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Multi-pesticides residue analysis of grains using modified magnetic nanoparticle adsorbent for facile and efficient cleanup

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ABSTRACT

A facile, rapid sample pretreatment method was developed based on magnetic nanoparticles for multi-pesticides residue analysis of grains. Magnetite (Fe_3O_4) nanoparticles modified with 3-(*N*,*N*-diethyla mino)propyltrimethoxysilane (Fe_3O_4-PSA) and commercial C18 were selected as the cleanup adsorbents to remove the target interferences of the matrix, such as fatty acids and non-polar compounds. Rice was used as the representative grain sample for method optimization. The amount of Fe_3O_4-PSA and C18 were systematically investigated for selecting the suitable purification conditions, and the simultaneous determination of 50 pesticides and 8 related metabolites in rice was established by liquid chromatography–tandem mass spectrometry. Under the optimal conditions, the method validation was performed including linearity, sensitivity, matrix effect, recovery and precision, which all satisfy the requirement for pesticides residue analysis. Compared to the conventional QuEChERS method with non-magnetic material as cleanup adsorbent, the present method can save 30% of the pretreatment time, giving the high throughput analysis possible.

1. Introduction

Pesticides are widely used in agricultural crops to prevent diseases and pests, leading to many benefits for the farmers. However, the pesticides residue cannot be ignored. It may be remained in the agro-products, transferred to the processed food, and consequently posing a potential risk to the human health. Rice, wheat, soybean and other grains are the largest consumed foods for billions of people all over the world with the increasing consumption in the recent decades (Pizzutti, de Kok, Hiemstra, Wickert, & Prestes, 2009). Thus, it is important to effectively monitor the pesticides residue in grains for ensuring the food safety and human health. Grains usually contain fatty acids, proteins, dietary fiber, vitamins and other micronutrients essential (González-Curbelo, Herrera-Herrera, Ravelo-Pérez, & Hernández-Borges, 2012; Walorczyk & Drożdżyński, 2012). The pesticides analysis of grains is thus considered to be a difficult task. For these reasons there is a clear need to develop reliable method for the multi-residue analysis in grains.

Until now, many sample preparation techniques have been utilized for extraction and purification of pesticides in grains. The main methodologies were based on solid phase extraction (SPE) (Chen, Shi, Shan, & Hu, 2007; Pareja, Fernández-Alba, Cesio, &

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Heinzen, 2011) and QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) (Malinowska, Jankowski, Sosnowski, & Wiśniewska-Kadżajan, 2015; Min et al., 2012; Nguyen et al., 2008) coupled with gas chromatography (GC), gas chromatography–tandem quadrupole (GC–MS/MS) or liquid chromatography–tandem mass spectrometry (LC–MS/MS). SPE can provide a good purification, but its procedure is usually tedious and labor intensive, limiting the speed of sample pretreatment. QuEChERS method can effectively shorten the procedure, decrease manual labor and the usage of organic reagents (Anastassiades, Lehotay, Štajnbaher, & Schenck, 2003), making it widely used in pesticides analysis for various kinds of samples. It involves extraction and purification procedure with dispersive solid phase materials as cleanup adsorbent. After purification, high-speed centrifugation was needed to separate the dispersive adsorbent and sample solution. This step is easy to operate for a small quantity of samples, but it may become a challenge for large quantity of samples. Therefore, it is a meaningful exploration to find an alternative approach for shortening the pretreatment time and improving the efficiency.

The magnetic materials went into our sights owing to its unique super paramagnetic property (Fan et al., 2012; Liu, Cai, & Feng, 2012; Sun, Liu, Sun, Wang, & Ding, 2012; Wang et al., 2012). If the magnetic materials were used as the dispersive adsorbent in the purification process, the extract would be separated with the dispersive adsorbent within 3 s under the external magnetic field. Compared with the centrifugation process in the traditional QuEChERS method, the magnetic separation is ten times quicker for one batch samples. If simultaneously dealing with two or three batch samples, the magnetic separation will be more efficient duo to leaping over the samples switch step. Besides, it is so simple that everyone can operate in every laboratory. You just need to put the samples on the magnet, and draw the supernatant solution. The large and expensive high-speed centrifuger is replaced by a smart magnet or magnetic frame.

Screening the ideal magnetic adsorbent is also important for developing a facile and efficient method. Ferroferric oxide (Fe_3O_4) is a good magnetic core owing to its surface hydroxyl group. This is attributed to the weak alkaline properties of the transition metal oxide. It can be easily modified and formed a series of meritorious magnetic adsorbent (Ahmadi, Rajabi, Faizi, Rahimi-Nasrabadi, & Maddah, 2014; Wang et al., 2015; Yu et al., 2010). The surface modification endues the novel materials with the advantages of super paramagnetic property and the selective adsorption capacity. Commercial PSA is widely used in the traditional QuEChERS method for removing the polar compounds (Hercegová, Dömötörová, & Matisová, 2007). In our previous research, Fe_3O_4 modified with 3-(*N*,*N*-diethylamino)propyltrimethoxysilane ($C_{10}H_{25}NO_3Si$, CAS No. 41051-80-3) (Fe_3O_4-PSA) was prepared and proved to be useful for removing organic acid in fruit samples (Qi et al., 2015). Therefore, a new attempt was carried out to explore its function on removing the fatty acids in grains.

The present work aimed at developing a simple, rapid and efficient method for the multi-pesticides analysis in grains coupled with LC–MS/MS. Selecting of the cleanup adsorbent was systematically discussed using rice as the representative sample. Based on the optimum conditions, the method was validated regarding the linearity, sensitivity, precision and further applied for wheat and soybean samples analysis. The satisfactory results demonstrated its feasibility for multi-pesticides residue analysis in grain samples.

