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Determination of Residual Flubendiamide in the Cabbage by QuEChERS-Liquid Chromatography–Tandem Mass Spectrometry

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Abstract Flubendiamide, which belongs to the new chemical class of phthalic acid diamides, is widely used against lepidopteron pests in a variety of vegetable and rice pests. It provides superior plant protection against a broad range of economically important lepidopterous pests, including *Spodoptera exigua* and *Plutella xylostella*. A determination method of flubendiamide in the cabbage was established in this paper. Flubendiamide in the cabbage was extracted with acetonitrile and ultrasonic extraction, and was purified by QuEChERS and analyzed by LC–MS/MS (liquid chromatography–tandem mass spectrometry). The results indicated that the average recovery of flubendiamide in the cabbage was 81.27 %–91.45 %, the coefficient of variation was 1.79 %–4.81 %, and the lowest detection concentration was 0.3 μg/kg. The extraction of flubendiamide from the cabbage and its analysis was in accordance with the pesticide residue criterion, i.e., simple, rapid, accurate, reproducible, stable, separatory, and convenient. It identifies and quantifies trace-level flubendiamide residues in the cabbage extracts using LC–MS/MS in the ESI negative mode coupled with the QuEChERS method.

Keywords Flubendiamide · QuEChERS · LC–MS/MS · Cabbage · Residue

Flubendiamide is one kind of phthalic acid diamide insecticides, which is mainly effective for controlling lepidopteron pests, including resistant strains in rice, cotton, corn, grapes, as well as other fruits and vegetables (Lahm et al. 2005). This insecticide has good, rapid, and long-term activity against both adults and larvae. As a new phthalic amide, it plays a role in the activation mechanism of intracellular calcium release channels (ryanodine receptors), a leading cause of uncontrolled storage of calcium release. Given the uniqueness of this mechanism, there is no cross-resistance to conventional insecticides, and it targets insects and mammals but not arthropods (Hoffmann et al. 2009; Lahm et al. 2009; Rhainds and Sadof 2009). Flubendiamide WDG (20 %) has been registered for controlling vegetable insects such as *Spodoptera exigua or Plutella xylostella* in China. When the flubendiamide preparation is sprayed in a cabbage field, it can remain in the cabbage or flow into the soil and waters.

Reports on the detection methods of flubendiamide in food matrices or other environmental media have been focused on the analysis of flubendiamide in different food matrices using HPLC–DAD (high performance liquid chromatography–photodiode array detector) (Battu et al. 2008), HPLC–UV (UV–visible detector) (Gopal and Mishra 2008; Sahoo et al. 2009; Mohapatra et al. 2010; Paramasivam and Banerjee 2011) and LC–MS/MS ion-switching in the ESI position mode (Caboni et al. 2008). However, there

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is no report on the detection of flubendiamide in vegetables using LC–MS/MS ion switching in the ESI negative mode coupled with the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method. Thus, the present study reported a simple and rapid method based on LC–MS/MS in the ESI negative mode coupled with QuEChERS method for identification and quantitation of flubendiamide residues in the cabbage.

Materials and Methods

The instruments were BS210S electronic balance, SB-1000 rotary vacuum evaporator and THZ-82A vibrating machine made by Sartorius company of Germany, Eyela company of Japan and FuHua Company of Jiangsu province, China, respectively. Agilent 1200 Series HPLC system equipped with an Agilent 6460 Triple Quadrupole LC/MS system was made by Agilent Co. (USA).

Flubendiamide (purify 98.52 %) and flubendiamide WDG (20 %) were purchased from Fluka Company (USA) and Nihon Nohyaku Co. Ltd. (Japan), respectively. Dehydrated magnesium sulfate ($MgSO_4$), *N*-propyl ethylenediamine (PSA), and C_{18} were obtained from DIMA Technology, Inc., USA, and all were analytical grade. Methanol and acetonitrile were obtained from DIMA Technology, Inc., USA, and both were HPLC grade.

