Determination of Aldicard and Its Metabolites Residues in Potato by Graphene SPE Combining with LC-MS/MS

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Abstract

A method was established to determine aldicard and its metabolites residues in potato by graphene solid phase extraction combining with liquid chromatography-mass spectrometry method. The sample was extracted by acetonitrile. After being cleaned up by graphene solid phase extraction column, the sample was performed on Poroshell 120 EC-C18，3.0×100 mm, 2.7 µm column. The analysis was carried out in the multi reaction monitoring mode and external standard method was used for quantification. The calibration curve of the three pesticides was good linear from 0.01 to 0.20 mg/L with the correlation coefficient r=0.9955-0.9989, the quantitation limit of this method was 0.01 mg/kg. In different concentrations, the average recoveries of this method were 80%-93% and the variability coefficient were 9.4%-14.1%.

Keywords

Graphene; SPE; Liquid chromatography-mass spectrometer; Aldicard; Metabolites.

1. Introduction

Aldicarb is a highly toxic, broad-spectrum carbamate insecticide widely used in the planting process of vegetables and fruits due to its high efficiency and economy. Aldicarb is easily oxidized to more toxic aldicarb sulfone and aldicarb sulfoxide under certain conditions. The national standard for food safety GB2763-2021 "National food safety standards-Maximum Residue Limits for Pesticides in Food" [1] stipulates that the maximum residue limit of aldicarb (contains its metabolites) in potato is 0.1 mg/kg. At present, the most reported detection method for aldicarb and its metabolites is liquid chromatography tandem mass spectrometry [2-5].

Solid phase extraction is a widely used sample pretreatment technology in recent years. Compared with traditional liquid-liquid extraction (LLE), it can improve extraction efficiency, simplify operation, reduce the amount of organic solvents used, and select appropriate adsorption fillers based on the properties of the analyte to better achieve sample purification. Commonly used fillers include C18, graphite carbon, silica gel, etc.

Graphene has a large specific surface area, with a theoretical value of 2630 m^2/g. It has good acid and alkali resistance, heat resistance, chemical stability, strong hydrophobicity, and is easy to functionalize. It can also generate strong π-π interactions with organic molecules [6]. Graphene is prepared from natural graphite, which has advantages such as low preparation cost, easy availability of raw materials, and easy implementation of large-scale preparation, making it more competitive than carbon nanotubes. At present, there have been relevant literature reports on graphene as a solid phase extraction filler [7-10], but there have been no reports on graphene solid phase extraction for the detection of aldicarb and its metabolites in

potato. This paper used graphene as a solid phase extraction filler and combined liquid chromatography tandem mass spectrometry to establish a new method for the detection of aldicarb and its metabolites residues in potato.

2. Experimental part

2.1. Instruments and reagents

Agilent 1290-6460 liquid chromatography-tandem mass spectrometer, United States; IKA GM200 high-speed homogenizer, German; Tokyo Physicochemical MMV-1000W oscillator, Japan; Hitachi CR22G III high-speed freezing centrifuge, Japan; Buchi R215 rotary evaporator, Swiss; IKA MS3 vortex mixer, German.

Methanol, acetonitrile, acetone, ethyl acetate and formic acid are all chromatographically pure; Anhydrous sodium sulfate (burned at 650℃ for 4 hours, cooled and stored in a dryer for backup), sodium chloride, and ammonia water are analytical pure; The experimental water is ultrapure water.

Standard products: aldicarb, aldicarb sulfone, and aldicarb sulfoxide are all 100 mg/L, 1 mL, purchased from the Environmental Protection Research and Monitoring Institute of the Ministry of Agriculture of China.

2.2. Analysis conditions

Chromatographic column: Poroshell 120 EC-C_{18}, 3.0×100 mm, 2.7 μm; Column temperature: 30℃; Injection volume: 1 μL; Mobile phase:A was methanol, B was water; The gradient elution conditions were: 0-4 min, 5-70% A; 4-5 min, 70% A; 5-7.1 min, 70-5% A; 7.1-10 min, 5% A. Flow rate: 0.3 mL/min.

Ion source: jet stream ion focusing electric spray ion source; Scanning mode: Positive ion mode; Detection method: multi reaction monitoring; Dry gas temperature: 325℃; Dry gas flow rate: 10 L/min; Atomizing gas pressure: 45 psi; Sheath gas temperature: 350℃; Sheath gas flow rate: 11 L/min; Capillary voltage: 4000 V; Collision gas: high-purity nitrogen(>99.999%); The residence time of each ion pair is 70 ms.