2. Experimental

2.1. Materials

50 pesticides and 8 related metabolites were purchased from the Agro-Environmental Protection Institute, Ministry of Agriculture (Tianjin, China) or Shanghai pesticide research institute (Shanghai, China). High performance liquid chromatography (HPLC) grade acetonitrile and methanol were purchased from Merck (New Jersey, USA). HPLC grade ammonium formate was from Tedia (Fairfield, USA). Sodium chloride, magnesium sulfate were all analytical reagent. C18 (50 μm), PSA (40–60 μm) were purchased from Agela Technologies Co. Ltd. (Tianjin, China). Purified water was obtained with a Millipore Milli-Q apparatus (Massachusetts, USA). Fe_3O_4-PSA was homemade with the particle size in the range of 531–955 nm. The detailed preparation procedure can refer to our previous report (Qi et al., 2015).

2.2. Sample extraction and purification

A 5 g grain sample was weighed into a 50 mL Teflon centrifuge tube, followed by addition of 5 g water and 10 mL acetonitrile for extraction. The mixture was vortexed for 1 min to ensure the solvent interact well with the sample. After adding anhydrous NaCl (1.5 g) and anhydrous $MgSO_4$ (4.0 g) to the above mixture, the sample was shaked vigorously for 1 min and centrifuged at 5000 r min^{-1} for 3 min using Thermo scientific biofuge Primo R centrifuge (Germany).

For the sample purification with magnetic adsorbent, 1 mL of the upper layer acetonitrile extracts was drawn into 2 mL centrifuge tube containing 30 mg Fe_3O_4-PSA, 10 mg C18 and 150 mg anhydrous $MgSO_4$. After shaking for 1 min, the samples were separated under outer magnetic field for 3 s. 0.5 mL of the supernatant was transferred into 2 mL centrifuge tube containing 0.5 mL water. The solution was filtered through 0.22 μm filter for LC–MS/MS analysis.

For comparison, the upper layer acetonitrile extracts were also purified by a traditional QuEChERS method with non-magnetic materials as adsorbent. 1 mL extract was added to 2 mL centrifuge tube containing 50 mg C18 and 50 mg PSA and 150 mg $MgSO_4$. The mixture was then shaken vigorously for 1 min and then centrifuged for 3 min at 7000 r min^{-1}. 0.5 mL of the supernatant was drawn into 2 mL centrifuge tube containing 0.5 mL water. The solution was filtered through 0.22 μm filter for LC-MS/MS analysis.

2.3. LC–MS/MS for determination of multi-pesticides

LC–MS/MS was used for determination of multi-pesticides residue in grain samples. It was performed on ultra high performance liquid chromatography LC-30A (Shimadzu, Kyoto, Japan) and AB 4500 triple-quadrupole mass spectrometer (ABSCIX Pte. Ltd., Massachusetts) with electro-spray ionization source (ESI). The chromatographic separation of the pesticides was performed on Waters BEH C18 (100 mm × 2.1 mm, 1.7 μm) analytical column (Waters Corporation, Massachusetts, USA). The mobile phase consisted of water and methanol (1:9, v/v), both methanol and water contained 5 mmol L^{-1} ammonium formate. The flow rate was kept at 0.25 mL min^{-1}. The sample volume was 2 μL. The column temperature was maintained at 40 °C. The tandem spectrometer was operated in the multiple reaction monitoring (MRM) mode. ESI-MS/MS detection was performed at positive ion mode for most of the pesticides, and negative mode for fluorine and its three metabolites. The MS parameters were as follows: ion spray voltage, 5500 V for positive ion mode and 4500 V for negative mode; temperature, 450 °C; ion source gas were air and collision gas was high purity nitrogen. All the gas was supplied by Peak Nitrogen (Peak Scientific, Scotland, UK). Each compound is determined by two pairs of parent ion and ion pairs. The MRM precursor ion, the product ions and the corresponding collision energy and declustering potential for all the pesticides were listed in Table S1.

3. Results and discussion

3.1. The choice of Fe_3O_4-PSA amount

Cleanup adsorbent plays an important role on achieving satisfactory analyte recoveries. It not only can remove the matrix co-extracted interference, but also can absorb target analytes in some degree, resulting in its low recoveries. In order to keep the balance between the analytes recovery and cleanup efficiency, the amount of Fe_3O_4-PSA was optimized.

Rice was selected as the typical sample for method optimization. 1 mL rice acetonitrile extract was spiked with pesticides at 100 μg L^{-1}, the extractant was transferred into 2 mL centrifuge tubes containing 150 mg $MgSO_4$ and different amounts of Fe_3O_4-

