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Analytical Methods

Chemometric-assisted QuEChERS extraction method for the residual analysis of thiacloprid, spirotetramat and spirotetramat's four metabolites in pepper: Application of their dissipation patterns

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ABSTRACT

Chemometric tools equipped with a Plackett–Burman (P–B) design, a central composite design (CCD) and a desirability profile were employed to optimise the QuEChERS (quick, easy, cheap, effective, rugged and safe) method for the quantification of thiacloprid, spirotetramat and spirotetramat's four metabolites in pepper. The average recoveries were in the range of 71.6–119.5%, with relative standard deviations ⩽12.1%. The limit of quantification for the method was less than 0.01 mg/kg. The method was applied to field samples to evaluate the residual characteristics of thiacloprid and spirotetramat. The data showed that the first + first-order model is a better fit than the first order model for the dissipation of thiacloprid and spirotetramat in pepper. The half-lives of thiacloprid and spirotetramat in pepper are 0.81 and 1.21 days, respectively. The final residues were between 0.016 mg/kg and 0.13 mg/kg for thiacloprid and 0.08 mg/kg and 0.12 mg/kg for spirotetramat.

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1. Introduction

Pepper is one of the most important crops in China due to its heavy consumption, high nutritional and medicinal value, and profitability (Xu, Li, & Wang, 2008). It is a rich source of capsaicin, dihydrocapsaicin, capsanthin, capsorubin, β-carotene, vitamin C and other compounds. However, pepper production is hampered by pest infestation (e.g., aphids, spider mites, thrips) during cultivation, which requires frequent use of pesticides to treat. Currently, pesticide and fungicide mixtures have been developed to broaden products' spectrum. In China, a combined product, 21.6% thiacloprid·spirotetramat suspension concentrate (SC), is being registered. Thiacloprid ((Z)-3-(6-chloro-3-pyridylmethyl)-1,3-thiazolidin-2-ylidenecyanamide) is the first chloronicotinyl insecticide that acts selectively on the insect nervous system by inhibiting nicotinic acetylcholine receptors. It has broad-spectrum efficacy against both sucking insects and chewing insects (Elbert, Ebbinghaus-Kintscher, Erdelen, Nauen, & Schnorbach, 2001). Spirotetramat, cis-3-(2,5-dimethylphenyl)-8-methoxy-2-oxo-1-azaspiro[4.5]dec-3-en-4-yl-ethyl carbonate (BYI08330), belongs to the chemical class of ketoenols. The compound also exhibits a unique property described as two-way systemicity (Nauen, Reckmann, Thomzik, & Thielert, 2008). Spirotetramat acts as an acetyl-CoA carboxylase inhibitor and is intended for use on a range of agricultural crops. Notably, spirotetramat is active against a broad spectrum of sucking insects (Brück et al., 2009; Kay & Herron, 2010; Kumar & Kuttalam, 2009; Kumar, Kuttalam, & Chandrasekaran, 2009; Smiley, Marshall, & Yan, 2011).

There is increasing concern that several toxicologically significant pesticide residues and metabolites left on or in the crops would be consumed by humans or livestock, causing health problems for non-target organisms (You, Liang, & Liu, 2014). Previous studies indicated that thiacloprid could produce delayed lethal and sub-lethal effects on freshwater arthropods at low concentrations (Beketov & Liess, 2008). It was also found to exhibit adverse effects on zebrafish and honey bees (Laurino, Porporato, Patetta, & Manino, 2011; Osterauer & Kohler, 2008). Spirotetramat is considered to harbour skin-sensitisation potential and be an eye irritant for animals and humans. BYI08330-enol (B-enol), BYI08330-ketohydroxy (B-keto), BYI08330-mono-hydroxy (B-mono) and BYI08330-enol-glucoside (B-glu) are the main metabolites of spirotetramat in plants. It was reported that maternal toxicity was observed at ⩾40 mg/kg bw/day for spirotetramat; further research demonstrated that male reproductive toxicity in rats is likely caused by the metabolite B-enol (APVMA, 2009). Additionally, spirotetramat, B-enol, B-keto, B-mono and B-glu are included in the definition for enforcement and risk assessment purposes in primary crops (United States Department of

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Agriculture, 2013). Therefore, it is necessary to establish a simultaneous determination method for thiacloprid, spirotetramat and spirotetramat's four metabolites to safeguard public health and the environment.

