Simultaneous Determination of Eight New Generation Amide Insecticide Residues in Complex Matrix Agri-food by Multi-walled Carbon Nanotube Cleanup and UPLC-MS/MS

Tao Lin
Quality Standards and Testing Technology Research Institute, Yunnan Academy of Agricultural Science

Xinglian Chen
Quality Standards and Testing Technology Research Institute, Yunnan Academy of Agricultural Science

Li Wang
Quality Standards and Testing Technology Research Institute, Yunnan Academy of Agricultural Science

Haixian Fang
Quality Standards and Testing Technology Research Institute, Yunnan Academy of Agricultural Science

Maoxuan Li
Quality Standards and Testing Technology Research Institute, Yunnan Academy of Agricultural Science

Yangang Li
Quality Standards and Testing Technology Research Institute, Yunnan Academy of Agricultural Science

Hongcheng Liu (liuorg@163.com)
Quality Standards and Testing Technology Research Institute, Yunnan Academy of Agricultural Science

Research Article

Keywords: Amide insecticides, Multi-walled carbon nanotubes, Agri-food

Posted Date: October 8th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-952153/v1

License: © This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

The simultaneous determination method of 8 amide pesticides by multi-walled carbon nanotubes (MWCNs) cleanup, combined with QuEChERS method and ultra-high performance liquid chromatography-triple quadrupole tandem mass spectrometry has been developed and successfully applied in complex matrix such as green onions, celery, leeks, citrus, lychees, avocado. The matric effect of MWCNs is optimized and compared with QuEChERS materials. The results show that MWCNs can effectively reduce the matrix effect in sample extraction. The mass spectrometry is optimized, through their chemical structure skeletons, the ESI+ and ESI- mode are simultaneously scanned in the method. The coefficient ($r$) is greater than 0.9990, the limit of quantification ranges from 0.03 to 0.80 μg/kg, the recovery rate ranges from 71.2% to 120%, and the relative standard deviation (RSD) ranges from 3.8% to 9.4%. The method is fast, simple, sensitive, and has good purification effect. It is suitable for the rapid determination of amide pesticides in complex matrix agri-food.

Introduction

New generate amide insecticides are a novel class of pesticides, because of their high efficiency and low toxicity. The early products include chlorantraniliprole, cyantraniliprole, flonicamid and flubendiamide[1], whereas cyclaniliprole and tetrachlorantraniliprole, bromoantraniliprole are the more recent members. They can activate insect ryanodine receptors, which play a critical role in muscle function.[2] They can be used to against a variety of insects, such as Cydia pomonella in apple, and Plutella xylostella (Linnaeus) in cabbage and pakchoi etc[3].

New amide insecticides are currently generally considered low-toxic pesticides. However, according to the test results of flubendiamide's environmental behavior and ecological toxicology, it has shown that it has unacceptable risks to invertebrates (Daphnia magna) and may have great risk to the aquatic ecological environment. Therefore, the Ministry of Agriculture and Rural Affairs of China banned the use of flubendiamide in rice in 2018. According to China's national food safety standard-Maximum residue limits for pesticides in food (GB 2763-2021), the ADI (Acceptable daily intake body weight) for tolfenpyrad is 0.006, indicating that it has a certain degree of toxicity. Due to the protection of foreign compound patents, pesticides such as chlorantraniliprole, bromoantraniliprole and cyclaniliprole have not been legally registered in European Union (the Annex of Regulation (EC) No 396/2005), but monitoring these insecticides are particularly important and urgent for assessing the food safety risk or for setting MRLs in plants. There have been a number of analytical methods for the diamide residue determination, Tian reported simultaneous determination of five diamide insecticides in mushroom[3], in food matrices[4-5], chlorantraniliprole and flubendiamide in vegetable[6], chlorantraniliprole and cyantraniliprole in food[7], and in soil[8]. The residue analysis of bromoantraniliprole is rarely reported to date. Some challenges are also identified for the analytic method for pesticide residue. The most complicated one is the influence of complex sample matrix on the extracted amount of target analyte. The effects of complex matrix generally will enhance or inhibit mass signal to cause the result error over the method, the complex matrix includes green onions, leeks, citrus.

At present, the QuEChERS method is widely used in the rapid determination of pesticides in agricultural products because of its rapidness, simplicity, low cost, high efficiency and environmental friendliness.[9-11] In conventional QuEChERS clean-up steps with graphitized carbon black (GCB)[11], PSA[9], C18[10] as adsorbent have also been reported. Multi-walled carbon nanotubes (MWCNTs) have also been widely used in the purification process of complex agricultural products in recent years, and they have a good effect on adsorbing pigments and reducing matrix effects. [12]
This study is to develop a method modified QuEChERS for simultaneous determination of eight new generate amide insecticides in fruits and vegetables by ultra-high performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). The complex matrix of green onions, celery, leeks, citrus, lychees, avocado, are extracted and cleaned by MWCNTs.

This method could be very practical for fast screening the new generate amide insecticides in vegetable and fruit to ensure food safety.

