

Optimization of a method for the determination of imidacloprid in cucumbers by liquid chromatography-tandem mass spectrometry

Zhongrui Guo

Abstract:

Objective: A method for the determination of imidacloprid pesticide residues in cucumber products by liquid chromatography tandem mass spectrometry was optimized and established. **Method:** According to the current national standard, the extraction solvent, extraction method, constant volume liquid and mobile phase of imidacloprid pesticide in cucumber samples were compared. After optimization, acetonitrile-water mixed solution (3:1) was used as extraction solvent, and the sample was pre-treated by QuChERS method. The constant volume of 50% methanol solution was carried out, and the mobile phase 0.1% acetic acid (phase A) and acetonitrile (phase B) were injected into the sample for detection and analysis. The standard curve method in external standard method was used for quantitative analysis to evaluate the precision and accuracy of the detected concentration of agricultural residues. The optimum condition was that the detection concentration (recovery rate) was close to 100%. **Results:** The detected concentrations of imidacloprid were $21.862\text{ng}\cdot\text{ml}^{-1}\sim 248.311\text{ng}\cdot\text{ml}^{-1}$, and the recoveries were 88.74%~99.32%. **Conclusion:** By using optimized method, the imidacloprid residue in cucumber was analyzed by sampling, and the results showed that the peak condition was good, the detection concentration was high, and the recovery rate was good, it can provide a new improvement and supplement to the analysis and detection process of GB23200.121-2021, and widen the path for the analysis and detection of imidacloprid in vegetables.

Keywords: Imidacloprid; Lc-ms; Cucumber; Pretreatment optimization; Mobile phase

Introduction

1. Research Background

In recent years, the increasing variety of pesticides has inevitably raised concerns regarding pesticide residues in agricultural products[1]. Imidacloprid, as a widely used insecticide, possesses multiple modes of action, including contact toxicity, stomach poisoning, and efficient uptake by crops without inhibiting normal growth. However, its application can pose significant threats to aquatic organisms and the safe consumption of agricultural products[2]. Due to the scarcity of certain sample types in routine monitoring, there is a strong need for a representative matrix for pesticide-residue testing. Martinez et al. suggested using cucumber as a universal vegetable matrix for pesticide analysis[3]. The high consumption and widespread cultivation of cucumber make its safety critical to public health. Meanwhile, its high water content and complex matrix can pose challenges to analytical procedures. Therefore, developing reliable detection methods for cucumbers can also provide valuable references for other agricultural products with similar characteristics and matrix complexities.

Currently, the main methods for determining imidacloprid residues include high-performance liquid chromatography (HPLC), gas chromatography–mass spectrometry (GC–MS), and liquid chromatography–tandem mass spectrometry (LC–MS/MS)[4]. Among these, LC–MS/MS offers high detection speed, excellent qualitative and quantitative accuracy, and superior sensitivity. Consequently, the present study employs LC–MS/MS, using cucumber as a representative matrix, to explore an efficient method for the determination of imidacloprid residues in agricultural products. With its simplicity, high sensitivity, and relatively short analysis time, this approach is well suited for rapid and accurate detection.

2. Research Progress

Among sample pretreatment techniques, the QuEChERS method has become the preferred choice for most pesticide-residue analyses due to its simple workflow and low methodological error. M. Zhang et al.[5] reported in 2016 that LC–MS/MS in MRM mode allows precise qualitative and quantitative analysis of various pesticides in complex matrices, highlighting its importance in pesticide-residue detection. Ultra-high-performance liquid chromatography (UHPLC)[6] has also been widely applied in pesticide analysis, such as G. Gervais's study on pesticide residues in water (2008)[7] and M. Arienzo's research on pesticide

residues in vegetables (2013)[8]. RP. Carneiro et al. further proposed a multi-residue method for pesticide analysis in bananas in 2013[9].

