Application of Multivariate Data Analysis to the Determination of Multiclass Pesticide Residues in Fruits and Vegetables using Headspace Solid-Phase Microextraction Gas Chromatography-Mass Spectrometry

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Abstract

Design of experiment (DOE) was employed to develop a headspace solid phase microextraction gas chromatography-mass spectrometry (HS-SPME/GC-MS) method for multiresidue analysis. The significance of SPME parameters was determined using Plackett-Burman (P-B) design. The main effect and the interaction effect of the significant factors were also determined followed by the optimization of the significant factors using central composite design (CCD). A Minitab® statistical software was used to generate both the 2^{7-4} Plackett-Burman and the central composite design matrix. The same statistical software was also employed in the determination of the optimum level of the significant parameters using surface response optimizer and desirability surface plot. The most significant factors are: extraction temperature (90%), extraction time (80%), the pH and stirring rate (50% and 60% respectively). The optimum parameters are: Temperature, 62 °C; time, 34 min; NaCl, 10%; stirring, 350rpm; pH, 6. The figures of merit of analytical methodologies was determined using an internal standard calibration method. The linearity of the developed method ranges from 1- 500 µg/kg and correlation coefficient (R^2) greater than 0.99. The average recovery was found between 74 – 115% and relative standard deviation ranges from 1.1 – 14%. The developed method was used to analyze 14 multiclass pesticide residues in two fruit (pear and grape) and two vegetable (lettuce and broccoli) samples, and the method was found to be satisfactory with LOD between 0.17 – 7.34 µg/kg and LOQ ranges from 0.55 – 24.50 µg/kg.

Keyword: Design of experiment (DOE), solid phase microextraction, response surface optimizer, pesticide residues, Central composite Design

Introduction

Pesticides refer to all natural and synthetic chemicals that are used to prevent, destroy, repel or fight crop pest and vector of plant diseases [1, 2]. They are mostly organic compounds with different functional groups, forming various types of isomeric compounds [3]. They differ in their substitution groups, degree of ionization, octanol/water coefficients, polarity, volatility and their solubility. The production and applications of pesticides in agriculture and non-agricultural purposes has in no doubt led to steady increase in food production, high food quality and reduced incidence of illness due to insect-borne diseases[4]. Pesticides are unavoidable inputs in agriculture and public health that are produced in large quantities, since the end of World War II [5, 6], and their worth have been demonstrated through increase in global agricultural production, eradication of insect borne and epidemic diseases and conservation of the ecosystem [7]. The use of pesticide increase significantly over the years, and

thus the immediate benefit of its uses overshadowed its toxicity. The occupational and accidental exposure to pesticide have been observed to lead to a wide variety of chronic effect such as endocrine disorder, blood disorder, genetic change, e. t. c. [4].

Solid phase microextraction is a solvent-free sample preparation method which combines sample preparation, isolation, concentration and enrichment into one step [8]. Although, pesticide residues analyses in fruit and vegetable samples have been investigated using different microextraction techniques and subsequent instrumental analysis using gas chromatography, liquid chromatography and capillary electrophoresis [9, 10]. QuEChERS method was used to analyze multiclass pesticide residues in fruits and vegetables produced in Shanghai [11], two-phase hollow fiber solid phase microextraction was also used for the analysis of pyrethroid pesticides in fruits and vegetables [12],

fungicides have been extracted in water and fruit samples using combination of solvents extractants for dispersive liquid-liquid microextraction coupled to liquid chromatography with tandem mass spectrometry [13]. However, other microextraction techniques involve the use of solvent (in microliter volume) some of which might be toxic [14].

Design of experiment is a chemometric approach used to design experimental runs, identifies significant factors, and estimate the main and interaction effect of various factors under study [15]. It requires few experimental runs that are carried out in an orderly manner and thus save analysis time, thereby improving sample throughput [16]. One factor at a time (OFAT) requires optimizing each factor at time, while other factors are kept constant while varying the factor being optimized [15] which has been shown to produce misleading results [17]. The use of chemometrics in the optimization of microextraction parameters for the analysis of pesticide residues in fruits and vegetables was also reviewed by our group [18].

In our present study, Plackett-Burman Design is employed to determine the factors that significantly affect the effective and efficient extraction of 14 multiclass pesticide residues in pear, grape, lettuce and broccoli.

Experimental

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-nitrobenzene (1000 µg/mL) used as internal standard with more than 95% purity, were purchased from AccuStandard Inc. New Haven CT, U.S.A. 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, 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.

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 [19].

