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Original Research

Detection of Frequently Used Pesticides in Apple Orchard Soil in China by High Resolution Mass Spectrometry

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Abstract

In this study, 10 frequently used pesticides in apple orchards from the major apple production area of China were identified through 150 questionnaires. Based on Orbitrap high-resolution mass spectrometry, a method for detecting these pesticides in soils was developed. Typically, these compounds can be extracted from dry soil samples using dichloromethane: acetone (2:1, v:v) and purified by dispersive solid-phase extraction (PSA and C18). The qualitative and quantitative analyses can be undertaken by monitoring the MS1 precursor ions under full scan mode within a mass error of <5 ppm (resolution 120000). The recoveries ranged from 68.4% to 102.4% (SD<10.5%), the matrix effects induced signal fluctuation was less than 10%, and the intra- and inter-day precisions were all below 5%. For 14 surface soil samples randomly collected from an apple orchard in China, most pesticides presented the highest detection frequencies (100%), with imidacloprid and tebuconazole showing the highest residue levels (4.1-39.1 and 9.8-350.2 ng/g dry weight, respectively). Compared with previous methods for analyzing individual or several pesticides, the proposed method represents a promising means of investigating these frequently used pesticide residues in apple orchards in China.

Keywords: pesticides, apple orchard, questionnaire, high resolution mass spectrometry, soil

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Introduction

According to information from the Apple IPM Information Network of China (http://www.apple-ipm.cn/yjk/list.asp), 16 plant diseases and insect pests are presented with their recommended pesticides, including tebuconazole, difenoconazole, pyridaben, carbendazim, chlorpyrifos, chlorbenzuron, acetamiprid, iprodione, flusilazole, and cyhalothrin. More than 50 pesticides have been used in the major apple production zones in China to control plant diseases and insect pests [1]. High amounts of pesticide use can lead to higher residue in apples and associated soils, which is closely associated with health and ecotoxicological risks [2]. For example, high levels of difenoconazole residue have been detected in apples and the corresponding soils (0.002-0.052 and 0.002-0.298 mg/kg, respectively) [3]. Meanwhile, pesticide use in apple orchards could also affect nearby surface runoff, especially for mountain streams [4].

Small-scale decentralized farms are the main production units in Chinese apple production areas, such as in Shaanxi, Gansu, Henan and Shandong provinces. To protect apple trees from the threat of pests and weeds, large amounts of pesticides have been widely used every year. It should be noted that pesticide use is in a disordered state because most Chinese fruit growers know little about pesticides, pests and weeds [5]. Farmers are mainly concerned about controlling pests and weeds, rather than usage amounts and ecological risks. To date, only scarce statistics on pesticide used in apple orchards have been reported by the authorities in China. In this study, a series of national key research and development programmes containing the status of pesticides used in apple orchards have been proposed and executed in China since 2016, specifically addressing the soil pesticide residues and the associated ecological risks.

Based on HPLC-UV, HPLC-MS, GC-MS and FTIR, many methods have been developed to detect the pesticide residues in soils [6-9]. The limits of detection (LODs) and limit of quantification (LOQs) of these methods were commonly at ppb levels (ng/g), which were sensitive and effective for analysing pesticide residues, such as acetamiprid, tebuconazole and difenoconazole [3, 10-13]. Generally, several kinds of pesticides, such as organophosphorus pesticides [14], organochlorine pesticides [15] and pyrethroids [16], were involved in the processes. However, few methods have been reported for analysing pesticides frequently used in apple orchards due to the knowledge gap on the species and amounts of pesticides [17].

In this paper, 150 questionnaires on pesticide use in apple orchards from major apple production areas in China were collected and summarized. Then a method based on ultra-high-performance liquid chromatography Orbitrap-high resolution mass spectrometry (UHPLC-Orbitrap-HRMS) was developed for analysing the most frequently used pesticides. The LODs, LOQs, recovery, matrix effects, accuracy and precision of the method have been studied. The validity has been evaluated by analysing 14 soil samples randomly collected from an apple orchard in Changwu County, Shaanxi Province.