Several analytical methods have been developed to measure thiacloprid, spirotetramat and spirotetramat's metabolites (Hengel, 2011; Jovanov et al., 2014; Mohapatra, Deepa, & Jagadish, 2012; Morales, Ruiz, Oliva, & Barba, 2011; Watanabe, Kobara, & Yogo, 2012; Zhu et al., 2013). Nevertheless, optimisation strategies for analytical methods are commonly based on the study of one-variable-at-a-time (OVAT) method, which often fails to achieve exact specifications because interactions between factors are not taken into account. Chemometrics is the chemical discipline that applies statistical and mathematical methods to chemistry (Brown, Sum, Despagne, & Lavine, 1996). Chemometrics-based techniques, such as multivariate experimental design and response surface methodology (RSM), could efficiently extract information from large amounts of chemical data with fewer resources. However, there is a paucity of published research on the application of chemometric experimental design to analytical methods (Alberti et al., 2014).

The dissipation rate of pesticides after application is one of the most important parameters in assessing of the fate of their residues. The residue dynamics of thiacloprid have been studied in different matrices, including medicinal herbs, tomatoes, eggplants, cabbage and soil (Dong et al., 2014; Omirou, Vryzas, Papadopoulou-Mourkidou, & Economou, 2009; Saimandir, Gopal, & Walia, 2009; Wang, Guan, & Zhang, 2011; Yu, Wu, Stahler, & Pestemer, 2007). Only two published papers were available on the behaviour of spirotetramat in plants (i.e., cotton, mango and soil) (Mohapatra, Deepa, Lekha, et al., 2012; Pandiselvi, Sathiyanarayanan, & Ramesh, 2010). However, the four metabolites of spirotetramat were ignored in those dissipation studies. In addition, no information is available on the behaviour of thiacloprid and spirotetramat in peppers.

This research applied a chemometric experimental design to identify a stacking modified QuEChERS method to analyse the residue of thiacloprid, spirotetramat and spirotetramat's four metabolites in pepper and to evaluate dissipation patterns and residue levels under field conditions.

2. Materials and methods

2.1. Materials and reagents

Standards of thiacloprid (99.0% purity), spirotetramat (99.2% purity), B-enol (99.1% purity), B-keto (94.8% purity), B-mono (98.2% purity), B-glu (98.6% purity) and 21.6% thiacloprid·spirotetramat suspension concentrate (SC) were kindly supplied by Bayer Crop Science Limited (Frankfurt, Germany). The stock solutions (100 mg L^{-1}) of individual standards were prepared in pure acetonitrile (MeCN). UHPLC grade MeCN and methanol were purchased from Sigma–Aldrich (Steinheim, Germany). Anhydrous magnesium sulphate ($MgSO_4$), sodium chloride (NaCl), formic acid and MeCN were of analytical grade and purchased from Bei-hua Fine-chemicals Co. (Beijing, China). PSA (40 μm), C18 (40 μm), GCB (40 μm) sorbents and 0.22-μm nylon syringe filters were purchased from Agela Technologies Inc. (Agela, Tianjin, PRC).

2.2. Field experiment design

The field trials, including the dissipation and residue experiments, were conducted in Beijing during the 2013 agricultural season. The field was divided into 30m^2-sized blocks for the dissipation rate studies and controls. Each treatment had three replicate plots. The peppers were sprayed with a 21.6% SC of thiacloprid·spirotetramat at a dosage of 216 g a.i. ha^{-1} (1.5 times the recommended dosage) with one spray. Then, 2 kg of samples were collected randomly from sampling plots at 0 (2 h after spraying), 1, 3, 7, 10, 14, 21 and 28 days after treatment. For the terminal residue experiment, the 21.6% thiacloprid·spirotetramat SC was applied at two dosage levels: 144 g a.i. ha^{-1} (the recommended dosage) and 216 g a.i. ha^{-1} (1.5 times the recommended dosage). Each dosage level was sprayed two and three times. Representative pepper samples were collected 3, 5 and 7 days after spraying. Collected pepper samples were chopped and kept at –20 °C until further analysis.

