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Simultaneous determination of 58 pesticides and relevant metabolites in eggs with a multi-functional filter by ultra-high performance liquid chromatography-tandem mass spectrometry

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ABSTRACT

A sensitive method for the simultaneous determination of 58 pesticides and relevant metabolites in eggs was developed using ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) after clean-up with a multi-functional filter (MFF) based on quick, easy, cheap, effective, rugged, and safe method (QuEChERS). The egg sample was extracted with 5 ml water and 10 ml 1% acetic acid in acetonitrile and then salt out with sodium chloride. The extracted solution was filtered directly through an MFF containing 50 mg PSA, 50 mg C18, and 150 mg magnesium sulphate before UHPLC-MS/MS analysis. The clean-up and filter procedures were integrated using the MFF to substantially improve the work efficiency. Good linearity was shown for each analyte, and all the correlation coefficients exceeded 0.99. The recoveries in the eggs at the five spiked levels were 74.4%–115.2%, and the relative standard deviations (RSDs) were less than 15.3%. The limit of detection (LOD) and the limit of quantitation (LOQ) of 58 pesticides and 8 metabolites in eggs were 0.1–1.0 μg/kg and 0.2–5.0 μg/kg, respectively. The decision limit (CCα) and detection capacity (CCβ) were 3.4–111.1 μg/kg and 6.8–122.1 μg/kg, respectively. This method has also been successfully applied in the determination of actual eggs samples. This developed method is more effective and faster in the monitoring of pesticide residue in eggs compared to the traditional analytical method.

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1. Introduction

Over the past two decades, eggs have represented the largest increase in protein demand among the world's population. China is one of the largest countries in producing and consuming eggs in the world. In addition to the affordable price, eggs are a good source of energy due to their rich nutrition. Eggs are a food with a wide range of edibility, consumed by people from four to five months old to older people. In addition, eggs can be added as a raw material to many foods. With the increasing production and consumption of eggs, their safety and quality has been regarded. At present, the detection of drugs in eggs is mainly focused on antibiotics and other veterinary drugs [1], but determination of pesticide residues is rare. Although pesticides are less used in livestock, they can enter poultry by other ways, then producing pesticide residues in eggs. In August 2017, the European food safety regulatory authority stated that fipronil was detected in eggs in an outbreak in the Netherlands. Fipronil is used in the prevention and treatment of pests on vegetables and other crops. It is forbidden in China, and high-dose intakes can cause liver, thyroid and kidney system damage [2]. Most astonishingly, eggs containing fipronil were sold to people, so causing a panic. Hence, people started to attach importance to pesticide residues in eggs.

Pesticides can be transported and circulated in the environment through transportation by the atmosphere, water, soil and other media [3]. Bioaccumulation through the food chain is easy, leading to chronic poisoning of the final recipient organism [4]. Food intake is the predominant route of pesticide exposure. The quality and safety of eggs is closely related to their production and processing. Although the European Union (EU) has banned the use of some pesticides, there are still cases of animal poisoning [5,6]. Poultry inhale pesticide-contaminated air or ingest contaminated soil and

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feed. This process produces eggs contaminated with drug residues, which can be hazardous to human health [7–9].

There is an unavoidable problem to solve in the technical bottleneck of pretreatments that require large amounts of time. We need a pretreatment method that saves time and improves the efficiency of extraction and purification. At present, the pretreatment methods that have been reported and widely used are mainly the QuEChERS method [10,11], solid-phase extraction (SPE) [12] and solid-phase micro-extraction (SPME) [13]. Of these methods, QuEChERS has been increasingly applied for the preparation of animal-derived food samples [14,15]. Detection methods include capillary gas chromatography (GC) [16,17], gas chromatography-mass spectrometry (GC–MS) [18] and UHPLC–MS/MS [19]. Among these detection methods, mass spectrometry is simple, rapid, and has a high sensitivity and wide detection range. It can be used for qualitative and quantitative determination of pesticide residues due to its good stability and reproducibility.

At present, there are relatively few reports on the detection of multiple pesticides in eggs. It was reported in the literature that ten to forty-two pesticides and herbicides were detected in eggs with liquid chromatography tandem mass spectrometry (LC–MS/MS) and GC–MS [20–23]. However, these methods have a long pretreatment time, the adsorbent still needs to be weighed by hand, and the methods detect fewer kinds of pesticides. Above all, to prevent pesticides abuse, ensure the safety of agricultural inputs and reduce the risks associated with egg safety, it's of great importance to establish a method for detecting pesticides in eggs. In this study, we developed a rapid, simple and reliable method for detection of 58 pesticides and relevant metabolites in eggs by a MFF based on QuEChERS and UHPLC-MS/MS analysis.

2. Materials and methods

2.1. Materials and chemical reagents

The mixed standard solutions of 58 compounds including 50 pesticides and 8 relevant metabolites were purchased from Alta Scientific Co. Ltd. (Tianjin, China). Chromatographic-grade acetonitrile (>99.5%) was purchased from Fisher Scientific (Fair Lawn, NJ, USA), and MS-grade methanol was obtained from Merck (Darmstadt, Germany). Acetic acid (>99%) and ammonium formate (>99%) were supplied by Sigma-Aldrich (St. Louis, MO, USA). Ultra-pure water was provided by a Milli-Q purification apparatus (Millipore Direct-Q UV, Bedford, MA, USA). Clean-up was performed using a QUICLEAR multi-function needle filter (MFF) 3202 developed by ourselves and produced by Alta Scientific Co. Ltd. The MFF 3202 contained 50 mg PSA, 50 mg C18, and 150 mg magnesium sulphate, along with a 0.22 μm filter. In addition, sodium chloride was also used. (≥99.5%, Sinopharm Chemical Reagent Co. Ltd, China).

2.2. Standard preparation

The mixed standard solutions contained 58 compounds was preserve in brown bottles and stored at −20 °C. Working standard solutions were diluted with acetonitrile to the concentrations of 10 μg/ml, 1 μg/ml and 0.1 μg/ml.

2.3. Sample preparation

5 g (accurate to 0.01 g) homogenized egg sample was weighed in a 50 ml centrifuge tube. The analytes were extracted with 5 ml water and 10 ml 1% acetic acid in acetonitrile, and the tube was mixed for 1 min, then ultrasonicated for 10 min. 3 g sodium chloride (NaCl) was added to the tube then vortexed for 1 min. After centrifugation at 4500 rpm for 10 min at 4 °C, 1 ml of the supernatant was directly filtered through the MFF 3202 (allowing the liquid to drip out) before UHPLC-MS/MS analysis.