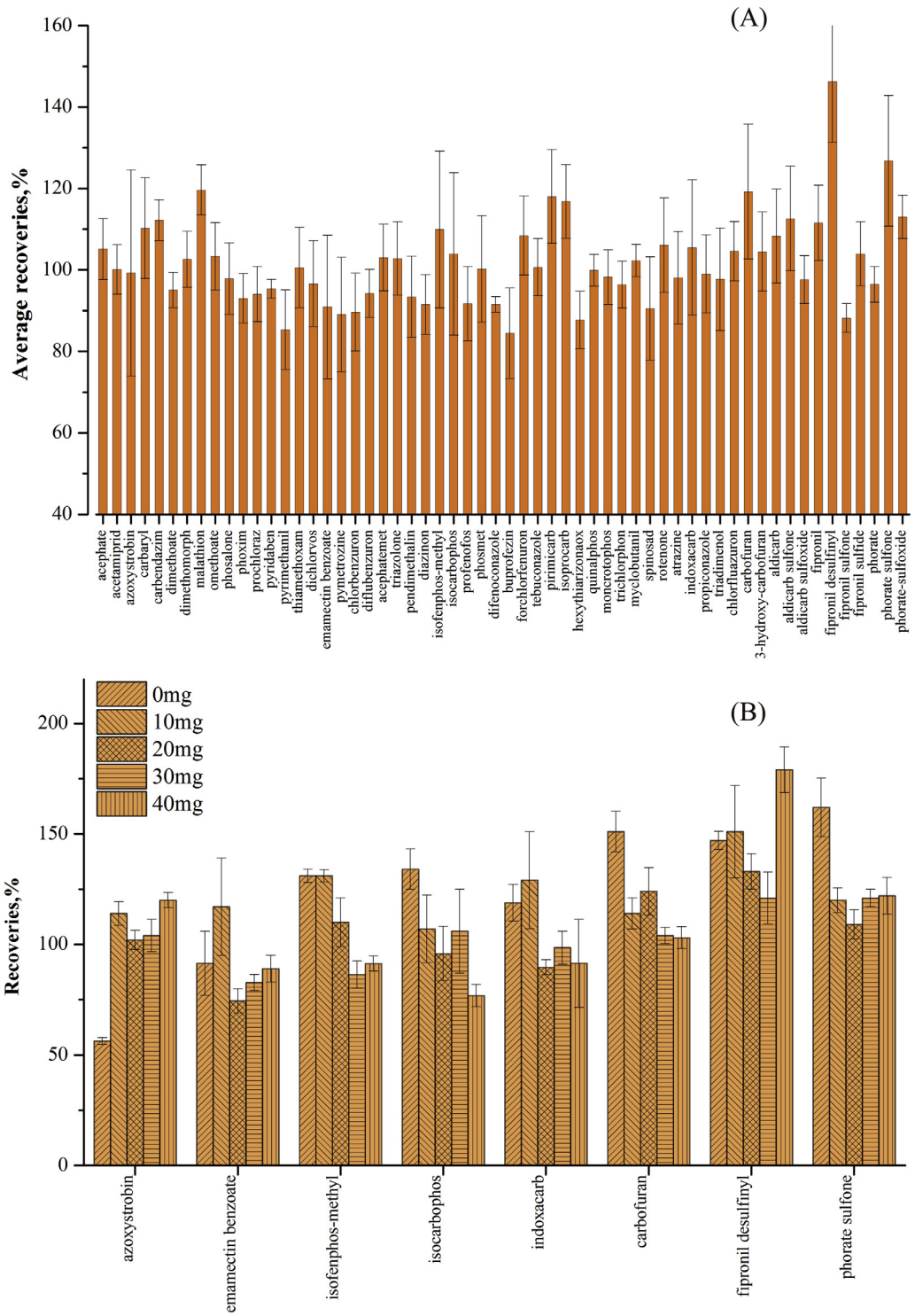

Fig. 1. (A) The average recovery and the corresponding RSD of each analyte based on its recovery value at different amount of Fe_3O_4-PSA; (B) The recoveries of the analytes which were largely affected by the Fe_3O_4-PSA amount.

PSA (0, 10, 20, 30, 40 mg). After shaking for 1 min, the sample solution was separated with the magnetic materials under the outer magnetic field. After LC–MS/MS analysis, the pesticides recoveries under different dosage of Fe_3O_4-PSA were calculated.

Liebig's law of the minimum tells us the importance of finding the key flaw among all the factors. Therefore, the first thing we need to do is find flaws. Relative standard deviation (RSD) is usually employed to evaluate the precision of the experiment. The lower RSD, the more reliable the result is. In the present experiment, the RSD is used to select the pesticides which are highly affected by the Fe_3O_4-PSA amount. For each pesticide, the average recovery and the corresponding RSD were calculated based on its recovery value at different amount of Fe_3O_4-PSA. The higher RSD for pesticide represents that the pesticide was highly influenced by the Fe_3O_4-PSA amount. As shown in Fig. 1A, it can be clearly seen that the average recovery of each pesticide was all higher than 80%. Generally, the recovery of each pesticide is required in the range of 70–130% with the RSD lower than 20% for pesticide