HPLC analysis was performed with an Agilent 1200 HPLC system equipped with a binary pump, auto platesampler, column oven, and diode-array detector. Separation was performed on Agilent Eclipse Plus chromatographic columns C_{18} (4.6 mm × 150 mm (i.d.), 5 μm) at 20°C, with mobile solvents consisting of methanol: ammonium acetate with 1 % 5 mmol/L acetic acid = 60:40(V:V), isocratic at 1 mL/min. Aliquots of 5 μL were injected directly to the LC–MS/MS system to test flubendiamide and quantified with external standard peak area. Mass spectra were recorded on an Agilent 6460 triple quadrupole (QQQ) mass spectrometer equipped with an ESI source. System control and data acquisition were controlled by Agilent Mass Hunter software. Detailed MS conditions were: cluster voltage: −120 V; gas temperature: 300°C, gas flow 10 L/min, nebulizer pressure: 15 psi, sheath gas temperature: 250°C; sheath gas flow: 7 L/min, capillary voltage: 4 kV, nozzle voltage: 500 V. ESI was operated in the negative ion mode in the MRM (multiple reaction monitoring).

A five-point standard curve was prepared for flubendiamide. Standard solutions containing 2.2702, 1.1351, 0.5676, 0.2838 and 0.1419 μg/mL flubendiamide were prepared in triplicate. All sample solutions were filtered through a 0.22 μm membrane and subjected to LC–MS/MS chromatographic analysis in the MRM mode. Calibration curves were constructed by plotting the concentration of flubendiamide against the standard peak area of the monitored transitions.

Fresh and uncontaminated cabbages were planted in a farm at the Yangzhou University, Yangzhou, China. Cabbage samples were placed in a blender/cutter and chopped for 30 s. About 5 g of the well-homogenized sample was weighed in a 50 mL screw-capped glass tube. Afterwards, 1.5 g of sodium chloride, 6 g of anhydrous magnesium sulfate, and 30 mL of acetonitrile were added. Flubendiamide was then added to the mashed cabbage samples until the final concentrations of flubendiamide were 5.00, 1.00 and 0.20 μg/g, respectively.

The tubes were agitated for 5 min in a rotary shaker at 4,000 r/min under room temperature. About 5 mL of the mixture was evaporated to dryness under a gentle nitrogen stream. About 1 mL of the mixture was collected and added to tubes containing $MgSO_4$(150 mg), PSA (25 mg), and C_{18} (25 mg). These tubes were centrifuged at 4,000 r/min for 5 min. 0.60 mL of the sample solution was diluted to 1.0 mL with distilled water. The sample solution was filtered through a 0.22 μm membrane and subjected to LC–MS/MS chromatographic analysis in the MRM mode. Six replicates for each level were analyzed by LC–MS/MS.

In the field, the cabbages were sprayed with 20 % flubendiamide WDG. About 5.0 g of cabbage was sampled according to QuEChERS extraction methods as above. The flubendiamide content in the cabbage was then determined. The sample solution was filtered through a 0.22 μm membrane and subjected to LC–MS/MS chromatographic analysis in the MRM mode. The experiments were repeated three times.

Results and Discussion

The parent compound of flubendiamide was subjected to collision-induced dissociation in the MRM negative mode. The electrospray capillary potential as well as the shield and needle voltage was optimized for flubendiamide. The collision energy was optimized to achieve the highest sensitivity. The observed mass transitions and collision energies used in the quantitation of flubendiamide was listed in Table 1. Fragmentation ions at *m/z* 254, 272, 274 and 214 were observed by the product ion scan of flubendiamide. The selected reaction monitoring of the precursor-product ion transition was *m/z* 254 for the quantitative ion transition of flubendiamide (Table 1).

Mobile solvent systems consisting of methanol–water, acetonitrile–water, acetonitrile-0.5 % formic acid, and methanol–ammonium acetate with 1 % 5 mmol/L acetic acid for separating flubendiamide standard were examined. The results indicated that the LC–MS/MS separation effects of flubendiamide achieved the highest sensitivity

Table 1 The LC–MS/MS parameters for flubendiamide

Ion transitions	Collision energy (eV)	Note
681/254	−22	Quantitative ion transition
681/272	−10	Qualitative ion transition
681/274	−10	Qualitative ion transition
681/214	−48	Qualitative ion transition

when the system comprising of methanol and ammonium acetate with 1 % 5 mmol/L acetic acid = 60:40 (v/v). In the MRM negative mode, the chromatographic separation of flubendiamide was achieved with isocratic elution in the reverse phase mode, yielding a flubendiamide retention time of 7.87 min (Figs. 1, 2, 3, 4, 5).