2.3. Sample pretreatment

Extraction: Weigh 2.00 g of the sample (accurate to 0.01 g) into a 50 mL centrifuge tube, add 20 mL acetonitrile, homogenize for 1 min, then add 4 g of anhydrous sodium sulfate and 1g of sodium chloride, shake and extract for 1 min, centrifuge at 4℃ and 9000 r/min for 5 min, collect the supernatant to a pear shaped bottle, and rotate and evaporate in a 40℃ water bath to concentrate until dry. Dissolve the residue in 5 mL of acetonitrile-0.1% formic acid aqueous solution (10+90, v/v) and wait for purification.

Purification: Activate the graphene solid-phase extraction columns with 3 mL methanol and 3 mL water, then pass the sample solution through the column at a rate of 1 mL/min, and finally elute with 3 mL acetonitrile. Collect the elution solution into a 15 mL centrifuge tube, dry it with nitrogen, and dissolve it in 1 mL mobile phase. After filtered with 0.22 μm organic phase filter membrane, the sample is tested by LC-MS/MS.

3. Results and Discussion

3.1. Optimization of Solid Phase Extraction Conditions

3.1.1. Determination of Graphene Quality

The amount of graphene used is an important factor affecting the extraction efficiency. The effects of graphene dosage of 5, 8, 10, 15, and 20 mg on the extraction efficiency (peak area) were investigated. The results showed that the peak areas of the three compounds showed an

overall upward trend from 5-15 mg; When the amount of graphene is 15 mg, aldicarb, aldicarb sulfone, and aldicarb sulfoxide could all reach their maximum peak areas. After that, increasing the amount of graphene, the peak areas of the three compounds showed a downward trend. Therefore, the amount of graphene used in this study was chosen as 15 mg. The effect of graphene dosage on extraction efficiency is shown in Fig. 1.
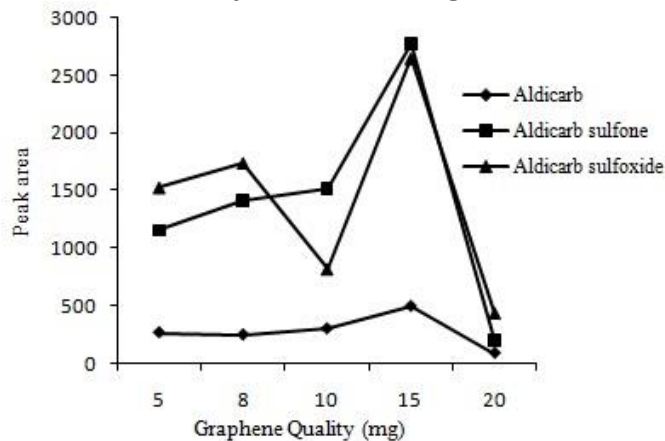

Fig. 1 The effect of graphene dosage on extraction efficiency

3.1.2. Determination of sample quality

Due to the complexity of the sample matrix, different sample loading amounts will inevitably affect the adsorption of graphene on the target substance and the final purification effect. This study investigated the effect of sample quality of 1, 2, 3, 4, and 5 g on extraction efficiency, and the results were measured by recovery rate. The results showed that there was no significant change in the recovery rate when 1-2 g sample quality; Continuing to increase the sample size resulted in a decrease in the recovery rates of all compounds, possibly due to interference from the sample matrix in the extraction process. In order to obtain the best response value and sensitivity, the sample quality for this study was set at 2 g.

3.1.3. Selection of elution reagents

The elution effects of methanol, ammonia containing methanol, acetonitrile, ammonia containing acetonitrile, acetone, ammonia containing acetone, ethyl acetate, and ammonia containing ethyl acetate (mixed with appropriate methanol) were investigated, see Fig. 2. The results showed that the ammonia reagent had an enhanced elution ability for aldicarb; The elution ability of ammonia containing methanol for the three target compounds is greater than that of methanol; acetonitrile can achieve good elution effects on aldicarb sulfone and aldicarb sulfoxide. Overall, acetonitrile was chosen as the elution reagent for this study.
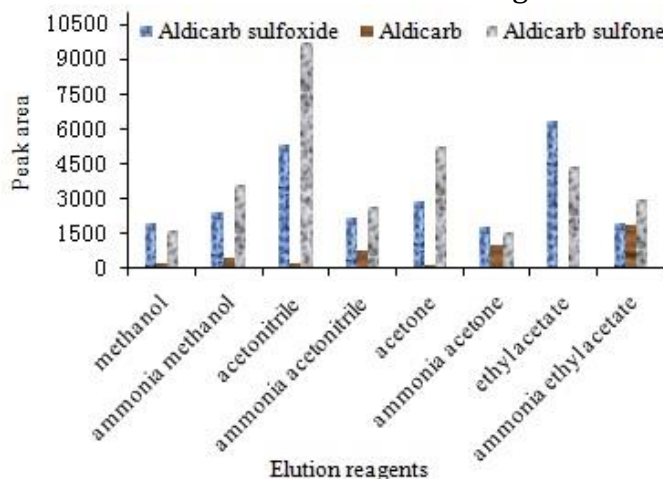