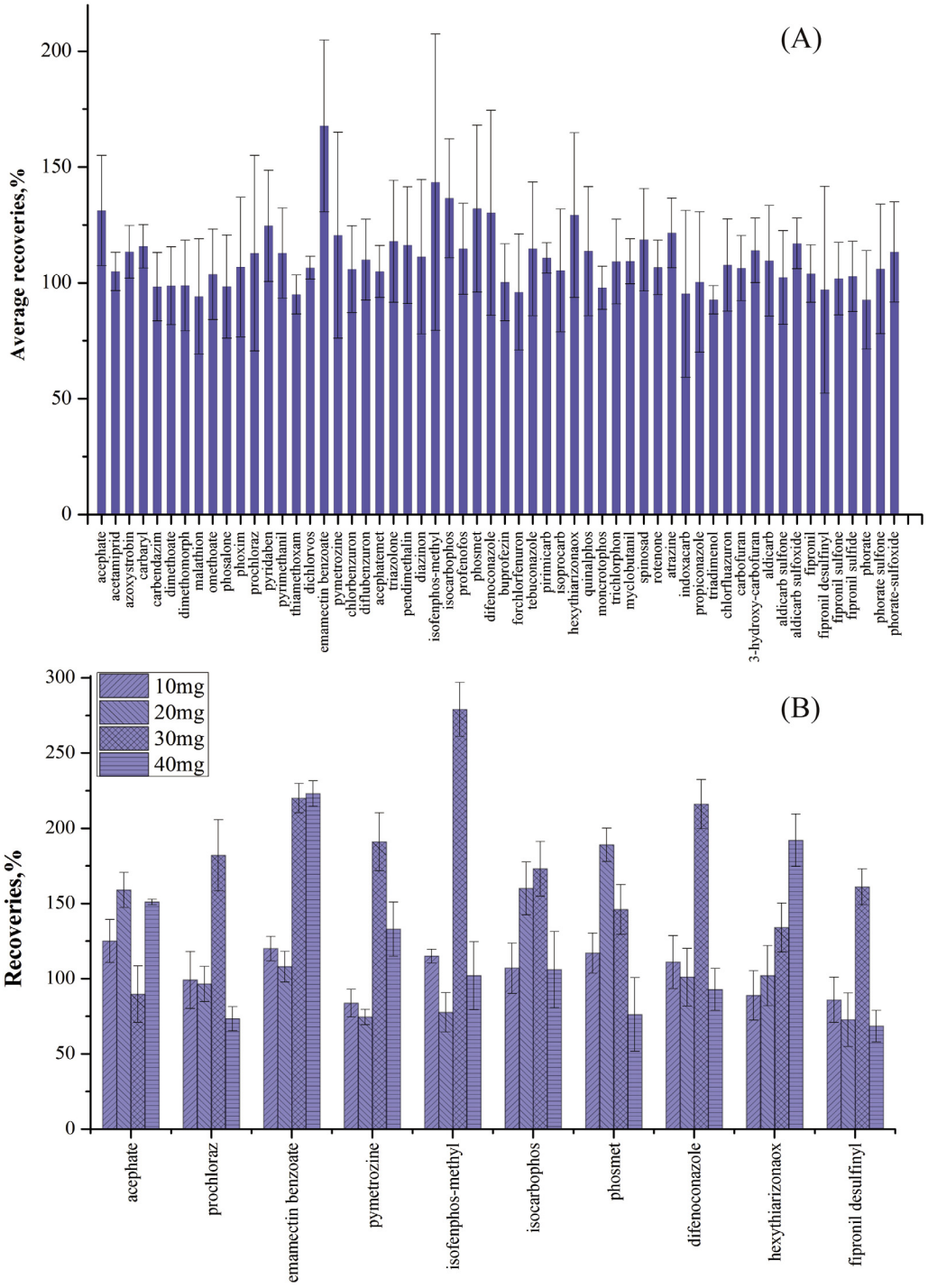

Fig. 2. (A) The average recovery and the corresponding RSD of each analyte based on its recovery value at different amount of C18; (B) The recoveries of the analytes which were largely influenced by the C18 amount.

Table 1
The method validation of the linearity, matrix effect, LODs and the recoveries of the analytes in rice spiked with different concentration (10 μg kg^{-1}, 100 μg kg^{-1}, 200 μg kg^{-1}).