A standard curve was drawn according to the concentration of flubendiamide (X-axis) and peak area (Y-axis). The calibration curve was found to be linear over the working range of 0.1419–2.2704 µg/mL, and the linear equation was $y = 16640\ x - 179$ with $r = 0.9999$.

Compared with LC–MS/MS figure of flubendiamide standard, LC–MS/MS figure of cabbage sample intense fragmentation ions at *m/z* 254, 272, 274, and 214 in the MRM negative mode. From that the LC–MS/MS figure of velvetleaf stems sample intense fragmentation ions were at m/z 272, 274 and 214 in the MRM negative mode. It was proved that the residue in the cabbage was flubendiamide (Fig. 6).

The samples were allowed to settle for 30 min before using the extract, and were processed later according to the above extraction procedure. To optimize the purification, different amounts of PSA (0, 10, 25, 50, 75, and 100 mg) and C_{18} (0, 10, 25, 50, 75, and 100 mg) were studied. The results showed no obvious improvement in the recovery of flubendiamide with increased amounts of PSA and C_{18}. When 1 mL of cabbage extract sample was purified with adsorbents (comprising 150 mg of $MgSO_4$, 25 mg of PSA, and 25 mg of $C_{18)}$, pesticide loss decreased and impurities were eliminated. Therefore, 25 mg of PSA, 25 mg of C_{18} and 150 mg of $MgSO_4$ were needed for the cabbage plant extracts purification.

Six replicates for each level were analyzed by LC–MS/MS. The average recoveries of flubendiamide were found to be 91.45 %, 84.74 %, and 81.27 % when the added concentrations of flubendiamide were 5.0, 1.00 and 0.20 µg/g, respectively. The coefficients of variation were 1.79 %, 1.92 % and 4.81 %, respectively (n = 6). These values were in accordance to the pesticide residue criterion at different fortification levels (Table 2). According to the noise signal of LC–MS/MS, the lower limit of detection of flubendiamide was 0.3 µg/kg.

After the cabbage samples was extracted and cleaned up by QuEChERS method, the flubendiamide content in the cabbage was 1.21 µg/g by LC–MS/MS.

A number of sample preparation techniques and methods of analyses have been developed for the pesticide residue determination in a wide range of food and other agricultural products. Conventional sample preparations include various solvent extraction methods, solid-phase extraction or gel permeation chromatography clean up, followed by gas chromatography analysis using a variety of detectors (Garrido et al. 2008; Guardia-Rubio et al. 2007). All these procedures are complex, using toxic solvents and time consuming, which results in consequent costs. An alternative technique called QuEChERS was developed by Anastassiades et al. (2003). The QuEChERS approach takes advantage of the wide analytical scope and high degree of selectivity and sensitivity (Anastassiades et al. 2003; Paula et al. 2007). Current developments include efforts to extend the method for other groups of food

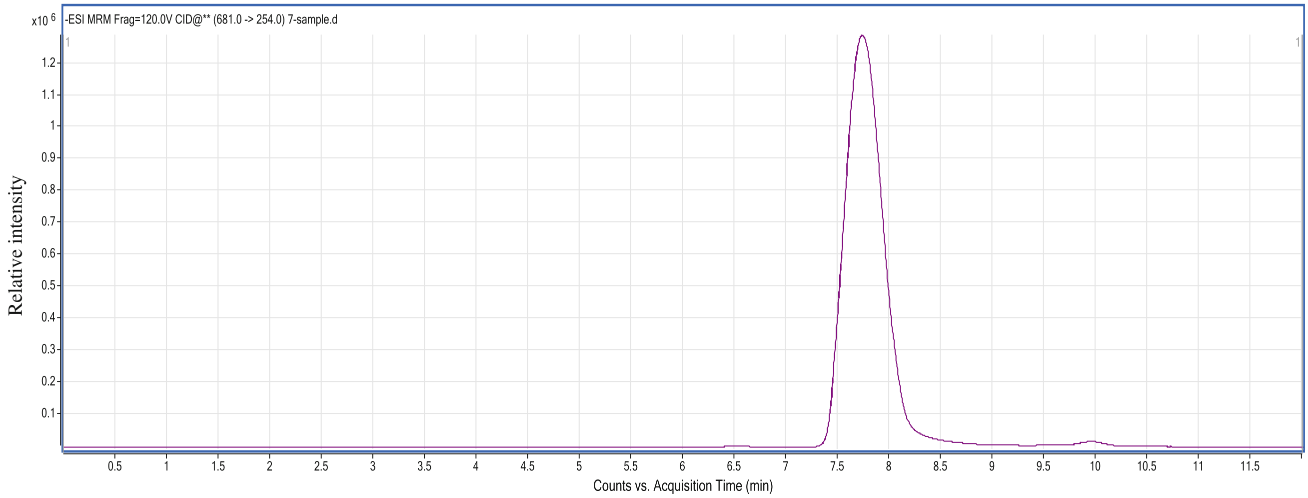

Fig. 1 Selected ionization chromatography of flubendiamide standard by MRM mode (m/z 254)

