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The determination of underivatized chlormequat, fosetyl-aluminium and phosphonic acid residues in maize and soybean by LC-MS/MS†

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In this study, a simple, rapid and sensitive method was developed for the simultaneous determination of chlormequat, fosetyl-aluminium and phosphonic acid residues in maize and soybean using liquid chromatography-triple quadrupole mass spectrometry (LC-MS/MS). Analytes were extracted with acetic acid solution, purified on an HLB column, and then filtered through a 0.2 µm hydrophilic microporous filter membrane. They were then separated on an IC column using a separation phase consisting of polyvinyl alcohol particles with quaternary ammonium groups. The mobile phase optimised with water was denoted as mobile phase A and that optimised with 200 mmol L⁻¹ ammonium bicarbonate solution containing 0.05% ammonium hydroxide was denoted as mobile phase B. The residues were detected by tandem mass spectrometry with negative electrospray ionization in a multi-reaction monitoring mode. The correlation coefficient ($R \geq 0.997$) showed good linear regressions for all analytes in water as well as in maize and soybean matrices with a wide dynamic range of 0.001 to 0.5 mg L⁻¹ for calibration. The mean recoveries (RSDs) of the analytes were in the range 85.0–106.4% (5.5–14.9%), 81.7–109.5% (2.7–11.0%) and 74.7–104.4% (2.9–6.1%) at three concentration levels (0.05, 0.1 and 1 mg kg⁻¹) for the interday test ($n = 15$). The limit of quantification (LOQ) and detection (LOD) of the method for different matrices were 0.01 and 0.003 mg kg⁻¹, respectively. In conclusion, the established analytical approach has high sensitivity and good accuracy and precision and is suitable for monitoring chlormequat, fosetyl-aluminium and phosphonic acid residues in maize and soybean.

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1 Introduction

Maize is an important food crop that can be used for human consumption and animal feed. In 2021, the planted area for maize was 4.332×10^7 hm² and the total yield reached 272.6 million tonnes in China, accounting for 43.3% and 43.1% of the world's total planted area and yield, respectively, making it the number one crop in China.¹ Soybean is an important oil crop that is rich in vegetable protein and fat, and China is the world's largest consumer of soybean. In 2021, China's soybean imports reached 96.52 million tonnes, which is 5.9 times China's total soybean production.² With the continuous progress of China's agricultural modernization, pesticides have become necessary to control insect pests, plant pathogens and weeds in the

agricultural production process owing to their advantages of low cost, high efficiency and convenience.³ However, the use of pesticides inevitably produces pesticide residues, which not only cause damage to the environment but also affect human health.⁴ The national food safety standard sets China's maximum residue limits for pesticides in food. Therefore, in order to protect human health, it is necessary to test for pesticide residues in maize and soybeans.

Chlormequat is a plant growth regulator that can inhibit cell elongation and promote cell division. Chlormequat shortens plants and increases yields by strengthening the stems and reducing branching in plants.⁵ Fosetyl-aluminium is a highly effective, broad-spectrum, systemic, organophosphate fungicide and previous studies suggest that the breakdown product of fosetyl-aluminium, phosphonic acid, may have significant antifungal activity.⁶ These pesticides play an important role in improving crop yield and quality; however, they pose a serious threat to the environment and human health. Animal toxicology studies show that chlormequat has adverse effects on animal reproduction, suggesting that chlormequat may also affect human fertility.^{7–9} Fosetyl-aluminium has low toxicity but can degrade to phosphonic acid in the matrix. Phosphonic acid poses a serious threat to human health, and long-term exposure

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to fosetyl-aluminium causes various diseases, such as tumour lesions and neuritis, and even affects future generations through malformations and genetic mutations. Therefore, the dosage of these chemicals must be strictly controlled. Residue testing is carried out to ensure the safe use of agricultural products and to protect human health.

Currently, LC-MS/MS is the main method used to detect chlormequat residues in food.^{10–13} The detection methods for fosetyl-aluminium residues are GC,^{14,15} LC-MS/MS,^{16,17} AAS,¹⁸ and LC.^{6,19} Previous studies have mostly tested individual pesticide residues and have not investigated the simultaneous detection of all three compounds. Some approaches are cumbersome, *e.g.*, derivatisation reaction prior to fosetyl-aluminium detection.^{15,20} Our research avoids the complicated operation of the conventional derivatisation method and saves up to 30 minutes (ESI Table S1†). Extracting chlormequat from pears and concentrated pear juice requires 100 mL of methanol, which uses a large amount of organic solvent and is less environmentally friendly.²¹ The method for detecting chlormequat has been applied in rice and flour,¹³ barley and wheat,²² and cottonseed oil.¹² To date, no residue detection methods have been reported for chlormequat, fosetyl-aluminium, and phosphonic acid in maize and soybean. A liquid chromatography-triple quadrupole mass spectrometry (LC-MS/MS) detection method has been developed for the simultaneous detection of the above three compounds in maize and soybean matrices. In the current study, the sample preparation is optimised, reducing the volume of organic solvents, significantly improving environmental performance, and enabling the simultaneous and accurate detection of highly polar compounds, namely chlormequat, fosetyl-aluminium, and phosphonic acid.

