CHAPTER 4

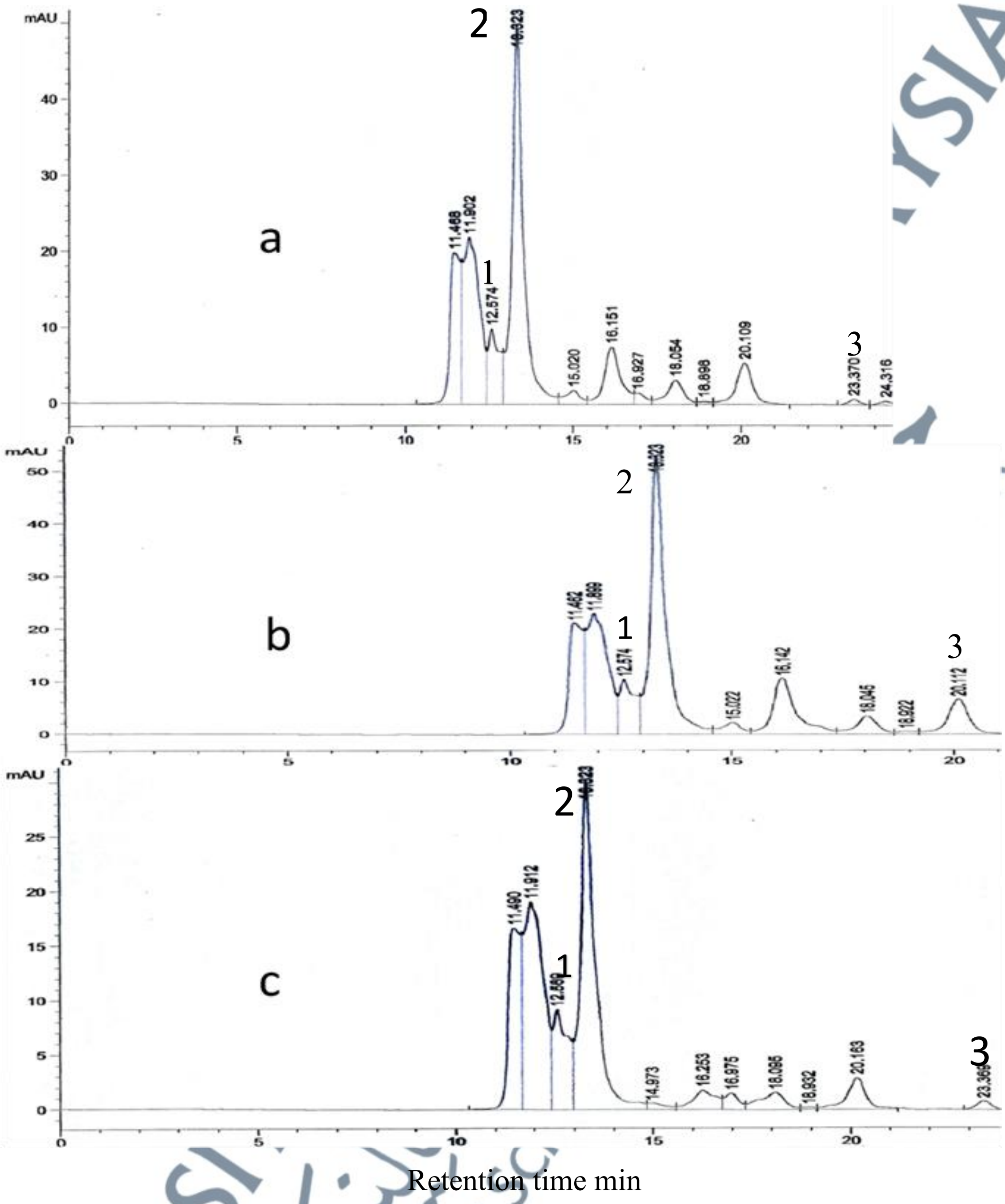
RESULTS AND DISCUSSION: OPTIMIZATION OF THE HPLC-DAD METHOD AND ACETATE BUFFERED METHOD FOR PESTICIDE EXTRACTION AND ANALYSIS

4.1 Optimization of HPLC Method for Pesticide Separation

To obtain the best resolution and peak purity for the separated pesticides, three important chromatographic factors were optimized such as, detection wavelength, mobile phase composition and flow rate.

4.1.1 Wavelength Optimization

The optimal wavelength for simultaneous determination of the three pesticides namely, dimethoate, carbendazim, and chlorpyrifos were determined using the standard pesticides solutions and isocratic elution as described in section 3.3.1.1. Detection wavelength was examined at 249, 254 and 270 nm while other chromatographic conditions, flow rate and mobile phase composition were kept constant. The wavelength of 254 nm showed the best chromatogram with less interferences and strongest absorption for each of the three tested pesticides while 249 and 270 nm showed very small intensities for chlorpyrifos thus they were not suitable for simultaneous determination of the three pesticides therefore, 254 nm was chosen as working wavelength. Results are shown in Figure 4.1.

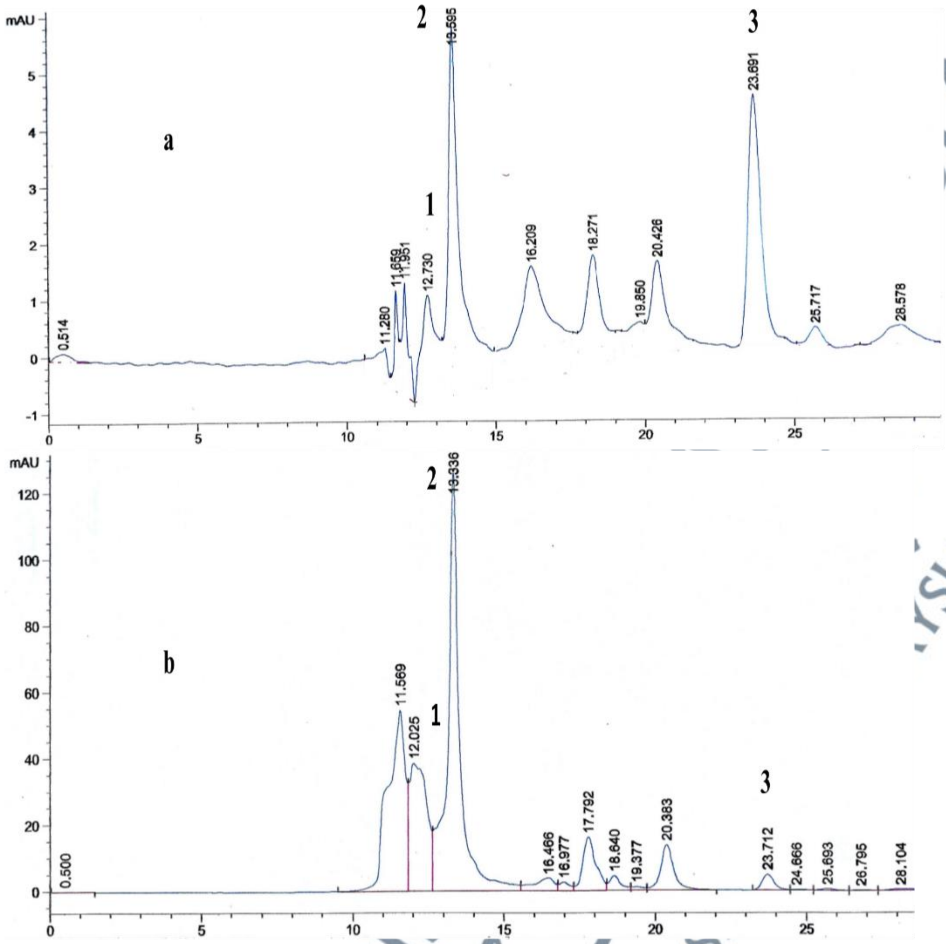


Note: 1) Dimethoate, (2) Carbendazim, (3) Chlorpyrifos

Figure 4.1: Chromatogram of Date Fruits Sample Spiked with 4 µg/mL Pesticide Standard Solution at Different Wave Lengths (a) 249 nm, (b) 254 nm and (c) 270 nm