Fig. 2 The effect of elution reagents

3.1.4. Determination of elution volume

After the completion of solid-phase extraction, elution experiments were conducted using 1, 2, 3, 4, 5, and 6 mL of acetonitrile respectively, see Fig. 3. In the range of 1-3 mL, as the elution volume increased, the peak areas of each compound gradually increased; When the elution volume was 3mL, the peak area of the target compound reached its maximum value, and increasing the elution volume afterwards had no contribution to the change in peak area of the target compound. Therefore, this study chose 3 mL as the final elution volume.
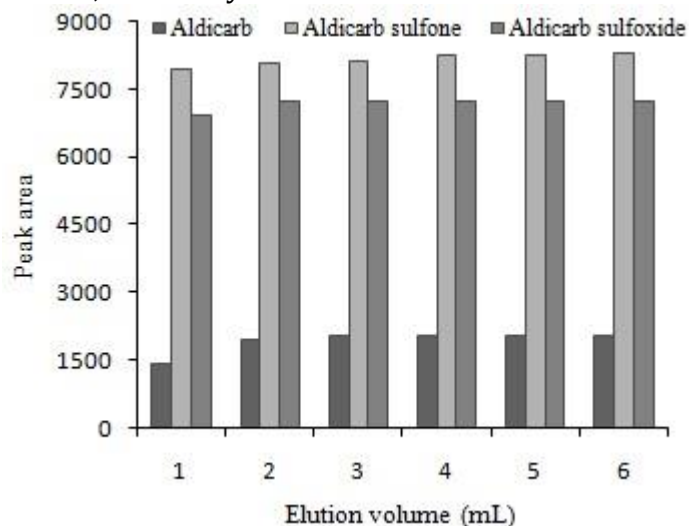

Fig. 3 The effect of elution volume

3.2.   Optimization of mass spectrometry conditions

Selected ESI+ as the ionization mode to optimize the parameters of the parent ion, daughter ion, collision energy of aldicarb and its metabolites. Firstly, performed a primary mass spectrometry scan to obtain the parent ion and optimal fragmentation voltage of the compound; Then, the selected parent ions were subjected to selective ion monitoring scanning (SIM) and Product scanning to obtain the corresponding fragmentation energy and collision energy of the compound, and to determine the quantitative and qualitative ions used for multiple reaction monitoring. Established multi reaction monitoring data parameters for the three compounds by optimizing the obtained mass spectrometry parameters, which were shown in Table 1. Under optimized mass spectrometry conditions, the standard of aldicarb and its metabolites were injected to obtain a MRM chromatogram, which is shown in Fig. 4.