2 Materials and methods

2.1 Chemicals and reagents

Phosphonic acid (97.5%) and fosetyl-aluminium (90.3%) standards were purchased from Dr Ehrenstorfer (Augsburg, Germany), while chlormequat (98.0%) was purchased from First Standard (MA, USA). Ultrapure water was obtained using a Milli-Q system (Millipore, Bedford, MA, USA). Ammonium bicarbonate was purchased from Sigma (purity \geq 99.0%, St. Louis, MO, USA). Ammonium hydroxide was supplied by Acros Organics (New Jersey, USA). Analytical grade dichloromethane was obtained from Tianjin Bohua Chemical Reagents Co., Ltd. (Tianjin, China).

Stock standard solutions (1000 mg L^{-1}) of chlormequat, fosetyl-aluminium, and phosphonic acid were prepared separately by accurately weighing the compounds and dissolving them in water and storing at $5 \pm 3^\circ\text{C}$ in high-density polypropylene (HDPE) narrow-mouth bottles (Thermo Scientific, Rochester, NY, USA). The standard calibration solutions consisting of chlormequat, fosetyl-aluminium and phosphonic acid (0.001, 0.002, 0.005, 0.01, 0.02, 0.05, 0.1, 0.2, and 0.5 mg L^{-1}) were prepared by diluting the stock solution with water. A further two sets of working standards were the matrix standards obtained by diluting the stock solution with the blank maize

and soybean matrices. Maize and soybeans, representatives of cereals and oilseeds, respectively, were used as starting materials and purchased from the market. The aforementioned maize and soybean matrices were free of interference from the analytes.

2.2 Sample preparation

2.2.1 Extraction. 2 g of the ground sample was weighed to an accuracy of 0.01 g. In a 50 mL centrifuge tube, add extraction solvent, which was optimised using seven solvents, including ultrapure water, methanol, acetonitrile, 1% formic acid solution, 1% formic acid in methanol, and 0.5% formic acid in methanol:water (50 : 50, v/v) solution and acetic acid solution (57.2 μL of acetic acid, make up to 10 mL with water, mix; then take 1 mL of the fixed solution and makeup to 1 L with water, mix). 20 mL of acetic acid solution was added to the ground sample and allowed to stand for 30 minutes, shaken at 200 rpm for 30 minutes, centrifuge at 5000 rpm for 5 minutes, and the supernatant was collected. 6 mL of the supernatant was transferred to a 50 mL centrifuge tube, add 30 mL of dichloromethane, vortex, and mixed and centrifuged at 5000 rpm for 5 minutes. The supernatant was then subjected to purification.

2.2.2 Clean up. Three kinds of HLB columns (60 mg, 3 mL) including Oasis HLB (Waters), Oasis PRiME HLB (Waters), and ASC Accurasil HLB (Ameritech) were compared for the purification of the maize and soybean analytes. 2 mL of the aqueous phase was taken, loaded onto the column and the extraction solution was passed through the HLB column drop by drop under negative pressure. After receiving the elute through a 0.2 μm hydrophilic microporous filter membrane, it was transferred to the sample vial for measurements.

2.3 UPLC-MS/MS conditions

UPLC-MS/MS determination was performed on a Waters Acquity™ Ultra Performance LC (Waters Corporation, Milford, MA, USA) coupled to a SCIEX QTRAP 4500 mass spectrometer (Framingham, MA, USA). All runs were acquired and processed using SCIEX Analyst™ software (version 1.6.2) and Multi-Quant™ 3.0 software. Chromatographic separations were performed on a Metrohm Metrosep A Supp 5 column (4.0 mm \times 150 mm \times 5 μm , Metrohm AG, Herisau, Switzerland) at 40°C . The flow rate was 0.6 mL min^{-1} and the injection volume was 10 μL . The elution effects of four different B mobile phases were compared, and the four mobile phases B were 50 mmol L^{-1} ammonium bicarbonate, 100 mmol L^{-1} ammonium bicarbonate, 200 mmol L^{-1} ammonium bicarbonate, and 200 mmol L^{-1} ammonium bicarbonate with 0.05% ammonium hydroxide. Finally, 200 mmol L^{-1} of ammonium bicarbonate with 0.05% ammonium hydroxide was selected as a mobile phase B coupled with a mobile phase A, which was water. The gradient programme was set as follows: 0–0.5 min, 90% A; 1 min, 60% A; 4 min, 20% A; 5 min, 17.5% A; 6–12 min, 5% A; 12.1–15 min, 90% A. The optimised ionisation source conditions were as follows: ion source type, electrospray ion source (ESI); ion source temperature, 600°C ; detection method, and multi-reaction monitoring (MRM). Two product ions of each