Table 1
Gradient elution program.

Time (min)	Volume fraction(V/V)%	
	A	B
initial	95	5
1	60	40
3	20	80
5	5	95
7	5	95
7.01	95	5
9	95	5

2.4. UHPLC–MS/MS analysis

The UHPLC-MS/MS analysis was performed on a Waters ultra-performance liquid chromatography system (UHPLC) connected to a Triple Quad™ 3500 Instrument (AB SCIEX, USA). MultiQuant™ 3.0.2 Software (AB SCIEX, USA) was used for data acquisition and analysis.

The 58 target compounds were separated on an Eclipse XDB-C18 column (3.5 μm, 2.1 × 150 mm, Agilent). The column temperature was 30 °C. The mobile phase consisted of 5 mM ammonium formate solution in water (A) and 5 mM ammonium formate in methanol (B). The mobile phase gradient elution programme is listed in Table 1.

The electrospray ion source (ESI) was operated in positive and negative-ion mode with multiple reaction ion monitoring (MRM). In addition to fipronil and its metabolites detected in negative ion scanning mode, all others were detected in positive ion scanning mode. The instrument conditions were as follows: ion-spray voltage (IS), 5.5 kV (ESI^+) and -4.5 kV (ESI^-); ion source temperature, 500 °C; curtain gas (CUR), 30 psi; collision gas (CAD), 8 psi; ion source gas 1.45 psi; ion source gas 2.60 psi.

2.5. Method validation

The method validation was conducted with mainly reference to the SANTE/11813/2017 [24], involving in investigation of parameters such as linearity, selectivity, LOD and LOQ, accuracy and precision. Moreover, the CCα and CCβ were also validated according to the European Commission Decision 2002/657/EC [25].

In this study, two transition ions were monitored for each analyte. The retention time of the analyte in the sample solution were corresponded to that of the calibration standard with a tolerance of ± 0.1 min. The average ion ratio and RSDs of each concentration point in solvent standard curve were calculated, and compared with the ion ratio of five fortified level in the samples to the reference ion ratio under the same conditions. The absolute value results were < 23.6% (Table S1), which met the SANTE/11813/2017 requirement that ion ratio from sample extracts should be within ±30% (relative) of average of calibration standards from same sequence.

The selectivity was verified by analysing blank samples from different sources to assess endogenous interference. The results showed that there was no obvious interference in the chromatograms of the blank samples. Seven concentration gradients were used to configure the standard solution curve and determine the linearity. The accuracy and reproducibility of this method were determined by the recoveries and RSDs. The LODs were defined as spiked concentrations that produced the signal-to-noise ratio (S/N) of 3, and LOQs as spiked concentrations with S/N of 10 under the acceptable accuracy and precision.

The matrix effects (MEs) were calculated from the slope ratio of the matrix calibration curve and standard solution calibration curve. The MEs were calculated as follows: MEs (%) = (b_{matrix} / $b_{solvent}$) × 100, where b_{matrix} represents the slope of the matrix and $b_{solvent}$ represents the slope of the solvent. If the value provided by

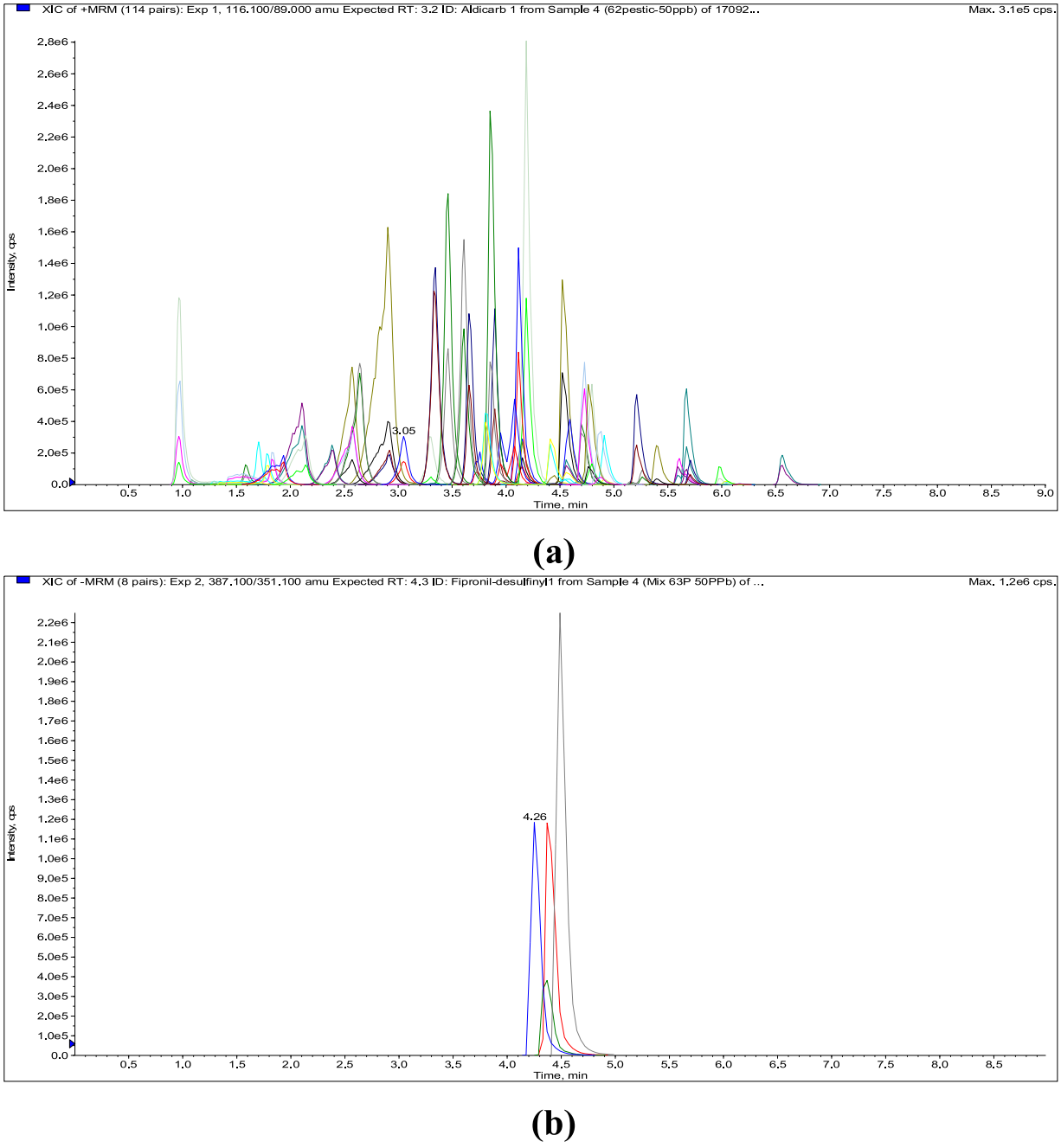

Fig. 1. Typical chromatograms of targeted pesticides by UHPLC-MS/MS in positive ion mode at 50 μg/L (a) and of fipronil and its metabolites in negative ion mode at 50 μg/L (b).

