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Analytical Methods

Chemometric approach to the optimization of HS-SPME/GC–MS for the determination of multiclass pesticide residues in fruits and vegetables

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ABSTRACT

An HS-SPME method was developed using multivariate experimental designs, which was conducted in two stages. The significance of each factor was estimated using the Plackett–Burman (P–B) design, for the identification of significant factors, followed by the optimization of the significant factors using central composite design (CCD). The multivariate experiment involved the use of Minitab® statistical software for the generation of a 2^{7-4} P–B design and CCD matrices. The method performance evaluated with internal standard calibration method produced good analytical figures of merit with linearity ranging from 1 to 500 μg/kg with correlation coefficient greater than 0.99, LOD and LOQ were found between 0.35 and 8.33 μg/kg and 1.15 and 27.76 μg/kg respectively. The average recovery was between 73% and 118% with relative standard deviation (RSD = 1.5–14%) for all the investigated pesticides. The multivariate method helps to reduce optimization time and improve analytical throughput.

1. Introduction

An estimate of 1 billion people went hungry in 2010, and with the ever increasing world population, there is need for 70% increase in global food production by the year 2050 (IUPAC, 2012). The increase in world population which has led to drastic increase in demand for food supply has also led to immeasurable rise in the application of chemical pesticides and fertilizers (Aulakh, Malik, Kaur, & Schmitt-Kopplin, 2005). To increase agricultural production and meet the growing demand for food, pesticides are used for control of pest and vector of plant diseases (Araoud, Douki, Rhim, Najjar, & Gazzah, 2007). Pesticides are also used in non-agricultural activities to control and eradicate carriers of vector borne diseases, such as malaria, yellow fever, typhoid fever and dengue, which are major public health concerns (Cabras, 2003; Chai, Tan, & Kumari, 2008; Maharaj, 2010; WHO, 1995). The production and applications of pesticides in agriculture and non-agricultural purposes have led to steady increase in food production, high food quality and reduced incidence of illness due to insect-borne diseases. However their continuous use has negative impact on the environment and their presence in soil, air, water and food pose a potential health risk due to their biocide activity (Araoud et al., 2007; Chai & Tan, 2010; Lambropoulou & Albanis, 2007). Therefore, pesticides must be used efficiently and effectively in order to strike a balance between their expected benefits and the possible risk to human health. This will enable their economic viability and environmental sustainability (Tadeo, Pérez, Albero, García-Valcárcel, & Sánchez-Brunete, 2012).

Therefore there is an urgent need for quality control monitoring of the use of such pesticides on fruits and vegetables for safety purposes (Abdulra'uf, Chai, & Tan, 2012). Analytical study is undertaken in order to obtain information on the quality and quantity of contaminants present in the sample. The extraction and subsequent analysis of pesticide residues and other contaminants in fruit and vegetable samples is becoming increasingly important due to the health hazards caused by their accumulation in human tissues (Tan & Abdulra'uf, 2012).

SPME is a very attractive extraction technique in sample preparation that results in high throughput analysis, and remarkable analytical characteristics, including linearity, reproducibility, repeatability, low and improved limits of detection and quantitation, high selectivity, sensitivity and versatility with minimum matrix interferences (De Fátima Alpendurada, 2000; Pawliszyn, 1997; Risticevic, Niri, Vuckovic, & Pawliszyn, 2009). It combines sampling, isolation, concentration and enrichment and sample introduction into analytical instruments in a single and uninterrupted sampling step, which results in high throughput analysis (Ouyang & Pawliszyn, 2008; Risticevic et al., 2009). SPME was developed to overcome the problems associated with the solvent-based, time consuming conventional techniques, which are

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multistep and usually requires a large amount of samples and solvents that can cause environmental pollution and be hazardous to human health.

There are different parameters that influence the partitioning of the analytes between the sample matrix and the SPME fiber. The amount of analytes extracted from fruit and vegetable samples depend on the nature of the stationary phase (fiber) and on the properties of the sample matrix. The most important method used in the optimization of extraction parameters is the consideration of the thermodynamic and theoretical models (Pawliszyn, 1995), in the selection of a particular procedure for the development of method for the determination of pesticides in fruit and vegetable samples.

The univariate optimization of SPME parameters involves optimizing each factor one at a time, in which other factors are kept constant except the one being optimized and it involves many experiments (Miller & Miller, 2010). This does not allow the estimation of possible interaction between the studied factors. Multivariate experimental design helps to identify the significant factors that maximize the response of an experiment. It also helps to improve the chromatographic response of the analytes, eliminate interference and improves the signal-to-noise ratio. It saves time and requires few experimental runs and can be used for quantitative modeling of mathematical relationships between factors and response (Brereton, 2003, 2007). Its use is aimed to understand the effect of each factor and model the relationship between the factors and response with a minimal number of experiments carried out in an orderly and efficient manner (Massart et al., 1997).

In this study, a fast and robust HS-SPME–GC–MS method was developed for the simultaneous determination of fourteen multiclass pesticide residues (Yeoh, 2000), in fruit and vegetable samples using Plackett–Burman (P–B) design to study the significance of various factors and its optimization using central composite design (CCD).

2. Materials and methods

2.1. Reagents and solutions

Pesticide standards (fenobucarb, ethoprop, diazinon, chlorothalonil, fenitrothion, methyl parathion, chlorpyrifos, thiobencarb, quinalphos, endosulfan I, endosulfan II, bifenthrin, fenpropathrin and permethrin) at 100 μg/mL and 1-chloro-3-nitrolbenzene (1000 μg/mL) used as internal standard with more than 95% purity, were purchased from AccuStandard Inc. New Haven CT, USA. A working standard solution containing the pesticides was prepared daily by diluting the stock solution in methanol to a concentration of 10 μg/mL, and kept at 4 °C before use. All solvents used were pesticide grade: methanol, acetone and acetonitrile were purchased from Fisher Scientific, Loughborough, U.K. Sodium chloride, sodium sulfate, ammonium chloride were purchased from Merck. The pH buffer solutions 4, 6, 8–10 and 5–7 were purchased from Fisher Scientific and Sigma–Aldrich respectively. Millipore filtered (0.45 μm) deionized water was used for method development.