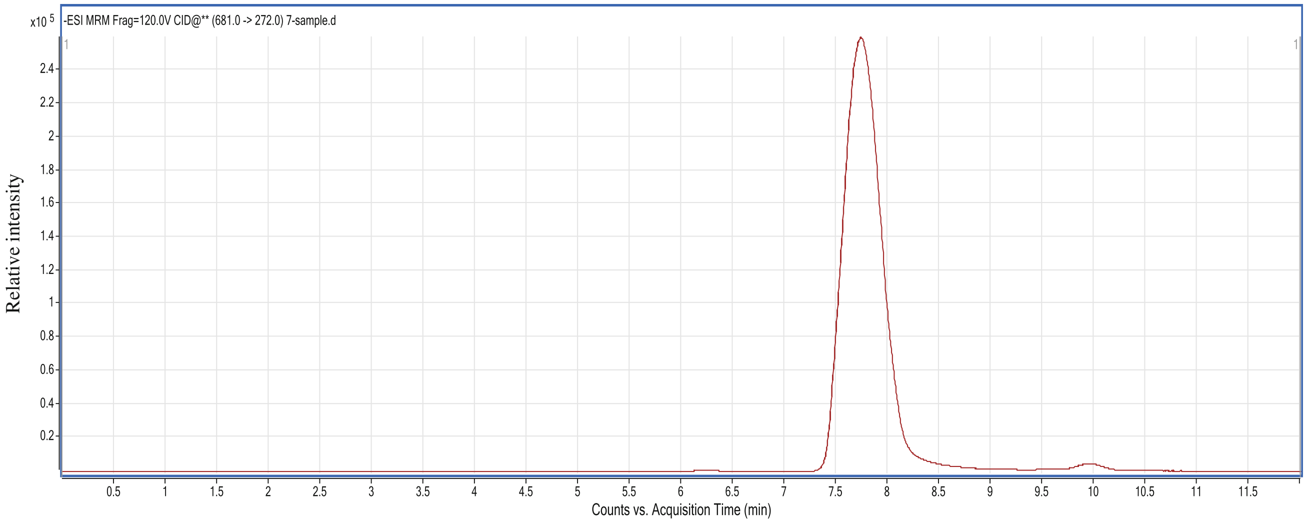

Fig. 2 Selected ionization chromatography of flubendiamide standard by MRM mode (m/z 272)