2.3. Sample preparation

A 10 g subsample of the homogeneous pepper sample was put into a 50-ml Teflon centrifuge tube with screw caps. Next, 10 of ml acetonitrile and 0.68 ml of formic acid were added. The mixtures were shaken vigorously for 3 min using a vortex mixer to ensure that the solvent contacted the entire sample. Subsequently, 2.5 g of sodium chloride was added, the tubes were capped and immediately shaken intensely for 1 min. After centrifuging the tubes at 2811×*g* for 5 min, a 3-ml aliquot was transferred to a single-use centrifuge tube containing 30 mg of PSA, 100 mg of C_{18}, 60 mg of GCB and 150 mg of anhydrous $MgSO_4$. The shaking step was repeated for 1 min and centrifuged at 2400×*g* for 5 min. The upper layer of the prepared sample was filtered through a 0.22-μm nylon syringe filter and transferred to an autosampler vial for UHPLC-MS/MS injection.

2.4. UHPLC–MS/MS conditions

The analyses were conducted on a Waters ACQUITY UHPLC system (Milford, MA) consisting of an ACQUITY UHPLC binary solvent manager, an ACQUITY UHPLC sample manager and an Acuity column heater equipped with a Waters Acquity UHPLC BEH C_{18} column (2.1 mm × 100 mm, 1.8 μm particle size; Milford, MA, USA). The mobile phase consisted of MeCN (A) and 0.2% (v/v) formic acid in water (B). The mobile phase was pumped at a flow rate of 0.3 mL/min. The following gradient program was run: an initial composition of 5% A; a ramp to 95% A over 3.0 min; a ramp to 95% A from 3.0–3.5 min; a decrease to 5% A from 3.5–3.6 min and column re-equilibration from 3.6 min to 5.0 min. The separation and stabilisation were achieved in 5.0 min. The column temperature was maintained at 45 °C to decrease the viscosity. The autosampler temperature was set to 5 °C.

Analyses of the six compounds were carried out on a triple–quadrupole mass spectrometer (TQD, Waters Corp., Milford, MA, USA) equipped with an electrospray ionisation (ESI) source, operating in positive and negative ionisation switching mode. Instrument control and data acquisition were performed using MassLynx software (version 4.1). Nebulizer gas was 99.95% nitrogen; the collision gas was 99.999% argon and was held at a pressure of 2×10^{-3} mbar in the T-wave cell. MS/MS monitoring conditions were optimised for the six target compounds. The typical conditions were as follows: The capillary voltage was set to 3.0 kV, while the source temperature and desolvation temperatures were held at 120 °C and 350 °C, respectively. The cone and desolvation gas flows were set to 50 and 600 L/h, respectively. Multi-reaction monitoring (MRM) mode was operated for each compound. The MRM transitions were *m/z* 253.0 → 126.0 (collision energy, 20 eV) for thiacloprid; *m/z* 374.1 → 302.2 (collision energy, 16 eV) for BYI08330; *m/z* 302.1 → 216.1 (collision energy, 28 eV) for B-enol; *m/z* 304.1 → 254.2 (collision energy, 20 eV) for B-mono; *m/z* 318.1 → 300.2 (collision energy, 16 eV) for B-keto and *m/z* 464.1 → 302.2 (collision energy, 12 eV) for B-glu.

2.5. Data analysis

To optimise the sample pre-treatment, the Statsoft Statistica 8.0 computer program was used to generate mock experimental matrix designs.

Dissipation kinetics for thiacloprid and spirotetramat residues in peppers were calculated by plotting thiacloprid and spirotetramat residue concentrations against time after application. It is well established that degradation patterns commonly follow first-order kinetics. However, some studies demonstrate that pesticides dissipation is sometimes insufficiently described by simple first-order kinetics and requires a nonlinear kinetic models instead (Gustafson & Holden, 1990; Jadhav et al., 2013; Sarmah & Close, 2009). In our study, the obtained data were analysed by a simple first-order as well as a first + first-order model using the following equations.