Regarding imidacloprid itself, the World Health Organization classifies it as a Class II (moderately hazardous) pesticide. Although the acute toxicity of this category is generally not high, HMAD. MI et al.[10] indicated in 2017 that long-term consumption of foods containing excessive pesticide residues may lead to potential health risks.

3. Research Objectives and Significance

The national standard GB 23200.121-2021 employs LC–MS/MS for detecting pesticide residues in plant-derived foods, yielding objective detection performance and recovery rates. However, the procedural descriptions for certain pesticides remain insufficiently detailed. The present experiment aims to optimize and supplement the existing method by establishing an efficient LC–MS/MS-based approach for the determination of imidacloprid residues in cucumbers. The goal is to refine extraction, pretreatment, and detection parameters, thereby providing methodological improvements and practical reference for pesticide-residue analysis in agricultural products.

Main Text

4. Experimental Instruments and Reagents

4.1 Materials and Reagents

Cucumber; chromatographic-grade acetonitrile, formic acid, acetic acid, ammonium formate, methanol; analytical-grade sodium chloride and n-hexane; ProElut QuE 15 mL Tube-EN, 150 mg PSA / 45 mg Carb / 900 mg MgSO₄ (DiKMA); Vac 6 cc (500 mg) NH₂ cartridges (Sep-Pak); 50 mL centrifuge tubes; 3 mL syringes; 0.22 μm nylon membrane filters.

4.2 Instruments and Equipment

Shimadzu LC-30A-8050 LC–MS/MS system; XH-C vortex mixer; electronic analytical balance; ASE-12 solid-phase extraction device; nitrogen evaporator; GT10-1 high-speed desktop centrifuge.

5. Experimental Methods

5.1 Preparation of Standard Solutions

A small amount of imidacloprid reference material (con-

taining 100 µg of active ingredient) was weighed and diluted to prepare an external standard stock solution of 1 µg/mL. The solution was stored below -25 °C.

5.2 Optimization of LC–MS/MS Sample Pretreatment

5.2.1 Pretreatment Methods

This study compared the QuEChERS method with solid-phase extraction (SPE).

In the QuEChERS procedure, PSA adsorbent is directly added to the extraction solution during the extraction step. In the SPE procedure, the cartridge is first activated, followed by filtration of the sample, repeated washing, and

collection of all eluates.

5.2.2 Optimization Procedure

Eleven portions of cucumber samples were spiked to a final imidacloprid concentration of 1 µg/mL. The study examined four experimental factors: extraction solvent, extraction method, volumetric solution, and mobile phase. In each group, three factors were kept constant while the remaining factor was varied. Six injections were conducted for each sample, and the averaged results were analyzed. Chromatographic profiles and detected concentrations were compared to evaluate optimal conditions for pesticide-residue detection.

The specific variable settings are shown in Table 1.

Table 1 Four groups of experimental factors and their control variables

Factor	Variable 1	Variable 2	Variable 3
Extraction solvent	Acetonitrile		
Extraction method	QuEChers Method	SPE	
Volumetric Solutions	Methanol	50%Methanol	75%Methanol
Mobile Phases	0.1%acetic acid (Phase A) / acetonitrile (Phase B)	Ammonium formate–formic acid solution (Phase A) / ammonium formate–formic acid methanol solution (Phase B)	0.1% formic acid (Phase A) / acetonitrile (Phase B)

Experimental Procedure

Five hundred grams of cucumber were homogenized thoroughly before pretreatment.

QuEChERS method:

A 10 g portion of homogenized sample was weighed into a 50 mL centrifuge tube. Then, 1 mL of 1 µg/mL imidacloprid spiking solution was added. After vortex-mixing, 20 mL of acetonitrile and 10 g of sodium chloride were added for salting-out extraction. The mixture was vortexed for 10 min and centrifuged at 8000 r/min for 5 min. An aliquot of 15 mL of the supernatant was collected, vortexed again, and centrifuged under the same conditions. Then, 10 mL of the resulting supernatant was transferred to a 50 mL centrifuge tube and evaporated to dryness under nitrogen at 50°C for subsequent volumetric dilution.

