

# Multiresidue determination of 29 pesticide residues in pepper through a modified QuEChERS method and gas chromatography–mass spectrometry

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**ABSTRACT:** This study describes the development and use of a modified quick, easy, cheap, effective, rugged and safe (QuEChERS) method coupled with gas chromatography with mass spectrometry to determine 29 pesticide residues in green, red and dehydrated red peppers. Pesticides were extracted with acetonitrile (1% acetic acid), partitioned with sodium chloride and purified with primary secondary amino and octadecyl silane in acetone. The QuEChERS extraction conditions were optimized, and the matrix effects that might influence recoveries were evaluated and minimized using matrix-matched calibration curves. Under the optimized conditions, the calibration curves for 29 pesticides showed good linearity in the concentration range of 0.1–10 µg/mL with determination coefficient  $R^2 > 0.998$ . The limits of quantification of the 29 pesticides were 0.006–0.06 mg/kg for green pepper, 0.005–0.039 mg/kg for red pepper and 0.014–0.25 mg/kg for dehydrated red pepper. These values are below the suggested regulatory maximum residue limits. The mean recoveries ranged between 70.1 and 110%, and the relative standard deviations were <13%. The developed method was successfully applied to commercial samples. Some samples were found to contain the 29 pesticides with levels below the legal limits. Copyright © 2016 John Wiley & Sons, Ltd.

**Keywords:** pepper; residue analysis; QuEChERS; gas chromatography–mass spectrometry (GC–MS)

## Introduction

Peppers (*Capsicum annum* L.) are rich in phytonutrients, including ascorbic acid (vitamin C), carotenoids (pro-vitamin A), tocopherols (vitamin E), flavonoids and capsaicinoids, all of which possess potential health-promoting properties (Kim *et al.*, 2015). Peppers have been used since ancient times as a seasoning to add and/or change food color, and it is one of the most common foods around the world (Kim *et al.*, 2002). With the increasing domestic and foreign demand for peppers, pesticides are increasingly being used to improve yield. Many pesticides are extensively used to control insects and diseases in peppers because of their excellent biological activities and high effectiveness. However, pesticide residues are an important source of pollution in agriculture and a potential public health threat (Klein and Alder, 2003; Oates and Cohen, 2009; Beyer and Biziuk, 2010; Koesukkiwat *et al.*, 2010; Park *et al.*, 2011). Therefore, the need for control of such substances and rapid and reliable analytical responses is unquestionable. The Chinese government has established maximum residue levels (MRLs) that set limits for the amount of 59 pesticides (including metalaxyl, cyprodinil and bifenthrin) in peppers (Ministry of Agriculture, 2014).

The development of sensitive and selective methods for the analysis of pesticide residues usually present in trace amounts in peppers is thus necessary. The quick, easy, cheap, effective, rugged and safe (QuEChERS) sample preparation method has been used for the multiresidue pesticide analysis of various plant-based matrices (Schenck and Hobbs, 2004; Payá *et al.*, 2007; Caldas *et al.*, 2011; Andraščíková and Hrouzková, 2013). The QuEChERS method presents some advantages, such as simplicity, fewer steps and

effectiveness for clean-up. The method is also environmentally friendly because of its lower consumption of organic solvents, which is in agreement with new trends in green analytical chemistry (Bolaños *et al.*, 2007; Balcke *et al.*, 2012; Gong *et al.*, 2012; Sobhanzadeh *et al.*, 2012). Many studies have reported various modified QuEChERS methods to determine pesticide residues in peppers (Yogendrarajah *et al.*, 2013; Kim *et al.*, 2015; Jang *et al.*, 2014; Morales *et al.*, 2011). Yogendrarajah reported an analytical method for the analysis of multiple mycotoxins in white, black and dehydrated red peppers using liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS), which involved the application of a QuEChERS procedure (Yogendrarajah *et al.*, 2013).

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**Abbreviations used:** GCB, graphitized carbon black; HAC, acetic acid; ME, matrix effect; MeCN, acetonitrile; MeOH, methanol; MRL, maximum residue level; PSA, primary secondary amine; QuEChERS, quick, easy, cheap, effective, rugged and safe; SIM, selected ion monitoring.

Kim developed a QuEChERS method to detect pyridaben in unprocessed and processed hot peppers by LC–MS/MS (Kim *et al.*, 2015). Jang applied an analytical method to detect etoxazole in red peppers using a modified QuEChERS method through gas chromatography coupled with a nitrogen phosphorus detector (Jang *et al.*, 2014). Morales determined 16 pesticides in peppers by high-performance liquid chromatography/mass spectrometry (Morales *et al.*, 2011). However, only a few of the 29 pesticides tested in the present paper were included in the above-mentioned multiresidue pesticide detection methods. Several studies used solid-phase or liquid–liquid extraction to analyze pesticide residues in peppers. However, to the best of our knowledge, no study has ever used the QuEChERS method to analyze multiresidues of the 29 tested pesticides in peppers.

The present study aims to develop a rapid, efficient and simple extraction and analysis procedure for 29 pesticides in samples of the main pepper products (i.e. green, red and dehydrated red peppers) in the Chinese market. Different clean-up procedures based on the QuEChERS preparation method were evaluated in accordance with their recoveries and relative standard deviation (RSD) values. Compared with existing reports on the analysis of pesticide residues in peppers, this study analyzed more commonly used pesticides and pepper types.

## Experimental section

### Chemicals and materials

Pesticide reference standards were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Anhydrous magnesium sulfate ( $\text{MgSO}_4$ ), sodium chloride (NaCl), acetonitrile (MeCN), methanol (MeOH), acetone and acetic acid (HAc) were analytical reagents purchased from Chengdu Jinshan Chemical Reagent Co. (Chengdu, China). Primary secondary amine (PSA, 40–60  $\mu\text{m}$ ), octadecyl silane ( $\text{C}_{18}$ , 40–60  $\mu\text{m}$ ) and graphitized carbon black (GCB, 40–60  $\mu\text{m}$ ) were purchased from Agela Technologies (Tianjin, China). Water used for the extraction was obtained directly from a Milli-Q system.

