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Analysis of organophosphorus and pyrethroid pesticides in organic and conventional vegetables using QuEChERS combined with dispersive liquid-liquid microextraction based on the solidification of floating organic droplet

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ABSTRACT

A simple, sensitive and environmentally-friendly method for determining organophosphorus and pyrethroid pesticides in vegetables was developed to better evaluate the risk of consuming them. The pesticides in vegetables were extracted, purified and concentrated by using the QuEChERS (quick, easy, cheap, effective, rugged and safe method) combined DLLME-SFO (dispersive liquid–liquid microextraction based on solidification of floating organic droplet) techniques. The key parameters were optimized through orthogonal array experimental design and statistical analysis. The linearity of the calibration curves was satisfied in matrix-matched standard solution with $R^2 \geq 0.99$. The limits of detection and limits of quantification were 0.3–1.5 and 0.9–4.7 μg/kg, respectively. The average recoveries of pesticides were 61.6–119.4% with relative standard deviations < 16.1%. Furthermore, the method was applied successfully to analyse the pesticides in 15 pairs of organic and conventional vegetables. These results reflect the efficiency, reliability and robustness of the developed method.

1. Introduction

Vegetables can supply essential nutrients, such as vitamins, minerals, dietary fibers, antioxidants and other benefits (Slavin & Lloyd, 2012). Regular consumption of vegetables is recommended because it can enhance human immunity and prevent certain diseases, e.g. diabetes, obesity, constipation, cardiovascular diseases, and cancer (Yu & Yang, 2017). To boost crop yield, various pesticides have been applied widely to prevent, repel, or control pests (Pang, Yang, & He, 2016). Among them, organophosphorus (OP) and pyrethroid (PYR) pesticides are the most extensively used (Chen et al., 2002). OP and PYR can penetrate the crop matrix and maybe converted to the oxidation and hydrolysis products which are more toxic to humans (Stratton & Corke, 1982; Wu, Luan, Lan, Hung Lo, & Chan, 2007). OP and PYR have been detected in human biological samples (Becker et al., 2006) and they have been demonstrated as neurotoxin insecticides. Usually, the main dysfunction caused in the human body by OP and PYR is inhibition of acetylcholinesterase and modulation of voltage gated ion channels, respectively. Furthermore, OP and PYR can also interfere with other biochemical targets (Babina, Dollard, Pilotto, & Edwards, 2012). For example, some PYR pesticides can disrupt estrogen function (Chen et al., 2002). Thus, there is increased concern regarding pesticide contamination on vegetables. Many countries and organizations have set maximum residue limits (MRLs) for OP and PYR pesticides in edible foods. For instance, the Singapore Food Agency has defined detailed MRLs for different OP and PYR pesticides in different of vegetable samples available in Singapore (https://www.sfa.gov.sg/ava/). Thus, the development of a simple, rapid, reliable and environmentally-friendly method to detect the pesticide residues in vegetable samples is urgently required.

The determination of pesticide residues in vegetables remains a challenge because of the trace amount of target analytes and complex interference components in vegetable matrices (Rizzetti et al., 2016). To eliminate interference and achieve good performance of the

Abbreviations: QuEChERS, quick, easy, cheap, effective, rugged and safe; DLLME–SFO, dispersive liquid–liquid microextraction based on solidification of a floating organic droplet; OP, organophosphorus; PYR, pyrethroid; MRLs, maximum residue limits; d-SPE, dispersive solid phase extraction; DLLME, dispersive liquid–liquid microextraction; GC–MS, gas chromatography-mass spectrometry; HPLC, high performance liquid chromatography; PSA, primary secondary amine; EI, electron impact; SIM, selected ion monitoring mode; LODs, limits of detection; LOQs, limits of quantification; ANOVA, analysis of variance; LSD, least significant difference; OAD, orthogonal array experimental design; RSDs, relative standard deviations

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analytical method, increasing efforts have been made to develop the effective, simple, and quick sample preparation techniques. In these techniques, QuEChERS (quick, easy, cheap, effective, rugged and safe) method developed by Anastassiades et al. in 2003 has received great attention and achieved great achievements (Camara, Barba, Cermeno, Martinez, & Oliva, 2017; Oliva, Cermeno, Camara, Martinez, & Barba, 2017; Paya, Anastassiades et al., 2007; Paya, Oliva, Camara, & Barba, 2007a, 2007b). The method involves an initial solvent extraction with acetonitrile and purification of the extract using dispersive solid phase extraction (d-SPE) (Yan et al., 2013). QuEChERS is one of the most prevalent sample preparation techniques for the extraction of pesticides in plant and animal matrices (Yan et al., 2013). Furthermore, it is an official AOAC method to determine pesticide residues in vegetables (Dashtbozorgi, Ramezani, & Waqif-Husain, 2013). However, the low enrichment factor of QuEChERS limits its sensitivity, leaving room for further improvement of this method (Cunha & Fernandes, 2011; Wang, Shu, Li, Yang, & Qiu, 2017). Dispersive liquid–liquid microextraction (DLLME) consists of an extraction solvent, a dispersive solvent and an aqueous phase. In this ternary system, the extraction solvent, dispersive solvent and aqueous phase demonstrate a very high contact area (You, Jiang, Liu, & Liu, 2012). The merits of DLLME include high enrichment factor, speed, and low reagent consumption (Wang et al., 2017). To date, DLLME has been commonly used to extract and concentrate pesticides in aqueous samples (Chen, Chen, & Li, 2010); however, it is difficult to extract solid food samples.

To overcome the drawbacks of QuEChERS and DLLME techniques and achieve better sample preparation, several researchers have attempted to combine the two methods (Dashtbozorgi et al., 2013). The conventional DLLME method uses organic solvents with a higher density than water (Ahmad, Al-Sibaai, Bashammakh, Alwael, & El-Shahawi, 2015). Most such solvents are toxic halogenated hydrocarbons, such as chlorobenzene, $CHCl_3$, CH_2Cl_2, and CCl_4, which are hazardous to health and cause serious pollution (You et al., 2012). Moreover, the microvolume of the sedimented organic layer is collected using the microsyringe, making the process difficult to handle and may cause sample loss and contamination during collection (March & Cerda, 2016). For these reasons, dispersive liquid–liquid microextraction based on solidification of a floating organic droplet (DLLME–SFO) was developed and has attracted much attention. In DLLME-SFO, the extraction solvent has a lower density than water, and its melting point around room temperature means that it can be rapidly solidified in an ice bath for easy collection (You, Wang, Liu, & Shi, 2013).