2.2. Sample preparation

For solid phase microextraction method development, 100 g of pesticide free fruits and vegetables obtained from Malaysian night and hypermarkets, were accurately weighed, finely chopped and homogenized in a blender. A known aliquot of the homogenized sample was then weighed into a separate 20 mL amber glass vial containing the internal standard and diluted accurately with Milli-Q filtered deionized water containing 10% of NaCl to make up a total mass of 5 g. The mixture was then spiked with a known amount of the working standard solution to prepare a concentration of 50 μg/kg, used for validation study. Fruit and vegetable samples used for method development, calibration and recovery studies were first analyzed to ensure the absence of the target pesticide residues (Melo, Aguiar, Mansilha, Pinho, & Ferreira, 2012; Souza-Silva, Lopez-Avila, & Pawliszyn, 2013).

2.3. Headspace-solid phase microextraction procedure

The SPME fibers (Supleco, Bellefonte, PA, USA) were conditioned in the GC/MS injection at 250 °C for 30 min (PDMS and PDMS/DVB) and 280 °C for 1 h (PA), prior to their first use as recommended by the manufacturer. Optimization of parameters and analysis were performed in a 20 mL amber glass vial containing 5 mL of Milli-Q filtered deionized water containing 10% of NaCl and spiked with 50 μL of the working standard solution to give a concentration of 0.1 μg/mL, used for method development. The vial containing the sample was shaken ultrasonically for 5 min, then agitated and incubated for 5 min at 60 °C in the autosampler agitator, followed by the exposure of the fiber to the headspace of the sample in the vial sealed with PTFE/silicone septum. The analytes were then extracted with 100 μm PDMS fiber (previously optimized using univariate experiment, data not shown), by exposing the fibre coating to the sample headspace, according to the Placket-Burman (P-B) design matrix (see Supplementary information Table S1).

2.4. GC–MS analysis

The extraction and analysis of pesticides were carried out with CTC CombiPAL autosampler equipped with agitator and needle heater (for fiber conditioning and inter-extraction clean up) coupled to a GC–MS (Shimadzu QP2010Series) and operated in the split/splitless mode at an injection temperature of 270 °C. The separation of target analytes were achieved on a DB-5MS fused capillary column containing 5% diphenyl and 95% dimethylpolysiloxane (30 m × 0.25 mm i.d. × 0.25 μm film thickness). The injection port of the GC was equipped with a high-pressure Merlin Microseal septumless injection kit and a silanized narrow bore liner (78.5 × 6.5 mm o.d. × 0.75 mm i.d.). Helium (carrier gas) was set to a constant flow rate of 1.3 mL/min with linear velocity of 42 cm/s. The GC column oven temperature program was set as follows. Initially set at 60 °C for 2 min, ramped at 30 °C/min to 180 °C, then ramped to 210 °C at 5 °C/min, and finally to 270 °C held for 5 min, for a total runtime of 24.50 min. The MS operation condition includes transfer line of 300 °C, ion source of 200 °C, electron ionization (EI) of 70 eV. The optimization of methods was done in scan mode while quantitation was done in selected ion monitoring (SIM) mode. A target ion (most abundance ion) and two other reference ions were monitored for the target analytes (Table 2). The investigated pesticides were identified by comparing the mass spectrum obtained for each analyte to that of the reference compound in GC–MS library using the US National Institute of Standard and Technology (NIST) and PESTANA libraries search. In case of co-elution, easy spectral identification and integration was achieved by using the deconvolution feature of the GC–MS system. The Placket–Burman (P–B) and the central composite design matrices were performed and estimated with Minitab® statistical software package version 16 (Minitab Inc., State College, USA).

3. Results and discussion

Several parameters affecting the efficiency of SPME of pesticide residues in fruits and vegetables were optimized. The optimization involved a two-step design: the screening design was used to determine the significant factors and the optimization design for estimating the best experimental conditions.

3.1. Placket–Burman (P–B) design

The Plackett–Burman (P–B) design matrix with a 2^{7-4} (resolution III) reduced factorial was generated for the screening of the most important factors affecting the SPME efficiency and recovery of pesticide residues from fruit and vegetable samples. It contains experimental runs of a multiple of four (4, 8, 12, 16, etc.) and the factors are one less than the number of experiments (Brereton, 2007; López, Goñi, Etxandia, & Millán, 2007). It helps for the estimation of the significant factors affecting extraction efficiency. It does not yield the exact quantity, but provides valuable information on each variable with relatively few and reasonable experimental runs (Khodadoust & Hadjmohammadi, 2011; López et al., 2007). The factors and level of variables (low and high respectively) was selected to cover the range of optimal conditions that was estimated using the univariate method. The factors selected for the 2^{7-4} Plackett–Burman design matrix include: extraction temperature, 30 and 60 °C; time, 30 and 60 min; salt addition; 5% and 10% (w/v); stirring rate, 300 and 600 rpm; pH, 4 and 8; desorption time, 5 and 10 min; desorption temperature, 250 and 270 °C (see Supplementary information Table S1). This was used to run the experiment for the determination of main effects of the factors under investigation. The P–B design consists of 12 runs, conducted in duplicate, to annul the effects of extraneous variables (Stalikas, Fiamegos, Sakkas, & Albanis, 2009).

