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Determination of 31 pesticide residues in wolfberry by LC-MS/MS and dietary risk assessment of wolfberry consumption

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Abstract

A modified QuEChERS method coupled with LC-MS/MS was developed and validated to detect 31 pesticides in wolfberry. The conditions for extraction solvent and QuEChERS purification were optimized. The validated method was applied to analyse pesticides in 200 wolfberry samples. The risk from chronic and acute dietary intake of the detected pesticide residues was assessed by the acceptable daily intake (*ADI*) and acute reference dose (*ARfD*), respectively, and the cumulative intake risk posed by the detected residues was assessed by the hazard index (*HI*). The results showed that 23 pesticides were detected in all wolfberry samples. The risk from chronic dietary intake was between 0.0001% and 1.6067%, and the risk from acute dietary intake was between 0.0010% and 0.4999%, which were all far below 100%. The *HI* was 0.02569 for chronic dietary intake and 0.015164 for acute dietary intake, which were both far below 1. The results indicated that the pesticide residues in wolfberry would not cause potential risk to human health. This work not only enhances our understanding of the potential exposure risks of pesticide residues in wolfberry, but also provides an effective method for the risk assessment of pesticide residues in other agricultural products.

Keywords: wolfberry; pesticide residues; modified QuEChERS; LC-MS/MS; risk assessment.

Practical Application: In China, wolfberry is considered as medicinal and edible plants, which are popular among consumers for their ability to improve human body function. However, due to the long planting history, the occurrence of diseases and insect pests is more and more serious, and the use of pesticides to control diseases and insect pests brings risk to the consumption of wolfberry. In this study, an LC-MS/MS method was established for the determination of 31 pesticide residues in wolfberry, and the pesticide residues of wolfberry in northwest China were determined, and dietary risk assessment was carried out. This has important significance to the safe consumption of wolfberry in the future. At the same time, it also provides a reference for the use of pesticides in the planting process of this plant.

1 Introduction

Wolfberry (*Lycium barbarum* L.) is a perennial deciduous shrub with ellipsoid orange-red berries (Zhao et al., 2015). It is a *Solanaceae* plant mainly found in Northwest China, including Xinjiang Province, Qinghai Province, Ningxia Province, Gansu Province and other provinces. Wolfberry has a long history in China and has been used for medicine and functional food, and it is listed in the Traditional Chinese Pharmacopeia (TCP) (Amagase & Farnsworth, 2011; Lu et al., 2014). To a certain extent, wolfberry has been one of the most important products exported from these provinces and has provided a significant contribution to the local economy (Ali et al., 2019).Wolfberry dried fruit contains many nutrients, such as polysaccharide, phenolic acid, carotene, betaine and flavonoids (Donno et al., 2016; Qian, 2004; Wang et al., 2010), which possesses many advantages, such as antioxidation, antiradiation, anticancer, anti-ageing characteristics, enhancement of haemopoiesis, brightening of the eyes, etc., which has increased its popularity among consumers (Chiu et al., 2010; Gan et al., 2004; Luo et al., 2004; Zhou et al., 2017). Therefore, wolfberry, as a kind of food with great health benefits, is not only loved by consumers in China and Southeast Asia but also consumed in European and American markets, which is expanding year by year (Potterat, 2010). However, due to its high sugar content, wolfberry is vulnerable to aphids, psyllids, gall mites and other pests and also susceptible to root rot, anthracnose and powdery mildew (Chawla et al., 2017).

In recent years, pesticide residues in wolfberry have become the focus of consumers' attention. To ensure the production and quality of wolfberry, insecticides, acaricides and fungicides are used in the process of wolfberry cultivation. Thus, a large amount of pesticide residue is contained in wolfberry, which represents hidden safety risks to consumers' human health (Huang et al., 2012). Therefore, carrying out risk assessments of pesticide residues in wolfberry is an important means to control the quality and safety of wolfberry and has important significance for protecting consumer health.

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The QuEChERS (quick, easy, cheap, effective, rugged and safe) method has been broadly applied for the analysis of pesticide multi-residues in fruits and vegetables because of its simplicity, low cost, speed and broad applicability to a wide range of analytes (Anastassiades et al., 2003). At present, there are few methods for simultaneous determination of pesticide residues in wolfberry by LC-MS/MS (Chen et al., 2019). In particular, there are a few studies on the risk assessment of pesticide residues in wolfberry. Liu et al. (2015) performed risk evaluations of the main pests and integrated management of Chinese Wolfberry. Li et al. (2020) performed a dissipation study and dietary risk assessment of dinotefuran, DN, and UF in wolfberry. Qin et al. (2020) determined pesticide residues of pyrethrins in *Lyciumbarbarum* (goji) by GC-MS/MS and analysed a dietary risk assessment of Chinese goji consumption. Fu et al. (2017) studied eight pesticides in *Lyciumbarbarum* by LC-MS/MS and analysed a dietary risk assessment. The above work mainly included the risk assessment of major diseases in wolfberry and risk assessment of a small part of pesticides, but there were no reports on the risk assessment of pesticide residues, such as triazole, carbendazine and amidine, which were often used in the cultivation process of wolfberry.

The main purpose of this work is to establish a modified QuEChERS method coupled with LC-MS/MS for the determination of 31 pesticide residues in wolfberry, and analyse the residual levels of 31 pesticides in these wolfberry samples from Northwest China. In addition, we also carried out chronic dietary intake risk assessment, acute dietary intake risk assessment and cumulative risk assessment of pesticides present in the wolfberry samples to assess the potential risks caused by human exposure to these pesticides. This study would establish a method for pesticide multi-residues analysis in wolfberry, and also support the risk managers for developing monitoring programs and further guide the rational use of pesticides.

2 Materials and methods

2.1 Chemicals, reagents and standards

Deionized water (> 18.2 MΩ) was prepared with a Milli-Q water purification system (Millipore Corp., USA). HPLC-grade formic acid, acetone, n-hexane and acetonitrile were purchased from Merck Co. (Darmstadt, Germany). Primary secondary amine (PSA), octadecylsilane (C18) and graphitized carbon black (GCB) for use as absorbents were all provided by CNW Technologies GmbH (Düsseldorf, Germany). Analytical reagent-grade sodium chloride (NaCl) and anhydrous magnesium sulfate ($MgSO_4$) were supplied by Tianjin Kemiou Chemical Reagent Co., Ltd. (Tianjin, China).

