tomato was 10.60 ± 0.66 days under foliar spraying and all the concentrations of thiacloprid in different days under root irrigation were < 10 µg/kg (LOQ). The T<sub>1/2</sub> in cucumber were 9.55 ± 0.78 days under foliar spraying and 20.44 ± 1.78 days under root irrigation, which were longer than that reported in cucumbers grown in open field (3.63 days) [20]. This phenomenon may be mainly caused by different planting environment. The difference between T<sub>1/2</sub> of thiacloprid in cucumber under different application modes was statistically significant (P < 0.05, Student's paired t-test). The trends of concentration changes and T<sub>1/2</sub> are different between different application modes. The thiacloprid is a strong systemic pesticide, comparing to the spraying method, the main reason for the longer half-life under root irrigation is that the thiacloprid doses continued to be transmitted from soil to plant during transpiration [40]. The different concentration in cucumber and tomato plants may be due to the difference of the fruit shapes, the structure of the epidermis, and degradation enzymes of the plant itself. Therefore, the difference in degradation rates was influenced by many factors including plant species, planting environment, and application modes.

Furthermore, all concentrations of thiacloprid in vegetables under

root irrigation were lower than that of foliar spraying. Moreover, the *Meloidogyne incognita* and *Bemisia tabaci* can be effectively controlled in cucumber by thiacloprid under soil application. Root irrigation can also reduce the droplet drift which are harmful to beneficial insects and elongate the persistent period of thiacloprid compared to foliar spraying [6]. These advantages may make root irrigation become a viable alternative especially in hydroponic system [41]. However, more experiments on field efficacy trials of thiacloprid under different application modes should be explored and compared in future research.

### 3.5. Degradation of thiacloprid in soil under different application modes

The changes of residues in surrounding soil had two stages under foliar spraying, the concentration increased to the maximum at 7 days at the first stage, and gradually decreased as the time elapsed at the second stage. Under root irrigation, the initial concentrations of thiacloprid in soil were arrived the maximum at 2 h, and then declined gradually. The potential force driving thiacloprid concentration increased before 7 days under foliar spraying mode is probably caused by the redeposition of pesticide in the greenhouse space. The advection, dispersion and sorption could affect the transport of thiacloprid from the top soil to the surrounding soil [42]. However, this is not the case under root irrigation method. The fitting equations for thiacloprid in soil were followed the first-order kinetics ( $R^2 = 0.8795\text{--}0.9213$ , Table 4), the  $T_{1/2}$  was  $9.14 \pm 0.12$  days under foliar spraying and  $3.74 \pm 0.03$  days under root irrigation. The difference between  $T_{1/2}$  of thiacloprid in soil under different application modes was statistically significant ( $P < 0.05$ , Student's paired *t*-test). The concentration of thiacloprid was decreased faster relatively under root irrigation than under foliar spraying. The difference may result from the difference in the original deposition of the pesticides. Generally, the higher the original deposition is, the lower the pesticide degradation rate and the longer its half-life is [43,44]. However, the microbial activity may be stimulated by higher concentration of thiacloprid, which would lead to shorter half-life under root irrigation [45,46].

## 4. Conclusion

In this paper, a sensitive and time-saving detection method for determination of thiacloprid by SFC-MS/MS was established for the first time. The parameters including the proportion of co-solvent, ABPR pressure and flow rate of compensation pump was optimized and analysis time of thiacloprid was achieved lower than 1.30 min. The method can significantly reduce the analysis time and decrease the amount of organic solvent compared to previous study. The method was applied to explore the degradation behavior of greenhouse vegetables and soil under foliar spraying and root irrigation. The degradation rates and concentrations in vegetables and soil were different under different application modes, which mainly caused by plant species and application modes. The research can provide a robust analytical method for thiacloprid and offer essential data for risk assessment and rational use of thiacloprid.

## Notes

Declarations of interest: none.

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