Analytes	Y = ax + b[a]	R	Sloperatio[b]	Linear range, μg L^{-1}	LOD, μg L^{-1}	Recoveries		
						LOQ (10 μg kg^{-1})	10 LOQ (100 μg kg^{-1})	20 LOQ (200 μg kg^{-1})
Acephate	y = 18,412x + 245,536[c]	0.9986	0.23	2–250	1.17	82.2(14.7)	83.2(6.8)	89.9(13.2)
	y = 79,142x + 1,232,050[d]	0.9793		2–250				
Acephatemet	y = 18,037x − 22,203	0.9992	0.26	2–250	0.39	112(13.9)	89.7(14.3)	102(10.8)
	y = 69,668x + 3,119,620	0.9547		2–250				
Acetamiprid	y = 68,192x − 140,541	0.9995	0.56	2–250	0.38	85.5(8.3)	83.6(6.9)	86.0(9.8)
	y = 121,100x + 5,261,730	0.9440		2–250				
Aldicarb	y = 94,113x + 192,161	0.9940	0.72	2–250	0.27	104(4.0)	82.9(11.1)	104(9.0)
	y = 131,118x + 2,326,090	0.9732		2–250				
Aldicarb sulfone	y = 7477x + 29,570	0.9987	0.45	2–250	1.59	85.5(14.9)	86.6(12.2)	114(5.6)
	y = 16,696x + 884,917	0.9226		2–250				
Aldicarb sulfoxide	y = 26,361x − 45,961	0.9988	0.35	2–250	0.24	84.5(9.3)	83.7(11.7)	89.6(9.1)
	y = 75,976x + 1,459,020	0.9690		2–250				
Atrazine	y = 10,2971x + 471,320	0.9923	0.83	2–250	0.46	106(8.7)	91.7(12.1)	93.8(2.6)
	y = 124,113x + 2,372,890	0.9785		2–250				
Azoxystrobin	y = 117,238x − 8659	0.9908	0.94	2–250	0.29	105(13.3)	90.3(9.8)	97.0(5.5)
	y = 124,585x + 2,012,320	0.9796		2–250				
Buprofezin	y = 142,058x − 511,678	0.9959	0.81	2–250	0.32	94.0(8.5)	88.4(8.9)	83.3(4.3)
	y = 176,285x + 1,192,790	0.9928		2–250				
Carbaryl	y = 78,042x + 152,964	0.9917	0.82	2–250	0.34	92.5(3.0)	83.2(14.3)	93.7(9.3)
	y = 95,114x + 1,695,390	0.9794		2–250				
Carbendazim	y = 4,833x + 1,985,770	0.9908	0.07	5–250	0.52	102(14.2)	84.2(10.5)	99.0(3.9)
	y = 68,134x + 2,969,460	0.9526		5–250				
Carbofuran	y = 14,3716x + 2,196,430	0.9621	0.92	2–250	0.39	105(10.1)	84.3(13.9)	107(12.5)
	y = 156,967x + 7,895,810	0.9174		2–250				
3-Hydroxy-carbofuran	y = 4,358x − 15,527	0.9992	0.46	2–250	1.43	121(12.0)	87.7(8.8)	91.2(13.1)
	y = 9,461x + 344,041	0.9463		2–250				
Chlorbenzuron	y = 68,319x − 98,819	0.9933	1.05	2–250	0.38	123(11.5)	87.1(14.0)	92.4(7.7)
	y = 65,130x + 1,061,690	0.9839		2–250				
Chlorfluazuron	y = 168,874x − 118,879	0.9975	1.18	2–250	0.27	110(4.2)	82.6(8.0)	93.2(11.1)
	y = 143,601x + 3,754,430	0.9934		2–250				
Diazinon	y = 122,172x − 291,719	0.9945	0.88	2–250	0.36	104(2.3)	102(14.5)	89.1(6.4)
	y = 139,412x + 1,621,140	0.9966		2–250				
Dichlorvos	y = 18,318x + 20,230	0.9966	0.71	2–250	1.26	84.6(12.3)	82.1(15.0)	84.8(9.4)
	y = 25,831x + 354,563	0.9901		2–250				
Difenoconazole	y = 45,764x − 36,095	0.9930	0.80	2–250	0.27	94.1(7.7)	83.7(7.8)	92.5(11.0)
	y = 56,989x + 675,794	0.9877		2–250				
Diflubenzuron	y = 66,996x − 266,215	0.9971	0.87	2–250	0.32	116(7.2)	85.8(13.3)	97.7(2.7)
	y = 76,741x + 743,888	0.9947		2–250				
Dimethoate	y = 98,348x − 105,865	0.9986	0.61	2–250	1.16	94.0(2.0)	89.0(11.6)	87.0(11.7)
	y = 161,888x + 6,600,810	0.9400		2–250				
Dimethomorph	y = 166,172x + 234,233	0.9920	0.87	2–250	0.34	97.4(14.4)	84.6(7.5)	95.2(5.2)
	y = 190,101x + 3,166,420	0.9883		2–250				
Emamectin benzoate	y = 5,405x − 34,045	0.9900	0.32	2–250	0.34	97.5(13.1)	89.1(15.0)	112(14.8)
	y = 16,804x + 67,616	0.9974		2–250				
Fipronil	y = 132,350x − 788,914	0.9983	1.16	2–250	0.39	102(2.6)	87.9(3.8)	90.5(7.8)
	y = 113,850x + 2,608,530	0.9928		2–250				
Fipronildesulfinyl	y = 159x + 1236	0.9972	1.18	2–250	1.65	107(11.2)	122(12.4)	106(12.4)
	y = 135x + 4389	0.9929		2–250				
Fipronilsulfide	y = 4,222x − 28,188	0.9977	1.14	2–250	0.92	105(8.9)	85.5(4.5)	102(6.6)
	y = 3704x + 54,354	0.9989		2–250				
Fipronil sulfone	y = 32,394x − 264,629	0.9970	1.04	2–250	0.79	107(6.2)	84.1(4.5)	96.9(10.9)
	y = 31,058x − 7559	0.9999		2–250				
Forchlorfenuron	y = 9,992x + 69,626	0.9926	0.96	2–250	0.28	85.1(10.2)	92.5(11.6)	84.1(6.5)
	y = 10,436x + 272,039	0.9701		2–250				
Hexythiarizonaox	y = 144,641x − 659,411	0.9950	1.04	2–250	0.34	101(9.6)	85.9(9.7)	89.2(14.4)
	y = 139,338x + 973,968	0.9907		2–250				
Indoxacarb	y = 16,468x − 72,388	0.9925	0.85	2–250	0.25	109(11.5)	84.8(13.8)	95.7(9.9)
	y = 19,333x + 98,851	0.9997		2–250				
Isocarbophos	y = 3259x − 717	0.9914	0.95	2–250	0.89	125(3.9)	82.9(13.1)	103(8.9)
	y = 3,434x + 55,153	0.9803		2–250				
Isofenphos-methyl	y = 54,633x − 61,940	0.9905	0.74	2–250	0.33	107(9.1)	93.7(11.2)	106(4.8)
	y = 73,735x + 9,721,280	0.9965		2–250				
Isoprocarb	y = 50,187x + 227,035	0.9912	0.93	2–250	0.34	111(10.6)	84.1(9.9)	96.0(10.2)
	y = 54,153x + 1,368,460	0.9590		2–250				
Malathion	y = 80,962x + 537,778	0.9940	0.77	5–250	1.70	109(15.0)	88.9(7.2)	92.2(8.4)
	y = 104,749x + 4,392,090	0.9842		5–250				
Moncrotophos	y = 46,112x − 68,494	0.9999	0.42	2–250	0.44	99.7(7.4)	83.6(10.5)	86.5(1.9)
	y = 108,894x + 3,555,150	0.9523		2–250				
Myclobutanil	y = 42,593x + 10,374	0.9906	0.89	2–250	0.37	102(4.7)	88.9(13.8)	88.5(14.9)
	Y = 47,979x + 1,232,760	0.9748		2–250				
Omethoate	y = 80,923x + 129,163	0.9995	0.40	2–250	0.54	82.6(7.2)	87.6(5.4)	82.0(5.5)
	y = 201,305x + 6,601,480	0.9564		2–250				