4.1.2 Optimization of Mobile Phase Composition

To optimize the chromatographic separation, mobile phases with varied ratios of acetonitrile, methanol, and water with and without ammonium format ($HCOONH_4$) which commonly used as separation modifier were tested as described in section 3.3.1.2. Acetonitrile water systems were found not suitable mobile phase because of poor separation for the target compounds which may refer to its relatively low polarity. Isocratic elution of methanol water 95:5 with 5 mM ammonium formate improved the selectivity and was found suitable for separation of dimethoate, carbendazim and chlorpyrifos in standard and the sample extract with acceptable resolution as shown in result Figure 4.2. However, ionic mobile phase modifier working on coating of the alkyl-bonded surface of C18 stationary phases with the modifier cation form a weak bilayer that competes with the solute molecules and facilitates the resolution enhancements to improve peak profiles by decreasing the peak tailing, minimizing the band broadening, and reducing the analysis time (Garcıa-Alvarez-Coque et al., 2015).

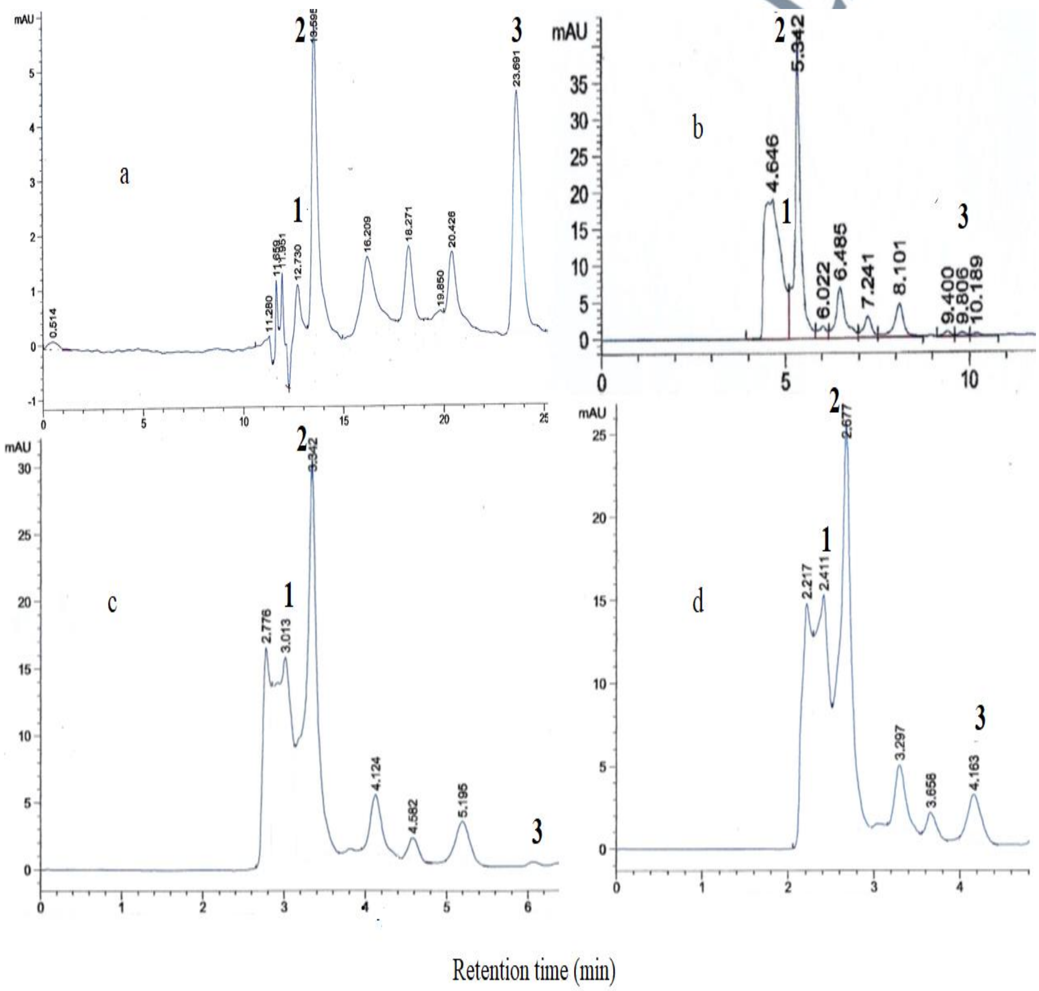


Note: 1) Dimethoate, (2) Carbendazim, (3) Chlorpyrifos

Figure 4.2: Chromatograms of Date Fruits Sample (Spiked) in Mobile Phase Methanol: Water (95:5) Mobile Phase (a) with 5 mM Ammonium Format and (b) without Ammonium Format

4.1.3 Flow Rate Optimization

The optimization of the flow rate was done by varying the flow rate at 1, 0.8 ,0.5 and 0.2 mL/min. while the other factors namely, (wavelength and mobile phase composition) were fixed. As shown in Figure 4.3, though increasing the flow rates significantly decrease the retention times of the pesticides, it negatively affects the resolution that causes bad separation for dimethoate and carbendazim at the flow rates of 1.0. 0.8 and 0.5 mL/min. The best resolution for the three examined pesticides was achieved with flow rate 0.2 mL/min. This can be explained by that, as the flow rate is decreased, the speed through the detection path decreases, the residence time of the analyte in the detection zoon increases, to give more chances for more photons to contribute to absorption. Following this fact, peak area will increase in proportion to the inverse of the flow rate (https://www.chromatographyonline.com).



Note: 1) Dimethoate, (2) Carbendazim, (3) Chlorpyrifos

Figure 4.3: Chromatograms of Spiked Date Samples Separation at Different Flow Rates. (a) flow rate 0.2 mL/min, (b) 0.5 mL/min, (c) 0.8 ml/min and (d) 1.0 ml/min

4.2 Validation of The Chromatographic Analysis

The developed HPLC- DAD method was validated via linearity, limit of detection (LOD), limit of quantification (LOQ) (sensitivity), accuracy, precision, and matrix effect.

