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Residues, degradation behavior of cyproconazole in Sichuan pepper and its dietary risk assessment

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Abstract: In this study, an efficient method was developed and validated to determine cyproconazole in Sichuan pepper samples using the QuEChERS procedure and ultrahigh-performance liquid chromatography-tandem mass spectrometry. The average recoveries of the method were 96.2%-104.5% with the relative standard deviation (RSDs) below 7.6%, the limit of detection (LOD), and the limit of quantitation (LOQ) was 0.001 mg/kg and 0.02 mg/kg. The field trial results showed that cyproconazole in two Sichuan pepper matrices was rapidly dissipated with half-lives less than 8.8 days, which could be regarded as an easy degradation pesticide. The terminal residues of cyproconazole in Sichuan pepper samples were lower than the maximum residue limit (MRL) set by China (0.2 mg/kg registered on wheat). The calculated hazard quotient and acute hazard index lower than 100% illustrated that the use of cyproconazole, even at 1.5 times the highest recommended dose, does not pose any hazards to consumers.

Keywords: Residue analysis; Degradation behavior; cyproconazole; Sichuan pepper (Zanthoxylum bungeanum Maxim.); Dietary risk assessment

1. Introduction

Sichuan pepper (*Zanthoxylum bungeanum Maxim.*) has been regarded as a common household seasoning since the Spring and Autumn Period and Warring States Period in ancient China (Xu et al., 2019). Besides, it has the effects of warming the middle gas, eliminating cold, and relieving pain. Therefore, Sichuan pepper can be widely used as traditional Chinese medicine (Fan et al., 2020). Meanwhile, it can relieve symptoms such as stomach pain, vomiting, diarrhea and can also treat schistosomiasis and ascariasis (Singh et al., 2013; Xiang et al., 2016). Given the fact that Sichuan pepper not only had the advantages of easy cultivation, convenient management, and widespread distribution in China, but also its yield and quality occupied a tremendous advantage in cash crops, Sichuan pepper therefore had excellent development potential (Wang et al., 2019). However, the biggest challenge encountered in production was mainly the appearance of several pests and diseases. In particular, the primary diseases of Sichuan pepper were bituminous coal disease, gum disease, rust disease, and anthracnose. Among them, gum disease and rust disease could be bought about the most severe damage to the yield and quality of Sichuan pepper. Currently, the most common and effective approach used for removing the loss was chemical control, that is, pesticide.

Cyproconazole, 2-(4-chlorophenyl)-3-cyclopropyl-1-(1H-1,2,4-triazol-1-yl)butan-2-ol, is a novel triazole fungicide with therapeutic and systemic effects developed by Syngenta in 1987 (Cao et al., 2019). Its action mechanism mainly inhibits the biosynthesis of sterols in bacteria (He et al., 2019). It is mainly used to prevent diseases

caused by powdery mildew, rust disease, ceratospora, sclerotia, and spores in cereals, wheat, vegetables, fruit tree stems, and leaves, especially for *Bipolaris sorokiniana* (Sacc.ex Sorok.) Shoem., *Erysiphe graminis* D.c.f.sp.*tritici* E. Marchal, and *Fusarium moniliforme* Sheld (Culbreath et al., 1993; Wang et al., 2010; Wang et al., 2011). Numerous literatures had reported on the detection methods of cyproconazole residues in varieties of crops, plants, vegetables, and fruits include mainly (Reichert et al., 2020; Schermerhorn et al., 2005; Dedola et al., 2014; Papadopoulou-Mourkidou et al., 1995; Miyauchi et al., 2005; Momohara and Ohmura, 2014). The methanol or acetone was chosen to be the extraction solvent, and the detection instruments were gas chromatography-nitrogen and phosphorus detector, liquid chromatography-diode array detector, gas chromatography-electron bombardment ion source mass spectrometry. However, the amounts of acetone extraction on the above methods were large, and many impurities existed in the extracts, which is not conducive to the purification procedure. In addition, the methanol extraction that required rotary evaporation had low efficiency. Moreover, the popularity of gas chromatography-electron bombardment ion source mass spectrometers was lower than the routine tandem mass spectrometers.

The chemical components of Sichuan pepper are complex, containing alkaloids, amides, lignans, coumarins, aromatic oils, fatty acids, triterpenes, sterols, alkanes, and flavonoid glycosides, and other compounds (Han et al., 2019). These impurities could generate severe matrix effects when using the different detection instruments, especially for liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) and gas chromatography-tandem mass spectrometry (GC-MS/MS), which might decrease

the stability and accuracy of the qualitative and quantitative analyses. In order to eliminate or reduce the matrix effects, pre-treatment technologies such as solid-phase matrix extraction and purification, gel permeation chromatography, and supercritical fluid extraction had been widely used (GB 23200.9-2016, 2016; Ruan et al., 2013; Wang et al., 2017; Li et al., 2010). However, these methods were cumbersome to operate, using large amounts of solvents, time-consuming, and not conducive to rapid screening of pesticide residues. QuEChERS, a quick, easy, cheap, effective, rugged, and safe pre-treatment technology, greatly simplified the sample pre-treatment process and became the preferred approach for pesticide multi-residue detection (Anastassiades et al., 2003). Therefore, it had been widely applied to analyze the pesticides on several substrates such as grains, oilseeds, tea, vegetables, and fruits (Huang et al., 2018; Fang et al., 2020; Fu et al., 2020). Moreover, Sufficient researches had been established the determination method coupled with QuEChERS for some pesticides in Sichuan pepper samples, including the high-performance liquid chromatography-mass spectrometry (HPLC-MS) method for the detection of flumetsulam (Wang et al., 2017), gas chromatography (GC) to detect pesticides such as BHC, DDT, fenvalerate, deltamethrin, fenthion, and malathion (Peng and Yao, 2019). From the perspective of substance characteristics and pesticide polarity, the QuEChERS technology had the advantages of better selectivity, higher sensitivity, and broader application range combining with the ultra-high performance chromatographic-tandem triple quadrupole mass spectrometry (UHPLC-MS/MS) than gas chromatography (GC) and high-performance liquid chromatography (HPLC). However, there is no report on the detection method of

cyproconazole residue in Sichuan pepper. Furthermore, a common phenomenon that blindly pesticides sprayed by farmers during the process of cultivating was observed in pursuit of efficacy and economic value, ignoring the safe use of pesticides and the reasonable harvest interval, thereby resulting in excessive pesticide residues and reducing the quality of Sichuan pepper, which affected the food safety and increased the burden of ecosystem protection. Therefore, it is of great significance to develop a novel, simple, reliable, and effective analytical method to determine the cyproconazole residue in Sichuan pepper samples.

Scientific analytical methods were used to assess the threat of pesticide residues quantitatively and qualitatively in edible agricultural products to human health or the environment. Besides, the degradation behavior and terminal residue deposits of a specific pesticide in/on some agricultural products were received more and more attention and interest among plenty of food chemistry scientists. Previous researches had been conducted on the prevention and control effects of cyproconazole on specific diseases, as well as the analysis of the half-life of cyproconazole on some crops and fruits, which pointed out that the half-lives of cyproconazole were 3-4 days in grapes (Papadopoulou-Mourkidou et al., 1995), 3.5-4.0 days in cucumbers (Wang et al., 2011), and 3.0-5.5 days in wheat plants (Wu et al., 2015), respectively. However, the residual degradation dynamics of cyproconazole in Sichuan pepper samples and the final residual deposits were still rarely reported. Therefore, understanding the distribution and dissipation of cyproconazole in the Sichuan pepper is essential to reduce the adverse effects on the environmental system and minimize the potential risks to human

health. Additionally, with the development of society and the improvement of living standards, food safety has received more and more attention. Maximum residue limits (MRLs) are formulated by various international authorities and government institutions to rule the lowest allowed pesticide residues levels in/on the related agricultural products. The European Union (EU) and the international Codex Alimentarius Commission (CAC) have not yet established the MRL value of cyproconazole in any crops and fruits. The MRLs of 0.2 mg kg^{-1} were set only in wheat regulated by the Chinese government (GB2763-2019, 2019). Besides, dietary intake risk assessment is an essential part of pesticide safety evaluation and an essential basis for scientific pesticide application to protect the ecological environment and human health.

Therefore, we focused on three specific aims in this study as follows: (1) to develop an analysis method based on the modified QuEChERS pre-treatment combined with an improved UHPLC-MS/MS conditions to determine the cyproconazole residues; whilst (2) to study the dissipation dynamic trend and final residues of cyproconazole in two Sichuan pepper matrices; finally (3) to evaluate the potential chronic and acute dietary hazards of the cyproconazole to consumers through Sichuan pepper intake by comparing international estimated long-term daily intake (IEDI) with the acceptable daily intake (ADI), and international estimated short-term daily intake (IESTI) with acute reference dose (ARfD), respectively. The research in this article could provide technical support for monitoring the cyproconazole residue levels and evaluating the risk assessment of cyproconazole in Sichuan pepper. Meanwhile, the results offered a data basis to formulate the rational use of cyproconazole in Sichuan pepper and its MRL

standards.

