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Development of an improved method to extract pesticide residues in foods using acetontrile with magnesium sulfate and chloroform

Guozhu Liu[a], Lei Rong[b], Bin Guo[a], Mingshan Zhang[a], Shengjun Li[a], Qing Wu[a], Jitao Chen[a], Bo Chen[a,*], Shouzhuo Yao[a]

[a] Key Laboratory of Chemical Biology & Traditional Chinese Medicine Research, Ministry of Education, Hunan Normal University, Changsha 410081, China
[b] School of Environment, Beijing Normal University, Beijing 100875, China

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ABSTRACT

A multiresidue method was developed based on extraction of 10 g sample with 10 mL acetonitrile and subsequent liquid–liquid partitioning formed by adding 4 g $MgSO_4$ plus 1 mL chloroform. During the partitioning process, the extraction recoveries of polar analytes were found to be essentially determined by the acetonitrile content in the aqueous phase. The use of $MgSO_4$ gave the least acetonitrile left in the aqueous phase (lower than 5%) and thus promoting complete partitioning of analytes into the organic phase. At the same time, removal of water from the acetonitrile phase was achieved by adding only a small amount of chloroform with no influence on the acetonitrile content in the aqueous phase, thus leading to decreasing the co-extraction of polar matrix components. The most complete mutual separation of acetonitrile and water was achieved by the joint use of $MgSO_4$ and chloroform and thus the optimal extraction recovery and analytical selectivity were obtained simultaneously. The new method, with higher recoveries of polar analytes, better analytical selectivity and simpler manipulation, is a claimed improvement to the original QuEChERS method. The proposed method was finally validated by the determination of 20 pesticides in a mixed food matrix by using liquid chromatography tandem mass spectrum (LC–MS/MS). Acceptable linearity, sensitivity, recovery, precision and selectivity results were obtained.

1. Introduction

Food safety, which is a worldwide public heath concern and is leading cause of trade problems internationally, has been achieved great concern by governments of different countries and relevant international organizations. Legislations have been established in which maximum limits of contaminants in food and environmental samples have been set. This has fostered the development of effective residue monitoring method in compliance with these legislations. During production, manufacturing, storage and transport of food, a variety of contaminants, e.g. pesticides, veterinary drugs, mycotoxins and illegal additives, may enter the food chain; the contaminant treatment history of a food sample is usually hard to be known. Thus, in theory, the food sample should be assayed for all suspect chemical hazards from different chemical families with quite distinct physicochemical characteristics. Without question, the multiclass, multiresidue method (MRM) is the best choice for this purpose.

The earliest multiclass MRM is the Mills method developed in 1960s [1]. This method is based on acetonitrile (MeCN) extraction and liquid–liquid partitioning between MeCN–water mixture and petroleum ether. The Mills method worked very well with the nonpolar organochlorine (OC) pesticides that were the main focus in 1960s; however, some of the relatively polar residues, such as organophosphorus (OP) pesticides developed in 1970s, were hardly recovered by this step. Thus, the Luke method was developed in 1970s to meet the demand of a wide analytical polarity range [2]. Produce sample was first extracted with acetone, and then both salt (NaCl or $MgSO_4$) and nonpolar solvent (dichloromethane, dichloromethane–petroleum ether or cyclohexane–ethyl acetate) were added to induce phase separation of the acetone–water mixture [2–4]. This multiclass MRM essentially differed from the Mills method by that a phase transition process was involved in the Luke method and as a result the water-miscible solvent, acetone, was transferred from water into the organic phase, which is essential for the successful extraction of the polar OPs. However, the main drawback of the Luke method is that a large amount of nonpolar solvents (usually at least equal volume to acetone) should be added to complete the phase separation since acetone is too miscible with water, thus diluting the analytes. Furthermore, the extract solution from the Luke approach is not compatible with reversed-

* Corresponding author. Tel.: +86 731 8865515; fax: +86 731 8865515.
E-mail address: dr-chenpo@vip.sina.com (B. Chen).

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phase liquid chromatography (LC); therefore, evaporation and solvent exchange to polar solvents is needed when analyzing LC-amenable contaminants, leading to the cost of more time, labor and expense.

MeCN is much more easily separated from water than acetone since almost all salts [5–7], all hydrophobic but MeCN-soluble organic solvents [8], and some saccharides (mainly monosaccharides and disaccharides) [9] can be used as inducers to induce phase separation of the MeCN–water mixture. In 2003, Anastassiades et al. proposed a highly streamlined multiclass MRM for analysis of pesticides in fruits and vegetables, which was called QuEChERS, standing for quick, easy, cheap, effective, rugged and safe [10]. This procedure involves initial single-phase extraction with MeCN followed by salting-out extraction/partitioning by addition of $MgSO_4$ plus NaCl, and finally using dispersive solid-phase extraction (d-SPE) for cleanup. The QuEChERS approach is obviously superior to previous ones in the following three aspects: (1) the novel use of $MgSO_4$ instead of other salts results in high-quality extraction results for a wide polarity range of analytes; (2) since the final extract solution is MeCN, the QuEChERS method can be well compatible with both GC–MS and LC–MS (MS/MS) which have become the main analytical tools in most contaminant monitoring laboratories to meet world standards; (3) all steps in this method have been well designed and highly streamed.

