

# Determination of Cyantraniliprole and its Major Metabolite Residues in Pakchoi and Soil Using Ultra-Performance Liquid Chromatography–Tandem Mass Spectrometry

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Received: 3 June 2012 / Accepted: 11 July 2012 / Published online: 30 August 2012  
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**Abstract** A rapid, simple and reliable analytical method was developed for the determination of cyantraniliprole and its major metabolite J9Z38 in pakchoi and soil by using ultra-performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS). The sample preparation approach is known as QuEChERS, which stands for quick, easy, cheap, effective, rugged and safe. Samples were extracted with acetonitrile, and cleaned up with dispersive primary and secondary amine sorbent before analysis by UPLC–MS/MS. The limit of quantitation for cyantraniliprole and J9Z38 was 0.01 mg/kg in both pakchoi and soil. Average recoveries of cyantraniliprole and J9Z38 at three fortified levels (0.01, 0.05, 0.1 mg/kg) ranged from 77.8 % to 102.5 % with relative standard deviation of 1.6 %–8.9 %. This method has been applied to the analysis of cyantraniliprole and J9Z38 residues in real pakchoi and soil samples selected from field. The results of the residue dynamic experiment showed that the half-life of cyantraniliprole ranged from 2.9 to 6.4 days in pakchoi and 8.7 to 18.2 days in soil, respectively. The final residual levels of cyantraniliprole in pakchoi and soil from Guangdong and Shanghai were below 0.20 and 0.10 mg/kg, respectively; similarly, the final residual levels of J9Z38 in pakchoi and

soil from Guangdong and Shanghai were <0.07 and 0.01 mg/kg. These results will be helpful in setting maximum residue limit guidance for cyantraniliprole in pakchoi in China.

**Keywords** Cyantraniliprole · J9Z38 · UPLC–MS/MS · Residue · Pakchoi

Cyantraniliprole(3-bromo-1-(3-chloro-2-pyridinyl)-*N*-[4-cyano-2-methyl-6-[(methyl-amino)carbonyl]phenyl]-1*H*-pyrazole-5-carboxamide, Fig. 1a) is an anthranilic diamide insecticide developed by DuPont Crop Protection that activate insect ryanodine receptors, causing calcium ions to pass rapidly out of muscle cells, resulting in feeding cessation followed by muscle paralysis, lethargy and regurgitation by affected insects (Jacobson and Kennedy 2011; Timo et al. 2011). Cyantraniliprole shows exceptional insecticidal activity on a broad range of *Lepidoptera*, *Coleoptera*, *Diptera*, and *Isoptera* insects, and it is reported to be active against a broader spectrum of insects than chlorantraniliprole (Chai et al. 2010). To the best of our knowledge, although cyantraniliprole residues have been reported in some vegetables, fruits and environmental materials (Timo et al. 2011; Dong et al. 2012; Sergio et al. 2011; Sergio 2011), we were unable to find any published information on cyantraniliprole and J9Z38 (Fig. 1b) residues in pakchoi.

In this study, the method to determine cyantraniliprole and J9Z38 residues in pakchoi and soil was established. And the dissipation dynamics of cyantraniliprole residues in pakchoi and soil was investigated. The purpose was to study the ultimate residue and dissipation of cyantraniliprole and J9Z38 in a pakchoi field ecosystem, help the Chinese government establish the MRL of cyantraniliprole

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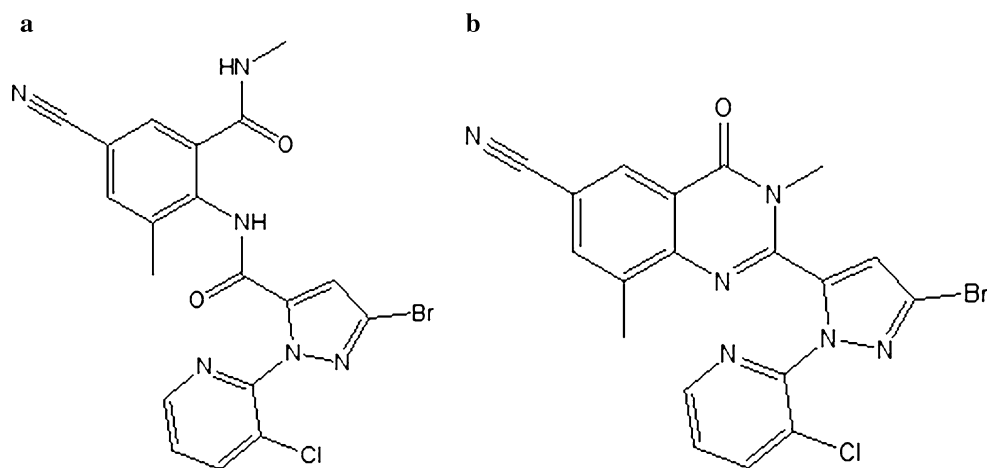
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**Fig. 1** The chemical structures of cyantraniliprole (a) and J9Z38 (b)



