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Determination of Multi - Pesticide Residues in Coconut Water by QuEChERS - dSPE Ionic Liquid - Based DLLME Couple with High Performance Liquid Chromatography - Tandem Mass Spectrometry (LC-MS/MS)

[1]Abubakar Lawal and [2]Isa Baba Koki
[1] Department of Chemistry, Umaru Musa Yar'adua University, P.M.B. 2218 Katsina, Nigeria.
[2] Department of Chemistry, Yusuf Maitama Sule University Kano, P.M.B. 3220 Kano, Nigeria.
Email: abubakarlawal360@yahoo.com

ABSTRACT

The use of pesticides in agricultural practices is very vital but continuously accumulates in water, moist soil and contaminate fruits and vegetables. This eventually leads to many health issues such as congenital diseases and different forms of cancers. For this reason, the validated (SANTE 11831-2017) method of quick, easy, cheap, effective, rugged and safe (QuEChERS) with the dispersive solid phase extraction (SPE) coupled with ionic liquid-based (IL-based) in dispersive liquid-liquid microextraction (DLLME) was used for sample preparation of coconut water to determine multiple pesticides (Dursban, Diazinon, Thiamethoxam, Metalaxyl, Thiobencarb, Baycarb, Carbaryl and Propamocarb) residue using high-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) instrument. The results of accuracy for relative recoveries (84 - 134%) were satisfactory because 87.5% of results were within the recommended range (70 - 120%). The precision results range (0-19%) of relative standard deviation (RSD) were also satisfactory as recommended ($\leq$ 20%). The resulted limit of detection (LOD) and quantitation (LOQ) was satisfactorily ranged 0.08-0.92 and 0.28-3.08µg/kg respectively. The linearity range results (5 – 500 µg/kg) were evaluated (linear) with regression coefficient (R^2) > 0.97. The matrix effects range ($\leq$ -72%) were very weak. The measurement uncertainties range (7 - 27%) estimated were satisfactory as recommended ($\leq$ 50%). Conclusively, the research indicates the reliability of the sample preparation method for multiple pesticides analysis in coconut water. Also, the concentration level for each of the pesticide analysed was found lower than the European Union maximum residue limits, which presumes that the analysed sample is safe for consumption to avoid health-related issues.

Keywords: Dispersive, Liquid-Liquid Microextraction, Solid-Phase Extraction, HPLC-MS, Pesticides, QuEChERS

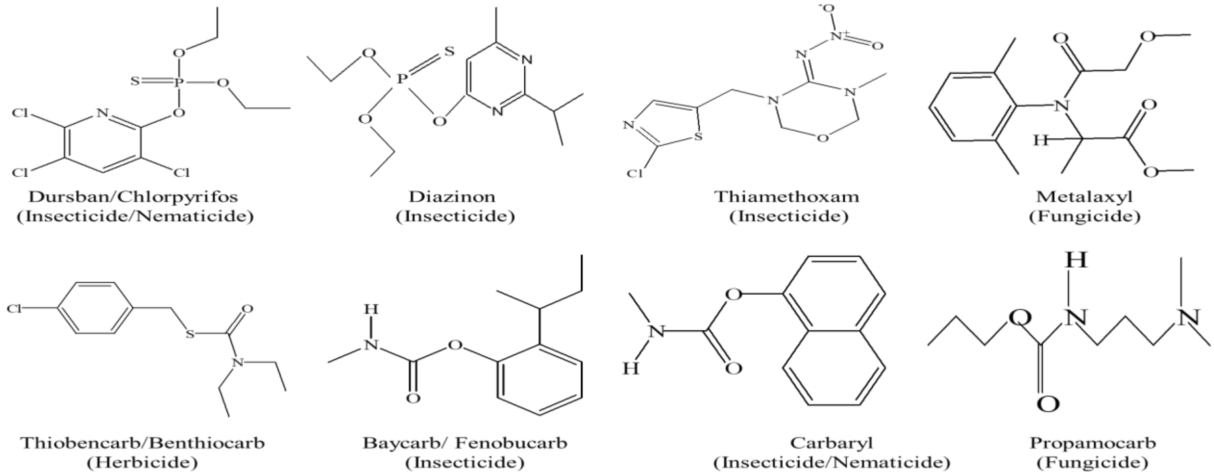
INTRODUCTION

Coconut water (Scheme 1) is a colourless liquid that is served naturally after harvest or processed as an isotonic beverage drink. The liquid is slightly acidic (4 - 6 pH range) and highly nutritionally sourced of carbohydrates, proteins, minerals, salts and vitamins (Young *et al*., 2009). For these reasons, a native of many South-Asia countries has been using coconut water for decades to quench their thirsts and satisfy their nutritional needs (Anjos & Andrade, 2014).