Standards of 31 pesticides were purchased from Dr.Ehrenstorfer (Augsburg, Germany), all with purity >98%. Individual pesticide stock solutions (1000 mg/L) were prepared in acetonitrile for LC-MS/MS analysis. All individual standard stock solutions were kept in a refrigerator at -20 °C. For optimization and calibration, working solutions were prepared daily by appropriate dilution of the stock standard solutions, which were kept at −4 °C. For optimization of the ion source parameters for LC-MS/MS, individual standard solutions of each pesticide were prepared at 10 μg/mL in acetonitrile. For the calibration studies, working standard solution mixtures were prepared at different concentrations in ACN:H_2O (10:90, *v*/*v*, containing 0.1% FA) for LC-MS/MS.

2.2 Samples

A total of 200 samples of dried wolfberry fruit were collected from the main cultivation areas in Xinjiang Province and Qinghai Province in Northwest China: 100 samples from Xinjiang and 100 samples from Qinghai. The samples were stored in sealed bags, refrigerated, transported to the laboratory, and stored at -20 °C until preparation and analysis.

2.3 Sample preparation

Wolfberry (2.00 g) was accurately weighed and transferred into a 50-mL plastic centrifuge tube. Then, 5 mL of water and 10 mL of acetonitrile were added sequentially to the tube. The mixture was vortexed for 30 s (Vortex Genie 2 vortex mixer, Scientific Industries Inc., USA) and extracted by ultrasonication for 15 min (KQ-1000 ultrasonic cleaner, Shanghai Baidian Instrument Factory, China). Then, 1.0 g of NaCl was added to the tube, and the mixture was vortexed for 1 min and then centrifuged for 5 min at 2500 g (3-30 K high-speed centrifuge, Sigma Laborzentrifugen GmbH, Germany). Five millilitres of the extract was transferred into a 15-mL plastic high-speed centrifuge tube preloaded with 400 mg C18, 400 mg PSA, 45 mg GCB and 1200 mg $MgSO_4$. The above mixture was vortexed for 1 min and centrifuged for 3 min at 10000g. Then, 1.0 mL of supernatant each was transferred into two clean 10-mL glass colorimetric tubes and dried under flowing nitrogen gas in a 40 °C water bath (nitrogen evaporator, Organomation Co., USA). Sample was redissolved in 1.0 mL of acetonitrile-0.1% FA water (10:90, *v*/*v*), filtered through a 0.22-μm organic filter membrane, and analysed by LC-MS/MS. If the pesticide concentrations in the wolfberry samples exceeded the linear range, the solutions were appropriately diluted with the corresponding solvent.

2.4 LC-MS/MS analysis

A UPLC-MS/MS system consisting of a Waters ACQUITY UPLC unit and a Xevo TQ-S mass spectrometer (Waters Co., USA) was used for the separation and quantitation of 25 pesticides. Chromatographic separation was performed on a BEH C18 analytical column (100 mm × 2.1 mm, 1.7 μm, Waters Co., USA), and the column temperature was maintained at 30 °C. The flow rate was maintained at 0.3 mL/min, and the injection volume was 5 μL. The mobile phase consisted of water (containing 0.1% FA, *v*/*v*) and acetonitrile. The following linear gradient elution procedure was adopted for separation of the 31 pesticides: 0.0-2.0 min, 10-50% acetonitrile; 2.0-2.1 min, 50-90% acetonitrile; 2.1-4.0 min, 90% acetonitrile; and 4.0-5.0 min, 90-10% acetonitrile.

The mass spectrometer contained a Z-spray electron spray ionization (ESI) source. The ion source parameters were as follows: positive mode, capillary voltage of 3.20 kV, source temperature of 150 °C, desolvation temperature of 400 °C, desolvation gas flow rate of 800 L/h, cone gas flow rate of 50 L/h and collision gas (Ar)

flow rate of 0.20 mL/min. The cone voltage (CV), parent ions, collision energy (CE) and fragment ions were optimized for each pesticide using MassLynxIntelliStar software. The 31 pesticides were analysed in multiple reaction monitoring (MRM) mode. Data acquisition and processing were accomplished using MassLynx TM 4.1 software.

2.5 Chronic risk assessment

The risk posed by the chronic dietary intake of each pesticide in wolfberry was calculated as the acceptable daily intake percentage (%*ADI*). The smaller the %*ADI* value is, the lower the risk. %*ADI*≤100% indicates acceptable risk; and a value above 100% indicates unacceptable risk. Formula 1 presents the %*ADI* calculation.

$$ADI\% = \frac{STMR \times P}{ADI \times \mathrm{bw}} \times 100 \quad (1)$$

Where *STMR* (mg/kg) is the supervised trial median residue, *ADI* (mg/kg) is the acceptable daily intake, *P* (kg) is the daily consumption of wolfberry for residents calculated based on the dry fruit consumption of wolfberry recommended by the Chinese Pharmacopoeia of 0.012 kg per day for adults, and *bw*(kg) is the human body weight (assumed to be 60 kg for an adult).

2.6 Acute risk assessment

The risk posed by acute dietary intake of each pesticide in wolfberry was calculated as the acute reference dose percentage (%*ARfD*). The smaller the %*ARfD* value is the lower the risk. %*ARfD*≤100% indicates acceptable risk; and a value above 100% indicates unacceptable risk. %*ARfD* and the safety margin (*SM*) of each pesticide were calculated by Formulas 2 and 3, respectively.

$$\%ARfD = \frac{HR \times P}{ARfD \times bw} \times 100 \quad (2)$$

$$SM = \frac{ARfD \times bw}{P} \quad (3)$$

Where HR (mg/kg) is the highest residue concentration and *ARfD* (mg/kg) is the acute reference dose.