Therefore, since its inception, the QuEChERS concept has got great worldwide popularity. The QuEChERS approach was initially developed for extraction of pesticides from high-moisture food matrices with low fat content, such as fresh fruits and vegetables [11–17]. However, this approach with minor modification has been subsequently proved to work well for both dry [18–20] and fatty [21–23] food matrices, and also for various non-food matrices, e.g. environmental samples such as soil [24–26] and waste water [27,28], and biological samples such as whole blood [29], muscle [30] and tissue [31]. Furthermore, besides pesticides analysis, the QuEChERS method has been widely applied for detection of veterinary residues [32–35], mycotoxins [20], pharmaceuticals [29], poisons [29] and even phytochemical compositions [36] in various matrices.

However, in the development of the QuEChERS method, Anastassiades et al. made a compromise between the extraction recovery and the selectivity (degree of cleanup) by using $MgSO_4$ combined with NaCl. The authors reported that using $MgSO_4$ alone gave the highest recoveries for the polar pesticides but resulted in poor selectivity because a large amount of water remained in the MeCN phase in this case. Considering that the use of NaCl could result in less water in the MeCN phase, the final method was decided to choose 4 g $MgSO_4$ in combination with 1 g NaCl to 10 g sample and 10 mL MeCN as the extraction/partitioning condition to decrease the co-extraction of polar matrix components. To some extent, the analytical selectivity was improved by this design, but the extraction recoveries of polar analytes decreased when NaCl was used. In addition, another drawback of the original QuEChERS is that the used salts ($MgSO_4$ and NaCl) can be partially transferred to the MeCN phase because a certain amount of water still remained in the MeCN phase. These nonvolatile salts are harmful to the MS detector and can result in ion suppression of the analytes with weak retention.

Therefore, the aim of this study was to improve upon the original QuEChERS method to realize the goal that better selectivity can be obtained without sacrificing recoveries of polar analytes. The two very polar OPs, i.e. methamidophos and acephate, were selected as the model analytes to develop the new method. The effectiveness of different extraction/partitioning conditions were systematically investigated and compared in terms of analytes recoveries, analytes matrix effects, and the background of the full scan MS chromatograms. Finally, method validation of the newly proposed QuEChERS method was conducted for the determination of 20 pesticides in a mixed matrix.

2. Experimental

2.1. Chemicals and reagents

All investigated pesticide standards, including methamidophos, acephate, aminocarb, methomyl, carbendazim, cymoxanil, thidiazuron, thiodicarb, atrazine, isoproturon, acibenzolar-S-methyl, myclobutanil, chromafenozide, diflubenzuron, famoxadone, benzoximate, clofentezine, fenoxaprop-ethyl, chlorpyrifos and pendimethalin, were purchased from Sigma (Poole, UK) and were of purity > 95% (w/w). All of these pesticides are commonly used agrochemicals and come from different structural groups and thus have diverse properties. HPLC-grade MeCN and dimethylformamide was purchased from Tedia Company (Fairfield, Ohio, USA). Ultrapure water was prepared by a Millipore Milli-Q purification system (Millipore Corp., Bedford, MA). Other common reagents, including dichloromethane, chloroform, acetic acid, magnesium sulfate, sodium sulfate, sodium carbonate, sodium chloride and potassium chloride, were all Analytical grade. All selected samples, including fruits, vegetables and meats, were purchased from a local food store. These samples were chopped and freezed by dry ice and then homogenized. Fortified samples were prepared by spiking the respective working standard solutions with the homogenized samples (mixtures of several foods) and then underwent a homogenization process again. All of the collected samples, homogenates and fortified samples were stored at −20 °C until extraction.

2.2. Newly proposed extraction procedure

10 g previously homogenized sample was weighed in a 50 mL teflon centrifuge tube. Then 10 mL MeCN was added, and the tube was vigorously shaken by using a vortex mixer for 1 min. After this, 4 g $MgSO_4$ and 1.0 mL chloroform were added, and the shaking process was repeated for 1 min. The extract was then centrifuged for 10 min at 4000 rpm (4500 × *g*). An aliquot of 1 mL of the extract was transferred into a vial and was analyzed by LC–MS/MS.

2.3. Determination of the MeCN content in the aqueous phase and the water content in the MeCN phase by gas chromatograph (GC) analysis

0.1 mL of each phase was diluted by 50 mL of dimethylformamide (DMF) containing 0.1% (v/v) ethanol (used as the internal standard), and then was analyzed by GC. For calibration, several MeCN aqueous solutions were prepared with MeCN content ranging from 5 to 100% (v/v). These calibration samples were also diluted using DMF (containing 0.1% ethanol) and were analyzed by GC. All calculations to measure MeCN contents were achieved by using a calibration plot of peak area ratio of MeCN with respect to the internal standard (ethanol). The MeCN content (C_{MeCN}) of the aqueous phase was directly determined by such a step.

For analysis of the water content of the organic phase, the MeCN content (C_{MeCN}) was first measured. Then, in the case of using chloroform or dichloromethane as the inducer to perform phase separation, the hydrophobic solvent content (C_{Hyd}) was also measured by a similar step to determine MeCN as described above (additional calibration samples were prepared with corresponding hydrophobic solvent). Thus, the water content (C_{Water}) in the MeCN phase can be clearly calculated as: $C_{Water} = 100\% - (C_{MeCN} + C_{Hyd})$.

