

Analytical Letters

ISSN: 0003-2719 (Print) 1532-236X (Online) Journal homepage: http://www.tandfonline.com/loi/lanl20

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To cite this article: Abubakar Lawal, Richard Chee Seng Wong, Guan Huat Tan & Lukman Bola Abdulra'uf (2018): Determination of Pesticide Residues in Fruit and Vegetables by High-Performance Liquid Chromatography–Tandem Mass Spectrometry with Multivariate Response Surface Methodology, Analytical Letters, DOI: 10.1080/00032719.2018.1459655

To link to this article: https://doi.org/10.1080/00032719.2018.1459655

Published online: 15 May 2018.

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ANALYTICAL LETTERS
https://doi.org/10.1080/00032719.2018.1459655

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Determination of Pesticide Residues in Fruit and Vegetables by High-Performance Liquid Chromatography–Tandem Mass Spectrometry with Multivariate Response Surface Methodology

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ABSTRACT

Multivariate response surface methodology optimization using Placket–Burman and Box–Behnken designs were respectively used for the screening and optimization of significant factors for liquid chromatography–tandem mass spectrometry. Consequently, the optimized instrument successfully improved the sample preparation protocol and the method was validated. However, modified QuEChERS dispersive solid phase extraction coupled with ionic liquid-based dispersive liquid–liquid microextraction were used for the determination of multi-pesticide residues in fruit and vegetable samples. The analysed samples were jackfruit, strawberries, cucumber, pears, and carrots. The resulting linearity range (5–400 µg/kg) and regression coefficient (>0.99) results were satisfactory. The 94.2 and 95.8% accuracy (89–138%) and precision (0–25%) results were satisfactory and within the recommended ranges (≤20%) and (70–120%), respectively. The limits of detection (0.01–0.54 µg/kg) and quantitation (0.03–1.79 µg/kg) were excellent. The matrix effects (≤−87%) for all analysed samples were not significant. The estimated measurement uncertainties (≤27%) were within the acceptable range (≤50%). Justifiably, the response surface methodology optimized instrument and sample treatment techniques were reliable and convenient for multi-pesticide residue determination in various fruits and vegetables.

ARTICLE HISTORY
Received 19 January 2018
Accepted 27 March 2018

KEYWORDS
Dispersive liquid–liquid microextraction; high-performance liquid chromatography–tandem mass spectrometry; multivariate response surface methodology; solid-phase extraction

Introduction

Historically, many mass spectrometry instruments have been produced from the periodical series modification beginning with the mass spectrometer pioneered by Francis Aston (1919) (Sharma 2013). Similarly, several modified chromatographic columns used for liquid and gaseous separation have been produced over the years. The columns were trailed to the previous attribution made by Mikhail Tsvet (1903) (Lundanes et al. 2013). Thus, the liquid and gas chromatography instruments have connected with various detectors.

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The detectors include diode array, photodiode array and mass spectrometry detectors, which is mostly supported by electron spray ionisation (Parejo et al. 2004). Examples of the liquid and gas chromatography instruments include high-performance liquid chromatography (Aulakh et al. 2005) and gas chromatography-atomic emission detector (Cook et al. 1998). Moreover, the new mass spectrometry detectors include gas chromatography–tandem mass spectrometry (Chang et al. 2016; Guan et al. 2018) and liquid chromatography–tandem mass spectrometry (Zhu et al. 2017).

Fortunately, the liquid chromatography–tandem mass spectrometry instrument has been widely used in analytical science and food quality for more than a decade in determining pesticide residues (Frenich et al. 2014). However, the instruments have been used in most cases by the technologists and researchers based on the initial factory settings (auto-tuning and Mass-Hunter optimization), without further optimizing the factors associated with the ion source, which maybe valid on the responses of the mass spectrometry detector (Szerkus et al. 2016). Moreover, further optimization would improve the sensitivity of the electron spray ionisation of the liquid chromatography–tandem mass spectrometry instrument Consequently, this increases the peak areas (recoveries) of the analytes (Leito et al. 2008; Szerkus et al. 2016).

In fact, several factors of the liquid chromatography–tandem mass spectrometry play essential roles for the favourable electron spray ionisation, thereby increasing the sensitivity and efficiency of the analytical method through excellent responses of the instrument. However, these could be achieved by chemometrics (multivariate) optimization approach using several factors of the liquid chromatography–tandem mass spectrometry instrument. The optimization is based on the use of response surface methodology (RSM) of experimental design to obtain optimal conditions for quantification of the extracted analytes. Thus, this study will inform users of liquid chromatography–tandem mass spectrometry on the benefits of optimization before the analytical instrumentation.

The liquid chromatography–tandem mass spectrometry instrument is one of the most recent instruments used for multi-pesticide analysis at the lowest concentration level in various matrices (Kittlaus et al. 2011). Notably, the fruits and vegetables may be contaminated in the food chain, during cultivation, transportation, and preservation (Gamble 2015). Therefore, the continuous analyses of multi-pesticide residues in vegetable and fruits is vital because of their health and environmental issues. These issues include congenital disabilities (Criswell et al. 2013), cardiovascular diseases (Mostafalou and Abdollahi 2013), and cancer (Parrón et al. 2014). Therefore conventional methods such as liquid–liquid extraction, solid phase extraction (SPE), and liquid-phase microextraction techniques such as dispersive liquid–liquid microextraction (DLLME) have been used previously for the sample preparation of pesticides (Lawal et al. 2016). Nevertheless, the reduced efficiency and selectivity of the methods are their significant drawbacks. Thus, the coupling modification of the conventional methods such as the quick, easy, cheap, effective, rugged, and safe (QuEChERS) with dispersive SPE (Anastassiades et al. 2003) and DLLME techniques may be the best efficient method. The developed technique may address the drawback challenges for the sample treatment and cleanups techniques in vegetable and fruit samples.