### Apparatus

An Agilent 6890 N gas chromatograph (GC) in tandem with an Agilent 5973 mass spectrometer (MS) (Agilent, California, USA) was used to perform all GC–MS analyses. Twenty-nine pesticide residues were separated in a capillary column (DB-17, 60 m  $\times$  0.25 mm  $\times$  0.25  $\mu\text{m}$ ).

### GC–MS analysis

All injections were splitless, and the volume was 1  $\mu\text{L}$ . The flow rate of the carrier gas ( $\text{N}_2$ , P 99.999%) was 1.3 mL/min. The temperature of the injector was 250 °C. The column temperature program used was from 140 °C (1 min) to 220 °C at 10 °C/min for 2 min, then to 240 °C at 3 °C/min for 3 min and to 290 °C at 5 °C/min for 5 min. The detector temperature was kept at 280 °C. The GC–MS interface temperature was 280 °C. The ion source temperature was 230 °C. Data were acquired in the electron impact mode at a voltage of 70 eV using the selected ion monitoring (SIM) mode.

### Standard solutions

Stock standard solutions (200  $\mu\text{g/mL}$ ) of the targeted pesticides were prepared separately in acetone. The stock standard solution

was diluted with acetone as required. Working standard mixtures of the 29 tested pesticides were prepared by diluting stock solutions with acetone to a concentration of 0.1–10  $\mu\text{g/mL}$ . A matrix-matched standard calibration was obtained by mixing working standard solutions with blank pepper extracts. All standard solutions were stored at –20 °C in dark amber bottles until further analysis.

### Extraction and clean up for green and red peppers

Samples of green and red peppers were obtained from different markets of Huaxi District, Guiyang city. The samples were chopped, labeled and placed in a freezer at –20 °C until analysis. Aliquots (10.0  $\pm$  0.01 g) of pepper samples were weighed in 50 mL Teflon centrifuge tubes, and 20 mL of the extraction solvent (MeCN–HAc, 99:1, v/v) was added. The tubes were vortex-mixed for 1 min. NaCl (4 g) was added, and the tubes were again vortex-mixed for 2 min. Then, the extract was centrifuged at 6000 rpm for 5 min at room temperature. Ten milliliters of the MeCN layers was pooled in a 50 mL pear-shaped bottle and concentrated using a rotary evaporator (40 °C, 0.08 MPa). The residues were dissolved in 1 mL of acetone, and then 50 mg of PSA and 50 mg of  $\text{C}_{18}$  were added. After the solution was vortexed for 1 min, the liquid supernatant was filtered into an autosampler vial with 0.45  $\mu\text{m}$  syringe filters (Millipore, MA, USA) and then analyzed by GC–MS without further clean-up.

### Extraction and clean up for dehydrated red peppers

Samples of dehydrated red peppers were obtained from different markets in Huaxi District, Guiyang city. All of the dehydrated red pepper samples were ground using a mortar. Finely ground samples (2  $\pm$  0.01 g) of dehydrated red pepper were weighed in 50 mL Teflon centrifuge tubes, and 5 mL of distilled water was added to each tube. The samples were soaked for 20 min. A 20 mL aliquot of the extraction solvent (MeCN–HAc, 99:1, v/v) was added, and the tubes were vortex-mixed for 1 min. The rest of the operation is the same as described above for green and red peppers.

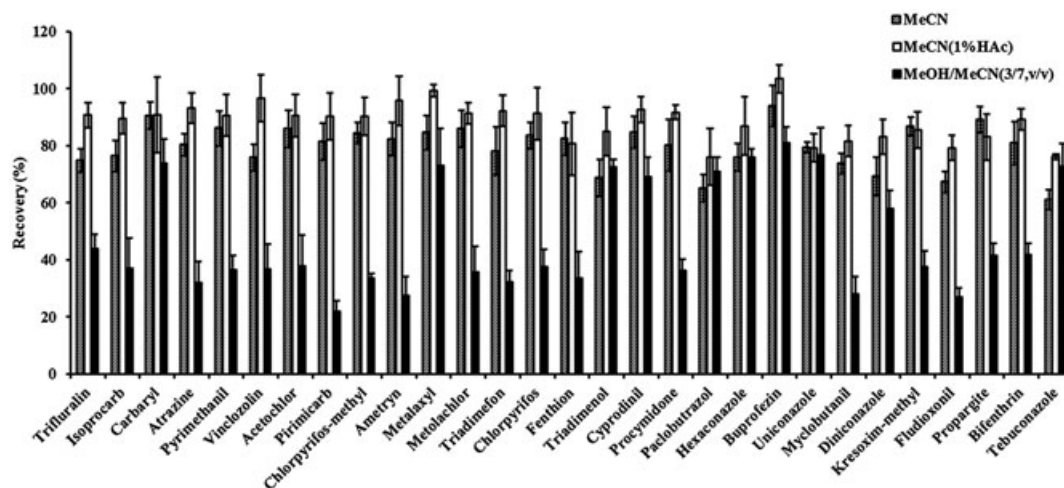
### Method validation

Validation was carried out to survey the following criteria: instrument linearity, recovery, specificity, precision, limit of detection (LOD) and limit of quantitation (LOQ). Matrix-matched standards, blanks and spiked blank extracts were injected to verify retention times and interfering peaks for selectivity and specificity, respectively. Matrix-matched standard solutions were used for quantitation and diluted in acetone. Linearity was assessed via the determination coefficient ( $R^2$ ) at five points (0.1, 0.5, 1, 5 and 10  $\mu\text{g/mL}$ ) for the 29 tested pesticides. Three and 10 times the signal-to-noise ratio were used to estimate the LOD and LOQ, respectively (Monbaliu *et al.*, 2009). Matrix effects in terms of signal suppression or enhancement owing to co-elution of matrix components were evaluated by post-extraction spiking and compared with the solvent standards. Higher and lower slopes of the matrix calibration equation with reference to the solvent-based calibration equation represented matrix-induced enhancement and suppression, respectively (Ferrer *et al.*, 2011; Kwon *et al.*, 2012; Orso *et al.*, 2014).