Material and Methods

Chemicals and Reagents

Methanol, acetone, dichloromethane (DCM), ethyl acetate and acetonitrile were all purchased from Merck (Darmstadt, Germany) and Fisher Scientific (Trinidad, UK). All chemicals were of chromatographic reagent grade. Formic acid was obtained from Dikma Technologies (USA). Analytical-grade standards (purities>98%), carbendazim, imidacloprid, acetamiprid, triadimenol, triadimefon, tebuconazole, chlorbenzuron, difenoconazole, chlorpyrifos, buprofezin, and pyridaben were purchased from Dr. Ehrenstorfer (Augsburg, Germany) and Sigma-Aldrich (Steinheim, Germany). Individual chemical stock solutions (2 mg/mL) were prepared in HPLC-grade methanol and stored in the dark at -20ºC. Anhydrous sodium sulfate (Na_2SO_4), primary-secondary amine (PSA) bonded silica bulk, octadecylsilane (LC-C18, 40-63 μm) and nylon syringe filters (0.22 μm) were obtained from Shanghai Anpel Scientific Instrument Co., Ltd. (Shanghai, China). Ultra-pure water was obtained from a Milli-Q system (Bedford, MA, USA).

Sample Collection

All of the 14 surface soil samples (0-20 cm depth) were collected from an apple orchard (350 × 150 m) in Changwu, Shaanxi Province, China in August 2017. At this time, tebuconazole, pyridaben and chlorbenzuron were often used to prevent trunk canker, European red mite and tan disease. Fourteen soil samples were randomly collected from the orchard and then quickly transported to the lab, freeze-dried, ground, sieved (100 mesh) and stored in a refrigerator at -20ºC until analysis.

The physical-chemical properties of the soil samples are shown in Table 1. The environment behaviours of the organic chemicals in soil are often influenced by organic matter and pH [18, 19]. The mean pH values (7.94±0.16) and organic matters (12.6±2.0 g/kg) of the soil samples (S1-S14) are similar to those of the main apple-producing areas of China (pH>7.5, organic matter>10.0 g/kg) [20, 21]. A blank soil sample (S0) (pH of 8.17 and organic matters of 11.2 g/kg) is chosen as the representative sample for method validation.

Sample Preparation

Before analysis, 5 g homogenized samples were weighed in a 50-mL glass centrifuge tube and spiked

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Table 1. Physical-chemical properties of the soils.

Parameter	S0	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14
pH	8.17	8.01	7.69	7.85	7.74	8.22	8.13	8.05	7.85	7.95	7.80	7.81	7.92	7.99	8.11
Organic matter (g/kg)	11.2	10.6	12.5	11.4	14.3	13.6	10.4	12.4	10.1	9.9	12.4	13.9	15.8	16.1	12.3

at three different levels for 11 pesticides. The soil samples were mixed uniformly after the addition of 3 g Na_2SO_4. Afterward, the mixture was mixed with 30 mL DCM/acetone (2/1, v/v, containing 0.1% formic acid). Then the mixture was vortexed vigorously for 5 min and ultrasonically extracted for 20 min. After centrifugation at 3000 rpm for 5 min, the supernatant was collected. The extraction processes were repeated another two times. The extracts were combined, rotary evaporated and re-dissolved in 1 mL of methanol. PSA (100 mg) and LC-C18 (50 mg) were weighed into the bottle, mixed with the solvent and vortexed vigorously for 3 min to purify the extracts. The supernatant was filtered through a 0.22-μm nylon filter and diluted with water (20%, v/v) before analysis using UHPLC-Orbitrap-HRMS.

Instrument Conditions

Sample analysis was performed by a Thermo Ultimate 3000 UHPLC coupled with an Orbitrap Fusion Tribrid mass spectrometer (Thermo Fisher Scientific, USA) and equipped with an ACQUITY UPLC BEH C18 column (100 × 2.1 mm, 1.7 μm, Waters). The mobile phase consists of 0.1% formic acid in acetonitrile (A) and water (B) at a flow rate of 0.3 mL/min. The initial gradient was set to 10% A and held for 3 min before starting a linear gradient that increased to 100% A in 17 min and held for 2 min. Then the gradient returned to the initial conditions for 2 min. The column temperature was set to 30ºC.