**Table 1** Retention times and UPLC–MS/MS parameters for cyantraniliprole and J9Z38

Pesticide	Retention times (min)	Parent mass (m/z)	Product mass (m/z)	Collision (V)	Tube lens (V)
Cyantraniliprole	1.82	475.2	286.0 <sup>a</sup>	33	223
			443.9	41	0
J9Z38	2.42	457	187.9 <sup>a</sup>	22	230
			298.9	18	0

<sup>a</sup> Quantitative ion

in pakchoi and provide guidance on the proper and safe use of this pesticide.

## Materials and Methods

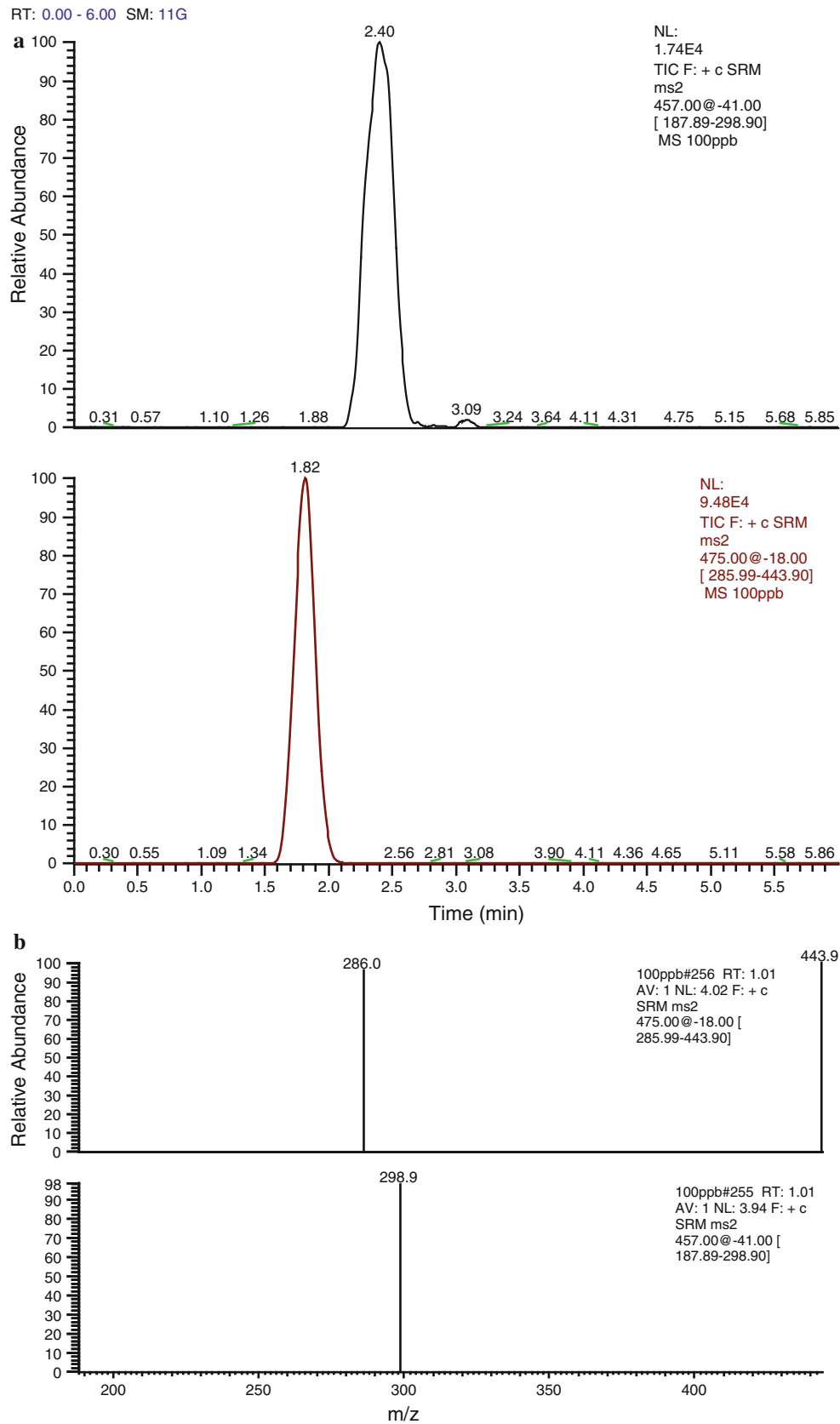
Cyantraniliprole standard (99.2 % purity) and its major metabolite (J9Z38, 97.2 % purity) were provided by DuPont Co. (DE, USA). Formulation of cyantraniliprole (suspension concentrate SC, 10 %) was supplied by Shanghai (China) Dubang Agrochemical Industry Co., Ltd. Acetonitrile for pretreatment and anhydrous magnesium sulfates were of analytical grade. And acetonitrile for ultra-performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS) analysis was chromatographic-grade. Ultrapure grade water was obtained from Millipore (Millipore, USA). Dispersive primary and secondary amine (PSA) sorbent was purchased from Waters Co. (MA, USA).