4.2.1 Linearity of Calibration Curves

Three sets of mixed standards solutions of dimethoate, carbendazim and chlorpyrifos were prepared and used to build up three calibration curves for each of examined pesticides as described in section 3.3.2.1. The linear regression analysis of the peak area versus concentration were used to obtain the calibration equation and correlation coefficient (R^2) for each calibration curve. The linearity of detector response was confirmed linear between 0.02-10 µg/mL because of convenience values of the correlation coefficients (R^2) obtained from all calibration curves as shown in Figure 4.4 which ranged from 0.975 – 0.998 for dimethoate, 0.996-0.999 for carbendazim and 0.998-0.999 for chlorpyrifos. The obtained R^2 values for each pesticide indicated that the developed method was very suitable for simultaneous analysis and determination of dimethoate, carbendazim and chlorpyrifos residues in date fruits.

Table 4.1: Data of Linear Range of Pesticides Calibration Curves

Pesticide	Concentration range (µg/mL)		Concentration range (µg/mL)		Concentration range (µg/mL)	
	0.02 – 0.10	**R^2**	**0.2- 1.0**	**R^2**	**2-10**	**R^2**
Dimethoates	Y= 178.07X-0.1752	0.998	Y=24.682X+2.185	0.975	Y=11.142X+ 3.689	0.979
Carbendazim	Y=588.91X+ 0.7176	0.997	Y=326.11X-4.558	0.999	Y=183.27X-34.970	0.996
Chlorpyrifos	Y= 157.43X +0.1077	0.998	Y=39.364X- 0.041	0.999	Y=37.911X-2.972	0.999

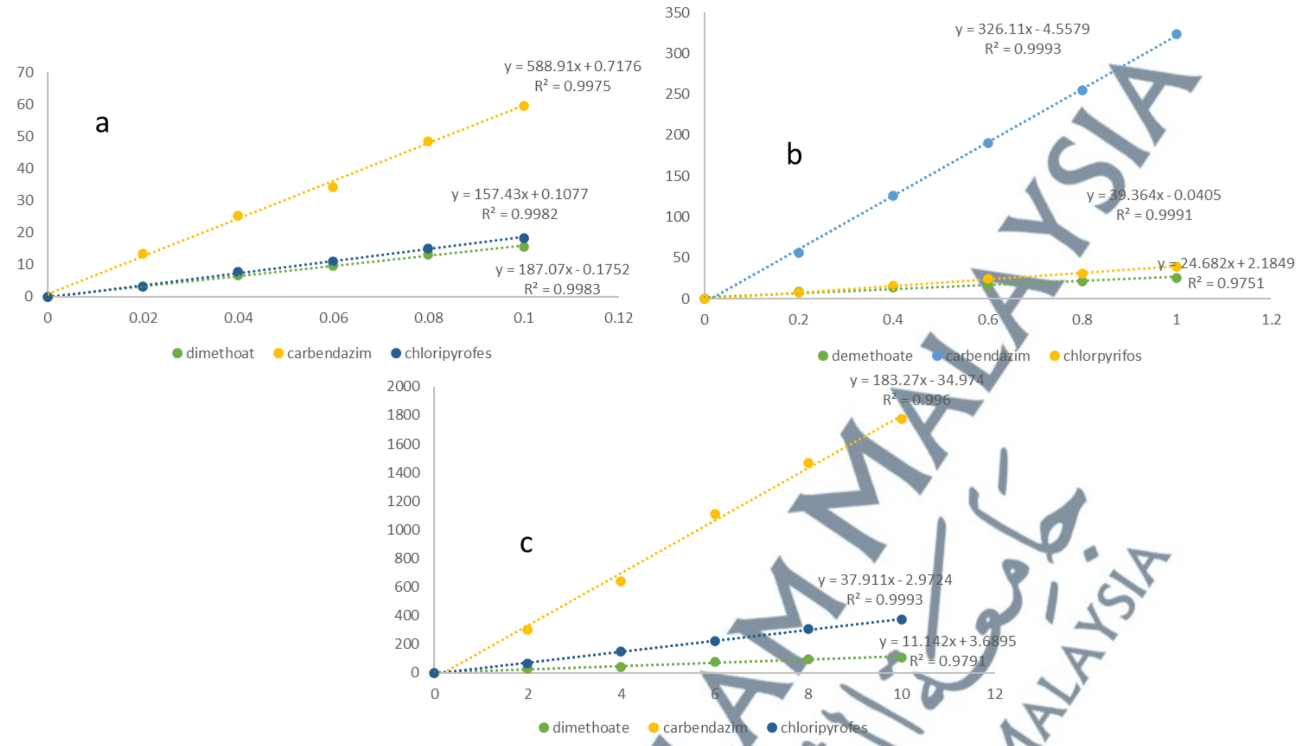


Figure 4.4: Calibration Curves of Linear Range (a) 0.02 -0.10, (b) 0.2-1.0 and (c) 2-10 µg/mL

4.2.2 Limit of Detection and Limit of Quantification

The sensitivity of HPLC- DAD method was determined from limit (LOD) of detection and limit of quantification (LOQ). Both LOD and LOQ were calculated from the calibration curves as following: LOD = 3.3*σ/S and LOQ = 10*σ/S where, σ is standard deviation of regression line and S is the slope the calibration curve. The limit of detection (LOD) for dimethoate, carbendazim and chlorpyrifos were 0.0065, 0.0073 and 0.0058 µg/mL, respectively. While the limit of quantification was found 0.0197 µg/mL (0.059 mg/kg), 0.022 µg/mL (0.066 mg/kg) and 0.0177 µg/mL (0.053 µg/kg) respectively. The obtained LOD and LOQ values for the tested pesticides are comparable to those reported by Fróes and Navickiene (2013) and correspond to the SANTE (2015) guideline, which states that the LOQ value must be equal to or less than the maximum residue limit (MRL) value defined for each analyte in a specific matrix.

4.2.3 Accuracy

The developed method's accuracy was determined by recovery study in which date samples were spiked with known concentrations of standard solutions, as detailed in Section 3.3.2.3. Unspiked samples (controls) were produced and analyzed in the same manner. Three standard doses of 0.05, 0.10, and 0.15 mg/kg were used in the recovery experiments. Table 4.2 summarizes the findings. Percent recovery varied between 98 and 102 percent with an RSD of 2.86–10.4% for dimethoate, 100.3 and 107 % with an RSD of 1.39–6.1 % for carbendazim, and 97.8–103% with an RSD of 1.2–4.5 % for chlorpyrifos. The obtained recoveries and RSDs show that the approach is suitable for evaluating the pesticides under investigation.