The DLLME technique is excellently known for its enrichment properties (Rezaee et al. 2006). Recently, DLLME was used with 1-hexyl-3-methylimidazolium hexafluorophosphate ionic liquid-based excellently for the determination of pesticides in fruits (Zhang

et al. 2012). Consequently, 1-hexyl-3-methylimidazolium hexafluorophosphate ionic liquid-based was chosen for this study in preference to 1-octyl-3-methylimidazolium hexafluorophosphate and 1-butyl-3-methylimidazolium hexafluorophosphate due to its excellent chromatographic properties (Xie et al. 2014). However, the QuEChERS-dispersive SPE (sample treatment) method has been known for its excellent extraction efficiency (Gunatilake et al. 2014). Therefore, the use of QuEChERS-dispersive SPE method coupled to 1-hexyl-3-methylimidazolium hexafluorophosphate ionic liquid-based DLLME may yield excellent results after quantitation with the optimized instrument for the determination of pesticide residues in fruit and vegetable samples.

To the best of our knowledge, there is no report accurately on the chemometric optimization to liquid chromatography–tandem mass spectrometry instrument for the determination of multi-pesticide residues. In fact, this article will serve as a reference for future studies and describe the best instrumental and efficient way for monitoring multi-pesticide residues in fruit and vegetables.

Therefore, this study is aimed at using Minitab-17 statistical software for the chemometric (multivariate optimization) approach to liquid chromatography–tandem mass spectrometry. However, the software generated the Placket–Burman design for screening and Box–Behnken design for optimising the significant factors of the liquid chromatography–tandem mass spectrometry. Subsequently, the optimized instrument was used for quantification of multi-pesticide residues in the prepared sample solutions by the use of the modified QuEChERS-dispersive SPE-ionic liquid-based-DLLME technique.

Experimental

Chemicals and reagents

The pesticide stock solution (100 mg/kg) for Propamocarb, Carbaryl, Baycarb (Fenobucarb), Thiobencarb, Metalaxyl, Thiamethoxam, Diazinon, and Dursban (Chlorpyrifos) was purchased from AccuStandard (New Haven, USA). Each of the solutions was diluted with methanol to 10 mg/kg working solution and refrigerated at 4°C. The organic solvents (liquid chromatography–mass spectrometry grade), salts and reagents purchased include methanol and acetonitrile (Merck, Germany), ethanol, acetic and formic acid (Fisher Scientific) and 1-hexyl-3-methylimidazolium hexafluorophosphate ionic liquid ($P \geq 97.0\%$) (Sigma-Aldrich, Germany). Other chemicals include Millipore ultra-pure water (Billerica, USA) and pH 5–7 buffer solutions (Sigma-Aldrich). The pH 4, 6, and 8–10 buffer solutions were obtained from Fisher Scientific. Sodium chloride (Hamburg Chemicals, France) and ProElut^{M} AOAC 2007.01 QuEChERS (extraction salt) and d-SPE (cleanup salt) kits (general fruits/vegetables) were purchased from Dikma Technologies (Lake Forest, USA).

Apparatus and equipment

A Dynamica centrifuge (Selangor, Malaysia), Vortexer-VTX-3000L (Copens Scientific, Japan), Microsyringe (Hamilton, USA), Supelco high-performance liquid chromatography column [Ascentis Express C_{18} (5 cm × 2.1 mm, 2.7 µm)] (Sigma-Aldrich, USA) and triple quadrupole liquid chromatography–mass spectrometry (G6490A) instrument (Agilent Technologies, Singapore) were used in this work.

Conditioning of the liquid chromatography–tandem mass spectrometry instrument

The total ion chromatography (Figure 1) and the supporting parameters of the multiple reactions monitoring scans were generated after the auto-tuning optimization and Mass-Hunter optimization of the instrument using 1 mg/kg multi-pesticides mixture of standard solutions. The parameters generated for each of the analytes include precursor, product ions and collision energies are shown in Table 1. Afterwards, the contributory factors were optimized further using RSM of the experimental design.

Experimentally, the multivariate RSM optimization runs were carried out on 100 µg/kg multi-pesticide mixture of standard solutions to provide the optimum condition of the instrumental electron spray ionisation in yielding better results. Furthermore, the data of the multiple reactions monitoring scans resulted in the total chromatographic peak areas, which was integrated by the use of Agilent (B.06.00) Acquisition Mass Hunter software. Thus, the total chromatographic peak areas were used by the two essential designs for the RSM screening (Placket–Burman) and optimization (Box–Behnken) of significant factors of the instrument. Notably, the total chromatographic peak areas used for the instrumental optimization corresponds to analytes concentration in the analysed samples (Abdulra'uf and Tan 2015).