2.7 Cumulative risk assessment

The cumulative risk assessment of each pesticide in wolfberry was carried out on the basis of the hazard index (*HI*). The *HI* is the sum of the hazard quotient (*HQ*) of each chemical, as shown in Formula 6. *HQ* is the ratio of the exposure (*EXP*) to reference (*RV*) values. The calculation of acute dietary exposure (*EXP a*) and chronic dietary exposure (*EXP c*) is shown in Formulas 4 and 5, respectively. When *HI* is less than 1, the cumulative exposure risk is acceptable; otherwise, the risk is unacceptable.

$$EXP\mathrm{a} = \frac{HR \times P}{bw} \quad (4)$$

$$EXPc = \frac{STMR \times P}{bw} \quad (5)$$

$$HI = \sum_{i-1}^{n} HQi = \frac{EXP}{RV} \quad (6)$$

The *ADI* is the reference value for chronic toxicity, and *ARfD* is the reference value for acute toxicity. Referring to the maximum daily consumption value of 97.5% proposed in the global environmental monitoring system/food pollution monitoring and assessment plan (GEMS/Food), the value of *P* in wolfberry in this study was 10.7 g/kg.

3 Results and discussion

3.1 Optimization of the extraction solvent

The target pesticides covered by this study mainly include 31 pesticides, including organophosphate, benzimidazole, carbamate, triazole, avermectin and methylcarbamate. To minimize the interference of the co-extracted materials and improve the extraction efficiency of the 31 pesticides, the extraction solvent was investigated. In this study, acetonitrile and ethyl acetate as the extraction solvents were compared. The results indicated that the recoveries of all the pesticides extracted with in acetonitrile were satisfactory and ranged between 80.4% and 110.4%, and the recoveries of all the pesticides extracted with in ethyl acetate were ranged between 68.2% and 114.6%. As shown in Figure 1, the extraction efficiencies for the 31 pesticides with acetonitrile as the extraction solvent were better than those obtained with ethyl acetate. acetonitrile extraction is commonly and widely applied in QuEChERS method since it leads to less interference, such as lipophilic compounds, waxes, lipids, and pigments, and other extract solvents, such as acetone and ethyl acetate (Anastassiades et al., 2003).

3.2 Optimization of the QuEChERS purification

After the wolfberry samples were extracted in acetonitrile, the extraction solution was not qualified for LC-MS/MS analysis due to a large amount of interference and need for purification (Oshita & Jardim, 2014). QuEChERS purification techniques have been widely applied in the agricultural products and food detection fields. Some sorbents, such as C18, PSA and GCB, are commonly employed in QuEChERS procedures. The purification efficiencies of different absorbents were evaluated in this study. Stock solutions were diluted with blank wolfberry extract to prepare a mixed standard working solution at a certain concentration. Two millilitres of the upper acetonitrile solution was accurately transferred into a 10 mL centrifuge tube pre-loaded with mixtures in different proportions of C18 adsorbent, PSA adsorbent and GCB adsorbent (nine levels). Then, the extraction solutions were vortexed and centrifuged. Next, 1 mL of the supernatant was transferred to a glass centrifuge tube and dried under flowing nitrogen in a 40 °C water-bath. Subsequently, the residue was redissolved 1.0 mL of 10% acetonitrile-0.1% FA in water (*v/v*) and filtered through a 0.22-μm organic filter membrane. Finally, the supernatant was determined by LC-MS/MS, and the recovery was calculated. The results are shown in Table 1. When 400 mg C18, 400 mg PSA, and 45 mg GCB are added, the purification efficiencies are best for the 31 pesticides. The purification efficiencies with the 400 mg adsorbents (C18 and PSA) were higher than

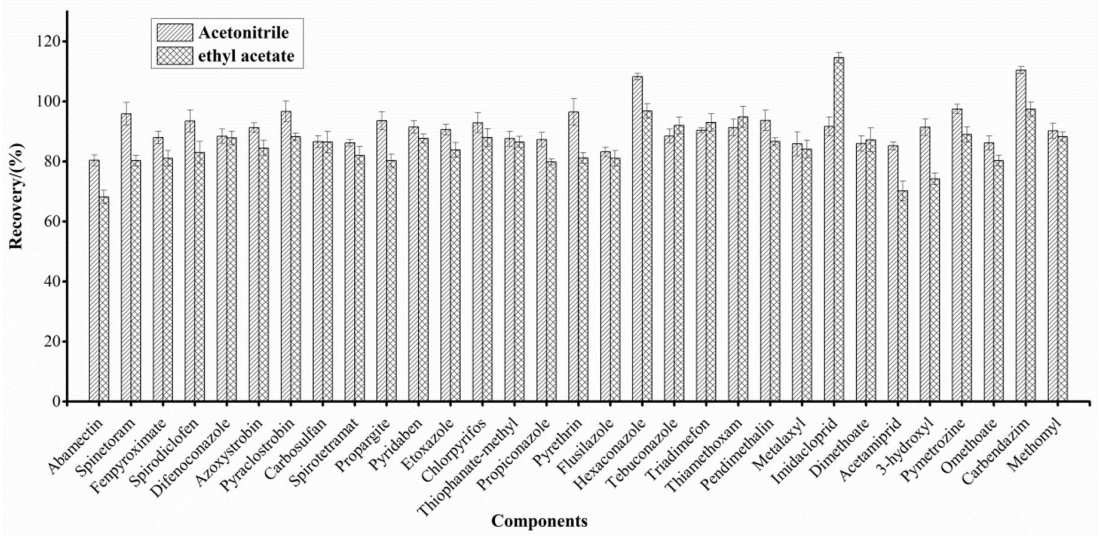

Figure 1. Comparison of extraction efficiency of different extraction solvents.

Table 1. Recoveries of 31 pesticides purified with different absorbent types and dosages (Spiked 20 μg/kg, n = 3).