Solid-phase extraction (SPE) method:

A 10 g sample was weighed into a 50 mL centrifuge tube, spiked with 1 mL of 1 µg/mL imidacloprid solution, and vortexed. Then, 20 mL of acetonitrile and 10 g of sodium chloride were added, followed by vortex extraction for 10 min and centrifugation at 8000 r/min for 5 min. Ten millilitres of the supernatant was evaporated to approximately 2 mL at 50°C under nitrogen.

An SPE cartridge was washed with 5 mL of an acetonitrile–toluene solution (3:1). After placing the cartridge on

a stand connected to a 100 mL flask, the 2 mL concentrated extract was loaded onto the cartridge and washed into the sorbent bed with 3 mL of the same acetonitrile–toluene solution. The cartridge was then eluted with 25 mL acetonitrile–toluene solution, and all eluates were collected in the 100 mL flask. The collected solution was evaporated nearly to dryness at 50°C under nitrogen.

Volumetric Dilution and Injection:

A total of 2.0 mL of the selected volumetric solution was added to reconstitute the residue, followed by vortex-mixing for 1 min. The solution was filtered through a 0.22 µm membrane prior to LC–MS/MS analysis.

Calculation of Recovery Rate

$$\text{Recovery(\%)} = \frac{\text{Measured concentration}}{\text{Theoretical concentration}} \times 100\%$$

Here, the measured concentration refers to the concentration of imidacloprid obtained by LC–MS/MS after sample extraction (unit: ng·mL⁻¹).

The theoretical concentration was 250 ng·mL⁻¹, assuming no loss of analyte during extraction and dilution.

5.2.3 Instrumental Conditions

Chromatographic Conditions

Column: C18 column, 2.1 mm × 100 mm, 1.8 µm;
Flow rate: 0.3 mL/min;

Column temperature: 40°C;
Injection volume: 2 μL.

The gradient elution parameters used for the mobile phase
are shown in Table 2.

Table. 2 Mobile phase elution gradient table

time/min	Phase A	Phase B
0	97	3
1.50	85	15
2.50	50	70
18.00	30	70
27.00	2	98
27.01	97	3

Ionization Source and MS Parameters

The ionization source was an electrospray ionization (ESI) source, operated in both positive- and negative-ion scanning modes. The ion source temperature was set at 350 °C. The electrospray voltage was 5500 V in positive-ion

mode. The nebulizing gas and auxiliary heating gas pressures were both maintained at 0.345 MPa.

The mass-spectrometric parameters for imidacloprid are shown in Table 3.

Table. 3 Spectrum parameters of compound

Analyte	Ion pair (m/z)	Q1(V)	CE(V)	Q3(V)
Imidacloprid	256.00>175.10	256.00	13	175.10
	256.00>209.00	256.00	17	209.00

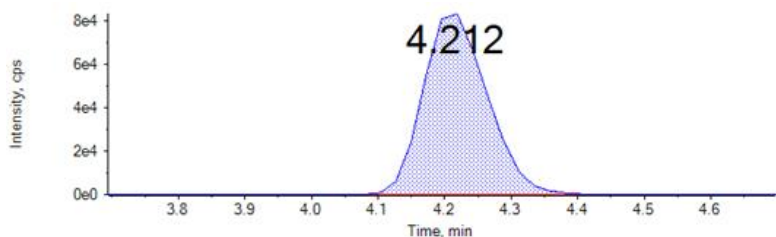
6 Results and Discussion

6.1 Optimization of Extraction Solvents

Imidacloprid contains multiple polar covalent bonds and nitrogen-containing heterocycles, enabling efficient extraction based on the principle of “like dissolves like.” In

this experiment, acetonitrile (control group) and two acetonitrile–water mixtures (2:1 and 3:1, v/v) were compared to evaluate their extraction performance.