The main effect of each factor was estimated using least square regression which indicates the significance in relation to the response (total chromatographic peak area, TCPA). In the Pareto chart (Fig. 1a), the length of the bar is proportional to the absolute value of the main effect (Khodadoust & Hadjmohammadi, 2011; López et al., 2007; Stalikas et al., 2009), while the vertical line indicates 95% confidence level. The normal plot (Fig. 1b) shows the significance of each factor (estimated using ANOVA test) and the magnitude of various effects, while the residual plots (Supplementary information, Fig. S1a) shows that the measurement deviation is randomly distributed around the mean. The main effect plot (Supplementary information, Fig. S1b), as indicated by the slope of the plots, when extraction temperature and extraction time increase from low value to high value, the extraction efficiency also increases, and the extraction efficiency increases with decrease in stirring rate and pH, Other factors such as salt addition, desorption time and desorption temperature show no significant effect, and thus can be fixed at any value, since their adjustment will have equal effect on the extraction efficiency. The extraction temperature is the most important factor, because it increases the headspace capacity and analyte diffusion coefficients (Risticevic, Lord,

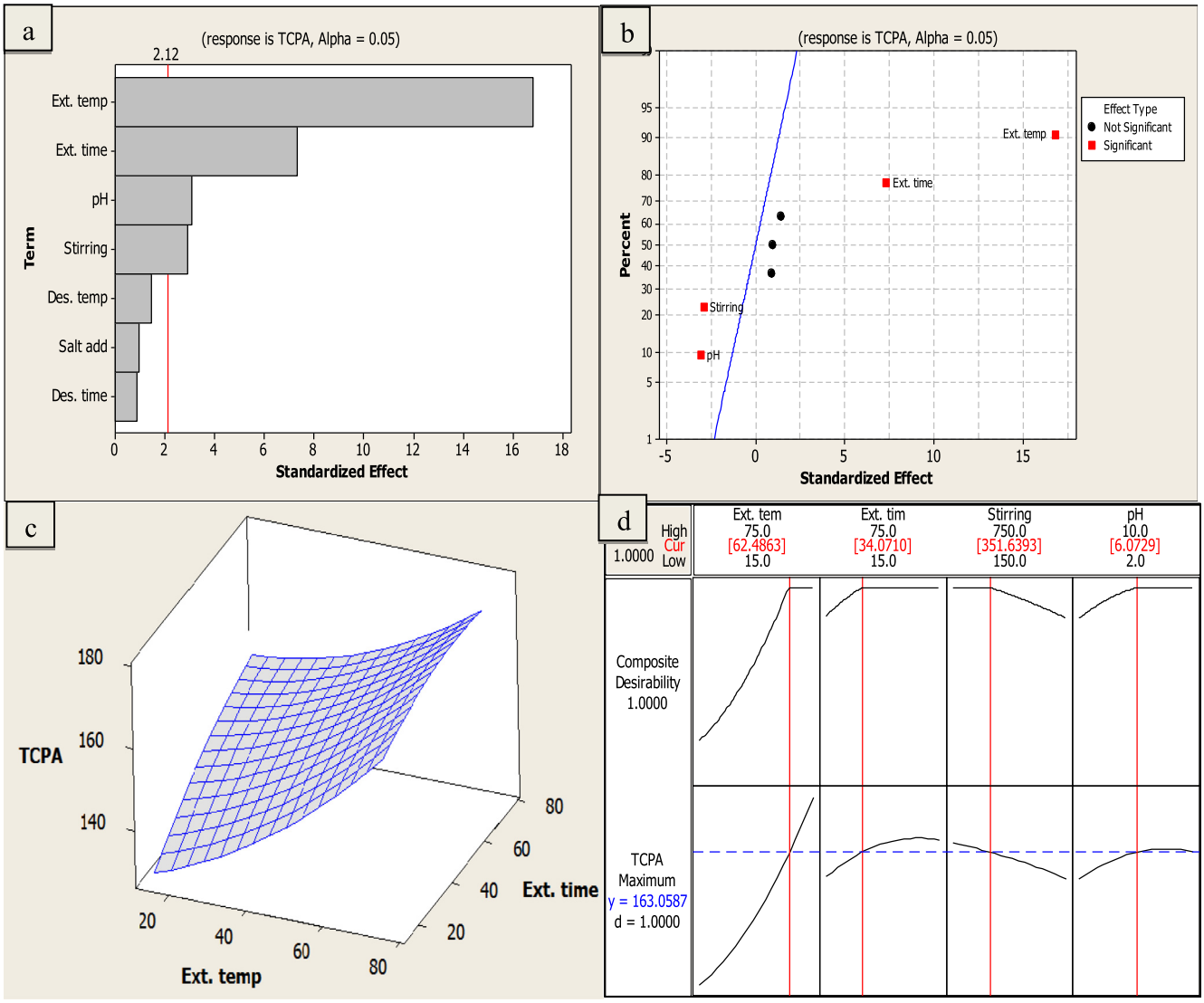

Fig. 1. (a) Pareto chart of standardized main effect, (b) normal plot of standardized main effect, (c) response surface optimizer and (d) desirability surface plot for TCPA.

Górecki, Arthur, & Pawliszyn, 2010), followed by the extraction time (more analytes are extracted with increase in extraction time). As can be observed from the normal plot (Fig. 1b) extraction temperature and time shows positive effects, while pH and stirring rate shows negative effect. Therefore, for the optimization step, all other factors were fixed, while extraction temperature, time, pH and stirring rate were considered for further optimization.

Table 1
Factors, levels and central composite design matrix[a].