First-order model

$$C_t = C_1 \exp^{-k_1 t}$$

First + first-order model (bi-exponential model)

$$C_t = C_1 \exp^{-k_1 t} + C_2 \exp^{-k_2 t}$$

where C_t represents the residue concentration after pesticide application at time t. C_1 and C_2 are the initial concentrations at time 0 that degrade through first-order processes 1 and 2, respectively. The half-life (DT50) is defined as the time required for the disappearance of 50% of the pesticide (based on initial residue levels after application). For the first-order model, the DT50 was calculated by the following equation: DT50 = ln(2)/k. Because the first + first-order model cannot be described in a differential form, DT50 was calculated by an iterative procedure using TableCurve 2D v 5.01 program (Jadhav et al., 2013; Mujawar, Utture, Fonseca, Matarrita, & Banerjee, 2014).

3. Results and discussion

3.1. Optimisation of sample pretreatment

The QuEChERS procedure is a combination of two steps (extraction and clean-up) during which a large number of factors can affect extraction efficiency. To evaluate and optimise the parameters that affect the QuEChERS procedure, P–B and CCD designs were used to search for the best experimental conditions.

3.1.1. Screening design

In this work, considering the complexity and long experimental time involved in the sequential study of all of the potential factors, an experimental P–B design was built to identify the main factors that elicit the responses. In this case, five factors, namely, the formic acid/MeCN ratio in the extraction solution (i.e., formic acid percentage, X_1, 0–10%), NaCl amount (X_2, 1–4 mg), PSA amount (X_3, 0–200 mg), C18 amount (X_4, 0–200 mg) and GCB amount (X_5,0–120 mg) were studied. The effects of the five selected parameters were investigated in 15 runs (12 + 3 centre points) that were performed randomly to eliminate the effects of extraneous or nuisance variables. An analysis of variance (ANOVA) was used to examine the main effects. Specifically, a t-test set at 95% confidence was employed(Vander Heyden et al., 1995). The effects of the studied factors in the P–B design were then illustrated in a standardised Pareto chart (Fig. 1) in which the significance of the variables on extraction yield is represented by bar length (the absolute values of the t-values). The vertical line delimits the 95% confidence interval. As can be concluded from Fig. 1, the formic acid percentage was the most significant variable, yielding a positive effect for all target analytes except BYI08330. The PSA amount was the next most significant variable followed by the amount of NaCl, and they exerted a negative effect. All other factors show no significant effect on extraction efficiency in the studied range and were thus eliminated from further studies.

3.1.2. Optimisation design

After the screening design, a CCD was used to evaluate the selected significant variables (i.e., formic acid percentage, NaCl amount and PSA amount) obtained from the P–B design and to find the optimal factor levels for each of the responses. A CCD consists of a two-level factorial design. Specifically, (2^f) with ($2f$) star points are at a distance α from its centre; Parameter f is the number of variables; at least one central point (N_0) is selected to establish the rotatability or orthogonality of the experimental design (Dejaegher & Heyden, 2011; Ferreira, Dos Santos, Quintella, Neto, & Bosque-Sendra, 2004). The central points were repeated three times to estimate the experimental error (pure error) and $\alpha = \sqrt[4]{2^f}$ (where f was set to 3). Thus, the total number of experimental points needed is 17 runs. The experiments were conducted randomly to minimise the effect of uncontrolled variables.

In this study, the mathematical relationship of responses to variables can be fitted with quadratic model, which can be expressed in the following polynomial equation:

$$y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2,$$

where y is the response and X_1, X_2, and X_3 correspond to formic acid percentage, NaCl amount and PSA amount, respectively. Parameter b_0 is the intercept. The variables b_1 to b_{33} indicate the coefficients of the polynomial equation. The data obtained were evaluated by ANOVA, which showed that the lack-of-fit (LOF, the variation of the data around the fit model) was not significant ($P > 0.05$). These results indicated that the model fitted the response well. As shown in Table 1, most model terms were significant ($P < 0.05$). At a 95% confidence level, the formic acid percentage was the most important factor affecting the extraction efficiency of all compounds except B-mono. The amount of NaCl affected the recoveries of thiacloprid, BYI08330, B-enol and B-mono. The PSA amount affected the recoveries of thiacloprid, BYI08330 and B-glu. For the quadratic terms, X_1^2, X_2^2 and X_3^2 were all significant except X_1^2 for thiacloprid and B-enol; X_2^2 for thiacloprid, B-enol and B-glu; and X_3^2 for BYI08330 and B-keto. For the interaction-terms, there was no significant correlation between the percentage of formic acid and the amount of PSA (X_1X_3) except with B-mono, demonstrating that the addition of formic acid to select solvents used to extract analytes (except B-mono) was independent of the amount of PSA. The percentage of formic acid and amount of NaCl amount showed a relatively strong interaction. Interaction of the NaCl and PSA amounts was significant for B-keto and B-glu.