this calculation is between 80% and 120%, it indicates that there is nearly no signal suppression or enhancement.

3. Results and discussion

3.1. Instrumental optimization

Methanol was chosen as the organic phase in this study because it could increase the peak area response of most pesticides. Due to the large individual differences in pesticides, the solution pH may affect their selectivity, peak shape and retention time. Pesticides are mostly acidic analytes. Therefore, ammonium formate should be used to prevent the analytes from ionizing. Under these conditions, good peak shape and higher detection sensitivity can be obtained.

The Eclipse XDB-C18 column offered high performance over a wide pH range and enabled the separation of 58 compounds. The optimized chromatograms for the 58 pesticides and relevant metabolites are shown in Fig.1. Optimization of the mass spectrometry conditions included the precursor ions, product ions, declustering potential (DP), and collision energy (CE), analysed by direct injection of pesticide standard solutions. The characteristic ions with high signal intensities, stable responses and large mass-to-charge ratios were selected as precursor ions (positive ion mode is preferred over hydrogenation or ammonium addition), then the productions were determined. Compound ion pair information and related parameters obtained in the MRM scanning mode are described in Table 2.

Table 2
Retention times and MRM conditions for UHPLC-MS/MS analysis.

Analyte	Retention time (min)	Q1 ion (m/z)	Q3 ion (m/z)	Declustering potential (V)	Collision energy (V)
3-Hydroxycarbofuran	2.59	238	181	65	14
	2.59	238	163	65	20
Acephate	1.77	184	143	50	10
	1.77	184	125	50	26
Acetamiprid	2.64	223	126	70	27
	2.64	223	99	70	47
Aldicarb	3.18	116.1	89	47	10
	3.18	116.1	70	47	10
Aldicarb sulfone	2.05	223	158	63	12
	2.05	223	76.1	63	10
Aldicarb sulfoxid	1.92	207	132	51	10
	1.92	207	89	51	20
Avermectin b1a	6.2	895.4	751.3	120	60
	6.2	895.4	449.2	120	60
Azoxystrobin	3.93	404.1	372	70	20
	3.93	404.1	344.1	70	34
Carbaryl	3.55	202.1	145	54	15
	3.55	202.1	127	54	40
Carbendazim	2.95	192	160	80	25
	2.95	192	132	80	41
Carbofuran	3.4	222.1	165	70	17
	3.4	222.1	123.1	70	29
Chlorantraniliprole	3.84	584	286	60	35
	3.84	584	453	60	35
Chlorbenzuron	4.62	309	156	75	20
	4.62	309	139	75	44
Chlorfluazuron	5.73	540	383	80	27
	5.73	540	158	80	24
Chlorpyrifos	5.62	350	198	82	29
	5.62	350	97	82	49
Diazinon	4.77	305	169	80	27
	4.77	305	153	80	28
Dichlorvos	3.31	221	109	70	23
	3.31	221	127	70	25
Diethofencarb	3.96	268.1	152.1	70	45
	3.96	268.1	226.2	70	45
Difenoconazole	4.92	406.1	251	120	37
	4.92	406.1	337	120	23
Diflubenzuron	4.47	311	141.2	72	47
	4.47	311	158	72	21
Dimethoate	2.68	230	125	56	29
	2.68	230	199	56	13
Dimethomorph	4.05	388.1	301	115	29
	4.05	388.1	165	115	43
Emamectin benzoate	5.49	886.5	158.1	120	37
	5.49	886.5	302.1	120	39
Fenitrothion	4.25	278	125	52	25
	4.25	278	246	52	22
Fenpropathrin	5.68	350.2	97.1	75	30
	5.68	350.2	125.1	82	40
Fipronil	4.4	434.8	329.9	−50	−15
	4.4	434.8	249.8	−50	−31
Fipronil desulfinyl	4.27	387.1	351.1	−65	−42
	4.27	387.1	281.9	−69	−58
Fipronil sulfide	4.42	419.1	383	−55	−35
	4.42	419.1	261.7	−40	−45
Fipronil sulfone	4.5	451	281.9	−82	−45
	4.5	451	415.1	−69	−50
Imazalil	4.59	297.1	155	80	25
	4.59	297.1	159	60	35
Imidacloprid	2.42	256.1	209	60	23
	2.42	256.1	175	60	26
Iprodione	4.49	330.1	245	55	20
	4.49	330.1	288	47	20
Isocarbophos	3.8	273.1	231	67	15
	3.8	273.1	121	67	32
Isofenphos methyl	4.53	273	231	39	13
	4.53	273	121.1	39	33
Malathion	4.17	331	127	64	17
	4.17	331	285	64	13
Methamidophos	1.58	142	94	54	19
	1.58	142	125	54	18
Methomyl	2.13	163	106	38	13
	2.13	163	88	38	13
Omethoate	1.84	214	182.9	56	16
	1.84	214	109	56	36

Table 2 (*Continued*)