**Experimental Procedure**

**Chemicals and Materials.**

Analytical standards of chlorantraniliprole, bromoantraniliprole, flubendiamide, tetrachlorantraniliprole, cyclaniliprole, flonicamid, cyantraniliprole and tolfenpyrad were bought from Tianjin Alta Scientific Ltd. (Tianjin, China), and the concentration of each compound was 100 µg/mL. HPLC grade methanol and acetonitrile were obtained from Merck KGaA. (Darmstadt, Germany). Water used in this study was prepared by a Milli-Q water purification system (Bedford, MA). Ammonium formate (≥99.995%) was purchased from MilliporeSigma Company (St Louis, MO). Analytical reagent grade anhydrous sodium chloride (NaCl) and magnesium sulfate (MgSO₄) were obtained from Sinopharm Chemical Reagent (Beijing, China). MWCNTs with length of 10-30 µm and diameter of 10-20 nm were provided by Nanjing XFNANO Materials Tech Co., Ltd. (Nanjing, China). PSA with diameter of 50 µm were bought from Dikma Technologies Inc. (Beijing, China).

**Instrumentation.** Agilent 1290 I Infinity UHPLC (Agilent Technologies, Santa Clara, USA) served for analyte separation. The UHPLC was equipped with an Waters ACQUITY UPLC BEH C18 column (2.1 × 50 mm, 1.7 µm), which was held at 35 °C. The LC mobile phase consisted of phase A, 1 mM ammonium formate in ultrapure water with 0.1% formic acid, and phase B, HPLC grade methanol. The flow rate was set at 0.2 mL·min⁻¹, and gradient elution was used as follows: 55% B → 95% B (3.0 min) → 95% B (4.5 min) → 55% B (4.7 min) → 55% B (6.0 min). The injection volume was 1 µL.

An AB Sciex API4000 tandem mass spectrometer (MS/MS) (Framingham, MS) was used for detection of the eight amide pesticides. The electrospray ionization (ESI) source was operated in both positive (ESI+) and negative (ESI−) mode for simultaneously forming [analyte + H]+ and [analyte − H]− ions. Analyte ion transitions used for qualification and quantitation were monitored by multiple reaction monitoring (MRM) mode. Ion source conditions were used as follows: ionspray voltage, 5500 V (ESI+)/−4500 V (ESI−); heating gas temperature, 550 °C; curtain gas flow rate, 20 L·h⁻¹; nebulizing gas flow rate, 55 L·h⁻¹; heating gas flow rate, 55 L·h⁻¹. The identification of proper ion transitions (precursor ion > product ion) of each amide pesticide and the optimization of a number of MS/MS parameters including declustering potential (DP) and collision energy (CE) were performed with a syringe pump providing a constant flow of the standard solution (0.1 µg·mL⁻¹) of amide pesticide to the MS/MS at a flow rate of 10 µL·min⁻¹. The parameters for the detection of the eight amide pesticides are shown in Table 1.

**Table 1. LC-MS/MS Parameters for Detection of the Eight Amide Pesticides**
### Compound Ionization Properties

| Compound                | Ionization mode | Precursor ion/\(m/z\) | Product ion/\(m/z\) | DP/(volts) | CE/(volts) |
|-------------------------|-----------------|------------------------|---------------------|------------|------------|
| Chlorantraniliprole     | ESI+            | 484.1                  | 453.0*/286.0        | 58         | 24/29      |
| Bromoantraniliprole     | ESI+            | 527.9                  | 286.0*/177.1        | 60         | 24/68      |
| Fionicamid              | ESI+            | 230.0                  | 98.0*/146.0         | 73         | 53/43      |
| Cyantraniliprole        | ESI+            | 475.0                  | 286.0*/177.1        | 63         | 19/28      |
| Tolfenpyrad             | ESI+            | 384.0                  | 197.1*/154.1        | 60         | 36/57      |
| Flubendiamide           | ESI−            | 681.0                  | 253.8*/272.0        | -90        | -37/-24    |
| Tetrachlorantraniliprole| ESI−            | 537.9                  | 203.9*/146.9        | -62        | -16/-47    |
| Cyclaniliprole          | ESI−            | 602.0                  | 257.9*/146.9        | -80        | -21/-47    |

*Quantitative ion

### Sample Preparation

All vegetable and fruit samples were collected from supermarkets or farmer’s markets with the permission of local management personnel. A thoroughly homogenized vegetable or fruit sample (10 g) was weighted into a 50 mL Teflon centrifuge tube. 10 mL acetonitrile was added and the tube was shaken vigorously for 2 min with vortex mixer ensuring that the solvent interacted well with the entire sample. Anhydrous NaCl (1 g) and anhydrous MgSO\(_4\) (4 g) were added into the mixture and the shaking step was repeated for 1 min. After centrifugation (5000 rpm, 3 min), 2 mL of the clarified supernatant was introduced into a 10 mL Teflon centrifuge tube containing 20 mg MWCNTs and 300 mg MgSO\(_4\). Then the mixture was shaken vigorously for 1 min and centrifuged for 3 min at 5000 rpm with a microcentrifuge. Finally the acetonitrile layer was filtered through a 0.22 \(\mu\)m filter membrane and determined by LC-MS/MS.

### Method Validation

According to the requirements of SANTE/11813/2017\(^{[13]}\), using different blank sample matrices, the quantification limits, linear ranges and correlation coefficients of each amide pesticide in different matrices were determined.

Recovery and reproducibility experiments was tested which were carried out for each sample matrix in 6 replicates each at three fortification levels (Limit of quantification, 5 times limit of quantification, 10 times limit of quantification).