The organic food industry is well known for its prohibition of the use of chemical pesticides and fertilizers (Bourn & Prescott, 2002). In recently years, because it is vulnerable to be infected by pathogens for using organic fertilizers, microbiological safety of organic food has received much attention (Zhao, Zhao, Phey, & Yang, 2019). However, the precise evaluation of chemical pesticide residues in organic food has rarely been reported (Baker, Benbrook, Groth, & Lutz Benbrook, 2002).

Hence, the aim of this work was to develop a simple, sensitive, environmentally-friendly method by combining QuEChERS and DLLME-SFO techniques for determining trace levels of OP and PYR pesticides in organic and conventional vegetables using gas chromatography-mass spectrometry (GC–MS). In this method, the purification and enrichment of extracted pesticides were completed in a single process. This procedure was simpler than many reported methods, where purification and enrichment processes were operated independently (Melo, Mansilha, Pinho, & Ferreira, 2012; Seebunrueng, Santaladchaiyakit, & Srijaranai, 2015). Moreover, the experimental conditions were optimized by using univariate analysis and orthogonal array experimental design. The significant effects on recoveries of pesticides were also discussed via statistical analysis. Finally, the proposed method was robust and successfully applied to analyze pesticide residues in 15 pairs of organic and conventional vegetable samples.

2. Materials and methods

2.1. Chemicals and reagents

High performance liquid chromatography (HPLC)-grade acetonitrile and methanol were obtained from Macron Fine Chemicals (Radnor, PA, USA). Ethanol and acetone were purchased from Fisher Chemical (Waltham, MA, USA). *n*-Hexadecane was purchased from Tokyo Chemical Industry Co., LTD (Tokyo, Japan). Deionized water was prepared using a Mill-Q purification system. Primary secondary amine (PSA) and Magnesium sulfate anhydrous ($MgSO_4$) were obtained from Sigma-Aldrich (St, Louis, MO, USA). All pesticides were at analytical grade (purity > 97.8%), including malathion, chlorpyrifos, parathion, bifenthrin, cyhalothrin, permethrin, fenvalerate, and deltamethrin were also purchased from Sigma-Aldrich. Stock solutions of each pesticide at 50 mg L^{-1} were prepared in acetone. Various concentrations of the matrix standard working solutions were obtained by dilution of the stock solutions with blank sample extracts. All solutions were stored at 0–4 °C.

2.2. Organic and conventional vegetable samples

Fifteen pairs of organic and conventional vegetables were purchased from local supermarkets in Singapore, which included lettuce, long bean, broccoli, tomato, carrot, pumpkin, siew pak choy, sweet choy sum, sweet pak choy, celery, amaranth, spinach, cabbage, mushroom, and cucumber. The vegetables were homogenized separately using an electric juicer. One batch of organic lettuce was analyzed following the procedure described below, and a sample showing the absence of the target pesticides was used as blank sample in the preparation of the standards and in the recovery study. The spiked samples were prepared by adding a certain amount of a mixture of pesticide standard solution into the homogenized vegetable paste, then shaking vigorously for 2 min, and equilibrating for 2 h in the dark at 4 °C in a refrigerator to permit the spiked solution to penetrate the sample matrix.

2.3. Sample preparation

The pesticides were extracted from vegetable sample using QuEChERS combined with DLLME-SFO. A schematic illustration of the method is shown in Fig. 1, and included the following steps: (1) 5.0 g of sample or spiked sample was weighed in a 50 mL centrifuge tube; (2) 5 mL of ethanol was added and the tube was immediately vortexed for 30 s; (3) 4 g of anhydrous $MgSO_4$ and 1 g of NaCl were added, the mixture was immediately shaken for 2 min, and then centrifuged at 4000 × *g* for 4 min; (4) 1 mL of the ethanol extract was transferred into a 15 mL screw cap centrifuge tube with a conical bottom containing 50 mg primary secondary amine (PSA); (5) 1 mL water and 20 μL of *n*-hexadecane were rapidly added to the tube, and the whole solution was incubated in an ultrasonic bath at 40 °C for 10 min; (6) after centrifugation for 4 min at 4000 × *g*, the tube was cooled in an ice bath for 6 min to solidify the organic extraction phase; (7) the solidified organic phase was transferred into a conical vial using tweezers and melted quickly at room temperature; the vial was then centrifuged for 2 min at 12000 × *g* to separate residual water; (8) 8 μL of supernatant organic phase was diluted with 80 μL of acetone because of the high boiling point of *n*-hexadecane. Finally, 1 μL of the mixture was used for GC–MS analysis.

2.4. GC–MS analysis

A Shimadzu GC 2010 gas chromatograph (Shimadzu, Kyoto, Japan) fitted with a BPX-5 fused silica capillary column (30 m, 0.25 mm i.d., 0.25 μm film thickness) and a Shimadzu GCMS-QP2010 Ultra mass selective detector was used to analyze pesticide residues in the organic and conventional vegetable extracts. Helium, with a purity of 99.999%,