Components	Recoveries (mean± SD, %)								
	A	B	C	D	E	F	G	H	I
Abamectin	77.6 ± 0.8	79.7 ± 1.3	74.1 ± 1.7	81.3 ± 1.2	83.5 ± 1.5	82.2 ± 1.6	64.3 ± 1.9	67.5 ± 2.6	72.2 ± 2.4
Spinetoram	80.1 ± 1.5	84.3 ± 1.0	86.2 ± 1.9	90.1 ± 0.9	94.3 ± 1.7	92.2 ± 0.9	77.1 ± 1.3	81.3 ± 2.1	78.2 ± 1.7
Fenpyroximate	81.8 ± 3.4	83.3 ± 1.5	81.5 ± 1.6	85.4 ± 1.4	93.7 ± 1.4	89.9 ± 1.5	73.5 ± 1.2	80.2 ± 1.7	75.9 ± 3.3
Spirodiclofen	80.7 ± 0.7	82.8 ± 2.6	80.4 ± 1.1	82.6 ± 1.5	86.4 ± 0.9	87.2 ± 1.2	77.9 ± 2.5	81.3 ± 1.3	74.8 ± 1.2
Difenoconazole	86.0 ± 0.8	88.4 ± 1.1	83.2 ± 0.7	90.2 ± 1.5	94.3 ± 0.5	91.8 ± 0.9	63.2 ± 3.9	66.1 ± 2.8	62.1 ± 2.7
Azoxystrobin	86.7 ± 1.6	89.4 ± 1.7	87.9 ± 0.9	89.3 ± 0.5	101.4 ± 1.1	95.3 ± 1.3	81.3 ± 2.3	84.7 ± 1.5	83.5 ± 1.3
Pyraclostrobin	80.2 ± 2.7	85.2 ± 0.5	80.1 ± 1.1	84.1 ± 1.0	93.6 ± 2.3	86.7 ± 1.0	72.7 ± 3.5	82.3 ± 1.8	77.6 ± 2.9
Carbosulfan	71.4 ± 2.2	78.3 ± 3.5	71.0 ± 1.5	75.4 ± 3.4	85.3 ± 0.8	87.0 ± 0.7	58.3 ± 4.6	73.6 ± 3.3	60.9 ± 2.4
Spirotetramat	78.5 ± 0.9	85.1 ± 0.7	87.9 ± 1.9	92.2 ± 2.0	95.5 ± 0.9	94.3 ± 2.4	77.2 ± 0.9	73.2 ± 1.2	75.8 ± 2.9
Propargite	78.3 ± 1.1	89.5 ± 2.1	87.1 ± 1.0	84.8 ± 0.6	104.5 ± 0.4	89.1 ± 0.6	73.5 ± 1.5	75.8 ± 0.9	71.8 ± 3.3
Pyridaben	83.6 ± 0.5	82.1 ± 1.2	80.9 ± 0.8	87.3 ± 1.1	92.7 ± 1.0	89.7 ± 0.2	81.2 ± 1.1	81.0 ± 2.4	79.8 ± 3.0
Etoxazole	79.5 ± 0.7	90.6 ± 1.1	89.6 ± 0.9	83.7 ± 1.0	95.6 ± 0.7	94.2 ± 1.0	76.3 ± 4.1	78.5 ± 2.5	72.4 ± 1.7
Chlorpyrifos	82.6 ± 1.4	82.8 ± 0.8	82.9 ± 1.4	86.2 ± 0.7	93.3 ± 1.2	90.2 ± 1.5	80.8 ± 1.7	81.9 ± 1.0	79.7 ± 3.0
Thiophanate-methyl	78.7 ± 1.0	81.9 ± 2.3	79.0 ± 0.7	84.4 ± 2.2	89.8 ± 0.5	85.7 ± 1.0	79.9 ± 1.8	77.0 ± 3.6	75.7 ± 2.2
Propiconazole	86.7 ± 1.7	85.2 ± 0.8	85.9 ± 1.1	90.5 ± 0.9	95.7 ± 0.8	106.5 ± 1.4	73.2 ± 0.7	73.9 ± 2.6	73.7 ± 1.4
Pyrethrin	81.2 ± 0.5	82.5 ± 0.6	82.4 ± 1.5	85.2 ± 1.3	95.3 ± 1.3	92.1 ± 0.7	58.4 ± 5.7	70.4 ± 2.7	70.5 ± 3.3
Flusilazole	78.3 ± 1.1	79.1 ± 1.1	77.7 ± 1.2	83.6 ± 1.1	90.2 ± 0.9	88.4 ± 0.9	75.3 ± 0.9	77.1 ± 2.9	75.7 ± 1.3
Hexaconazole	80.7 ± 0.6	82.3 ± 1.5	86.9 ± 1.7	87.4 ± 1.4	93.5 ± 1.5	93.5 ± 2.3	75.7 ± 1.3	75.4 ± 2.5	76.3 ± 0.9
Tebuconazole	78.7 ± 1.2	78.3 ± 3.6	77.4 ± 3.6	80.8 ± 3.1	88.9 ± 2.4	86.3 ± 1.5	75.5 ± 2.9	72.6 ± 3.4	78.9 ± 1.9
Triadimefon	81.9 ± 1.5	77.5 ± 0.9	74.6 ± 0.7	82.7 ± 0.6	87.8 ± 0.8	86.1 ± 0.6	70.2 ± 1.5	79.2 ± 1.4	72.0 ± 2.6
Thiamethoxam	83.2 ± 0.8	81.2 ± 1.7	84.0 ± 1.2	82.7 ± 1.7	92.1 ± 1.1	90.1 ± 1.1	73.1 ± 1.9	72.9 ± 2.6	72.8 ± 1.6
Pendimethalin	76.1 ± 2.3	84.9 ± 1.3	84.8 ± 1.8	82.8 ± 1.3	99.4 ± 0.6	93.6 ± 1.3	77.7 ± 1.4	80.3 ± 1.7	84.9 ± 0.9
Metalaxyl	86.3 ± 1.5	86.0 ± 0.9	86.1 ± 0.8	92.1 ± 0.9	97.6 ± 1.6	94.3 ± 0.8	84.2 ± 1.0	83.5 ± 2.1	81.4 ± 1.2
Imidacloprid	79.1 ± 3.4	87.4 ± 1.8	89.5 ± 2.8	83.2 ± 2.5	94.7 ± 0.7	95.6 ± 1.2	86.4 ± 2.4	84.6 ± 1.6	85.7 ± 2.1
Dimethoate	72.9 ± 1.7	70.6 ± 4.1	76.9 ± 0.9	79.6 ± 1.2	81.3 ± 1.3	82.2 ± 0.5	67.4 ± 3.5	68.5 ± 2.3	69.4 ± 3.4
Acetamiprid	84.7 ± 0.6	84.7 ± 1.2	83.8 ± 2.2	87.8 ± 0.9	97.4 ± 0.5	90.4 ± 1.4	78.4 ± 2.5	83.2 ± 1.8	81.3 ± 1.5
3-hydroxyl Carbofuran	84.3 ± 0.9	85.2 ± 2.8	83.4 ± 0.6	83.8 ± 2.9	95.7 ± 2.0	94.1 ± 2.1	81.8 ± 1.0	81.7 ± 0.7	77.7 ± 1.5
Pymetrozine	79.9 ± 1.8	85.1 ± 0.9	85.7 ± 0.8	89.9 ± 1.0	94.6 ± 0.8	97.2 ± 0.9	74.5 ± 2.7	73.3 ± 1.9	76.1 ± 1.7
Omethoate	76.5 ± 2.1	83.0 ± 0.7	84.4 ± 2.3	80.5 ± 1.3	90.3 ± 1.7	91.2 ± 1.7	75.3 ± 1.1	80.1 ± 0.8	79.2 ± 2.4
Carbendazim	82.3 ± 1.4	88.8 ± 1.4	87.0 ± 0.5	96.3 ± 1.4	97.8 ± 0.9	96.0 ± 2.9	89.7 ± 2.3	88.6 ± 2.8	83.9 ± 0.9
Methomyl	83.5 ± 0.8	83.3 ± 0.8	82.2 ± 1.0	95.7 ± 0.7	93.9 ± 0.6	89.3 ± 2.0	70.3 ± 1.9	71.5 ± 1.3	73.7 ± 1.0