Searching for optimal working conditions for the six responses requires the use of Derringer's desirability function, which can convert a multi-response problem into a single-response one. Derringer's desirability function (Derringer & Suich, 1980), a multi-criterion decision-making method proposed by Derringer and Suich in 1980, is the most current and frequently used technology for multiple response optimisation. The procedure involves the transformation of each individual response to a desirability function (d_i), defined as a dimensionless partial desirability function that varies from 0 (considered a completely undesirable response) to 1 (considered a fully desired response). The individual desirability functions are then combined into an overall desirable function (D) by calculating the geometric average of different d_i values:

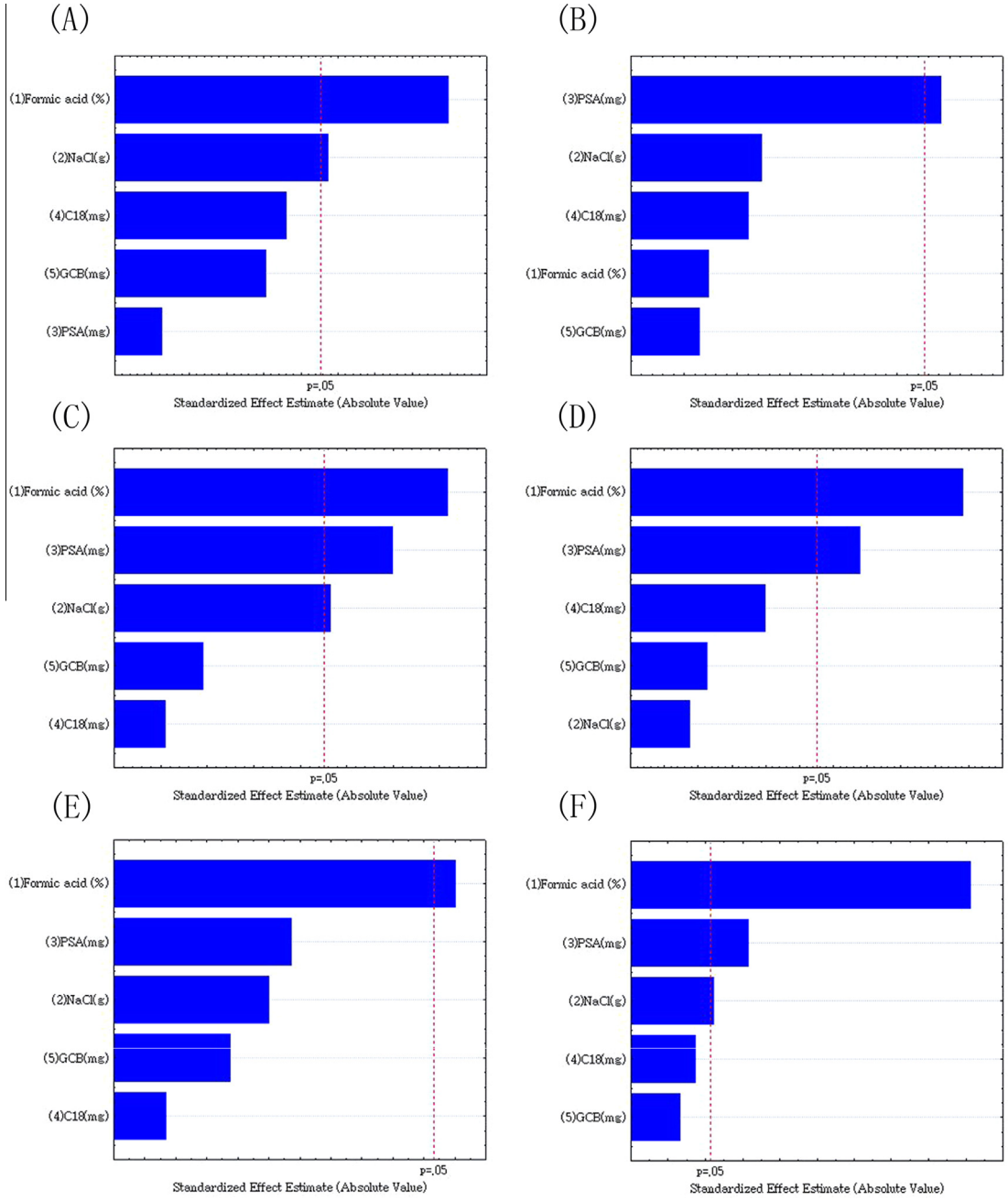

Fig. 1. Standardised main effect Pareto charts obtained from the Plackett–Burman design. (A) thiacloprid, (B) BYI08330, (C) B-enol, (D) B-mono, (E) B-keto and (F) B-glu.

Table 1
Analysis of variance (ANOVA) for the central composite design.

Source	Thiacioprid		BYI08330		B-enol		B-mono		B-keto		B-glu	
	Coefficients	*P*-value	Coefficients	*P*-value	Coefficients	*P*-value	Coefficients	*P*-value	Coefficients	*P*-value	Coefficients	*P*-value
Intercept	97.969	0.000	99.991	0.000	84.977	0.000	90.704	0.000	100.303	0.000	77.874	0.001
X_1	−9.024	0.006	−3.737	0.046	11.921	0.020	0.469	0.515	−10.621	0.010	22.215	0.010