Analyte	Retention time (min)	Q1 ion (m/z)	Q3 ion (m/z)	Declustering potential (V)	Collision energy (V)
Parathion	4.57	292	236	80	20
	4.57	292	264	80	15
Parathion methyl	4	264	231.9	65	23
	4	264	124.9	65	23
Pendimethalin	5.68	282.1	212	45	15
	5.68	282.1	194	45	25
Phorate	4.94	261.1	75.2	34	14
	4.94	261.1	199	34	11
Phorate sulfone	3.71	293	171	55	15
	3.71	293	143	55	25
Phorate sulfoxide	3.61	277	143	55	30
	3.61	277	199	40	15
Phosalone	4.82	368	182	71	20
	4.82	368	322	71	13
Phosmet	3.95	318	160	55	35
	3.95	318	133	47	45
Phoxim	4.74	299.1	77	67	46
	4.74	299.1	129	67	16
Prochloraz	4.81	376.2	308	65	17
	4.81	376.2	70.1	65	43
Profenofos	5.25	373	302.9	80	25
	5.25	373	345.2	80	18
Propoxur	3.31	210	111	80	40
	3.31	210	168	81	40
Pyridaben	6.06	365	309	110	17
	6.06	365	147	110	31
Pyrimethanil	4.17	200	107	91	34
	4.17	200	82	91	37
Tau-fluvalinate	5.97	503	181	82	25
	5.97	503	208	75	15
Tetramethrin	5.26	332.2	135	82	40
	5.26	332.2	288	83	40
Thiamethoxam	2.16	292	211	60	18
	2.16	292	181	60	32
Triadimefon	4.21	294	197	81	21
	4.21	294	225	81	17
Triazophos	4.23	314	162	70	25
	4.23	314	119.1	70	47
Tricyclazole	5.26	332.4	135	80	40
	5.26	332.4	164	80	40

3.2. Optimization of sample preparation

3.2.1. Extraction

In pesticide residue analysis, the choice of solvent for extraction must take into account the polarity of the solvent, the characteristics of the pesticide and the nature of the sample. Acetonitrile has been commonly used as an extraction solvent [26–32]. In the analysis of pesticides, it is used in the Association of Official Analytical Chemists (AOAC) method based on QuChERS, such as for organic phosphorus, organic chlorine, pyrethroid and carbamate. Acid can promote the dissolution of pesticides from tissue to improve the extraction efficiency.

In our study, the effects of three extractive reagents were investigated (10 ml 1% acetic acid in acetonitrile, 5 ml water and 10 ml 1% acetic acid in acetonitrile, and 5 ml water and 10 ml acetonitrile). When the fortified concentration was 100 μg/kg, the extraction efficiencies of 58 compounds by the three extractive reagents were shown in Fig.2a. The number of compounds which satisfied the recovery range of 70.0% to 120.0% was calculated. By comparing the effects of the three extractive reagents, it could be seen that water and 1% acetic acid in acetonitrile was the optimum way to extract pesticides from eggs, followed by 1% acetic acid in acetonitrile, because most pesticides are acidic and under these conditions the protein deposition is the best.

Furthermore, to provide a better extraction environment, we used sodium chloride (NaCl) or magnesium sulphate ($MgSO_4$) to separate the water layer from the egg matrix. The two different processing steps were as follows. After the extraction reagent was added, 4 g $MgSO_4$ and 1 g NaCl was added to the centrifuge tube, vortex mixed for 1 min and oscillated for 10 min, then centrifuged for 10 min at 4500 rpm. Another treatment was vortexed in the tube for 1 min after the extraction reagent was transferred. The tube was put in a sonic bath for 10 min. After 3 g NaCl was added to the tube, it was centrifuged again as described above.

When the above steps were performed sequentially, the recoveries were in the range of 40.0–126.6% and 53.2–121.6%. As shown in Fig.2b, in the analysis of different levels of analyte (5, 20, 100 μg/kg), the recoveries of the two treatments were almost between 70.0% and 120.0% at higher levels. The difference was significant when the concentration was low, and the data showed that the recovery of compounds with NaCl alone was generally higher than those with the use of $MgSO_4$. The results indicated that when the $MgSO_4$ absorbed water, some of the pesticides were also lost. At the same time, the effects of oscillation extraction and ultrasonic extraction were also compared. The recoveries for the two methods were 58.6–142.9% and 59.7–140.8%. Despite the results showing little difference, the samples after ultrasonic were more stable, so we selected ultrasonic extraction for use.

3.2.2. Clean-up

Because eggs contain many fat and protein, they are complex to clean up. A simple and effective purification method is needed to remove the interfering substances in eggs. Thus, MFF based on QuEChERS was used for clean-up.

The role of primary secondary amine (PSA) is to effectively remove organic acids, pigments and carbohydrate impurities in the sample matrix, but the graphitized carbon blacks (GCB) pigment adsorption performance is more obvious. The C18 sorbent (C18)