Electrospray ionization was conducted in the positive ionization mode. The recommended values were used in the full scan acquisition from m/z 100 to 800 without further optimization. Collision-induced dissociation (CID) in Orbitrap Fusion HRMS was applied to collect the MS2 pesticide information. The parameters used for the mass spectrometer were as follows: spray voltage, 3500 V; sweep gas flow rate, 1 respective arbitrary units; sheath gas flow rate, 30 respective arbitrary units; aux gas flow rate, 8 respective arbitrary units; ion transfer tube temperature, 350ºC; vaporizer temperature, 200ºC; MS1 detector, Orbitrap; MS1 resolution, 120,000; MS1 scan range, 100-800; MS1 maximum injection time, 100 ms; MS1 automated gain control (AGC) target, 100,000; S-lens RF level, 60 V; MS2 CID collision energy, 30%; MS2 detector, Orbitrap; MS2 resolution, 30,000; MS2 AGC target, 50,000; MS2 maximum injection time, 100 ms; and MS2 start mass, 50. Xcalibur Qual and Quan Browser software were used for the qualitative and quantitative analyses.

Method Validation

The linearity, LODs, LOQs, recovery, matrix effects and precision were evaluated for this method. Serial standard pesticide dilutions (0.5, 1, 2, 5, 10, 20, 50, 100, and 500 ng/mL) with methanol/water (80:20, v:v) were prepared using a standard stock solution (2 mg/mL in methanol) mixture of the 11 pesticides. Correspondingly, matrix-matched standard solutions were prepared (50, 500 ng/mL) by mixing the corresponding standards with concentrated sample extracts. The matrix-induced signal suppression or enhancement was determined by comparison with the signals of pure standards [22]. The LODs and LOQs were established based on the lowest concentration with a signal-to-noise ratio (S/N) of 3:1 and 10:1. The recovery of soil samples was conducted to evaluate the accuracy, precision and feasibility of the method. The intra-day precision (repeatability) was performed at the same two concentration levels as the recovery studies. The intermediate precision (inter-day precision) was studied by spiking blank samples at 10 and 100 ng/g in different days. Three replicates of the spiked samples at three levels (10, 100 and 500 ng/g dw of each pesticide) were prepared to evaluate the recoveries.

Results and Discussion

Questionnaire Survey Results

The recommended pesticides (60 kinds) for pest and weed prevention in a Chinese apple orchard were involved in the self-designed questionnaire, which was used for investigating the type, quantity and time of application of pesticides. The questionnaires were focused on the local farmers and the agricultural machine stations in Shaanxi Province (120 questionnaires) and Shanxi Province (30 questionnaires). The used frequencies (UFs) of 60 pesticides were counted by the 150 completed questionnaires (Table 2). The results showed that there were 10 pesticides presenting higher UFs (> 85%) among all of the 60 inquisitional pesticides. These pesticides were carbendazim (UF = 100.0%), tebuconazole (UF = 100.0%), chlorbenzuron (UF = 91.3%), chlorpyrifos (UF = 92.0%), buprofezin (UF = 90.0%), difenoconazole (UF = 88.7%), triadimefon (UF = 89.3%, metabolite triadimenol), acetamiprid (UF = 88.7%), imidacloprid (UF = 87.3%) and pyridaben (UF = 86.0%). To a certain extent, the residues of these

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Table 2. Use frequencies of the pesticides from the questionnaires.