X_2	2.971	0.048	−4.657	0.030	−15.262	0.012	2.644	0.047	2.514	0.146	8.191	0.065
X_3	−4.435	0.022	4.393	0.034	−2.745	0.249	−0.142	0.834	−3.559	0.082	−13.423	0.026
X_1^2	−0.530	0.550	−7.509	0.015	−3.287	0.224	3.296	0.038	6.125	0.036	−13.317	0.032
X_2^2	0.075	0.929	4.111	0.047	7.463	0.059	13.939	0.002	8.530	0.019	−6.804	0.107
X_3^2	4.794	0.023	0.850	0.453	18.258	0.011	9.903	0.004	4.228	0.072	−10.847	0.047
X_1X_2	−0.350	0.729	3.077	0.105	32.747	0.005	4.548	0.028	−6.489	0.044	−5.263	0.208
X_1X_3	2.300	0.120	3.054	0.107	−0.102	0.968	6.397	0.015	−3.095	0.160	4.349	0.268
X_2X_3	−2.300	0.120	2.610	0.138	4.071	0.209	2.203	0.106	11.695	0.014	22.903	0.015

Table 2
Method validation data for thiacloprid, BYI08330 and its four metabolites in pepper.

Compound	Fortified level (mg/kg)	Average recovery (%)	RSD (%)	LOD (mg/kg)	LOQ (mg/kg)
Thiacioprid	0.005	99.8	4.9	0.001	0.002
	0.1	100.4	11.3		
	1	96.3	4.1		
BYI08330	0.005	89.4	5.0	0.0002	0.001
	0.1	99.4	4.6		
	1	89.1	3.4		
B-enol	0.005	88.3	7.6	0.001	0.004
	0.1	100.1	3.7		
	1	94.5	4.4		
B-mo2no	0.005	97.8	6.5	0.001	0.003
	0.1	109.7	4.3		
	1	91.4	3.8		
B-keto	0.005	88.93	9.9	0.001	0.002
	0.1	114.2	7.3		
	1	119.5	2.7		
B-glu	0.01	75.7	4.3	0.003	0.01
	0.1	77.3	12.1		
	1	71.6	6.7		

$$D = \left(\prod_{i=1}^{n} d_i\right)^{\frac{1}{n}}$$

where d_i is the partial desirability function of each response and n is the number of responses. According to the overall results calculated from the desirability function, the optimum working conditions are as follows: 6.8% formic acid, 2.5 g NaCl and 29.7 mg PSA.