GC analysis was performed based on a Shimadzu GC-2010 system equipped with a FID detector (Tokyo, Japan). A fused-silica capillary GC column of 30 m × 0.53 mm i.d. coated with 0.25 μm film thickness (HP-5, Agilent Technologies) was used to separate

Table 1
Parameters for the LC–MS/MS analyses of the twenty pesticides in the validation study.

No.	Compound	t_R (min)	MRM transitions (Q1 → Q3)		Compound-devendent MRM conditions			
			MRM1 (qualitation)	MRM2 (quantitation)	DP (V)	EP (V)	CE (Ev) MRM1, MRM2	CXP (V) MRM1, MRM2
1	Methamidophos	2.63	142.1 → 94.1	142.1 → 125.1	20.0	10.0	30.0, 30.0	15.0, 15.0
2	Acephate	2.72	184.2 → 143.1	184.2 → 125.1	35.0	10.0	42.0, 37.0	2.0, 2.0
3	Aminocarb	9.46	209.2 → 137.1	209.2 → 152.1	30.0	10.0	32.0, 18.0	3.0, 3.0
4	Methomyl	11.22	163.1 → 88.1	163.1 → 106.0	15.0	10.0	15.0, 13.0	3.0, 3.0
5	Carbendazim	12.04	192.0 → 160.0	192.0 → 132.0	27.0	10.0	25.0, 41.0	6.0, 6.0
6	Cymoxanil	14.22	199.0 → 128.0	199.0 → 111.0	46.0	10.0	13.0, 25.0	6.0, 6.0
7	Thidiazuron	16.28	221.2 → 102.1	221.2 → 127.9	46.0	4.0	21.0, 21.0	4.0, 4.0
8	Thiodicarb	17.49	355.1 → 88.1	355.1 → 108.0	20.0	10.0	27.0, 31.0	3.0, 3.0
9	Atrazine	19.01	216.0 → 174	216.0 → 104.1	46.0	4.5	23.0, 39.0	4.0, 4.0
10	Isoproturon	19.25	207.2 → 72.1	207.2 → 165.1	26.0	10.0	25.0, 19.0	12.0, 10.0
11	Acibenzolar -S-methyl	22.04	211.0 → 136	211.0 → 140.0	26.0	10.0	39.0, 31.0	6.0, 6.0
12	Myclobutanil	22.73	289.0 → 70.0	289.0 → 125.0	36.0	10.0	33.0, 41.0	4.0, 6.0
13	Chromafenozide	23.34	395.0 → 175.1	395.0 → 339.0	50.0	10.0	30.0, 18.0	15.0, 15.0
14	Diflubenzuron	24.75	311.2 → 158.1	311.2 → 141.0	76.0	4.5	21.0, 37.0	4.0, 4.0
15	Famoxadone	26.36	392.0 → 331.0	392.0 → 238.0	11.0	10.0	15.0, 23.0	12.0, 8.0
16	Benzoximate	27.41	364.0 → 199.0	364.0 → 105.0	30.0	10.0	17.0, 35.0	7.0, 6.0
17	Clofentezine	27.60	303.0 → 138.0	303.0 → 102.0	56.0	10.0	21.0, 57.0	6.0, 6.0
18	Fenoxaprop-ethyl	29.67	362.0 → 288.0	362.0 → 121.0	46.0	10.0	23.0, 37.0	10.0, 6.0
19	Chlorpyrifos	31.80	350.0 → 125.0	350.0 → 97.0*	41.0	10.0	19.0, 43.0	6.0, 4.0
20	Pendimethalin	31.99	282.0 → 212.0	282.0 → 194.1	6.0	10.0	15.0, 23.0	4.0, 4.0

MeCN, ethanol (I.S.) and the hydrophobic solvents. A gradient column temperature program was used for the oven, starting at 40 °C for 5 min and increasing up to 250 °C at a rate of 15 °C/min. The temperatures of the FID detector and injector were held at 300 and 280 °C, respectively. Nitrogen was used as the carrier gas.

2.4. Liquid chromatography tandem mass spectrometry (LC–MS/MS) analysis

Chromatographic separation of the analytes was based on an Agilent 1100 series HPLC system (Agilent Technologies, USA) consisting of a quaternary pump, a vacuum degasser and a thermostatted autosampler. In the proposed modified QuEChERS method, the sample solution is MeCN containing low content of chloroform; thus, an ultimate XB C18 column (5 μm, 250 mm × 4.6 mm) (Welch Materials, Ellicott, USA) with high carbon loading (17%), which is insensitive to the presence of hydrophobic solvents with low concentration, was used with the new QuEChERS method to avoid broadening peaks of polar analytes. A binary mobile phase composed of 0.1% (v/v) acetic acid in water (eluent A) and 0.1% (v/v) acetic acid in MeCN (eluent B) pumped at a total flow rate of 1 mL/min was used for separation of analytes. The gradient elution program started with 20% B, and increased linearly to 40% B in 10 min, further to 90% B in 25 min, and kept at 90% B for 5 min, and then returned to the initial composition (20% B) in 1 min and held for 5 min to re-equilibrate the column prior to the next injection. The injection volume was 5 μL.