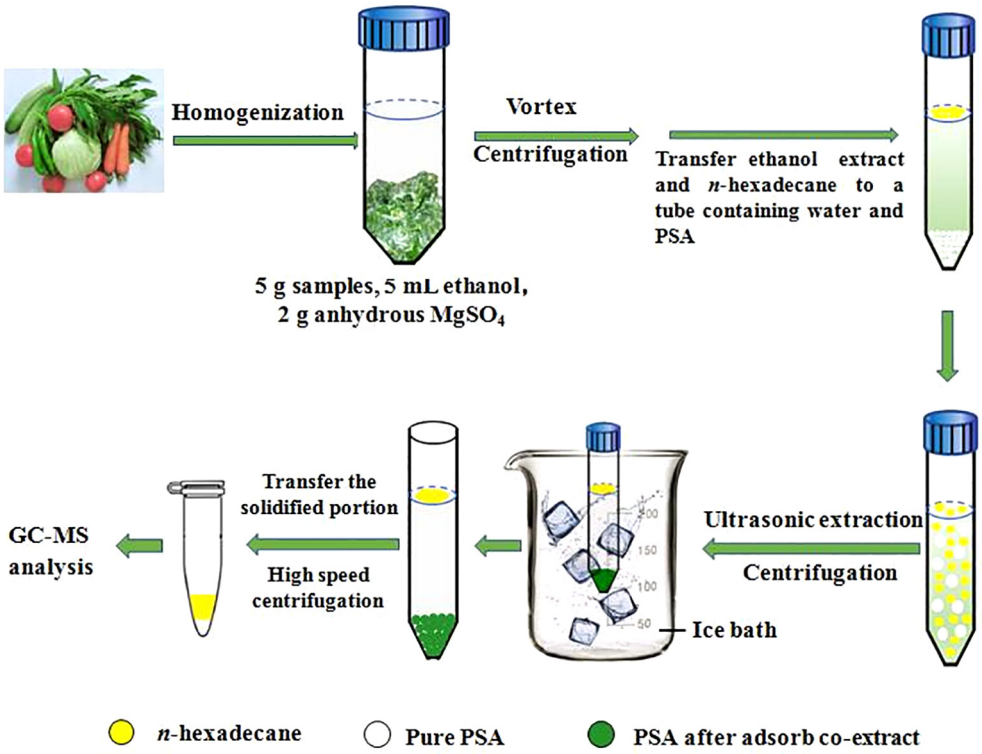

Fig. 1. Schematic illustration of the developed method.

was used as the carrier gas at a flow rate of 1.0 mL/min. 1 μL of sample was injected into the inlet in splitless mode at 260 °C using a Shimadzu AOC-5000 plus autosampler. The mass spectrometer was operated in electron impact (EI) mode at 70 eV. The ion source and transfer-line temperature were set at 280 °C and 280 °C, respectively. The oven temperature program was initially at 100 °C; increasing at a rate of 20 °C/min up to 220 °C, held for 2 min; and then increased at a rate of 15 °C/min up to 280 °C, and held for 12 min. Solvent delay was 10 min. Analysis was performed in the selected ion monitoring mode (SIM), based on the use of one target and two qualifier ions. Target and qualifier ions were determined by injection of individual pesticide standards under the same chromatographic conditions in full-scan mode, with the mass/charge ratio ranging from *m/z* 50 to 500. Groupings were defined to increase the sensitivity of the MS analysis. Chemical structures, retention times, the target and qualifier ions, and the SIM program of the target pesticides are shown in Table S1.

2.5. Method validation

Validation of the developed method was performed using the following parameters: Linearity, limits of detection (LODs), limits of quantification (LOQs), method accuracy, and precision. Linearity was studied using matrix-matched calibrations by spiking blank vegetable extract at seven different concentration levels from 5 to 500 μg/kg. The LOD and LOQ values were estimated based on a signal-to-noise ratio of 3 and 10, respectively. To assay the accuracy of the method, recoveries were investigated in the vegetable samples spiked at three concentration levels of 20, 50, and 100 μg/kg. The spiked samples were processed according to the above sample preparation procedures. The recoveries were determined by comparing the calculated amounts of pesticides in the samples with the spiked amounts. The precision of the method, expressed as repeatability (intra-day) and reproducibility (inter-day), was assessed by determining the spiked samples at three concentration levels.

2.6. Statistical analysis

The preliminary trials were performed in triplicate to achieve reliable and accurate results. The data were analyzed statistically using analysis of variance (ANOVA) in IBM SPSS software (Version 18, IBM Corp., Armonk, NY, USA), and means were compared using the least significant difference (LSD) method to assess the significant differences. Differences with $P < 0.05$ were considered significant. The results are presented in histograms. Within each pesticide, means with different capital letters are significantly different ($P < 0.05$) among different groups. Furthermore, ANOVA was used to test the significance of the factors in the orthogonal array experimental design (OAD). Finally, analyses of the differences between the means of different levels were carried out using LSD multiple comparisons. The significance level was set at $P < 0.05$.

3. Results and discussion

3.1. Optimization of experimental parameters

In this study, 5.00 g of blank vegetable sample spiked with pesticides at 0.010 mg/kg was used for optimization. Parameters comprising the amount of PSA and $MgSO_4$ were assessed following a previously reported QuEChERS method (Wang et al., 2017). The parameters of the DLLME-SFO step were optimized and investigated. The initial extract containing target analytes and vegetable matrix was obtained by the QuEChERS method, and was used as the dispersive solvent in the DLLME-SFO step. The dispersive solvent volume was fixed at 1 mL to investigate the influence of extraction solvent, water volume, and other parameters on extraction efficiency in DLLME-SFO step.

3.1.1. Selection of the extraction solvent

Selection of extraction solvent is one of most vital process in DLLME-SFO because the physical properties of the solvent determine its ability to extract the target analytes. In general, extraction solvent should possess several characteristics: (1) Lower density than water, (2)