3.2. Method performance

The linearity of the method was evaluated by spiking pepper samples with the six target analytes over the concentration range of 0.005–1.0 mg/L. Good linearity was obtained in the studied concentration range for all six pesticides with correlation coefficient (r) values ranging from 0.9959 to 0.9999. The matrix effect was evaluated using a Student's t-test to compare the slopes of the standard and matrix-matched calibration curves (Biluca et al., 2014). The results proved no marked matrix-induced suppression or enhancement effects for thiacloprid ($P = 0.054$), B-keto ($P = 0.051$) and B-glu ($P = 0.256$), while significant differences were observed for BYI08330 ($P = 0.004$), B-enol ($P = 0.002$) and B-mono ($P = 0.028$) demonstrating that there exit obvious matrix effects for these three compounds. Thus, matrix-matched solution calibration curves were used to quantify the investigated compounds. The limits of detection (LODs) were determined at a signal-to-noise ratio (S/N) of 3; limits of quantification (LOQs) were calculated at 10 times the above-mentioned ratio. As shown in Table 2, LOD and LOQ values ranged from 0.0002 to 0.003 mg/kg and between 0.001 and 0.01 mg/kg, respectively. For the recovery experiment, pepper samples were spiked at three different concentrations and analysed in five replicates. As observed in Table 2, recoveries of thiacloprid, BYI08330, B-enol, B-mono, B-keto and B-glu were in the range of 96.3–100.4%, 89.1–99.4%, 88.3–100.1%, 91.4–109.7%, 88.93–120.1% and 71.6–77.3% with RSDs less than 12.1%. The results indicated that the developed QuEChERS method coupled to UHPLC/MS/MS analysis is suitable for the determination of thiacloprid, spirotetramat and spirotetramat's four metabolites in pepper.

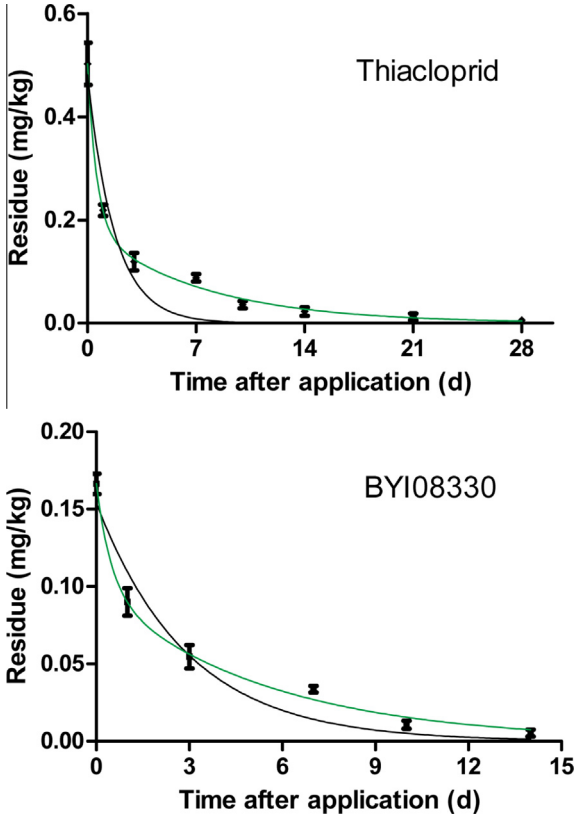

Fig. 2. Dissipation of thiacloprid and BYI08330 in pepper. (1) black line: data fit with the first-order model and (2) green line: data fit with the first + first-order model. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.3. Dissipation and residue

The dissipation of thiacloprid and spirotetramat residues was simulated by both a first-order model and a first + first-order model. Better fits to the field data were obtained using the first + - first-order model, which yielded correlation coefficient (R^2) values of 0.9904 for thiacloprid and 0.9891 for spirotetramat (Table 3). Therefore, the data obtained was evaluated using a first + first-order model. Fig. 2 shows that a fast initial decrease in both pesticides was followed by a slower decline. This pattern is referred to as a bi-phasic pattern of pesticide degradation, where one part was immediately available in a free form while the other fraction was adsorbed on cellular components, remaining in dynamic equilibrium and degrading slowly over time (Jadhav et al., 2013). The initial thiacloprid and spirotetramat concentrations were

Table 3
Regression equation, correlation coefficient and half-life of thiacloprid and BYI08330 in pepper.