Unfortunately, persistence and accumulation of hazardous pesticide residues in moistened soil and waterbodies due to their lower vapour pressure and higher molecular weight, solubility and half-life ($t_{1/2}$) have been the issue of concern (Lawal *et al*., 2018c). This is because Agricultural produce such as coconut water gets contaminated through the absorption-transportation processes in plants from the roots to the leaves and fruits due to various forms of pesticides mishandlings and applications on moist farmlands (Lawal *et al*., 2018c). Consequently, these results in many health issues at a long run after human consumption. These include congenital disabilities, cardiovascular diseases and cancer risks in both adults and children (Lawal *et al*., 2018b). Therefore, these issues could be addressed objectively by routine determination of the concentration level of the targeted pesticide residues (analytes) in the fruit samples using advanced methods and instruments for better environmental and food safety (Lawal *et al*., 2016; Xu *et al*., 2012).

Scheme 1: Sample of coconut water

Moreover, this research focused on the analysis of multi-pesticide residues that include Dursban, Diazinon, Thiamethoxam, Metalaxyl, Thiobencarb, Baycarb, Carbaryl and Propamocarb (Fig. 1) in coconut water procured from the Kuala Lumpur (Malaysia) area (Fig. 2). The analysis is carried out using quick, easy, cheap, effective, rugged and safe (QuEChERS) with the dispersive solid phase extraction (SPE) coupled with 1-hexyl-3-methylimidazolium hexafluorophosphate ([C_6MIM][PF_6]) ionic liquid-based (IL-based) in dispersive liquid-liquid microextraction (DLLME) method. We hope that it will serve as a reference guide for the future studies of pesticides in food samples.



Dursban/Chlorpyrifos (Insecticide/Nematicide)

Diazinon (Insecticide)

Thiamethoxam (Insecticide)

Metalaxyl (Fungicide)

Thiobencarb/Benthiocarb (Herbicide)

Baycarb/ Fenobucarb (Insecticide)

Carbaryl (Insecticide/Nematicide)

Propamocarb (Fungicide)

Fig. 1: Structural formula of the analysed pesticide residues

Fig. 2: Map of study area

MATERIALS AND METHODS

Sampling and treatments

Ten coconut fruits were purchased from a local market located at Petaling Jaya (Kuala Lumpur), Malaysia. The 300 mL water mixture of the coconut fruits were refrigerated at 4 ºC. Meanwhile, the stock standard solutions (100 mg/kg) for the analysed pesticides were purchased from AccuStandard® (New Haven, USA). The standard solutions were further singly diluted from 100 to10 mg/kg and subsequently to 0.5 mg/kg (500 µg/kg), 0.4 mg/kg (400 µg/kg), 0.3 mg/kg (300 µg/kg), 0.2 mg/kg (200 µg/kg), 0.1 mg/kg (100 µg/kg) and 0.005 mg/kg (5 µg/kg) (working standard solutions) with the estimated volume of methanol and preserved (4 ºC).

Procedure for QuEChERS-dSPE coupled with IL-DLLME method

The QuEChERS-dSPE coupled with IL-DLLME method used by Lawal *et al*. (2018a) was adopted for the research. The developed QuEChERS extraction method started by transferring 20 mL of five parted portions of the coconut water sample into 50 mL centrifuge tubes. Each of the tube's content was spiked serially with the estimated volumes of working standard solutions (5, 100, 200, 300, 400 & 500 µg/kg) respectively, for the calibration curve and validation of the method. The tube was vortexed for 1 min after the addition of 15 mL acetonitrile. The sachet of ProElut™ AOAC 2007.01 QuEChERS extraction salt mixture (6 g $MgSO_4$ and 1.5 g NaOAc) was added and shaken vigorously for 1 min before a 2 min centrifugation (4000 rpm). Then, the d-SPE methodology was carried out after occupying 2 mL centrifuge tube with 1.5 mL supernatant from the QuEChERS extraction. Then, the cleanup salt mixture (150 mg $MgSO_4$ and 50 mg PSA) was added into the tube, vortexed for 30 sec and centrifuged for 5 min at 4000 rpm.

Subsequently, the IL-DLLME cleanup procedure took place by the transfer of 1 mL extract from the d-SPE cleanup into 15 mL centrifuge tube which contained 10% NaCl (w/v) in 9 mL of distilled water. The 130 µL of IL-based was added and centrifuged (7000 rpm) for 5 min. Then, 100 µL of the collected sedimental extract was mixed with 0.5 mL methanol in 2 mL HPLC auto-sampler vial that was vortexed for 1 min and finally, the extract was analysed using high-performance liquid chromatography-tandem mass spectrometry (Agilent G6490A LC-MS/MS) instrument. The method was repeated on the other tubes with different spiked concentration.