	Pesticides	Shaanxi (120)	Shanxi (30)	Average frequency (%)
		Frequency (%)	Frequency (%)	
1	Carbendazim	100.0	100.0	100.0
2	Tebuconazole	100.0	100.0	100.0
3	Chlorbenzuron	91.7	90.0	91.3
4	Chlorpyrifos	90.0	100.0	92.0
5	Buprofezin	90.0	90.0	90.0
6	Difenoconazole	89.2	86.7	88.7
7	Triadimefon	88.3	93.3	89.3
8	Acetamiprid	88.3	90.0	88.7
9	Imidacloprid	88.3	83.3	87.3
10	Pyridaben	85.0	90.0	86.0
11	Cyhalothrin	83.3	76.7	82.0
12	Thiophanate-mythyl	83.3	80.0	82.7
13	Polyoxins	75.0	76.7	75.3
14	Ammonium glyphosate	75.0	66.7	73.3
15	Mancozeb	70.8	63.3	69.3
16	Bordeaux mixture	70.0	63.3	68.7
17	Atrazine	61.7	60.0	61.3
18	Pymetrozine	54.2	60.0	55.3
19	Copper sulfate	54.2	53.3	54.0
20	Pyraclostrobin	48.3	40.0	46.7
21	Chlorantraniliprole	30.0	23.3	28.7
22	Carbosulfan	15.0	10.0	14.0
23	Tetracycline/ Oxytetracycline/ Streptomycin	8.3	6.7	8.0
24	Thiram•asomate	5.0	10.0	6.0
25	Chlorothalonil	5.0	6.7	5.3
26	Benomyl	3.3	6.7	4.0
27	Amobam	3.3	6.7	4.0
28	Nitenpyram	0	0	0
29	Dinotefuran	0	0	
30	Clothianidin	0	0	0
31	Sulfoxaflor	0	0	0
32	Thiamethoxam	0	0	0
33	Dichlorvos *	0	0	0
34	Isocarbophos *	0	0	0
35	Triazophos	0	0	0
36	Profenofos *	0	0	0
37	Diflubenzuron	0	3.3	0.7
38	Phoxim *	0	0	0
39	Acephate *	0	0	0
40	Bifenthrin	0	0	0
41	Dipterex *	0	0	0
42	Dimethoate/omethoate *	0	0	0
43	Ethofenprox	0.8	0	0.7
44	Thiprole	0	0	0
45	Monosultap *	0	0	0
46	Dimehypo *	0	0	0
47	Methomyl *	0	0	0
48	Indoxacarb	0	0	0
49	Propineb• Tebuconazole	0	0	0
50	Vinclozolin	0	0	0
51	Fosetyl-aluminum	0	3.3	0.7
52	Metalaxyl	0	0	0
53	Pretilachlor	1.7	0	1.3
54	Butachlor	0	0	0
55	Acetochlor	0	3.3	0.7
56	Paraquat *	0	0	0
57	Quizalofop-p-ethyl	0	0	0
58	Oxyfluorfen	0	0	0
59	Thiram	0	0	0
60	Asomate *	0	0	0

* , banned pesticides in China

10 compounds and triadimenol might represent the entire usage situation of Chinese pesticides. In this study, a method for monitoring these 11 pesticides based on Orbitrap-HRMS was developed to enable large-scale soil residue investigations in apple orchards.

Optimizing UHPLC-Orbitrap-HRMS Conditions

Benefiting from the high resolution of UHPLC-Orbitrap-HRMS (120,000), the extracted mass ranges could be strictly limited according to the theoretical values with a mass error of <5 ppm. The sample interferences were always excluded by higher

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Table 3. Chemical information of 11 pesticides by using UHPLC-Orbitrap-HRMS.

Compounds	Molecular formula	Molecular weight	MS^1 (m/z)	Isotope ratio (Cl)	Error (ppm)	MS^2 (m/z)	RTs (min)
Carbendazim	$C_9H_9N_3O_2$	191.19	192.07681	----	0.057	160.05069	2.1
Imidacloprid	$C_9H_{10}ClN_5O_2$	255.66	256.05954/258.05649	3:1	-0.039	210.06723/175.09827	7.3
Acetamiprid	$C_{10}H_{11}ClN_4$	222.67	223.07454/225.07149	3:1	0.039	126.01070, 196.06374	8.0
Triadimenol	$C_{14}H_{18}ClN_3O_2$	295.76	296.11597/298.11301	3:1	-0.061	279.23190, 227.08345	12.6
Triadimefon	$C_{14}H_{16}ClN_3O_2$	293.75	294.10043/296.09735	3:1	0.049	197.07294, 225.06786	13.5
Tebuconazole	$C_{16}H_{22}ClN_3O$	307.82	308.15240/310.14935	3:1	-0.017	290.14188, 151.03113	13.8
Buprofezin	$C_{16}H_{23}N_3OS$	305.44	306.16345	-----	-0.010	201.10582, 116.05310	13.9
Chlorbenzuron	$C_{14}H_{10}Cl_2N_2O_2$	309.15	309.01920/311.01624/313.01324	9:6:1	-0.010	156.02122, 138.99472	14.4
Difenoconazole	$C_{19}H_{17}Cl_2N_2O_3$	406.26	406.07187/408.06885/410.06589	9:6:1	-0.103	337.03934, 251.00253	15.2
Chlorpyrifos	$C_9H_{11}Cl_3NO_2PS$	350.59	349.93359/351.93060/353.92758/355.92429	27:27:9:1	0.030	321.90228, 197.92754	17.5
Pyridaben	$C_{19}H_{25}ClN_2OS$	364.93	365.14487/367.14166	3:1	-0.018	309.08234	18.5