For the stability and precision of the established method to be evaluated, intra- and inter-day recovery experiments were carried out. The intra-day repeatability of the method was evaluated by

spiking working standard mixtures into blank pepper samples at three different spike levels (green and red pepper, 0.01, 0.05 and 1 mg/kg; dehydrated red pepper, 0.05, 0.25 and 5 mg/kg) in sextuplicate and analyzing them in the same run of the day on the GC–MS. The spiked samples were left to equilibrate for 2 h before extraction to allow the spiked solution to penetrate the matrix and

then extracted according to the procedures described above. The recovery percentage was calculated by comparing the peak area of the analyte in the spiked samples with those of the standard solutions at identical concentrations. The inter-day reproducibility of the method was determined by repeating this experiment consecutively for three different days.



**Figure 1.** Extraction efficiency of 29 pesticides with different extraction solvents in green peppers (spiked at 0.05 mg/kg).

**Table 1.** Retention times, qualitative ions and selected ion monitoring (SIM) group start times of 29 pesticides.

No.	Pesticides	Retention time (min)	Qualitative ion 1	Qualitative ion 2	Qualitative ion 3	SIM group start times
1	Trifluralin	9.537	306	224	335	6.00
2	Isoprocarb	10.327	121	136	103	9.80
3	Carbaryl	10.486	144	115	116	
4	Atrazine	13.571	200	215	173	12.50
5	Pyrimethanil	14.416	198	199	200	14.00
6	Vinclozolin	15.281	285	212	198	14.90
7	Acetochlor	15.453	146	162	223	15.35
8	Pirimicarb	16.076	166	238	138	15.80
9	Chlorpyrifos-methyl	16.488	286	288	290	16.30
10	Ametryn	17.041	227	212	185	16.60
11	Metalaxyl	17.21	206	249	220	17.13
12	Metolachlor	17.388	162	238	240	17.29
13	Triadimefon	17.484	208	181	210	
14	Chlorpyrifos	17.756	314	197	258	17.65
15	Fenthion	19.332	278	169	153	19.00
16	Triadimenol	19.574	112	168	128	19.45
17	Cyprodinil	19.937	224	225	210	19.80
18	Procymidone	20.696	236	125	238	20.45
19	Paclobutrazol	21.103	283	285	255	20.97
20	Hexaconazole	22.15	214	216	231	22.00
21	Buprofezin	23.273	105	172	305	22.95
22	Uniconazole	23.269	234	236	235	
23	Myclobutanil	24.266	179	150	288	23.90
24	Diniconazole	24.937	268	270	269	24.80
25	Kresoxim-methyl	25.692	116	206	131	25.54
26	Fludioxonil	26.421	248	127	154	26.10
27	Propargite	27.301	135	150	197	26.90
		27.458				
28	Bifenthrin	27.659	181	166	165	27.53
29	Tebuconazole	27.751	125	250	252	

**Table 2.** Name, correlation coefficient ( $R^2$ ) in matrix-matched standards and limits of quantitation (LOQs) of the tested pesticides.