2. Materials and methods

2.1. Chemicals and reagents

The commercial product of 40% cyproconazole suspension concentrate (SC) was supplied by the Jiangsu seven continents green chemical Co., Ltd (Jiangsu, China). The cyproconazole standard (purity 98.56%) was purchased from the Dr. Ehrenstorfer GmbH (Augsburg, Germany). HPLC grade organic solvent acetonitrile was obtained from the TEDIA chemical reagents Co., Ltd (Ohio, USA). Other analytical-grade reagents, such as acetone, formic acid, acetic acid, anhydrous magnesium sulfate ($MgSO_4$), and sodium chloride (NaCl), were purchased from the Sinopharm Chemical Reagent Co., Ltd (Shanghai, China) and Shanghai Lingfeng Chemical Reagent Co., Ltd (Shanghai, China), respectively. Ultra-pure water was obtained from the Youpu Instrument Equipment Co., Ltd (Xi'an, China). The purification agents were purchased from Agela Technologies (Tianjin, China) and Nanjing Chemical Reagent Co., Ltd (Nanjing, China), respectively, including 40-60 μm 60A primary secondary amine (PSA), graphitized carbon black (GCB), and C18.

2.2. Standard solutions preparation

0.0110 g solid portions of standard cyproconazole (purity 98.56%) were accurately taken into a 10 mL volumetric flask, diluted with acetone to prepare 1084 mg/L standard stock solution. Subsequently, the standard solutions of 100 mg/L and 10 mg/L were

obtained by pipetting the appropriate stock solution volume with acetonitrile. A series concentration of solvent-matched and matrix-matched solutions of 0.002, 0.004, 0.02, 0.04, 0.2, 0.4, and 1 mg/L was prepared by gradient dilution with acetonitrile and different extracts of Sichuan pepper matrix, respectively. All of the solutions were stored at -4 °C in sealed vials, protected from light, and brought to room temperature before use. Besides, these standard solutions were filtered through 0.22 μm membrane syringe filters before injecting into the chromatographic system.

2.3. Field trials

The supervised field trials were designed in accordance with the reference of Pesticide Residue Test Guidelines (NY/T 788-2018, 2018). In this study, a commercial product of 40% cyproconazole SC was applied to investigate the degradation patterns and final residual deposits on Sichuan pepper in four different cultivation regions, Mengzhou City, Henan Province (temperate continental climate, 113.21°E and 35.24°N), Xuzhou City, Jiangsu Province (north-subtropical monsoon climate, 117.18°E and 34.27°N), Chengdu City, Sichuan Province (subtropical monsoon climate, 102.54°E and 30.05°N), and Qianxinan Prefecture, Guizhou Province (subtropical monsoon humid climate, 104.91°E and 25.09°N). Treatments with cyproconazole were applied in triplicate. Each plot has four trees, and the amount of water added is 2 L per Sichuan pepper tree (it can be increased or decreased according to the size of the trees). It is necessary to ensure uniform spraying, and the application sequence was from the control plot to the treatment plot.

The Sichuan pepper of the four sites were the common local cultivation varieties (Mengzhou, *dahongpao*; Xuzhou, *dahongpao*; Chendu, *jiuyeqing*; Qianxinan, *dingtan*). The average daily temperature during the trials was as follows: Mengzhou, 20-39 °C from 4th July to 22nd August 2019; Xuzhou, 16-35 °C from 24th May to 12th July 2019; Chendu, 19-34 °C from 6th June to 18th July 2019; Qianxinan, 18-31 °C from 3rd July to 14th August 2019. The mean rainfalls during the experiment period were 239.8 mm (Mengzhou), 119.3 mm (Xuzhou), 315.4 mm (Chendu), 286.7 mm (Qianxinan), respectively. Mengzhou soil belongs to the cinnamon soil type (pH 7.2, organic matter content 2.0%). Xuzhou soil is clay (pH 6.8, organic matter content 2.0%). Chendu soil is the loam (pH 6.7, organic matter content 3.3%). Qianxinan soil belongs to the yellow-brown earth type (pH 6.6, organic matter content 2.3%). The previous crops of these sites are Sichuan pepper without any pesticide applications.

During the dissipation experiment, the 40% SC was sprayed at the 1.5 times recommended maximum dosage (200 g a.i./hm^2) twice during the early onset period of pepper rust, with the application interval of 7 days. Meanwhile, a separate plot was maintained with the water as a control. Representative fresh Sichuan pepper samples were collected separately, at intervals of 0.08 (2 h), 1, 7, 14, 21, 28, 35, and 42 days after the last session of spraying. For the final residue experiment, the cyproconazole was applied at the two dosages of 133 g a.i./hm^2 (highest recommended dosage) and 200 g a.i./hm^2 with two- or three-times application, respectively. Representative fresh Sichuan pepper samples were collected randomly from each plot, at intervals of 28 and 35 days after the last spraying.

Subsequently, the supervised field trial samples were strictly followed with the Guideline on sampling for pesticide residue analysis issued by the Chinese Ministry of Agriculture and Rural Affairs (NY/T 789-2004, 2004). Briefly, at least 0.5 kg of fresh peppers samples were harvested at different directions and positions from top to bottom and utilized to prepare at least 0.2 kg of dried pepper samples and put into sample bags and wrapped properly. The fresh and dried pepper samples were mixed thoroughly in a stainless-steel basin, separately. Two samples of 150 g for different matrices, one for routine experiment and the other for storage stability testing, were transferred into ziplock bags and pasted laboratory sample labels. The prepared field trial samples should be transferred into the laboratory following frozen (below -20 °C) and stored in the freezer or cold storage. In addition, the storage temperature shall be continuously monitored and recorded.

2.4. Laboratory sample preparation

2.0 g of blank and unprocessed Sichuan pepper sample was accurately placed into a 50 mL centrifuge tube. 10 mL of 0.1% acetic acid and acetonitrile were added. The tubes containing the target compound were vortexed for 1min, followed by the ultrasonic extraction for 20min. The capped tubes were immediately vortexed intensively for 1 min and then centrifuged at 5000 rpm for 5min. Subsequently, 1.0 mL of the upper organic phase was transferred into a 1.5 mL single-use centrifuge tube containing 25 mg PSA, 100 mg GCB, and 50 mg anhydrous $MgSO_4$. Then, the mixture was again vortexed for 30 s and centrifuged at 10000 rpm for 5min. The resulting

supernatant was passed through a 0.22 μm filter membrane into an autosampler vial for instrument injection.

2.5. Instrumental parameters

Chromatographic separation of cyproconazole was carried out on a Waters Acquity UPLC binary solvent manager equipped with an Agilent Eclipse Plus-C_{18} column (2.1 mm×100 mm, 3.5 m particle size) (Agilent Technologies, USA). The column temperature was kept at 30 °C during the experiments. The mobile phases A and B were 0.1% (v/v) formic acid and acetonitrile, respectively, operating under gradient elution. Elution was carried out as follows: 0–1 min, 90% A; 1–1.5 min, 90–5% A; 1.5–2.5 min, 5% A; 2.5–2.6 min, 5–90% A; 2.6–3.5 min, 90% A. The flow rate was 0.35 mL/min, and the injection (5 μL) was conducted using an autosampler. The total run time was 3.5 min. The temperature of the autosampler vial holder was maintained at 4 °C. Under these conditions, the retention time for cyproconazole was found at 2.45 min.

A triple quadrupole (TQD) mass spectrometer (Waters Corp., Milford, MA, USA) equipped with an electron spray ionization (ESI) source was applied to quantify these compounds. The nebulizer gas was 99.95% nitrogen, and the collision gas was 99.99% argon with a pressure of 3.2×10^{-3} mbar in the T-Wave cell. The positive ionization-switching mode was selected, and the monitoring conditions optimized for cyproconazole were as follows: the capillary voltage was set at 3.0 kV, and the source temperature and desolvation temperature were held at 110 °C and 450 °C, respectively. 99.95% nitrogen and 99.99% argon were applied as nebulizer gas and collision gas. A

50 L/h cone gas flow and 500 L/h desolvation gas flow were used. Multi-reaction monitoring (MRM) was used to detect all compounds with a dwell time of 106 ms. Transition mass-to-charge ratio (m/z) of 292.10>125.05 was used for quantification, m/z of 292.10>70.05 was used for identification, and the corresponding cone energy and collision voltage were 40 and 20, 25 eV, respectively. The MassLynx NT V.4.1 (Waters, USA) software was used to collect and analyze the data obtained.