resolutions. In this study, the recommended condition for UHPLC-Orbitrap-HRMS was used directly, and both full scan (MS1) and fragmentation (MS2) data were obtained at the same time. MS1 data of the 11 pesticides were used for identification and quantitation by comparing the mass information and retention time with that of pure standards (Table 3). More information, including accurate MS1 m/z values, isotopic ratios and MS2 characteristics, could be given by one injection. The identification of the target chemicals by UHPLC-Orbitrap-HRMS displays more finger information than HPLC-MS/MS.

For optimizing UHPLC conditions, mobile phase composition, flow rate and solvent composition were evaluated. The mobile phase containing acetonitrile and water was more likely to elute the pesticides from the column than that consisting of methanol and water. Additionally, the addition of 0.1% formic acid is in favour of the positive ionization of pesticides and their separation on column, which is in accordance with the results in the literature [23, 24]. An ACQUITY UPLC BEH C18 (2.1 × 100 mm, 1.7 μm, Waters) was used for optimization of the elution conditions. When the flow rate increased from 0.2 mL/min to 0.4 mL/min, the tailing peaks decreased, but peak kurtosis was not improved, especially for polar chemicals of carbendazim, imidacloprid and acetamiprid. This finding can be attributed to the higher elution capacity of a substance in a solvent than that of the mobile phase. The addition of a certain amount of water to the standard solution could solve this problem. However, high levels of water (e.g., 50%) reduce pesticide solubility. Finally, a solvent containing 20% water in methanol is used for dissolving the pesticides and as the sample solvent. Finally, the solvent of methanol/water (80/20, v/v) and the mobile phase of acetonitrile and water (containing 0.1% formic acid) at a flow rate of 0.3 mL/min were applied for separating these 11 pesticides in 25 min with a rare peak overlap (Fig. 1).

Optimizing Extraction and Clean-Up Procedures

To obtain good recoveries, a series of organic solvents, including acetonitrile, methanol, acetone, ethyl acetate, DCM and their mixtures were tested. Both extraction efficiencies and co-extracted interferences were used as the evaluation items. The results showed that there were more interferences when using acetonitrile, methanol and acetone as the sole solvents. For DCM or ethyl acetate, this approach could lead to low extraction efficiencies for polar analytes, such as carbendazim and imidacloprid. The combination of solvents, including acetone/ethyl acetate (2/3, v/v), acetone/DCM (1/2, v/v), and DCM/ethyl acetate (1/2, v/v) were also tested. The extraction efficiencies using acetone/DCM (1/2) presented the best performance, with the recoveries ranging from 72.0% to 102.4% at a spiking level of 100 ng/g dw. The addition of 0.1%