Subsequently, the RSM optimized instrument was operated starting from 15 and 85% of mobile phase B (acetonitrile + 0.1% formic acid) and A (deionised H_2O + 0.1% formic acid +1% acetonitrile) which were transported by a nitrogen stream through the column. The Supelco high-performance liquid chromatography column was injected with the analyte solution (5 µL) at a flow rate of 0.1 mL/min and 30°C column temperature. The estimated gradient of the elution time for mobile phase B was 15% (0–1.6 min); 15–100% (1.6–10.4 min); 100–15% (10.4–12.0 min). The Mass Hunter setup was operated at 200°C (gas temperature), 14 L/min (gas flow), 45 psi (nebulizer gas), 400°C (sheath gas temperature), 11 L/min (sheath gas flow), 3000 V (capillary voltage), and 200 V (Delta$^{(+)}$ electron multiplier voltage).

Sample preparation

The 250 g individually homogenised samples purchased from the night market located in Petaling Jaya (Malaysia) were preserved (4°C). Then, some parts of the preserved samples

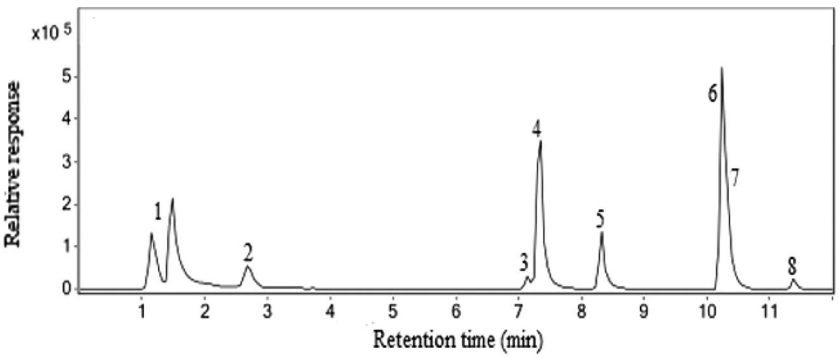


Figure 1. Total ion chromatography (TIC) responses of the eight pesticide analytes present at 1 mg/kg (ppm).

Table 1. Auto-tuning and Mass-Hunter optimization of the instrumentation.

Chromatographic peak	Pesticides	Precursor ion, *m/z*	Product ions, *m/z*	Collision energies, eV
1	Propamocarb	189	74;102	26;14
2	Carbaryl	202	127;145	30;6
3	Baycarb	208	77;95	42;10
4	Thiobencarb	258	89;125	54;26
5	Metalaxyl	280	160;220	26;10
6	Thiamethoxam	292	132;211	26;10
7	Diazinon	305	97;169	42;22
8	Dursban	350	97; 198	34;22

were equally weighed and spiked serially with estimated volumes of a known mixture of standard analyte solutions. These were used for calibration curves and validation studies.

QuEChERS-dispersive solid phase extraction method

Each of the five 50 mL centrifuge tubes was occupied with 20 g of the refrigerated sample and 15 mL acetonitrile was added. Then the tubes were spiked serially with the volumes of known concentration mixture of the multi-pesticides standard solutions, respectively. Each of the tubes was vortexed for 1 min after covering. The extraction salt (6 g $MgSO_4$ and 1.5 g sodium acetate) was added and shaken vigorously for 1 min before centrifugation (4000 rpm) for 2 min. Afterwards, the 1.5 mL supernatant was transferred to 2 mL centrifuge tube, which contained the cleanup salt (150 mg $MgSO_4$ and 50 mg PSA). The tube was centrifuged (4000 rpm) for 5 min.

Ionic liquid-based DLLME method

The resulted supernatant from d-SPE cleanup was transferred into 15 mL centrifuge tube containing 10% NaCl (w/v) in 9 mL of distilled water. Centrifugation (7000 rpm) took place for 5 min on the tube after 130 µL of ionic liquid-based was added. Eventually, the 2 mL high-performance liquid chromatography vial was occupied with the sedimental extract (100 µL), and 500 µL methanol was added. The analyte solution was analysed with the RSM optimized liquid chromatography–tandem mass spectrometry instrument after the vial was vortexed for 1 min.

Validation

Validation expresses the desirability, effectivity and certifiable accord of the RSM optimized liquid chromatography–tandem mass spectrometry instrument and the sample preparation technique for determination of multi-pesticide residues in the analysed fruits and vegetables (Li et al. 2017). Unfortunately, the RSM optimized liquid chromatography–tandem mass spectrometry instrument was not validated because there is no specific (official) guideline for its validation because a consensus has not been reached on how the protocol should be conducted (Kruve et al. 2015). However, the sample treatment and cleanup techniques of this work were validated using the guidelines of the European Union Commission (SANTE-11813, 2017).