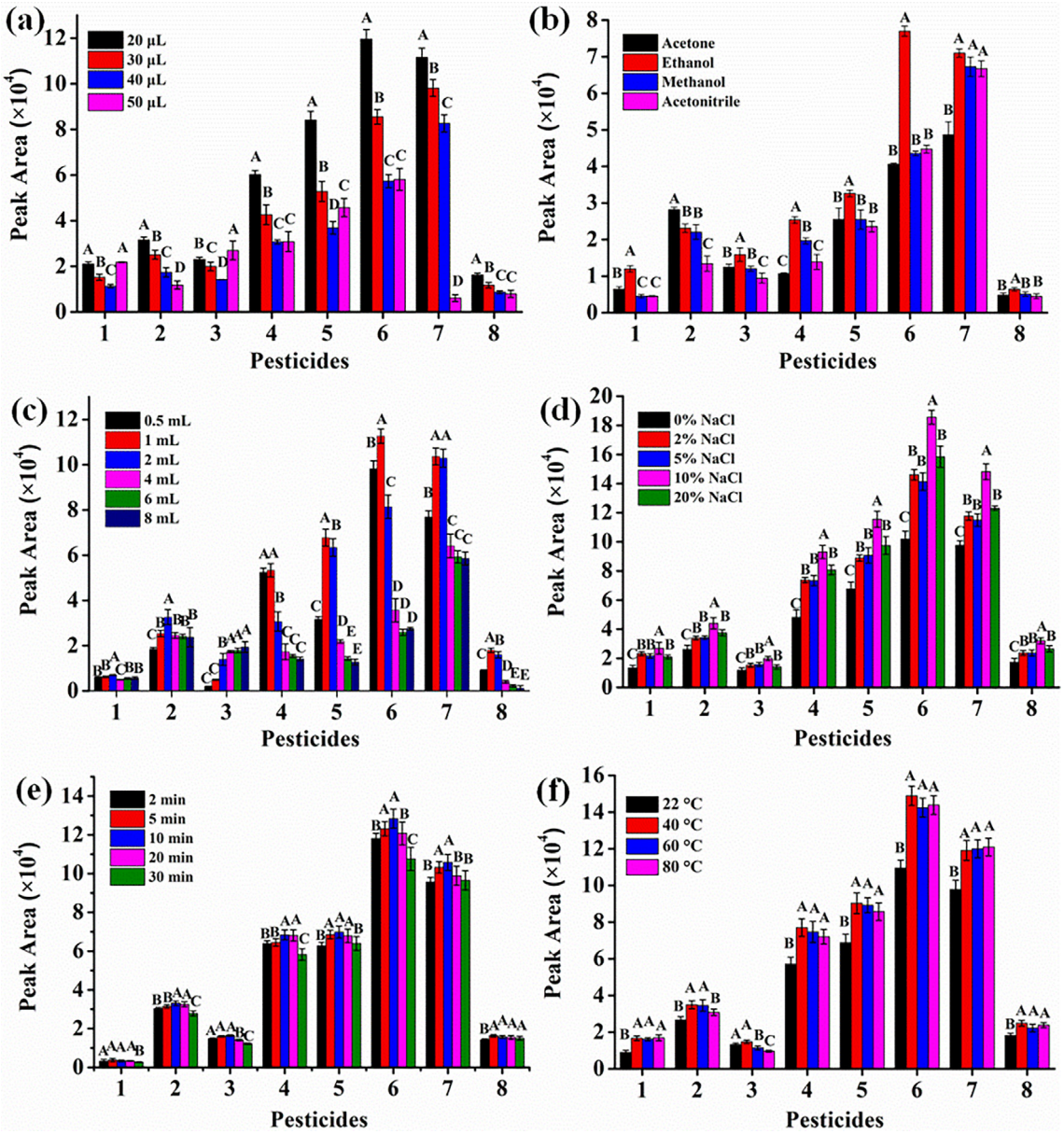

Fig. 2. Optimization of the pretreatment conditions: (a) Volume of the extraction solvent, (b) Selection of the disperser solvent, (c) Selection of the water volume, (d) Salt addition, (e) Extraction time, (f) Effect of temperature. Within each pesticide, means with different capital letters are significantly different ($P < 0.05$) among different groups. Peak identification: 1. Malathion, 2. Chlorpyrifos, 3. Parathion, 4. Bifenthrin, 5. Cyhalothrin, 6. Permethrin, 7. Fenvalerate, 8. Deltamethrin.

superior extraction ability for analytes, (3) immiscible with water, and (4) satisfactory chromatographic behavior. Thus, four organic solvents, namely oleylalcohol, dodecanol, cyclohexane, and *n*-hexadecane were selected and evaluated. When oleylalcohol and dodecanol were selected as extraction solvents, serious emulsification was found and an obvious layer between the water and organic solvents could not be achieved. For cyclohexane, the solidified cyclohexane divided into small drops and was difficult to collect or transfer. Therefore, *n*-hexadecane was used as the extraction solvent in the subsequent experiments.

3.1.2. Volume of the extraction solvent

To optimize the extraction volume, volumes of *n*-hexadecane ranging from 20 to 50 μL were evaluated. The experiment results (Fig. 2a) show that the peak areas of the analytes decreased as the volume of the extraction solvent (*n*-hexadecane) increased from 20 to 50 μL because of the dilution effect (Leong & Huang, 2009). However, if the extraction solvent volume was less than 20 μL, the floating organic phase would divide into small drops and was difficult to collect. Therefore, 20 μL of extraction solvent was selected for subsequent experiments.

3.1.3. Selection of the dispersive solvent

The dispersive solvent in the DLLME-SFO step was the initial extract obtained from the QuEChERS step, thus the dispersive solvent should be able to extract the target analytes from the sample. For this purpose, ethanol, methanol, acetonitrile, and acetone, displaying this ability, were tested for sample preparation. A comparison of the effect of these solvents is shown in Fig. 2b, which shows that the highest peak area was obtained when ethanol was used as the dispersive solvent. In addition, ethanol is more environmentally friendly and much cheaper than the other solvents. Thus, ethanol was selected as the dispersive solvent.

3.1.4. Selection of the water volume

In DLLME-SFO process, the aqueous phase was used as an intermediate phase, which can disperse the extraction solvent to enrich the target analytes. Therefore, different volumes of deionized water (0.5, 1,

2, 4, 6, and 8 mL) were evaluated. As observed in Fig. 2c, peak areas increased as the volume of deionized water increased up to 1 mL, and then decreased at higher volumes. So, 1 mL of water was chosen in the subsequent experiments.

3.1.5. Salt addition

Salt addition is commonly used in liquid-liquid extraction processes because it can increase the ionic strength and affect the extraction efficiency. However, a high ionic strength could lead to an inefficient mass transfer rate and low recovery (Martin, Santos, Aparicio, & Alonso, 2015). The effect of salt addition was investigated by adjusting the concentration of NaCl in the aqueous phase within the range of 0 to 20% (w/v). As shown in Fig. 2d, the analytical signals for the target analytes was highest when concentration of NaCl as 10%; therefore, 10% NaCl was chosen in subsequent experiments.

3.1.6. Type of extraction and extraction time

Vortex agitation and ultrasound assisted extraction are efficient techniques to enhance liquid-liquid microextraction efficiency (March & Cerda, 2016). Therefore, the two techniques were evaluated and compared. When vortex agitation was applied to the extraction system, a cloudy and stable dispersion of fine droplets in the dispersive solvent was formed after 5 min. The comparable phenomenon was also observed in ultrasonic treatment after 10 min. Compared with vortex agitation, ultrasound assisted extraction can process many samples simultaneously. Thus, ultrasound assisted extraction was adopted. A suitable extraction time can improve the extraction efficiency. Therefore, the extraction time was investigated in the range of 2 to 30 min. As shown in Fig. 2e, the peak areas of almost all the pesticides were the highest at an extraction time of 10 min. Thus, 10 min was chosen as the optimum extraction time for subsequent experiments.

3.1.7. Effect of temperature

The temperature alters the mass transfer rates of analytes. Especially, in DLLME, temperature helps to disperse the extraction solvent in the aqueous solution, allowing the target analyte to migrate into the extraction solvent. The temperature of ultrasound treatment was studied from 22 °C (room temperature) to 80 °C. As shown in Fig. 2f, the analytical signals for the target analytes increased as the temperature increasing to 40 °C, and then decreased when further rising the temperature. This phenomenon could be explained by the decrease in the distribution coefficient at high temperature (Berijani, Assadi, Anbia, Milani Hosseini, & Aghaee, 2006). According to the test results, 40 °C was a suitable temperature to conduct the extraction procedure.