Nine representative matrices were selected for matrix effects evaluation. Onions, shallots, leeks and garlic were selected as representative commodity with high irritating sulfide content, orange as high acidic compounds and volatile oils content and lychee and mango as high sugar content. Matrix effects (ME) were measured according to the equation: \(ME = (\text{slope of solvent standard} / \text{slope of matrix matched standard} - 1) \times 100\%\)^\([14]\). When the result of \(ME<0\), it means matrix inhibitory effect, >0 means matrix enhancement effect, \(ME\) absolute value is 0%-20% means weak matrix effect, 20%-50% means medium matrix effect, more than 50% means a strong matrix effect.

### Results And Discussion

**Mass spectrometry Optimization.**
In the ESI+ and ESI− modes, the precursor and product ions were scanned using eight pesticide standards (0.1 μL/mL) respectively, and the decluttering potential and collision energy were optimized (Table 1).

The chemical structure skeletons of chlorantraniliprole, bromoantraniliprole, flonicamid, and cyantraniliprole are similar and their mass spectrum signals in the ESI+ mode were better than those in the ESI− mode. In the positive ion mode, both m/z 286.0 and 177.1 appeared in the product ions of the four compounds. The amide bonds of chlorantraniliprole and bromoantraniliprole were broken to form product ions m/z 286.0, and then the amide bond and C-Br bond of bromoantraniliprole were broken to form product ions m/z 177.1. The both sides of the C=N bond of cyantraniliprole were to form product ions m/z 286.0 and 177.1. The fragmentation of the secondary mass spectrometry of chlorantraniliprole, bromoantraniliprole and cyantraniliprole were speculated as shown in Figure 1.

In the negative ion mode, product ions m/z 146.9 produced by tetrachlorantraniliprole and cyclaniliprole, which may be the 3-bromo-1H-pyrazole produced after fragmentation. The fragmentation of the secondary mass spectrometry of tetrachlorantraniliprole and cyclaniliprole were speculated as shown in Figure 2.

In mass spectrometry analysis, the response of the compound was enhanced and the peak shape was improved when a certain amount of volatile salt and acid was added to the mobile phase \[11, 15, 16\]. Therefore, the response intensity of 0.1% formic acid-water, 0.1% formic acid-water (containing 1 mmol/L ammonium acetate) and pure water as the mobile phase were compared in the experiment (Figure 3). Generally in the positive ion mode, the ionization efficiency of the compound was improved and the response intensity was enhanced when the acid and ammonium salt are added. Therefore, after adding formic acid and ammonium acetate to the pure water phase, the response intensity of chlorantraniliprole, bromoantraniliprole, cyantraniliprole and tolfenpyrad was signicantly improved compared to pure water as the mobile phase. However, the response of flonicamid in formic acid solution is not as strong as in pure water, but the response is also significantly enhanced with the addition of ammonium acetate. For the pesticides tested in the negative ion mode such as flubendiamide, tetrachlorantraniliprole and cyclaniliprole, their response was reduced to a certain extent with the addition of formic acid and ammonium acetate. In the negative ion mode, the [M-H]− ion in the compound was inhibited by excess hydrogen ion and ammonium ion, which reduces its response. The chromatograms are shown in Figure 4.

**Matrix effects**

As shown in Figure 5, for some fruits and vegetables with complex matrices, most of the eight amide compounds have strong matrix effects in the absence of purification, especially the avocado with higher oil content, leeks and garlic with more sulfur compounds have a strong matrix effect (the range of matrix effect was: -44.1%~748.5%). Pesticides such as flubendiamide, tetrachlorantraniliprole, chlorantraniliprole, flonicamid were present strong matrix effect in various matrices (range of matrix effect: -44.1%~748.5%). However, with the addition of multi-walled carbon nanotubes, the matrix effect can be effectively suppressed, and better results have been obtained.

**Modified cleanup process**

Optimization of the amount of the MWCNTs

It was found in the experiment that the amount of multi-walled carbon nanotubes added had a greater impact on the recovery effect. To evaluate the effect of this parameter, the experiment was performed using 2 mL of the acetonitrile extract at the spiked level of 0.01 mg/kg that was placed into 2.0 mL centrifuge tubes containing 300 mg MgSO4 and different amounts of MWCNTs (i.e., 10, 20, 30 mg). With the increase in the amount of multi-walled carbon nanotubes, the recoveries rate of the 8 amide pesticides had decreased to a certain extent. When the added amount of MWCNTs
was 30 mg, the recoveries of chlorantraniliprole, bromoantraniliprole and cyantraniliprole in onions were 53.3%, 45.9% and 54.5%, fonicamid in leek was 64.0%, and cyantraniliprole in garlic was 62.1%. Consequently, 20 mg of MWCNTs was selected to be added to 2 mL of acetonitrile extract to obtain a better recovery effect.

Comparison of purification effects between MWCNTs and PSA

In order to compare the purification effects of MWCNTs and PSA, 20 mg MWCNTs and 20 mg PSA were added to 2 mL of the acetonitrile extract at the spiked level of 0.01 mg/kg to compare the purification effects, respectively. The results are shown in Figure 6, when PSA were added, except for the low recovery of fonicamid in chives and shallots, the low recovery of tolfenpyrad in avocado, and the high recovery of cyantraniliprole in celery, the recoveries of other pesticides were at the acceptable range. However, the purification effect of flubendiamide, tetrachlorantraniliprole and cyclaniliprole was poor, and the recoveries were higher than 120%. On the other hand, for substrates with higher pigment content such as oranges, shallot, chives, mangoes, and celery, as shown in Figure 7, the samples processed by MWCNT looked transparent, while the PSA-cleanup sample had deeper color. Compared with PSA, the pigment in the sample can be effectively removed by the MWCNT, which can further eliminate the matrix effect caused by pigment.