A: 200 mg C18+200 mg PSA+15 mg GCB; **B**: 200 mg C18+200 mg PSA+45 mg GCB; **C**: 200 mg C18+200 mg PSA+90 mg GCB; **D**: 400mg C18+400 mg PSA+15 mg GCB; **E**: 400mg C18+400 mg PSA+45 mg GCB; **F**: 400 mg C18+400 mg PSA+90 mg GCB; **G**: 600mg C18+600 mg PSA+25 mg GCB; **H**: 600mg C18+600 mg PSA+45 mg GCB; **I**: 600mg C18+600 mg PSA+90 mg GCB. SD: standard deviation

those obtained with the 200 mg adsorbents (C18 and PSA) and the 600 mg adsorbents (C18 and PSA). However, GCB strongly adsorbed some pesticides leading to low recoveries of these analytes (less than 70%). Therefore, 400 mg C18, 400 mg PSA and 45 mg GCB was selected for purification in this experiment.

3.3 Method validation

Matrix effects

It has been reported that matrix effects (ME) are common when analysing pesticide residues by LC-MS/MS. ME are caused by the influence of co-eluting compounds on the ionization efficiency of the electrospray interface in the LC-MS/MS analysis, and the effects manifest as ion enhancement or inhibition (Chawla et al., 2017; Galani et al., 2018). The wolfberry is rich in mineral substances, proteins, polysaccharose, amino acids, carotinoid, flavonoids (Gong et al., 2018). In this work, the matrix effect was calculated by the following Equation 7:

$$ME = A_{Matrix} / A_s \quad (7)$$

Where A_{Matrix} is the peak area of matrix standard sample and A_s is the peak area of pure solvent standard sample.

The ME of 31 pesticide residues were determined, and the ME values were split into three groups based (0.8-1.2, higher than 1.2 and less than 0.8). ME values between 0.8 and 1.2 were classified as low ME, which can be ignored; ME values higher than 1.2 were deemed matrix enhancements; and ME values less than 0.8 were classified as matrix suppression (Fan et al., 2013). As shown in Table 2, the ME values of 28 pesticide residues were between 0.8 and 1.2, and could be ignored. The ME values of 2 pesticide residues were less than 0.8, indicating matrix suppression. The ME values of carbendazim were higher than 1.2, indicating matrix enhancement. The above experimental results showed that although the extractant solution was purified, some interfering substances that inhibited the analysis of the target analytes remained in the solution. This phenomenon was consistent with the results found by other researchers (Prodhan et al., 2016). To compensate for the matrix inhibition effects, a matrix standard curve was used to quantify 31 pesticide residues in the wolfberry samples.

Table 2 Performance characteristics of the optimized method

Components	ME (%)	Liner range (μg/L)	Linear equation	R^2	LODs (μg/kg)	LOQs (μg/kg)
Abamectin	0.86	1~100	y = 10918x - 589	0.9991	0.5	1
Spinetoram	1.04	1~100	y = 97035x + 4682	0.9986	0.5	1
Fenpyroximate	0.84	1~100	y = 21453x + 7294	0.9967	0.5	1
Spirodiclofen	0.82	1~100	y = 17456x - 1384	0.9957	0.5	1
Difenoconazole	0.95	1~100	y = 74291x - 8792	0.9978	0.5	1
Azoxystrobin	0.88	1~100	y = 59640x + 2843	0.9956	0.5	1
Pyraclostrobin	0.89	1~100	y = 89751x - 3927	0.9969	0.5	1
Carbosulfan	0.84	1~100	y = 113544x + 9390	0.9976	0.5	1
Spirotetramat	0.91	1~100	y = 42842x + 8253	0.9988	0.5	1
Propargite	0.68	1~100	y = 60124x + 5764	0.9987	0.5	1
Pyridaben	0.74	1~100	y = 109223x + 9823	0.9986	0.5	1
Etoxazole	0.79	1~100	y = 25389x - 1946	0.9988	0.5	1
Chlorpyrifos	1.01	1~100	y = 30321x - 2043	0.9987	0.5	1
Thiophanate-methyl	0.77	1~100	y = 94742x + 3809	0.9988	0.5	1
Propiconazole	0.89	1~100	y = 173024x - 17645	0.9958	0.5	1
Pyrethrin	0.74	1~100	y = 87356x + 10362	0.9979	0.5	1
Flusilazole	0.93	1~100	y = 21443x + 1974	0.9979	0.5	1
Hexaconazole	0.86	1~100	y = 123154x + 6532	0.9978	0.5	1
Tebuconazole	0.88	1~100	y = 51837x + 4071	0.9958	0.5	1
Triadimefon	0.92	1~100	y = 89374x - 7163	0.9968	0.5	1
Thiamethoxam	1.06	1~100	y = 12584x - 1487	0.9986	0.5	1
Pendimethalin	0.83	1~100	y = 151656x - 16158	0.9957	0.5	1
Metalaxyl	0.98	1~100	y = 73625x + 3729	0.9988	0.5	1
Imidacloprid	0.87	1~100	y = 123722x - 8645	0.9989	0.5	1
Dimethoate	0.89	1~100	y = 185697x + 6719	0.9957	0.5	1
Acetamiprid	0.95	1~100	y = 129413x + 30723	0.9989	0.5	1
3-hydroxyl Carbofuran	1.09	1~100	y = 98735x - 9547	0.9988	0.5	1
Pymetrozine	0.91	1~100	y = 60124x + 5764	0.9986	0.5	1
Omethoate	0.76	1~100	y = 49485x + 9897	0.9979	0.5	1
Carbendazim	1.48	1~100	y = 60747x + 3085	0.9984	0.5	1
Methomyl	0.65	1~100	y = 83519x + 2785	0.9994	0.5	1

ME: matrix effect; R^2: linear correlation coefficient; LOD: limit of detection; LOQ: Limit of Quantification.