MS detection was carried out on an API 4000 triplestage quadrupole (Q1–Q2–Q3) tandem mass spectrometer (Applied Biosystems/MDS Sciex, Toronto, Canada) equipped with a Turbolonspray electrospray ionization (ESI) source. The outlet of the column was split, and only 0.2 mL/min portion of the effluent was delivered into the ESI source. The ESI source was operated in the positive ion mode. Capillary voltage and turbo ion spray interface source temperature were set at 4.5 kV and at 450 °C, respectively. Nitrogen was used as the nebulizer gas, auxiliary gas and curtain gas with values at 10, 50 and 50 psi, respectively. MS/MS experiments were performed in the MRM acquisition mode for simultaneous detection of all selected analytes. All parameters for each MRM transition were tuned to efficiently produce its characteristic fragment ions. The compound-dependent ion transition parameters for each analyte, including precursor ion (Q1), product ion (Q3), declustering potential (DP), collision energy (CE) and collision cell exit potentials (CXP), were used the optimized values as shown in Table 1.

2.5. Method validation

An eight-level series in triplicate for matrix-matched calibration curves were prepared with nominal concentrations at 10–500 μg/kg for 20 pesticides spiked in a mixed blank matrix (apple–grape–squash–pork–beef, 1 + 1 + 1 + 1 + 1). All fortified calibration samples were processed with the newly proposed QuEChERS procedure and analyzed by LC–MS/MS as described above. The calibration curves were constructed using weighed ($1/X$) linear regression of the analyte peak areas (Y) versus the analyte concentrations (X) with the corresponding correlation coefficients (r).

To evaluate the matrix effect and the extraction recovery of the proposed method, the blank mixed matrices were fortified with corresponding analytes in five replicates at the level of 50 μg/kg. In addition, nonfortified samples were included in the test set and the obtained blank extracts were used to prepare the matrix matched calibration standards of which the concentrations were equal to 50 μg/kg in the samples. Finally, corresponding solvent (50% MeCN/water (v/v)) standards of which the concentrations were also equal to 50 μg/kg in the samples were prepared. Average recoveries and relative standard deviations (RSDs) were calculated by comparing the peak area of each analyte in the fortified sample with that of the matrix-matched standard. Matrix effects and relative standard deviations (RSDs) were calculated by comparison of the response obtained for each compound in the matrix-matched standard with that of the solvent standard.

To assess the precision of the method, five replicate extractions and measurements of the fortified samples at 50 μg/kg were conducted during five consecutive days. Intraday precision was calculated as the average value of the five daily RSDs, while interday precision was calculated as the RSD of the total twenty-five data ($n = 5 \times 5$).

Table 2
Influence of different inducers on the phase separation process and the partitioning of two polar organophosphorus pesticides.

Inducer	Added amount[a] (mL or g)	Volume of the MeCN Phase (mL)	C_{H_2O}[b] in MeCN phase (% v/v)	C_{MeCN}[c] in aqueous phase (% v/v)	Partition coefficient[d]	
					Methamidophos	Acephate[e]
Chloroform	0.5	3.3	3.9	42.0	0.4	0.6
	1	5.4	< 2	38.5	0.6	1.1
	1.5	6.6	<2	35.3	0.6	1.0
	2	7.2	<2	34.8	0.7	1.0
Dichloromethane	1	5.6	<2	39.2	0.5	0.7
	2	7.5	<2	34.1	0.8	0.9
NaCl	0.5	3.6	27.0	43.5	0.6	0.9
	1	5.9	10.2	29.9	1.6	1.2
	2	6.6	3.9	19.5	2.5	3.5
	3	8.1	2.9	15.3	3.5	2.9
KCl	1	6.3	12,3	28.4	1.3	2.0
	2	6.8	4.7	20.1	2.8	3.7
Na_2SO_4	0.5	11.6	31.2	25.4	1.4	2.1
	1	10.5	20.3	15.5	2.4	3.8
	2	10.7	16.3	10.0	4.5	5.5
Na_2CO_3	1	10.3	22.5	17.1	3.5	4.3
	2	10.5	17.4	12.5	5.4	6.2
$MgSO_4$	0.5	16.2	43.0	3.9	26.6	36.2
	1	14.0	35.4	4.8	25.8	35.9
	2	11.5	21.4	5.1	27.5	34.7
	3	11.0	15.4	4.4	31.0	37.4
	4	10.8	13.7	4.2	29.8	39.6

[a] To 10 mL water and 10 mL MeCN.
[b] The water content.
[c] The MeCN content.
[d] The ratio of the concentration of analyte in the MeCN (upper) phase to that in the aqueous (lower) phase.
[e] Methamidophos and acephate were added to the mixture of MeCN and H_2O before phase separation with the same concentration of 5 μg/mL.

3. Results and discussion

3.1. Characteristics to control the partitioning process of polar analytes

MeCN is easily and effectively separated from water by adding polar substances including salts [5–7] and sugars [9], and also hydrophobic solvents [8]. In the present study, phase separation experiments induced by different amounts of various salts and also two hydrophobic solvents (chloroform and dichloromethane) were conducted. The two very polar OPs, methamidophos and acephate, were selected as the model analytes and their partition coefficients in MeCN/aqueous system were determined by LC–MS analysis of the two separated phases. Furthermore, the solvent compositions of both the organic and the aqueous phase were determined by GC analysis with the purpose to explain the partitioning process with respect to analytes recoveries. All experiment data were summarized in Table 2.