Method Validation.

Linearity

Linearity was studied in the range 0.15–20 ng/mL for eight amide pesticides by matrix-matched standard calibration in blank extracts of orange, chives, shallot, garlic, mango, onion, avocado, celery, litchi. As shown in Table 3, good linear range was found for all pesticides with $R^2$ values better than 0.999.

Limits of quantification

The described method was tested for simultaneous extraction and determination of eight amide pesticides in nine representative matrices. Table 2 showed the LOQs for the eight pesticides studied in orange, chives, shallot, garlic, mango, onion, avocado, celery, litchi. The LOQs for eight pesticides ranged from 0.03 to 0.8 μg/kg. Tolfenpyrad also had lower LOQs than the other seven pesticides.

Table 2 Linear ranges, correlation coefficients ($r$), limits of quantitation (LOQs) and Matrix effect of amide pesticides in different matrices
| Compound         | Matrix     | Linear ranges/(ng/mL) | $r$  | LOQ/(μg/kg) | ME/% |
|------------------|------------|-----------------------|------|-------------|------|
| Chlorantraniliprole | Orange     | 0.15-20.0             | 0.9997 | 0.15        | 7.2  |
|                   | Celery     | 0.15-20.0             | 0.9996 | 0.15        | 2.7  |
|                   | Onion      | 0.15-20.0             | 0.9998 | 0.15        | -0.3 |
|                   | Litchi     | 0.15-20.0             | 0.9994 | 0.15        | 6.9  |
|                   | Mango      | 0.15-20.0             | 0.9996 | 0.15        | 3.0  |
|                   | Shallot    | 0.15-20.0             | 0.9992 | 0.15        | -2.7 |
|                   | Chives     | 0.15-20.0             | 0.9996 | 0.15        | 9.0  |
|                   | Avocado    | 0.15-20.0             | 0.9994 | 0.15        | 2.7  |
|                   | Garlic     | 0.15-20.0             | 0.9999 | 0.15        | 1.2  |
| Bromoantraniliprole | Orange     | 0.30-20.0             | 0.9992 | 0.30        | 5.4  |
|                   | Celery     | 0.30-20.0             | 0.9995 | 0.30        | 6.9  |
|                   | Onion      | 0.30-20.0             | 0.9993 | 0.30        | 1.0  |
|                   | Litchi     | 0.30-20.0             | 0.9996 | 0.30        | -0.5 |
|                   | Mango      | 0.30-20.0             | 0.9997 | 0.30        | 8.4  |
|                   | Shallot    | 0.30-20.0             | 0.9996 | 0.30        | -1.0 |
|                   | Chives     | 0.30-20.0             | 0.9996 | 0.30        | 8.9  |
|                   | Avocado    | 0.30-20.0             | 0.9994 | 0.30        | 8.4  |
|                   | Garlic     | 0.30-20.0             | 0.9996 | 0.30        | 3.4  |
| Flonicamid        | Orange     | 0.80-20.0             | 0.9993 | 0.80        | -8.8 |
|                   | Celery     | 0.80-20.0             | 0.9997 | 0.80        | -9.6 |
|                   | Onion      | 0.80-20.0             | 0.9996 | 0.80        | -8.1 |
|                   | Litchi     | 0.80-20.0             | 0.9994 | 0.80        | -2.2 |
|                   | Mango      | 0.80-20.0             | 0.9999 | 0.80        | 1.5  |
|                   | Shallot    | 0.80-20.0             | 0.9991 | 0.80        | -5.1 |
|                   | Chives     | 0.80-20.0             | 0.9993 | 0.80        | -6.6 |
|                   | Avocado    | 0.80-20.0             | 0.9992 | 0.80        | 8.8  |
|                   | Garlic     | 0.80-20.0             | 0.9995 | 0.80        | -8.1 |
| Cyantraniliprole  | Orange     | 0.20-20.0             | 0.9996 | 0.20        | 5.5  |
|                   | Celery     | 0.20-20.0             | 0.9996 | 0.20        | 2.3  |
|                   | Onion      | 0.20-20.0             | 0.9997 | 0.20        | 2.9  |
|                   | Litchi     | 0.20-20.0             | 0.9998 | 0.20        | 6.2  |
| Fruit      | Tolfenpyrad   | Flubendiamide  | Tetrachlorantraniliprole | Cyclaniliprole |