Pesticides	Green pepper			Red pepper			Dehydrated red pepper		
	Regression equation	LOQ (mg/kg)	$R^2$	Regression equation	LOQ (mg/kg)	$R^2$	Regression equation	LOQ (mg/kg)	$R^2$
Trifluralin	$y = 28338x - 4017.4$	0.009	0.9994	$y = 25587x - 2397.2$	0.005	0.9997	$y = 29845x - 3403.1$	0.076	0.9998
Isoproc carb	$y = 26226x - 96.17$	0.011	0.9997	$y = 25930x + 1501.7$	0.009	0.9998	$y = 28034x + 1716.6$	0.22	0.9997
Carbaryl	$y = 19641x - 680.57$	0.008	0.9997	$y = 17052x - 1360$	0.005	0.9996	$y = 17177x + 229.49$	0.23	0.9998
Atrazine	$y = 21487x - 668.37$	0.006	0.9998	$y = 19087x - 351.07$	0.01	0.9999	$y = 19564x - 189.78$	0.22	0.9999
Pyrimethanil	$y = 74033x - 2445.6$	0.006	0.9998	$y = 65876x - 997.3$	0.009	0.9999	$y = 69304x - 690.68$	0.069	0.9999
Vinclozolin	$y = 7323.8x + 295.4$	0.009	0.9999	$y = 6922.2x - 311.43$	0.011	0.9995	$y = 7284x - 297.14$	0.032	0.9992
Acetochlor	$y = 9188.5x - 262.4$	0.01	0.9998	$y = 8652.5x - 49.956$	0.006	0.9999	$y = 9101.2x + 469.39$	0.25	0.9995
Pirimicarb	$y = 55126x - 1826.5$	0.06	0.9999	$y = 49679x - 634.12$	0.005	0.9999	$y = 52911x + 409.6$	0.23	0.9999
Chlorpyrifos-methyl	$y = 19200x - 875$	0.008	0.9996	$y = 15913x - 1090.2$	0.008	0.9995	$y = 13980x - 961$	0.059	0.9996
Ametryn	$y = 28117x - 777.6$	0.006	0.9998	$y = 25044x - 179.32$	0.005	0.9999	$y = 26650x + 1625.7$	0.093	0.9999
Metalaxyl	$y = 14342x - 433.4$	0.01	0.9998	$y = 12861x - 14.858$	0.018	0.9999	$y = 13225x + 1049.1$	0.23	0.9991
Metolachlor	$y = 33962x - 374.9$	0.006	0.9999	$y = 31777x - 232.97$	0.013	0.9999	$y = 34302x - 221.14$	0.14	0.9997
Triadimefon	$y = 11939x - 529.8$	0.01	0.9997	$y = 10370x - 668.5$	0.025	0.9997	$y = 10828x - 261.47$	0.23	0.9999
Chlorpyrifos	$y = 11086x - 305.5$	0.01	0.9997	$y = 10046x + 2524.2$	0.013	0.9996	$y = 9553.7x + 572.07$	0.023	0.9990
Fenthion	$y = 37963x - 1110.6$	0.006	0.9998	$y = 31787x - 960.92$	0.01	0.9999	$y = 30452x + 1564.1$	0.083	0.9995
Triadimenol	$y = 11723x - 991.7$	0.011	0.9996	$y = 9593.2x - 833.57$	0.01	0.9999	$y = 10213x - 782.7$	0.21	0.9997
Cyprodinil	$y = 49074x - 1538.1$	0.008	0.9998	$y = 41135x - 942$	0.014	0.9999	$y = 42772x - 349.05$	0.16	0.9999
Procymidone	$y = 12624x - 248.75$	0.013	0.9998	$y = 10519x + 450.6$	0.022	0.9999	$y = 9870x + 608.3$	0.061	0.9991
Paclobutrazol	$y = 22985x - 2334.2$	0.013	0.9996	$y = 18314x - 1843.5$	0.02	0.9997	$y = 19157x - 1174.1$	0.052	0.9999
Hexaconazole	$y = 11828x - 1094.9$	0.011	0.9998	$y = 9470.3x - 991.73$	0.026	0.9996	$y = 9299.3x + 245.59$	0.034	0.9989
Buprofezin	$y = 11693x - 18.947$	0.012	0.9999	$y = 9763.7x - 62.422$	0.008	0.9999	$y = 10248x + 192.91$	0.13	0.9999
Uniconazole	$y = 24529x - 3030.8$	0.018	0.9994	$y = 19211x - 2390.7$	0.029	0.9996	$y = 19065x - 2344.3$	0.046	0.9996
Myclobutanil	$y = 15352x - 1211$	0.011	0.9996	$y = 11933x - 1051.1$	0.011	0.9997	$y = 12213x - 469.69$	0.096	0.9989
Diniconazole	$y = 15968x - 1790.4$	0.012	0.9997	$y = 12595x - 1348.2$	0.01	0.9998	$y = 12026x - 1688.1$	0.066	0.9994
Kresoxim-methyl	$y = 25969x - 286.55$	0.012	0.9999	$y = 21755x - 350$	0.01	0.9999	$y = 23469x + 52.817$	0.086	0.9999
Fludioxonil	$y = 16686x - 660.36$	0.008	0.9998	$y = 11748x - 454.08$	0.027	0.9999	$y = 11107x + 95.895$	0.11	0.9992
Propargite		0.013	0.9993		0.027	0.9990		0.2	0.9989

(Continues)



**Table 2.** (Continued)

Pesticides	Green pepper			Red pepper			Dehydrated red pepper		
	Regression equation	LOQ (mg/kg)	$R^2$	Regression equation	LOQ (mg/kg)	$R^2$	Regression equation	LOQ (mg/kg)	$R^2$
Bifenthrin	$y = 11015x + 2397$	0.006	0.9999	$y = 9910.5x + 1819.3$	0.007	0.9999	$y = 9110.6x + 2100.4$	0.014	0.9999
	$y = 54580x - 944.26$			$y = 44653x - 93.825$			$y = 48003x + 713.12$		
Tebuconazole	$y = 7213.8x - 1027.5$	0.014	0.9995	$y = 5283.6x - 1086.5$	0.039	0.9991	$y = 5453.6x - 662.43$	0.1	0.9996

## Results and discussion

### Selection of extraction solvent

The following combinations of extraction solvents (v/v) were initially investigated to achieve acceptable recoveries for each analyte in pepper: MeCN, MeCN (1% HAc) and MeOH–MeCN (3:7). When 20 mL of MeOH–MeCN (3:7) was used as the extraction solvent, the extracts obtained were too dark in color compared with the MeCN extract because of the absence of salt-induced partitioning with MeOH-containing solvents. In addition, the extracts containing a significant amount of water were difficult to evaporate. Better liquid–liquid partitioning with salts was only obtained with the solvent containing 100% MeCN. When 20 mL of MeCN was used as the extraction solvent, the average recovery range in the low level (0.05 mg/kg) for all 29 pesticides was 61.1–94.0%. The repeatability RSD ranged from 3.3 to 8.9%. The addition of 1% HAc to the extraction solvent (MeCN containing 1% HAc) increased the recovery of all 29 pesticides, and the average recovery was 76.0–103%; the repeatability RSD was <13% (Fig. 1). Figure 1 showed that MeCN (1% HAc) was the most efficient extraction solvent, and it was therefore used in subsequent experiments.

The QuEChERS method was initially developed for fruit and vegetables that have high water content; thus, the addition of water to dry food products prior to analysis was generally recommended. In the present study, water was added to the dehydrated red peppers to hydrate them prior to extraction. Soaking the dehydrated red peppers in water helped to swell the matrix, weaken the interactions of the analyte with the matrix components and assist in efficient extraction. Different ratios of water to extraction solvent were investigated (3:20, 5:20, 7:20, water–MeCN containing 1% HAc, v/v mL) per 2 g of the dehydrated pepper matrix. The recoveries for all 29 pesticides were 68.4–88.6% when using 3:20 (v/v mL) water-to-solvent ratio; the recoveries were 85.3–97.1% when using 5:20 (v/v mL) water-to-solvent ratio; and the recoveries were 86.4–91.5% when using 7:20 (v/v mL) water-to-solvent ratio. Considering the large volume of water need to consume more  $\text{MgSO}_4$  and NaCl, the optimized water-to-solvent ratio was 5:20 (v/v mL) for most of the analytes.