2.6. Analytical method validation and calculation

The validation of the method was evaluated based on the principle of SANTE guidelines (SANTE, 2017), which included a series of parameters: specificity, linearity, matrix effect, the limit of quantitation (LOQ), accuracy, and precision.

Blank samples were analyzed to identify the absence of interfering species during the retention time of the analyte. The linearity of calibration curves was evaluated both in the solvent-based and matrix-based standard solution at a concentration range of 0.002–1 mg L^{-1}. Matrix effects (%ME) were calculated as follows:

$$\%ME = \frac{K_M - K_S}{K_S} \times 100\%$$

Where K_m refers to the calibration curve slope in the matrix; K_s refers to the calibration curve slope of the solvent-based standard concentration. When ME > 20%, it indicates a significant matrix enhancement effect; when ME < -20%, it indicates a significant matrix inhibitory effect; when -20% < ME <20%, it indicates that the matrix effect is not significant.

The LOQ was defined as the lowest spiked concentration that could meet the analysis

criterion. Method accuracy refers to the degree to which the result measured by the developed method is close to the actual value or reference value, expressed in terms of recovery rate. Method precision refers to the degree of closeness between the results obtained after multiple sampling and determination of the same homogeneous sample under specified conditions, expressed by the relative standard deviation. Briefly, the appropriate volumes of the cyproconazole standard working solution were added into two blank Sichuan pepper matrices, thereby preparing the method validation samples at fortification levels of 0.02, 0.2, and 1 mg/kg, with five replicates for each level on three different days. The average recovery and relative standard deviation were calculated based on the previous report (Xu et al., 2021). The LOQ for cyproconazole was defined as the lowest validated spiked level in the Sichuan pepper matrix, meeting the requirements of a recovery within the range of 70–120% and the RSD lower than 20%.

2.7. Storage stability experiment

The experiment was designed according to the reference of "Test Guidelines for the Storage Stability of Pesticide Residues in Plant Origin Agricultural Products" (NY/T 3094-2017, 2017). The Sichuan pepper samples were thoroughly mixed in a stainless-steel basin to prepare a storage stability sample of Sichuan pepper with cyproconazole at a concentration of 0.2 mg/kg. 2.0 g of each sample of Sichuan pepper was weighed accurately, followed by putting it into a 50 mL centrifuge tube. Subsequently, the tubes contained the matrix and pesticide were stored in a freezer with a temperature lower

than -20°C. The storage time was set to 0 days, 31 days, 92 days, and 206 days. Besides, two quality control samples for each matrix at each storage time were prepared to evaluate the accuracy and reproducibility of the method. Sample extraction and detection methods are based on established analytical methods. The degradation rate of the cyproconazole was calculated according to Eq. (1) as follows:

$$D = \frac{C_0 - C_t}{C_0} \times 100\% \quad (1)$$

Here, D was the degradation rate; C_0 was the initial concentration of the sample (mg/kg); C_t was the detected concentration of the sample (mg/kg); During the storage test, the degradation rates are less than 30%, indicating that the cyproconazole is stable. By contrast, if the degradation values are more than 30%, which indicates that it is unstable during this period.

2.8. Statistical analysis

The dissipation pattern and degradation half-lives (DT_{50}) of cyproconazole on Sichuan pepper were evaluated by fitting the first-order kinetic model as follows:

$$C_t = C_0 \times e^{-kt} \quad (2)$$

$$DT_{50} = \frac{ln2}{k} \quad (3)$$

Here, C_t and C_0 are the residual level of cyproconazole (mg/kg) at time point t (day) and the initial concentration (mg/kg), respectively. k is the degradation rate constants of cyproconazole.

The SPSS 20.0 software was utilized to perform a one-way analysis of variance on the data. When $P<0.05$, it was considered that there was a significant difference. The

Excel 2019 software was applied to calculate the results and make related figures.

2.9. Dietary risk assessment

Dietary exposure and risk assessment are mainly used to evaluate the possible exposure routes and doses in daily life, to clarify the sensitive groups that may be harmed and the actual and expected exposure dose levels. In this study, Sichuan pepper was used as a single pesticide residue exposure route, and chronic intake risk assessment was conducted based on the data obtained from terminal residue experiments. The chronic intake risk quotient (RQ_c) was calculated according to formulas (4) and (5):

$$NEDI = \frac{\sum STMR_i \times F_i}{bw} \quad (4)$$

$$RQ_c = \frac{NEDI}{ADI} \times 100\% \quad (5)$$

The acute dietary exposure risk assessment (RQ_a) was evaluated by Eq. (5) and (6) as follows:

$$NESTI = HR \times F_i \quad (6)$$

$$RQ_a = \frac{NESTI}{ARfD \times bw} \times 100\% \quad (7)$$

Here, NEDI refers to the national estimated daily intake (mg/kg bw). $STMR_i$ represents the supervised trials median residual level of cyproconazole on Sichuan pepper or other crops regulated in China (mg/kg). The corresponding MRLs were utilized for calculating NEDI when no available $STMR_i$. F_i (kg) is the general population's consumption of a particular food in China. bw is the average body weight of a Chinese

adult (63 kg). ADI (mg/kg bw) is the acceptable daily intake (ADI) of cyproconazole. NESTI refers to the international estimated short-term intake (mg/kg bw). HR (mg/kg) is the highest terminal residue level. ARfD (mg/kg bw) is the acute reference dose. The RQ_c is determined by comparing NEDI and ADI values, while RQ_a is obtained by comparing NESTI and ARfD values. An RQ < 100% demonstrates that the evaluated food poses an acceptably minor health hazard to consumers. Therefore, Smaller RQ values, lower intake risk; (Xu et al., 2021).

3. Results and discussion

3.1. Analytical method development

3.1.1. Optimization of the instrument conditions

Suitable chromatographic separation and ionization conditions for the cyproconazole are essential to achieve accuracy and sensitivity in the analysis. The cyproconazole structure contains a hydroxyl group and is easy to ionize, so it is suitable for ESI sources. In this study, a 1.0 mg/L cyproconazole standard solution was directly injected into mass spectrometry. Then full scan for the precursor ion of the analyte was performed in the electrospray positive and negative ion (ESI+/−) modes, respectively. The results showed when scanning in ESI+ mode, the response intensity of the molecular ion peak $[M+H]^+$ of cyproconazole was higher than that of ion peak $[M-H]^-$. Meanwhile, the molecular ion of cyproconazole was obtained in the ESI+ mode as m/z 292.1. Subsequently, the product ion, cone voltage, and collision energy were optimized, the two transitions with the highest response intensity were selected as well as the

corresponding optimal cone voltage and collision energy were obtained. The quantitative transition was set with the most prominent response intensity (292.1 > 125.05), while the qualitative transition was chosen with relatively weak response intensity (292.1 > 70.05). Once the mass spectrum conditions were optimized, the liquid chromatographic parameters were modified to obtain a suitable peak shape and quick retention time. A C18 chromatographic column (100 mm × 2.1 mm, 3.5 μm) was applied to separate the analyte for the preliminary experiment at a flow rate of 0.3 mL/min with an isocratic elution condition. The effects of four mobile phase systems (methanol-water, methanol-0.1% formic acid water, acetonitrile-water, and acetonitrile-0.1% formic acid water) on the separation abilities of the target compound were investigated, respectively. The results showed that the retention behavior of cyproconazole on the C18 column was weak under the mobile phase system without adding formic acid, which might be due to the low ionization rate of cyproconazole under neutral condition, thereby resulting in poor signal peak shape. When acetonitrile and 0.1% formic acid aqueous solution were used as mobile phases, cyproconazole had satisfactory retention on the chromatographic column, the peak shape and detection sensitivity were significantly improved (Fig. 2). Therefore, acetonitrile-0.1% formic acid aqueous solution was selected as the mobile phase simultaneously with modified gradient elution, bearing in mind that gradient elution can reduce the accumulation of strongly retained impurities on the chromatographic column. Under these conditions, the retention time of cyproconazole was 2.45 min, and the analysis time was less than 3.5 min.

3.1.2. Optimization of the sample extraction procedure

Previous reports had proposed several extraction methods, including oscillation, sonication, and homogenization (Angioni et al., 2003; Wang et al., 2011; Wu et al., 2015; Xu et al., 2021). It should be noted that the high lipid contents in Sichuan pepper might bind the target analyte up to form greasy drops, which made it difficult for a polar solvent to extract cyproconazole through the non-polar environment. Therefore, a more robust extraction method would take effect by vibrational energy with continuous frequencies to break the lipid drops. Given the above assumptions and inferences, the effects of cyproconazole recoveries in Sichuan pepper matrices with different extraction methods were investigated. As clearly shown in Figure 3d, ultrasonic extraction contributed to the satisfactory recoveries for cyproconazole compared to the rest extraction without the clean-up process in Sichuan pepper samples. More concretely, the significant enhancement of cyproconazole recoveries in Sichuan pepper samples was observed from 50.9% ± 1.8% to 70.1% ± 3.3% in fresh Sichuan pepper, 53.9% ± 2.4% to 66.9% ± 3.9% in dried Sichuan pepper when using the sonication extraction.