(*continued on next page*)

Table 1 (*continued*)

Analytes	Y = ax + b[a]	R	Sloperatio[b]	Linear range, μg L^{-1}	LOD, μg L^{-1}	Recoveries		
						LOQ (10 μg kg^{-1})	10 LOQ (100 μg kg^{-1})	20 LOQ (200 μg kg^{-1})
Pendimethalin	y = 81,006x − 472,296	0.9958	0.87	2–250	0.44	117(2.5)	84.5(12.5)	88.0(13.0)
	y = 92,608x − 105,967	0.9998		2–250				
Phorate	y = 13,179x − 104,960	0.9950	1.02	2–250	0.32	118(14.4)	91.2(12.7)	107(13.1)
	y = 12,952x + 55,868	0.9974		2–250				
Phorate sulfone	y = 82,127x + 3656	0.9923	0.10	2–250	0.66	100(3.7)	96.4(13.1)	107(4.8)
	y = 803,715x + 2,255,510	0.9903		2–250				
Phorate sulfoxide	y = 148,638x + 875,814	0.9909	0.88	2–250	0.71	106(12.8)	85.1(11.0)	102(10.3)
	y = 169,503x + 4,320,740	0.9749		2–250				
Phosalone	y = 118,371x − 256,786	0.9916	0.96	2–250	0.31	109(3.2)	88.1(12.8)	84.9(7.8)
	y = 122,828x + 1,619,390	0.9975		2–250				
Phosmet	y = 105,868x − 275,244	0.9910	3.60	2–250	0.34	93.1(11.6)	84.8(13.6)	90.6(7.7)
	y = 29,365x − 306,365	0.9956		2–250				
Phoxim	y = 137,618x − 173,556	0.9909	0.96	2–250	0.63	104(11.7)	89.3(11.8)	88.6(13.8)
	y = 142,944x + 3,010,150	0.9951		2–250				
Pirimicarb	y = 35,851x + 176,251	0.9943	0.82	2–250	0.26	97.7(5.0)	83.8(8.1)	95.0(2.4)
	y = 43,964x + 727,984	0.9806		2–250				
Prochloraz	y = 26,323x + 44,754	0.9912	0.83	2–250	0.38	106(14.1)	83.9(12.7)	90.5(4.1)
	y = 31,552x + 390,072	0.9935		2–250				
Profenofos	y = 74,298x + 33,879	0.9918	0.67	2–250	0.39	114(11.2)	86.0(8.4)	82.8(6.1)
	y = 110,238x + 1,053,410	0.9876		2–250				
Propiconazole	y = 15,332x + 17,733	0.9937	0.85	2–250	0.28	94.1(3.0)	105(9.8)	83.6(12.4)
	y = 18,119x + 276,274	0.9982		2–250				
Pymetrozine	y = 24,764x + 16,524	0.9995	0.40	2–250	0.29	108(8.9)	88.2(11.5)	121(14.9)
	y = 61,694x + 1,441,460	0.9782		2–250				
Pyridaben	y = 137,618x − 173,556	0.9909	0.39	2–250	2.05	96.4(14.8)	83.4(11.6)	92.1(10.4)
	y = 352,971x + 6,982,080	0.9903		2–250				
Pyrimethanil	y = 51,437x − 175,879	0.9971	0.93	2–250	0.42	118(11.6)	88.6(9.3)	87.7(3.3)
	y = 55,443x − 140,301	0.9990		2–250				
Quinalphos	y = 88,871x − 120,179	0.9958	0.87	2–250	0.39	94.3(1.3)	87.9(12.4)	83.5(8.6)
	y = 101,954x + 2,002,980	0.9924		2–250				
Rotenone	y = 46,081x − 122,220	0.9941	0.92	2–250	0.35	95.6(1.1)	82.2(3.5)	85.6(9.1)
	y = 50,051x + 113,590	0.9991		2–250				
Spinosad	y = 105,992x − 150,779	0.9999	1.05	2–250	0.26	114(14.2)	83.0(11.8)	94.5(4.1)
	y = 101,197x + 11,656	0.9999		2–250				
Tebuconazole	y = 72,700x + 207,302	0.9911	0.90	2–250	0.37	90.8(12.2)	82.8(9.5)	98.5(5.8)
	y = 80,793x + 965,166	0.9915		2–250				
Thiamethoxam	y = 21,025x − 32,783	0.9993	0.48	2–250	0.51	84.7(3.7)	93.5(14.7)	94.5(11.2)
	y = 43,380x + 2,427,600	0.9063		2–250				
Triadimenol	y = 67,388x − 282,507	0.9986	0.80	2–250	0.75	103(7.0)	84.4(9.0)	99.6(9.4)
	y = 84,625x + 966,798	0.9941		2–250				
Triazolone	y = 60,105x − 146,892	0.9953	0.88	2–250	0.41	93.9(10.8)	82.5(10.4)	99.7(3.4)
	y = 67,988x + 694,236	0.9902		2–250				
Trichlorphon	y = 20,879x + 12,021	0.9992	0.65	2–250	0.67	97.7(11.8)	94.6(12.8)	86.8(5.0)
	y = 32,191x + 902,017	0.9584		2–250				

[a] Calibration curves are expressed as regression lines (y = a x + b), where y is the integrated peak area and x is the concentration (μg L^{-1}), a is the slope, b is the intercept and r is the correlation coefficient.
[b] The value of slope ratio is calculated by the slope of the matrix-matched calibration curve to the slope of the standard calibration curve in solvent.
[c] The first calibration curve for each pesticide represents the matrix-matched calibration curve.
[d] The second calibration curve for each pesticide represents the standard calibration curve in solvent.

residue analysis. Herein, the pesticides which average recoveries are higher than 130% or the RSDs are higher than 15% were selected and shown in Fig. 1B.