### Selection of sorbent materials

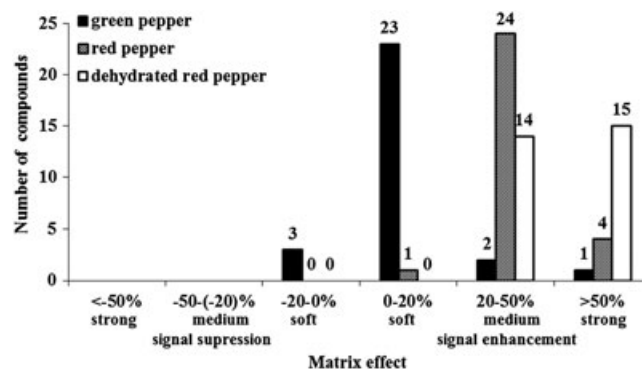
Different sorbent materials, namely,  $\text{C}_{18}$ , PSA and GCB, were investigated to achieve the most effective clean-up. PSA is a weak anion exchange sorbent that retains carboxylic acids, such as fatty acids, from extracts.  $\text{C}_{18}$  is a nonpolar sorbent that effectively retains a trace amount of lipids from the extract. Furthermore, GCB is usually

used in pigment clean up (Kinsella *et al.*, 2009; Sobhanzadeh and Nemati, 2013). Initially, 50 mg of PSA, 50 mg of  $\text{C}_{18}$  and 30 mg of GCB were tested in mixed standard solutions to determine whether they affect pesticide recoveries. Then, the effect of different sorbent materials was studied to remove the impurity peak in the dissolved residues. After the study, we used 50 mg of PSA and 50 mg of  $\text{C}_{18}$  for purification because of their capability to remove impurities and low matrix levels.

### Linearity and matrix effect

Twenty-nine pesticides of different functions (herbicides, fungicides and plant growth regulators) acted as digestive disorders or endocrine disruptors were selected. The retention times, qualitative ions and SIM group start times of the 29 pesticides are shown in Table 1.

Matrix effects (MEs) are a common problem in GC or GC–MS and thus adversely affect the analytical results. The ME was calculated by comparing the slope of the matrix-matched standard curve with the slope of the solvent standard curve. Standard curves at a concentration range of 0.1–10  $\mu\text{g/mL}$  for all tested pesticides in both solvent and matrix-matched standards were generated to evaluate the MEs. The results revealed a satisfactory linearity for all analytes with excellent determination coefficients  $R^2 > 0.998$  (Table 2). When the signal suppression or enhancement effect is between –20 and 20%, the matrix effect is weak (between –50 and –20% or 20 and 50%), the matrix effect is medium, and <–50% or >50%, the matrix effect is strong (Kmetz *et al.*, 2008). The ME distributions in the three pepper matrix extracts for different analytes are shown in Fig. 2. Approximately 80% of the pesticides presented a very soft signal enhancement effect in