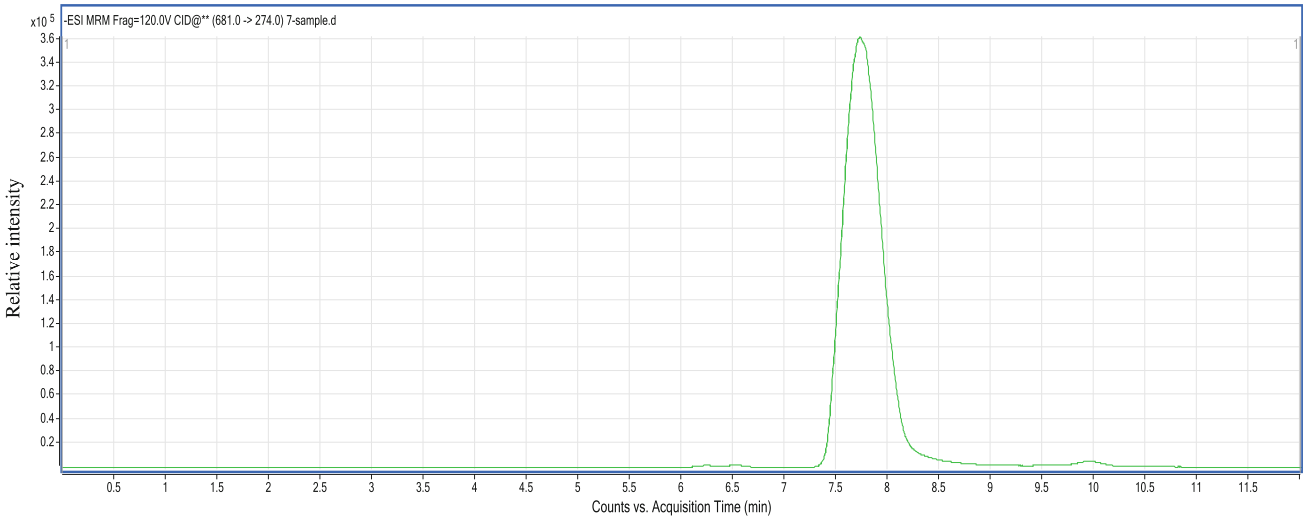

Fig. 3 Selected ionization chromatography of flubendiamide standard by MRM mode (m/z 274)

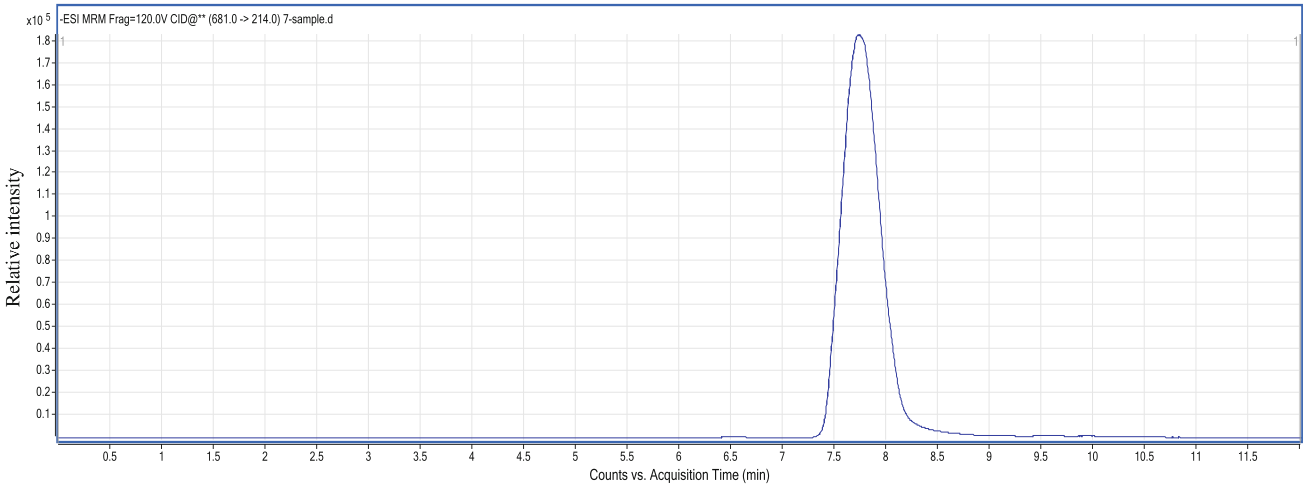

Fig. 4 Selected ionization chromatography of flubendiamide standard by MRM mode (m/z 214)