Headspace-Solid Phase Microextraction Procedure

The SPME fibers (100 µm PDMS) purchased from Supleco, Bellefonte, PA USA, was conditioned in the GC/MS injection at 250 °C for 30 min, 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. To extract the target pesticides, the vial containing the sample spiked with pesticide standard 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 extract onto the SPME coated fiber were desorbed into the injection port for GC-MS analysis.

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 x 0.25 mm i.d. x 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 m x 6.5 mm o.d x 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/sec. 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. A target ion (most abundance ion) and two other reference ions were monitored for the target analytes (Table 1). 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. The Plackett-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).

Results and Discussion

The significant of factors affecting the SPME analysis of pesticide residues in fruit and vegetables samples were developed in our previous study, using Plackett-Burman Design followed by the optimization of the significant factors [20]. The ruggedness of the developed method was determined by using the developed method for the determination of 14 multiclass pesticide residues in pear, grape, lettuce and broccoli. The interaction effect of the significant factors was also analyzed.

Plackett-Burman (P-B) Design

The normal plot of standardized effect (Fig. 1a) shows that extraction temperature has the most significant effect with about 90 %, followed by extraction time (approx.. 80%), the pH and stirring rate has an average effect (50 % and 60 % respectively). Extraction temperature has been observed to have dual effect on extraction efficiency. It enhances the transport of analytes and also causes decrease in distribution coefficient, [21] and therefore must be carefully optimized.

The interaction plot (Fig. 1b) of the significant factors shows that the change in the total chromatographic peak area, when extraction time changed from low level to high level is different depending on the extraction temperature, which is a positive effect. The extraction temperature showed negative effect on the stirring rate and pH at higher temperature (i.e. the total chromatographic peak area (TCPA) reduced as the stirring rate and pH moved from low level to high level), while it shows positive effect at lower temperature. The extraction time also showed negative effect on stirring rate and pH at high level, while it shows positive effect at low level. The stirring rate also showed negative effect on the pH at high level, while the effect was negative at low level. It can be concluded that any interaction between the extraction parameters can either diminish or magnify the total chromatographic peak area (TCPA). Hence, the need to carefully optimized the significant factors. The residual plot (Fig. 1c) shows that the measurement deviation is randomly distributed around the mean.

N. B: Ext. time, Extraction time; Ext. temp, Extraction temperature; TCPA, Total Chromatographic Peak Area
 Fig 1: (a) Normal Plot of Standardized Effect of TCPA, (b) Interaction Plot of TCPA (c) Residual Plots for TCPA in Plackett-Burman Design (d) Desirability Surface Plots of TCPA.

Central Composite Design (CCD)

The significant factors were optimized using CCD. The residual plot (Fig 1c), shows that the measurement deviation is randomly distributed around the mean, and the global desirability surface response in 3D plot was obtained for the optimized parameters (Fig. 1d). In the contour plot (Fig. 2) of two-way interactions, the factors that are not in the plot were held constant. The counter plot of pH and stirring rate showed that the higher response (TCPA) lies between pH of 2-5 and a stirring rate greater than 700 rpm. The higher TCPA for stirring rate vs extraction temperature lies at stirring rate between 200 – 600 rpm and extraction temperature of approximately 70 °C. Optimal TCPA for pH vs extraction temperature was between temperature greater than

70 °C and pH 2 -10. Extraction time vs extraction temperature best TCPA was between 30 – 70 mins and greater than 70 °C respectively. The highest TCPA response for pH vs extraction time was between 2 – 10 and 50 – 60 °C respectively, while the contour plot for stirring rate vs extraction time shows the best TCPA at rate greater than 600 rpm and time greater than 60 min. The second order response was utilized because of its flexibility, the ability to give an approximation of the true value and the parameters can easily be estimated [18]. Taking into account the univariate and multivariate results, the optimal extraction conditions were: Temperature, 62 °C; time, 34 min; salt addition, 10 %; stirring rate, 350 rpm; pH, 6; desorption time, 7 min; desorption temperature, 270 °C.

Fig 2: Contour Plot of TCPA

Validation of Analytical Parameters

The analytical data (Tables 1 and 2) of the optimized SPME method was validated for the determination of 14 target pesticides in pear, grape, lettuce and broccoli samples. The limit of quantitation (LOQ) was calculated experimentally from a signal-to-noise ratio of 3, while the limit of detection (LOD) was calculated from a signal-to-noise ratio of 10, using the standard deviation of the y-intercept of the regression line of the calibration curve. The repeatability (n=9) was estimated by performing three extractions per day for three days. The

calibration curve was obtained using internal standard method by plotting the peak area ratio (i. e. chromatographic peak area of each target analytes to the chromatographic peak area of internal standard) against the concentration of each analyte and the linearity ranges from 1- 500 µg/kg. The relative recoveries ranges from 74 – 115.7%, the LOD ranges from 0.17 – 7.34 µg/kg while the LOQ ranges from 0.55 – 24.50 µg/kg. Fig. 3 shows the chromatogram of pear sample spiked at 50 µg/kg, of the pesticide standards, containing the target analytes indicating no matrix effect.