Sufficient studies had demonstrated that acetonitrile had an appropriate polarity for most analytes, thereby generated higher recoveries and more minor co-extracted matrix components, such as pigments, proteins, lipids, and waxes (Xu et al., 2021; Fang et al., 2020; Fu et al., 2020). Consequently, acetonitrile was selected as the extraction solvent in this study. Acidified acetonitrile is a routine and effective method to increase extraction efficiency and minimize the degradation of labile analytes. Therefore, a certain proportion (0.1%, 0.2%, and 1%) of formic acid and acetate acid was added to

acetonitrile separately to optimize the extractant acidity. As shown in Fig. 3a, an unsatisfactory recovery (< 62%) was obtained by adding formic acid into acetonitrile. Meanwhile, decreased cyproconazole recoveries (70.5-71.7%) were acquired when 1% acetate acid was added. An acceptable recovery (79.2–80.5%) of cyproconazole on Sichuan pepper was obtained when adding 0.2% acetate acid. The results demonstrated that the buffer salt system formed by acidified acetonitrile (0.2% acetic acid acetonitrile solution) was more conducive to the dissolution of pesticides with acidic groups in acetonitrile. Thus, acetonitrile with 0.2% acetate acid was selected as the extraction solvent for cyproconazole in further research.

3.1.3. Optimization of the sample purification procedure

Given the fact that cyproconazole was a polar compound and the matrix contained a large amount of limonene, cumicalcohol, geraniol, phytosterols, unsaturated organic acids, and other impurities, which might decrease the chromatographic separation efficiency and instrument detection intensity, therefore, the clean-up procedure was necessary to increase the accuracy of the analysis method. The PSA, GCB, and C18 were currently widely applied to remove the impurities in the varieties of matrices with the combination or separation form. The PSA adsorption structure contains two amino groups, which can remove organic acids, fatty acids, sugars, and other substances through hydrogen bonds. C18 is a non-polar, broad-spectrum purifier, which can effectively remove non-polar impurities such as trace fats and esters in the extracts. The surface of GCB has strong adsorption, which can remove pigments, sterols, and non-polar interferences. In this study, the effects of cyproconazole recoveries in Sichuan

pepper matrices with six different forms of PSA, C18, and GCB were investigated, respectively. The results showed that when using GCB separately or with combination forms, the recovery rates of cyproconazole in the Sichuan pepper samples were between 55.2% and 65.6%, while over 82.2% were obtained with the rest adsorbents, possibly due to the fact that the GCB could remove the pesticide with planar structures. Moreover, the combination of the PSA and C18 was selected in this study based on the satisfactory cyproconazole recovery, ranged from 88.5% to 90.9%, which met the requirements of quantitative pesticide residue analysis issued by the Chinese Ministry of Agriculture and Rural Affairs (NY/T 788-2018, 2018). Furthermore, taking the effectiveness and cost of each adsorbent into consideration, six groups of the experiment were set to evaluate the suitable amounts of purification mixture agents and anhydrous $MgSO_4$. The ideal result had been yielded in Sichuan pepper matrices using the 100 mg of mixture adsorbents per 2 mL extract with equal amounts of PSA and C18, ranged from 87.5% to 96.5%. In terms of the amount of anhydrous $MgSO_4$, although 200 mg and 300 mg could enough to receive satisfactory results, the recoveries were slightly decreased, thereby could be more suitable with 150 mg of anhydrous $MgSO_4$ among others.

3.2. Analytical method validation

Linearity, LOD, LOQ, and matrix effect. A good linearity with correlation coefficients (R^2) exceeding 0.9953 was obtained in all Sichuan pepper matrices, ranging from 0.002 to 1 mg kg^{-1}. The limit of detection (LOD) of cyproconazole calculated by

three times the signal-to-noise (S/N) ratio was 0.001 mg kg^{-1}. The lowest fortification level of 0.02 mg kg^{-1} was regarded as the LOQs were validated with acceptable recovery in two Sichuan pepper matrices. The LOQ for cyproconazole was lower than the MRLs formulated by China on wheat (0.2 mg kg^{-1}).

For tandem mass spectrometry, since the interference or co-eluting matrix part affected the ionization efficiency of the electrospray interface, resulting in ion suppression or enhancement, false negative or false positive or inaccurate quantification. The matrix effect, therefore, is an essential parameter for analytical method verification. As shown in Table 2, the apparent signal suppression was observed for cyproconazole in Sichuan pepper matrices as the slope ratios of matrix/acetonitrile were ranged from -73.6% to -74.0%, indicating that the matrix effect still existed in Sichuan pepper despite the inclusion of a clean-up procedure, which was mainly in the form of ion suppression. In this study, the matrix-matched calibration curve was used to eliminate the matrix effect and obtain more accurate results for cyproconazole in the Sichuan pepper samples.

Precision and accuracy. Recoveries and RSD of cyproconazole were studied by spiking matrix spike samples with different concentrations (0.02, 0.2, and 1 mg kg^{-1}). On each of three different days, five replicate trials were performed for each fortification concentration of cyproconazole. A series of matrix-matched gradient calibration standard solutions was used to calculate the recovery, ranging from 0.002-1 mg/kg. The precision of the developed method was expressed as repeatability (RSD_r) and reproducibility (RSD_R). As shown in Table S1, the intra-day recovery rate of

cyproconazole in fresh Sichuan pepper sample was 96.2%~101.7%, the RSD_r was 2.6%~4.4%; the inter-day recovery rate was 94.6%~98.7%, and the RSD_R was 4.1%~6.7%. The intra-day recovery rate of cyproconazole in the dried pepper matrix was 98.7%~104.5%, and the RSD_r was 4.9%~7.6%; the inter-day recovery rate was 94.8%~99.3%, and the RSD_R was 3.8%~6.3%. Therefore, the established method can meet pesticide residue analysis requirements and be reliable and suitable for the routine analysis of the cyproconazole residues in Sichuan pepper. The typical chromatogram was shown in Figure S1. It can be seen that there was no apparent impurity interference at the peak retention time of cyproconazole.

3.3. Dissipation behavior of the cyproconazole on Sichuan pepper

In the degradation dynamics experiments, the pesticide was applied twice at 1.5 times of recommended high dose. The developed method was successfully performed for the analysis of the harvested samples. The dissipation curves of cyproconazole in two Sichuan pepper samples at two different cultivation areas were presented in Fig. 3. In accordance with the relevant literature, the residue levels in the samples 2 h after pesticide application were defined as the initial residue levels. In this study, the initial residue levels reached 0.84 mg/kg and 0.74 mg/kg in the fresh Sichuan pepper samples for Mengzhou and Xuzhou. The cyproconazole residues in two Sichuan pepper matrices were gradually degraded with time increases. The degradation rate is relatively fast in the early stage and relatively slow and flattened in the later stage. The dissipation dynamics of cyproconazole in fresh Sichuan pepper could be described using the first-

order kinetics equation: $C_t=0.9041e^{-0.097t}$ (Mengzhou, $R^2=0.9694$) and $C_t=0.6376e^{-0.081t}$ (Xuzhou, $R^2=0.9802$). The data indicated 97.6% and 97.3% of cyproconazole dissipated from Mengzhou and Xuzhou after 42 days, respectively. For dried Sichuan pepper samples, the initial residues of cyproconazole were 0.57 mg/kg and 0.62 mg/kg for Mengzhou and Xuzhou. The dissipation dynamics of cyproconazole could be described by the following first-order kinetics equation: $C_t=0.4489e^{-0.079t}$ (Mengzhou, $R^2=0.9616$) and $C_t=0.6855e^{-0.082t}$ (Xuzhou, $R^2=0.9062$). The degradation rate of cyproconazole was more than 50% in both Sichuan pepper samples 14 days after spraying. The data indicated 96.5% and 96.8% of cyproconazole dissipated from Mengzhou and Xuzhou after 42 days, respectively. The degradation half-lives of cyproconazole were 7.1-8.6 days in fresh Sichuan pepper samples and 8.5-8.8 days in dried Sichuan pepper samples. Previous studies showed that cyproconazole had the half-lives of 16 days in field peaches (without any other treatments), while the half-lives were 6 days when the surface was coated with wax in the photo-degradation model, and 2.5 days without wax (Angioni et al., 2003). Besides, the half-lives of cyproconazole in cucumber were 3.5-4.0 days, 3.9-5.2 days in soil (Wang et al., 2011); the dissipation half-lives in the wheat plant were 3.0-5.5 days (Wu et al., 2015), and 3-4 days in grapes (Papadopoulou-Mourkidou et al., 1995). Therefore, the half-lives of cyproconazole on Sichuan pepper in this study were longer compared to the above results. During the planting process of Sichuan pepper, the soil type, plant growth, cultivation management level, environmental factors all played a complicated role in the degradation of cyproconazole. It is necessary to understand in detail that the

translocation and residual degradation patterns of cyproconazole in Sichuan pepper samples, as well as the specific effects of individual factors, such as temperature, humidity, rainfall, wind speed, light, microorganisms, and plant growth on the dissipation of cyproconazole, which remain to be further studied.