3.2. Experimental design

Based on the results obtained in preliminary trials detailed in Section 3.1 “Optimization of Experimental Parameters”, the experimental parameters affecting more to recovery efficiency were further evaluated using the cost-effective optimization method, $L_9(3^4)$ orthogonal array experimental design (OAD) (Mao, Yan, Wan, Luo, & Yang, 2019). The allocation of factors and levels is illustrated in Table S2. Factors A, B, and C represent the volume of extraction solvent, the volume of water, and the concentration of NaCl, respectively.

For each test, triplicate samples were employed. Table S2 also provides data on the average recoveries of the eight pesticides used in each trial, and the mean effects (K1, K2, and K3) for each factor at different levels. The effects of the factors were evaluated using range values (the difference between the maximal and minimal mean effect within three levels of each factor). A variable with a greater R value was more important to the extraction efficiency. According to the ranges calculated (Table S2), the sequence of importance of the three factors in the extraction efficiency was A > B > C. Subsequently, ANOVA was used to assess the OAD results (Sobhi, Yamini, Esrafili, & Abadi, 2008; Zhong, Li, Zhong, Luo, & Zhu, 2013). The results of ANOVA for the effects of three tested factors on recovery are listed in Table S3. The level of significance was set at $P < 0.05$. From Table S3, it could be seen that the effect of factor A (extraction solvent) and factor B (volume of water) were statistically significant for extraction recovery ($p < 0.05$), while the effects of factor C (concentration of NaCl) was not significant ($P > 0.05$). This meant that the concentration of NaCl could be ignored during the optimization process to simplify the sample extraction procedure.

Furthermore, the effect of different levels for significant factors A and B were investigated using LSD multiple comparison (Yan, Zhang, Liu, & Li, 2009). The results are listed in Tables S4 and S5, respectively. For factor A, the extraction recovery from Table S2 showed that level 1 gave higher results than the other two levels. The calculated data from Table S4 showed a significant difference between level 1 and the other two levels for factor A ($P < 0.05$). In addition, there was no significant difference between level 2 and level 3 ($P > 0.05$). Thus, the optimal level of factor A was level 1. Similarly, the calculated data from Table S2 showed that the average recovery of level 2 and level 3 were higher than that of level 1 for factor B. In addition, through the LSD multiple comparison results listed in Table S5, no significant difference was found between level 2 and level 3 ($P > 0.05$). Ultimately, level 2 of factor B was selected for higher recovery and less water consumption. From the results of the statistical analysis of the OAD results, the optimal extraction conditions were as follows: Volume of extraction solvent: 20 µL, volume of water: 1 mL; and concentration of NaCl: 10%.

3.3. Method evaluation

3.3.1. Linearity and LOD

The linearities were evaluated using vegetable samples with amounts of pesticides, for which blank vegetable samples were spiked with standard pesticides at 5, 10, 20, 50, 100, 200 and 500 µg/kg, respectively. The calibration curves of all analytes demonstrate good linearity, with correlation coefficients ranging from 0.9914 to 0.9985. The LOD values ranged from 0.3 to 1.5 µg/kg, and the LOQ ranged from 0.9 to 4.7 µg/kg (Table 1). These LOQ values are lower than the

Table 1
Analytical performance for organophosphorus and pyrethroid pesticides in a vegetable matrix using the proposed method.

Pesticide	Linear range (ng/g)	Calibration data in matrix	R^2	LOD (µg/kg)	LOQ (µg/kg)	MRL[a] (µg/kg)	MRL[b] (µg/kg)
Malathion	5–500	y = 103.99x + 275.62	0.9914	1.4	4.7	2000–8000	–
Chlorpyrifos	5–500	y = 742.79x + 2595.8	0.9959	1.3	4.1	50–1000	50–200
Parathion	5–500	y = 430.84x + 993.52	0.9957	1.5	4.5	500–700	–
Bifenthrin	5–500	y = 2221.2x + 12355	0.9985	0.5	1.6	50–300	50–300
Cyhalothrin	5–500	y = 1841.4x + 13967	0.9961	0.4	1.4	10–500	10–500
Permethrin	5–500	y = 2412.7x + 26939	0.9968	0.4	1.3	50–5000	50–5000
Fenvalerate	5–500	y = 1764.4x + 33200	0.9922	0.6	2.1	50–5000	50–3000
Deltamethrin	10–500	y = 503.22x + 1891.3	0.9985	0.3	0.9	10–200	10–300

[a] Source: SFA, Singapore Food Agency, Food with maximum amounts of pesticides.
[b] Source: CAC, Codex Alimentarius Commission, Codex Alimentarius Commission Pesticide Residues in Food Online Database.

Table 2
Intra- and inter-day method precisions at three spiked levels.

Pesticides	Spiked level (μg/kg)	Intra-day (n = 6)		Inter-day (n = 36)	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Malathion	20.0	109.1	12.0	119.4	14.7
	50.0	79.3	12.6	78.8	11.7
	100.0	86.6	14.0	79.0	14.2
Chlorpyrifos	20.0	62.7	11.8	61.6	13.7
	50.0	79.0	7.4	80.6	5.4
	100.0	76.2	8.0	72.4	8.9
Parathion	20.0	88.6	14.4	85.7	13.3
	50.0	81.0	11.0	85.3	8.7
	100.0	79.7	6.3	75.6	6.9
Bifenthrin	20.0	76.5	3.6	78.9	5.1
	50.0	86.4	6.1	89.9	5.5
	100.0	88.5	7.5	82.6	8.8
Cyhalothrin	20.0	82.7	4.6	84.7	6.2
	50.0	83.1	6.5	86.2	5.9
	100.0	86.5	9.1	78.6	11.9
Permethrin	20.0	77.5	6.0	81.4	7.8
	50.0	87.9	9.1	86.4	10.7
	100.0	86.8	10.0	79.7	11.1
Fenvalerate	20.0	92.0	5.5	96.1	5.9
	50.0	95.6	5.7	94.2	9.8
	100.0	93.1	8.7	85.7	11.2
Deltamethrin	20.0	109.4	16.0	114.7	16.1
	50.0	79.7	9.3	79.9	7.3
	100.0	87.6	11.3	76.3	14.3

maximum residue limits set by Singapore Food Agency and Codex Alimentarius Commission (shown in Table 1). These results demonstrated that the LOQs of the present work are sufficient to safeguard public health.