Chopped and homogenized pakchoi samples (10 g) were accurately weighed into a 50 mL PTFE centrifuge tube and 10 mL acetonitrile was added. The centrifuge tube was tightly capped and vigorously mixed for 2 min using a vortex mixer. Anhydrous magnesium sulfate (6 g) and sodium acetate (1.5 g) were added to the tube and immediately mixed with a vortex mixer for 2 min. The tube was centrifuged for 8 min at 5,000 rpm.

Homogenized soil samples (10 g) were accurately weighed into a 50 mL PTFE centrifuge tube and 10 mL acetonitrile was added. The centrifuge tube was tightly capped and agitated on a shaker for 30 min. After adding 6 g anhydrous magnesium sulfate and 1.5 g sodium acetate, the sample was mixed vigorously by vortexing for 2 min and centrifuged for 8 min at 5,000 rpm.

For both pakchoi and soil, a 2 mL aliquot of the upper acetonitrile layer was transferred into EP tube containing 50 mg PSA sorbent and 150 mg anhydrous magnesium sulfate. The sample was mixed vigorously using a vortex mixer for 1 min and then centrifuged for 5 min at 5,000 rpm. Acetonitrile layer was filtered through a 0.22  $\mu$ m filter membrane for UPLC–MS/MS analysis.

A Thermo TSQ Quantum UPLC–MS/MS equipped with a 2.1 mm  $\times$  100 mm  $\times$  5  $\mu$ m Betasil C18 column (Thermo Inc., USA) was used for pesticide analysis. The operation of the LC gradient involved the following two eluent components: A: acetonitrile/0.2 % acetic acid; B: water/0.1 % acetic acid. The gradient elution was as follows: 0–1.0 min isocratic 20 % B, 1.0–2.0 min linear from 20 % to 90 % B, 2.0–2.1 min linear from 90 % to 0 % B, and 2.1–2.5 min linear from 0 % to 95 % B, 2.5–3.0 min linear from 95 % to 20 % B, with 3.0–5.1 min for initial conditions of 20 % B. The flow rate remained constant at 0.2 mL/min and injection volume was 10  $\mu$ L. The column temperature was kept at 40°C.



**Fig. 2** **a** The SRM chromatograms of cyantraniliprole and J9Z38 in standard solution (0.1 mg/kg); **b** mass spectra of cyantraniliprole and J9Z38

**Table 2** Average recovery and RSD of cyantraniliprole in samples spiked at different levels

Sample	Spike levels (mg/kg)	Recovery (%)					Average	RSD (%)
		1	2	3	4	5		
Soil	0.01	99.3	88.9	99.1	102.4	98.4	97.6	4.7
	0.05	98.3	87.8	83.2	89.8	83.3	88.5	6.3
	0.1	89.4	89.5	91.3	90.6	93.3	90.8	1.6
Pakchoi	0.01	100.4	91.1	81.6	99.3	86.5	91.8	7.9
	0.05	85.4	81.5	86.4	100.3	87.8	88.3	7.2
	0.1	84.7	84.7	98.6	89.2	90.4	89.5	5.7

**Table 3** Average recovery and RSD of J9Z38 in samples spiked at different levels

Sample	Spike levels (mg/kg)	Recovery (%)					Average	RSD (%)
		1	2	3	4	5		
Soil	0.01	94.7	93.8	93.4	90.5	98.6	94.2	2.8
	0.05	92.3	99.3	87.8	87.8	99.3	93.3	5.5
	0.1	87.6	97.7	91.3	90.6	102.5	93.9	5.7
Pakchoi	0.01	87.4	98.9	89.8	96.5	87.8	92.1	5.1
	0.05	89.4	89.3	87.8	88.2	79.5	86.8	4.3
	0.1	86.9	79.4	94.3	77.8	97.4	87.2	8.9

MS/MS detection was performed in the selected reaction monitoring (SRM) mode using electrospray ionization (ESI) source in the positive ion mode. Ion spray potential was 3.1 kV and MS transfer line temperature was 350°C. Sheath gas pressure was 10 arb and aux gas pressure was 25 arb. Other parameters of analyte were shown in Table 1.