3.4. Storage stability experiment

Under normal circumstances, the field trial samples needed to be stored for a period of time before analyzing. During this period, the target pesticide may be degraded due to metabolism, oxidation, hydrolysis, and other reactions, all of which would affect the authenticity and accuracy of the residue detection data. Therefore, it is of great significance to study the stability of pesticides during storage systematically. Food and Agriculture Organization (FAO), Organization for Economic Cooperation and Development (OECD), United States Environmental Protection Agency (EPA), and the Chinese Ministry of Agriculture and Rural Affairs had all made detailed regulations and requirements for storage stability tests. The degradation rates and recoveries of cyproconazole and in the two Sichuan pepper samples were presented in Table 1. The quality control sample with a concentration of 0.2 mg/kg was prepared by adding the blank pepper sample extracts to standard working solutions. Subsequently, the sample extraction, purification, and determination steps were performed following the modified analysis method. The average recoveries of quality control of cyproconazole in Sichuan pepper matrices were in the range of 93%~108%, which met the requirements of pesticide registration experiment residue analysis method (NY/T 3094-

2017, 2017), and the maximum average degradation rate of cyproconazole in two Sichuan peppers was 10.1% (< 30%), indicating that its storage stability is acceptable. During the period of the storage experiment, it was shown that there was no noticeable degradation for cyproconazole in Sichuan pepper matrices when the storage time was lower than 206 days and under the background temperature condition of lower than -18°C. The degradation rate of cyproconazole in different matrices fluctuated with the extension of storage time, a common trend in research on pesticide storage stability. Numerous studies had found that several different factors could bring about significant effects on the storage stability of pesticides, such as substrate types (JMPR, 2011; FAO, 2012), water content (Afridi et al., 2001), and pH values (Mastovská et al., 2004; Sun et al., 2003).

3.5. Terminal residue of the cyproconazole on Sichuan pepper

The results of cyproconazole residues in Sichuan pepper samples were shown in Table S2. The terminal residual mass fractions of cyproconazole at different harvest intervals were <0.02-0.17 mg/kg in fresh Sichuan pepper samples, <0.02-0.10 mg/kg in dried Sichuan pepper samples, which were all lower than the MRLs of 0.2 mg/kg formulated in wheat (GB2763-2019, 2019). The selection criteria for the MRLs value are: China is preferred, then Codex Alimentarius Commission (CAC), the United States, Australia, South Korea, the European Union, and Japan (Xu et al., 2021). Moreover, the choice of the MRL values followed the principle of maximizing risk, among which none of the countries or organizations except China had established the MRL value for

cyproconazole. It can be seen that with the increase of the application dose and the number of applications, the final residue of cyproconazole in two Sichuan pepper samples also increased. In other words, the cyproconazole residues detected in two Sichuan pepper samples collected when the application dosage was 133 g a.i./hm^2 were generally lower than the cyproconazole residues when the application dosage was 200 g a.i./hm^2. Similarly, the final residual amounts of cyproconazole in two Sichuan pepper samples after three times of application were higher than that of the twice application. Additionally, pre-harvest interval (PHI) is the time required after the last application of the target pesticide to dissipate to the safe level (lower than MRL). In this study, the terminal residual deposits of cyproconazole in two Sichuan pepper samples showed a gradually decreasing trend as the sampling interval increased under the premise of keeping other conditions consistent. Therefore, the frequency of application, the interval between harvests, and the dosage of application all significantly affected the final residue of cyproconazole in Sichuan pepper samples. However, it is worth noting that when the harvest interval was 35 days, the final residues of the harvested field pepper samples mainly were less than the LOQ even with the highest recommended dosage and application times, which suggested that 28-35 days could be the appropriate harvest intervals for cyproconazole in Sichuan pepper samples. Moreover, this work provides important data for establishing the tested pesticide MRLs for Sichuan pepper in China.

3.6. Risk assessment

Dietary exposure assessment originated from chemical safety assessment. From the theory of zero threshold to the concept of acceptable risk under certain probability conditions, it then evolved into the maximum allowable daily intake in food. The Codex Committee on Pesticide Residues (CCPR) believes that the maximum daily intake Amount (ADI) is not the only criterion for toxicological risk assessment (Li et al., 2016). In 1995, the World Health Organization Joint Conference on Pesticide Residues (JMPR) began to study the risk assessment of acute dietary exposure to pesticides. The specific method is to set the food consumption of the population according to the actual situation, determine it as a fixed value, and then multiply it with the fixed pollutant concentration in the food. Finally, the Dietary exposure risk quotient was obtained by comparing the dietary exposure estimates with the relevant health guidance values (such as ADI and ARfD) for the chemicals of concern (Boon et al., 2015). In this study, the chronic and acute dietary intake risk assessments were evaluated based on the terminal residue results of 1.5 times the highest recommended application dosage in two Sichuan pepper samples.

3.6.1. Chronic dietary intake risk assessment

The process of conducting chronic dietary exposure assessment was as follows: Firstly, the median distribution (STMR) of pesticide residues in agricultural products was obtained through the residue monitoring of substance samples and combined with the recommended dietary structure data to calculate the NEDI; and then the chronic risk quotient (RQ_c) of the test pesticides was obtained by comparing the NEDI with the ADI. In this study, The ADI value for cyproconazole is 0.02 mg/kg bw (GB 2763-2019, 2019).

Currently, the registered crop of cyproconazole was wheat only in China. Emphatically, Sichuan peppers are in soy source classification. Thus, the STMR of cyproconazole in two Sichuan pepper matrices was applied as the reference residue limits to calculate the NEDI. MRL of wheat (0.2 mg/kg, China) was applied to calculate the NEDI of the crop group for the sake of taking account of the maximum dietary hazard principle. As displayed in Table 2, the STMR values of cyproconazole in fresh Sichuan pepper was 0.082 mg/kg (PHI, 28 days) and 0.020 mg/kg (PHI, 35 days) with twice spraying treatment, while 0.16 mg/kg (PHI, 28 days), and 0.056 mg/kg (PHI, 35 days) with the third application, respectively. Similarly, the STMRs of cyproconazole in dried Sichuan pepper with twice or third application and different PHIs were far lower than the MRLs (< 0.2 mg/kg). Thus, the total NEDI of cyproconazole in various food classifications that are subject to registered crops was ranged from 0.0279 mg to 0.0291 mg. Therefore, the RQ_c values of cyproconazole in the two Sichuan pepper matrices were far below 100%, indicating a minor and acceptable chronic dietary intake hazard (Table 2).

3.6.2. Acute dietary intake risk assessment

The ARfD value for cyproconazole is 0.06 mg kg^{-1} bw (GB 2763-2019, 2019). The HR values of cyproconazole in two Sichuan pepper samples were 0.065-0.097 mg kg^{-1} (PHI, 28 days), 0.020-0.027 mg/kg (PHI, 35 days) with twice spraying treatment, while 0.10-0.17 mg kg^{-1} (PHI, 28 days) and 0.020-0.058 mg kg^{-1} (PHI, 35 days) with the third application, respectively. Therefore, the total NESTI values of cyproconazole in two Sichuan pepper matrices were far below the ARfD, which was ranged from 0.00018

mg to 0.00153 mg. Moreover, the RQ_a values were corresponding to 0.015%-0.023% (PHI, 28 days), 0.005%-0.006% (PHI, 35 days) with twice application, and 0.024%-0.040% (PHI, 28 days), 0.005%-0.014% (PHI, 35 days) with third treatment, respectively (Table 3). The results indicated that acute dietary intake risk of cyproconazole was negligible with consumption of Sichuan pepper

Risk assessment has a certain degree of uncertainty, which is mainly reflected in three aspects in this study. Firstly, the processing factors such as cleaning and cooking are not considered. Secondly, the safety of other isomers of cyproconazole is not considered, since the standard isomers are not readily available, and their toxicity and structure have still not been studied in depth (He et al., 2019), there may be certain unknown risks to human health. Finally, some limitations are still existed to assess the overall cyproconazole exposure risk of residents. The Sichuan pepper matrices were applied in this study as the single exposure route to cyproconazole, and residents would also be exposed to cyproconazole through various routes such as ingesting other fruits, vegetables, and grains. Therefore, uncertain factors such as processing factors, average residues, metabolites, and total dietary intake routes should be studied in-depth. Meanwhile, more precise guidelines for safe and proper use should be formulated in future researches.