Different results were obtained by using different types and different amounts of inducers. However, by carefully examining the data in Table 2, it is very interesting to note that the investigated inducers can be clearly sorted into four groups, i.e. two hydrophobic solvents; NaCl and KCl; Na_2SO_4 and Na_2CO_3; and finally $MgSO_4$. The inducers from the same group with the same added amounts resulted in very similar both solvent composition and volume of the two layers (organic and aqueous), and thus providing very similar partitioning results for the two OPs. When using the same amount of different inducer, the MeCN content in the aqueous phase decreased in the following order: two hydrophobic solvents–NaCl and KCl–Na_2SO_4 and Na_2CO_3–$MgSO_4$. This result is consistent with that the partition coefficients of the two OPs dramatically increased in the same order. In addition, besides in the case of using $MgSO_4$, the MeCN content in the aqueous phase decreased when larger amount of inducer was added, which results in the partition coefficients of the two OPs clearly increased. So, it is obvious that the partitioning behavior of polar compounds is essentially determined by the MeCN content in the aqueous phase: the less MeCN remained in water results in more complete partitioning of polar analytes into the MeCN phase.

During the development of the original QuEChERS method, Anastassiades et al. stated that the recoveries of polar analytes would primarily correlated with the water content in the MeCN phase based on the hypothesis that more water dissolved in the MeCN phase would lead to more polar analytes recovered because the MeCN phase would become more polar and thus more receptive for polar compounds [15]. We believe this is an incomplete argument because the polar analytes that can be extracted by MeCN must give better solubility in MeCN than in water. As shown in Table 2, such a viewpoint is not supported by the fact that partition coefficients of polar OPs increased when larger amount of inducer was used which resulted in less water in the organic phase.

3.2. Development of the new QuEChERS method

As discussed above, the extraction recovery tightly correlates with the MeCN content in the aqueous phase, independent of the water content in the MeCN phase. However, the water content in the organic phase also should be controlled since more water remaining in the organic phase are expected to lead to more water-soluble matrix substances to be co-extracted. Thus, the best extraction/partitioning process requires the most complete mutual separation of MeCN and water to simultaneously obtain high extraction recovery and good analytical selectivity. Based on recoveries, $MgSO_4$ is the best choice due to the lowest MeCN content in the aqueous phase, but the water content in the MeCN phase was very high in this case, suggesting reduced selectivity would be obtained. The use of NaCl or chloroform resulted in very low water content in the MeCN phase, but a large amount of MeCN remained in the aqueous phase and thus resulting in poor extraction recoveries of polar analytes. Considering the joint use of different inducers

Table 3
Influence of combining chloroform/NaCl with $MgSO_4$ on the phase separation process and the partitioning of two polar organophosphorus pesticides.

Modifier[a]		Volume of the MeCN phase (mL)	C_{H_2O}[b] in MeCN phase (% v/v)	C_{MeCN}[c] in aqueous phase (% v/v)	Partition coefficient[d]	
					Methamidophos	Acephate[e]
$MgSO_4$ (g)	Chloroform (mL)	Combination of chloroform and $MgSO_4$				
4	0	10.8	13.7	4.2	29.8	39.6
4	0.5	10.1	5.7	6.1	30.3	38.3
4	1	10.2	<2	5.5	25.7	37.5
4	1.5	10.1	<2	5.4	29.8	36.6
4	2	10.4	<2	4.7	28.0	34.2
0	1	5.4	<2	38.5	0.6	0.9
0.5	1	7.5	<2	28.5	1.2	2.1
1	1	8.6	<2	20.2	3.8	3.2
2	1	10.2	<2	9.4	6.8	12.5
3	1	10.4	<2	4.9	25.0	31.2
$MgSO_4$ (g)	NaCl (g)	Combination of NaCl and $MgSO_4$				
4	0	10.8	13.7	4.2	29.8	39.6
4	0.5	9.8	10.8	5.5	23.6	35.5
4	1	8.9	7.4	6.9	14.3	25.2
4	1.5	7.7	4.9	7.5	12.6	17.7
4	2	7.6	<2	9.1	8.0	8.5
4	3	6.6	<2	10.7	5.0	7.5

[a] To 10 mL water and 10 mL MeCN.
[b] The water content.
[c] The MeCN content.
[d] The ratio of the analyte responsive in the MeCN (upper) phase to that in the aqueous (lower) phase.
[e] Methamidophos and acephate were added to the mixture of MeCN and H_2O before phase separation with the same concentration of 5 μg/mL.

may have the potential to achieve the good extraction result, the effects of using $MgSO_4$ combined with different amount of both NaCl and chloroform were evaluated.

The MeCN/water partitioning result by the joint use of $MgSO_4$ and NaCl is similar to the result in the study by Anastassiades et al. [10]. As Table 3 indicated, the addition of increasing amounts of NaCl along with constant amount of $MgSO_4$ (4 g to 10 mL water and 10 mL MeCN) yields: (1) the water content in the MeCN phase dramatically decreased; (2) the MeCN content in the aqueous phase increased and thus the partition coefficients of polar OPs decreased; (3) the volume of the MeCN phase obviously decreased. It is thus clear that the partitioning effect with the combination of $MgSO_4$ and NaCl is a compromised result between the effects by using the two salts alone. However, the joint use of $MgSO_4$ and chloroform provided different results: (1) the MeCN content in aqueous phase maintained the lowest value when using different amounts of chloroform in combination with 4 g $MgSO_4$, and thus very high partition coefficients of the two polar OPs were obtained in all these cases; (2) the water content in the MeCN phase was lower than 2% when the added amount of chloroform was higher than 1 mL; (3) the volume of the MeCN phase only varied slightly when more chloroform was used. It seems that the additional use of chloroform has no influence on the MeCN content in the aqueous phase but effectively removes the water from the MeCN phase. The optimal partitioning result was thus achieved by the joint use of $MgSO_4$ and chloroform.