The method described for sample preparation was validated by a recovery investigation. Untreated pakchoi and soil samples were fortified with known amount of working standard solution to reach concentration of 0.01, 0.05, and 0.1 mg/kg. And they were processed according to the above procedure. Five replicates for each concentration were analyzed. Blank analyses were also performed in order to check interference from the matrix.

The field trials were carried out in Guangdong and Shanghai including the residue dynamic experiment and final residue experiment in 2009 and 2010. Each experiment field consisted of three replicate plots with an area of 15 m<sup>2</sup>. Another untreated plot was sprayed water without any insecticide and maintained as controls.

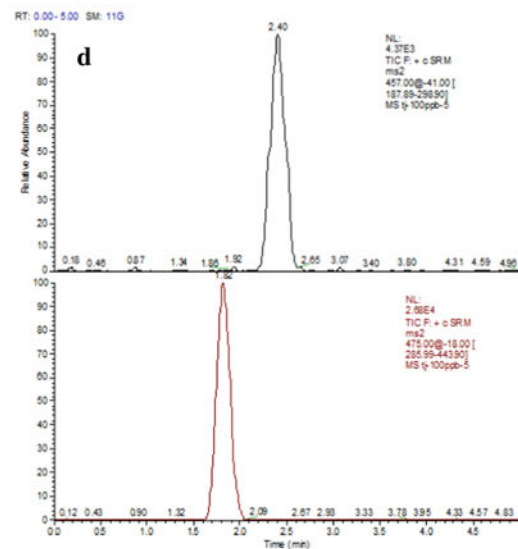
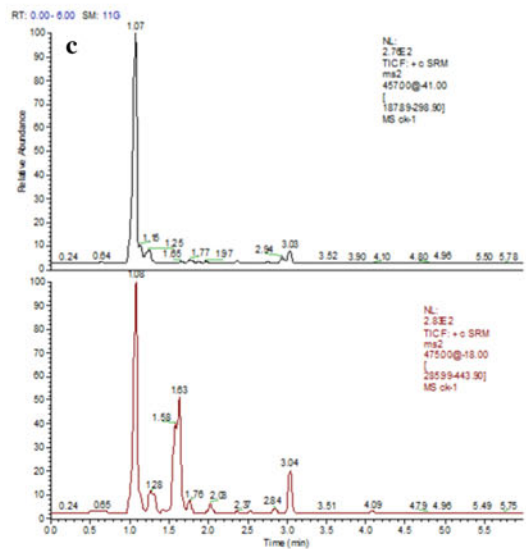
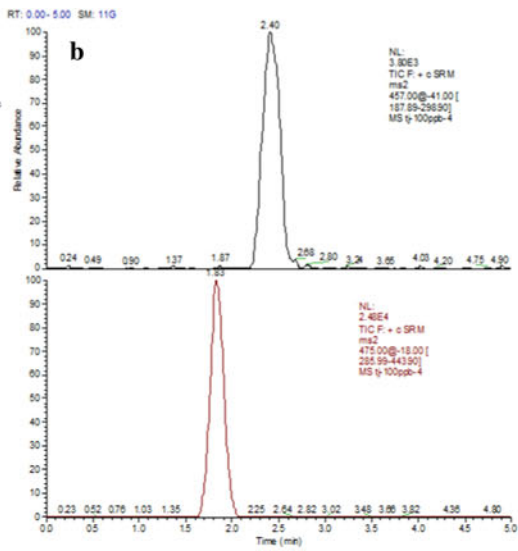
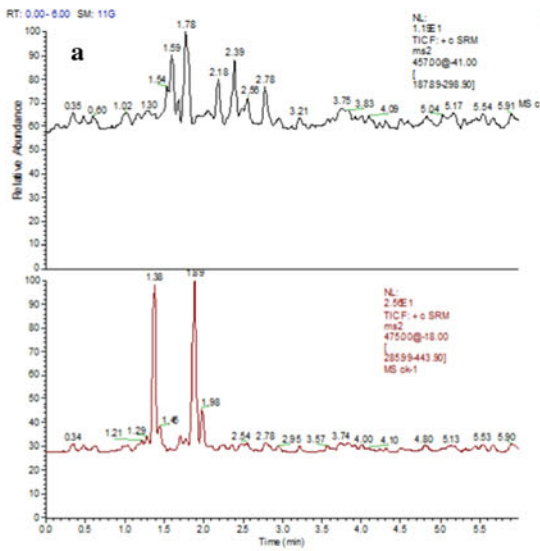
In order to study the dissipation trends of cyantraniliprole in pakchoi and soil, cyantraniliprole (SC, 10 %) was applied at 1.5 times recommended high dosage of 90 g active ingredient per hectare (90 g a.i. ha<sup>-1</sup>, spray 1 time) for pakchoi and 2 times recommended high dosage (120 g a.i. ha<sup>-1</sup>, spray 1 time) for soil. Cyantraniliprole was dissolved in water and sprayed in the growing pakchoi and soil with no insecticide using a knapsack sprayer. Representative pakchoi samples were collected randomly from each treatment plot at different intervals before and (2 h, 1,