Table 4.2: Recovery Study of the Examined Pesticides

	Dimethoate			**Carbendazim**			**Chlorpyrifos**		
Amount added mg/kg	0.05	0. 1	0.15	0.05	0. 1	0.15	0.05	0. 1	0.15
Amount measured mg/kg ± SD	0.0506 ± 0.001	0.098 ± 0.0053	0.1503 ± 0.0043	0.0508 ± 0.0031	0.1071 ± 0.0042	0.1505 ± 0.0021	0.0489 ± 0.0022	0.0995 ± 0.0012	0.1497 ± 0.0031
Recovery (%)	101.2	98	102	101.6	107	100.3	97.8	99.5	100.3
(RSD)	10.4	5.4	2.86	6.1	3.91	1.39	4.5	1.2	2.07

4.2.4 Precision

Inter-day and intra-day precision were used to determine the suitability of the established method, as described in Section 3.3.2.4. One concentration from each pesticide (2 µg/mL) was spiked and evaluated five times on the same day and three days later. As stated in Table 4.3, the standard deviation RSD of the pesticides spiked was determined to be as follows: Inter-day RSD values for dimethoate ranged from 1.68 to 3.07 %, while intra-day RSD values were 2.02 ± 0.045 %. Inter-day RSD for carbendazim varied from 1.6 to 2.58%, but intra-day RSD was 2.013 ± 0.051. For chlorpyrifos, the RSD for inter-day measures varied from 1.5 to 2.17 %, while the RSD for intra-day was 2.57 %. The fact that the relative standard deviations (RSDs) were less than 4 % supported the established method's viability for the examined pesticide analysis (Green,1996).

Table 4.3: Intra-day and Inter-day Precision for Examined Pesticides

	Inter- day (n = 5)			Intra-day (n = 5)
Compound (µg/mL)	**Day 1**	**Day2**	**Day 3**	
Dimethoate 2 µg/mL	1.898 ± 0.055	2.015 ± 0.062	2.018 ± 0.043	2.02 ± 0.045
RSD (%)	2.28	3.07	1.68	2.22
Carbendazim 2 µg/mL	1.996 ± 0.032	2.011 ± 0.052	2.016 ± 0.033	2.013 ± 0.051
RSD (%)	1.6	2.58	1.63	2.53
Chlorpyrifos 2 µg/mL	1.979 ± 0.03	1.887 ± 0.041	1.876 ± 0.033	1.978 ± 0.051
RSD (%)	1.5	2.17	1.76	2.57

4.2.5 Matrix Effect (ME)

Matrix effect (Figure 4.5) was measured by the method reported by Abdallah et al., (2018) which described in Section 3.3.2.5. A calibration curve was used to determine the effect of the date fruits matrix on the detector response (suppression or enhancement) by comparing the slope of the solvent calibration curve (SCC) of each pesticide and the slope of the matrix-matched calibration curve (MMCC), which were both created using five concentration levels of the examined pesticides (0.5, 2, 4, 6, 8 µg/mL) using the equation (3.1).

The results in Figure 4.5 showed that during extraction step, one of the tested pesticides (Chlorpyrifos) had a low matrix effect of 1 %, and two pesticides dimethoate and carbendazim had a medium matrix effect of 36.7 % and 29 % respectively. Clean up stage showed strong ability on reduce the matrix effect to become, 0 %, 18 % and 11 % for chlorpyrifos, carbendazim and dimethoate, respectively. The matrix effect less than 20 % indicated that a clean-up step is very important and necessary for pesticide analysis in date fruits matrix to increase the degree of certainty. Interference problems in complex matrices such as date fruits are mostly due to hydrophilic co-extractives like sugars and phenolic acids, or hydrophobic co-extractives like amino acids, proteins, and lipids. When these compounds are present in the sample extract, they can generate the matrix effect which strongly influences the parameters such as accuracy, linearity, LOD, and LOQ, which are all critical factors that are assessed through the validation process (Lambert, 2004). The level of ME is usually variable and random. It strongly depends on the interactions between the analyte and the interfering co-elution (Cortese et al., 2020). A specific analyte may respond differently in different matrices, and the same matrix may affect each analyte differently. Most of the time, diluting the sample or reducing the injection volume does not provide satisfactory keys for overcoming the matric effect. Sample preparation and cleanup techniques are required to selectively eliminate or reduce co-extractive interferences (Cortese et al., 2020). To completely eliminate the matrices effect, a selective extraction should be planned and carried out (Kataoka, 2010).

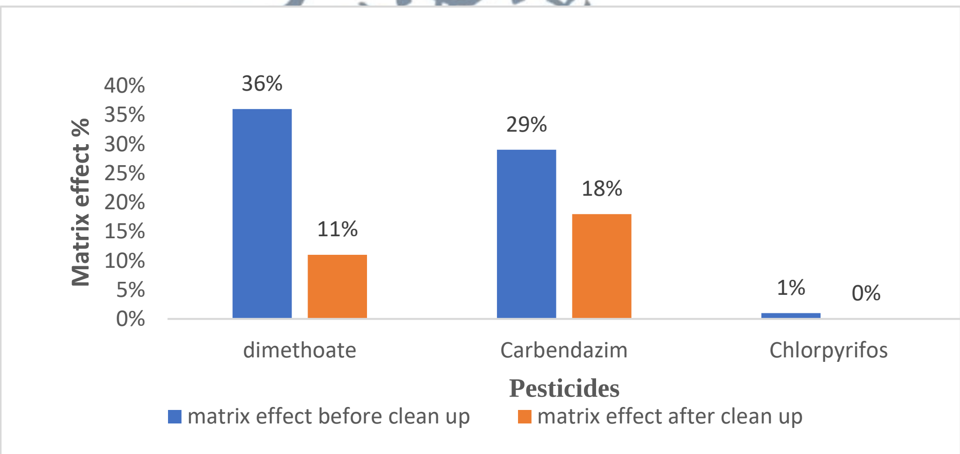


Figure 4.5: Matrix Effect Levels Before and After Clean-up. (ME) 0-2 Soft, 20-50 Medium and >50 Strong.

4.3 Optimization of Extraction and Clean-up of the Acetate Buffered QuEChERS Methodology

4.3.1 Screening Design for Method Described in Section

In this work, Plakett-Borman design (P-B design) was applied to screen the most significant factors that affect the separation and cleanup stages to improve efficiency and the recovery of the target pesticides as described in Section 3.2.1. As some pesticides are relatively strong acids and more stable at pH values of 3.5, it is important to maintain pH control in the solvent extraction. Moreover, highly polar pesticides, are soluble in aqueous solutions and less soluble in water-immiscible organic solvents, whereas others as carbendazim, dimethoate and chlorpyrifos are good soluble in most organic solvents (i.e., methanol, acetonitrile, and acetone). Therefore, the solvent extraction was examined to improve the recoveries. The main effect of each factor was investigated in 13 runs (12+ 1 center point), and recoveries of the examined pesticides from every experiment was calculated. The results are shown in Table 4.4. The experiment with the best recoveries and lower RSD was selected for analysis of real samples.