3.3.2. Accuracy and precision

Accuracy and precision were evaluated using the average recoveries and relative standard deviations (RSDs) in recovery experiments, respectively. The RSDs for intra-day experiments were conducted on the same day and comprised six replicates at each spiked level. For inter-day precision experiments, six replicates at each fortified level were analyzed on six successive days. The results of the average recoveries and RSDs are listed in Table 2. The values for recovery varied from 61.6 to 119.4%. Intra and inter-day RSD values ranged from 3.6 to 16.0 and 5.1 to 16.1, respectively. These results showed that the developed method had good accuracy and precision.

3.4. Real sample analysis

The validated method was applied to determine pesticide residues in 15 pairs of organic and conventional vegetable samples. To achieve reliable results, each experiment was performed in triplicates, and spiked samples at a concentration of 50 μg/kg were also carried out. The results of analysis are listed in Table 3. The conventional vegetable samples contained multiple residues compared with those in the organic vegetable samples. Most residues were detected at higher levels in the conventional vegetable samples than in the organic vegetables. Luckily, the amounts of pesticide residues in conventional and organic vegetables were lower than the limit set by the Singapore Food Agency, and Codex Alimentarius Commission (listed in Table 3). This analysis supplies some basic data about differences of pesticide residue profiles between organic and conventional vegetables. The results were consistent with previous findings on the point that organic foods contain fewer residues than conventional foods in the market (Baker et al., 2002; Saba & Messina, 2003).

3.5. Method comparison

To evaluate the developed method objectively, a comparison between this method and several other reported methods to determine pesticide residues in vegetables was carried out. Important factors, such as the amount of sample, the extraction solvent, the solvent volume, the extraction time, LODs, recoveries, and RSD, are listed in Table 4. As shown in Table 4, the LODs, recoveries and RSD values obtained using the present method were comparable to or better than those of previously reported methods (Camara et al., 2017; Dashtbozorgi et al., 2013; Oliva et al., 2017; Paya, Anastassiades et al., 2007; Sang, Wang, Tsoi, & Leung, 2013; Wang et al., 2016; Zawiyah et al., 2007; Zhou et al., 2015). These results indicated that the present method provides satisfactory accuracy and sensitivity. In addition, much smaller sample and solvent volumes, and less complicated equipment and pretreatment are required in this method than the SPE method (Zawiyah et al., 2007). The SPME method involved a longer equilibration and extraction time (Sang et al., 2013) as compared with the developed method. Compared with QuEChERS and traditional DLLME method (Camara et al., 2017; Dashtbozorgi et al., 2013; Oliva et al., 2017; Paya, Anastassiades et al., 2007; Wang et al., 2016), the developed method uses more economical and environmentally friendly solvents were adopted. In short, the developed method is a reliable, simple, and environmental-friendly method to determine pesticide residue in vegetable samples.

4. Conclusion

The current work established a method for determining the trace levels of OP and PYR pesticides in vegetables by combining the QuEChERS and DLLME-SFO pretreatment techniques coupled with GC–MS analysis. In this method, the purification and enrichment processes are integrated in one step, which reduces the time and simplifies the operation in sample preparation. Moreover, the present method is slight or even no harmful to our health and environment due to the use of low toxicity ethanol and *n*-hexadecane as the disperser and extraction solvents. In addition, the sample preparation conditions were optimized using OAD, and the significant effects of the parameters were analyzed statistically using SPSS software. The experimental results show that the method had desirable sensitivity, and satisfactory precision and accuracy for the target pesticides. The LOD and LOQ values for the target pesticides ranged from 0.3 to 1.5 μg/kg and from 0.9 to 4.7 μg/kg, respectively. The recoveries were between 61.6 and 119.4%. Intra-day and inter-day RSD values were less than 16.1%. These results demonstrated that the developed method was reliable, simple and environmentally-friendly. Furthermore, this method was also robust and successfully applied to detect the pesticides in 15 pairs of organic and conventional vegetable samples.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 3
Real sample analysis of organic and conventional vegetables.