4. Conclusions

In this study, a simple and efficient method for the residual analysis of cyproconazole on Sichuan pepper was established, extracted, and purified by QuEChERS pre-

treatment and dispersive solid-phase extraction followed by detected by ultrahigh-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS). Subsequently, a supervise field experiment of 40% cyproconazole suspension concentrate on Sichuan pepper was conducted to study the dissipation dynamics and terminal residual amount of cyproconazole. Finally, the potential dietary intake risk assessment of cyproconazole in Sichuan pepper matrices was evaluated. The results showed that the average recovery of cyproconazole in Sichuan pepper was 98.7%-107.5%, and the relative standard deviation (RSD) was 4.4%-6.3% at the fortification level of 0.02, 0.2, and 1 mg/kg. The limit of detection (LOD) and limit of quantification (LOQ) of cyproconazole are 0.001 mg/kg and 0.02 mg/kg, respectively. The field trial results demonstrated that the degradation of 40% cyproconazole suspension concentrates in Sichuan pepper complied with the first-order reaction kinetic equation. The half-lives of cyproconazole were 7.7-8.6 days in the fresh Sichuan pepper samples and 8.5-8.8 days in the dried Sichuan pepper samples. The final residual deposits of cyproconazole in Sichuan pepper matrices were less than 0.2 mg/kg. The dietary intake risk assessment results showed that the acute dietary intake risk quotient of cyproconazole in Sichuan pepper samples to the population was the range from 0.005%-0.040%. Meanwhile, the chronic dietary intake risk quotient was far below 100%, indicating that even spraying 1.5 times the highest recommended dosage with three times, a 40% cyproconazole suspension concentrate had a negligible dietary intake hazard and was safe and acceptable for consumers' health.

5. Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Data availability

All data generated or analysed during this study are included in this published article [and its supplementary information files].

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

XF performed the data analyses, manuscript preparation and wrote the manuscript;

WH performed the experiments of Field experiments and sample collection section;

XD and QY performed the experiments of Sample preparation and UPLC-MS/MS analysis section;

JF helped perform the analysis with constructive discussions;

XB contributed to the conception of the study and provided the assistants through all the experiments.

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Figure Legends

Fig. 1. The chemical structure of cyproconazole.

Fig. 2. The effect of acidity of extraction solvent (a), purification agent (b), the amount of adsorbents and anhydrous $MgSO_4$ (c), and the extraction approach (d) on the recoveries of cyproconazole from the two Sichuan pepper matrices at the 0.02mg/kg (n=3).

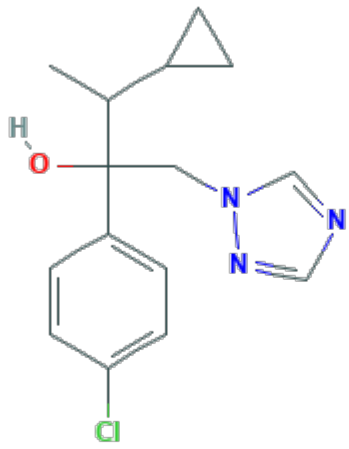
Fig. 3. The dissipation behaviors of cyproconazole in two Sichuan pepper matrices from Mengzhou and Xuzhou.

Table captions

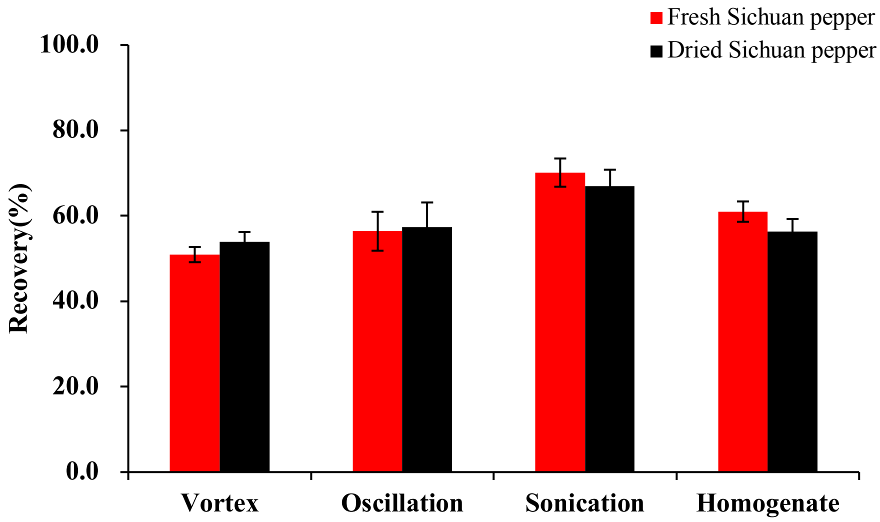
Table 1. The degradation rate and recoveries of cyproconazole and in two Sichuan pepper samples.

Table 2. The chronic dietary exposure risk of cyproconazole in Sichuan pepper samples.

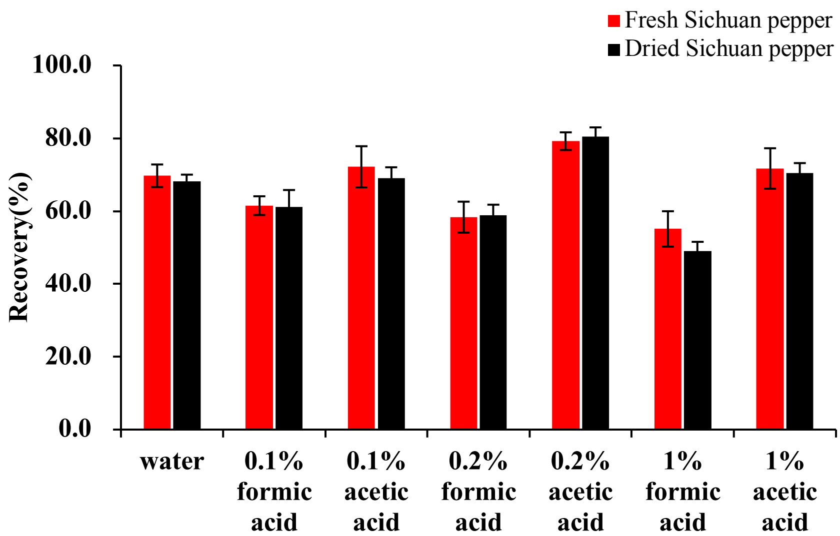
Table 3. The acute dietary exposure risk of cyproconazole in Sichuan pepper samples.



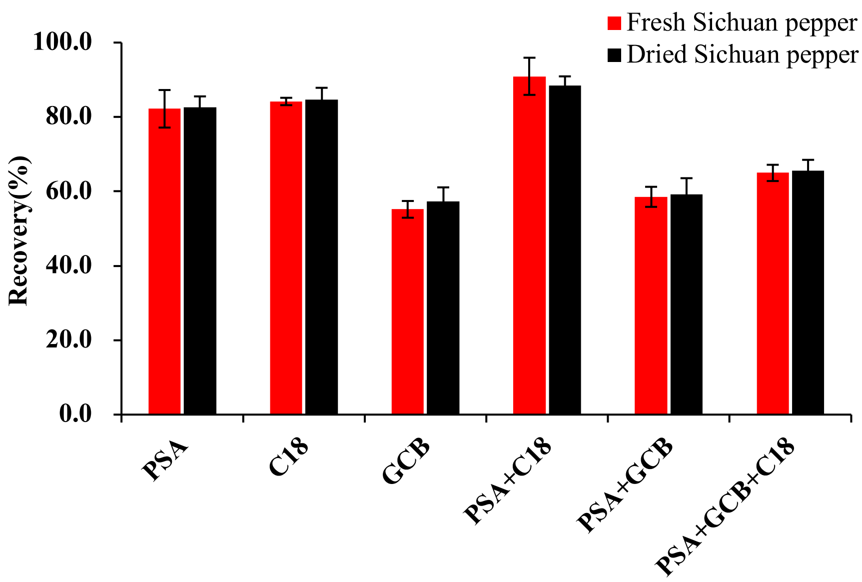
(Fig. 1)


Fresh Sichuan pepper
Dried Sichuan pepper
Recovery(%)
100.0
80.0
60.0
40.0
20.0
0.0
Vortex
Oscillation
Sonication
Homogenate