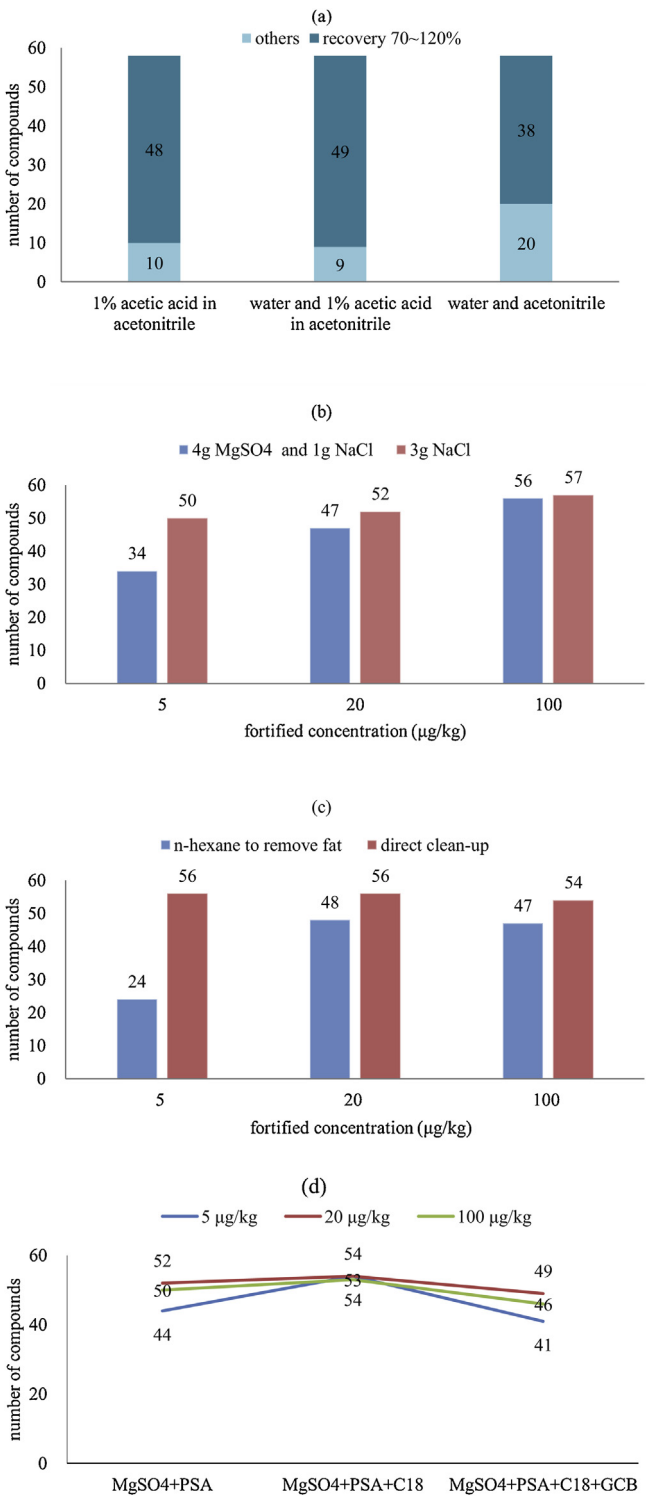

Fig. 2. Comparison of the number of compounds with recoveries of 70–120% using different extractive reagents (a), magnesium sulphate and sodium chloride (b), purification methods (c), and purification materials (d).

grease removal effect is very significant, but it can also remove some non-polar impurities.

Hence, in the process of clean-up, 1 ml supernatant was added to three kinds of MFF filled with different combinations of adsorption materials to investigate the purification effects of PSA, C18 and GCB on eggs. Specific types and adsorption material combinations used were as follows: (1) MFF 3201 ($MgSO_4$ 150 mg, PSA 50 mg); (2) MFF 3202 ($MgSO_4$ 150 mg, PSA 50 mg, C18 50 mg); and (3) MFF 3305 ($MgSO_4$ 150 mg, PSA 50 mg, C18 50 mg, GCB 5 mg).

Before proceeding with the MFF procedure, 4 ml extracting solution was first directly added into the n-hexane solution (1/1, V/V) to obtain an acetonitrile layer. The reason for this step is that n-hexane has a high fat solubility. Due to the weak miscibility between n-hexane and acetonitrile, the n-hexane should be saturated with acetonitrile to avoid loss of the target substance in the acetonitrile extract during the liquid-liquid separation. After the above operation, 1 ml of the acetonitrile layer was filtered using MFF 3202.

It was found that the addition of n-hexane to remove fat had a great effect on the recovery of compounds, especially with the analyte concentration of 5 μg/kg, for which the number of compounds with recoveries ranging from 70.0% to 120.0% was only about half. In summary, n-hexane removed fat but also obviously reduced the compound recovery. After analysing the results, it was decided to adopt the method of using 1 ml extraction solution for MFF directly (Fig.2c).

As shown in Fig.2d, at high, intermediate and low fortified levels, the recovery of pesticides with GCB was lower than the recoveries of the other two purification methods. The recoveries ranged from 42.8% to 142.8%, and ten drugs were only approximately 50%. At the low fortified level of 5 μg/kg, when $MgSO_4$ combined with PSA (MFF 3201) and $MgSO_4$ combined with PSA and C18 (MFF 3202) were added, the numbers of compounds with recoveries in the range of 70.0–120.0% were 44 and 54, respectively. For higher levels of analytes, when only $MgSO_4$ and PSA were added, the number of compounds in the desired recovery range significantly decreased. When C18 was added, the result was the most satisfactory and stable, with recoveries between 63.0% and 124.0%. The number of compounds with better recoveries was above 53. Therefore, in this study, the purification method using MFF 3202 ($MgSO_4$ 150 mg; PSA 50 mg; C18 50 mg) was selected as the optimal clean-up method.

3.3. Matrix effects

As the matrix often causes significant interference with the analytic process and affects the accuracy of analytical results, it is necessary to examine the matrix effect. The solvent standard curve and matrix standard curve using concentrations of 0.5, 1, 2.5, 5, 10, 25, 50 and 100 μg/L were configured. The matrix standard curve was prepared by diluting the mixed standard solution step by step, using blank matrix solution that was obtained by extraction and clean-up of blank eggs with the developed method. The MEs of the method was investigated by the ratio of its slope. By calculating the MEs of each compound (Fig.3), the MEs of most compounds were within 82.6%–118.9%, which were not obvious. Only parathion-methyl had slight matrix inhibition, thus we could use the solvent standard calibration curve for quantification.

3.4. Method validation

3.4.1. Calibration curve and linear range

The mixed standard solution was accurately measured and diluted with acetonitrile to prepare a series of standard working solutions at concentrations of 0.5, 1, 2.5, 5, 10, 25, 50, and 100 μg/L for UHPLC-MS/MS. The correlation coefficients (r) of the 58 compounds were greater than 0.99 (Table S2). This result indicated that all tested pesticides had a good linear range.

3.4.2. Accuracy and precision

The accuracy and precision of the method was calculated using the recoveries obtained by spiking blank egg samples at five con-

Table 3
Recovery, intra-day, inter-day precisions for 50 pesticides and 8 metabolites.