Pesticide	Organic Long bean		Conventional Long bean		Organic Lettuce		Conventional Lettuce		Organic Broccoli		Conventional Broccoli	
	Detected (μg/kg)	Recovery (%)	Detected (μg/kg)	Recovery (%)	Detected (μg/kg)	Recovery (%)	Detected (μg/kg)	Recovery (%)	Detected (μg/kg)	Recovery (%)	Detected (μg/kg)	Recovery (%)
Malathion	9.3 ± 1.0	85.3 ± 8.6	ND	72.6 ± 9.4	ND	78.9 ± 6.6	42.2 ± 6.0	86.5 ± 5.8	32.2 ± 7.8	72.3 ± 4.6	37.0 ± 1.6	105.9 ± 4.5
Chlorpyrifos	8.0 ± 0.2	82.3 ± 1.6	28.7 ± 1.3	74.9 ± 7.3	ND	80.6 ± 5.8	14.6 ± 1.6	73.6 ± 2.7	28.5 ± 0.6	99.6 ± 2.3	32.7 ± 2.6	114.1 ± 7.2
Parathion	ND	90.6 ± 7.0	ND	76.8 ± 12.9	ND	83.2 ± 6.7	8.0 ± 0.3	90.3 ± 4.5	ND	81.7 ± 7.4	ND	116.5 ± 5.3
Bifenthrin	ND	90.8 ± 4.5	ND	83.6 ± 6.4	ND	78.0 ± 8.6	ND	85.0 ± 10.3	ND	81.3 ± 1.2	ND	92.6 ± 9.3
Cyhalothrin	ND	85.6 ± 6.9	ND	79.7 ± 6.7	ND	76.2 ± 5.3	ND	71.2 ± 3.7	ND	97.2 ± 1.0	ND	104.6 ± 8.9
Permethrin	ND	91.0 ± 9.6	ND	82.2 ± 7.6	ND	70.1 ± 7.8	ND	79.1 ± 7.1	ND	89.2 ± 8.8	ND	91.1 ± 11.0
Fenvalerate	ND	97.4 ± 5.6	ND	94.6 ± 5.3	ND	88.2 ± 5.6	ND	93.4 ± 7.7	ND	99.4 ± 3.9	ND	124.2 ± 6.6
Deltamethrin	ND	78.7 ± 12.7	ND	74.1 ± 7.4	ND	78.2 ± 4.8	ND	80.3 ± 3.9	ND	110.7 ± 5.2	ND	80.4 ± 14.4
Pesticide	Organic Tomato		Conventional Tomato		Organic Carrot		Conventional Carrot		Organic Pumpkin		Conventional Pumpkin	
	Detected (μg/kg)	Recovery (%)	Detected (μg/kg)	Recovery (%)	Detected (μg/kg)	Recovery (%)	Detected (μg/kg)	Recovery (%)	Detected (μg/kg)	Recovery (%)	Detected (μg/kg)	Recovery (%)
Malathion	ND	70.1 ± 6.2	ND	85.2 ± 2.6	ND	107.1 ± 10.2	ND	115.1 ± 4.9	ND	117.7 ± 18.7	ND	89.3 ± 8.6
Chlorpyrifos	ND	77.9 ± 9.1	ND	71.9 ± 7.8	33.2 ± 1.2	72.4 ± 9.4	30.7 ± 0.5	79.4 ± 7.2	9.2 ± 0.5	93.2 ± 8.9	13.1 ± 0.7	78.9 ± 6.1
Parathion	ND	83.0 ± 13.1	ND	92.3 ± 10.2	ND	109.5 ± 1.5	ND	102.5 ± 4.9	ND	125.8 ± 11.3	ND	99.3 ± 5.2
Bifenthrin	ND	75.6 ± 7.2	ND	80.5 ± 4.8	ND	97.0 ± 2.4	ND	86.3 ± 6.3	ND	104.0 ± 7.8	ND	89.5 ± 4.7
Cyhalothrin	ND	73.9 ± 6.9	21.2 ± 0.7	93.9 ± 9.1	ND	82.0 ± 0.4	ND	88.1 ± 5.9	ND	80.7 ± 10.6	ND	93.9 ± 11.1
Permethrin	ND	78.0 ± 6.2	ND	72.3 ± 4.6	ND	88.5 ± 4.6	ND	78.1 ± 8.0	ND	81.4 ± 12.9	ND	77.3 ± 4.9
Fenvalerate	ND	83.4 ± 5.8	ND	79.4 ± 7.3	ND	123.1 ± 1.7	ND	103.1 ± 3.5	ND	116.5 ± 14.5	ND	89.4 ± 9.1
Deltamethrin	ND	70.9 ± 3.4	ND	82.0 ± 7.9	ND	73.5 ± 10.0	ND	84.9 ± 12.3	ND	75.0 ± 14.9	ND	89.0 ± 7.2
Pesticide	Organic Siew Pak choy		Conventional Siew Pak choy		Organic Sweet choy sum		Conventional Sweet choy sum		Organic Sweet Pak choy		Conventional Sweet Pak choy	
	Detected (μg/kg)	Recovery (%)	Detected (μg/kg)	Recovery (%)	Detected (μg/kg)	Recovery (%)	Detected (μg/kg)	Recovery (%)	Detected (μg/kg)	Recovery (%)	Detected (μg/kg)	Recovery (%)
Malathion	ND	65.2 ± 14.4	9.2 ± 1.0	82.3 ± 13.0	ND	89.8 ± 5.2	ND	112.1 ± 12.0	23.8 ± 4.5	102.3 ± 14.0	74.0 ± 6.7	90.1 ± 7.5
Chlorpyrifos	43.0 ± 3.5	94.2 ± 1.0	13.7 ± 0.5	92.0 ± 6.1	25.0 ± 1.2	74.5 ± 13.1	36.5 ± 3.2	92.1 ± 7.3	28.2 ± 2.0	90.0 ± 6.3	36.8 ± 1.3	87.0 ± 3.2
Parathion	ND	112.5 ± 6.3	4.4 ± 0.5	108.4 ± 9.3	ND	107.1 ± 13.9	ND	88.2 ± 9.1	6.8 ± 0.1	78.4 ± 9.7	ND	89.6 ± 1.9
Bifenthrin	ND	86.3 ± 3.6	ND	77.2 ± 9.7	ND	90.1 ± 11.6	ND	87.3 ± 5.1	ND	77.2 ± 3.7	ND	98.1 ± 9.3
Cyhalothrin	ND	82.9 ± 1.9	48.9 ± 4.6	75.2 ± 5.9	ND	95.0 ± 10.8	ND	79.7 ± 5.9	ND	70.1 ± 2.9	ND	84.5 ± 9.4
Permethrin	ND	85.5 ± 2.6	ND	79.5 ± 7.7	ND	88.3 ± 11.5	ND	90.8 ± 13.4	ND	70.8 ± 5.4	ND	74.3 ± 7.8
Fenvalerate	ND	111.2 ± 1.7	ND	88.3 ± 3.4	ND	69.0 ± 12.5	ND	82.3 ± 7.3	ND	78.3 ± 3.5	ND	93.0 ± 10.4
Deltamethrin	ND	71.7 ± 11.9	ND	78.9 ± 8.6	ND	84.5 ± 14.8	ND	90.5 ± 9.2	ND	70.5 ± 5.9	186.9 ± 6.1	84.7 ± 7.0
Pesticide	Organic Celery		Conventional Celery		Organic Amaranth		Conventional Amaranth		Organic Spinach		Conventional Spinach	
	Detected (μg/kg)	Recovery (%)	Detected (μg/kg)	Recovery (%)	Detected (μg/kg)	Recovery (%)	Detected (μg/kg)	Recovery (%)	Detected (μg/kg)	Recovery (%)	Detected (μg/kg)	Recovery (%)
Malathion	ND	105.9 ± 10.7	ND	85.7 ± 12.1	ND	95.7 ± 1.5	ND	108.1 ± 10.5	ND	106.3 ± 2.4	ND	103.8 ± 8.1
Chlorpyrifos	ND	105.9 ± 4.2	ND	93.2 ± 7.2	14.9 ± 1.0	87.2 ± 6.3	48.6 ± 9.6	89.8 ± 12.4	26.2 ± 0.8	74.0 ± 8.4	9.8 ± 0.2	75.7 ± 8.7
Parathion	ND	117.1 ± 8.8	ND	101.1 ± 5.4	14.5 ± 0.8	99.6 ± 1.3	15.7 ± 1.5	100.8 ± 8.0	ND	83.2 ± 5.2	ND	81.2 ± 7.3
Bifenthrin	ND	110.9 ± 10.2	ND	100.9 ± 9.8	ND	108.1 ± 10.2	ND	77.3 ± 10.2	ND	71.3 ± 10.2	ND	90.1 ± 5.3
Cyhalothrin	ND	110.5 ± 11.9	ND	98.5 ± 7.2	ND	74.5 ± 11.0	ND	97.5 ± 10.0	ND	77.9 ± 5.5	19.8 ± 0.5	119.3 ± 9.5
Permethrin	ND	108.6 ± 11.2	ND	101.1 ± 12.5	ND	114.3 ± 11.8	ND	92.6 ± 9.9	ND	74.9 ± 7.1	ND	85.2 ± 7.6
Fenvalerate	ND	119.7 ± 15.5	ND	89.7 ± 14.3	ND	93.0 ± 2.2	ND	103.5 ± 8.1	ND	83.5 ± 5.7	ND	96.5 ± 5.3
Deltamethrin	ND	110.7 ± 12.6	ND	79.9 ± 13.5	ND	73.3 ± 2.5	ND	101.1 ± 16.5	ND	77.6 ± 4.6	12.5 ± 0.8	99.6 ± 4.7
Pesticide	Organic Cabbage		Conventional Cabbage		Organic Mushroom		Conventional Mushroom		Organic Cucumber		Conventional Cucumber	
	Detected (μg/kg)	Recovery (%)	Detected (μg/kg)	Recovery (%)	Detected (μg/kg)	Recovery (%)	Detected (μg/kg)	Recovery (%)	Detected (μg/kg)	Recovery (%)	Detected (μg/kg)	Recovery (%)
Malathion	16.9 ± 2.1	103.7 ± 13.1	21.3 ± 2.4	104.4 ± 10.8	ND	84.9 ± 4.8	144.9 ± 9.0	82.8 ± 8.1	ND	96.4 ± 10.7	ND	104.4 ± 8.5
Chlorpyrifos	15.9 ± 1.4	104.9 ± 10.5	6.2 ± 0.2	101.7 ± 12.4	49.8 ± 4.7	123.3 ± 8.5	ND	114.7 ± 5.1	14.8 ± 1.2	86.3 ± 13.9	15.9 ± 0.1	96.0 ± 7.6