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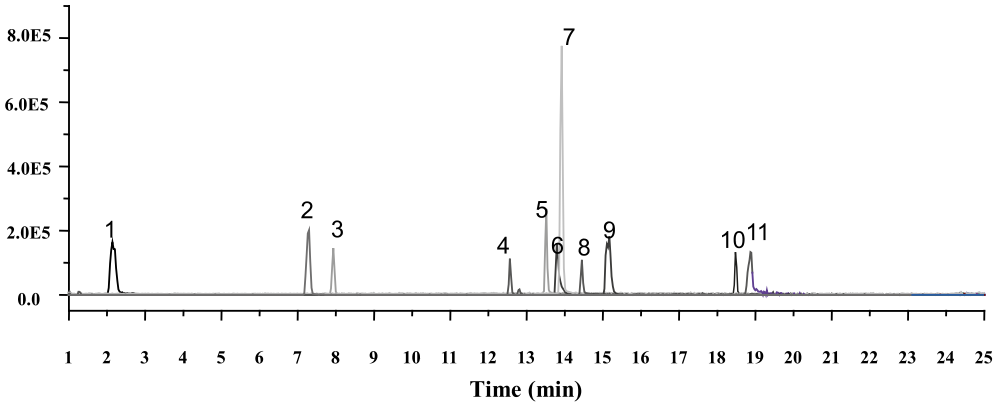

Fig. 1. HRMS chromatogram of 11 pesticides.
1, Carbendazim (10 ng/mL); 2, Imidacloprid (20 ng/mL); 3, Acetamiprid (10 ng/mL); 4, Triadimenol (50 ng/mL); 5, Triadimefon (10 ng/mL); 6, Tebuconazole (10 ng/mL); 7, Buprofezin (10 ng/mL); 8, Chlorbenzuron (50 ng/mL); 9, Difenoconazole (10 ng/mL); 10, Chlorpyrifos (100 ng/mL); 11, Pyridaben (20 ng/mL).

formic acid improved the method performance with the recovery efficiencies of the analytes increasing by 1-10%, especially for carbendazim. Ultimately, acetone/DCM (1/2, containing 0.1% formic acid) was chosen as the extraction solvent.

LC-C18, PSA and ENVI-Carb SPE cartridges are the commonly used clean-up materials for analysing pesticide residues, especially for eliminating pigment matters [4, 25-27]. The recoveries of pesticides (100 ng/g dw) eluted from the ENVI-Carb SPE cartridge (6 cc, 500 mg, Supelco) ranged 10-30%. This result can be attributed to the higher absorption capacity between the carbon packing and the pesticides [28]. PSA and C18 are often used in the QuEChERS method to remove the polar matrix components by absorption or extraction [29, 30]. Similar to that in most QuEChERS methods [31, 32], PSA (100 mg) and C18 (50 mg), which presented a better elimination performance, were chosen as the clean-up media.

Analytical Performance

Under the optimized conditions, the performance of the developed method was systematically evaluated (Table 4). Calibration curves of most pesticides were in the range of 0.5 to 500 ng/mL, except triadimenol (1-500 ng/mL), chlorbenzuron (1-500 ng/mL), pyridaben (2-500 ng/mL) and chlorpyrifos (10-500 ng/mL). There was good linearity for the 11 pesticides (r^2>0.99). The LODs (S/N = 3) ranged from 0.2 to 1.5 ng/g dw, and the LOQs (S/N = 10) ranged from 0.6 to 4.0 ng/g dw. LODs and LOQs were comparable to that of HPLC-ESI-MS/MS methods (1-20 ng/g) developed for food (pomegranate, coconut and wheat, etc.) residue analysis [31-34], but considerably lower than the methods based on HPLC-MS/MS, GC-ECD/MS and HPLC-UV (5-79 ng/g) for soil residue analysis [35-37].

When spiked at levels of 10, 100 and 500 ng/g dw in soil samples, recoveries ranged from 68.4 to 102.4%, and SD values were lower than 10.5%. The intra-day RSD and inter-day RSD at levels of 10 and 100 ng/g dw were 1.0-4.5% and 2.3-4.4%, respectively.

The matrix effects (ME), which often induce signals enhancement or suppression in MS analysis, have been studied using the following equation:

$$\mathrm{ME} = \frac{A_{matrix}}{A_{solvent}} \times 100\% \quad (1)$$

…where $A_{solvent}$ is the detected peak area of the standard solution and A_{matrix} is the detected peak area of the same chemical, which is prepared in the extracted matrix of the blank sample with the same levels of standard solutions. The numerical value of 100% of ME represents no matrix effect, while lower or higher than 100% represents signal suppression or enhancement, respectively.