Similarly, the blank portion of the sample was analysed in triplicates to determine the concentration levels of analytes in the prepared sample solution from the calibration curves, individually.

Validation of the sample treatment method

The desirability, effectivity and certainty of a method can be expressed by the use of a specific validation approach (Abbott *et al*., 2010). However, the SANTE-11813 (2017) guideline was employed for the method validation that includes relative recovery (accuracies) (70-120%), precisions (repeatability) (≤ 20%) and linearity range with the coefficient of regression (R^2). The accuracies and precision [relative standard deviation (RSD)] were estimated by performing three repeatable (n = 3) extractions/cleanup of blanks spiked at three concentration levels (5, 100 and 300 µg/kg). The linearity for each of the targeted analytes was evaluated from their respective calibration curves at five concentration levels ranging from 5 – 500 µg/kg. In addition, the LOD and LOQ were estimated (equation 1) from the calibration curve based on the signal-to-noise (S/N) ratio corresponding to a factor of 3 and 10, respectively (Xiu-Ping *et al*., 2017).

$$\text{LOD or LOQ } (\mu\text{g/kg}) = \frac{F \times \text{STEYX}}{m} \qquad (1)$$

Where; F: LOD and LOQ are having factors of 3 and 10, respectively.
STEYX: standard error estimated on Microsoft Excel 2013.
m: Slope of the linear regression

Moreover, the matrix effects (equation 2) in the samples were estimated (Dias *et al*., 2016). The matrix effect indicates suppression (-20%) or enhancement (20%) of analyte recoveries.

$$\text{ME (\%)} = \left[\left(\frac{\text{Slope of a calibration curve for the analyte in a matrix}}{\text{Slope of a calibration curve for analyte standard solution in ACN}}\right) - 1\right] \times 100 \qquad (2)$$

LC-MS/MS Analysis

The LC-MS/MS instrument was used for the analysis of multi-residue of pesticides in the prepared analyte solution of coconut water. The analyses were carried out after good acquisition of the instrumental setups were attained such as stabilised gas flow, nebulizer, temperature and capillary voltage. However, the instrument was set up at 15% starting organic mobile phase B (ACN + 0.1% FA) coupled with mobile phase A (deionized H_2O + 0.1% FA + 1% ACN) pushed by a stream of nitrogen gas. The 5 µL analyte solution was injected, passed and runs through Supelco HPLC column [Ascentis® Express C_{18} (5 cm x 2.1 mm, 2.7 µm)] (Sigma-Aldrich, USA) at 30 ºC and the flow rate of 0.1 mL/min. The gradient path was estimated: 0 – 1.6 min, 15% B; 1.6 - 10.4 min, 15 – 100% B; 10.4 – 12.0 min, 15% B.

Notably, the total chromatographic peak areas (TCPA) or total ion chromatography (TIC) (Fig. 3) of the multiple reactions monitoring (MRM) scans (Fig. 4) obtained from LC-MS/MS analysis were used for the analysis of the multiple pesticides. It is because TCPA is an index that corresponds to the number of analytes present in analysed samples (Lawal *et al*., 2018a).

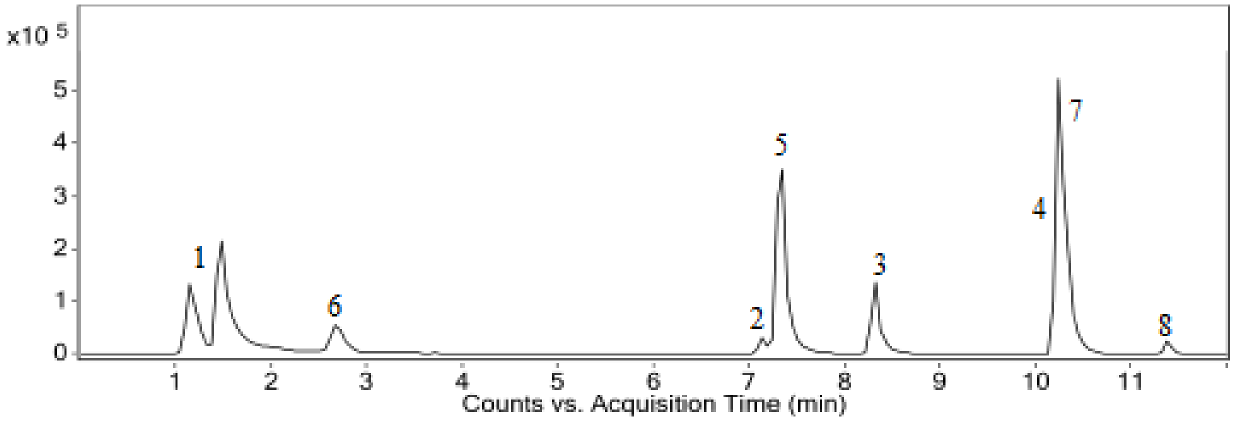


Fig. 3: Schematic presentation of total ion chromatography (TIC) chart for the multi-pesticide analytes