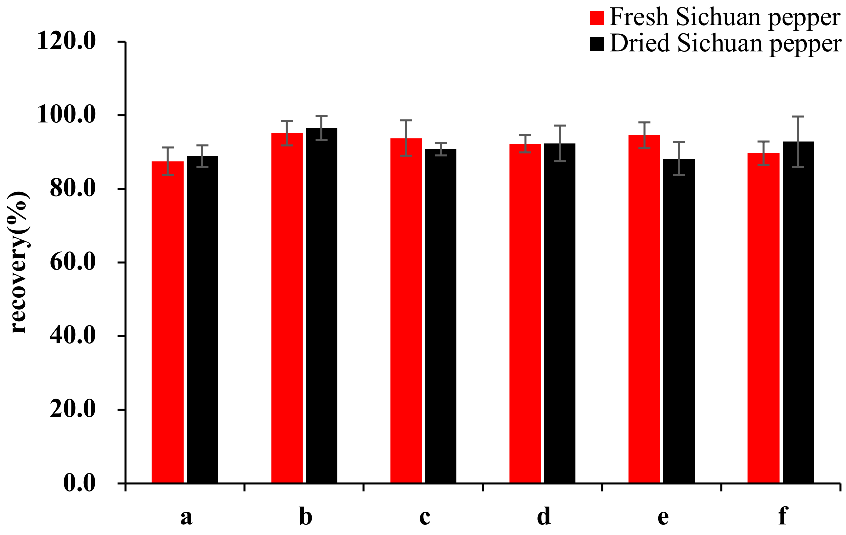
871


Fresh Sichuan pepper
Dried Sichuan pepper
Recovery(%)
100.0
80.0
60.0
40.0
20.0
0.0
water
0.1% formic acid
0.1% acetic acid
0.2% formic acid
0.2% acetic acid
1% formic acid
1% acetic acid

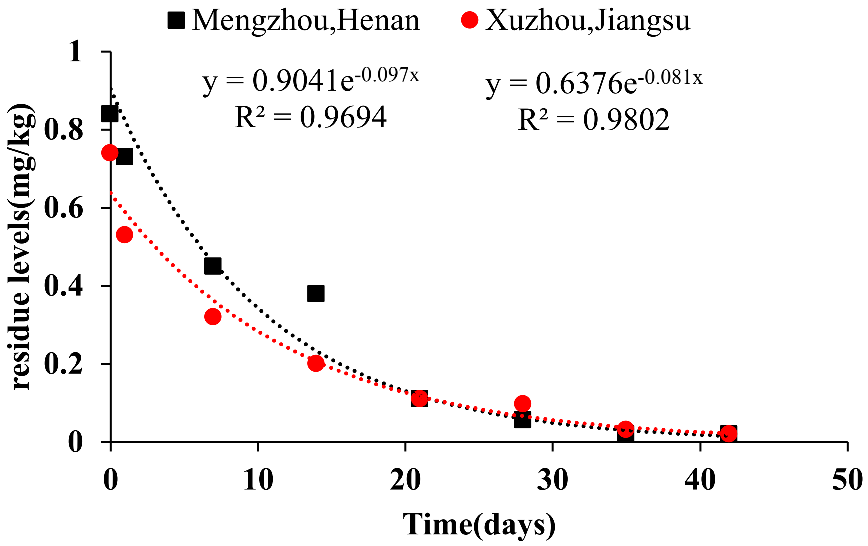
872

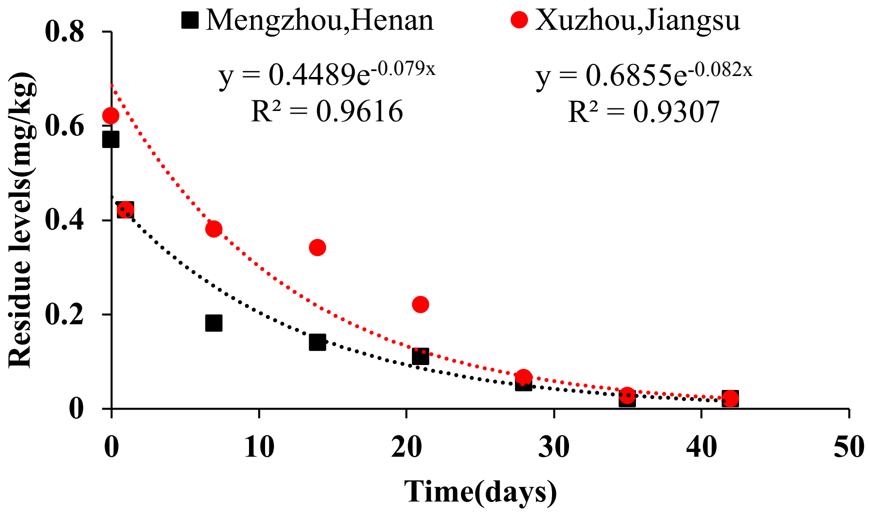

Fresh Sichuan pepper
Dried Sichuan pepper
Recovery(%)
100.0
80.0
60.0
40.0
20.0
0.0
PSA
C18
GCB
PSA+C18
PSA+GCB
PSA+GCB+C18

873


Fresh Sichuan pepper
Dried Sichuan pepper
120.0
100.0
80.0
60.0
40.0
20.0
0.0
recovery(%)
a
b
c
d
e
f

(Fig. 2)


Mengzhou,Henan
Xuzhou,Jiangsu
y = 0.9041e-0.097x
R² = 0.9694
y = 0.6376e-0.081x
R² = 0.9802
residue levels(mg/kg)
0
0.2
0.4
0.6
0.8
1
0
10
20
30
40
50
Time(days)


Mengzhou,Henan
Xuzhou,Jiangsu
y = 0.4489e-0.079x
R² = 0.9616
y = 0.6855e-0.082x
R² = 0.9307
Residue levels(mg/kg)
0
0.2
0.4
0.6
0.8
0
10
20
30
40
50
Time(days)

(Fig. 3)

Table 1. Degradation rate and recoveries of cyproconazole and in two Sichuan pepper samples.

Matrices	Storage times (d)	Storage experiment samples						Quality control samples			
		Spiked levels (mg/kg)	Residue levels (mg/kg)		Degradation rate (%)			Spiked levels (mg/kg)	Recovery (%)		
			1	2	1	2	Average		1	2	Average
Fresh Sichuan pepper	0	0.20	0.21	0.20	N/A	N/A	N/A	0.20	106	110	108
	31	0.20	0.20	0.19	4.8	5.0	4.9	0.20	103	105	104
	92	0.20	0.19	0.18	9.5	10.0	9.8	0.20	98	102	100
	206	0.20	0.18	0.19	14.3	5.0	9.7	0.20	105	110	108
Dried Sichuan pepper	0	0.20	0.20	0.19	N/A	N/A	N/A	0.20	100	95	98
	31	0.20	0.19	0.19	5.0	0.0	2.5	0.20	102	100	101
	92	0.20	0.17	0.18	15	5.3	10.1	0.20	105	108	107
	206	0.20	0.20	0.16	0.0	15.8	7.5	0.20	100	103	102

Table 2. The chronic dietary exposure risk of cyproconazole in Sichuan pepper samples.

The registered crops	Food classification	Fi (kg)	Reference residue limits (mg kg^{-1})		Sources from different application times		NEDI (mg)		ADI (mg)	RQc[a] (%)	RQc[b] (%)
			2	3	2	3	2	3		2	3
Fresh Sichuan pepper	Soy sauce	0.009	0.082	0.16	$STMR_1$ (28d)	$STMR_1$ (28d)	0.000738	0.00144	0.02×63		
			0.020	0.056	$STMR_2$ (35d)	$STMR_2$ (35d)	0.00018	0.000504			
Dried Sichuan pepper			0.049	0.087	$STMR_1$ (28d)	$STMR_1$ (28d)	0.000441	0.000783			
			0.024	0.020	$STMR_2$ (35d)	$STMR_2$ (35d)	0.000216	0.00018			
Wheat	Flour and its products	0.1385	0.2		MRL, China	MRL, China	0.0277	0.0277			
	Total						0.0284 (28d)	0.0291 (28d)	1.26	2.25 (28d)	2.31 (28d)
							0.0279 (35d)	0.0282 (35d)		2.21 (35d)	2.24 (35d)
							0.0281 (28d)	0.0285 (28d)		2.23 (28d)	2.26 (28d)
							0.0279 (35d)	0.0279 (35d)		2.21 (35d)	2.21 (35d)

Note: the F_i is the dietary intake for a certain kind of food of healthy Chinese people. 0.02 (mg/kg) is the acute reference dose of cyproconazole. HR is the highest residue level of cyproconazole in different Sichuan peppers. PHI is the pre-harvest interval. 63 (kg) is the average body weight of a Chinese adult. RQ_c is the acute dietary exposure risk probability.

Table 3. The acute dietary exposure risk assessment of cyproconazole in Sichuan pepper samples.