Table 1. Mass spectrometry parameters of aldicarb and its metabolites

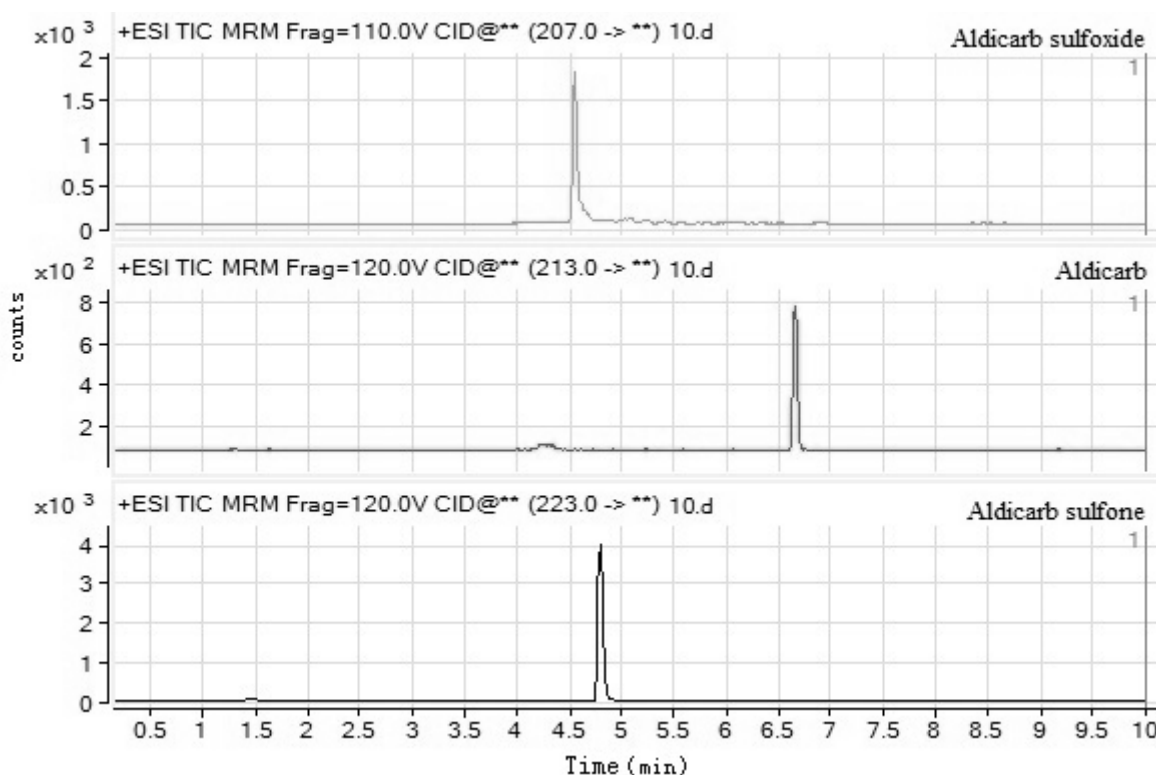| Serial Number	Name	Qualitative Ion Pair (m/z)	Quantitative Ion Pair (m/z)	Fragmentation Voltage (V)	Collision Energy (eV)
1	Aldicarb	213.0/89.1 213.0/98.2	213.0/89.1	120	15 11
2	Aldicarb sulfone	223.0/86.3 223.0/148.2	223.0/86.3	120	13 6
3	Aldicarb sulfoxide	207.0/89.1 207.0/132.2	207.0/132.2	110	14 6

Fig. 4 MRM chromatogram of aldicarb and its metabolite standard

3.3. Linear range, quantitation limit, recovery rate and precision

Prepare standard solutions with concentrations of 0.01, 0.02, 0.05, 0.10, 0.15, and 0.20 mg/L for LC-MS/MS testing. Draw a standard curve with concentration as the horizontal axis and quantitative ion peak area as the vertical axis to obtain a linear regression equation (Table 2). Within the concentration range of 0.01 to 0.20 mg/L, there was a good linear relationship for each compound's peak area, with a correlation coefficient (r) of 0.9955 to 0.9989. The quantitation limit (LOQ) of the method is determined to be 0.01 mg/kg using a 10 times signal-to-noise ratio. Weigh 2.00 g (accurate to 0.01 g) of blank sample and conduct spiked recovery experiments according to the method established in this article at three concentration levels of 0.01, 0.05, and 0.1 mg/kg. Perform 6 parallel measurements at each level of addition. The average recovery rate of the method was 80%-93%, and the relative standard deviation was 9.4%-14.1%.

Table 2 Method Linear Regression Equation, quantitation limit, recovery rate and precision

Name	Linear regression	Correlation coefficient	LOQ (mg/kg)	Recovery (RSD)/%		
				0.01mg/kg	0.05 mg/kg	0.1 mg/kg
Aldicarb	y=458856.6x+279.2	0.9955	0.01	85 (9.7)	89 (9.4)	80 (10.0)
Aldicarb sulfone	y=352302.2x-388.1	0.9988	0.01	90 (13.4)	93 (9.5)	86(10.8)
Aldicarb sulfoxide	y=118119.0x-334.5	0.9989	0.01	89 (12.2)	87(14.1)	90(11.1)

4. Conclusion

This paper used graphene as a solid phase extraction material and established a method for the detection of aldicarb, aldicarb sulfone, and aldicarb sulfoxide residues in potato with liquid

chromatography tandem mass spectrometry. The conditions affecting the detection of solid phase extraction, such as graphene dosage, sample quality, eluent reagents, elution volume, as well as instrument conditions such as liquid phase and mass spectrometry, were optimized. The method had good linear relationship and precision, high recovery rate, detection and quantification limits could meet the detection needs. It was suitable for the detection of aldicarb and its metabolites residues in potato.

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