Selectivity

The selectivity of the detection method is important for the qualitative and quantitative analysis of 31 pesticide residues because the blank matrix solution did not contain parent ions and fragment ions of the target analytes at a detectable level. Based on a comparison of the chromatograms of the matrix-matched standard solution and the blank matrix solution (Figure 2), after QuEChERS purification, the peaks of residual co-extracting compounds did not interfere, as they appeared at different retention times, confirming the excellent selectivity of the established method.

Linearity and detectability of the method

In the linearity studies, all the standard working solutions were determined under the optimal chromatography and mass spectrometry conditions. Linear regression analysis was performed on a plot with concentration on the X-axis, and the peak area on the Y-axis. The results shown in Table 2 indicate that suitable linearities were obtained in the corresponding concentration range of each pesticide residue, and the coefficients of determination (R^2 values) were higher than 0.99.

The LODs and LOQs of the method were calculated according to the validated experimental results. The results showed that the LODs and LOQs of this method the LODs were 0.5 μg/kg and 1.0 μg/kg, respectively (Table 2), which are consistent with those of the methods reported for pesticide residue analysis in cereals (Bordin et al., 2016), vegetables (Xiu-Ping et al., 2017), fruits (Stachniuk et al., 2017), juices (Rizzetti, et al., 2016) and other foods (Kasiotis et al., 2014).

Accuracy and precision of the method

The accuracy and precision of the method were assessed for each pesticide residue by determining the recoveries and the RSDs from blank wolfberry samples spiked at three different levels. The results are shown in Table 3. The average recoveries were in the range of 73.8%-111.5%, and the RSDs were less than 10%. Thus, the accuracy and precision of the 31 pesticide residues in wolfberry are acceptable.

3.4 Actual sample ananlysis and risk assessment

Pesticide residues in wolfberry

Among the 200 wolfberry samples from Northwest China, 23 pesticides were detected and samples containing pesticide residues were in 83.5% of the wolfberry samples. The levels of individual pesticides in the samples are shown in Table 4. The detection rate exceeded 5% for of 14 pesticides and ranged from 7.5-79% for all 23 pesticides, with acetamiprid, carbendazim and imidacloprid being detected in 79%, 50% and 43% of the samples, respectively. In these samples, the residues of 10 pesticides exceed the maximum residue limit (MRL) in GB 2763-2019 (China, 2019) for wolfberry. Among them, pyridaben, acetamiprid and difenoconazole exceeded the MRL the most frequently, exceeding the MRL in 19.5%, 12% and 11% of the wolfberry samples, respectively. In this work, it was found that abamectin, pymetrozine, spinetoram, etoxazole, omethoate, dimethoate, triadimenol, chlorpyrifos, pendimethalin and fenpyroximate were detected in wolfberry samples for the first time, and the detection rates of etoxazole, triadimenol, and pendimethalin were more than 10%. The amounts and types of pesticide residues in wolfberry differed in different regions, but acetamiprid, imidacloprid carbendazim, pyridaben, propargite and thiamethoxam were detected in samples from all regions.

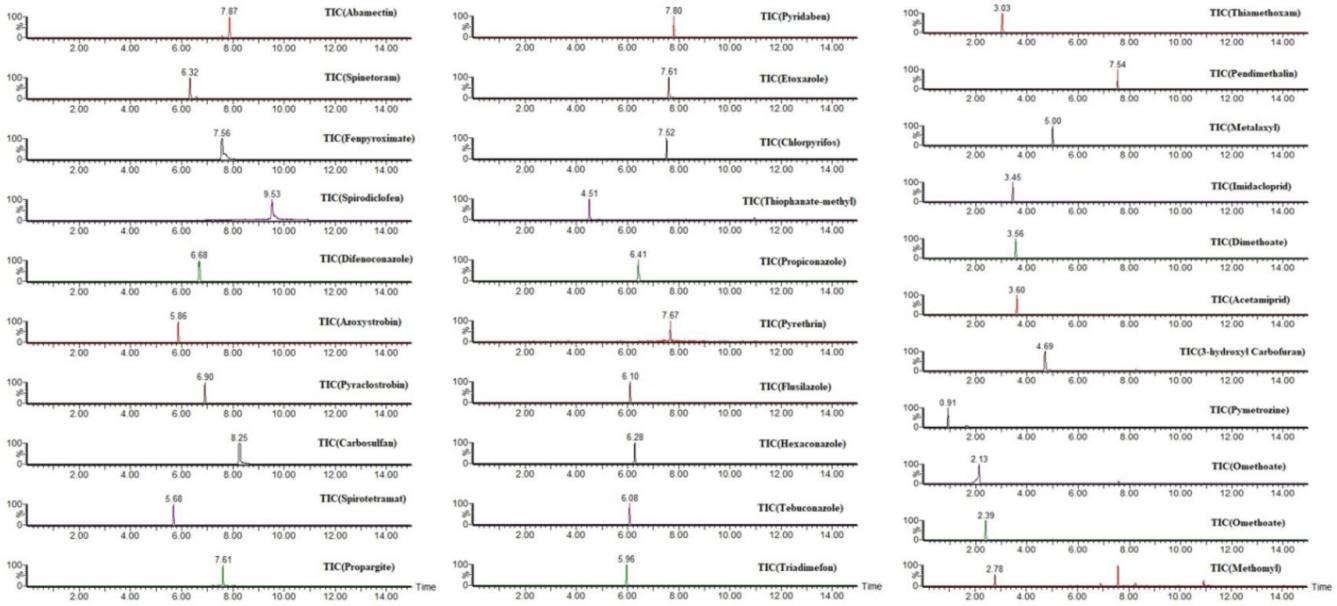

Figure 2. TIC of LC-MS/MS containing the 31 pesticides (Spiked 20 μg/L).