Matrices	Food classification	Application time	PHI	HR (mg kg^{-1})	F_i (kg)	NESTI (mg)	ARfD (mg)	RQa (%)
Fresh Sichuan Pepper	Soy sauce	2	28	0.097	0.009	0.000873	0.06×63	0.023
			35	0.020		0.00018		0.005
		3	28	0.17		0.00153		0.040
			35	0.058		0.000522		0.014
Dried Sichuan Pepper		2	28	0.065		0.000585		0.015
			35	0.027		0.000243		0.006
		3	28	0.10		0.0009		0.024
			35	0.020		0.00018		0.005

Note: the F_i is the dietary intake for a certain kind of food of healthy Chinese people. 0.06 (mg/kg) is the acute reference dose of tebufenozide. HR is the highest residue level of cyproconazole in different Sichuan peppers. PHI is the pre-harvest interval. 63 (kg) is the average body weight of a Chinese adult. RQ_a is the acute dietary exposure risk probability.

Figures

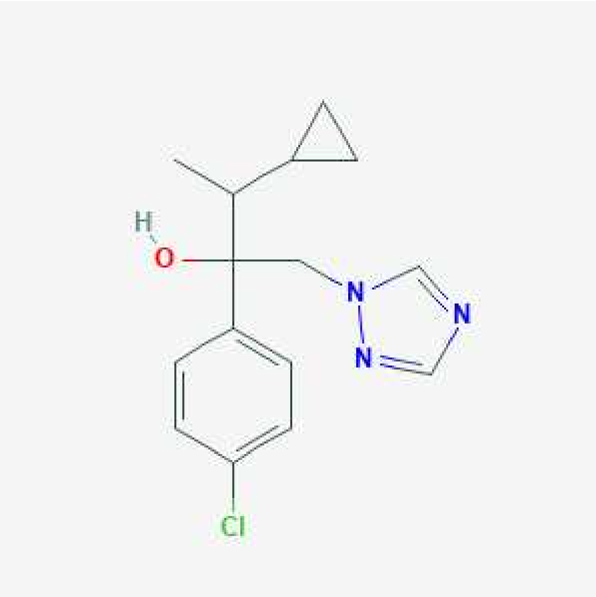


Figure 1

The chemical structure of cyproconazole.

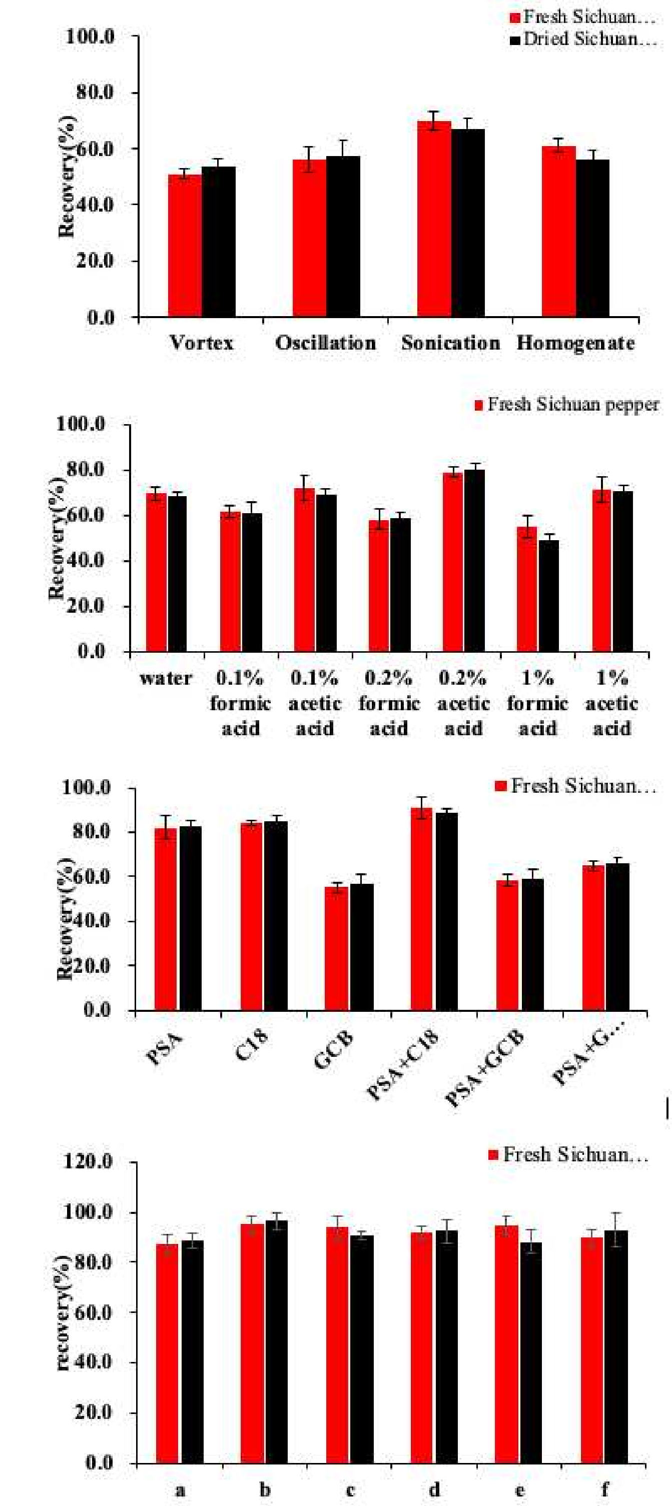


Figure 2

The effect of acidity of extraction solvent (a), purification agent (b), the amount of adsorbents and anhydrous MgSO4 (c), and the extraction approach (d) on the recoveries of cyproconazole from the two Sichuan pepper matrices at the 0.02mg/kg (n=3).

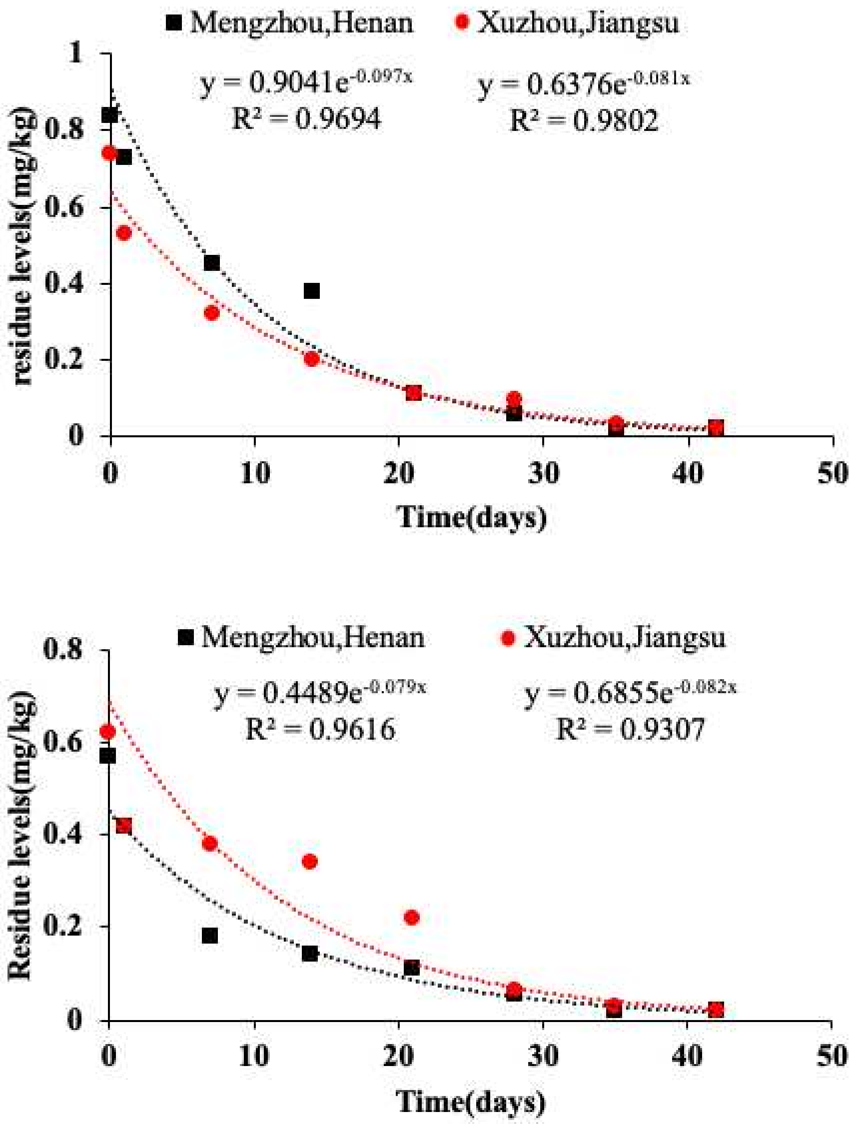


Figure 3

The dissipation behaviors of cyproconazole in two Sichuan pepper matrices from Mengzhou and Xuzhou.

Supplementary Files

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- Supplmentarymaterial.docx