The LC chromatograms of the three solvent systems are shown in Figure 1. All three extraction solvents produced well-shaped peaks without interfering signals. The experimental results are summarized in Table 4.



Acetonitrile:Water = 3:1

Fig. 1 The spectra of imidacloprid were extracted with Acetonitrile, acetonitrile-water mixed solution (2:1), acetonitrile-water mixed solution(3:1)

Table. 4 Data of imidacloprid extraction from three kinds of extracts

Evaluate extraction solvent	retention time (min)	peak area	detected concentration(ng·mL ⁻¹)
Acetonitrile	4.24	4.067×10 ⁵	203.438
Acetonitrile:Water = 3:1	4.21	5.022×10 ⁵	248.311
Acetonitrile:Water = 2:1	4.22	4.584×10 ⁵	229.345

The results indicated that when the acetonitrile-to-water ratio reached 3:1, the detected concentration of imidacloprid was the highest and approached the theoretical concentration of 250 ng·mL⁻¹, yielding the best recovery performance. According to relevant studies[11], the addition of an appropriate amount of water to the extraction solvent can improve extraction efficiency. As the proportion of water increases, the extraction efficiency first increases and then decreases. These findings serve as a useful refer-

ence for interpreting the results of this experiment.

3.2 Optimization of Extraction Methods

The extraction efficiency of the QuEChERS method was compared with that of the solid-phase extraction (SPE) cartridge method. The LC chromatograms of the two extraction methods are shown in Figure 2, both exhibiting well-defined peaks without interference. The experimental data are summarized in Table 5.

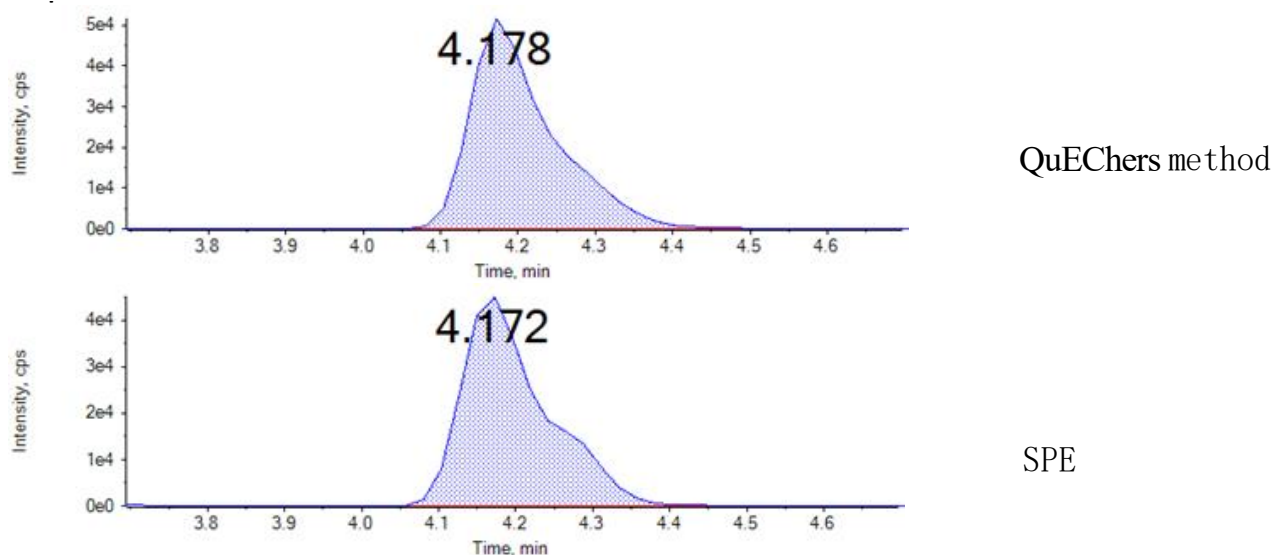


Fig. 2 The spectra of two kinds of purifying solution by QuEChers method and solid phase extraction column