Table 1 Mass spectrometry parameters for the detection of the three targets

Compound	CAS	Molecular formula	Molecular weight (g mol ⁻¹)	Ionisation mode	Retention time (min)	Precursor ion (m/z)	Product ion (m/z)	CE (V)
Chlormequat	999-81-5	C ₅ H ₁₃ Cl ₂ N	158.069	Positive	1.69	122	58	41
						122	63	29
Fosetyl-aluminium	39148-24-8	C ₆ H ₁₈ AlO ₉ P ₃	354.1045	Negative	3.35	109	81	-21
						109	79	-34
Phosphonic acid	13598-36-2	H ₃ O ₃ P	81.9958	Negative	4.1	81	79	-20
						81	63	-39

pesticide were selected and the product ions to be detected were each detected in time segments according to the order of the peak production. The mass spectrum parameters of each pesticide are given in Table 1 below. Chlormequat was already a charged cationic species in the solution, resulting in the precursor molecular ion [M]⁺ at m/z 122 in the positive ESI mode, the product ion at m/z 58 assigned to [M-C₂H₅Cl]⁺ was used for chlormequat quantification and the product ion at m/z 63 corresponding to [M-N(CH₃)₃]⁺ was selected for confirmation. Fosetyl-aluminium was already a charged anionic species in the solution, resulting in the precursor molecular ion [M]⁻ at m/z 109 under the negative ESI mode, the product ion at m/z 81 assigned to [H₂PO₃]⁻ was used for the fosetyl-aluminium quantification, and the product ion at m/z 79 corresponding to [PO₃]⁻ was selected for confirmation. The mass transition for phosphonic acid observed as m/z 81 → 79 was used for quantification, and the product ion at m/z 63 corresponding to [PO₂]⁻ was selected for confirmation. The ion pairs used in this study were identical to those used in the previous reports.^{10,23,24}

2.4 Method validation

The method has been validated, including linearity, accuracy, precision, and matrix effect (ME). The linearity of the method was verified by analysing working and matrix-matching standards at concentrations ranging from 0.001 to 0.5 mg L⁻¹. Three sets of the calibration solutions were injected in the same sequence to evaluate the effects of the maize and soybean matrices. One set was prepared using water and two other sets using the maize and soybean blank extracts as matrix-matched standards. The ME was calculated according to the following eqn (1):

$$ME (\%) = \frac{slope_{matrix} - slope_{water}}{slope_{water}} \times 100\% \quad (1)$$

where slope_{matrix} and slope_{water} are the calibration curve slopes of the maize or soybean matrix and water standards, respectively.

The accuracy and precision of the method were determined by the recovery test and the relative standard deviation (RSD), which were performed on each matrix in 5 replicates at three different concentration levels: 0.05, 0.1, and 1 mg kg⁻¹ for chlormequat, fosetyl-aluminium, and phosphonic acid in maize and soybean. The intraday RSD (RSD_I) was determined on the same day and the interday RSD (RSD_R) was determined on three consecutive days.

3 Results and discussion

3.1 Optimization of the mobile phase

In this experiment, the effect of four different mobile phases B on the separation efficiency of three compounds was investigated. The peak area and retention time of the three compounds under different mobile phase B conditions are shown in Fig. 1. The experimental results show that when the mobile phase B is 200 mmol L⁻¹ of ammonium bicarbonate containing 0.05% of ammonium hydroxide, the response signal is high and the peak shape is symmetrical. With increasing ammonium bicarbonate concentration in the mobile phase B, the retention time of chlormequat increases, while the retention time of fosetyl-aluminium and phosphonic acid decreases. Therefore, 200 mmol L⁻¹ of the ammonium bicarbonate solution with 0.05% ammonium hydroxide was chosen as the mobile phase B. By gradient elution, a better chromatogram could be obtained and the analysis time was reduced. The use of organic solvents was avoided, which is very environmentally friendly. In previous studies, the instrumental analysis took 15 min,²⁵ 16 min,²² 17.5 min,¹⁶ 20 min,⁸ 24 min,²⁶ 25 min,²⁴ 30.5 min,²⁰ and 30.8 min.¹⁵ Martin Vahl *et al.*²⁵ have reported the detection of chlormequat residues using methanol and acetonitrile as the mobile phase, which is less environmentally friendly. This experiment can detect three compounds at the same time, the instrumental analysis time is 15 min, and no organic solvent is used. Our research methods are more efficient and environmentally friendly. Therefore, 200 mmol L⁻¹ of the ammonium bicarbonate solution containing 0.05% ammonium hydroxide was selected as the mobile phase B. By the gradient elution, a better chromatogram can be obtained, the analysis time is reduced, and the environment is improved.