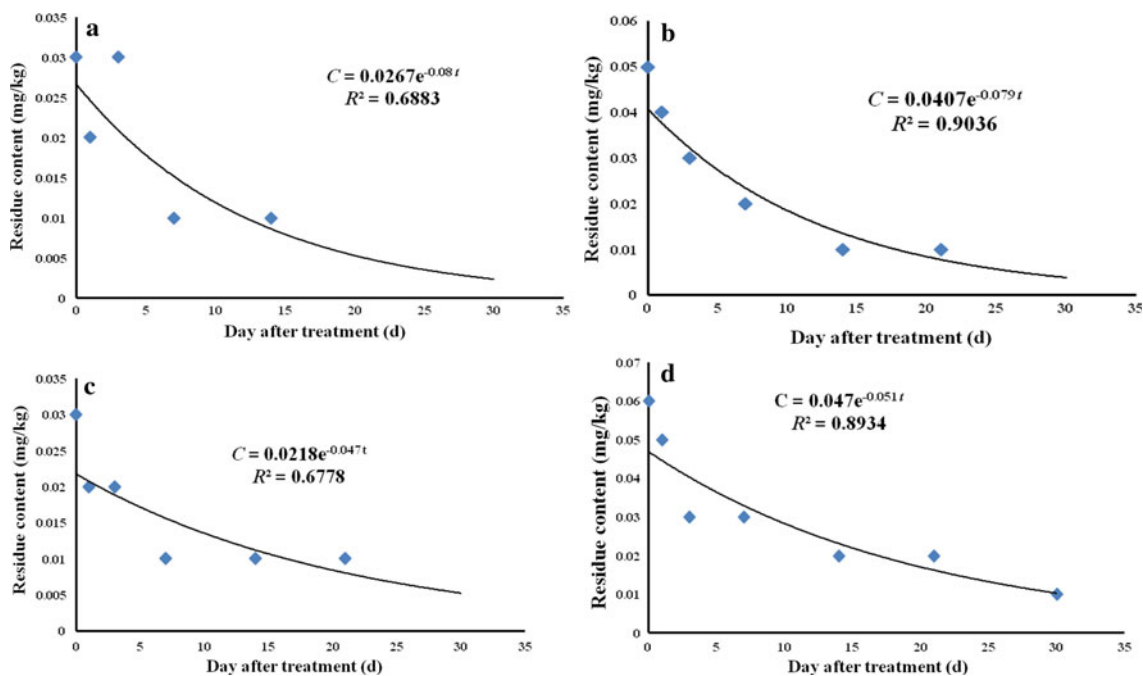
**Fig. 3** Chromatograms of cyantraniliprole (Rt = 1.82 min) and J9Z38 (Rt = 2.42 min) in the studied samples. **a** Control (untreated whole soil); **b** spiked whole soil with cyantraniliprole and J9Z38 working solutions at 0.1 mg/kg levels; **c** control (untreated pakchoi); **d** spiked pakchoi with cyantraniliprole and J9Z38 working solutions at 0.1 mg/kg levels; **e** soil (21-day); **f** pakchoi (5-day)

3, 7, 14, 21, 30 and 45 days) after spraying. Soil samples were collected from each plot using a soil sampling device from the surface to a depth of 10 cm at different intervals before and (2 h, 1, 3, 7, 14, 21, 45 and 60 days) after spraying. Each pakchoi sample was chopped and homogenized, and then took 300 g for residue analysis. Little stones and other unwanted materials were removed from each soil samples and then homogenized and took 300 g for residue analysis.