Table 4.4: Optimization of Extraction Clean-up Condition

Exp.No.	Acetic acid %	CH_3CN mL	H_2O mL	$MgSO_4$ mg	NaOAc mg	PSA mg	C18 mg	Chlorpyrifos R %	Carbendazim R %	Dimethoate R %	Mean R%	SD
1	0.75	15	5	4000	1000	50	50	96	85.8	84.04	88.61	6.45
2	1.25	15	5	6000	1000	20	20	86	35	27.86	49.61	31.70
3	1.25	15	10	4000	3000	50	20	73	41.96	31.59	48.85	21.54
4	1	12.5	7.5	5000	2000	35	35	163	99.4	99.4	120.6	36.72
5	1.25	15	5	6000	3000	20	50	142.8	84	91.14	105.9	32.08
6	0.75	15	10	6000	1000	50	50	101	102.4	104.2	102.5	1.58
7	1.25	10	10	6000	1000	50	20	132	43.15	58.6	77.94	47.45
8	0.75	10	5	4000	1000	20	20	108	35.7	42.62	62.10	39.89
9	0.75	10	10	6000	3000	20	50	108	128	126.0	120.7	11.02
10	0.75	10	5	6000	3000	50	20	104	65.5	50.19	73.23	27.72
11	0.75	15	10	4000	3000	20	20	82.8	25.3	35.66	47.92	30.66
12	1.25	10	5	4000	3000	50	50	208	91.7	101.8	133.8	64.41
13	1.25	10	10	4000	1000	20	50	92	129.7	114.4	112.0	18.96

From Table, experiment number 6 showed the best recoveries values for the examined pesticides as follow; dimethoate 104.2 %, carbendazim 102.4 % and chlorpyrifos 101 % with lower RSD (1.58) so, it was chosen as the extraction method for determination of dimethoate, carbendazim and chlorpyrifos in the real date fruits samples. For extraction step, 5.0 mL of 0.75 % (v/v) acetic acid in acetonitrile was added to 5 g of dates sample hydrated with 10mL water and homogenized in 50 mL centrifuge tube. The tubes were shaken for 1 min. Next, anhydrous magnesium sulphate $MgSO_4$ (6.0 g) and sodium acetate CH_3COONa (1.0 g) were added and vortexed for 2 min. Then samples were immediately centrifuged for 5min at 4500 rpm.

For clean-up: 1- 2 mL of the supernatant was put into another 15 ml centrifuge tube. For each 1 mL of extract, mixture of 50 mg of PSA, 50 mg C18 and 150 mg anhydrous $MgSO_4$ were added. The mixture was vortexed and then centrifuged for 5 min at 4500 rpm. Finally, 0.5 to 1.0 mL of the supernatant was filtered through 0.2 µm syringe filter and transferred to HPLC vials for analysis.

4.3.2 Comparison of the Efficiency the Modified Acetate Buffered QuEChERS and Original Unbuffered QuEChERS

The modified acetate buffered method described in Table 4.4 experimental run no.6 was compared with the original unbuffered method regarding the recoveries of the three examined pesticides. The results in Table 4.5 indicated that the modifies acetate buffered QuEChERS is superior to the original unbuffered method because of pH adjustment (5.25) which improved the distribution coefficients of the extract contents between water and organic layer according to their polarity. Acidic pesticides at low pH tend to separate in the organic phase, while basic extractives ionize at this pH and tend to separate in the water phase. This action decreases the matrix effect and improves the recoveries of pesticides. In the original unbuffered method (pH 8), acidic extractives would have a lower polarity and would separate in the organic phase rather than the water phase, increasing the matrix effect.

Table 4.5: Comparison of Pesticide Recoveries from Samples Extracted by Original Unbuffered QuEChERS and Modified Acetate Buffered QuEChERS

Pesticide	% Recovery (original unbuffered QuEChERS)	% Recovery (modified acetate buffered QuEChERS)
Dimethoate	38 %	96 %
Carbendazim	137 %	99.7 %
Chlorpyrifos	79.8 %	4.8 %

4.4 Quantification of Pesticides in Real Sample Using The Modified Buffered QuEChERS

The developed high-performance liquid chromatography with diode array detector (HPLC-DAD) method described in Sections 3.3-3.3.2.5 was used for simultaneous determination of dimethoate, carbendazim and chlorpyrifos in date fruit samples extracted by modified buffered QuEChERS extraction clean up methodology described in Table 3.3. HPLC-DAD chromatogram of date fruits sample and pesticide standards is shown in Figure 4.6. The calibration curves were built up from standard solution of the mixture of the three pesticides with concentration ranged from 0.5 – 10.0 μg/mL as shown in Figure 4.7, data Table A1 appendix1 were used for quantification of the examined pesticides. Result of pesticide residue analysis in 20 date fruit samples is shown in Table 4.6.