Compound	Spike Level ($n = 6$)														
	5 μg/kg			10 μg/kg			25 μg/kg			50 μg/kg			100 μg/kg		
	Recovery (%)	Intra-day RSD(%)	Inter-day RSD(%)	Recovery (%)	Intra-day RSD(%)	Inter-day RSD(%)	Recovery (%)	Intra-day RSD(%)	Inter-day RSD(%)	Recovery (%)	Intra-day RSD(%)	Inter-day RSD(%)	Recovery (%)	Intra-day RSD(%)	Inter-day RSD(%)
3-Hydroxycarbofuran	100.1	8.5	9.1	88.7	9.2	10.8	95.5	5.9	8.1	97.3	6.3	7.7	109.5	6.6	7.9
Acephate	77.4	8.8	9.3	81.3	4.3	5.5	83.8	5.1	6.4	86.3	7.3	8.3	78.1	5.2	6.4
Acetamiprid	79.6	3.5	4.4	99.1	8.0	9.7	100.2	4.8	5.8	107.7	8.8	10.3	89.5	4.6	5.4
Aldicarb	88.1	7.0	8.2	101.0	9.2	11.0	110.2	5.5	6.7	104.9	5.5	6.3	95.4	7.0	9.3
Aldicarb sulfone	83.6	4.3	5.5	100.2	6.8	8.2	101.3	4.3	5.3	91.2	8.8	9.8	90.2	7.7	9.2
Aldicarb sulfoxid	75.4	2.7	3.5	90.2	6.2	7.3	95.9	8.5	10.6	92.1	8.0	9.9	85.6	10.2	11.6
Avermectin b1a	87.6	8.5	10.0	93.1	7.3	8.1	82.2	8.2	11.6	99.7	7.3	8.8	86.3	9.9	11.7
Azoxystrobin	99.2	4.0	4.8	100.3	8.9	10.6	98.3	4.5	5.7	93.6	3.6	4.9	90.0	3.3	4.4
Carbaryl	83.7	11.5	12.4	113.7	6.2	7.6	109.4	5.0	6.6	101.1	6.4	7.8	103.5	5.7	6.9
Carbendazim	85.6	5.2	6.3	88.0	5.8	8.5	89.2	2.4	3.2	83.0	5.5	6.9	82.0	5.9	7.2
Carbofuran	102.8	5.4	5.8	98.5	8.5	9.9	109.0	6.0	7.4	93.7	2.8	4.0	96.4	6.4	7.7
Chlorantraniliprole	108.3	6.0	7.2	107.9	3.4	4.4	96.1	3.1	3.9	100.7	9.4	11.1	96.6	7.0	8.3
Chlorbenzuron	84.9	9.0	10.6	91.0	8.1	10.3	97.1	6.2	7.6	86.0	3.5	4.4	87.6	3.2	4.1
Chlorfluazuron	102.9	8.9	9.4	86.7	6.0	7.2	84.2	2.5	3.3	88.8	6.8	7.6	93.2	4.2	5.0
Chlorpyrifos	83.2	9.6	10.7	93.0	5.9	7.0	80.5	1.3	2.1	84.4	4.1	5.1	85.8	7.2	8.0
Diazinon	86.2	9.5	11.4	81.7	3.4	4.5	87.8	4.0	5.2	89.0	7.2	8.0	89.1	3.6	4.6
Dichlorvos	103.6	8.6	9.5	103.9	2.4	3.3	95.8	6.9	8.0	87.7	9.4	11.3	85.4	8.9	10.5
Diethofencarb	77.4	3.5	4.6	110.1	7.1	9.1	97.1	11.2	12.5	109.0	6.9	7.9	100.2	7.8	9.3
Difenoconazole	87.4	4.4	5.7	83.7	3.2	4.0	81.4	4.3	5.5	86.0	6.5	7.3	86.6	6.6	8.1
Diflubenzuron	76.8	3.0	4.1	88.5	6.0	7.4	90.5	3.5	5.4	83.3	5.9	7.2	92.8	9.5	11.4
Dimethoate	77.8	3.9	4.7	80.5	3.6	4.4	85.5	4.9	5.8	101.6	5.2	6.3	91.3	4.6	5.8
Dimethomorph	91.2	3.4	4.2	101.1	6.8	7.8	99.2	7.8	9.4	83.5	2.7	3.7	91.4	6.2	7.3
Emamectin benzoate	80.2	3.6	4.6	86.5	5.9	7.3	85.4	8.4	10.1	76.9	3.6	4.4	79.1	9.5	10.6
Fenitrothion	97.6	7.5	8.9	93.3	12.4	13.9	103.9	10.2	11.8	106.1	11.4	13.4	104.4	7.2	8.6
Fenpropathrin	83.6	6.6	8.0	84.5	9.6	11.0	75.6	2.4	3.5	84.1	2.1	3.1	90.3	4.1	5.4
Fipronil	106.8	6.4	7.5	102.2	5.9	7.1	111.3	3.8	5.0	115.2	1.7	2.9	104.3	9.2	12.5
Fipronil desulfinyl	101.0	2.8	4.3	109.0	4.3	5.5	107.0	4.2	5.7	107.9	9.3	10.6	112.5	3.8	4.7
Fipronil sulfide	95.3	6.9	8.0	109.5	6.0	7.1	109.3	3.9	5.1	112.0	4.1	5.0	110.7	5.4	6.7
Fipronil sulfone	106.9	4.6	5.9	105.5	6.2	8.0	107.4	3.7	4.5	102.7	9.5	11.4	112.3	4.7	5.8
Imazalil	97.0	5.5	6.8	82.1	2.3	3.2	81.6	2.5	3.5	86.8	3.5	4.6	96.2	2.7	3.5
Imidacloprid	89.4	8.0	8.9	97.7	3.0	4.0	96.1	6.4	8.2	104.6	8.9	9.8	90.8	6.5	7.9
Iprodione	106.2	13.6	15.3	97.5	8.7	9.8	94.9	3.2	4.0	88.4	10.9	11.8	88.9	7.2	8.7
Isocarbophos	101.4	11.4	13.1	89.2	6.3	8.3	86.2	5.3	7.5	95.2	5.2	7.2	86.8	6.3	7.8
Isofenphos methyl	105.8	8.4	9.7	96.2	3.4	5.5	91.6	6.2	7.5	101.9	5.4	6.7	101.3	7.9	8.9
Malathion	92.3	2.7	3.5	94.1	2.8	3.8	94.0	1.7	2.8	98.9	5.8	6.6	105.1	4.2	5.3
Methamidophos	75.2	5.9	7.2	77.0	4.9	6.6	79.5	9.6	11.1	100.4	7.8	9.4	83.6	6.8	8.3
Methomyl	87.3	8.4	9.2	98.7	7.3	8.3	105.8	7.7	9.1	93.5	7.6	8.7	101.6	9.9	11.6
Omethoate	76.0	5.4	6.7	77.6	8.3	9.8	78.8	4.2	5.7	84.4	4.3	5.4	78.2	6.1	7.0
Parathion	94.7	5.6	6.5	106.6	8.8	9.7	98.7	5.8	7.8	86.2	8.4	9.6	82.6	4.5	5.8
Parathion methyl	109.0	9.4	11.1	90.3	10.9	13.1	103.5	8.3	9.9	102.5	8.2	9.8	95.0	3.2	4.3
Pendimethalin	94.6	8.2	9.5	88.1	3.7	4.5	103.7	6.6	7.6	94.3	7.1	8.0	77.3	3.5	4.3
Phorate	108.2	6.1	8.1	85.2	7.9	9.3	90.3	3.4	4.2	98.0	7.4	8.9	92.0	5.1	7.4
Phorate sulfone	107.4	4.3	5.7	98.5	12.4	13.3	98.4	12.1	13.7	96.8	9.7	11.7	91.1	12.0	14.8
Phorate sulfoxide	105.3	3.3	4.3	107.6	3.9	5.8	99.5	8.0	10.3	93.1	9.7	11.3	88.9	10.2	12.2
Phosalone	90.2	6.9	8.3	83.7	4.8	5.9	91.0	5.5	6.8	98.8	10.0	11.9	101.0	7.2	8.7
Phosmet	102.3	2.5	3.7	102.5	12.4	14.3	101.7	10.7	12.0	108.3	1.8	2.6	99.4	3.3	4.1
Phoxim	99.3	5.8	7.2	91.8	8.5	9.5	99.3	4.6	5.6	94.0	9.5	11.3	91.4	3.2	4.0
Prochloraz	74.9	4.9	5.7	81.5	8.4	9.5	79.1	2.1	3.1	88.4	5.4	6.5	95.5	2.4	3.4
Profenofos	96.2	3.8	4.6	74.4	3.2	4.9	76.6	3.8	4.9	93.9	3.5	4.6	87.4	7.2	8.3
Propoxur	101.9	9.3	11.0	95.5	12.1	13.6	90.0	13.9	14.2	100.1	10.4	12.3	115.2	7.1	9.4
Pyridaben	88.3	11.4	12.3	97.5	8.6	9.6	79.9	4.3	5.4	79.7	11.0	14.1	76.1	4.1	5.2
Pyrimethanil	95.5	8.9	10.0	108.2	4.6	5.7	108.1	7.3	8.5	93.6	3.5	4.6	89.7	7.2	8.7
Tau-fluvalinate	88.5	5.7	7.1	99.2	8.8	10.3	92.1	9.6	10.8	81.2	5.6	6.6	75.5	4.7	6.0
Tetramethrin	107.0	8.3	9.1	102.2	12.2	13.5	88.2	7.5	8.9	98.3	6.3	7.2	108.2	5.7	7.1
Thiamethoxam	75.7	5.2	6.4	93.0	1.9	3.0	103.9	5.8	7.1	95.4	12.9	14.5	76.5	4.5	6.0
Triadimefon	87.6	7.5	8.5	84.9	4.6	5.7	83.7	2.3	3.3	88.0	5.5	6.5	89.2	5.8	6.6
Triazophos	89.6	4.0	5.1	90.9	4.5	6.5	93.1	4.7	6.0	96.8	5.9	6.8	93.4	7.3	8.5
Tricyclazole	99.7	6.6	7.5	80.8	5.5	7.8	81.9	5.5	6.4	84.0	6.4	7.8	83.9	6.6	7.9