A simple medium effect theory proposed previously [37–39] can be used to explain the above interesting experiment results. According to the medium effect theory, the mechanism of the MeCN/water salting-out phenomenon can be explained as that the added salts are preferentially solvated by water, thus making water less available (becoming much more polar) for dissolving MeCN and therefore leading to that some of the MeCN molecules are "pushed out" from water. At the same time, the mechanism of the hydrophobic solvent induced MeCN/water partitioning phenomenon can be explained by a reversed process: the hydrophobic solvents are preferentially solvated by MeCN, thus making MeCN too nonpolar to dissolve water and therefore resulting in that water was "pushed out" from the MeCN phase. These two "pushing out" effects are two independent processes and can be performed simultaneously with no influence on each other. Thus, the joint use of $MgSO_4$ and chloroform can achieve the best partitioning result since the use of $MgSO_4$ could ensure the MeCN content in the aqueous phase below 5% while the use of chloroform could effectively drive water from the MeCN phase. However, the joint use of $MgSO_4$ and NaCl yielded an unsatisfied result due to the reason that the two salts ($MgSO_4$ and NaCl) all act on the aqueous phase and thereby the partitioning result obtained must be a compromised one.

Thus, with no question, the joint use of $MgSO_4$ and chloroform is the better choice to develop a multiclass MRM. As discussed above, 1 mL chloroform (to 10 mL water and 10 mL MeCN) is enough to drive water from the MeCN phase. The effect of different amount of $MgSO_4$ combined with 1 mL chloroform on the partitioning of the two OPs was evaluated as well. Table 3 shows that the use of more $MgSO_4$ resulted in less MeCN remaining in the aqueous phase and thus gave higher partition coefficients of the two polar OPs. Therefore, in the final method, we chose 4 g $MgSO_4$ combined with 1 mL chloroform (to 10 g sample and 10 mL MeCN) as the extraction/partitioning condition.

3.3. Selectivity evaluation

As discussed above, the redesigned QuEChERS method is characterized by high extraction recovery of polar analytes. Here, the analytical selectivity of the new method was evaluated and compared with the original one. Fig. 1 shows the full-scan LC/MS chromatograms of the extracts from a mixture of fruits, a mixture of vegetables and a mixture of meats, by using the new and the original QuEChERS method. Several peaks with high abundance appeared in the first 4 min of the chromatograms of all investigated mixed matrices when using $MgSO_4$ in combination with NaCl. Such peaks should be the signals of salts dissolved in the MeCN phase and the very polar co-extractives. Fabulously, these signals disappeared or significantly decreased when chloroform was used in combination with $MgSO_4$. The reason for this is that the addition of chloroform would drive water from the MeCN phase and thus effectively remove both the salts and the very polar matrix components from the extract.