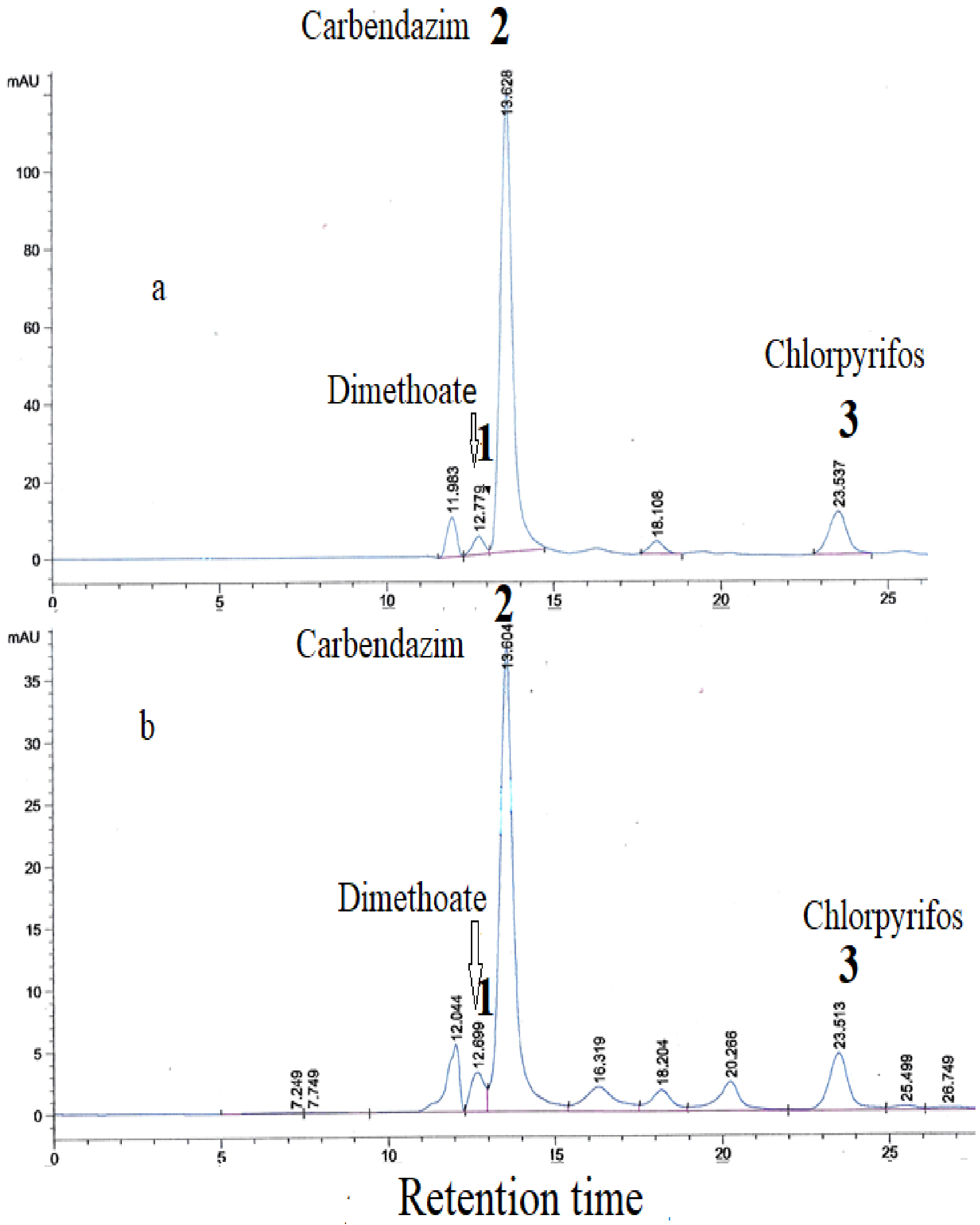


Figure 4.6: Chromatogram of Pesticide's Standards 4µg/ml (a) Spiked Date Fruit Sample (b) 1- Dimethoate (RT = 12.77) min, (2)-Carbendazim (RT = 13.62 min), and (3)- Chlorpyrifos (RT = 23.53 min)

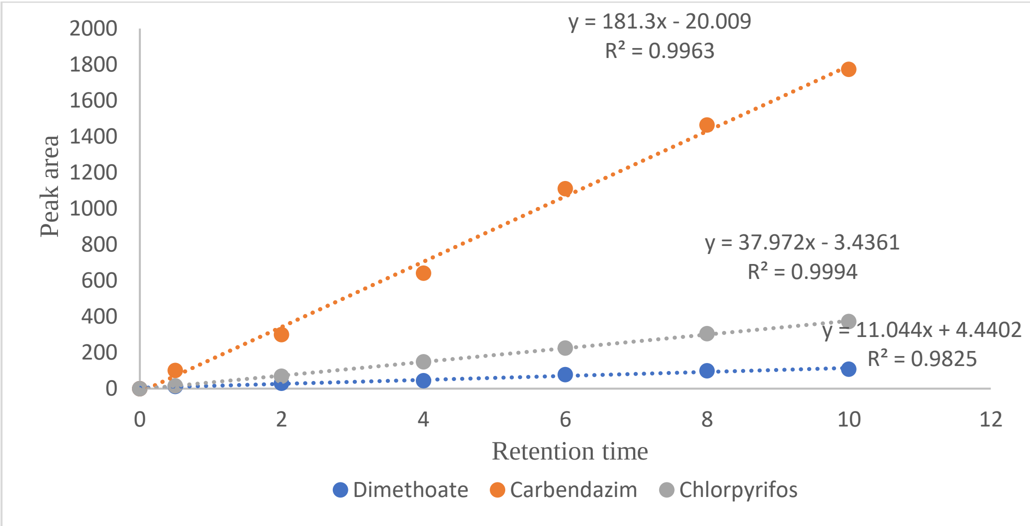


Figure 4.7: Calibration Curves for Determination of the Examined Pesticides in the Real Date Sample

Table 4.6: Result of Pesticide Residue Analysis of 20 Date Fruit Samples

EU MRL	**(0.01 mg/kg)**	**(0.1 mg/kg)**	**(0.01 mg/kg)**
Date's species	**Dimethoate**	**Carbendazim**	**Chlorpyrifos**
Ajwa	0.01594 ± 0.00014	0.05685± 0.0083	ND
Deglet Nour	0.01956 ± 0.00398	0.02850± 0. 0021	ND
Farad	ND	0.20270 ± 0.0016	0.1935 ± 0.0021
Khalas-Ihsa	ND	0.18201 ± 0.0035	1.5431± 0.0112
Khasouei	0.02009 ± 0.00272	0.15367 ± 0.0294	0.1635 0.0149
Khudri	ND	0.27870± 0.01230	0.9726 ± 0.0636
Lamry	ND	0.30750 ± 0.0255	0.5129 ±0 .0035
Lulu	0.01323 ± 0.0032	0.02730± 0.0140	ND
Mabroom	ND	0.20715 ± 0. 0380	0.6480 ± 0.0297
Mariami	0.01603 ± 0.0002	0.07910 ± 0.0032	ND
Mashrook	0.05307 ± 0.0012	0.31000 ± 0.0403	ND
Medjool	ND	0.10545 ±0 .01930	0.2061 ± 0.0038
Nomades (Promel)	0.01889 ± 0.0017	0.18930 ±0 .01655	ND
Palm dates	0.01644 ± 0.0013	0.32070 ± 0.0449	0.3090 ± 0 .03394
Rabi	ND	0.20840 ± 0. 01421	ND
Roshdi	0.01835 ± 0.0002	0.11160 ± 0.01655	0.5895 ± 0.0064
Safawi-1	0.01636 ± 0.0700	0.13760 ± 0.01548	0.1770 ± 0.0042
Safawi-2	0.01059 ± 0.0012	0.07820 ± 0.0134	ND
Zahdi-1	0.01616 ± 0.0012	0.014850± 0.0023	0.0324 ± 0.0009
Zahdi-2	0.01469 ± 0.0005	0.2109 0± 0.0462	0.0522 ± 0.0212

Table 4.6 shows the pesticides residue concentrations of dimethoate, carbendazim and chlorpyrifos in the examined imported dates fruits collected from Selangor stores. However, dimethoate was detected in 13 samples (65 %). The concentration ranged from 0.053 mg/kg to 0.0106 mg/kg. Dimethoate residue exceeding EU- MRLs (0.01 mg/kg) in most samples namely, Ajwa, Deglet Nour, Lulu, Mariami, Zahdi-1, Zahdi-2, Palm dates, Nomades (Promel), Safawi-1, Khasouei, Roshdi and Mashrook. Mashrook was exceeding dimethoate MRls of Gulf technical committee too. (0.05 mg/kg). While carbendazim was detected in all examined samples (20) with concentration ranged from 0.0148 to 0.321 mg/kg. 14 samples (70 %) exceeded EU-MRL (0.1 mg/kg) namely, Zahdi-2, Mabroom, Khudri, Lamry, Palm-dates, Nomades (Promel), Safawi-1, Khasouei, Khalas- Ihsa, Farad, Mashrook and Rabi, Roshdi and Medjool. Chlorpyrifos was detected in 12 sample (60 %) with concentration ranging from 0.0324 to 1.543 mg/kg, all of them are exceeding EU-MRL (0.01 mg/kg), namely, Zahdi-1, Medjool, Zahdi-2, Mabroom, Khudri, Lamry, Palm dates, Safawi-1, Khasouei, Khalas-Ihsa, Farad and Roshdi. Except Zahdi-1 sample, they are also exceeding chlorpyrifos MRls of Gulf technical committee for sector standards of food and agriculture products (0.05 mg/kg). Khalas-Ihsa from KSA showed the highest concentration of chlorpyrifos among the examined samples. However, the results agree with that reported by El-saeid et al., (2010) who reported that, dimethoate, chlorpyrifos, Lindan, and several acaricide residues in date fruit samples were above the maximum residue levels (MRLs), indicating a potentially hazardous trend in date palm agriculture. Furthermore, the study carried out by Abdallah et al., (2018) on 200 date sample (Var. Sukkari) from Al-Qassim, KSA reported, pesticide residues in 18 % of the examined samples,7.5 % of them exceeded the MRLs. HHI of carbendazim in this study that ranged from 8.4×10^{-4} to 1.8×10^{-1} is higher than that reported by Abdallah et al., 2018 (2.6×10^{-4}).