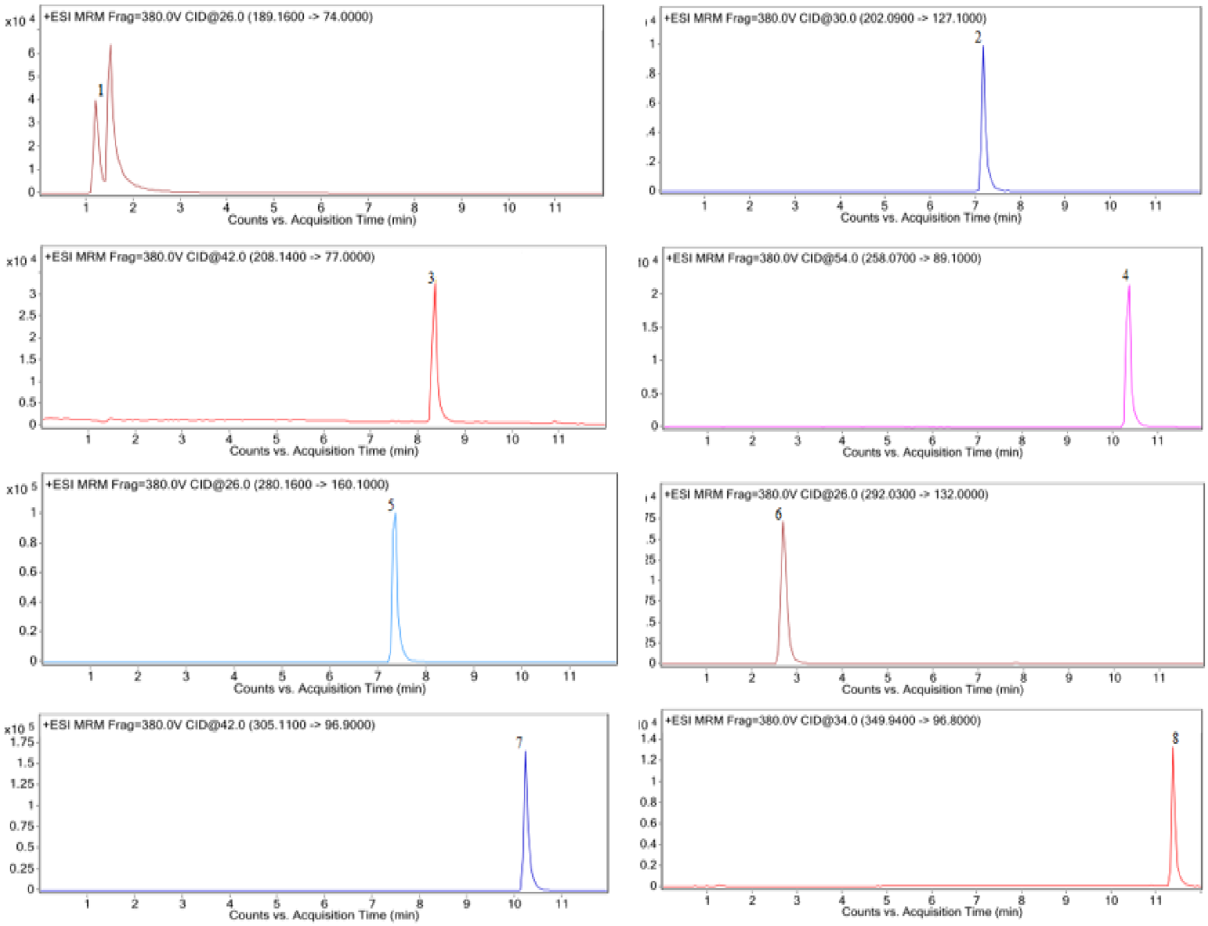


Fig. 4: Schematic presentation of the multiple reaction monitoring (MRM) chart for the multi-pesticide analytes

RESULTS AND DISCUSSION

Table 1 presented the relative recovery and precision results of the analysed residue of multi-pesticides in coconut-water. The 87.5 % of relative recovery range (84–294%) and 100% of RSD range (0–19%) at three spiked concentration levels were found within the guidelines of 70-120% and 0-20% respectively as recommended by SANTE-11813 (2017).