Without the usage of Fe_3O_4-PSA, the recovery of azoxystrobin was 56.3%, and the recoveries of phorate sulfone, carbofuran and fipronil desulfinyl were as high as 163%, 151% and 148%, respectively. This is attributed to the existence of matrix interference. The usage of Fe_3O_4-PSA made the pesticides recoveries range from 74.4% to 136% except fipronil desulfinyl. This metabolite of fipronil can obtain a relatively better recovery at 30 mg Fe_3O_4-PSA. Under other conditions, its recoveries were all too high to be accurately quantified. For the other seven analytes, their recoveries were in the range of 82.7–122% with the amount of Fe_3O_4-PSA at 30 mg. This result demonstrated that Fe_3O_4-PSA could remove the impurities and thus improved the accuracy of the analysis. The amount of Fe_3O_4-PSA can obviously influence the accuracy of pesticides analysis, and 30 mg Fe_3O_4-PSA was selected for the further experiment.

3.2. Influence of C18 amount on the pesticides recoveries

C18 is normally used as dispersive adsorbent in traditional QuEChERS method (Correia-Sá, Fernandes, Calhau, Domingues, & Delerue-Matos, 2012; Georgakopoulos et al., 2011; Gilbert-López, García-Reyes, Lozano, Fernández-Alba, & Molina-Díaz, 2010). As reported, C18 is a reversed phase sorbent, which is effective at trapping and removing starch and sugar from rice samples and gives rise to the cleanest extract (Koesukwiwat, Sanguankaew, & Leepipatpiboon, 2008; Pareja et al., 2011). But C18 can also absorb the relatively weaker polar pesticides. Therefore, it is needed to optimize the amount of C18 to achieve good recovery and cleanup efficiency.

1 mL acetonitrile extract was firstly spiked with each pesticide at the concentration of 100 μg L^{-1}. The spiked extract was then placed into 2.0 mL centrifuge tubes which containing 150 mg anhydrous $MgSO_4$, 30 mg Fe_3O_4-PSA and different amount of C18

(10, 20, 30, 40 mg). The samples were purified, separated and determined by LC–MS/MS. The RSDs were used again for finding the limit factor (Fig. 2A). Obviously, the average recoveries of the pesticides are higher than 92.6% with the highest recovery of emamectin benzoate at 168%. This result displayed that too higher recoveries is the problem for addition of C18. The pesticides which average recoveries are higher than 130% or the RSDs are higher than 30% were selected and shown in Fig. 2B.

From Fig. 2B, it can be clearly seen that the amount of C18 seriously affected the pesticides recoveries. When its amount was set at 20, 30 or 40 mg, too higher recoveries are a general phenomenon. Of course, the low recoveries also existed. Overall, when the amount of C18 was 10 mg, the ten pesticides recoveries ranging from 83.7% to 125%. Therefore, the amount of C18 was set at 10 mg.

3.3. Method validation

The developed method was validated by evaluating the linearity, sensitivity, precision and matrix effects. For the linearity, both solvent and matrix-matched calibration curves were constructed by plotting the peak area versus concentration. The concentration of the analytes was set at 2, 5, 10, 25, 50, 100 and 250 μg L^{-1}. As demonstrated in Table 1, the analytes displayed good linearity between 2 and 250 μg L^{-1} for most of the pesticides and between 5 and 250 μg L^{-1} for carbendazim and malathion. Their correlation coefficient varied from 0.9174 to 0.9999. The LODs were determined to be the concentration that was the three times of the signal to noise ratio. The results demonstrated that the LODs ranged from 0.24 to 2.05 μg L^{-1} (Table 1).

The LOQs were determined based on the European Commission guidance document SANTE/11945/2015. The LOQs was required to be validated by recovery test. It is the lowest spiked level that met demand of the method performance for accuracy and precision. In the present work, the lowest spiked concentration was 10 μg kg^{-1}, the recoveries of all the pesticides were in the range of 82.2–125% (Table 1), indicating that this method can meet monitoring requirements for the grain samples under trace concentration level. Besides, related regulations on maximum residue limit (MRL) of the pesticides were consulted. It was proved that the MRLs of all the pesticides were not lower than 10 μg kg^{-1}. Therefore, LOQs were set at 10 μg kg^{-1} for all the pesticides in the present method.

Matrix effect is evaluated by the slope ratio of the matrix-matched calibration curve to the solvent standard calibration curve (Gosetti, Mazzucco, Zampieri, & Gennaro, 2010). If the slope ratio value is 1, there is no matrix effect; if the ratio value is lower than 1, it represents signal suppression; and if the ratio value is higher than 1, it means signal enhancement. As displayed in Table 1, the slope ratio ranged from 0.07 to 3.6, demonstrating the existence of matrix effect. However, for about 60% of the pesticides, there is no obvious matrix effect with the slope ratio ranging from 0.8 to 1.18. Regarding the other pesticides, ion suppression was widely existed, especially for carbendazim (0.07), phorate sulfone (0.1), acephate (0.23), methamidophos (0.26) and so on. In contrast, ion enhancement was only found in phosmet (3.60) analysis. Therefore, the matrix-matched standard calibration curve was used to calculate the pesticide concentration.