Table. 5 The data of imidacloprid were extracted by two purification methods

Purifying method	Retention time(min)	Peak area	Detected concentration(ng·mL ⁻¹)
QuEChers method	4.18	3.751×10 ⁵	233.864
SPE	4.17	3.381×10 ⁵	210.850

The results showed that the QuEChERS method produced higher detected concentrations and better recovery of imidacloprid than the solid-phase extraction method. Therefore, the QuEChERS approach was selected as the purification method for subsequent experiments.

were compared to evaluate their effectiveness as volumetric solvents. The LC chromatograms of the three solvents are presented in Figure 3, and all three produced well-shaped peaks without interference. The experimental results are shown in Table 6.

3.3 Optimization of Volumetric Solutions

Methanol, 75% methanol–water solution, and 50% methanol–water solution (as specified in the national standard)

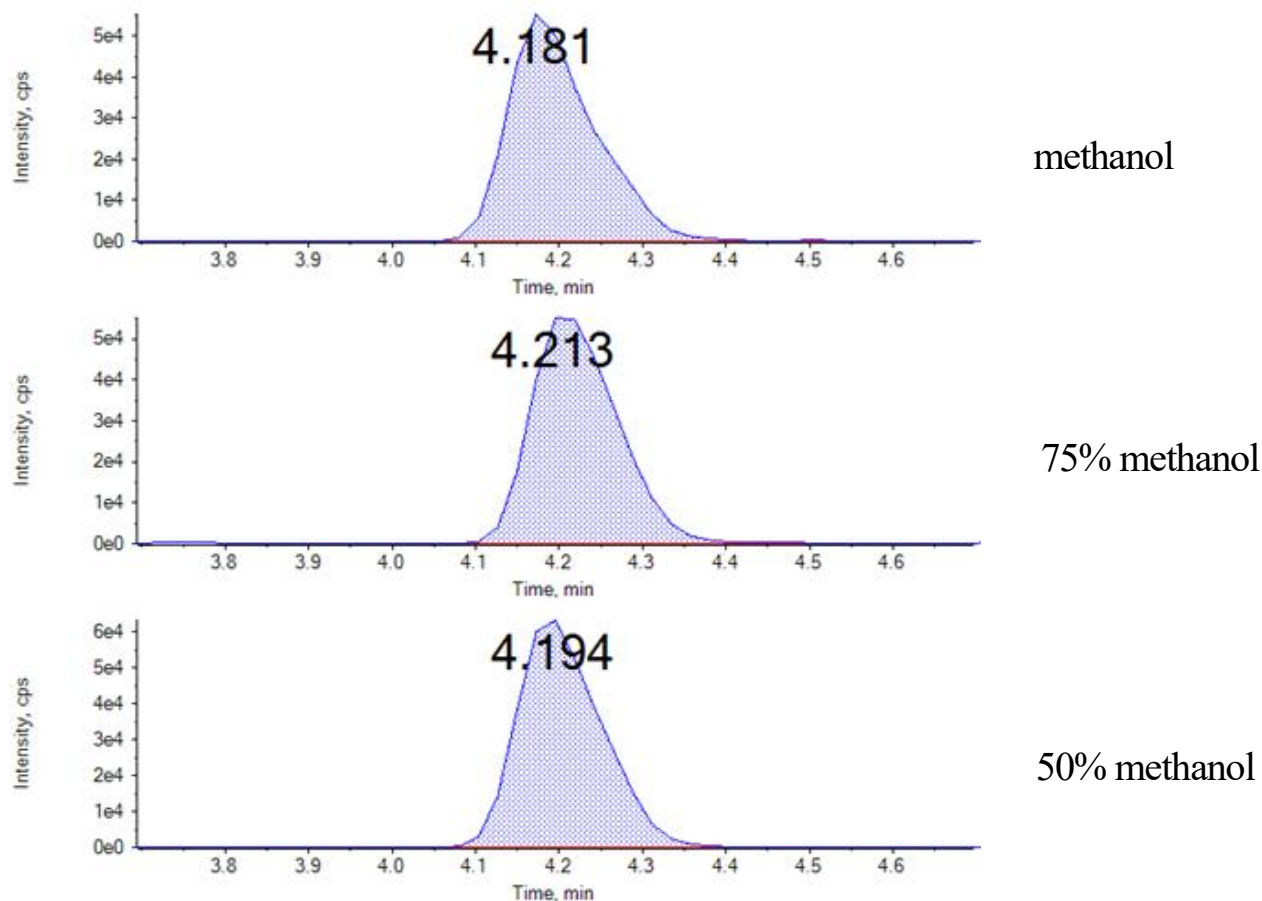


Fig. 3 The chromatograms of three volumetric solutions of methanol, 75% methanol and 50% methanol were determined

Table. 6 Three groups of volumetric data of imidacloprid

Volumetric Solution	Retention Time(min)	Peak Area	Detected Concentration (ng·mL ⁻¹)