We evaluated the matrix effects at two spiking levels (50 and 500 ng/mL). As shown in Table 4, the matrix effects of 11 targeted pesticides were 93.6-104.6% at 50 ng/mL and 92.4-104.2% at 500 ng/mL. A signal fluctuation of less than 10% suggested that the matrix effects can be neglected in this study.

Application in Real Samples

The most commonly used method in China for applying pesticides is hydraulic pressure atomization. This approach often causes uncovered soil under trees to be exposed to pesticides [38]. To investigate the residue levels of these frequently used pesticides in apple orchards, 14 surface soil samples (S1-S14) were randomly collected and analyzed by the developed method (Table 5). Tebuconazole, carbendazim, imidacloprid, difenoconazole, chlorpyrifos, buprofezin and pyridaben presented the highest detection frequency of 100%. These pesticides were followed by chlorbenzuron (92.8%), triadimefon (85.7%), acetamiprid (42.8%) and triadimenol (28.6%). Tebuconazole showed the highest

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Table 4. Method performance results of 11 target pesticides.

Pesticides	Calibration curves	Recovery (%) 3 spiking levels (ng/g dw) n = 3			Matrix effects (%) 2 spiking levels (ng/mL) n = 3		Intra-day RSD (%) 2 tested levels (ng/g dw) n = 6		Inter-day RSD (%) 2 tested levels (ng/g dw) n = 3		LOD (ng/g dw)	LOQ (ng/g dw)
		10.0	100.0	500.0	50.0	500.0	10.0	100.0	10.0	100.0		
Carbendazim	Y = 40477+50540X r^2 = 0.996	90.6±5.4	101.4±3.7	95.4±6.5	97.2±10.6	96.6±10.2	1.5	2.4	2.6	3.0	0.2	0.6
Imidacloprid	Y = -8163+31161X r^2 = 0.993	97.8±3.9	94.6±5.8	68.4±3.8	96.2±9.8	97.9±6.5	2.2	2.1	3.1	2.6	0.8	2.0
Acetamiprid	Y = -9348+72771X r^2 = 0.994	95.6±3.5	100.8±3.6	77.5±3.1	98.5±11.6	97.7±5.3	1.2	1.7	2.5	2.5	0.2	0.6
Triadimenol	Y = -8633+10002X r^2 = 0.996	85.4±6.7	72.0±7.2	71.8±3.0	93.6±8.3	95.6±8.7	4.5	4.3	3.2	2.9	1.2	3.5
Triadimefon	Y = 23816+210165X r^2 = 0.992	87.6±4.6	82.8±1.1	100.1±4.8	94.3±12.8	98.9±6.6	2.1	1.0	3.1	2.6	0.4	1.3
Tebuconazole	Y = 45839+202059X r^2 = 0.992	99.5±4.3	102.4±10.5	92.8±3.1	104.6±9.5	103.8±4.9	2.8	3.6	3.1	2.4	0.3	1.2
Chlorbenzuron	Y = -1440+5551X r^2 = 0.991	95.2±7.6	93.6±1.4	101.6±3.1	99.8±8.7	104.2±5.3	3.8	4.0	2.7	3.8	1.1	3.5
Difenoconazole	Y = -4351+185002X r^2 = 0.994	90.4±6.4	85.8±2.1	97.1±3.1	96.7±13.8	92.4±4.7	2.9	3.6	2.9	3.6	0.2	0.8
Chlorpyrifos	Y = -33762+4387X r^2 = 0.996	89.4±4.8	90.6±5.2	85.4±8.1	98.5±11.3	97.9±9.5	1.3	2.7	2.4	4.4	1.5	4.0
Buprofezin	Y = 511700+552126X r^2 = 0.999	76.2±5.9	83.8±4.4	93.3±2.5	94.6±12.9	96.7±5.1	2.1	1.9	4.1	3.7	0.3	1.0
Pyridaben	Y = -164778+102472X r^2 = 0.999	90.5±3.8	90.4±9.5	98.2±2.9	96.6±14.7	96.7±5.4	2.0	1.2	2.3	2.7	0.9	3.2

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Table 5. Contents of pesticides (ng/g dw) in 14 soil samples analyzed by UHPLC-Orbitrap HRMS.