3.2 Optimization of sample preparation

In previous studies, methanol,^{9,24,27,28} acetonitrile,^{23,29} and acetone¹² were usually used to extract chlormequat and fosetyl-aluminium. However, methanol is more toxic, and can pass through the digestive tract, respiratory tract, and skin to pose a threat to human health. Acetonitrile causes damage to the human liver and gastrointestinal function and affects female fertility, acetone affects nerve function in the human body. The extraction effects of seven different extractants were investigated on chlormequat, fosetyl-aluminium and phosphonic acid, namely ultrapure water (E1), methanol (E2), acetonitrile (E3), 1% aqueous formic acid solution (E4), 1% formic acid methanol



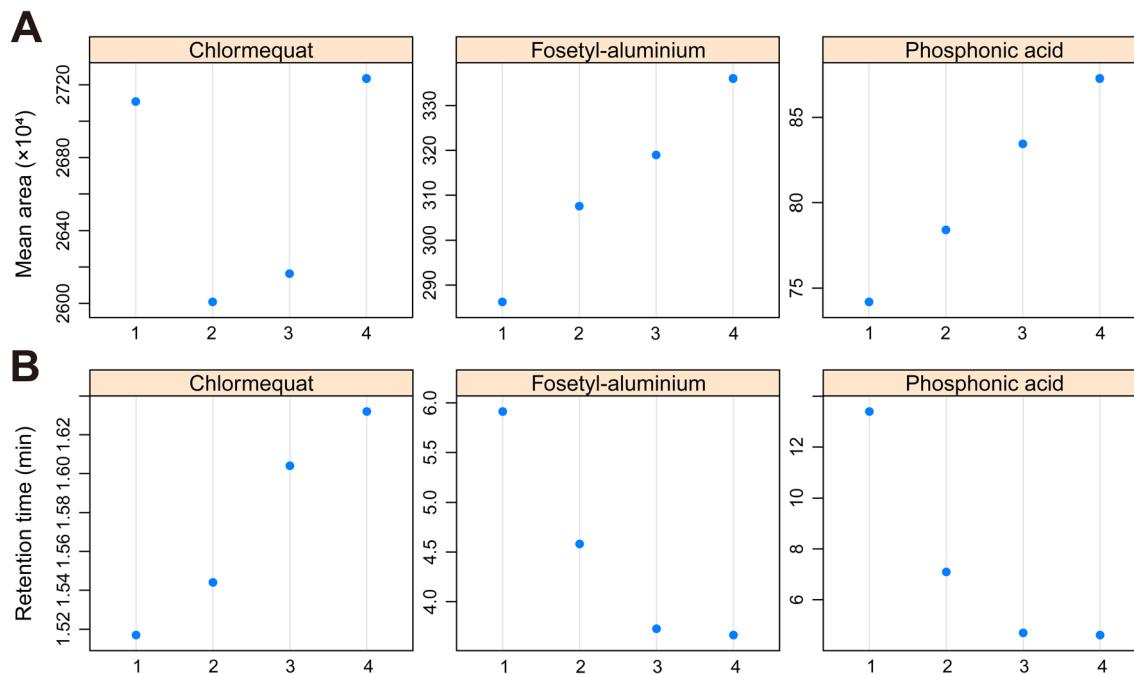


Fig. 1 The peak area (A) and retention time (B) of chlormequat, fosetyl-aluminium and phosphonic acid under different mobile phase conditions; the four mobile phases are as follows: 1 : 50 mmol L⁻¹ ammonium bicarbonate, 2 : 100 mmol L⁻¹ ammonium bicarbonate, 3 : 200 mmol L⁻¹ ammonium bicarbonate, and 4 : 200 mmol L⁻¹ ammonium bicarbonate with 0.05% ammonium hydroxide.

solution (E5), 0.5% formic acid methanol:water (50:50, v/v) solution (E6), and acetic acid solution (E7). The comparison of the extraction effects of the different extraction solvents is shown in Fig. 2. The ultrapure water (E1), 1% aqueous formic acid solution (E4), and acetic acid solution (E7) gave acceptable recoveries of analytes. It was found that the acetic acid solution had the best effect on the extraction of three compounds in maize and soybean. In maize, the extraction rates of acetic acid solution for chlormequat, fosetyl-aluminium, and phosphonic acid were 82%, 94.8%, and 86.1%. The RSD ranged from 1.2% to 11.1%. In soybeans, the extraction rates of the three compounds using acetic acid solution were 120.6%, 81.6%, and 114.6%. The RSD ranged from 4.7% to 7.8%. In addition, in order to better reduce the co-extraction of protein from the sample, aqueous acetic acid solution (pH ~ 4.5, close to the isoelectric point) was finally chosen as the extraction solvent.