Methanol	4.18	3.975×10^5	198.862
75% Methanol–water	4.21	3.381×10^5	202.877
50% Methanol–water	4.19	4.495×10^5	224.908

The results indicate that the 50% methanol–water solution produced the highest detected concentration and the best recovery performance. Thus, 50% methanol was selected as the optimal volumetric solvent.

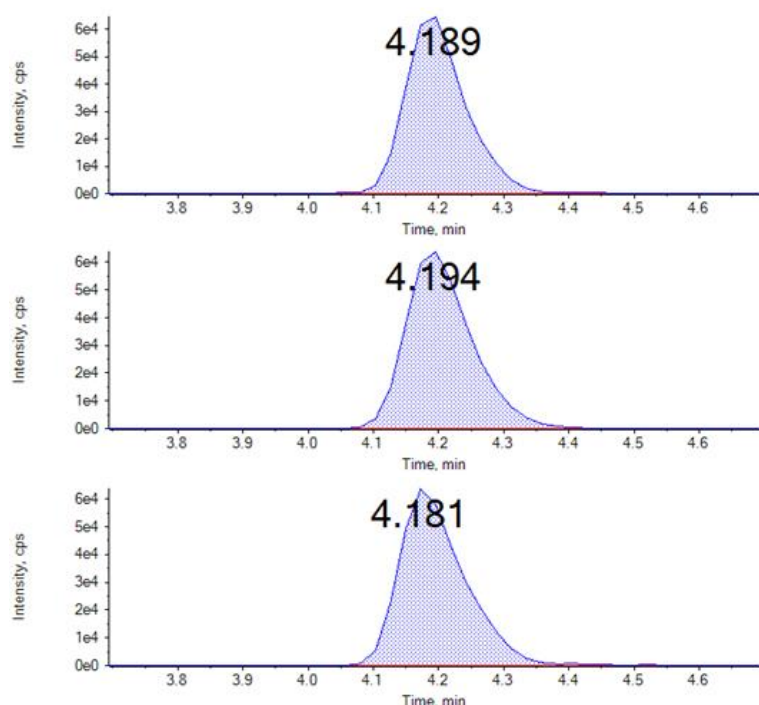
3.4 Optimization of Mobile Phases

In several studies involving LC detection of drug residues, weakly acidic mobile phases—typically containing low concentrations of formic acid or acetic acid—are frequently used to improve chromatographic peak shape[12].

In this experiment, three mobile-phase systems were compared:

1. A $10 \text{ mmol} \cdot \text{L}^{-1}$ ammonium formate–formic acid solution (Phase A) and acetonitrile (Phase B) (national standard)
2. 0.1% acetic acid (Phase A) and acetonitrile (Phase B)
3. 0.1% formic acid (Phase A) and acetonitrile (Phase B)

The LC chromatograms of the three mobile phases are shown in Figure 4. All three yielded clear and well-defined peaks without interference. The experimental data are summarized in Table 7.