The presence of residue levels that are significantly higher than the present standards is attributable to soil contamination or drift applications from surrounding fields as well as diversion from Good Agriculture Practice (GAP) such as failure to observe pre-harvest intervals or failure to apply the recommended rate and concentration in accordance with GAP (Abdallah et al., 2018).

The obtained data of this study point to the need to implement recommendations regarding the use of the sanctioned pesticides. This could be accomplished through the education and licensing of farmers and pesticide applicators, with a particular emphasis on the licensing of high-risk pesticide application operations (Abdallah et al., 2018).

The pesticide chlorpyrifos was found to be the most frequently detected in both market and farm samples, according to a study conducted by Kumari and John (2019) on the main fruits and vegetables produced in the Western Himalayan region. There is a possibility that it has a larger concentration because of its persistence in food matrices and accumulation (Angioni et al., 2011).

4.5 Pesticide's Health Risk Assessment

Health risk assessment was calculated using the method reported by Kumari, & John, (2019) and described in section 3.5. Based on the mean concentration of pesticide residues in each induvial date fruit sample, a health hazard index was calculated. The predicted health hazard index (HHIs) associated with the examined pesticides residue in the 20 date fruit samples are presented in Table 4.7. Although the concentrations of examined pesticides in most of the samples are exceeding European Commission maximum residue limits (EU-MRLs) of examined pesticides, there was no health risk associated with any of the studied pesticides as no samples exceeded the acceptable HHI limit (<1) for adults or children. Mashrook sample from KSA exerted the highest

HHI values of dimethoate (0.04 and 0.09) for adults and children, respectively. While the lowest HHI of dimethoate was found in Safawi-2 from KSA (0.009 and 0.018) for adults and children, respectively. The highest HHI values of carbendazim was in Palam dates sample (0.018 and 0.036) for adults and children, respectively, while the lowest HHI value of carbendazim was found in Safawi sample from KSA which ranged from 0.0008 to 0.0017 for adults and children, respectively. Chlorpyrifos showed the highest HHI values among the three examined pesticides, (0.2572 and 0.5144) in Khalas-Ihsa from KSA and (0.0087 and 0.0174) in Zohdi-1from UAE for adults and children, respectively. Therefore, it can be assumed that children are more susceptible to health risk due to pesticide consumption through food. However, according to the findings of the study conducted by El-seaid et al. (2010) on three cultivars of date fruits and their seeds purchased from the KSA Riyadh markets regarding the residues of lindane, dieldrin, dimethoate, chlorpyrifos, and some acaricide, were found higher than the maximum residue limits (MRLs), indicating a potentially hazardous trend in date palm cultivation.

The present findings provide critical information about the current contamination status of date fruits on the Malaysian market, as well as the actions that must be taken to prevent the overuse of pesticides in the production of date fruits.

4.6 Conclusion

HPLC- DAD analysis method was developed for simultaneous determination of dimethoate, carbendazim and chlorpyrifos residues in date fruits using column Agilent Zorbax XDB C18 5um 4.6 x 250 5-µm. Mobile phase: 95 % MeOH + 5 mM ammonium formate and 5 % H_2O + 5 mM ammonium formate. Injection volume 20 µL and flow rate 0.2 mL/min. The HPLC method validation was done via the following parameters intra-day and inter-day (precision), recovery (accuracy), sensitivity (LOD and LOQ) and linearity. The obtained results indicated the effectiveness of the developed method for analysis and quantification of the examined pesticides in date fruits. Extraction and cleanup of date fruit samples was carried out using a modified acetate buffered QuEChERS method which was found to be more superior when compared with the original unbuffered QuEChERS regarding the obtained pesticide recoveries (Table 4.5). The importance of the clean-up step was confirmed via matrix effect study which showed significant reduction in matrix effect measured before and after cleanup step (Figure 4.6). The targeted pesticides were simultaneously determined in 20 date fruit samples collected from Malaysian stores.

The peak of dimethoate were observed at retention time 12.79 min and identified by comparison with the peak of the authentic sample (standard) which appears at 12.77 min (Fig 4.6). The dimethoate calibration curve equation (Fig 4.7) was Y = 37.972X – 3.436. The correlation coefficient of linear calibration was 0.9994. The method was validated by determining the following analytical and statistical parameters; the linearity of the detector response was confirmed between 0.02 - 10 µg/mL (Table 4.1). The limit of detection and the limit of quantification were found 0.0065 µg/mL and 0.0197 µg/mL (0.0591 mg/kg), respectively. Accuracy of the method was confirmed by a recovery experiment (Table 4.2). Compared to the theoretical mounts (spiked) of 0.05, 0.10 and 0.150 mg/kg, the obtained recovery rates were 101.2 % (RSD 10.4 %), 98 % (RSD 5.4 %) and 102 % (RSD 2.86 %) respectively. Intra and inter-day variation of the assay was determined and the resulting RSD ranging from 1.68 to 2.28 % confirmed the precision of the method (Table 4.3). Dimethoate was detected in 13 sample (65%). The quantity was ranged between 0.01059 - 0.053 mg/kg of fresh weight (Table 4.6). dimethoate residues in 13 (65 %) sample were exceeding EU- MRLs.

The peak of carbendazim was at retention time 13.596 min and it was identified by comparison with the peak of the authentic sample (standard) which appears at 13.63 min (Figure 4.6). The calibration curve equation (Figure 4.7) was Y = 181.3X – 20. 009. The correlation coefficient of linear calibration was 0.9963. The method was validated by determining the following analytical and statistical parameters; the linearity of the detector response was confirmed between 0.02 - 10 µg/mL. The limit of detection and the limit of quantification (LOQ) were found 0.0073 µg/mL and 0.022 µg/mL (0.066 mg/kg), respectively. Accuracy of the method was confirmed by a recovery experiment (Table 4.2). Compared to the theoretical mounts (spiked) of 0.05, 0.10 and 0.150 mg/kg, the obtained recovery rates were 101.6 % (RSD 6.1 %), 107 % (RSD 3.91%) and 100.3 % (RSD 1.39 %) respectively. Intra and inter-day variation of the assay was determined and the resulting RSD ranging from 1.6 to 2.53 % confirmed the precision of the method (Table 4.3). Carbendazim was detected in all examined date fruit samples. The quantity was ranged between 0.01485 - 0.3207 mg/kg of fresh weight (Table 4.4). Carbendazim exceeded EU-MRL in 14 samples (70 %).