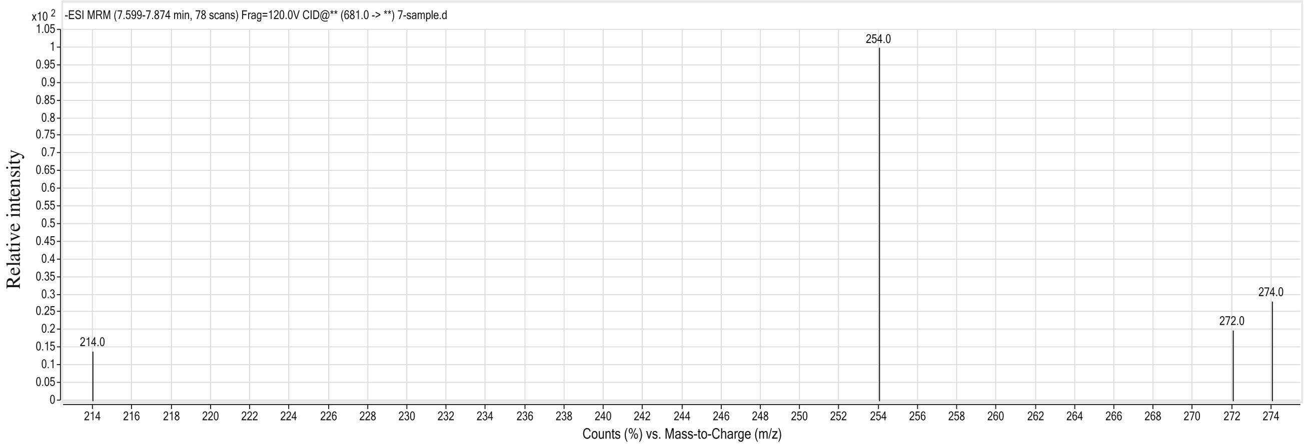

Fig. 5 MS–MS spectrum of flubendiamide standard by MRM mode

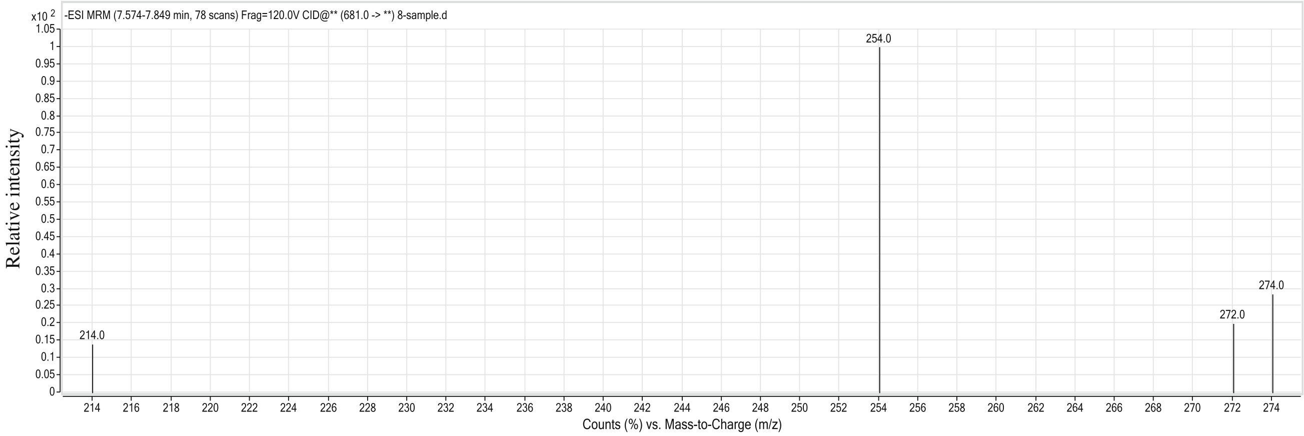

Fig. 6 MS–MS spectrum of residue flubendiamide in the cabbage sample by MRM mode

Table 2 The recovery of flubendiamide from the cabbage (n = 6)

Fortification levels (μg/g)	Average recoveries (%)	Standard deviation	Coefficient of variation (%)
5.00	91.45	0.0818	1.79
1.00	84.74	0.0163	1.92
0.20	81.27	0.0078	4.81

Mean of six replicates

products insofar that it has also become one of the approved official test methods by the European Committee for Standardization (Lehotay 2007; Cieslik et al. 2011). In December 2008, the QuEChERS method was adopted as the official Polish Standard of sample preparation (PN-EN15662 2008). QuEChERS method was applied in this study, which could provide high-quality results with a minimum number of steps and low solvent and glassware consumption. Flubendiamide in the cabbage was extracted with acetonitrile and ultrasonic extraction, and was purified by QuEChERS and analyzed by LC–MS/MS. In present study, the QuEChERS method analysis was proved to be a rapid, simple and highly sensitive method.

In conclusion, determination method of flubendiamide in the cabbage was established in this study. The acetonitrile was used as extracted solvent and the residual flubendiamide in the cabbage was extracted with the ultrasonic extracted method, purified by QuEChERS, and analyzed by LC–MS/MS. The results indicated that the average recovery of flubendiamide in the cabbage was 81.27 %–91.45 %; the coefficient of variation was 1.79 %–4.81 %, and the lowest detection concentration was 0.3 μg/kg. The presented method is simple, convenient to operate and conforms with pesticide residues criterion.

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