|------------|---------------|---------------|---------------------------|---------------|
| Mango      | 0.20-20.0     | 0.20-20.0     | 0.10-20.0                 | 0.50-20.0     |
| Shallot    | 0.20-20.0     | 0.20-20.0     | 0.10-20.0                 | 0.50-20.0     |
| Chives     | 0.20-20.0     | 0.20-20.0     | 0.10-20.0                 | 0.50-20.0     |
| Avocado    | 0.20-20.0     | 0.20-20.0     | 0.10-20.0                 | 0.50-20.0     |
| Garlic     | 0.20-20.0     | 0.20-20.0     | 0.10-20.0                 | 0.50-20.0     |
| Orange     | 0.03-20.0     | 0.03-20.0     | 0.03-20.0                 | 0.03-20.0     |
| Celery     | 0.03-20.0     | 0.03-20.0     | 0.03-20.0                 | 0.03-20.0     |
| Onion      | 0.03-20.0     | 0.03-20.0     | 0.03-20.0                 | 0.03-20.0     |
| Litchi     | 0.03-20.0     | 0.03-20.0     | 0.03-20.0                 | 0.03-20.0     |
| Mango      | 0.03-20.0     | 0.03-20.0     | 0.03-20.0                 | 0.03-20.0     |
| Shallot    | 0.03-20.0     | 0.03-20.0     | 0.03-20.0                 | 0.03-20.0     |
| Chives     | 0.03-20.0     | 0.03-20.0     | 0.03-20.0                 | 0.03-20.0     |
| Avocado    | 0.03-20.0     | 0.03-20.0     | 0.03-20.0                 | 0.03-20.0     |
| Garlic     | 0.03-20.0     | 0.03-20.0     | 0.03-20.0                 | 0.03-20.0     |
| Orange     | 0.20-20.0     | 0.20-20.0     | 0.10-20.0                 | 0.50-20.0     |
| Celery     | 0.20-20.0     | 0.20-20.0     | 0.10-20.0                 | 0.50-20.0     |
| Onion      | 0.20-20.0     | 0.20-20.0     | 0.10-20.0                 | 0.50-20.0     |
| Litchi     | 0.20-20.0     | 0.20-20.0     | 0.10-20.0                 | 0.50-20.0     |
| Mango      | 0.20-20.0     | 0.20-20.0     | 0.10-20.0                 | 0.50-20.0     |
| Shallot    | 0.20-20.0     | 0.20-20.0     | 0.10-20.0                 | 0.50-20.0     |
| Chives     | 0.20-20.0     | 0.20-20.0     | 0.10-20.0                 | 0.50-20.0     |
| Avocado    | 0.20-20.0     | 0.20-20.0     | 0.10-20.0                 | 0.50-20.0     |
| Garlic     | 0.20-20.0     | 0.20-20.0     | 0.10-20.0                 | 0.50-20.0     |
| Orange     | 0.03-20.0     | 0.03-20.0     | 0.03-20.0                 | 0.03-20.0     |
| Celery     | 0.03-20.0     | 0.03-20.0     | 0.03-20.0                 | 0.03-20.0     |
| Onion      | 0.03-20.0     | 0.03-20.0     | 0.03-20.0                 | 0.03-20.0     |
| Litchi     | 0.03-20.0     | 0.03-20.0     | 0.03-20.0                 | 0.03-20.0     |
| Mango      | 0.03-20.0     | 0.03-20.0     | 0.03-20.0                 | 0.03-20.0     |
| Shallot    | 0.03-20.0     | 0.03-20.0     | 0.03-20.0                 | 0.03-20.0     |
| Chives     | 0.03-20.0     | 0.03-20.0     | 0.03-20.0                 | 0.03-20.0     |
| Avocado    | 0.03-20.0     | 0.03-20.0     | 0.03-20.0                 | 0.03-20.0     |
| Garlic     | 0.03-20.0     | 0.03-20.0     | 0.03-20.0                 | 0.03-20.0     |
| Orange     | 0.20-20.0     | 0.20-20.0     | 0.10-20.0                 | 0.50-20.0     |
| Celery     | 0.20-20.0     | 0.20-20.0     | 0.10-20.0                 | 0.50-20.0     |
| Onion      | 0.20-20.0     | 0.20-20.0     | 0.10-20.0                 | 0.50-20.0     |
| Litchi     | 0.20-20.0     | 0.20-20.0     | 0.10-20.0                 | 0.50-20.0     |
| Mango      | 0.20-20.0     | 0.20-20.0     | 0.10-20.0                 | 0.50-20.0     |
| Shallot    | 0.20-20.0     | 0.20-20.0     | 0.10-20.0                 | 0.50-20.0     |
| Chives     | 0.20-20.0     | 0.20-20.0     | 0.10-20.0                 | 0.50-20.0     |
| Avocado    | 0.20-20.0     | 0.20-20.0     | 0.10-20.0                 | 0.50-20.0     |
| Garlic     | 0.20-20.0     | 0.20-20.0     | 0.10-20.0                 | 0.50-20.0     |
Recovery and reproducibility