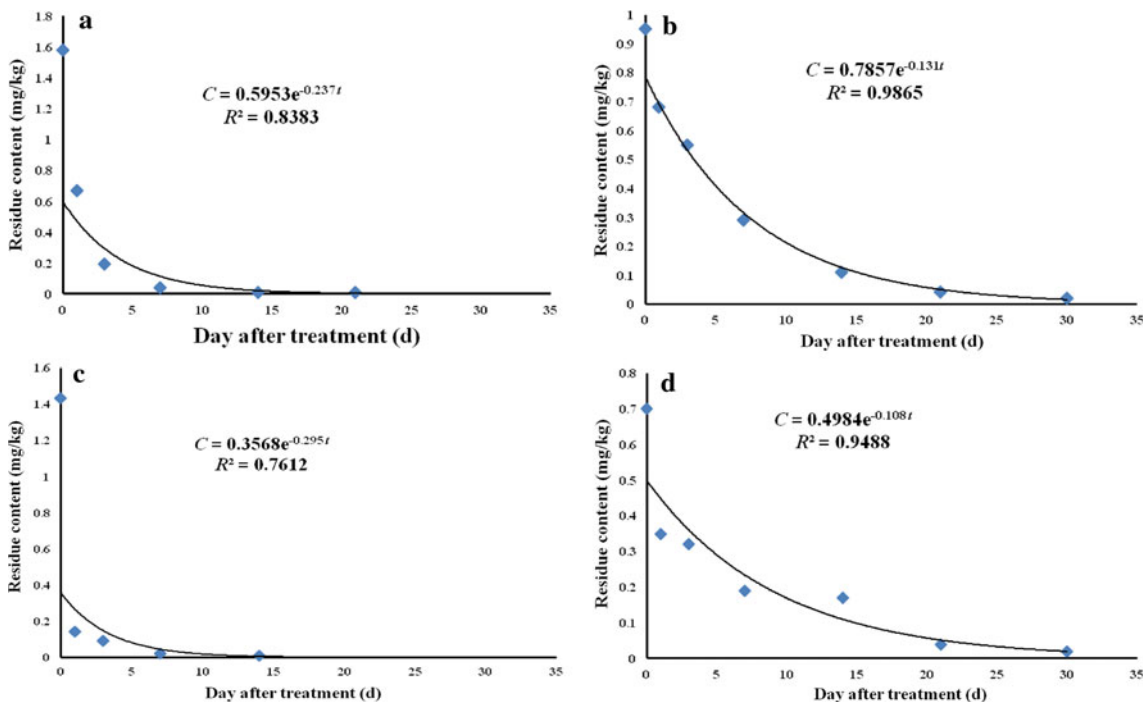
To investigate the terminal residue of cyantraniliprole and its major metabolite J9Z38 in pakchoi and soil, cyantraniliprole was applied at low dosage of 60 g a.i. ha<sup>-1</sup> (recommended high dosage) three and four times and high dosage of 90 g a.i. ha<sup>-1</sup> (1.5 times the recommended high dosage) three and four times with interval of 7 days between each application, respectively. After the last application, the soil in which the pakchoi had been grown and pakchoi was sampled on day 3, 5, and 7 after spraying, respectively. Each pakchoi sample was chopped and homogenized, and then took 300 g for residue analysis. Little stones and other unwanted materials were removed from soil samples and then homogenized and took 300 g for residue analysis.

All collected samples were stored in a deep freezer at -18°C until analysis.





**Fig. 4** The dissipation curves of cyantraniliprole in soil. **a** Guangdong soil (2009); **b** Shanghai soil (2009); **c** Guangdong soil (2010); **d** Shanghai soil (2010)



**Fig. 5** The dissipation curves of cyantraniliprole in pakchoi. **a** Guangdong pakchoi (2009); **b** Shanghai pakchoi (2009); **c** Guangdong pakchoi (2010); **d** Shanghai pakchoi (2010)

## Results and Discussion

Quantification was accomplished using the standard curve constructed by plotting analyte concentrations against peak

areas under the proposed chromatographic conditions. A good linearity was achieved from 0.005 to 1 mg/kg. The regression and correlation coefficients ( $R^2$ ) for cyantraniliprole and J9Z38 were as follows:  $y = 1.578x + 53.25$ ,