Fig 3. GC-MS Chromatogram of Pear Sample spiked at 50 µg/kg of pesticide standard
1. I.S (Internal Standard); 2. Fenobucarb; 3. Ethoprophos; 4. Diaxinon; 5. Chlorothalonil; 6. Parathion Methyl; 7. Fenitrothion; 8. Chlorpyrifos; 9. Thiobencarb; 10. Quinalphos; 11. Endosulfan I; 12. Endosulfan II; 13. Bifenthrin; 14. Fenpropathrin; 15. Permethrin.

Table 1: Linearity range, LOD and LOQ ($\mu\text{g/kg}$) of the developed HS-SPME method in pear, grape, lettuce and broccoli

Analytes	Ret. Time (min)	Ion (m/z)	Linearity ($\mu\text{g/kg}$)	r^2				LOD ($\mu\text{g/kg}$)				LOQ ($\mu\text{g/kg}$)			
				Pear	Grape	Lettuce	Broccoli	Pear	Grape	Lettuce	Broccoli	Pear	Grape	Lettuce	Broccoli
Fenobucarb	8.81	121, 91, 150	2.5 – 500	0.9918	0.9979	0.9955	0.9976	2.19	2.17	2.47	2.44	7.31	7.22	8.22	8.13
Ethoprophos	9.13	158, 97, 139	2.5 – 250	0.9979	0.9980	0.9961	0.9945	2.51	1.20	0.34	0.21	8.36	4.00	1.14	0.70
Diazinone	11.04	179, 137, 152	2.5 – 250	0.9980	0.9989	0.9978	0.9958	0.51	1.05	0.23	0.21	1.84	3.50	0.77	0.68
Chlorothalonil	11.31	266, 263, 268	10 – 500	0.9989	0.9964	0.9973	0.9978	4.76	0.43	0.51	7.34	15.86	1.44	1.84	24.50
Parathion-m	12.81	109, 79, 125	1 – 250	0.9964	0.9952	0.9963	0.9973	0.27	0.22	0.59	0.55	0.89	0.72	0.59	0.55
Fenitrothion	13.70	125, 79, 109	2.5 – 200	0.9952	0.9985	0.9983	0.9963	0.23	0.20	0.20	0.27	0.88	0.66	0.67	2.24
Chlorpyrifos	14.34	97, 125, 197	5 – 500	0.9985	0.9985	0.9983	0.9985	3.17	2.79	3.52	3.32	10.58	9.29	11.75	11.08
Thiobencarb	14.50	100, 125, 127	5 – 250	0.9977	0.9978	0.9981	0.9988	3.42	3.19	3.19	3.67	11.40	10.62	10.62	12.23
Quinalphos	16.37	146, 118, 156	2.5 – 125	0.9964	0.9968	0.9990	0.9964	2.41	2.24	2.05	1.86	8.03	7.47	6.83	6.20
Endosulfan I	17.26	195, 207, 241	5 – 250	0.9976	0.9976	0.9970	0.9976	2.76	3.45	2.27	2.93	9.20	11.50	7.57	9.77
Endosulfan II	18.61	195, 159, 207	10 – 250	0.9988	0.9987	0.9980	0.9988	2.71	3.28	3.06	2.34	9.03	10.95	10.20	7.80
Bifenthrin	20.14	181, 166,	1 – 500	0.9982	0.9983	0.9985	0.9982	0.17	0.75	0.64	0.67	0.60	2.50	2.14	2.22
Fenpropathrin	20.31	97, 125, 181	1 – 50	0.9972	0.9972	0.9986	0.9927	0.22	0.55	0.34	0.49	0.74	1.83	1.13	1.65
Permethrin	22.21	183, 91, 163	5 – 100	0.9976	0.9973	0.9990	0.9973	2.03	1.94	1.65	1.95	6.78	6.44	5.50	6.50

Table 2: Accuracy (relative recoveries) and precisions (relative standard deviation) of the pesticides in pear, grape, lettuce and broccoli samples