Analytes	Models	Dynamic equation	R^2	DT50
Thiacloprid	First-order model	$C_t = 0.4811e^{-0.5761x}$	0.9303	1.20
	Bi-exponential model	$C_t = 0.3181e^{-1.718x} + 0.1846e^{-0.1365x}$	0.9904	0.81
BYI08330	First-order model	$C_t = 0.1541e^{-0.3402x}$	0.9412	2.04
	Bi-exponential model	$C_t = 0.0692e^{-2.0550x} + 0.0974e^{-0.1834x}$	0.9891	1.21

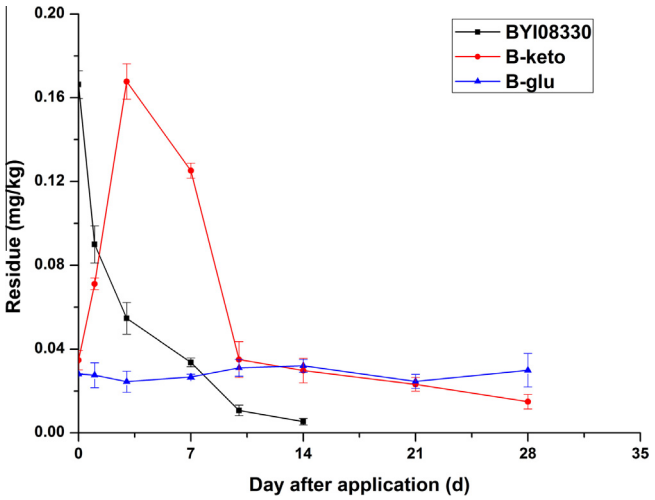

Fig. 3. Dissipation of BYI08330 and its metabolites in pepper.

0.503 mg/kg and 0.166 mg/kg. The thiacloprid and spirotetramat half-lives were 0.81 and 1.21 days, respectively. In this field experiment, B-enol, B-keto and B-glu were detected in pepper samples. Cleavage of the ester group in spirotetramat yields B-enol. Hydroxylation of the tetramic acid moiety resulted in B-keto, B-enol conjugated to glucose yielded B-glu. However, the detected B-enol concentration was below the LOD. Trace levels of B-enol in pepper indicate its rapid degradation and conversion in pepper. B-keto increased first and then decreased continuously over the entire period, whereas B-glu was constant throughout the course of the entire period (Fig. 3).

The terminal residues of thiacloprid, spirotetramat and spirotetramat's metabolites (B-enol, B-keto and B-glu) were positive after applying the thiacloprid·spirotetramat formulation (21.6%, SC) at 144 g a.i. ha^{-1} (the recommended dosage) and 216 g a.i. ha^{-1} (1.5 times the recommended dosage). The residue levels of thiacloprid and spirotetramat (sum of spirotetramat, B-enol, B-keto and B-glu, expressed as spirotetramat) in pepper were 0.016–0.13 and 0.080–0.12 mg/kg, respectively. These values are much lower than the MRL established by Switzerland (1.0 mg/kg for thiacloprid and spirotetramat). These results demonstrated that the 21.6% thiacloprid·spirotetramat formulation (SC) was safe at the recommended dosage to protect pepper from insects in China.

4. Conclusion

This study represents the first application of a simple and reliable QuEChERS approach combined with UHPLC/MS/MS to simultaneously determine thiacloprid, spirotetramat and spirotetramat's four metabolites in pepper. Furthermore, in this research, experimental parameters influencing the efficiency of the QuEChERS method were optimised using a chemometric procedure equipped with a P–B design (to investigate the main variables affecting the QuEChERS procedure) and a CCD combined with a DF (to evaluate the selected variables and find the optimal experimental conditions). This simple extraction method is robust considering the linearity, accuracy, precision, LOD and LOQ values. The extraction method also enables high-throughput quantification in terms of convenience, low cost and celerity. The applicability of the proposed analytical method was demonstrated with the analysis of field pepper samples. The trial results showed that the half-lives of thiacloprid and spirotetramat in pepper were 0.81 and 1.21 days, respectively. The metabolite B-enol was detectable, but below the LOQ. Notably, B-glu levels remained constant throughout the course of the experiment. In pepper, B-keto levels increased first and then decreased. The terminal residues of thiacloprid and spirotetramat in pepper were far below the MRLs.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2015.07.122.

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