10 mmol·L⁻¹ ammonium formate solution and 6 mmol·L⁻¹ formic acid solution mixed at a volume ratio of 1:1 (Phase A) and acetonitrile (Phase B).

0.1% acetic acid (Phase A) and acetonitrile (Phase B).

0.1% formic acid (Phase A) and acetonitrile (Phase B).

Fig. 4 10 mmol·L⁻¹ ammonium formate solution and 6 mmol·L⁻¹ formic acid solution were mixed at a volume ratio of 1:1(phase A) and acetonitrile (phase B) ; 0.1% acetic acid (phase A) and acetonitrile (phase B) ; 0.1% formic acid (phase A) and acetonitrile (phase B)

Table. 7 Three groups of flow relative imidacloprid injection test data

Mobile Phase	Retention time(min)	Peak area	Detected concentration (ng·mL ⁻¹)
Ammonium formate–formic acid (A) / Acetonitrile (B)	4.19	4.205×10 ⁵	210.358
0.1% Acetic acid (A) / Acetonitrile (B)	4.19	4.501×10 ⁵	225.189
0.1% Formic acid (A) / Acetonitrile (B)	4.18	4.435×10 ⁵	221.862

The results show that the mobile phase consisting of 0.1% acetic acid (Phase A) and acetonitrile (Phase B) provided the highest detected concentration and best recovery performance.

3.5 Quantification by External Standard Method (Calibration Curve)

Under the chromatographic conditions described above,

external standard quantification was performed using methanol as the solvent. Imidacloprid standards ranging from 1 to 1000 ng·mL⁻¹ were prepared, and the peak areas corresponding to each concentration were measured. The results demonstrated a strong linear relationship between imidacloprid concentration (x) and peak area (y), meeting the requirements for quantitative analysis, as shown in Figure 5. The regression equation and correlation coefficient are listed in Table 8.

e 5. The regression equation and correlation coefficient are listed in Table 8.