Table 1. Accuracy and precision measured at three concentration levels in the coconut water sample

Pesticides	Spike (µg/kg)	RR (%)	RSD (%)
Durban	5	87	10
	100	105	1
	300	102	3
Diazinon	5	84	15
	100	103	0
	300	99	2
Thiamethoxam	5	88	16
	100	129	1
	300	85	1
Metalaxyl	5	123	19
	100	99	5
	300	101	16
Thiobencarb	5	134	17
	100	96	7
	300	98	3
Baycarb	5	117	8
	100	103	1
	300	102	1
Carbaryl	5	89	6
	100	110	4
	300	95	11
Propamocarb	5	105	3
	100	99	10
	300	101	1
Ranges	5-300	84-134	0-19

RR, relative recoveries; RSD, relative standard deviation

The results are in the same range reported by Ferreira *et al.* (2016) for the analysis of pesticides in the sample of coconut water procured from Brazil. Furthermore, the precision results of the analytes in coconut water were satisfactory (≤ 20%) as recommended by SANTE-11813 (2017) and found within the documented range (Ferreira *et al.*, 2016).

Furthermore, the results of LOD & LOQ, regression coefficient, matrix effect, measurement uncertainty, as well as the detected analyte residues in coconut water are presented in Table 2.

Table 2. The quantitation & detection limits, regression coefficient, matrix effect, measurement uncertainty, detected residue and European Union maximum residue limits of analytes in the coconut water sample

Pesticides	LOD (µg/kg)	LOQ (µg/kg)	R^2	ME (%)	MU (%)	DR (µg/kg) ± RSD (%)	EU-MRL (µg/kg)
Dursban	0.29	0.96	0.9979	-72	9	112 ± 2	300
Diazinon	0.08	0.28	0.9998	-97	12	7 ± 2	20
Thiamethoxam	0.92	3.08	0.9790	-100	12	8 ± 2	10
Metalaxyl	0.26	0.88	0.9981	-99	27	19 ± 1	50
Thiobencarb	0.23	0.77	0.9980	-90	18	18 ± 4	20
Baycarb	0.17	0.56	0.9992	-98	7	< LOQ	10
Carbaryl	0.27	0.91	0.9981	-100	15	< LOQ	10
Propamocarb	0.09	0.31	0.9997	-100	9	< LOQ	10
Ranges	0.08-0.92	0.28-3.08	> 0.97	≤ -72	7-27		

LOD, limit of detection; LOQ, limit of quantitation; R^2, regression coefficient; ME, matrix effect; MU, measurement of uncertainty; DR, detected residue; ND, not detected; EU-MRL, European Union maximum residue limits

The ranges of the LOD (0.08-0.92 µg/kg) and LOQ (0.28-3.08 µg/kg) results were lower than the lowest calibration level (5 µg/kg) and the EU recommended maximum residue limits (MRLs) (EU, 2016). The linear results of the regression coefficient (R^2) for the linearity range are greater than 0.9 (0.9790 - 0.9998), which agrees with the documentation of Ferreira *et al*. (2015) for the determination of pesticides in coconut-trunk. The matrix effects of the sample is very weak (≤ –72%) and less effective in accordance to the guideline indicating suppression (-20%) or enhancement (+20%) of analyte recoveries. Reasonably, it is due to the extra-cleanup of matrix interferences performed by the treatment method. The MU was estimated at the range of 7-27% and it is acceptable because it's within the recommended range (≤ 50%). Ultimately, the concentration level of Dursban, Diazinon, Thiamethoxam, Metalaxyl and Thiobencarb in the unspike samples of coconut water were detected quantitatively and were found lower than the EU-MRL. While, the concentration level obtained for Baycarb, Carbaryl and Propamocarb were less than the LOQ. This could be as a result of the proper handlings and management of the pesticides during agricultural production of the farm produce.

CONCLUSIONS

The QuEChERS – dSPE – IL – based - DLLME technique successfully provided an optimum condition for the determination of multi-residue of pesticides in a sample of coconut water. Fortunately, the technique could maximally cleanup matrix interferences with less consumption of organic solvents which improve simplicity, rapidity, better detectability and selectivity of targeted analytes. Therefore, the sample preparation technique proved reliable and suitable for routine determination of multi-residue of pesticides in coconut waters. Also, this research justifies the safe consumption of coconut water sourced from the sampling area due to the level of analyte concentration found below that of the EU MRLs.

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