**Table 4** Final residues of cyantraniliprole in soil and pakchoi in Guangdong and Shanghai

Dosage (g a.i. ha <sup>-1</sup> )	Spray times	Interval (days)	Cyantraniliprole residue (mg/kg)							
			Soil				Pakchoi			
			2009		2010		2009		2010	
			Guangdong	Shanghai	Guangdong	Shanghai	Guangdong	Shanghai	Guangdong	Shanghai
60.00	3.00	7.00	0.03	0.02	0.05	0.03	0.07	0.04	0.10	0.09
		5.00	0.02	0.03	0.02	0.03	0.08	0.11	0.11	0.09
		3.00	0.01	0.03	0.04	0.04	0.50	0.19	0.39	0.19
	4.00	7.00	0.04	0.03	0.06	0.03	0.08	0.06	0.08	0.10
		5.00	0.03	0.04	0.04	0.05	0.12	0.13	0.15	0.21
		3.00	0.02	0.06	0.03	0.06	0.65	0.28	0.69	0.53
90.00	3.00	7.00	0.03	0.02	0.04	0.04	0.13	0.04	0.09	0.19
		5.00	0.02	0.03	0.04	0.04	0.12	0.12	0.18	0.38
		3.00	0.02	0.05	0.03	0.08	0.83	0.23	0.77	0.69
	4.00	7.00	0.06	0.04	0.07	0.05	0.16	0.08	0.20	0.18
		5.00	0.05	0.06	0.07	0.06	0.25	0.17	0.35	0.26
		3.00	0.03	0.10	0.06	0.08	1.07	0.28	0.95	0.42
CK			<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

**Table 5** Final residues of J9Z38 in soil and pakchoi in Guangdong and Shanghai

Dosage (g a.i. ha <sup>-1</sup> )	Spray times	Interval (days)	J9Z38 residue (mg/kg)							
			Soil				Pakchoi			
			2009		2010		2009		2010	
			Guangdong	Shanghai	Guangdong	Shanghai	Guangdong	Shanghai	Guangdong	Shanghai
60	3	7	0.02	<0.01	0.03	<0.01	0.02	<0.01	0.01	<0.01
		5	0.02	<0.01	0.02	<0.01	0.01	<0.01	0.01	<0.01
		3	0.01	<0.01	0.01	<0.01	0.04	<0.01	0.02	<0.01
	4	7	0.05	<0.01	0.07	<0.01	0.01	<0.01	0.01	<0.01
		5	0.03	<0.01	0.05	<0.01	0.01	<0.01	0.02	<0.01
		3	0.03	<0.01	0.04	<0.01	0.05	<0.01	0.05	0.01
90	3	7	0.03	<0.01	0.04	<0.01	0.03	<0.01	0.01	<0.01
		5	0.03	<0.01	0.04	<0.01	0.03	<0.01	0.02	0.01
		3	0.03	<0.01	0.03	<0.01	0.06	<0.01	0.04	0.02
	4	7	0.04	<0.01	0.05	<0.01	0.01	<0.01	0.01	<0.01
		5	0.04	<0.01	0.05	<0.01	0.02	<0.01	0.02	<0.01
		3	0.04	<0.01	0.05	<0.01	0.08	<0.01	0.05	0.01
CK			<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

$R^2 = 0.9921$  (cyantraniliprole);  $y = 0.3011 x + 5.639$ ,  $R^2 = 0.9984$  (J9Z38). The retention times of cyantraniliprole and J9Z38 were 1.82 and 2.42 min, respectively (Fig. 2).

The efficiency of pesticide residue analytical method is usually expressed as the recovery. The recovery studies were carried out five replicates at different spike levels (0.01, 0.05, and 0.1 mg/kg) of different substrates. The

average recoveries obtained were shown in Tables 2 and 3. The fortified recoveries of cyantraniliprole in the pakchoi and soil samples were 83.3 %–102.4 % and 81.5 %–100.4 %, respectively, and the corresponding relative standard deviations (RSDs) of the recovery data were 1.6 %–6.3 % and 7.2 %–7.9 %. The average recoveries of J9Z38 in the pakchoi and soil samples were 87.6 %–102.5 % and 77.8 %–98.9 %, respectively, and the corresponding RSDs of the recovery data were

2.8 %–5.7 % and 4.3 %–8.9 %. Meanwhile blank pakchoi and soil samples were analyzed and the results indicated the blank extracts did not contribute any interference with the target compounds. The typical UPLC–MS/MS chromatograms were shown in Fig. 3.

The limit of detection (LOD) for cyantraniliprole and J9Z38 was estimated to be 0.1 and 0.5 ng, respectively. The limit of quantification (LOQ) of cyantraniliprole and J9Z38 for both was 0.01 mg/kg in this method.