The peak of Chlorpyrifos was at retention time 23.53 min and it was identified by comparison with the peak of the authentic sample (standard) which appears at 23.51 min (Fig 4.6). The calibration curve equation (Figure 4.7) was Y = 11.044X + 4. 4402. The correlation coefficient of linear calibration was 0.983. The method was validated by determining the following analytical and statistical parameters; the linearity of the detector response was confirmed between 0.02 - 10 µg/mL. The limit of detection and the limit of quantification were found 0.0058 µg/mL and 0.0177 µg/mL (0.053 mg/kg), respectively. Accuracy of the method was confirmed by a recovery experiment (Table 4.2). Compared to the theoretical amounts (spiked) of 0.05, 0.10 and 0.150 mg/kg, the obtained recovery rates of chlorpyrifos were 97.8 % (RSD 4.5%), 99.5 % (RSD 1.2 %)

and 103 % (RSD 2.07 %) respectively. Intra and inter-day variation of the assay was determined and the resulting RSD ranging from 1.5 to 2.57 % confirmed the precision of the method (Table 4.3). Chlorpyrifos was detected in 12 sample (60 %). The quantity was ranged between 0.0324 - 1.543 mg/kg of fresh weight (Table 4.6). Chlorpyrifos residues were exceeding EU-MRL in 12 sample.

The mean concentration of pesticide residues in each induvial date fruit sample was used to calculate the level of health risk associated with the fruit consumption. Predicted health hazard indexes (HHIs) for the evaluated pesticide residues in the 20 date fruit samples suggested that there was no health risk associated with any of the studied pesticides, since all HHI values were determined to be less than (1) for either adults or children (Table 4.7). However, as pesticide residues found in most of the investigated samples was exceeding the EU-MRLS threshold, it is obvious that continual monitoring of pesticide residues in imported date fruits is necessary to minimize the pesticide daily intake levels from food and drinks.

Table 4.7: Risk Assessment of the Pesticides in each 20 Date Fruits Sample Built Up on Consumption Rate of (100g) A Day and Average Body Weight 60 kg for Adults and 30 kg for Children

Dates	**Dimethoate mg/kg**	**60Kg and 30kg BW for 100g/day consumption rate**		**EDI/ADI (ADI= 0.002)**		**Carbendazim mg/kg**	**60Kg and 30kg BW for 100g/day consumption rate**		**EDI/ADI (ADI= 0.03)**	
		EDI adult	**EDI child**	**HHI adult**	**HHI child**		**EDI adult**	**EDI child**	**HHI adult**	**HHI child**
Ajwa	0.01594	2.66E-05	5.31E-05	0.013	0.026	0.05685	9.48E-05	1.9 E-04	0.0032	0.0063
Deglet Nour	0.01956	3.26E-05	6.52E-05	0.016	0.033	0.0285	4.75E-05	9.5 E-05	0.0016	0.0031
Frad	ND	ND	ND	ND	ND	0.20269	3.38E-04	6.76 E-04	0.011	0.023
Khalas-Ihsa	ND	ND	ND	ND	ND	0.18201	3.03E-04	6.07 E-04	0.01	0.02
Khasouei	0.02009	3.35E-05	6.70E-05	0.017	0.033	0.15366	2.561 E-04	5.12 E-04	0.008	0.017
Khudri	ND	ND	ND	ND	ND	0.2787	4.645 E-04	9.29 E-04	0.015	0.03
Lamry	ND	ND	ND	ND	ND	0.3075	5.125 E-04	1.03E+03	0.017	0.034
Lulu	0.01323	2.21E-05	4.41E-05	0.011	0.022	0.0273	4.55 E-05	9.1 E-05	0.0015	0.003
Mabroom	ND	ND	ND	ND	ND	0.20715	3.45E-04	6.91E-05	0.012	0.023
Mariami	0.01603	2.67E-05	5.34E-05	0.013	0.027	0.07905	1.32E-04	2.64E-04	0.0044	0.0087
Mashrook	0.05307	8.85E-05	1.77E-05	0.04	0.09	0.31	5.17E-04	1.03E-03	0.017	0.0344
Medjool	ND	ND	ND	ND	ND	0.10545	1.757 E-04	3.52E-04	0.0058	0.012
Nomades (Promel)	0.01889	3.15E-05	6.30E-05	0.016	0.031	0.1893	3.155 E-04	6.31 E-04	0.01	0.021
Palm dates	0.01644	2.74E-05	5.48E-05	0.014	0.027	0.3207	5.345 E-04	1.07E-03	0.018	0.036
Rabi	ND	ND	ND	ND	ND	0.20835	3.47E-05	6.95 E-04	0.012	0.0232
Roshdi	0.01835	3.06E-05	6.12E-05	0.015	0.03	0.1116	1.86 E-04	3.72 E-04	0.0062	0.0124
Safawi-1	0.01636	2.73E-05	5.45E-05	0.014	0.027	0.13755	2.292 E-04	4.59 E-04	0.0076	0.015
Safawi-2	0.01059	2.00E-05	0.00004	0.009	0.018	0.07815	1.30E-05	2.61 E-04	0.0043	0.0086
Zahdi -1	0.01616	2.69E-05	5.39E-05	0.013	0.027	0.01485	2.475 E-05	4.95E-05	0.00083	0.0017
Zahdi. -2	0.01469	2.45E-05	4.90E-05	0.012	0.024	0.2109	3.515 E-04	7.03E-04	0.012	0.0234
Average	0.0125	2.08E-05	4.157E-05	0.01	0.02	0.1736	0.00027	0.00054	0.00892	0.0178

Table 4.7, continued

Dates	Chlorpyrifos mg/g	60 Kg and 30kg BW for 100g/day consumption rate		EDI/ADI (ADI= 0.01)	
		EDI Adult	EDI child	HHI adult	HHI child
Ajwa	ND	ND	ND	ND	ND
Deglet Nour	ND	ND	ND	ND	ND
Frad	0.1935	3.225 E-04	6.45 E-04	0.03225	0.0645
Khalas-Ihsa	1.543	2.6 E-03	5.14 E-04	0.257175	0.51435
Khasouei	0.1635	2.73 E-04	5.45 E-04	0.02725	0.0545
Khudri	0.9726	1.621 E-03	3.242 E-04	0.1621	0.3242
Lamry	0.51294	8.549 E-04	1.7098 E-03	0.08549	0.17098
Lulu	ND	ND	ND	ND	ND
Mabroom	0.648	1.08 E-03	2.16 E-03	0.108	0.216
Mariami	ND	ND	ND	ND	ND
Mashrook	ND	ND	ND	ND	ND
Medjool	0.2061	3.44 E-04	6.87 E-04	0.03435	0.0687
Nomades (Promel)	0	0	ND	ND	ND
Palm dates	0.309	5.15 E-04	1.03 E-03	0.0515	0.103
Rabi	ND	ND	ND	ND	ND
Roshdi	0.5895	9.83 E-04	1.97 E-03	0.09825	0.1965
Safawi-1	0.177	2.95 E-04	5.9 E-04	0.0295	0.059
Safawi-2	ND	ND	ND	ND	ND
Zahdi -1	0.0324	5.4 E-05	1.08E-04	0.0054	0.0108
Zahdi -2	0.0522	8.7 E-05	1.74 E-04	0.0087	0.0174
Average concentration	0.23	0.00045	0.0009	0.045	0.09

Note: EDI (estimated daily intake), ADI (acceptable daily intake), HHI (health hazard index), ND (not detected).