Table 4
LOQ, LOD, CCα, and CCβ for 50 pesticides and 8 metabolites.

Analyte	MRL (μg/kg)	LOQ (μg/kg)	LOD (μg/kg)	CCα (μg/kg)	CCβ (μg/kg)
3-Hydroxycarbofuran	10	0.2	0.1	23.35	36.69
Acephate	20	5	0.2	27.05	34.09
Acetamiprid	20	1	0.2	27.95	35.89
Aldicarb	10	0.2	0.1	25.23	40.47
Aldicarb sulfone	10	1	0.2	21.12	32.25
Aldicarb sulfoxid	10	1	0.2	19.17	28.34
Avermectin b1a	10	5	1	21.18	32.35
Azoxystrobin	10	0.2	0.1	24.57	39.14
Carbaryl	50	0.2	0.1	60.58	71.15
Carbendazim	50	0.2	0.1	57.55	65.09
Carbofuran	10	1	0.2	23.80	37.59
Chlorantraniliprole	100	2	0.2	111.03	122.06
Chlorbenzuron		0.2	0.1	5.80	11.60
Chlorfluazuron		0.2	0.1	18.43	36.87
Chlorpyrifos	10	0.2	0.1	19.03	28.06
Diazinon	10	0.2	0.1	14.59	19.17
Dichlorvos		2	1	10.44	20.88
Diethofencarb	10	2	0.2	22.79	35.58
Difenoconazole	50	1	0.2	59.23	68.45
Diflubenzuron	50	1	0.2	58.07	66.13
Dimethoate		0.2	0.2	13.66	27.31
Dimethomorph	10	1	0.2	21.30	32.61
Emamectin benzoate	10	2	0.2	18.39	26.77
Fenitrothion	10	2	1	28.91	47.82
Fenpropathrin		1	0.2	4.81	9.61
Fipronil	5	1	0.2	16.26	27.53
Fipronil desulfinyl	5	1	0.2	9.59	14.18
Fipronil sulfide	5	1	0.2	15.75	26.51
Fipronil sulfone	5	1	0.2	13.05	21.10
Imazalil	50	1	0.2	54.96	59.92
Imidacloprid	50	0.2	0.1	65.25	80.51
Iprodione	100	2	1	110.49	120.99
Isocarbophos		1	0.2	18.83	37.66
Isofenphos methyl		0.2	0.1	3.41	6.83
Malathion	20	0.2	0.1	22.64	25.28
Methamidophos	10	2	1	16.19	22.39
Methomyl	10	1	0.2	21.75	33.50
Omethoate		2	0.2	4.84	9.68
Parathion	50	2	0.2	61.94	73.88
Parathion methyl	10	2	0.2	26.17	42.34
Pendimethalin	10	0.2	0.1	15.31	20.62
Phorate	50	1	0.2	61.95	73.90
Phorate sulfone	50	0.2	0.1	65.41	80.82
Phorate sulfoxide	50	0.2	0.1	64.80	79.60
Phosalone	10	0.2	0.1	16.56	23.12
Phosmet	50	1	0.2	53.21	56.42
Phoxim	60	1	0.2	74.63	89.27
Prochloraz	100	0.2	0.1	103.78	107.56
Profenofos	20	0.2	0.1	24.77	29.54
Propoxur	50	2	0.2	67.05	84.11
Pyridaben	20	2	0.2	25.66	31.31
Pyrimethanil	10	1	0.2	18.13	26.27
Tau-fluvalinate	10	1	0.2	24.40	38.79
Tetramethrin		2	0.2	10.89	21.78
Thiamethoxam	10	0.2	0.1	12.95	15.89
Triadimefon	10	0.2	0.1	16.34	22.68
Triazophos	10	0.2	0.1	16.74	23.48
Tricyclazole	10	1	0.2	17.35	24.71

centrations of 5, 10, 20, 50, 100 μg/kg, with six replicates at each level. As shown in Table 3, the recoveries for each compound at five different spiked levels were between 74.4–115.2%. The precision of this method was demonstrated in term of repeatability and reproducibility, which were expressed as the intra-day RSD(%) and inter-day RSD(%) values. The determination of reproducibility was carried out on three different days. In egg the intra-day RSD(%) range from 1.3% to 13.9% and the inter-day RSD(%) range from 2.1% to 15.3%. All RSDs were less than 20%, indicating that this method could detect 50 pesticides and 8 metabolites in eggs with good recovery and reproducibility.