Figure 4 showed the dissipation curves of cyantraniliprole in soil under field conditions. The initial concentrations of cyantraniliprole in soil in 2009 and 2010 were 0.03 and 0.05 mg/kg from Guangdong, and 0.03 and 0.06 mg/kg from Shanghai. The half-life time of cyantraniliprole was 8.7 and 8.8 days in Guangdong and Shanghai in 2009 and the dissipation dynamics of cyantraniliprole could be described by the following first-order kinetics equation:  $C = 0.0267e^{-0.08 t}$  with coefficient  $R^2 = 0.6883$  (Guangdong) and  $C = 0.0407e^{-0.079 t}$  with coefficient  $R^2 = 0.9036$  (Shanghai). The half-life time of cyantraniliprole was 14.7 and 18.2 days in Guangdong and Shanghai in 2010 and the dissipation dynamics of cyantraniliprole could be described by the following first-order kinetics equation:  $C = 0.0218e^{-0.047 t}$  with coefficient  $R^2 = 0.6778$  (Guangdong) and  $C = 0.0428e^{-0.038 t}$  with coefficient  $R^2 = 0.8586$  (Shanghai).

Figure 5 showed the dissipation curves of cyantraniliprole in pakchoi under field conditions. The initial concentration of cyantraniliprole in pakchoi from Guangdong and Shanghai were 1.58 and 0.95 mg/kg in 2009 and 1.43 and 0.70 mg/kg in 2010, which were higher than in soil. The half-life time of cyantraniliprole was 2.9 and 5.3 days in Guangdong and Shanghai in 2009 and the dissipation dynamics of cyantraniliprole could be described by the following first-order kinetics equation:  $C = 0.5953e^{-0.237 t}$  with coefficient  $R^2 = 0.8383$  (Guangdong) and  $C = 0.7857e^{-0.131 t}$  with coefficient  $R^2 = 0.9865$  (Shanghai). The half-life time of cyantraniliprole was 3.6 and 6.4 days in Guangdong and Shanghai in 2010 and the dissipation dynamics of cyantraniliprole could be described by the following first-order kinetics equation:  $C = 0.2529e^{-0.193 t}$  with coefficient  $R^2 = 0.6912$  (Guangdong) and  $C = 0.4984e^{-0.108 t}$  with coefficient  $R^2 = 0.9488$  (Shanghai).

The final residues of cyantraniliprole in pakchoi and soil from Guangdong and Shanghai were listed in Tables 4 and 5. The residues concentration of cyantraniliprole from 3 to 7 days ranged 0.01–0.11 mg/kg in the soil and 0.02–1.27 mg/kg in the pakchoi in Guangdong and Shanghai. The residues concentration of J9Z38 from 3 to 7 days ranged 0.01–0.07 mg/kg in the soil and 0.01–0.10 mg/kg in the pakchoi in Guangdong and Shanghai. Because the maximum residue limits (MRLs) of cyantraniliprole in the pakchoi have not been legislated by

Chinese legislation or Food and Agriculture Organization (FAO)/World Health Organization (WHO) and other countries yet, the data generated from this work would be helpful for the Chinese government to establish MRLs of cyantraniliprole in pakchoi and provide guidance on safe and proper use of the pesticide.

In conclusion, the QuEChERS method with HPLC–MS/MS detection was successfully validated for the determination of cyantraniliprole and its major metabolite J9Z38 in pakchoi and soil with a LOQ of 0.01 mg/kg. The average recoveries in all matrices for the pesticide ranged from 77.8 % to 102.5 % with RSD of 1.6 %–8.9 %. The proposed analytical procedure is fast easy to perform and could be utilized for regular monitoring of cyantraniliprole residues in pakchoi field. The results of the dissipation study of cyantraniliprole in Guangdong and Shanghai in 2009 and 2010 under field conditions showed that cyantraniliprole dissipated rapidly in pakchoi (half-lives were 2.9–6.4 days) and in the soil was relatively slow (half-lives were 8.7–18.2 days). The final residual levels of cyantraniliprole in pakchoi and soil from Guangdong and Shanghai were below 0.20 and 0.10 mg/kg; similarly, the final residual levels of J9Z38 in pakchoi and soil from Guangdong and Shanghai were <0.07 and 0.01 mg/kg, respectively. From the results of the experimental data that cyantraniliprole is easy-degraded pesticide. This work will aid in the establishment of a maximum residue limit and the safe and proper use of cyantraniliprole in pakchoi in China.

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