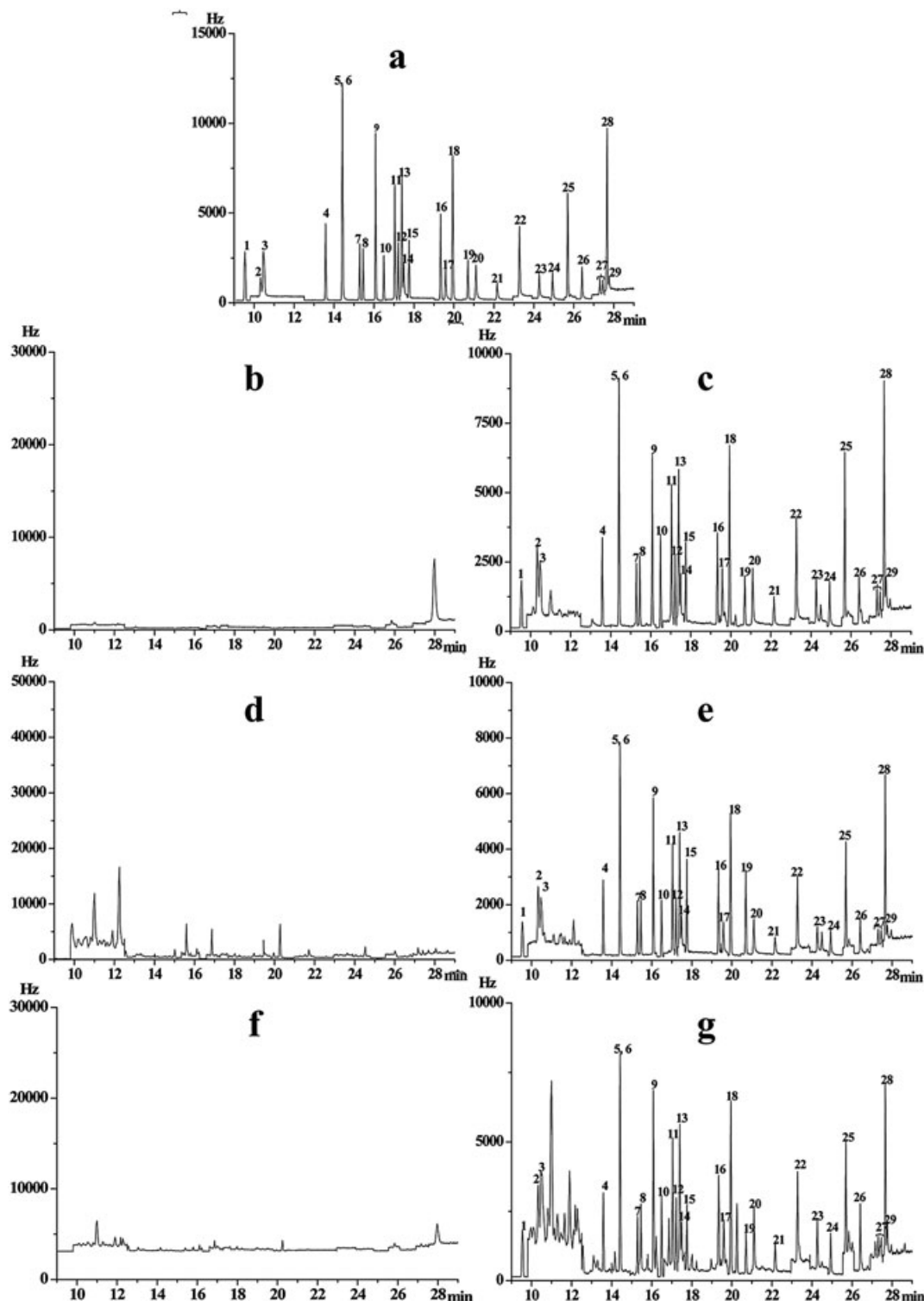


**Figure 2.** Matrix effect (ME) distributions of 29 pesticides in green, red and dehydrated red peppers.

green peppers. More than 80% of the pesticides in red peppers presented a medium signal enhancement effect. For dehydrated red pepper, 50% of the pesticides showed a medium signal enhancement effect and the other 50% showed a strong signal enhancement effect.

### Method performance

The standard, blank and spiked sample chromatograms showed identical retention times without interference (Fig. 3), indicating that the method was selective and specific. The LOD values of



**Figure 3.** Chromatograms of standard (a), blank (b) and spiked (c) green pepper at 0.05 mg/kg; blank (d) and spiked (e) red pepper at 0.05 mg/kg; and blank (f) and spiked (g) dehydrated red pepper at 0.25 mg/kg (1, trifluralin; 2, isoprocarb; 3, carbaryl; 4, atrazine; 5, pyrimethanil; 6, vinclozolin; 7, acetochlor; 8, pirimicarb; 9, chlorpyrifos-methyl; 10, ametryn; 11, metalaxyl; 12, metolachlor; 13, triadimefon; 14, chlorpyrifos; 15, fenthion; 16, triadimenol; 17, cyprodinil; 18, procymidone; 19, paclobutrazol; 20, hexaconazole; 21, buprofezin; 22, uniconazole; 23, myclobutanil; 24, diniconazole; 25, kresoxim-methyl; 26, fludioxonil; 27, propargite; 28, bifenthrin; 29, tebuconazole).