Variables		Level			Star points ($\alpha = 2$)	
		Low (−)	Central (0)	High (+)	−α	+α
Extraction temp. (°C)		30	45	60	15	75
Extraction time (min)		30	45	60	15	75
pH		4	6	8	2	10
Stirring rate (rpm)		300	450	600	150	750
StdOrder	RunOrder	PtType	Blocks	*A*	*B*	*C*	*D*
10	1	1	1	60	30	300	8
6	2	1	1	60	30	600	4
8	3	1	1	60	60	600	4
13	4	1	1	30	30	600	8
18	5	−1	1	75	45	450	6
30	6	0	1	45	45	450	6
26	7	0	1	45	45	450	6
11	8	1	1	30	60	300	8
14	9	1	1	60	30	600	8
29	10	0	1	45	45	450	6
15	11	1	1	30	60	600	8
20	12	−1	1	45	75	450	6
24	13	−1	1	45	45	450	10
5	14	1	1	30	30	600	4
27	15	0	1	45	45	450	6
12	16	1	1	60	60	300	8
31	17	0	1	45	45	450	6
2	18	1	1	60	30	300	4
3	19	1	1	30	60	300	4
7	20	1	1	30	60	600	4
22	21	−1	1	45	45	750	6
9	22	1	1	30	30	300	8
25	23	0	1	45	45	450	6
4	24	1	1	60	60	300	4
16	25	1	1	60	60	600	8
17	26	−1	1	15	45	450	6
19	27	−1	1	45	15	450	6
23	28	−1	1	45	45	450	2
21	29	−1	1	45	45	150	6
28	30	0	1	45	45	450	6
1	31	1	1	30	30	300	4

[a] Generated using Minitab® statistical software.

3.2. Central composite design

The screening experiment obtained by the use of Plackett–Burman design indicates that, desorption time, desorption temperature and salt addition do not affect extraction efficiency to a significant extent. Therefore, they were fixed according to the optimal value estimated using the univariate experiments (desorption time, 7 min; desorption temperature, 270 °C; salt addition, 10%) (Data not shown). The extraction time, extraction temperature, stirrin rate and pH, which are the significant variables were further optimized by the use of second-order central composite design (CCD) utilizing a response surface methodology (RSM). The number of points in CCD contains a factorial run of 2^k, axial runs of $2k$ and C_o center point runs. Therefore the total experimental runs (N) of CCD is given by: $N = 2^k + 2k + C_o$, where k and C_o are the number of variables and the number of center points respectively (Stalikas et al., 2009; Stoyanov & Walmsley, 2006). In order to reduce the effect of uncontrolled variables, the CCD experiments were run in a random manner. The CCD design includes 16 cube points, 7 center points in cube, 8 axial points and 0 center point in axial with $\alpha = 2$ (selected to establish rotatability conditions) and a total of 31 randomized runs. The significant variables involved in the generation of CCD, their levels and the design matrix are as shown in Table 1.

The total chromatographic peak area (TCPA) corresponding to the 14 investigated pesticides was used as the yield/response for the experimental runs presented in Table 1, were used to obtain the response surface plot as shown in Fig. 1c. The desirability function was first fixed by assigning values of 0.0 (undesirable), 0.5 (medium desirability) and 1.0 (very desirable). The global desirability surface response in 3D plot was obtained for the optimized parameters as shown in Fig. 1d. The second order response is utilized because of its flexibility, the ability to give an approximation of the true value and the parameters can easily be estimated (Myers, Montgomery, & Anderson-Cook, 2009).

The surface plot (Fig. 1c) and response optimizer plot (Fig. 1d) were used to indicate the optimal conditions and it can be observed that the overall response desirability of the independent variables in the experimental domain was obtained at extraction temperature greater than or equal to 62 °C, while optimum extraction time can be obtained at 34 min or higher, while the stirring rate was at 351 rpm or lower and pH of 6. The result is in good agreement with the P–B design as represented by the main effect plot (Supplementary information, Fig. S1a), where increase in extraction temperature and time increases extraction efficiency while extraction efficiency is increased with decreasing stirring rate and pH value. This also helps to determine the robustness of

Table 2
Linearity range (μg/kg) of the developed HS-SPME method.

Pesticides	Ret. time (min)	Ion (*m*/*z*)	Range (μg/kg)	Apple r^2	Tomato r^2	Cucumber r^2	Cabbage r^2
Fenobucarb	8.81	121, 91, 150	2.5–500	0.9975	0.9996	0.9985	0.9976
Ethoprophos	9.13	158, 97, 139	2.5–250	0.9981	0.9986	0.9975	0.9979
Diazinon	11.04	179, 137, 152	2.5–250	0.9987	0.9948	0.9981	0.9980
Chlorothalonil	11.31	266, 263, 268	10–500	0.9987	0.9975	0.9978	0.9989
Parathion-methyl	12.81	109, 79, 125	1–250	0.9986	0.9994	0.9988	0.9964
Fenitrothion	13.70	125, 79, 109	2.5–200	0.9989	0.9995	0.9983	0.9952
Chlorpyrifos	14.34	97, 125, 197	5–500	0.9980	0.9979	0.9981	0.9985
Thiobencarb	14.50	100, 125, 127	5–250	0.9982	0.9950	0.9984	0.9977
Quinalphos	16.37	146, 118, 156	2.5–125	0.9985	0.9991	0.9981	0.9968
Endosulfan I	17.26	195, 207, 241	5–250	0.9980	0.9967	0.9990	0.9976
Endosulfan II	18.61	195, 159, 207	10–250	0.9988	0.9992	0.9978	0.9987
Bifenthrin	20.14	181, 166,	1–500	0.9985	0.9989	0.9983	0.9982
Fenpropathrin	20.31	97, 125, 181	1–50	0.9976	0.9938	0.9984	0.9978
Permethrin	22.21	183, 91, 163	5–100	0.9969	0.9976	0.9989	0.9973

the method, as slight increase or decrease (as the case may be), in the optimized factors, based on the desirability surface plot (Fig. 1d), will not significantly affect the measured response (TCPA). Consequently, and taking into account the univariate results, the optimal extraction conditions are: temperature, 62 °C; time, 34 min; salt addition, 10%; stirring rate, 350 rpm; pH, 6; desorption time, 7 min; desorption temperature, 270 °C.