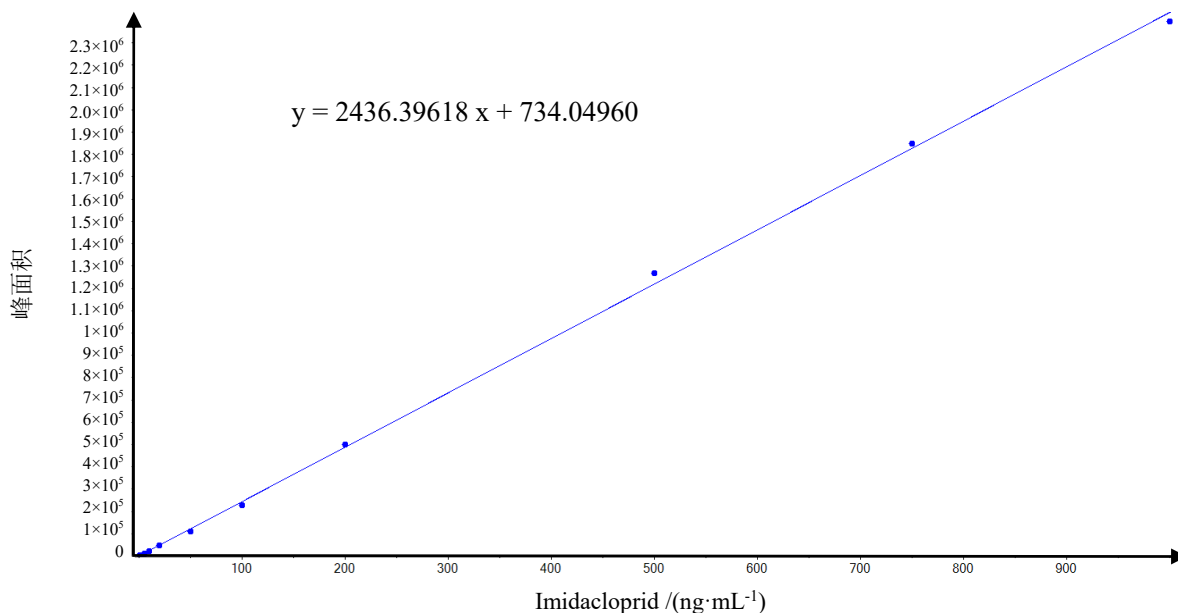


Fig. 5 Standard curve of imidacloprid

Tab. 8 Regression equation of imidacloprid

Analyte	Regression equation	Correlation coefficient
Imidacloprid	$y = 2436.39618 x + 734.04960$	0.99935

3.6 Accuracy Verification

For each optimized parameter with the highest recovery, six parallel determinations were performed. According to the laboratory quality-control specifications in GB/T 27404-2008 (Appendix F, physicochemical testing of food), for analyte concentrations between 0.1–1 mg/kg, the acceptable recovery range is 80%–110%, and the coef-

ficient of variation (CV) must be ≤11% when the analyte concentration is ≤1 mg/kg.

The measured analyte concentration in this study was 0.4 mg/kg (equivalent to post-dilution levels). The calculated results are shown in Table 9. All optimized conditions met the required accuracy and precision standards.

Tab. 9 Parallel data and coefficient of variation of imidacloprid optimization levels

Parallel concentrations		1	2	3	4	5	6	Mean	Mean recovery (CV/%)
Optimization factor	Detected concentration (ng/mL)	248.523	239.352	258.742	260.774	235.669	246.806	248.311	4.05
	recovery rate (%)	99.41	95.74	103.49	104.31	94.26	98.72	99.32	
Extraction solvent	Detected concentration (ng/mL)	230.589	235.147	228.976	226.612	238.729	243.131	233.864	2.69
	recovery rate (%)	92.23	94.06	91.59	90.64	95.49	97.25	93.55	

Volumetric solution	Detected concentration (ng/mL)	232.862	221.573	228.694	229.702	215.652	220.965	224.908	2.90
	recovery rate (%)	93.14	88.63	91.48	91.88	86.26	88.39	89.96	
Mobile phase	Detected concentration (ng/mL)	233.675	224.328	219.486	226.503	218.463	228.679	225.189	2.55
	recovery rate (%)	93.47	89.73	87.79	90.60	87.38	91.47	90.07	

4 Discussion

4.1 Selection of Extraction Solvent

The primary extraction method used in this study was the QuEChERS approach. Common extraction solvents for pesticide-residue analysis include methanol, acetone, and acetonitrile. Previous studies have shown that acetonitrile provides superior extraction efficiency[13]. Because imidacloprid is relatively polar and susceptible to co-extracted impurities, using acetonitrile mixed with water helps reduce interference and precipitate unwanted matrix components.

4.2 Selection of Extraction Method

Purification methods commonly used in pesticide-residue analysis include QuEChERS and SPE cartridges. The QuEChERS method offers high detected concentrations, excellent recovery, low analytical error, and reduced labor and material cost. SPE cartridges can concentrate and purify samples simultaneously, provide high sensitivity, shorten processing time, reduce solvent consumption, and allow for automation and batch processing.

4.3 Selection of Mobile Phase

Acetonitrile and methanol are widely used organic solvents in chromatographic detection of drug residues due to their polarity, elution strength, and low UV absorbance. They facilitate efficient elution during injection. In most pesticide-residue analyses, weakly acidic mobile phases are used to ensure satisfactory chromatographic peak shapes.

5 Conclusion

This study optimized the pretreatment conditions and mobile-phase selection based on the national standard GB 23200.121-2021. Using cucumber as the test matrix and LC-MS/MS for detection, this work provides additional

reference data and supplements the applicability of the national method. The detected concentrations of imidacloprid ranged from 221.862 to 248.311 ng·mL⁻¹, with recoveries of 88.74%–99.32%, meeting standard analytical requirements.

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