Notwithstanding, the validated parameters such as relative recovery (accuracy) and relative standard deviation (precision) were estimated at three (spiked) concentrations

(5, 100, and 300 µg/kg). The accuracy and precision results of the prepared samples of the triple analyses ($n = 3$) were estimated. The linearity range for each of the analytes calibration curve at five levels (spiked) concentrations is 5–400 µg/kg. Moreover, the estimated matrix effect [Eq. (1)] (Li et al. 2016) for each sample was measured and tabulated. The limits of detection and quantitation were estimated corresponding to 3 and 10 factors of the signal-to-noise ratio, respectively (Xiu-Ping et al. 2017). Also, the measurement uncertainties were conducted on the validated parameters and shown to be certifiable with the obtained results. The measurement uncertainties were estimated at the 95% confidence level with the use of an empirical model and coverage factor ($k = 2$) (Kaczyński 2017).

$$\text{Matrix effect}(\%) = \left[\left(\frac{\text{slope of calibration curve for analyte in matrix}}{\text{slope of calibration curve for analyte in organic solvent}}\right) - 1\right] \times 100 \quad (1)$$

Results and discussion

Placket–Burman design

The Placket–Burman design was operated on 24 experimental runs at 2-levels of variables [low (−) and high (+)] as reported for pesticides determination (Cortada et al. 2009). Apparently, the 100 µg/kg standard solutions of the eight pesticides mixture were used for screening the significant factors of the liquid chromatography–tandem mass spectrometry instrument at the 95% confidence interval (0.05 confidence limits). The total chromatographic peak areas of the resulted multiple reactions monitoring were recorded accordingly in the Minitab generated design before undergoing statistical analysis. Therefore, Figures 2 and 3 respectively illustrate the Pareto chart and normal plot of standardized effects for the screened factors. The Pareto chart shows horizontal bars with a red line vertically across the bars to show a significant difference. Also, the normal plot expresses the significant extent (ANOVA) for each of the screened parameter is distributed along the mean (straight line).

Eventually, the eleven factors screened are: (A) starting mobile phase B, 10 and 20%; (B) column temperature, 25 and 35°C; (C) flow rate, 0.1 and 0.2 mL/L; (D) injection volume, 1 and 5 µL; (E) gas temperature, 150 and 250°C; (F) gas flow, 11 and 17 L/min; (G) nebulizer, 30 and 60 psi; (H) sheath gas temperature, 200 and 400°C; (J) sheath gas flow, 10 and 12 L/min; (K) capillary voltage, 2000 and 4000 V; and (L) Delta$^{(+)}$ electron multiplier voltage, 100 and 200 V. The results showed that the flow rate, injection volume, sheath gas temperature, and Delta$^{(+)}$, electron multiplier voltage had the largest influence on the instrumental response.

Box–Behnken design

The Box–Behnken design was employed for optimization of the four significant factors: flow rate, injection volume, sheath gas temperature, and Delta$^{(+)}$ electron multiplier voltage. Similarly, the total chromatographic peak areas results of the generated design (Table 2), which was used for the 27 runs at three levels [low (−), medium (0), and high (+)] were analysed statistically, as supported by Dong et al. (2009). Later on, the optimization responses

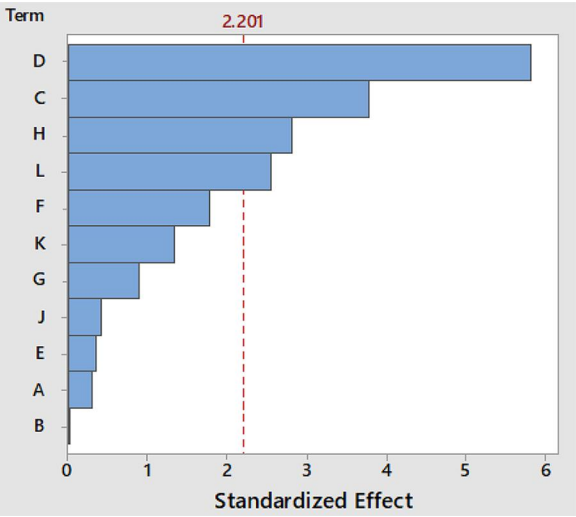


Figure 2. Pareto chart of standardized effects at 0.05 confidence limit (2.201 bar chart length). The screened factors were (A) starting mobile phase B, 10 and 20%; (B) column temperature, 25 and 35°C; (C) flow rate, 0.1 and 0.2 mL/L; (D) injection volume, 1 and 5 µL; (E) gas temperature, 150 and 250°C; (F) gas flow, 11 and 17 L/min; (G) nebulizer, 30 and 60 psi; (H) sheath gas temperature, 200 and 400°C; (J) sheath gas flow, 10 and 12 L/min; (K) capillary voltage, 2000 and 4000 V; and (L) Delta$^{(+)}$ electron multiplier voltage, 100 and 200 V.

were demonstrated in the contour plot and desirability chart. However, the response surface of the contour (Figure 4) plot was expressed in a two-dimensional plane for the two significant factors, which were proportional to each other. The generated lines of the contour plot were based on total chromatographic peak areas interception against the two significant

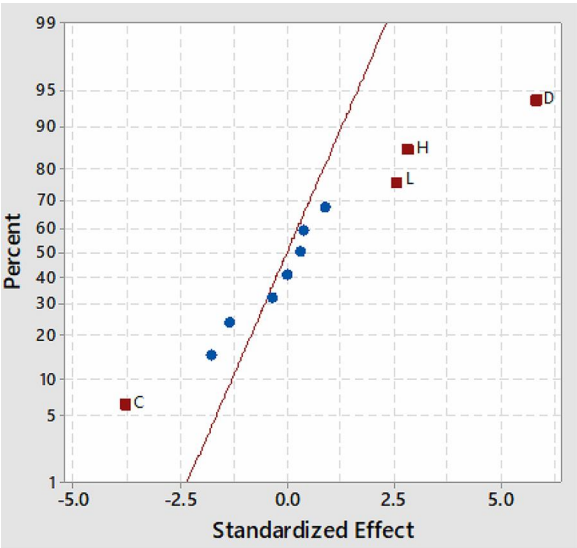


Figure 3. Normal plot of the standardized effects at the 0.05 confidence limit. The most significant parameters were (C) flow rate, 0.1 and 0.2 mL/L; (D) injection volume, 1 and 5 µL; (H) sheath gas temperature, 200 and 400°C; and (L) Delta$^{(+)}$ electron multiplier voltage, 100 and 200 V.