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	CN-MRLs	EU-MRLs
Carbendazim	1.8	0.8	0.9	1.4	1.8	0.3 [a]	1.7	0.7	5.0	3.9	0.6	1.3	2.0	0.3 [a]	5	0.2
Imidacloprid	18.3	5.9	10.7	30.1	17.1	13.4	11.9	5.2	14.2	39.1	15.4	22.2	5.3	4.1	0.5	0.5
Acetamiprid	ND	ND	ND	0.3 [a]	0.1 [b]	0.1 [b]	ND	ND	0.1	0.3 [a]	ND	0.1 [b]	ND	ND	0.8	0.8
Triadimenol	0.8 [a]	ND	0.8 [a]	ND	ND	0.8 [a]	ND	ND	ND	0.8 [a]	ND	ND	ND	ND	1	0.2
Triadimefon	0.2 [b]	0.2 [b]	0.2 [b]	0.6 [a]	ND	0.2 [b]	0.2 [b]	0.2 [b]	0.6 [a]	0.2 [b]	0.2 [b]	0.2 [b]	0.2 [b]	ND	1	0.01
Tebuconazole	40.8	18.3	90.7	112.5	107.7	48.4	31.4	9.8	117.6	350.2	11.2	236.7	259.6	20.7	2	0.3
Chlorbenzuron	13.7	18.9	3.3	3.6	6.0	1.5 [a]	6.6	24.7	1.5 [a]	5.8	ND	4.3	1.5 [a]	1.5 [a]	3 (crop)	----
Difenoconazole	18.7	1.7	5.1	12.2	9.5	3.6	3.7	1.3	13.1	27.0	2.0	8.1	3.6	0.4 [b]	0.5	0.8
Chlorpyrifos	13.5	9.9	7.8	14.6	14.3	4.5	8.4	6.2	9.6	43.2	4.7	7.6	4.1	2.0 [a]	1	0.01
Buprofezin	1.9	2.1	0.5 [a]	0.2 [b]	0.5 [a]	0.2 [b]	2.4	0.5 [a]	0.5 [a]	1.4	0.5 [a]	0.5 [a]	0.2 [b]	0.2 [b]	0.5 (fruit)	3
Pyridaben	1.6 [a]	1.6 [a]	5.7	47.2	1.6 [a]	4.3	1.6 [a]	1.6 [a]	6.4	20.0	1.6 [a]	4.2	4.2	1.6 [a]	2	0.5

[a] the contents higher than LOD and lower than LOQ was represented by 1/2 LOQ; [b] the contents lower than LOD was represented by 1/2 LOD; CN-MRLs (mg/kg), Maximum residue limits for pesticides in apple and other food of China (GB 2763—2016); EU-MRLs (mg/kg), Maximum residue limits in apple of European Union.

levels (9.8-350.2 ng/g dw), which coincide well with its higher-use frequency. Among these detected pesticides, both imidacloprid and chlorpyrifos also presented higher levels, with contents ranging from 4.1 to 39.1 and 2.0 to 43.2 ng/g dw, respectively, which were slightly lower than imidacloprid in crude pollen samples (3.2-4516 ng/g) [39] and chlorpyrifos in fresh vegetable samples (5-500 ng/g) [40]. The higher contents of imidacloprid in soils could be attributed to their longer half-lives in soil ($t_{1/2}$>20 days) [41]. The other 9 detected pesticides in most of the soil samples were approximately 1-20 ng/g dw, which is comparable to values previously reported in food samples [25, 42]. The soil pesticide contents were all below the MRLs of China (0.5-5 mg/kg) and the European Union (0.01-3 mg/kg), indicating their low acute toxic risks.

Conclusions

In this study, 150 questionnaires were collected to determine the most-used pesticides in apple orchards. The results showed that there were 10 highly frequently used chemicals. Based on the questionnaires, a sensitive method using UHPLC-Orbitrap-HRMS was developed for simultaneously detecting these 10 pesticides and one metabolite. Good performance in analyzing 14 soil samples randomly collected from an apple orchard in Shaanxi Province was obtained. The developed method based on questionnaires should be significant for monitoring pesticide residues in Chinese apple orchards.

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Conflict of Interest

The authors declare no conflict of interest

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