

CHAPTER 4

CONCLUSIONS

The simultaneous extraction was achieved to transfer of dimethoate, carbaryl and fenvalerate which came from different pesticide class in orange sample peels by using a mixture of ethyl acetate-acetone-ethanol in ratio of 1:1:1 (v/v) with sonication time of 15 minutes. After that the SPE C18 clean-up step is applied for sample extract to protect the chromatographic system from matrix which caused the performance of the chromatographic separation to deteriorate rapidly or impair the sensitivity of the UV detector that as limited selectivity. In SPE procedure acetone or deionized water was used as dissolved solvent. It can be noticed that most of dimethoate and carbaryl was not retained on C18 sorbent when using acetone as dissolved solvent. Thus deionized water was used as dissolved solvent in this step. The unadsorbed dimethoate using deionized water as diluent resulted from the hydrophilic structure or due to relatively polar thus dimethoate was preferred to solubility in deionized water than retained on C18 sorbent whereas carbaryl and fenvalerate were retained. Since dimethoate was not retained on column as mentioned earlier, the fraction was collected immediately after loaded the sample extract. The elution of carbaryl adsorbed on C18 sorbent was mostly eluted with a mixture of acetone-water (7:3, v/v) because the van der Waals force between C18 sorbent and carbaryl was lower than dipolar attraction and/or hydrogen bonding between carbaryl and acetone. At the same reason, fenvalerate was also almost eluted with acetonitrile. It is noteworthy that using SPE method is superior to conventional liquid-

liquid extraction (LLE) in terms of low solvent consumption and reducing analysis time. The unspiked sample was also extracted following the same extraction procedure to eliminate the effect of matrix interference and to obtain true value of detector response from standard spiking. In order to obtain the most accurate results in pesticide analysis, the fortification for orange peels should make as near as possible in sample but the suggestion for less sensitivity compound like dimethoate in HPLC detection should be used at a high level of concentration or over in concentration in sample being analyzed. Due to lack in sensitivity by HPLC technique, the dimethoate was also determined by GC-FPD to investigate the optimum extracting solvent and sonication time. The GC obtained results were also considered together with HPLC results and their results were in the same trend. However the GC-FPD is recommended for dimethoate analysis because it is selective for compound containing phosphorus or sulfur atom thus most of the sample matrix could not response. In addition, by using HPLC, the dimethoate pesticide might lose during extraction procedure it would be better analyze as its nature of volatile property.

The chromatographic separation was performed on reverse phase C18 column using a mobile phase of methanol-water (1:9, v/v) and methanol-water (9:1, v/v) in solvent A and solvent B, respectively, and both of solvents were contained 5 mM of ammonium acetate buffer with a constant flow rate of 0.3 mL min⁻¹. The total analysis time was 80 minutes and then returned to initial conditions in 10 minutes for another analysis run. The gradient conditions for pesticides analysis followed as separation mode (Table 2.3). The retention time (t_R) of dimethoate, carbaryl and fenvalerate were approximately 14, 22

and 63, respectively. Although the background of pesticide was cleaner with C18-SPE cartridge than crude extraction, the presence of co-extractives still disturbed the pesticides determination after clean-up step using in HPLC but did not in the MS detection without clean-up step. In the other word, despite the incomplete recoveries of HPLC, the LC/MS without clean-up step was encouraging. The LC/MS, an efficiency technique, provides higher accuracy, less matrix interferences, reducing of extraction time and more powerful sensitivity than HPLC for quantitative determination of dimethoate, carbaryl and fenvalerate pesticide residues in complex matrices that as orange peels. Moreover the advantages of LC/MS are eliminating sample clean-up step leading to rapid, efficient sample preparation and economics saving. The molecular ion $[M+H]^+$ appeared at m/z 202 and 230 were carbaryl and dimethoate, respectively, in both of standard solution and sample. The ammonium adducted ion $[M+NH_4]^+$ of fenvalerate appeared at m/z 437 in sample meanwhile in standard solution fenvalerate shown clearly the $[M+H]^+$ at m/z 421.

By using SPE-HPLC, the lowest detectable concentrations are 0.20, 0.0051 and 0.00020 $mg L^{-1}$ for dimethoate, carbaryl and fenvalerate, respectively, which are not in agreements with Miller-Miller method (Table 3.14) owing to fluctuation in signal from each injection. The calibration curve of dimethoate, carbaryl and fenvalerate are in the range of 0.13 – 1.0, 0.24 – 1.2 and 0.20 – 1.2 $mg L^{-1}$, respectively. The linear regression equation ($y = ax + b$) and the correlation coefficients (R^2) obtained were $y = 170.99x - 17.67$ with $R^2 = 0.9965$ for dimethoate, $y = 3967.52x - 181.98$ with $R^2 = 0.9973$ for carbaryl and $y = 272.55x + 13.99$ with $R^2 = 0.9980$ for fenvalerate. The repeatability

with relative standard deviation (R.S.D) ranged from 0.63 - 4.19% (n = 8) and from 0.57 - 4.90% for the reproducibility (n = 8, 6 days). The recoveries using orange sample peels spiked with 50, 15 and 20 mg L⁻¹ of dimethoate, carbaryl and fenvalerate were 28.0, 54.7 and 31.0, respectively. In addition the low percentage of recovery obtained might be from the effect of different diluents used in the calibration curve (methanol) and in the sample (a mixture of deionized water, acetone and acetonitrile), but this effect did not observe in the LC/MS method.

By using LC/MS without clean-up step, the limits of detection (LODs) are 0.50, 0.030 and 0.20 mg L⁻¹ for dimethoate, carbaryl and fenvalerate, respectively, which are not in agreements with Miller-Miller method (Table 3.27) owing to fluctuation in signal from each injection. The calibration curve of dimethoate, carbaryl and fenvalerate are in the range of 1.0 – 5.0, 0.30 – 1.5 and 1.2 – 2.8 mg L⁻¹, respectively. The linear regression equations obtained were $y = 71,449.20x + 17,744.40$ with $R^2 = 0.9994$ for dimethoate, $y = 660,357.00x + 2,311.90$ with $R^2 = 0.9984$ for carbaryl and $y = 104,062.50x - 5,052.20$ with $R^2 = 0.9980$ for fenvalerate. The repeatability with relative standard deviation (R.S.D) ranged from 3.52 - 4.60% (n = 8) and from 1.55 - 4.60% for the reproducibility (n = 8, 2 days). The recoveries of dimethoate, carbaryl and fenvalerate were 69, 71 and 105, respectively, which are approximately in the range of 70 - 120% meeting EU guidelines method. According to the results obtained from unspiked orange sample peels expressed invalid values as it reported not detected under the optimum conditions, thus there was no need to subsequently investigate the real samples. That is fortunately

indicated none of dimethoate, carbaryl and fenvalerate pesticide residues remaining on Tangerine (*Citrus reticulata blanco*) leading to a safe consumption in Thailand.



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