Table 2. Four most significant factors of liquid chromatography–tandem mass spectrometry and the Box–Behnken design response.

Factor				Level		
				Low (–)	Medium (0)	High (+)
Flow rate (mL/L)				0.1	0.15	0.2
Injection volume (μL)				1	3	5
Sheath gas temperature (°C)				200	300	400
Delta$^{(+)}$ electron multiplier voltage (V)				100	150	200
Run order	Pt type	Flow rate (mL/L)	Injection volume (μL)	Sheath gas temperature (°C)	Delta$^{(+)}$ electron multiplier voltage (V)	Total chromatographic peak area
1	2	0.15	1	300	100	3212880
2	2	0.1	3	300	200	45948949
3	2	0.15	3	400	100	21226211
4	2	0.15	5	300	100	31639197
5	0	0.15	3	300	150	27412061
6	2	0.1	5	300	150	59341689
7	2	0.2	3	300	100	13545963
8	2	0.15	1	300	200	10281586
9	2	0.15	3	200	100	13676817
10	0	0.15	3	300	150	25328649
11	2	0.15	5	400	150	53961209
12	2	0.2	5	300	150	31894779
13	2	0.2	3	400	150	20830312
14	2	0.2	1	300	150	5171605
15	2	0.15	3	400	200	46590534
16	2	0.15	3	200	200	29513573
17	2	0.15	5	300	200	51031714
18	2	0.1	1	300	150	11273447
19	2	0.15	1	400	150	10495997
20	2	0.2	3	200	150	13190386
21	2	0.2	3	300	200	22668595
22	0	0.15	3	300	150	25890246
23	2	0.15	5	200	150	33968671
24	2	0.1	3	400	150	12506025
25	2	0.1	3	300	100	47552062
26	2	0.1	3	200	150	29940610
27	2	0.15	1	200	150	6706898

factors. For example, the most shaded (colored) line in the contour plot in between the two significant factors (sheath gas temperature against flow rate) signified the best-optimized area (value) that could provide the maximum total chromatographic peak areas (>35,000,000). Furthermore, the resulted total chromatographic peak areas was at its maximum when parameter sheath gas temperature approaches 400°C, and parameter flow rate was at 0.1 mL/L.

On the other hand, the desirability chart illustrated the optimized values for each of the significant factors, which yielded the best analytical result at the composite desirability fit of 1.0 (Figure 5). Thus, the figure indicates optimum values of a flow rate of 0.1 mL/L (A), 5 µL injection volume, 400°C sheath gas temperature, and 200 V Delta$^{(+)}$ electron multiplier voltage. These results are in the same range for pesticides analysis documented by Vázquez et al. (2015) except that the reported flow rate is higher than 0.1 mL/L, which may be due to the different column size used. Subsequently, the setup of optimized and unoptimized factors was compared through instrumental analysis of the analyte solution (100 µg/kg). Resultantly, the RSM (experimental design) optimization of the instrumental setup yielded a better result of 68% compared to the 32% average total chromatographic peak areas for the unoptimized setup (Figure 6).

Similarly, comparative investigation of the sample treatment techniques for this analysis (Figure 7) favoured the RSM optimized QuEChERS-ionic liquid-based-DLLME technique (50.10%) over the RSM optimized QuEChERS-dispersive solid-phase extraction-ionic liquid-based-DLLME (48.17%) and RSM optimized QuEChERS-dispersive SPE (1.73%) techniques after analysis of the distilled water (sample). However, the RSM optimized QuEChERS-dispersive solid-phase extraction-ionic liquid-based-DLLME technique (65.90%) was selected and used for the sample treatment of fruits and vegetables due to the weak recoveries by QuEChERS-ionic liquid-based-DLLME technique (34.10%) in a real sample of fruits (Figure 8). Therefore, the QuEChERS-dispersive SPE-ionic liquid-based-

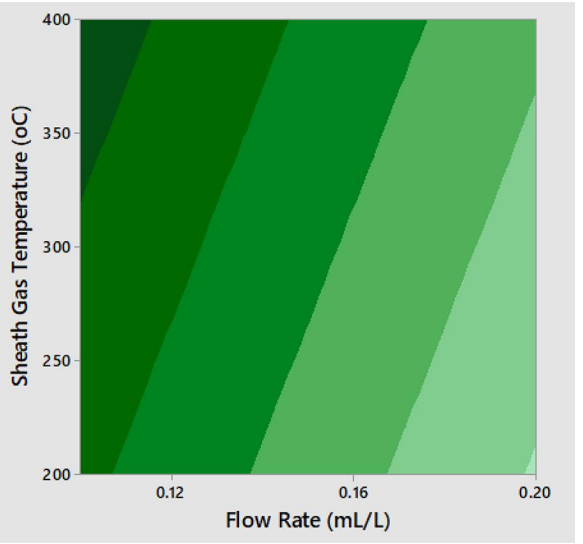


Figure 4. Contour plot response interaction between sheath gas temperature against flow rate at the 0.05 confidence limit. The point or area between the two interactive factors with more coloration signifies higher total chromatographic area.