3.3. Validation of analytical figures of merit

The validation of analytical methodology has been observed to be a quality assurance step in method development (Thompson, Ellison, & Wood, 2002), used to confirm the method performance and its suitability for the intended purpose. In the present study, the figures of merit of analytical methodology of the developed method was validated in terms of linearity, accuracy (reported as the relative recovery), intra- and inter-day precisions, limits of detection (LOD) and quantification (LOQ) (ICH-Topic Q2(R1), 2006), using the optimized HS-SPME parameters. Although, validation of figures of merit of analytical methodology has been described to be a time consuming activity, it is very essential in order to ensure optimal utilization of analytical resources (Chan, 2008, 2011).

The intra-day precision ($n = 3$) was estimated by performing three extractions in a single day, and inter-day precision ($n = 9$)

Table 3a
Accuracy, intra- and inter-day precisions of the pesticides in fruit and vegetable samples.

Pesticides	Added (μg/kg)	Apple			Tomato			Cucumber			Cabbage		
		Intra (%)	Inter (%)	Accuracy (%)	Intra (%)	Inter (%)	Accuracy (%)	Intra (%)	Inter (%)	Accuracy (%)	Intra (%)	Inter (%)	Accuracy (%)
Fenobucarb	50	11.5	12.9	80.9	2.8	6.0	105.0	7.4	10.4	76.40	9.1	12.6	76.8
	100	8.2	9.0	96.2	2.2	2.4	75.6	5.1	6.1	80.70	4.9	6.1	77.4
	150	2.74	4.0	103.5	2.2	3.1	95.5	3.1	4.4	89.19	4.9	6.8	92.6
Ethoprophos	50	13.6	14.9	79.1	13.2	14.5	80.0	8.9	9.6	83.11	12.2	14.3	89.2
	100	8.3	8.9	95.0	3.0	3.3	75.4	3.0	3.5	86.89	7.1	8.0	81.8
	150	4.3	4.5	102.4	3.2	5.0	91.6	5.3	5.3	88.59	5.1	5.7	89.4
Diazinon	50	5.5	9.2	77.8	10.4	13.7	82.3	8.4	12.4	88.94	9.9	10.8	85.8
	100	5.1	6.0	88.7	3.7	5.6	75.5	5.0	6.4	91.72	9.0	9.3	87.6
	150	7.4	6.3	101.9	3.2	6.0	102.8	2.8	3.2	103.85	8.3	8.7	106.1
Chlorothalonil	50	11.5	12.4	76.2	8.8	10.2	112.0	11.4	13.2	81.86	12.9	14.8	113.8
	100	5.1	9.8	81.0	7.1	7.6	109.3	2.7	4.5	105.11	9.0	10.2	113.6
	150	6.1	7.0	104.2	3.8	4.7	115.0	4.2	4.5	117	7.8	8.7	90.5
P. methyl	20	6.8	10.7	73.3	10.5	11.0	80.3	10.7	12.0	77.66	12.5	14.1	76.0
	50	3.2	10.6	80.4	6.1	6.7	96.9	8.6	9.4	78.43	10.5	12.0	78.4
	100	4.3	6.4	98.0	4.4	4.7	105.0	4.4	4.8	78.56	8.8	10.0	103.3
Fenitrothion	50	5.6	6.6	102.4	12.1	13.6	85.5	10.8	11.4	90.71	12.7	14.4	117.9
	100	4.4	7.0	106.4	4.0	6.4	99.8	4.6	5.3	109.55	9.3	10.8	106.6
	150	4.3	4.3	107.6	5.5	7.4	107.6	3.7	4.2	93.06	5.6	6.4	105.1
Chlorpyrifos	50	7.8	14.3	91.6	7.4	13.6	92.2	8.0	10.8	108.24	12.3	13.9	118.5
	100	4.4	8.9	96.6	4.6	6.4	109.8	3.2	5.6	105.72	7.9	8.5	110.2
	150	6.6	6.6	98.2	3.4	7.4	107.5	3.0	5.4	116.41	9.7	11.2	117.0

Table 3b
Accuracy, intra- and inter-day precisions of the pesticides in fruit and vegetable samples (cont'd).