**Table 3.** Precision and accuracy of the method for determining 29 pesticides in pepper.

Pesticide	Spiked level (mg/kg)	Green pepper			Red pepper			Spiked level (mg/kg)	Dehydrated red pepper		
		AR <sup>a</sup> (%)	RSD <sub>r</sub> <sup>b</sup> (%)	RSD <sub>R</sub> <sup>c</sup> (%)	AR <sup>a</sup> (%)	RSD <sub>r</sub> <sup>b</sup> (%)	RSD <sub>R</sub> <sup>c</sup> (%)		AR <sup>a</sup> (%)	RSD <sub>r</sub> <sup>b</sup> (%)	RSD <sub>R</sub> <sup>c</sup> (%)
Trifluralin	0.01	101	9.0	9.5	102	8.9	9.4	0.05	96.2	9.5	9.9
	0.05	94.3	2.1	4.2	90.0	6.2	6.0	0.25	92.4	5.9	8.7
	1	79.2	6.6	3.9	79.2	7.5	6.2	5	77.5	3.0	5.2
Isoprocab	0.01	104	5.7	6.0	102	5.9	6.2	0.05	95.8	6.2	6.5
	0.05	102	8.1	5.6	105	3.3	10.1	0.25	96.0	6.4	9.4
	1	85.2	9.0	7.0	99.4	5.2	8.2	5	83.2	4.3	8.0
Carbaryl	0.01	110	6.6	7.0	103	7.1	7.4	0.05	97.3	7.5	7.9
	0.05	107	2.6	3.1	95.7	3.7	4.0	0.25	88.6	5.1	8.4
	1	88.0	5.1	2.3	88.4	4.7	4.5	5	82.0	2.2	6.2
Atrazine	0.01	105	6.5	6.8	103	6.6	7.0	0.05	97.2	7.0	7.4
	0.05	104	4.1	3.5	104	2.9	2.1	0.25	92.9	5.6	8.2
	1	91.2	5.2	2.3	86.8	4.7	4.4	5	78.2	2.0	7.1
Pyrimethanil	0.01	106	6.3	6.7	104	6.5	6.8	0.05	98.3	6.9	7.2
	0.05	102	3.7	3.5	98.6	2.2	3.9	0.25	92.4	5.8	7.5
	1	87.8	5.3	2.3	87.6	4.4	4.7	5	79.1	1.6	7.0
Vinclozolin	0.01	102	3.5	3.7	103	3.5	3.7	0.05	97.4	3.7	3.9
	0.05	99.1	5.6	3.5	104	2.6	4.0	0.25	88.8	4.2	5.6
	1	92.6	4.9	2.2	86.9	4.6	4.3	5	78.4	1.7	7.3
Acetochlor	0.01	103	6.1	6.4	105	6.0	6.3	0.05	98.8	6.3	6.6
	0.05	100	4.9	3.5	105	3.4	2.6	0.25	95.9	6.4	6.9
	1	90.5	5.0	2.3	89.9	4.9	4.7	5	75.2	1.8	10.6
Pirimicarb	0.01	106	5.0	5.2	108	4.8	5.1	0.05	102	5.1	5.4
	0.05	102	4.2	3.7	104	4.2	3.5	0.25	97.8	5.6	6.5
	1	90.2	4.1	1.9	87.0	5.0	6.2	5	80.9	2.3	7.6
Chlorpyrifos-methyl	0.01	102	5.1	5.4	104	5.0	5.2	0.05	98.5	5.3	5.5
	0.05	99.7	6.3	3.6	106	3.4	5.7	0.25	92.2	5.4	5.1
	1	85.2	5.4	2.8	91.4	4.7	4.8	5	75.2	2.4	11.6
Ametryn	0.01	102	4.5	4.7	105	4.4	4.6	0.05	99.2	4.6	4.8
	0.05	103	6.8	2.4	101	4.5	3.4	0.25	96.1	6.3	6.4
	1	86.1	5.3	2.5	85.6	4.7	6.2	5	75.6	2.3	10.1
Metalaxyl	0.01	105	6.0	6.2	103	6.1	6.4	0.05	97.1	6.4	6.8
	0.05	102	3.7	3.6	95.4	3.7	4.0	0.25	98.8	5.9	6.1
	1	87.8	5.2	2.3	87.3	4.9	6.1	5	80.5	2.1	8.3
Metolachlor	0.01	103	3.5	3.7	106	3.4	3.6	0.05	100	3.6	3.8
	0.05	102	4.9	4.0	105	2.5	2.3	0.25	94.0	5.7	8.0
	1	85.1	4.8	2.1	87.0	5.1	4.7	5	73.9	2.0	10.0
Triadimefon	0.01	102	4.3	4.6	106	4.2	4.4	0.05	99.5	4.5	4.7
	0.05	98.0	5.0	3.6	100	6.2	4.5	0.25	88.7	6.1	5.6
	1	94.4	4.9	2.5	84.6	4.9	4.8	5	72.3	2.1	10.2
Chlorpyrifos	0.01	103	3.9	4.1	101	4.0	4.2	0.05	95.3	4.3	4.5
	0.05	103	3.5	3.7	97.9	6.5	5.7	0.25	93.0	5.7	9.8
	1	89.3	5.7	3.0	87.0	6.1	5.5	5	75.6	2.1	8.0
Fenthion	0.01	93.3	3.3	3.4	99.3	3.1	3.2	0.05	91.5	6.5	6.8
	0.05	85.2	2.9	3.9	97.0	4.3	5.0	0.25	91.9	12.2	10.1
	1	81.2	4.0	1.9	86.4	5.4	4.8	5	75.2	2.0	8.6
Triadimenol	0.01	103	9.4	9.8	104	9.3	9.8	0.05	88.1	9.9	10.4
	0.05	96.9	5.3	5.3	96.9	5.9	5.4	0.25	87.2	7.3	7.1
	1	94.5	4.8	2.2	83.5	4.4	5.0	5	71.2	2.3	10.4
Cyprodinil	0.01	104	7.0	7.4	110	6.6	7.0	0.05	99.9	1.6	1.7
	0.05	99.4	5.6	3.7	101	2.7	2.5	0.25	90.4	5.9	7.9
	1	86.3	4.8	1.8	83.7	4.6	5.1	5	73.0	2.4	9.2
Procymidone	0.01	108	5.3	5.5	114	5.0	5.2	0.05	94.7	6.0	6.3

(Continues)

**Table 3.** (Continued)

Pesticide	Spiked level (mg/kg)	Green pepper			Red pepper			Spiked level (mg/kg)	Dehydrated red pepper		
		AR <sup>a</sup> (%)	RSD <sub>r</sub> <sup>b</sup> (%)	RSD <sub>R</sub> <sup>c</sup> (%)	AR <sup>a</sup> (%)	RSD <sub>r</sub> <sup>b</sup> (%)	RSD <sub>R</sub> <sup>c</sup> (%)		AR <sup>a</sup> (%)	RSD <sub>r</sub> <sup>b</sup> (%)	RSD <sub>R</sub> <sup>c</sup> (%)
Paclobutrazol	0.05	101	5.6	3.4	103	3.2	4.2	0.25	88.8	5.4	7.5
	1	94.2	4.5	2.0	86.4	4.5	4.2	5	71.0	2.0	12.2
	0.01	93.7	3.5	3.7	92.7	3.6	3.8	0.05	90.0	8.3	8.8
	0.05	93.4	5.5	4.2	93.1	5.4	4.7	0.25	84.7	6.0	6.0
Hexaconazole	1	95.6	5.6	2.9	81.2	4.8	7.3	5	70.8	1.5	12.6
	0.01	98.8	4.8	5.0	96.8	4.9	5.1	0.05	90.8	5.2	5.5
	0.05	93.0	6.3	5.6	94.9	7.6	6.1	0.25	83.7	5.0	5.5
	1	92.7	5.3	2.4	81.3	4.3	6.3	5	71.7	1.2	11.2
Buprofezin	0.01	104	10.1	10.6	107	9.8	10.3	0.05	90.8	7.7	8.1
	0.05	90.6	4.3	4.4	101	3.7	3.1	0.25	84.0	10.0	8.0
	1	92.3	4.6	2.2	82.1	4.9	5.8	5	72.4	2.0	9.8
	0.01	101	9.1	9.6	100	9.2	9.7	0.05	85.3	5.9	6.2
Uniconazole	0.05	92.0	7.6	5.0	90.3	3.5	3.8	0.25	81.2	6.5	6.1
	1	95.6	5.0	2.3	79.8	4.4	6.3	5	70.1	4.3	11.8
	0.01	99.8	7.0	7.3	94.8	7.4	7.7	0.05	94.6	7.7	8.1
	0.05	96.2	6.8	4.9	90.2	6.3	6.9	0.25	85.8	6.0	6.0
Myclobutanil	1	98.2	4.8	2.2	81.0	4.6	6.7	5	72.7	3.1	10.7
	0.01	103	8.6	9.0	102	8.7	9.1	0.05	88.6	5.0	5.3
	0.05	92.2	9.1	5.3	92.8	3.5	3.6	0.25	75.1	7.2	10.2
	1	94.2	4.6	2.2	80.0	4.8	7.4	5	85.5	2.6	5.4
Kresoxim-methyl	0.01	102	4.4	4.7	109	4.2	4.4	0.05	97.2	4.7	4.9
	0.05	98.6	6.5	4.3	102	2.5	2.4	0.25	81.2	5.6	9.7
	1	91.5	4.4	2.1	82.6	5.0	4.8	5	73.8	5.0	8.5
	0.01	92.4	4.4	4.6	101	4.0	4.2	0.05	88.4	4.6	4.8
Fludioxonil	0.05	83.1	9.7	5.4	103	2.6	2.6	0.25	84.2	6.6	6.5
	1	86.7	6.6	2.5	85.0	4.8	5.2	5	71.3	2.9	11.9
	0.01	102	4.7	5.0	111	4.4	4.6	0.05	98.4	4.9	5.2
	0.05	101	7.2	6.6	108	5.0	4.4	0.25	99.5	7.1	8.6
Propargite	1	105	3.7	5.2	92.7	5.5	5.8	5	86.4	3.0	6.1
	0.01	101	1.7	1.8	104	1.6	1.7	0.05	94.6	3.2	3.4
	0.05	99.7	6.6	3.6	98.7	2.2	3.2	0.25	92.7	7.1	11.5
	1	94.1	4.4	3.6	76.9	5.6	5.9	5	76.0	4.0	6.0
Tebuconazole	0.01	99.3	1.0	1.1	101	7.0	7.3	0.05	83.6	7.4	7.8
	0.05	95.1	4.0	6.4	93.1	8.0	5.7	0.25	77.3	5.8	8.5
	1	92.6	5.1	2.5	80.9	5.0	6.1	5	73.2	3.5	7.0