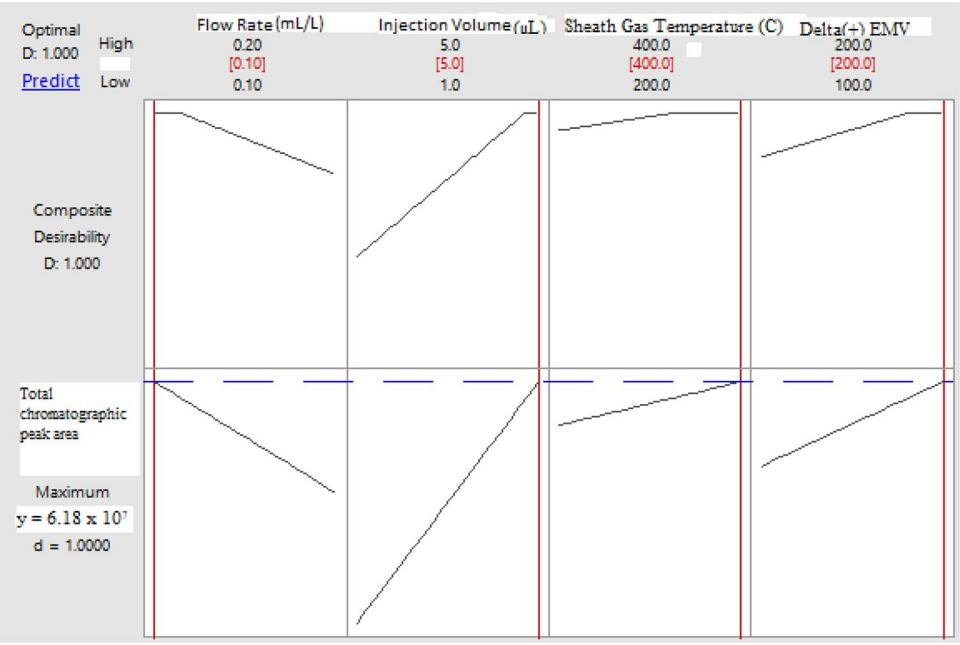


Figure 5. Composite desirability optimization chart at the 0.05 confidence limit. The best predicted optimized values were flow rate, 0.1 mL/L; injection volume, 5 µL; sheath gas temperature, 400°C; and Delta$^{(+)}$ electron multiplier voltage (EMV), 200 V.

based-DLLME technique coupled with the RSM optimized liquid chromatography–tandem mass spectrometry instrument was used for determination of multi-pesticide residues in the fresh samples of jackfruit, strawberries, cucumber, pear, and carrot.

Therefore, the impact of the optimized settings of the instrument successfully increases the instrumental efficiency, which contributed to improving the selectivity and robustness

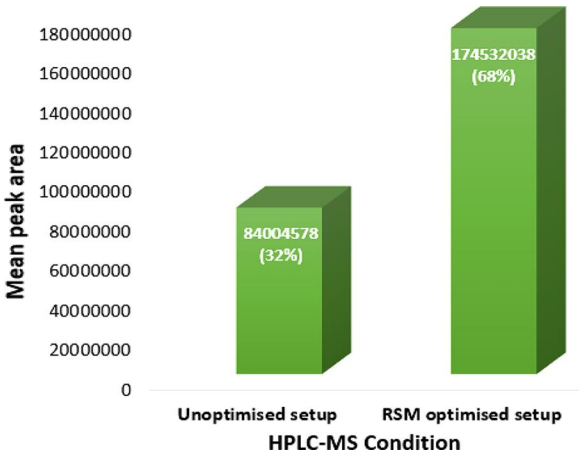


Figure 6. Comparison of unoptimized and RSM optimized setups of high performance liquid chromatography–tandem mass spectrometry using a 100 µg/kg (ppb) mixed pesticide standard solution.

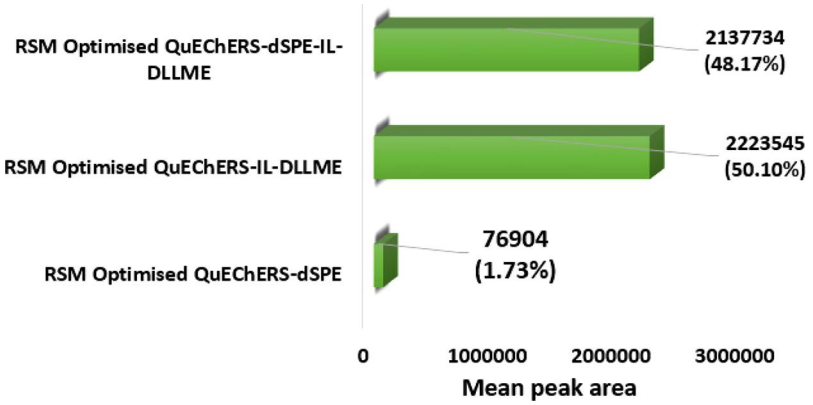


Figure 7. Comparative analysis of the RSM optimized QuEChERS sample treatment methods after spiking a distilled water samples with 100 µg/kg (ppb) mixed pesticide standard solution.

of the sample preparation technique as reported by Jansson et al. (2004). Accordingly, the sample preparation succeeded in detecting and quantifying the targeted analytes at lower concentration levels and provided satisfactory results of the accuracy and precision in the analysed fruit and vegetable samples.