3.3 Optimization of the purification SPE column

Three purification columns were selected to perform preliminary purification experiments on three compounds in maize and soybean, namely Oasis HLB, Oasis PRiME HLB, and ASC Accurasil HLB. The purification effect is shown in Fig. 3. The recoveries of compounds in maize were 88.0 to 96.4%, 82.8 to 89.4%, and 82.5 to 96.3% under 3 different HLB columns. The RSDs were 2.0 to 4.5%, 2.5 to 5.8%, and 4.3 to 5.9%. The recoveries of compounds in soybeans were 70.3 to 95.6%, 70.0 to 90.0%, and 70.2 to 94.3% under 3 different HLB columns. The RSDs were 2.0 to 5.1%, 4.4 to 5.9%, and 2.5 to 7.6%. The results show that Oasis HLB (Waters) has a better recovery. It can effectively remove the interference from pigments and proteins in maize and soybeans.

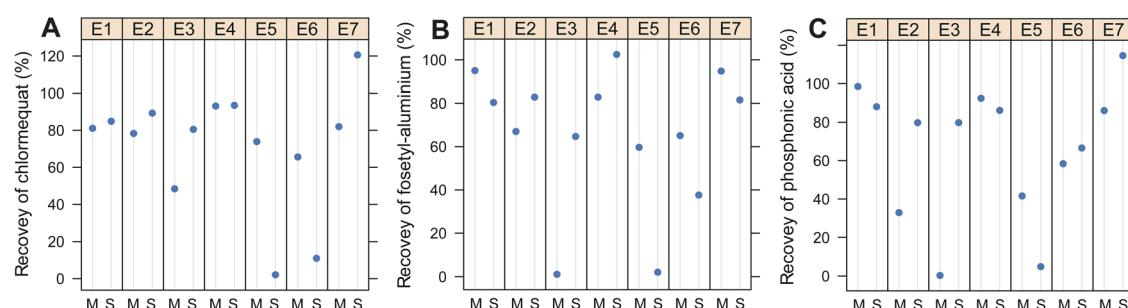


Fig. 2 The extraction effects of chlormequat (A), fosetyl-aluminium (B) and phosphonic acid (C) in maize and soybean; letter M and S stand for maize and soybean, respectively. The seven extractants are as follows: E1 (ultrapure water), E2 (methanol), E3 (acetonitrile), E4 (1% formic acid solution), E5 (1% formic acid in methanol), E6 (0.5% formic acid in methanol:water 1:1 solution) and E7 (acetic acid solution).

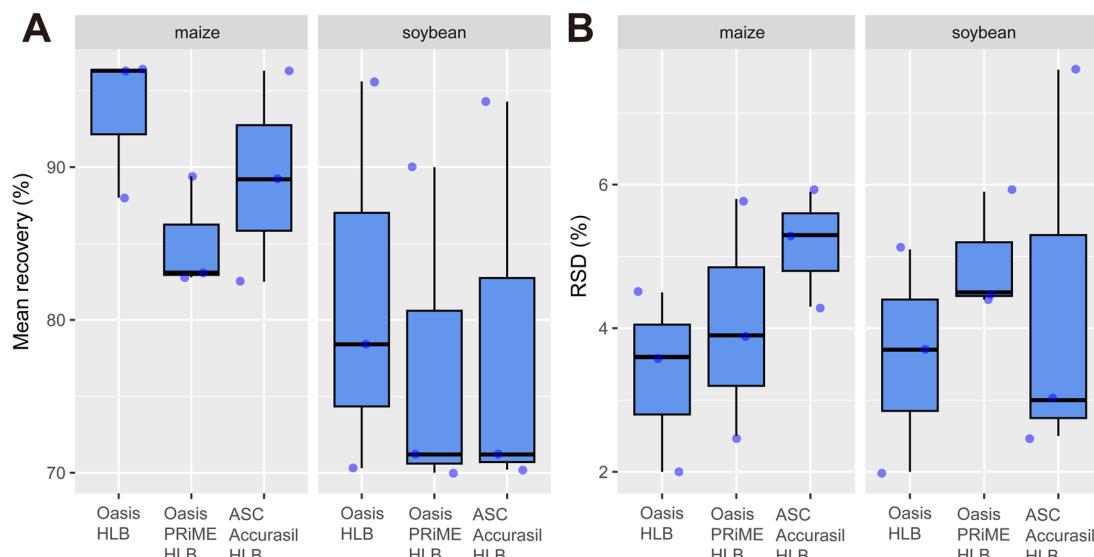


Fig. 3 Mean recoveries (A) and RSDs (B) of the three compounds from maize and soybean using Oasis HLB (Waters), Oasis PRIME HLB (Waters) and ASC Accurasil HLB (Ameritech).