(*continued on next page*)

Table 3 (*continued*)

Pesticide	Organic Long bean		Conventional Long bean		Organic Lettuce		Conventional Lettuce		Organic Broccoli		Conventional Broccoli	
	Detected (μg/kg)	Recovery (%)	Detected (μg/kg)	Recovery (%)	Detected (μg/kg)	Recovery (%)	Detected (μg/kg)	Recovery (%)	Detected (μg/kg)	Recovery (%)	Detected (μg/kg)	Recovery (%)
Parathion	ND	106.1 ± 12.4	2.1 ± 0.5	105.1 ± 15.4	ND	85.8 ± 1.0	ND	87.6 ± 4.2	ND	63.3 ± 4.9	ND	78.3 ± 7.5
Bifenthrin	ND	101.4 ± 13.1	ND	101.3 ± 8.3	ND	74.0 ± 4.4	ND	76.3 ± 6.5	ND	108.0 ± 7.8	ND	88.3 ± 4.9
Cyhalothrin	ND	101.6 ± 11.6	ND	104.1 ± 8.9	ND	70.6 ± 7.8	ND	67.4 ± 2.2	ND	109.6 ± 9.0	ND	96.7 ± 10.3
Permethrin	ND	101.0 ± 13.3	ND	104.2 ± 11.0	ND	75.5 ± 4.3	ND	75.5 ± 3.7	ND	82.7 ± 7.1	ND	76.5 ± 7.2
Fenvalerate	ND	100.3 ± 13.3	ND	97.6 ± 9.9	ND	68.2 ± 6.2	ND	86.6 ± 5.5	ND	73.1 ± 8.5	ND	83.7 ± 4.1
Deltamethrin	ND	92.8 ± 5.5	ND	100.5 ± 16.2	8.4 ± 1.0	81.9 ± 11.1	ND	64.6 ± 10.7	ND	93.7 ± 9.8	ND	78.9 ± 6.5

Table 4
Comparison of the QuEChERS (quick, easy, cheap, effective, rugged, and safe)-dispersive liquid-liquid microextraction (DLLME)-solidification of floating organic droplet (SFO) method with other reported methods to determine multiple pesticide residues in vegetable samples.

Methods	Sample amount (g)	Extraction solvent	Solvent volume (mL)	Extraction time (min)	LODs (μg/kg)	Recovery (%)	RSD (%)	Reference
SPE-GC-ECD	20	1. ethyl acetate 2. acetone/*n*-hexane	140	–	3–15	54.0–104.1	3.4–27.5	Zawiyah et al. (2007)
HS-SPME-GC–MS	1	1. methanol/acetone 2. 10% Nacl solution	–	56	0.08–2.88	66–120	0.7–9.8	Sang et al. (2013)
QuEChERS-DLLME-HPLC-MS/MS	15	1. MeCN (10% AC), 2. carbon tetrachloride	15.25	2	3.4–10.4	86–104	2.1–19.7	Dashtbozorgi et al. (2013)
DLLME-GC–MS	10	1. ACN 2. $CHCl_3$	10.1	16.62	2.4–14.2	70.8–93.2	4.1–10.6	Wang et al. (2016)
dSPE-UHPLC-QTRAP	10	1. hexane/ethyl acetate 2. ACN/ammonium acetate	21	11	0.04–4.16	76.0–120.0	0.3–14.8	Zhou et al. (2015)
1.QuEChERS-GC–MS 2.QuEChERS-LC-MS	10	1. ACN 2. ACN (5% formic acid)	10.01	2.5	3	75.2–109.4	0.8–14.3	Oliva et al. (2017)
1.QuEChERS-GC–MS/MS 2.QuEChERS-LC-MS/MS	10	1. ACN 2. 5% formic acid	10.01	2.5	3	86.0–108.0	0.2–12.9	Paya, Anastassiades et al. (2007)
1.QuEChERS-GC–MS 2.QuEChERS-LC-MS/MS	10	1. ACN 2. 5% formic acid	10.01	2.5	–	72.4–102.0	1.98–18.32	Camara et al. (2017)
QuEChERS-DLLME-SFO-GC–MS	5	1. Ethanol 2. *n*-hexadecane	5.02	12.5	0.3–1.5	61.6–119.4	3.6–16.1	This work

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2019.125755.

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