All the recoveries were determined from the analyses of eight amide pesticides in the matrices, orange, chives, shallot, garlic, mango, onion, avocado, celery, litchi by carrying out six consecutive extractions (n = 6) of spiked matrices at three concentration levels (LOQs, 5 × LOQs, 10 × LOQs). The values were calculated using matrix-matched calibration standards, as Table 3 shows detailed recovery and relative standard deviation data for eight pesticides analyzed in the nine matrices. The recoveries of eight pesticides were in the range 71.2–120.0% with the relative standard deviations (RSDs) were in the range 3.8–9.4% for all cases.

Table 3 Recoveries and relative standard deviations of amide pesticides

|       | Concentration | Recovery (%) | RSD (%) |
|-------|---------------|--------------|---------|
| Celery| 0.50-20.0     | 0.9994       | 0.50    | 6.5    |
| Onion | 0.50-20.0     | 0.9996       | 0.50    | 6.5    |
| Litchi| 0.50-20.0     | 0.9997       | 0.50    | 2.5    |
| Mango | 0.50-20.0     | 0.9998       | 0.50    | 1.5    |
| Shallot| 0.50-20.0   | 0.9995       | 0.50    | -8.0   |
| Chives| 0.50-20.0     | 0.9997       | 0.50    | 1.0    |
| Avocado| 0.50-20.0   | 0.9998       | 0.50    | 8.0    |
| Garlic| 0.50-20.0     | 0.9999       | 0.50    | 6.0    |
| Compound          | Matrix | added | Recovery/% | RSD/% | added | Recovery/% | RSD/% | added | Recovery/% | RSD/% |
|-------------------|--------|-------|------------|-------|-------|------------|-------|-------|------------|-------|
|                   |        | μg/kg |            |       | μg/kg |            |       | μg/kg |            |       |
| Chlorantraniliprole | Orange | 0.15  | 99.9±6.4%  | 0.75  | 94.3±5.6% | 1.50 | 96.7±5.9% |       |       |            |       |
|                   | Celery | 0.15  | 106±7.2%   | 0.75  | 101±7.7% | 1.50 | 103±6.7%  |       |       |            |       |
|                   | Onion  | 0.15  | 73.7±6.9%  | 0.75  | 77.7±5.3% | 1.50 | 83.5±6.2% |       |       |            |       |
|                   | Litchi | 0.15  | 93.8±5.9%  | 0.75  | 97.3±4.6% | 1.50 | 95.6±4.8% |       |       |            |       |
|                   | Mango  | 0.15  | 92.8±4.8%  | 0.75  | 95.3±5.1% | 1.50 | 98.2±4.5% |       |       |            |       |
|                   | Shallot| 0.15  | 94.0±9.1%  | 0.75  | 97.3±6.8% | 1.50 | 98.5±7.5% |       |       |            |       |
|                   | Chives | 0.15  | 113±8.4%   | 0.75  | 111±5.8% | 1.50 | 108±6.0%  |       |       |            |       |
|                   | Avocado| 0.15  | 106±5.3%   | 0.75  | 110±5.9% | 1.50 | 108±6.8%  |       |       |            |       |
|                   | Garlic | 0.15  | 73.5±5.9%  | 0.75  | 78.6±5.3% | 1.50 | 86.8±4.5% |       |       |            |       |
| Bromoantraniliprole| Orange | 0.30  | 98.5±8.7%  | 1.50  | 96.7±7.8% | 3.00 | 99.4±5.6% |       |       |            |       |
|                   | Celery | 0.30  | 103±7.7%   | 1.50  | 100±5.4% | 3.00 | 105±4.5%  |       |       |            |       |
|                   | Onion  | 0.30  | 99.0±9.1%  | 1.50  | 95.7±8.4% | 3.00 | 99.7±6.3% |       |       |            |       |
|                   | Litchi | 0.30  | 91.8±9.4%  | 1.50  | 96.4±7.5% | 3.00 | 95.3±8.8% |       |       |            |       |
|                   | Mango  | 0.30  | 95.0±8.4%  | 1.50  | 94.2±4.7% | 3.00 | 96.7±5.2% |       |       |            |       |
|                   | Shallot| 0.30  | 100±8.8%   | 1.50  | 98.3±6.1% | 3.00 | 103±6.9%  |       |       |            |       |
|                   | Chives | 0.30  | 117±9.2%   | 1.50  | 114±7.5% | 3.00 | 118±6.7%  |       |       |            |       |
|                   | Avocado| 0.30  | 111±6.5%   | 1.50  | 115±6.9% | 3.00 | 113±5.5%  |       |       |            |       |
|                   | Garlic | 0.30  | 75.1±5.8%  | 1.50  | 78.3±5.6% | 3.00 | 76.7±4.5% |       |       |            |       |
| Flonicamid        | Orange | 0.80  | 72.0±6.4%  | 4.00  | 75.4±7.5% | 8.00 | 77.9±4.6% |       |       |            |       |
|                   | Celery | 0.80  | 78.0±6.3%  | 4.00  | 79.1±6.9% | 8.00 | 88.3±4.8% |       |       |            |       |
|                   | Onion  | 0.80  | 78.1±7.9%  | 4.00  | 88.7±6.7% | 8.00 | 85.3±7.5% |       |       |            |       |
|                   | Litchi | 0.80  | 78.5±6.7%  | 4.00  | 77.8±7.3% | 8.00 | 83.1±5.4% |       |       |            |       |
|                   | Mango  | 0.80  | 99.0±8.3%  | 4.00  | 94.5±7.2% | 8.00 | 95.6±6.6% |       |       |            |       |
|                   | Shallot| 0.80  | 74.1±4.8%  | 4.00  | 77.4±6.3% | 8.00 | 79.6±4.0% |       |       |            |       |
|                   | Chives | 0.80  | 77.5±5.3%  | 4.00  | 75.2±5.9% | 8.00 | 79.3±4.7% |       |       |            |       |
|                   | Avocado| 0.80  | 73.6±5.1%  | 4.00  | 78.9±6.3% | 8.00 | 79.4±4.5% |       |       |            |       |
|                   | Garlic | 0.80  | 71.9±6.8%  | 4.00  | 83.4±7.4% | 8.00 | 84.5±6.0% |       |       |            |       |
| Cyantraniliprole  | Orange | 0.20  | 101±8.2%   | 1.00  | 91.3±6.4% | 2.00 | 95.4±6.9% |       |       |            |       |
|                   | Celery | 0.20  | 120±8.3%   | 1.00  | 110±6.7% | 2.00 | 107±7.5%  |       |       |            |       |