Analytes	Spike (µg/kg)	Pear		Grape		Lettuce		Broccoli	
		Accuracy (%)	RSD ⁱ (%)	Accuracy (%)	RSD (%)	Accuracy (%)	RSD (%)	Accuracy (%)	RSD (%)
Fenobucarb	50	99.1	8.9	90.4	4.9	85.5	12.5	79.9	7.2
	100	104.0	5.0	80.7	6.9	80.7	10.9	76.8	1.7
	150	103.5	5.8	86.8	5.0	94.8	4.2	83.4	1.8
Ethoprophos	20	106.7	7.1	76.0	9.2	105.9	8.0	75.8	4.9
	50	103.9	3.6	79.9	5.1	85.2	4.1	75.6	1.8
	100	106.4	4.7	81.9	3.1	93.1	3.7	93.9	2.3
Diazinone	50	97.8	7.7	86.7	9.0	92.5	5.0	88.0	5.9
	100	99.4	8.4	87.3	4.9	86.4	4.6	88.3	2.4
	150	104.2	5.8	96.7	2.7	88.3	4.3	94.0	1.9
Chlorothalonil	50	89.6	5.6	80.7	8.6	82.1	8.1	90.3	5.9
	100	82.0	10.6	78.9	5.4	88.1	4.4	86.4	2.7
	150	100.5	6.0	89.4	6.3	87.5	4.0	97.2	1.5
Parathion-m	50	76.6	7.1	83.2	5.5	79.3	5.6	78.4	6.1
	100	76.4	14.0	87.5	5.2	74.2	5.0	88.5	4.5
	150	88.6	4.1	87.3	2.2	80.1	4.2	86.0	2.6
Fenitrothion	5	109.1	5.4	95.8	6.0	75.7	8.1	76.4	2.8
	10	108.7	3.9	103.2	4.7	86.2	3.4	86.88	3.1
	20	107.5	4.1	103.5	3.7	89.5	3.1	93.4	2.5
Chlorpyrifos	20	99.8	7.9	100.7	7.6	74.0	6.7	85.0	2.9
	50	104.1	4.0	105.8	5.4	78.6	5.0	94.9	1.8
	100	102.2	3.6	111.0	2.5	86.6	3.7	98.9	1.2
Thiobencarb	50	88.0	9.7	94.8	6.2	77.7	9.3	94.7	3.1
	100	95.4	4.7	92.1	2.9	80.0	4.9	95.8	2.7
	150	95.0	5.4	98.2	3.2	85.3	2.2	97.4	1.7
Quinalphos	20	105.8	5.1	103.0	8.0	82.1	6.1	86.5	7.6
	50	105.9	2.6	108.5	1.9	82.7	5.2	94.7	4.5
	100	102.9	2.8	110.3	1.7	78.0	4.4	92.8	4.6
Endosulfan I	50	99.1	7.0	101.6	3.9	74.4	5.4	93.4	4.5
	100	102.4	5.6	102.4	2.2	76.4	2.6	95.6	2.0
	150	104.1	3.4	104.2	1.9	91.3	2.1	98.6	2.6
Endosulfan II	50	97.6	5.4	101.8	3.3	75.6	5.7	90.4	3.4
	100	103.1	4.4	105.6	2.7	85.1	3.1	95.3	3.1
	150	102.4	4.3	102.22	2.4	91.0	1.4	96.1	3.8
Bifenthrin	50	101.8	4.7	108.0	3.3	75.3	2.4	115.7	5.1
	100	104.6	3.0	107.5	3.1	85.1	2.9	101.3	5.4
	150	103.9	2.5	110.4	1.5	91.0	1.6	94.2	4.4
Fenpropathrin	5	79.9	3.4	93.3	2.9	113.6	4.0	109.8	9.4
	10	93.9	8.1	95.1	8.8	95.5	9.6	99.8	11.1
	20	96.2	9.8	98.5	7.9	83.9	7.3	95.8	2.4
Permethrin	20	95.7	11.2	102.4	8.8	80.6	8.0	95.4	8.0
	50	103.4	7.0	107.9	6.6	76.0	5.2	79.8	4.7
	100	104.6	3.3	109.9	3.1	83.5	5.8	99.7	4.1
Range	5-150	76.4-108.9	2.4-14	76-111	1.5-9	74-113.6	1.4-12.5	75.6-115.7	1.1-11.1

^aRSD, Relative standard deviation**Analysis of Real Samples**

The developed method was applied in the analysis 20 samples each of pear, grape lettuce and broccoli, purchased from Malaysian local and hypermarket markets. This is done in order to further verify the reliability and robustness of the developed method. All samples were devoid of target analytes and therefore were not detected.

Conclusions

It has been shown that the use of chemometrics combined with SPME method is cheap, simple, robust and fast. It enhances recoveries and improves method validation. The figures of merit were comparable or better than other methods of pesticide analysis as reported in our previous review [21]. 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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