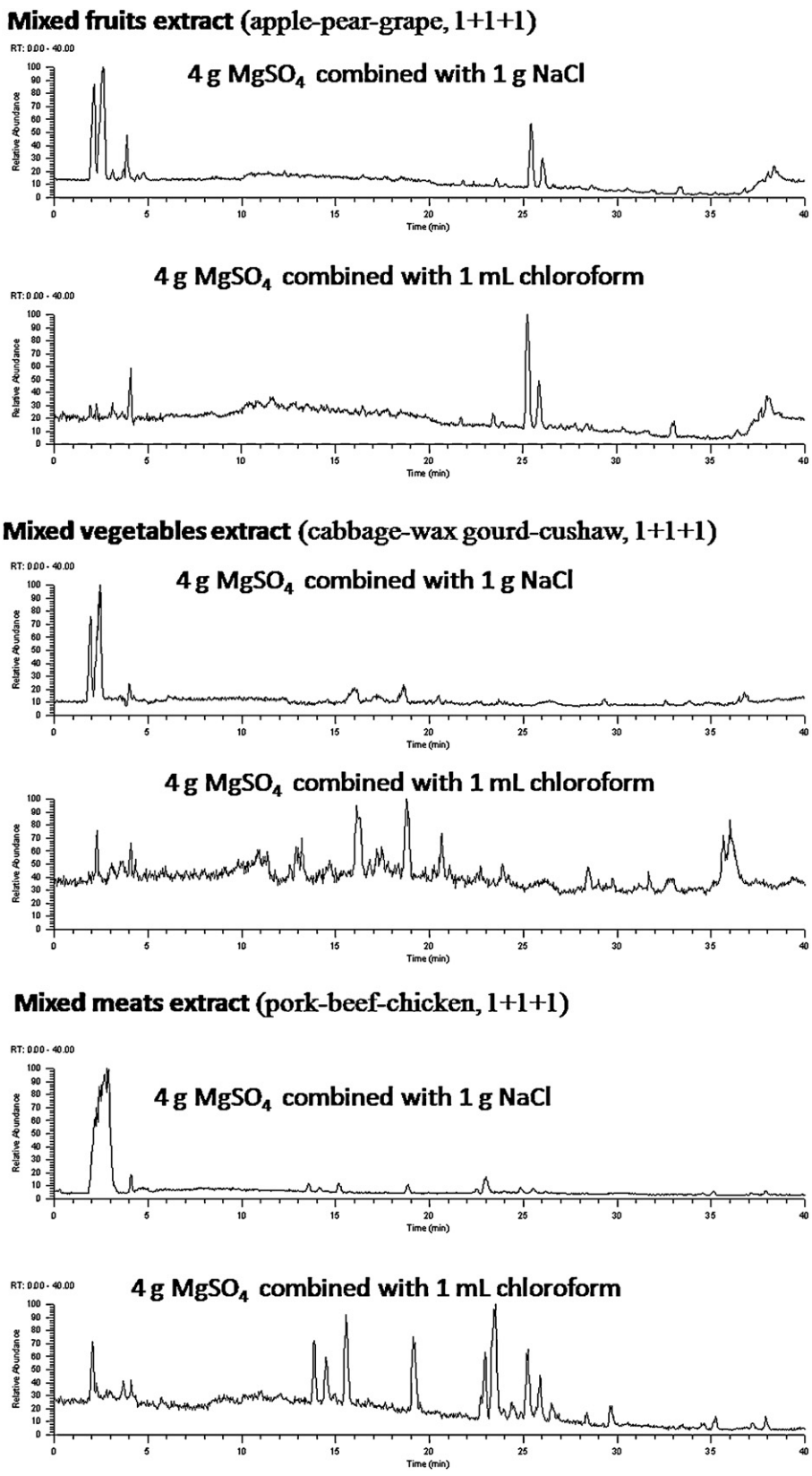

Fig. 1. LC–MS chromatograms (full scan mode) of three mixed extracts by using the conventional QuEChERS method and the new QuEChERS method.

The matrix effect, which reflects the presence or absence of MS signal suppression or enhancement of analytes, was also evaluated for the two OPs in the three mixed samples by using the two different partitioning methods, and the results are compared in Fig. 2. Matrix effects were obviously observed for the two OPs in all three mixed samples by using $MgSO_4$ in combination with NaCl. However, matrix effect values for the two OPs are close to 1 when using $MgSO_4$ in combination with chloroform,

Table 4
Validation results for determination of the twenty pesticides fortified in a mixed matrix by using the new QuEChERS method.

No.	Compound	Linearity r^2[a]	LOD μg/kg	Recovery % (RSD)[b]	Matrix effect % (RSD)[c]	Intraday precision	Interday precision
1	Methamidophos	0.9932	5	91.2% (8%)	89 (10)	7	17
2	Acephate	0.9954	5	93.5% (7%)	85 (11)	4	15
3	Aminocarb	0.9994	1	98.2% (3%)	95 (6)	4	7
4	Methomyl	0.9988	5	99.0% (2%)	96 (2)	7	9
5	Carbendazim	0.9994	1	102.4% (3%)	108 (9)	4	9
6	Cymoxanil	0.9961	5	101.4% (3%)	107 (3)	4	13
7	Thidiazuron	0.9973	1	95.5% (11%)	75 (12)	3	7
8	Thiodicarb	0.9933	1	87.6% (12%)	73 (5)	7	14
9	Atrazine	0.9991	1	88.5% (4%)	85 (4)	9	14
10	Isoproturon	0.9918	5	101.3% (3%)	94 (7)	6	7
11	Acibenzolar-S-methyl	0.9990	5	91.4% (9%)	94 (7)	8	10
12	Myclobutanil	0.9982	1	95.4% (11%)	104 (9)	4	6
13	Chromafenozide	0.9937	1	96.5% (2%)	106 (1)	5	5
14	Diflubenzuron	0.9985	5	106.9% (6%)	93 (4)	4	5
15	Famoxadone	0.9988	5	90.2% (7%)	95 (7)	7	12
16	Benzoximate	0.9925	5	98.2% (8%)	86 (1)	5	11
17	Clofentezine	0.9994	5	92.2% (3%)	87 (5)	7	15
18	Fenoxaprop-ethyl	0.9981	5	87.8% (4%)	87 (7)	3	9
19	Chlorpyrifos	0.9989	5	98.6% (7%)	105 (3)	5	6
20	Pendimethalin	0.9974	5	95.6% (9%)	104 (3)	6	11

[a] Linearity evaluated in the range from 10 to 500 μg/kg.
[b] Data obtained at 50 μg/kg ($n = 5$).
[c] Data obtained at 50 μg/kg ($n = 5$).

meaning no obvious matrix effect in this case. Thus, the newly designed extraction/partitioning method gives excellent analytical selectivity.

In the conventional QuEChERS method, a dispersive-SPE cleanup step with primary secondary amine (PSA) and a drying step with $MgSO_4$ were normally employed to reduce some polar matrix interferents and the residual water in the extract solution. The drying step should be removed in the redesigned QuEChERS method because very little water remained in the MeCN phase. The PSA material can effectively remove the polar matrix interferents, such as sugars, organic acids and polar pigments [10], but also has the potential to adsorb some acidic analytes [40] and to degrade some base-sensitive substances [41]. Thus, the dispersive-SPE step with PSA was not recommended in our design since cleaner extract can be obtained. However, for special application, the dispersive-SPE step with PSA or other sorbent materials, such as C18, GCB and graphitized carbon, can be conveniently adopted in the new QuEChERS method, if necessary.