Method validation

The parameter results from the sample treatment and cleanup techniques were validated according to the official guideline (SANTE-11813, 2017) for the determination of pesticide residues in the analysed samples. These include accuracy, precision, limit of detection, limit of quantitation, matrix effect, and measurement uncertainties. Approximately, the 94.2% of

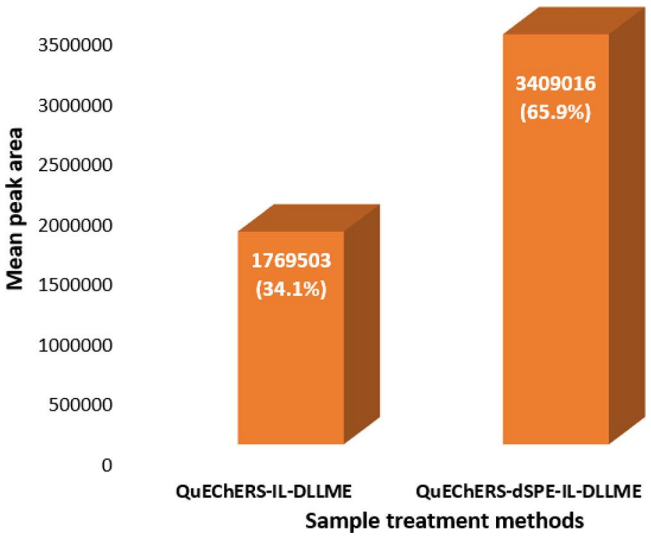


Figure 8. Comparison of recoveries between the RSM optimized QuEChERS-IL-DLLME and QuEChERS-dSPE-IL-DLLME sample preparation methods after spiking fruit samples with 100 µg/kg (ppb) mixed pesticide standard solutions.

Table 3. Accuracy and precision for the three spiked concentrations of the analyzed samples.

		Jackfruit		Strawberries		Cucumber		Pear		Carrot	
Pesticides	Spike, µg kg^{-1}	Relative recovery, %	Relative standard deviation, %	Relative recovery, %	Relative standard deviation, %	Relative recovery, %	Relative standard deviation, %	Relative recovery, %	Relative standard deviation, %	Relative recovery, %	Relative standard deviation, %
Durban	5	98	5	120	3	121	9	117	15	107	3
	100	100	0	98	1	97	6	99	9	100	1
	300	98	3	100	2	99	13	97	0	100	14
Diazinon	5	101	13	92	7	90	7	100	2	114	3
	100	100	1	99	3	99	2	101	13	101	5
	300	100	2	99	1	99	8	101	1	101	5
Thiamethoxam	5	105	8	119	4	101	5	112	8	114	3
	100	99	13	95	2	104	2	96	13	98	2
	300	101	2	100	2	100	11	98	2	94	2
Metalaxyl	5	131	12	105	4	121	10	115	11	99	0
	100	100	7	99	9	102	4	100	4	100	12
	300	100	10	98	7	98	4	100	6	101	9
Thiobencarb	5	138	25	109	1	126	19	93	12	111	6
	100	101	1	100	4	103	2	96	6	102	4
	300	100	1	100	1	101	4	101	2	101	4
Baycarb	5	120	21	112	24	96	23	98	6	121	1
	100	98	2	110	1	99	4	102	4	99	1
	300	99	1	95	1	101	12	101	4	99	2
Carbaryl	5	124	24	91	14	105	13	105	3	105	2
	100	98	1	89	1	99	1	97	8	105	5
	300	99	4	98	4	98	19	100	5	100	16
Propamocarb	5	96	21	106	13	90	6	90	16	112	4
	100	98	5	94	5	105	4	101	4	112	2
	300	100	0	100	1	100	18	99	5	100	18
Ranges	5-300	96-138	0-25	89-120	1-24	90-126	1-23	90-117	0-16	94-121	0-18

Table 4. Pesticides detection and quantitation limits in the analyzed samples.

	Jackfruit		Strawberries		Cucumber		Pear		Carrot	
	Limit of detection, μg kg^{-1}	Limit of quantitation, μg kg^{-1}	Limit of detection, μg kg^{-1}	Limit of quantitation, μg kg^{-1}	Limit of detection, μg kg^{-1}	Limit of quantitation, μg kg^{-1}	Limit of detection, μg kg^{-1}	Limit of quantitation, μg kg^{-1}	Limit of detection, μg kg^{-1}	Limit of quantitation, μg kg^{-1}
Dursban	0.13	0.45	0.09	0.29	0.08	0.26	0.18	0.61	0.01	0.03
Diazinon	0.04	0.13	0.07	0.24	0.07	0.25	0.06	0.20	0.15	0.48
Thiamethoxam	0.07	0.24	0.15	0.50	0.12	0.41	0.19	0.63	0.39	1.29
Metalaxyl	0.08	0.27	0.13	0.42	0.12	0.39	0.04	0.13	0.08	0.27
Thiobencarb	0.11	0.35	0.02	0.07	0.14	0.46	0.14	0.46	0.10	0.34
Baycarb	0.06	0.21	0.37	1.24	0.08	0.28	0.07	0.23	0.07	0.22
Carbaryl	0.06	0.21	0.54	1.79	0.11	0.36	0.09	0.31	0.16	0.53
Propamocarb	0.07	0.25	0.20	0.67	0.16	0.52	0.04	0.13	0.38	1.27
Ranges	0.04-0.13	0.13-0.45	0.02-0.54	0.07-1.79	0.07-0.16	0.25-0.52	0.04-0.19	0.13-0.63	0.01-0.39	0.03-1.29

Table 5. Matrix effects and European Union (2016) maximum residue limits of the analyzed samples.