Table 3. Average recovery and RSD of 31 pesticides in the wolfberry (n = 6).

Components	0.01 mg/kg		0.02 mg/kg		0.1 mg/kg	
	Average recovery (%)	RSD (%)	Average recovery (%)	RSD (%)	Average recovery (%)	RSD (%)
Abamectin	71.2	2.9	78.9	4.1	79.2	1.9
Spinetoram	94.8	6.9	91.5	4.7	111.7	2.5
Fenpyroximate	86.9	2.2	82.3	2.5	91.7	2.3
Spirodiclofen	82.4	3.8	84.2	3.6	87.4	4.5
Difenoconazole	87.3	5.5	99.1	2.1	100.4	1.1
Azoxystrobin	90.2	2.7	89.7	4.4	93.6	5.9
Pyraclostrobin	95.6	3.6	89.5	1.1	96.3	2.5
Carbosulfan	85.5	2.1	87.7	4.0	88.3	1.1
Spirotetramat	85.1	6.2	83.2	2.9	93.2	3.5
Propargite	80.5	3.1	81.5	2.0	84.9	3.1
Pyridaben	90.0	6.2	88.9	2.3	92.1	3.8
Etoxazole	89.5	4.9	85.1	2.3	86.2	4.5
Chlorpyrifos	91.8	6.4	90.2	2.9	95.8	2.6
Thiophanate-methyl	86.5	2.6	95.7	4.8	97.6	2.1
Propiconazole	86.2	5.0	91.1	4.9	94.2	3.4
Pyrethrin	95.4	4.6	82.4	1.7	85.1	2.9
Flusilazole	80.0	5.0	82.3	2.5	92.1	1.9
Hexaconazole	107.2	1.2	98.0	2.3	104.2	2.7
Tebuconazole	87.4	5.4	93.2	3.7	92.3	2.3
Triadimefon	89.3	3.9	94.2	2.8	96.4	2.6
Thiamethoxam	90.2	2.9	96.1	3.4	98.7	1.2
Pendimethalin	82.6	4.5	87.9	4.1	91.8	2.7
Metalaxyl	84.8	4.0	85.3	4.9	93.7	3.1
Imidacloprid	90.6	3.3	100.3	6.0	100.0	4.1
Dimethoate	84.9	2.7	88.4	3.9	89.9	4.1
Acetamiprid	84.1	4.0	91.4	4.2	92.6	3.8
3-hydroxyl Carbofuran	90.3	2.8	95.4	1.9	100.3	2.7
Pymetrozine	96.4	4.0	90.2	3.5	97.5	1.6
Omethoate	85.1	4.5	91.5	3.7	95.3	1.7
Carbendazim	93.0	1.3	98.6	2.0	109.7	3.9
Methomyl	89.1	3.7	89.5	1.5	96.6	3.5

RSD: relative standard deviation.

On the one hand, wolfberry is susceptible to pests during its growth, and correspondingly, more and more pesticides will be used. On the other hand, overuse of pesticide has resulted in excessive pesticide residues.

Risk posed by chronic dietary intake of pesticide residues in wolfberry

The risk of the chronic dietary intake of the 23 detected pesticide residues was calculated. As shown in Table 5, the chronic dietary intake risk (%*ADI*) was much less than 100%, ranging from 0.0001% to 1.6067%, with an average of 0.1254%. The %*ADI* of omethoate was slightly higher than 1%, at 1.6067%, while the %*ADI* of difenoconazole, acetamiprid, dimethoate and 3-hydroxyl carbofuran ranged from 0.10% to 0.60%. The %*ADI* values of the other 18 pesticides were below 0.1%. These results showed that the risk posed by chronic dietary intake of the pesticide residues in wolfberry produced in Northwest China is acceptable. The *HQ* for the cumulative chronic dietary risk assessment of wolfberry was 0.02569, which is much less than 1 and indicates that the cumulative risk posed by chronic dietary intake is also acceptable.

Risk posed by acute dietary intake of pesticide residues in wolfberry

According to the World Health Organization (WHO) database (Philippe et al., 2020), the *ARfD* data for thiophanate methyl, spinetoram, propargite, thiophanate-methyl and azoxystrobin are "inconclusive", and there are no *ARfD* data for avermectin, pyridaben, any bactericide and omethoate. The *ARfD* values for the other 10 pesticides are shown in Table 6. As shown in Table 6, the risk posed by acute dietary intake of these 10 pesticides is much less than 100%, ranging from 0.0010% to 0.4999%, with an average of 0.26001%. These results showed that the risk posed by acute dietary intake of the pesticide residues in

Table 4. Residue levels of 23 pesticides in wolfberry.

Pesticide	% Positive	Range (mg/kg)	MRL(mg/kg)	%ex MRL
Avermectin	1.0	0.0243~0.0289	0.01	1.0%
Difenoconazole	20.0	0.0011~0.3200	0.01	11.0%
Imidacloprid	43.0	0.00222~1.4118	1.00	2.0%
Pymetrozine	0.5	0.0757	—	—
Pyridaben	33.0	0.00187~0.5601	0.01	19.5%
Acetamiprid	79.0	0.00168~5.7647	2.00	12.0%
Carbendazim	50.0	0.0008~1.9272	1.00	3.5%
Spinetoram	2.0	0.00624~0.0184	—	—
Thiophanate-Methyl	0.5	0.0154	—	—
Spirodiclofen	20.5	0.0033~1.0459	—	—
Propargite	25.5	0.0015~4.0889	—	—
Tebuconazole	25.0	0.0013~2.2664	—	—
Etoxazole	15.5	0.0009~0.4672	—	—
Omethoate	2.0	0.0040~0.3103	0.01	1.5%
Thiamethoxam	13.0	0.0017~2.1038	—	—
Dimethoate	3.5	0.0014~0.0297	—	—
3-hydroxyl Carbofuran	7.5	0.0010~0.0594	0.01	2.0%
Azoxystrobin	3.0	0.0012~0.0435	—	—
Triadimefon	4.0	0.0025~0.0900	1.00	0.0%
Triadimenol	18.0	0.0025~2.0588	—	—
Chlorpyrifos	7.5	0.0015~0.6741	0.10	1.5%
Pendimethalin	10.0	0.0018~0.0178	—	—
Fenpyroximate	3.0	0.0022~0.0600	0.50	0.0%

MRL: maximum residue limit.