|                   | Onion  | 0.20  | 76.4±7.6%  | 1.00  | 86.5±5.6% | 2.00 | 84.7±5.1% |       |       |            |       |
|                   | Litchi | 0.20  | 87.6±7.4%  | 1.00  | 89.5±4.9% | 2.00 | 90.4±5.0% |       |       |            |       |
| Ingredient | Tolfenpyrad (µg/ml) | Orange (µg/ml) | Celery (µg/ml) | Onion (µg/ml) | Litchi (µg/ml) | Mango (µg/ml) | Shallot (µg/ml) | Chives (µg/ml) | Avocado (µg/ml) | Garlic (µg/ml) |
|------------|---------------------|---------------|--------------|--------------|---------------|--------------|---------------|--------------|----------------|---------------|
| Mango      | 0.20                | 101.79        | 1.00         | 91.97.7      | 2.00          | 96.36.6      |                |              |                |               |
| Shallot    | 0.20                | 91.36.4       | 1.00         | 94.68.5.5    | 2.00          | 98.45.3      |                |              |                |               |
| Chives     | 0.20                | 108.66.0      | 1.00         | 105.67.6     | 2.00          | 109.55.5     |                |              |                |               |
| Avocado    | 0.20                | 99.56.5       | 1.00         | 96.85.6      | 2.00          | 97.74.6      |                |              |                |               |
| Garlic     | 0.20                | 84.95.8       | 1.00         | 89.33.9      | 2.00          | 90.54.9      |                |              |                |               |
| Tolfenpyrad| Orange              | 0.03          | 98.96.4      | 0.15         | 94.65.7      | 0.30         | 96.76.0      |                |                |               |
| Celery     | 0.03                | 87.75.7       | 0.15         | 89.54.9      | 0.30          | 94.55.2      |                |              |                |               |
| Onion      | 0.03                | 82.15.3       | 0.15         | 87.35.9      | 0.30          | 89.94.9      |                |              |                |               |
| Litchi     | 0.03                | 92.74.8       | 0.15         | 90.84.3      | 0.30          | 95.94.4      |                |              |                |               |
| Mango      | 0.03                | 96.14.3       | 0.15         | 95.86.5      | 0.30          | 97.44.8      |                |              |                |               |
| Shallot    | 0.03                | 93.04.9       | 0.15         | 96.25.3      | 0.30          | 95.66.7      |                |              |                |               |
| Chives     | 0.03                | 82.95.8       | 0.15         | 88.64.7      | 0.30          | 89.75.0      |                |              |                |               |
| Avocado    | 0.03                | 76.56.3       | 0.15         | 79.75.7      | 0.30          | 87.94.8      |                |              |                |               |
| Garlic     | 0.03                | 71.25.6       | 0.15         | 73.65.6      | 0.30          | 86.54.9      |                |              |                |               |
| Flubendiamide| Orange              | 0.20          | 99.45.0      | 1.00         | 101.54.2     | 2.00         | 99.74.2      |                |                |               |
| Celery     | 0.20                | 92.55.8       | 1.00         | 97.36.4      | 2.00          | 95.84.6      |                |              |                |               |
| Onion      | 0.20                | 81.97.2       | 1.00         | 83.66.8      | 2.00          | 88.65.4      |                |              |                |               |
| Litchi     | 0.20                | 101.74.1      | 1.00         | 100.57      | 2.00          | 97.88.3      |                |              |                |               |
| Mango      | 0.20                | 100.64.1      | 1.00         | 108.57      | 2.00          | 105.56.1     |                |              |                |               |
| Shallot    | 0.20                | 91.25.9       | 1.00         | 96.05.5      | 2.00          | 95.64.5      |                |              |                |               |
| Chives     | 0.20                | 100.90.1      | 1.00         | 98.05.6      | 2.00          | 95.95.7      |                |              |                |               |
| Avocado    | 0.20                | 103.89.1      | 1.00         | 109.54      | 2.00          | 105.65.1     |                |              |                |               |
| Garlic     | 0.20                | 83.18.6       | 1.00         | 88.86.8      | 2.00          | 89.65.0      |                |              |                |               |
| Tetrachlorantraniliprole| Orange | 0.10 | 111.55.8 | 0.50 | 106.69 | 1.00 | 117.46 | | | |
| Celery | 0.10 | 110.63.3 | 0.50 | 112.39 | 1.00 | 107.47 | | | |
| Onion | 0.10 | 90.26.8 | 0.50 | 98.85.0 | 1.00 | 96.95.4 | | | |
| Litchi | 0.10 | 116.69 | 0.50 | 112.46 | 1.00 | 117.52 | | | |
| Mango | 0.10 | 111.75 | 0.50 | 115.63 | 1.00 | 112.59 | | | |
| Shallot | 0.10 | 114.72 | 0.50 | 115.65 | 1.00 | 118.48 | | | |
| Chives | 0.10 | 113.74 | 0.50 | 116.50 | 1.00 | 119.55 | | | |
| Avocado | 0.10 | 112.69 | 0.50 | 105.42 | 1.00 | 107.59 | | | |
| Garlic | 0.10 | 95.75.7 | 0.50 | 97.24.5 | 1.00 | 98.84.3 | | | |
| Cyclanilprole| Orange | 0.50 | 98.35.2 | 2.50 | 95.76.1 | 5.00 | 97.95.6 | | | |
## Conclusion