	Jackfruit		Strawberries		Cucumber		Pear		Carrot	
	Matrix effects, %	European Union maximum residue limit, μg kg^{-1}	Matrix effects, %	European Union maximum residue limit, μg kg^{-1}	Matrix effects, %	European Union maximum residue limit, μg kg^{-1}	Matrix effects, %	European Union maximum residue limit, μg kg^{-1}	Matrix effects, %	European Union maximum residue limit, μg kg^{-1}
Dursban	−98	10	−88	200	−97	50	−87	10	−91	100
Diazinon	−96	–	−97	10	−99	10	−100	10	−100	10
Thiamethoxam	−100	10	−100	300	−100	500	−100	300	−100	300
Metalaxyl	−99	50	−99	500	−99	500	−100	1000	−100	100
Thiobencarb	−92	10	−92	10	−98	10	−99	10	−98	10
Baycarb	−90	10	−89	10	−97	10	−87	10	−97	10
Carbaryl	−100	10	−100	10	−100	10	−100	10	−100	10
Propamocarb	−100	–	−100	1000	−100	5000	−100	10	−100	10
Ranges	≤− 90		≤−88		≤−97		≤−87		≤−91	

Table 6. Regression coefficients and measurement uncertainties of the analyzed samples.

	Jackfruit		Strawberries		Cucumber		Pear		Carrot	
	Regression coefficient	Measurement uncertainty, %	Regression coefficient	Measurement uncertainty, %	Regression coefficient	Measurement uncertainty, %	Regression coefficient	Measurement uncertainty, %	Regression coefficient	Measurement uncertainty, %
Dursban	0.9993	5	0.9993	4	0.9996	18	0.9987	16	0.9999	12
Diazinon	0.9998	11	0.9998	7	0.9998	11	0.9998	11	0.9991	9
Thiamethoxam	0.9997	15	0.9991	5	0.9994	12	0.9987	16	0.9949	5
Metalaxyl	0.9995	19	0.9993	13	0.9993	13	0.9998	14	0.9997	12
Thiobencarb	0.9993	18	0.9998	4	0.9991	16	0.9993	13	0.9995	9
Baycarb	0.9997	17	0.9954	17	0.9997	27	0.9998	10	0.9996	2
Carbaryl	0.9996	19	0.9906	13	0.9995	22	0.9996	10	0.9991	15
Propamocarb	0.9997	18	0.9986	12	0.9992	19	0.9999	17	0.9951	16
Ranges	>0.999	≤19	>0.99	≤17	>0.999	≤27	>0.99	≤16	>0.99	≤16

all accuracy (Table 3) results (89–138%) are within the recommended range (70–120%). The 95.8% precision (Table 3) results were within acceptable range (≤20%). The range results of limit of detection (0.01–0.54 µg/kg) (Table 4) and limit of quantitation (0.03–1.79 µg/kg) (Table 4) were less than the least level of calibration (5 µg/kg), and the European Union maximum residue limits (Table 5) (European Union 2016).

Furthermore, the capability and performance of the technique were estimated based on the measurement of matrix effects, which shows the effects of the sample matrix inferences on the recovery of the analytes (Wang et al. 2017). Thus, the matrix effect (Table 5) in all the analysed samples were very weak (≤−87%). This may be due to the extensive extraction/cleanup of matrix interferences during sample preparation (Oulkar, Goon, and Banerjee 2017). Also, the matrix effect enhancement in liquid chromatography–mass spectrometry is not easy to achieve compared to the gas chromatography–mass spectrometry instrumentation because of the co-elution of the individual analytes (SANTE-11813, 2017). Ultimately, the estimated measurement uncertainties (Table 6) range (≤27%) were within the accepted recommendation (≤50%). Moreover, the linearity results were obtained with regression coefficients greater than 0.99 (Table 6). Eventually, analyses were carried out on the real (unspike) samples to further certified reliability and credibility of the research technique. Therefore, the outcomes respectively detected multi-residue of pesticides in the analysed samples. Although, most of the analytes detected were lower than the limit of quantitation and European Union maximum residue limits.

Conclusion

The chemometric approach to liquid chromatography–tandem mass spectrometry was successful, leading to attainment of the maximum optimum condition for the analyte quantification and increasing the sensitivity of the QuEChERS-dispersive SPE-ionic liquid-based-based-DLLME method. Therefore, the optimized instrument was rapid with outstanding detectability and quantification of the analytes (multi-pesticide residues) determined in the analysed fruit and vegetable samples. Therefore, the optimized instrument could be reliable and convenient for the determination of multi-pesticide residues in various samples of fruits and vegetables.

Funding

The Postgraduate Research Project (IPPP) supported this research under the grant no. PG 174-2014B, University of Malaya Kuala Lumpur, Malaysia.

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