Pesticides	Added (μg/kg)	Apple			Tomato			Cucumber			Cabbage		
		Intra (%)	Inter (%)	Accuracy (%)	Intra (%)	Inter (%)	Accuracy (%)	Intra (%)	Inter (%)	Accuracy (%)	Intra (%)	Inter (%)	Accuracy (%)
Thiobencarb	20	8.2	12.9	102.4	9.50	13.0	89.1	9.8	11.9	98.9	11.8	12.1	108.9
	50	5.6	9.0	104.3	6.62	7.0	91.6	8.5	10.5	109.6	9.1	9.8	113.3
	100	4.9	4.0	106.0	3.49	6.0	101.6	5.9	6.6	113.1	5.9	6.6	113.5
Quinalphos	50	8.9	13.2	79.1	6.31	12.1	86.5	11.7	12.7	102.7	11.0	12.4	88.3
	100	7.5	11.2	95.0	9.16	11.0	88.5	10.4	11.3	115.1	1.1	13.0	108.6
	150	3.9	4.4	102.4	3.36	8.2	103.3	8.3	8.9	112.7	4.5	7.5	111.5
Endosulfan I	50	5.2	15.5	77.8	11.83	12.0	87.5	8.3	9.3	80.8	10.0	10.2	96.8
	100	6.0	6.8	88.7	7.03	8.0	98.2	6.4	7.2	89.5	7.2	9.2	94.6
	150	4.6	5.1	101.9	1.93	6.7	98.3	6.0	7.1	87.9	4.4	4.9	90.0
Endosulfan II	50	7.8	8.2	76.2	5.30	5.8	87.0	10.3	10.8	76.7	11.0	11.9	96.7
	100	5.8	6.5	81.0	2.68	3.9	96.2	7.5	8.3	81.8	9.7	10.8	97.1
	150	3.3	4.2	104.2	2.74	3.0	95.4	6.2	6.4	85.6	5.3	6.2	107.5
Bifenthrin	5	6.4	7.4	73.3	6.84	9.3	90.9	12.4	13.1	84.7	10.3	13.3	91.3
	10	6.5	6.5	80.4	4.3	6.0	96.6	6.9	7.8	83.4	6.4	7.0	88.8
	20	4.1	4.1	98.0	1.56	5.3	90.9	6.4	6.7	87.2	6.8	7.2	98.5
Fenpropathrin	20	10.8	10.6	102.4	7.21	9.1	94.8	13.9	14.2	77.4	12.5	14.4	106.9
	50	10.0	10.8	106.4	4.95	9.1	97.3	11.7	12.5	83.3	11.2	14.1	90.7
	100	10.7	11.4	107.6	7.09	8.3	97.8	7.0	8.1	87.2	8.6	9.6	113.2
Permethrin	20	8.7	8.8	91.6	11.74	13.1	102.3	11.6	12.8	111.3	11.0	12.4	112.8
	50	5.8	6.4	96.6	6.18	7.2	104.9	7.3	10.9	108.5	10.5	12.0	88.3
	100	3.4	5.01	98.2	4.71	14.7	98.4	5.1	7.7	110.6	3.4	4.6	112.2

was estimated based on three extractions per day for 3 days, while the accuracy was reported in terms of the average recoveries of the spiked sample at three different concentration levels (Table 3a and 3b). The limits of detection and quantification values were estimated experimentally at a signal-to-noise ratio of 3 and 10 respectively using the standard deviation of the *y*-intercept of the regression line of the calibration curve.

The linearity was estimated using set of calibration curves prepared with concentrations ranging from 1 to 500 μg/kg, with an internal standard calibration method. The peak area ratio which is the ratio of the total chromatographic peak area of the analytes to the total chromatographic peak area of internal standard was plotted against the concentration of analytes. As shown in Table 2, the calibration curves were linear over the tested concentration range and the correlation coefficients (r^2) were greater than 0.99 for all the investigated pesticides. Tables 3a and 3b shows the precisions and accuracies (relative recoveries) of the developed method in fruit and vegetable samples. The intra-day precision varies from 1.56% to 13.9% while the intermediate precision varies from 2.4% to 14.9%. The relative recoveries of the spiked fruit and vegetable samples range from 73.3% to 118.5% which were acceptable according to the SANCO guideline (SANCO, 2011).

The recoveries obtained in vegetable samples were slightly higher than those obtained in fruit samples, this could be attributed to the presence of suspended solid particles and high molecular mass substances such as pectin and sugar present in the fruit samples (Lambropoulou & Albanis, 2003; Sang, Wang, Tsoi, & Leung, 2013; Simplício & Vilas Boas, 1999), although matrix interference were completely eliminated by appropriate dilution of the samples. It was also observed that, better recoveries and precisions were achieved at higher spiked levels as shown in Table 3. All the parameters validated in this study were based on the method validation requirements of the European Union (SANCO, 2011). The LOQ (S/N = 10) and LOD (S/N = 3) values obtained (Table 4) are in most cases below the first calibration level and are lower than the maximum residue levels (MRL) allowed by Codex Alimentarius and the European Union (EU, 2005). The LOD range from 0.35 to 8.33 μg/kg, while the LOQ was between 1.15 and 27.76 μg/kg.

Table 4
Figures of merit of the developed method in fruit and vegetable samples.

Pesticides		Apple (μg/kg)	Tomato (μg/kg)	Cucumber (μg/kg)	Cabbage (μg/kg)
Fenobucarb	LOD	2.41	2.49	1.74	2.49
	LOQ	8.03	8.33	5.81	8.33
	MRL	300	1000	300	1500
Ethoprophos	LOD	1.31	0.23	0.35	0.23
	LOQ	4.36	0.77	1.15	0.77
	MRL	20	20	20	20
Diazinon	LOD	0.88	0.21	0.32	0.21
	LOQ	2.92	0.68	1.05	0.68
	MRL	10	10	10	10
Chlorothalonil	LOD	2.16	6.94	8.33	6.80
	LOQ	7.21	23.12	27.76	22.67
	MRL	1000	2000	1000	1000
P. methyl	LOD	0.24	0.62	0.50	0.53
	LOQ	0.79	2.24	1.65	1.76
	MRL	10	10	10	10
Fenitrothion	LOD	0.53	1.35	0.26	0.68
	LOQ	1.77	4.48	0.85	2.25
	MRL	10	10	10	10
Chlorpyrifos	LOD	3.30	3.71	2.96	3.30
	LOQ	11.01	12.36	9.87	11.00
	MRL	500	500	500	500
Thiobencarb	LOD	3.48	4.34	4.03	3.77
	LOQ	11.58	14.47	13.43	12.57
	MRL	100	100	100	100
Quinalphos	LOD	2.16	1.94	1.94	1.97
	LOQ	7.37	6.48	6.48	6.60
	MRL	50	50	50	50
Endosulfan I	LOD	2.30	3.91	3.25	3.13
	LOQ	7.67	13.14	10.83	10.43
	MRL	50	50	50	50
Endosulfan II	LOD	2.17	3.19	2.08	2.93
	LOQ	7.23	10.63	6.92	9.77
	MRL	50	50	50	50
Bifenthrin	LOD	0.11	0.99	0.89	0.74
	LOQ	0.38	3.31	2.96	2.47
	MRL	300	300	300	100
Fenpropathrin	LOD	0.14	0.52	0.75	0.47
	LOQ	0.47	1.72	2.50	1.57
	MRL	10	10	10	10
Permethrin	LOD	1.01	1.50	2.42	1.80
	LOQ	3.36	5.00	8.05	6.00
	MRL	50	50	50	50