3.4 Linear range and limit of quantification

Blank maize and soybean samples were analysed to evaluate the specificity of the method. No interferences were observed at the RT of each analyte. The correlation coefficient ($R \geq 0.997$) showed good linear regressions for all analytes in water and in maize and soybean matrices (Table 2). All calibration curves ranged from 0.001 to 0.5 mg L⁻¹ for each analyte with a wide dynamic range. Co-elution from the sample solution may affect the response of the analytes to suppression or enhancement, known as the ME. The ME was assessed by comparing standards dissolved in aqueous solvent alone with those dissolved in blank sample matrices. A significant ion suppression was observed from -56.9% to -40.4% for maize and from -84.6% to -71.9% for soybean. To eliminate the ME, the matrix-matched standards were used in the present study. The limit of quantification (LOQ) and detection (LOD) of the method in different matrices were 0.01 and 0.003 mg kg⁻¹, respectively.

3.5 Method performance

Two grams of maize and soybean samples were weighed and mixed with standard solutions of chlormequat, fosetyl-

aluminium and phosphonic acid with spiked mass fractions of 0.05, 0.1, and 1 mg kg⁻¹, respectively. The standard recovery experiment was carried out. Samples were treated and assayed according to the above conditions, three days were selected to measure each treatment. The results are presented in Table 3. The recoveries at 0.05, 0.1, and 1 mg kg⁻¹ for chlormequat, fosetyl-aluminium, and phosphonic acid were 70.3% to 120.4% and RSDs were 1.1% to 15.1%, respectively. It shows that the method has high accuracy, high sensitivity, good generality, and acceptable recovery. These results were satisfactory, meeting the requirements of the SANTE/11312/2021 guideline (mean recovery between 70 and 120% with RSD less than 20%).³⁰

3.6 Application of the method

Two samples of green maize and two samples of mature maize were taken from Shanxi Province and Hainan Province, and one sample of green soybean and one sample of the mature soybean were taken from Henan Province and Hainan Province in China (12 samples in total). The established method was then used to detect residues of chlormequat, fosetyl-aluminium and phosphoric acid in the samples (Table 4). Chlormequat was not detected in green and mature maize; however, fosetyl-

Table 2 Calibration curve and matrix effect (ME) for the analytes

Matrix	Chemical	Equation	R	ME (%)
Maize	Chlormequat	$y = 27\ 417.1x + 5963.2$	0.9999	-56.9
	Fosetyl-aluminium	$y = 14\ 335.7x + 12\ 665.0$	0.9992	-40.4
	Phosphonic acid	$y = 1600.6x + 6471.7$	0.9992	-52.1
Soybean	Chlormequat	$y = 9800.4x + 24\ 172.5$	0.9995	-84.6
	Fosetyl-aluminium	$y = 6763.1x + 9205.4$	0.9995	-71.9
	Phosphonic acid	$y = 912.2x + 7869.9$	0.9994	-72.7
Water	Chlormequat	$y = 63\ 570.2x - 5913.3$	0.9978	
	Fosetyl-aluminium	$y = 24\ 045.3x + 4123.6$	0.9981	
	Phosphonic acid	$y = 3344.8x + 10\ 606.6$	0.9976	

Table 3 Mean recoveries (%) and RSDs (%)

Matrix	Compound	Level, mg kg ⁻¹	Day 1 (N = 5)		Day 2 (N = 5)		Day 3 (N = 5)		3 Days (N = 15)	
			Recovery%	RSD _R %	Recovery%	RSD _R %	Recovery%	RSD _R %	Recovery%	RSD _R %
Maize	Chlormequat	0.05	97.9	6.2	92.9	5.1	92.2	3.1	94.3	5.5
		0.1	88.6	4.2	86.2	1.7	87.4	1.1	87.4	2.7
		1	88	2	86.3	3.4	86.3	3.4	86.8	2.9
	Fosetyl-aluminium	0.05	90.6	9.3	92.1	6.4	91.8	6.5	91.5	7
		0.1	92	5.2	84.4	1.9	84.4	1.9	87	5.4
		1	96.4	3.5	93.9	3.1	93.9	3.1	94.7	3.3
	Phosphonic acid	0.05	104.2	10.7	93.1	15	112.1	15.1	103.2	14.9
		0.1	90.2	7.5	99	3.4	99	3.4	96.1	6.5
		1	102.1	2.7	97.5	4.1	97.5	4.1	99	4
Soybean	Chlormequat	0.05	84.4	3.7	86.3	7.6	84.2	7.4	85	6.1
		0.1	80.1	5.2	86.3	4.2	78.7	3.8	81.7	5.9
		1	78.5	5.1	80.3	4.9	78.9	4.1	79.2	4.4
	Fosetyl-aluminium	0.05	97	5.5	80.3	9.2	100.9	9.5	92.7	12.5
		0.1	95.9	5.1	80.7	9.4	79.1	4.8	85.2	11
		1	70.3	3.7	76.6	5.6	77.2	4.1	74.7	6
	Phosphonic acid	0.05	109.5	14.5	101.9	4.3	107.8	6.9	106.4	9.6
		0.1	106.8	6.6	101.3	9.4	120.4	2.4	109.5	9.6
		1	105.6	6.2	100	5.5	107.7	5.4	104.4	6.1