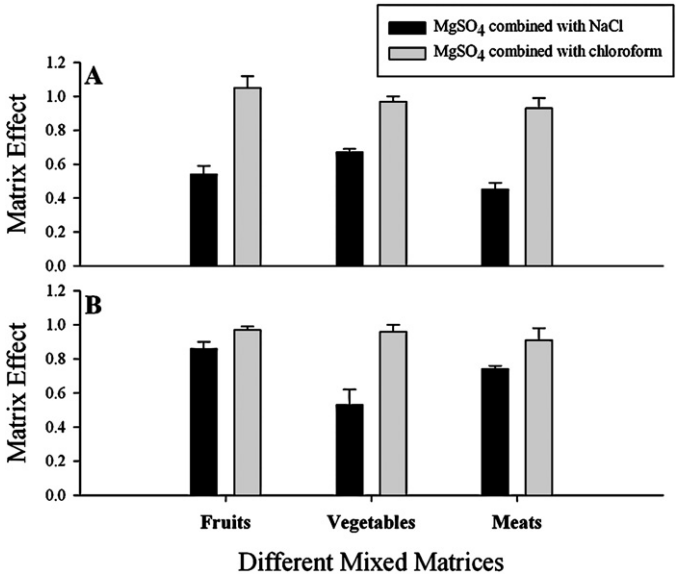

Fig. 2. Matrix effect of methamidophos (A) and acephate (B) in the extract from the three mixed matrices.

3.4. Method validation

Method validation of the newly proposed QuEChERS method was conducted for the determination of 20 pesticides in a mixed matrix (apple–grape–squash–pork–beef, 1 + 1 + 1 + 1 + 1). HPLC–MS/MS was used during this validation study because of its good quantitative performance characteristics (high sensitivity and wide dynamic range). Several validation parameters, i.e. linearity, sensitivity, extraction recovery, matrix effect and precision, were determined and were summarized in Table 4. The typical LC–MS/MS MRM chromatogram of the 20 pesticides spiked in the mixed sample was shown in Fig. 3.

Response linearity for each pesticide in the mixed matrix was evaluated by matrix-matched calibration standards at eight concentration levels in the range from 10 to 500 μg/kg. Good linearity results with regression coefficients higher than 0.99 were obtained for all pesticides. The limit of quantitation (LOD) was settled as the value where the signal for the analyte was significantly higher than the background (the signal-to-noise ratio higher than 10). The achieved LOD values for the pesticides in the mixed sample were all below or equal to 5 μg/kg. This sensitivity is sufficient to verify compliance of foodstuffs with legal tolerances.

The extraction recovery and the matrix effect for the 20 analytes in the mixed matrix were evaluated at the 50 μg/kg fortifying level. As expected, good extraction results (recovery higher than 85%) were obtained for all investigated analytes, further proving the characteristic of high extraction recovery for the new QuEChERS method. For most of the investigated analytes, the matrix effect values are close to 1 (in the range from 0.85 to 1.1), meaning no obvious matrix effect. However, obvious matrix-induced suppressions were observed for thidiazuron and thiodicarb (75% and 73%, respectively). Thus, the use of matrix matched calibration solutions is still necessary to compensate errors associated with matrix induced suppression or enhancement effects in the new QuEChERS method.

Precision of the proposed method was also evaluated based the fortified samples at 50 μg/kg level. Both the intraday and interday precision values were lower than 20% for all pesti-

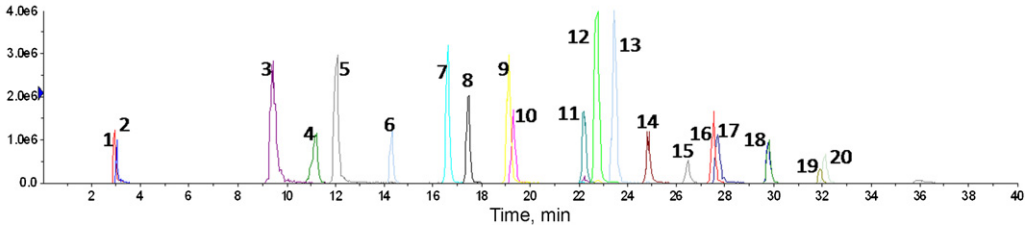

Fig. 3. Overlaid LC–MS/MS MRM chromatograms of extracted ion transitions for twenty pesticides spiked at 100 μg/kg in a mixed matrix (apple–grape–squash–pork–beef, 1 + 1 + 1 + 1 + 1).

cides, confirming the good reproducibility and repeatability of this method.

4. Conclusion

In this study, the improvement of the original QuEChERS method to obtain increased analytical selectivity without sacrificing the extraction recoveries of polar analytes, was demonstrated to be successful. The effectiveness of different extraction/partitioning conditions was systematically investigated, and the joint use of $MgSO_4$ and chloroform was recommended in our final method. During the phase separation process, the MeCN can be "pushed out" from water by adding very polar substances, such as salts; at the same time, the water can also be "pushed out" from MeCN by adding nonpolar ones, such as hydrophobic solvent. These two processes can be performed simultaneously to obtain mutually promoted results. Thus, the most complete mutual separation of MeCN and water was achieved by adding $MgSO_4$ in combination with chloroform. The little MeCN left in the aqueous phase promotes the analytes to be completely extracted, while little water in the MeCN phase decreases the co-extraction of salts and very polar matrix components and thus the analytical selectivity is enhanced and the harmful effect to the MS instrument may be reduced. Certainly, one drawback of the proposed method is the use of chloroform which would lead to environmental and safety concerns. However, the low usage amount of chloroform weakens this negative effect.

Acknowledgments

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