3.4. Analysis of real samples

The HS-SPME method developed in this study was subsequently applied to the analysis of apple, tomato, cucumber and cabbage samples purchased from a local Malaysian wet markets. The real sample analysis was conducted in order to further verify the reliability and robustness of the developed method. A total of 220 samples were analyzed, and three samples each of tomato and cabbage were found to contain chlorothalonil, while one sample of tomato contains permethrin. One sample of apple was also found to contain chlorpyrifos. However, all fruits and vegetables found to contain the target pesticides were far below the maximum residue levels allowed by the European Union and the Codex Alimentarius Commission (EU, 2005). All other pesticides investigated in the selected commodities were either not detected or were detected below the limits of quantification and thus were not quantified.

4. Conclusion

The use of chemometrics approach to the screening and subsequent optimization of extraction parameters has helped to reduce analysis time and also help to determine the best optimized parameters. It is also a cheap, simple, robust and fast method development, which enhances better recoveries and precisions and also improves detectability of the target analytes and an improved method validation. The analytical figure of merit obtained in the present study were comparable or better when compared with other method of pesticide residues analysis in fruits and vegetables reviewed in our previous paper (Abdulra'uf et al., 2012). Further studies should be focused on the use of sol–gel prepared, ionic liquid, nanomaterials, supramolecular molecules and molecularly imprinted polymer coatings as the extraction phase to increase the range of analytes that can be qualitatively and quantitatively analyzed in a wide range of environmental samples.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2015.01.031.

References

Abdulra'uf, L. B., Chai, M. K., & Tan, G. H. (2012). Applications of solid-phase microextraction for the analysis of pesticide residues in fruits and vegetables: A review. *Journal of Association of Official Analytical Chemists International, 95*(5), 1272–1290.

Araoud, M., Douki, W., Rhim, A., Najjar, M. F., & Gazzah, N. (2007). Multiresidue analysis of pesticides in fruits and vegetables by gas chromatography–mass spectrometry. *Journal of Environmental Science and Health B, 42*(2), 179–187. http://dx.doi.org/10.1080/03601230601123474.

Aulakh, J. S., Malik, A. K., Kaur, V., & Schmitt-Kopplin, P. (2005). A review on solid phase micro extraction–high performance liquid chromatography (SPME–HPLC) analysis of pesticides. *Critical Reviews in Analytical Chemistry, 35*, 71–85.

Brereton, R. G. (2003). *Chemometrics: Data analysis for the laboratory and chemical plants*. West Sussex, England: Wiley.

Brereton, R. G. (2007). *Applied chemometrics for scientists*. West Sussex, England: Wiley.

Cabras, P. (2003). Pesticides: Toxicology and residues in food. In J. P. F. D'Mello (Ed.), *Food safety: Contaminants and toxins* (pp. 91–124). Cambridge, MA, USA: CABI Publishing.

Chai, M. K., & Tan, G. H. (2010). Headspace solid-phase microextraction for the evaluation of pesticide residue contents in cucumber and strawberry after washing treatment. *Food Chemistry, 123*(3), 760–764. http://dx.doi.org/10.1016/j.foodchem.2010.05.038.

Chai, M. K., Tan, G. H., & Kumari, A. (2008). Application of solid-phase microextraction for the determination of pesticides in vegetable samples by gas chromatography with electron capture detector. *Malaysian Journal of Analytical Science, 12*(1), 1–9.

Chan, C. C. (2011). Principles and practices of analytical method validation: Validation of analytical methods is time-consuming but essential. *Quality Assurance Journal, 14*, 61–64.

Chan, C. C. (2008). Analytical method validation: Principles and practices. In S. C. Gad (Ed.), *Pharmaceutical manufacturing handbook: Regulations and quality* (pp. 727–742). Hoboken, NJ: John Wiley.

De Fátima Alpendurada, M. (2000). Solid-phase microextraction: A promising technique for sample preparation in environmental analysis. *Journal of Chromatography A, 889*(1–2), 3–14.

ICH-Topic Q2(R1). (2006). *Validation of analytical procedures: Text and methodology*. U. S Department of Health and Human Services.

IUPAC. (2012). A vision for chemistry for 2050. *Chemistry International*.

EU. (2005). Regulation (EC) No 396/2005. Retrieved on 24/11/2012 from <http://ec.europa.eu/sanco_pesticides/public/index.cfm>.

Khodadoust, S., & Hadjmohammadi, M. (2011). Determination of N-methylcarbamate insecticides in water samples using dispersive liquid–liquid microextraction and HPLC with the aid of experimental design and desirability function. *Analytica Chimica Acta, 699*(1), 113–119. http://dx.doi.org/10.1016/j.aca.2011.04.011.