<sup>a</sup>Average recoveries.<sup>b</sup>Average accuracy of six replicates in one day.<sup>c</sup>Average accuracy of 18 replicates over three different days.

the 29 tested pesticides were 0.0018–0.018, 0.0015–0.012 and 0.004–0.075 mg/kg in green, red and dehydrated red peppers, respectively. The LOQs of different analytes ranged from 0.006–0.06 mg/kg for green peppers, 0.005–0.039 mg/kg for red peppers and 0.014–0.25 mg/kg for dehydrated red peppers (Table 2).

Intra- and inter-day recovery experiments were carried out to evaluate the stability and precision of the established method. The spiked levels were set at 0.01, 0.05 and 1 mg/kg for green and red peppers and at 0.05, 0.25 and 5 mg/kg for dehydrated red pepper. For green peppers, the average recoveries ranged from 79.2 to 110% with RSD<sub>r</sub> (intra-day RSD) of 1.0–10.1% and RSD<sub>R</sub> (inter-day RSD) of 1.1–10.6%. For red peppers, the average recoveries ranged from 76.9 to 114% with RSD<sub>r</sub> of 1.6–9.8% and RSD<sub>R</sub> of 1.7–10.3%. For dehydrated red pepper, the average recoveries

ranged from 70.1 to 102% with RSD<sub>r</sub> of 1.2–12.2% and RSD<sub>R</sub> of 1.7–12.6%. The data are summarized in Table 3. The mean recoveries ranged between 70.1 and 110%, and the RSDs were <13% for all matrices. The results indicated that the newly established method was satisfactory and repeatable for the determination of 29 pesticides in green, red and dehydrated red peppers.

#### Analysis of real samples

The proposed analytical method was used to determine the 29 tested pesticide residues in marketed peppers. Four batches peppers were sampled and analyzed (three green pepper samples, three red pepper samples and three dehydrated red pepper samples for each batch). The 29 tested pesticides were undetectable



(less than the LOQs) in two batches of samples, and the amounts of the 29 tested pesticides in the other two batches were less than the MRLs (0.01–10 mg/kg for fresh peppers, 2–50 mg/kg for dried peppers). For example, three of the 29 pesticides, metalaxyl (MRL 0.5 mg/kg, EU), fludioxonil (MRL 1 mg/kg, Codex Alimentarius Commission (CAC)), and bifenthrin (MRL 0.5 mg/kg, EU) were found in one sample of green pepper at 0.02, 0.15 and 0.02 mg/kg, respectively. Another four of the 29 pesticides, metalaxyl (MRL 0.5 mg/kg, EU), chlorpyrifos (MRL 0.5 mg/kg, Japan), kresoxim-methyl (MRL 0.8 mg/kg, EU) and bifenthrin (MRL 0.5 mg/kg, EU), were found in one sample of red pepper at 0.02, 0.02, 0.01 and 0.01 mg/kg, respectively. In one sample of dehydrated red pepper, carbaryl (MRL 2 mg/kg, CAC), chlorpyrifos (MRL 20 mg/kg, EU) and paclobutrazol (MRL 0.5 mg/kg, Japan) were found at 0.71, 0.26 and 0.26 mg/kg, respectively. The results showed that the established method was satisfactory for the analysis of real pepper samples.

## Conclusion

A simple and rapid modified QuEChERS method was developed for the routine analysis of 29 pesticides by GC–MS in peppers. The residues were dissolved in acetone and cleaned with 50 mg of PSA and 50 mg of C<sub>18</sub>, providing a clean chromatogram. Validation results showed that the GC–MS method had good accuracy, precision, sensitivity, selectivity and stability with RSDs <13% and recoveries between 70.1 and 110%. The proposed method was successfully used to screen the 29 tested pesticide residues in actual peppers on the Chinese market.

## Conflicts of interest

The authors have declared no conflicts of interest.

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