Table 4 Residues of analytes in maize and soybean from the different provinces of China

No.	Province	Matrix	Chlormequat, mg kg ⁻¹	Fosetyl-aluminium, mg kg ⁻¹	Phosphonic acid, mg kg ⁻¹
1	Shanxi	Green maize	<0.01	<0.01	0.025
2	Shanxi	Mature maize	<0.01	<0.01	0.025
3	Hainan	Green maize	<0.01	<0.01	0.018
4	Hainan	Mature maize	<0.01	<0.01	0.024
5	Shanxi	Green maize	<0.01	0.048	0.032
6	Shanxi	Mature maize	<0.01	<0.01	<0.01
7	Hainan	Green maize	<0.01	<0.01	0.03
8	Hainan	Mature maize	<0.01	0.015	0.039
9	Henan	Green soybean	0.012	0.013	<0.01
10	Henan	Mature soybean	0.012	<0.01	0.025
11	Hainan	Green soybean	<0.01	<0.01	<0.01
12	Hainan	Mature soybean	0.013	<0.01	2.451

aluminium was detected in the range 0.015–0.048 mg kg⁻¹ and phosphoric acid in the range 0.018–0.039 mg kg⁻¹. All three compounds were detected in soybean samples at the levels of 0.012–0.013 mg kg⁻¹, 0.013 mg kg⁻¹ and 0.025–2.451 mg kg⁻¹, respectively.

4 Conclusions

A method was developed for the determination of residues of chlormequat, fosetyl-aluminium, and phosphonic acid in maize and soybean. The mobile phase was optimised. The mobile phase A was water, and the mobile phase B was 200 mmol L⁻¹ ammonium bicarbonate solution containing 0.05% ammonium hydroxide. All the analytes were eluted within 15 minutes and the peak shape was good. The extraction solvent was optimised, and aqueous acetic acid solution was selected as the extraction agent, the extraction yields of the three compounds in maize

and soybean were 81.6–120.6% and the RSDs were 1.2–11.1%. Oasis HLB (Waters) was selected to optimise the clean-up column. The mean recoveries of the three compounds in maize and soybean under the same purification conditions were 88.0 to 96.4% and 70.3 to 95.6% and the RSDs were 2.0 to 4.5% and 2.0 to 5.1%. The purification effect was good and the impurities could be effectively removed. Using the method of this experiment, the recoveries of chlormequat, fosetyl-aluminium, and phosphonic acid at three spiking levels of 0.05, 0.1, and 1 mg kg⁻¹ were between 70.3% and 120.4%, and the RSDs were between 1.1% and 15.1%. The sensitivity, recovery, and precision of the method can meet the requirements of pesticide residue analysis and is easy to use. This method provides an accurate and reliable pretreatment method and detection method for the determination of chlormequat, fosetyl-aluminium, and phosphonic acid residues in maize and soybean, which is more economical and reliable than



conventional detection methods and can be used as a determination and confirmation method for chlormequat, fosetyl-aluminium, and phosphonic acid in cereals and oilseeds.

Author contributions

Hao Yang: data curation, formal analysis, investigation, validation, visualization, writing – original draft. Yue Geng: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, supervision, writing – review and editing. Shu Lin: investigation. Lu Wang: investigation, resources. Yi Peng: data curation, investigation. Yaping Xu: resources. Jingran Zhang: investigation, validation. Xiaowei Liu: resources, supervision.

Conflicts of interest

There are no conflicts to declare.