Table 5. %*ADI*, *HQ* and *HI* of pesticide residues in wolfberry.

Pesticide	*STMR* (mg/kg)	*ADI* (mg/kg)	*ADI*%	*HQ*	*HI*
Avermectin	0.0266	0.001	0.5320	0.00474	
Difenoconazole	0.0642	0.01	0.0360	0.00032	
Imidacloprid	0.2733	0.06	0.0480	0.00043	
Pymetrozine	0.0757	0.03	0.0505	0.00045	
Pyridaben	0.0465	0.01	0.0296	0.00026	
Acetamiprid	0.8091	0.07	0.1031	0.00092	
Carbendazim	0.2027	0.03	0.0114	0.00010	
Spinetoram	0.0121	0.02	0.0119	0.00011	
Thiophanate-Methyl	0.0154	0.09	0.0034	0.00003	
Spirodiclofen	0.1371	0.01	0.0593	0.00053	
Propargite	0.3854	0.01	0.0237	0.00021	
Tebuconazole	0.19	0.03	0.0154	0.00014	
Etoxazole	0.0428	0.05	0.0038	0.00003	
Omethoate	0.0906	0.0003	1.6067	0.01432	
Thiamethoxam	0.1258	0.08	0.0024	0.00002	
Dimethoate	0.0137	0.002	0.1030	0.00092	
3-hydroxyl Carbofuran	0.0129	0.001	0.1333	0.00119	
Azoxystrobin	0.0084	0.2	0.0001	0.00000	
Triadimefon	0.0327	0.03	0.0133	0.00012	
Triadimenol	0.2129	0.03	0.0371	0.00033	
Chlorpyrifos	0.0804	0.01	0.0326	0.00029	
Pendimethalin	0.0054	0.1	0.0009	0.00001	
Fenpyroximate	0.0209	0.01	0.0246	0.00022	
					0.02569

STMR: supervised trial median residue; *ADI*: acceptable daily intake; %*ADI*: acceptable daily intake percentage; *HQ*: hazard quotient; *HI*: hazard index.

Table 6. %*ARfD*, *SM*, *HQ* and *HI* of pesticide residues in wolfberry.

Pesticide	*HR* (mg/kg)	*ARfD* (mg/kg)	*ARfD*%	*SM* (mg/kg)	*HQ*	*HI*
Avermectin	0.0289	—	—	—		
Difenoconazole	0.3200	0.3	0.3005	106.5	0.0026787	
Imidacloprid	1.4118	0.4	0.3999	353.0	0.0035654	
Pymetrozine	0.0757	0.1	—	—		
Pyridaben	0.5601	—	—	—		
Acetamiprid	5.7647	0.1	—	—		
Carbendazim	1.9272	0.1	0.4999	385.5	0.0044569	
Spinetoram	0.0184	—	—	—		
Thiophanate-Methyl	0.0154	Unnecessary	—	—		
Spirodiclofen	1.0459	Unnecessary	—	—		
Propargite	4.0889	Unnecessary	—	—		
Tebuconazole	2.2664	0.3	—	—		
Etoxazole	0.4672	Unnecessary	—	—		
Omethoate	0.3103	—	—	—		
Thiamethoxam	2.1038	1	—	—		
Dimethoate	0.0297	0.02	0.0200	148.5	0.0001784	
3-hydroxyl Carbofuran	0.0594	0.001	0.0010	5950.0	0.0000089	
Azoxystrobin	0.0435	Unnecessary	—	—		
Triadimefon	0.0900	0.08	0.0800	112.5	0.0007132	
Triadimenol	2.0588	0.08	0.0800	2575.0	0.0007128	
Chlorpyrifos	0.6741	0.1	0.999	675.0	0.0008903	
Pendimethalin	0.0178	1	0.1998	8.9	0.0017808	
Fenpyroximate	0.0600	0.02	0.0200	300.0	0.0001783	
						0.015164

HR: highest residue; *ARfD*: acute reference dose; %*ARfD*: acute reference dose percentage; *SM*: safety margin; *HQ*: hazard quotient; *HI*: hazard index.

wolfberry from Northwest China is acceptable and very low. The *HQ* for the cumulative acute dietary risk assessment of wolfberry is 0.015164, which is far less than 1 and indicates that the cumulative acute dietary risk is also acceptable. As shown in Table 6, the maximum concentration of each pesticide was far less than the MRL, which confirms that the risk posed by acute dietary intake of these pesticides is very low.

4 Conclusions

A modified QuEChERS method coupled with LC-MS/MS was established and validated for the determination of 31 pesticides residues in wolfberry. A sorbent of 400 mg C18, 400 mg PSA and 45 mg GCB was used as the dSPE sorbent for sample cleanup; 200 wolfberry samples were analysed; and 23 pesticides were detected. The most frequently detected pesticides were carbendazim, pyridaben, propargite, thiamethoxam, acetamiprid and imidacloprid. These results were compared with pesticide residues detected in samples from the other three main wolfberry production areas. The pesticides detected in different areas differed, although some pesticides were common to all regions. This finding illustrated that a greater number of pesticides are present in wolfberry from Northwest China, and the increase in pesticide residues in wolfberry necessitate continuous monitoring to ensure the safety of wolfberry consumption. The chronic dietary intake, acute dietary intake and cumulative risk of 23 pesticides in wolfberry were estimated for adults, and the results showed that the exposure to pesticides was quite low. The results of the risk assessment in this study demonstrated that the pesticide residues in wolfberry do not represent a potential risk to human health.

Conflict of interest

The authors declare no conflicts of interest.

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Author contributions

Conceptualization, Lijie Xing and Yuan Wang; sample collection, Ruifeng Luo and Xianyi Li; sample analysis, Liangjun Zou and Yuan Wang; statistical analysis, Lijie Xing and Ruifeng Luo; writing-original draft preparation, Lijie Xing and Yuan Wang; writing-review and editing, Yuan Wang. All authors have read and agreed to the published version of the manuscript.

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