Lambropoulou, D. A., & Albanis, T. A. (2003). Headspace solid-phase microextraction in combination with gas chromatography–mass spectrometry for the rapid screening of organophosphorus insecticide residues in strawberries and cherries. *Journal of Chromatography A, 993*, 197–203.

Lambropoulou, D. A., & Albanis, T. A. (2007). Liquid-phase micro-extraction techniques in pesticide residue analysis. *Journal of Biochemical and Biophysical Methods, 70*(2), 195–228. http://dx.doi.org/10.1016/j.jbbm.2006.10.004.

López, R., Goñi, F., Etxandia, A., & Millán, E. (2007). Determination of organochlorine pesticides and polychlorinated biphenyls in human serum using headspace solid-phase microextraction and gas chromatography-electron capture detection. *Journal of Chromatography B, 846*(1–2), 298–305.

Maharaj, R. (2010). Use of insecticides for malaria control and needs for reversion of resistance. *Indian Journal of Medical Research, 132*, 248–250.

Massart, D. L., Vandaginste, B. G. M., Buydens, L. M. C., De Jong, S., Lewi, P. J., & Smeyers-Verbeke, J. (1997). *Handbook of chemometrics and qualimetrics: Part A*. Amsrterderm, The Netherlands: Elsevier.

Melo, A., Aguiar, A., Mansilha, C., Pinho, O., & Ferreira, I. M. P. L. V. O. (2012). Optimisation of a solid-phase microextraction/HPLC/diode array method for multiple pesticide screening in lettuce. *Food Chemistry, 130*(4), 1090–1097. http://dx.doi.org/10.1016/j.foodchem.2011.07.137.

Miller, J. N., & Miller, J. C. (2010). *Statistics and chemometrics for analytical chemistry* (6th ed.). Essex, England: Pearson.

Myers, R. H., Montgomery, D. C., & Anderson-Cook, C. M. (2009). *Response surface methodology: Process and product optimization using designed experiments* (3rd ed.). USA: Wiley.

Ouyang, G., & Pawliszyn, J. (2008). A critical review in calibration methods for solid-phase microextraction. *Analytica Chimica Acta, 627*(2), 184–197.

Pawliszyn, J. (1995). New directions in sample preparation for analysis of organic compounds. *TrAC – Trends in Analytical Chemistry, 14*(3), 113–122.

Pawliszyn, J. (1997). *Solid phase microextraction: Theory and practice*. New York: VCH.

Risticevic, S., Lord, H., Górecki, T., Arthur, C. L., & Pawliszyn, J. (2010). Protocol for solid-phase microextraction method development. *Nature Protocols, 5*, 122–139.

Risticevic, S., Niri, V. H., Vuckovic, D., & Pawliszyn, J. (2009). Recent developments in solid-phase microextraction. *Analytical and Bioanalytical Chemistry, 393*, 781–795.

SANCO. (2011). Method validation and quality control procedures for pesticide residues analysis in food and feed. SANCO/12495/2011.

Sang, Z. Y., Wang, Y. T., Tsoi, Y. K., & Leung, K. S. Y. (2013). CODEX-compliant eleven organophosphorus pesticides screening in multiple commodities using headspace-solid phase microextraction-gas chromatography–mass spectrometry. *Food Chemistry, 136*(2), 710–717.

Simplício, A. L., & Vilas Boas, L. (1999). Validation of a solid-phase microextraction method for the determination of organophosphorus pesticides in fruits and fruit juice. *Journal of Chromatography A, 833*(1), 35–42.

Souza-Silva, É. A., Lopez-Avila, V., & Pawliszyn, J. (2013). Fast and robust direct immersion solid phase microextraction coupled with gas chromatography–time-of-flight mass spectrometry method employing a matrix compatible fiber for determination of triazole fungicides in fruits. *Journal of Chromatography A, 1313*, 139–146. http://dx.doi.org/10.1016/j.chroma.2013.07.071.

Stalikas, C., Fiamegos, Y., Sakkas, V., & Albanis, T. (2009). Developments on chemometric approaches to optimize and evaluate microextraction. *Journal of Chromatography A, 1216*(2), 175–189. http://dx.doi.org/10.1016/j.chroma.2008.11.060.

Stoyanov, K. S., & Walmsley, A. D. (2006). Response surface modelling and experimental designs. In P. Gemperline (Ed.), *Practical guide to chemometrics* (2nd ed. Boca Raton, FL, USA: Taylor and Francis.

Tadeo, J. L., Pérez, R. A., Albero, B., García-Valcárcel, A. I., & Sánchez-Brunete, C. (2012). Review of sample preparation techniques for the analysis of pesticide residues in soil. *Journal of AOAC International, 95*(5), 1258–1271.

Tan, G. H., & Abdulra'uf, L. B. (2012). Recent developments and applications of microextraction techniques for the analysis of pesticide residues in fruits and vegetables. In R. P. Soundararajan (Ed.), *Pesticides – Recent trends in pesticide residue assay* (pp. 171–190). Rijeka: InTech.

Thompson, M., Ellison, S. L. R., & Wood, R. (2002). Harmonized guides for single-laboratory validation of method of analysis. *Pure and Applied Chemistry, 74*(5), 835–855.

WHO. (1995). Vector control for malaria and other mosquito-borne diseases. Retrieved on 29/11/2012 from <http://apps.who.int/iris/bitstream/10665/41726/1/WHO_TRS_857.pdf>.

Yeoh, N. S. (2000). Pesticide residues in food, maximum residue limits (MRLs) and food safety. paper presented at the DOA, Malaysia-pesticides, health. Ipoh, Malaysia: You and The Law Conference.