The pesticides were spiked in rice samples under the concentration levels of 10, 100 and 200 μg kg^{-1}. The recoveries and the corresponding RSDs were displayed in Table 1. From the results, it can be clearly seen that the recoveries for all the pesticides ranged from 82.0% to 125% with the RSDs lower than 15.0%. The results verified the feasibility of the developed methods.

3.4. Comparison with the conventional QuEChERS method

As described above, the advantage of the present method is easy operation and rapid separation. Hence, the comparative experiment was performed with the conventional QuEChERS method, in which the non-magnetic materials were used as dispersive adsorbent. The commercial cleanup adsorbents combination of 50 mg PSA, 50 mg C18 and 150 mg $MgSO_4$ was employed. The present magnetic method contained 30 mg Fe_3O_4-PSA, 10 mg C18 and 150 mg $MgSO_4$.

The pesticides were spiked in blank rice samples at the concentration of 100 μg kg^{-1}. After the extraction with acetonitrile, 1 mL supernatant was drawn for cleanup with two approaches. The recovery distribution of the pesticides by the two approaches was illustrated in Fig. 3. It can be obviously seen that the recoveries of the pesticides purified by the magnetic adsorbent focused in the range of 80–100%. The pesticides purified by the non-magnetic adsorbent had a wide recovery distribution between 40% and 130%. This result can display that the present developed method can give rise to a more stable and better recoveries for the pesticides in grain samples.

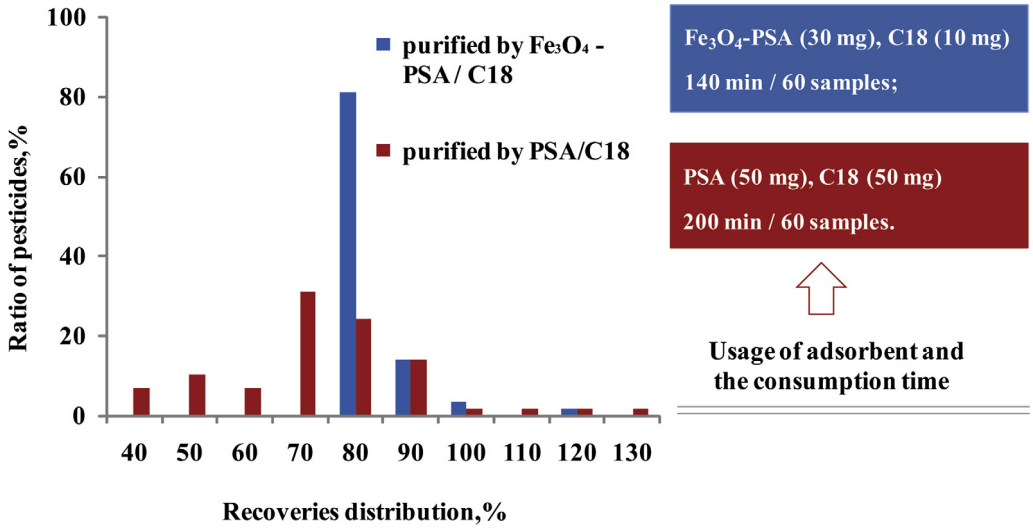

Fig. 3. Comparison of the present method with the purification method with non-magnetic PSA / C18 as adsorbent.

Regarding the amounts of the cleanup adsorbent, the present method seems more economical due to the fewer amounts of reagents than the conventional QuEChERS method. Besides, the sample pretreatment consumption time was compared for simultaneous analysis of sixty samples by the two approaches. The result was also shown in Fig. 3. It can be seen that 140 min was needed for sample pretreatment of sixty samples, while 200 min was necessary for the conventional QuEChERS method with non-magnetic adsorbent. This result demonstrated that the present developed method, which does not include time needed for preparation of nanoparticles Fe_3O_4-PSA, can save at least 30% time in the sample pretreatment procedure, fully exhibiting its advantage in improving the operation efficiency and giving a new approach for high-throughput sample pretreatment technique.

3.5. Application to the other kinds of grains

Under the optimized conditions, the developed method was applied to the analysis of the pesticide residues in wheat and soybean samples. The pesticides of 58 target analytes were spiked at 100 μg kg^{-1}. As listed in Table S2, the recoveries of the analytes in wheat and soybean samples were ranged from 71.2% to 127% with the RSDs less than 17.8%. In contrast, using the conventional QuEChERS method, the recoveries of the analytes in wheat and soybean samples ranged from 47.7% to 132% with the RSDs less than 20.0%. These results are in accord with the pesticides recoveries distribution in rice, fully displaying the advantage of the present method. It also demonstrated its feasibility in analysis of pesticides residue in grain samples.

4. Conclusions

The present work aimed at developing a facile and rapid method for the multi-pesticide residue analysis in grain samples. Magnetic Fe_3O_4-PSA and the commercial C18 were collectively used as cleanup adsorbents to remove the interferences in grain samples. The results displayed that the optimized methods can give rise to a satisfactory recoveries for all the analytes in grains under different fortification concentration level. The more important to be mentioned, the advantage of the present method is easy operation and high efficiency owing to the use of magnetic material. Compared with the conventional method, the magnetic separation is ten times quicker for one batch samples without extra large equipment. Consequently, the present developed method can save at least 30% time in the sample pretreatment procedure, fully exhibiting its advantage in improving the operation efficiency and giving a new approach for high-throughput sample pretreatment technique.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2017.03.082.

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