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References

- 1 X. Ma, J. Zhao, Z. Yu and J. Li, *J. Maize Sci.*, 2023, **31**, 54–58.
- 2 Z. Wang, M. Yuan, D. Han, D. Zhang, H. Sun, Z. Zhu, L. Wang, H. Gao, Z. Tang and B. Gao, *Soybean Sci. Technol.*, 2023, 6–9.
- 3 L. Mo, B. Yuan, J. Zhu, L. Qin and J. Dai, *Chin. J. Struct. Chem.*, 2022, **41**, 166–177.
- 4 S. Chen, H. Zhao, L. Jiao, Z. Wang, M. Zhao, L. Tian, Y. Xiu and S. Liu, *Chin. Chem. Lett.*, 2023, **34**, 107286.
- 5 X. Guo, Y. Xu, F. Zhang, S. Yu, L. Han and S. Jiang, *Ecotoxicol. Environ. Saf.*, 2010, **73**, 642–646.
- 6 H. Karasali, K. M. Kasiotis and K. Machera, *Chem. Pap.*, 2014, **68**, 725–731.
- 7 Z. Yu, F. Jin, J. Hu, X. Zhang, J. Sun and M. Yang, *Anal. Chim. Acta*, 2010, **678**, 90–95.
- 8 M. E. Poulsen, H. B. Christensen, M. T. Sorensen, H. Leffers and J. H. Andersen, *Anal. Bioanal. Chem.*, 2007, **389**, 1799–1804.
- 9 H. Zhang, Z. Ma, H. Yang and L. Kong, *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.*, 2017, **1064**, 75–84.
- 10 X. Esparza, E. Moyano and M. T. Galceran, *J. Chromatogr. A*, 2009, **1216**, 4402–4406.
- 11 S. Riediker, H. Obrist, N. Varga and R. H. Stadler, *J. Chromatogr. A*, 2002, **966**, 15–23.
- 12 J. Zhang and P. Du, *J. Food Saf. Food Qual.*, 2014, **5**, 3684–3690.
- 13 L. Lu, X. Luo, Y. Wang and J. Hao, *J. Instrum. Anal.*, 2011, **30**, 321–325.
- 14 H. Sun, Y. Yang, G. Xu, A. Cao, X. Si, Y. Wang, C. Wang, Y. Li, J. Xu and Q. Yang, *Chin. Tobac. Sci.*, 2010, **31**, 59–62.
- 15 C. Gou, Q. Ge, Y. Liu and R. Jiang, *J. Anhui Agric. Sci.*, 2014, 1686–1687.
- 16 N. Chamkasem, *J. Agric. Food Chem.*, 2017, **65**, 7535–7541.
- 17 X. Zhang, C. Huang, H. Zhang, F. Xi, M. Lv, Q. Wang, B. Wang, C. Song, Y. Wang and L. Wang, *China Food Addit.*, 2021, **32**, 51–56.
- 18 Z. Fan, N. Ding, W. Zhao, S. Jia and S. Wang, *Chin. J. Anal. Lab.*, 2009, **28**, 113–115.
- 19 Z. Fan, N. Ding, W. Zhao, S. Jia and S. Wang, *Chin. J. Chromatogr.*, 2009, **27**, 849–851.
- 20 L. Sun, Z. Chi, X. Liu and X. Yu, *Agrochemicals*, 2009, **48**, 747–748+759.
- 21 J. Z. Francesquett, T. M. Rizzetti, T. J. Cadaval, O. D. Prestes, M. B. Adaime and R. Zanella, *J. Chromatogr. A*, 2019, **1592**, 101–111.
- 22 A. Bauer, J. Luetjohann, S. Rohn, J. Kuballa and E. Jantzen, *Food Anal. Methods*, 2018, **11**, 2237–2243.
- 23 J. Gao, J. Wang, M. Zuo, L. Ma, Y. Cui, T. Yang and M. Ding, *RSC Adv.*, 2015, **5**, 5895–5903.
- 24 A. Bauer, J. Luetjohann, S. Rohn, J. Kuballa and E. Jantzen, *J. Agric. Food Chem.*, 2018, **66**, 346–350.
- 25 M. Vahl, A. Graven and R. K. Juhler, *Fresenius' J. Anal. Chem.*, 1998, **361**, 817–820.
- 26 S. Marchese, D. Perret, E. Bafile, A. Gentili, F. Caretti and M. Berardino, *Chromatographia*, 2009, **70**, 761–767.
- 27 T. Henriksen, R. K. Juhler, G. Brandt and J. Kjær, *J. Chromatogr. A*, 2009, **1216**, 2504–2510.
- 28 Y. Zhao, K. Lazou, M. Schelfaut, L. De Reu and P. Sandra, *Chromatographia*, 2000, **51**, 531–535.
- 29 B. Han, Y. He, B. Lin, H. Huang, L. Chen and L. Xu, *Chin. J. Pestic. Sci.*, 2017, **19**, 403–408.
- 30 E. Commission, *Guidance Document on Analytical Quality Control and Method Validation Procedures for Pesticide Residues and Analysis in Food and Feed*, SANTE/11312/2021, 2021.