In this work, a modified QuEChERS method was developed for the purification by MWCNTs and determination of eight amide pesticides in fruits and vegetables by UHPLC-MS/MS. The validation parameters of the method in terms of analytical range, precision, recovery and precision showed that the proposed method meets the requirements for pesticide analysis (average recovery values were in the range 71.2–120.0% for eight pesticides with RSDs lower than 10%). MWCNTs have been shown to effectively reduce the matrix effect and remove pigments in the matrix.

## Declarations

### Acknowledgments

The authors are grateful for the support from Yunnan Key Research and Development (No.202102AE090021, 202002AE3200053, 2019ZG001) and Quality and Safety Risk Assessment for Agro-Products of China (No. GJFP2019013).

## References

1. Tu, Y. *et al.* Determination of matrix effect of five amide pesticides in vegetables using UPLC-MS/MS. *Journal of Zhejiang Agricultural Sciences* **60**, 1216-1220 (2019).
2. Ding, J., Chunmei, X. U., Zhang, W., Zou, N. & Wei, M. U. Determination of cyantraniliprole and its metabolite in maize plant and soil by QuEChERS and ultra-performance liquid chromatography tandem mass spectrometry. *Chinese Journal of Pesticide Science* **20**, 83–89 (2018).
3. Tian, F. *et al.* Development and validation of a method for the analysis of five diamide insecticides in edible mushrooms using modified QuEChERS and HPLC-MS/MS. *Food Chemistry* **333**, 127468 (2020).
4. Pan, X. *et al.* Simultaneous determination of chlorantraniliprole and cyantraniliprole in fruits, vegetables and cereals using ultra-high-performance liquid chromatography–tandem mass spectrometry with the isotope-labelled internal standard method. *Analytical and bioanalytical chemistry* **407**, 4111-4120 (2015).
5. Lu, Z. *et al.* Simultaneous determination of five diamide insecticides in food matrices using carbon nanotube multiplug filtration cleanup and ultrahigh-performance liquid chromatography–tandem mass spectrometry. *Journal of agricultural and food chemistry* **67**, 10977-10983 (2019).
6. Caboni, P. *et al.* Liquid chromatography-tandem mass spectrometric ion-switching determination of chlorantraniliprole and flubendiamide in fruits and vegetables. *Journal of agricultural and food chemistry* **56**,
7696-7699 (2008).

7. Schwarz, T. et al. QuEChERS multiresidue method validation and mass spectrometric assessment for the novel anthranilic diamide insecticides chlorantraniliprole and cyantraniliprole. *Journal of agricultural and food chemistry* **59**, 814-821 (2011).

8. Pastor-Belda, M. et al. Dispersive liquid–liquid microextraction for the determination of new generation pesticides in soils by liquid chromatography and tandem mass spectrometry. *Journal of Chromatography A* **1394**, 1-8 (2015).

9. Dong, Y. et al. Determination of 38 kinds of pesticide residues in nuts by QuEChERS-ultra performance liquid chromatography-tandem mass spectrometry. *Chinese Journal of Analytical Chemistry* **45**, 1397-1404 (2017).

10. Liang, X. et al. Simultaneous determination of residues of 38 pesticides in fruits by QuEChERS combined with high performance liquid chromatography-tandem mass spectrometry. *Food Science* **41**, 288-296 (2020).

11. Huang, Y. et al. Determination of multi-pesticide residues in green tea with a modified QuEChERS protocol coupled to HPLC-MS/MS. *Food chemistry* **275**, 255-264 (2019).

12. Gu, B. et al. Determination of 8 kinds of cephalosporins in aquatic products by multi-walled carbon nanotubes solid phase extraction-ultra high performance liquid chromatography-mass spectrometry. *Chinese Journal of Analytical Chemistry* **45**, 381-388 (2017).

13. European Commission Directorate General for Health and Food Safety (DG SANTE) Guidance document on analytical quality control and method validation procedures for pesticide residues and analysis in food and feed SANTE/11813/2017 (2017).

14. Stahnke, H., Kittlaus, S., Kempe, G. n. & Alder, L. Reduction of matrix effects in liquid chromatography–electrospray ionization–mass spectrometry by dilution of the sample extracts: how much dilution is needed? *Analytical chemistry* **84**, 1474-1482 (2012).

15. Cao, X. et al. Determination of 10 preservative residues in fruits by ultra-high performance liquid chromatography-tandem mass spectrometry. *Food Science* **41**, 319-324 (2020).

16. Liu, Z. Z. et al. Development, validation, comparison, and implementation of a highly efficient and effective method using magnetic solid-phase extraction with hydrophilic-lipophilic-balanced materials for LC-MS/MS analysis of pesticides in seawater. *Science of The Total Environment* **708**, 135221 (2020).

**Figures**
Figure 1

Chemical structures of amide pesticides
Figure 2

The main fracture mode of tetrachlorantraniliprole and cyclaniliprole
Figure 3

Response intensity of amide pesticides in different mobile phases
Figure 4

Chromatograms of amide pesticides
Figure 5

Matrix effect of amide pesticides under clean and unpurified conditions (A) cleaned up, (B) without cleanup
Figure 6

Different recovery effects of PSA purification materials on amide pesticides
Figure 7

The effect of different purification materials to remove pigment (A) without cleanup, (B) cleaned up by Multi-wall carbon nanotubes, and (C) cleaned up by PSA.