3.4.3. Limit of detection (LODs) and limit of quantitation (LOQs)

As shown in Table 4, the LODs for most compounds were around 0.1 μg/kg, and the LOQs ranged from 0.2 to 5 μg/kg (The detailed data was shown in Table S3), with both being lower than the corresponding maximum residue limits (MRLs) according to EU. Compared with the methods reported in current literature for detection of pesticides in chicken eggs by LC–MS/MS [10,14,22], approximately 70.0% of the compounds had significantly lower LOQs and LODs values. Particularly, the values for thiamethoxam, malathion and imidacloprid were all approximately 0.1 μg/kg, indicating that the method has higher sensitivity for these compounds.

3.4.4. Decision limit (CCα) and detection capacity (CCβ)

The CCα indicates the limit at and above which it can be concluded that a sample is non-compliant with an error probability of α. The CCβ indicates the smallest content of a substance that may be detected, identified and/or quantified in a sample with an error probability of β. [25]

According to previous reports, we calculated CCα and CCβ in combination with European Commission Decision 2002/657/EC and other approaches [33–35]. In our validation, CCα and CCβ were calculated from the matrix matched calibration curve. The blank sample was fortified at and below the maximum residue limit (for analytes with MRL) or at and above the lowest possible level (for analytes without MRL) in equidistant steps. According to EU's MRLs, 49 pesticides in this study have been set MRLs in eggs. For compounds with specific MRLs, CCα and CCβ can be calculated as follows: $CC\alpha = MRL + 1.64 \times SD_{MRL}$ and $CC\beta = CC\alpha + 1.64 \times SD_{MRL}$, Where SD_{MRL} is the standard deviation at the MRL level that determined by laboratory reproducibility conditions. If the MRL for a compound has not been established, the MRL value in above formula was replaced by the LOQ [36], which is $CC\alpha = LOQ + 1.64 \times SD_{LOQ}$ and $CC\beta = LOQ + 1.64 \times SD_{LOQ}$. As seen in Table 4, the CCα and CCβ values of 58 target compounds in eggs were 3.4–111.0 μg/kg and 6.8–122.1 μg/kg, respectively. Data showed that the compounds without established MRLs which had lower CCα and CCβ values were closer to the limit of detection of the method, although in other case, these concentrations were always above the MRL. Considering that each compound has a different established MRL value, this large range of values may be justified.

3.5. The actual sample application

Seventy-two egg samples were obtained from ten different markets in China to validate the method. A total of 3 pesticides were detected in 6 samples, and the total incidence was 8.33%, including acetamiprid, pyrimethanil and fipronil sulfone at detected concentrations of 0.68–1.56, 4.94, 1.75–7.50 μg/kg, respectively; the corresponding percentages were 2.78%, 1.39% and 4.17%, respectively. Among them, one sample detected 7.50 μg/kg of fipronil sulfone, which exceeds the EU's MRL of 5 μg/kg but under the Codex Alimentarius Commission (CAC)'s MRL of 20 μg/kg. In addition, Guo Q et al reported that fipronil sulfone was detected in egg with concentration of 9.57 μg/kg [37], Which is higher than present results of 1.75–7.50 μg/kg, indicated that more attention should be paid to the illegal use of fipronil in breeding of laying hens. However, to our knowledge, it has not been reported that acetamiprid and pyrimethanil were detected in actual samples of eggs. The application to actual samples proved that the method has good practicality.

4. Conclusions

In this study, an accurate and reliable determination of pesticide residues in eggs using MFF based on QuEChERS by UHPLC-MS/MS was developed. Extraction was performed with 5 ml water and

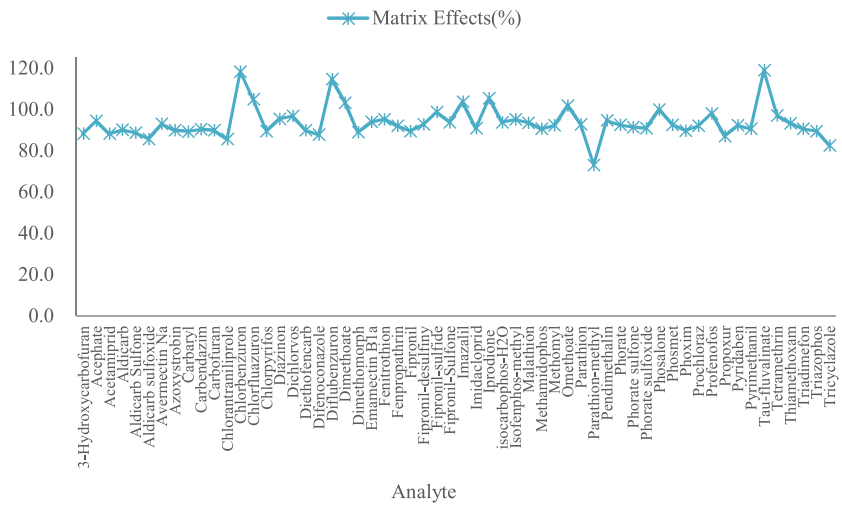

Fig. 3. Matrix effects of 50 pesticides and 8 Metabolites.

10 ml 1% acetic acid in acetonitrile, salting out by sodium chloride, and clean-up with MFF 3202, which saved pretreatment time and reduced the matrix interference significantly. Furthermore, the precision, RSDs, LODs, LOQs and other verification parameters all satisfied the requirements of pesticide detection. In addition, the method was also successfully applied to real samples.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.chroma.2019.01.074.

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