CHAPTER 4

DISCUSSION AND CONCLUSIONS

4.1 Discussion

Preparation of standard solution is one of the important step in this analysis. Normally, stock solutions are prepared in a very high level of standard substance. For stock solution of cypermethrin and fenvalerate, 1000 mg/L are a typical stock solution. In this work, 1000 mg/L solution of insecticides were prepared because it was easy to weigh such a weight by an available analytical balance and it could be kept for quite a long time at 4 °C without significant changes in measurement of insecticide levels. Preparation of working standard solutions by successive dilution is a simple method in which available glassware is used. However, it has a disadvantage in losing the standard especially when several dilution steps are involved.

GC analysis was carried out as primary screening method and for insecticides residue quantitation. Using GC-ECD for this analysis, it was necessary to try to find suitable conditions for running. Results shown that GC-ECD and with a 30.0 m × 320 μm I.D., 0.25 μm nominal film thickness capillary column coated with cross linked HP-5 5% phenyl methyl siloxane was used. The column temperature was programmed as follows: the initial temperature of 100 °C was increased at a rate of 8 °C/min up to 180 °C and held 3 min, from 180 to 260 °C at a rate of 10 °C/min was used and held for 3 min, then to 300 °C at a rate of 6 °C/min and held 5 min. The temperature of injector and detector were maintained at 250 °C and 300 °C respectively. Helium was used as a carrier gas at a flow rate of 1.6 mL/min, and kept

at constant flow and the injections were made in the splitless mode. The extract was injected and the peak area was compared (sum of the peak areas of all isomers of cypermethrin and fenvalerate insecticides) to that of the calibration standards to determine the residue quantitatively. Under these conditions, the retention time of cypermethrin was 28.186, 28.379, 28.529, 28.603 minutes and retention time of fenvalerate was 30.025, 30.453 minutes. The identification of these compounds in the cabbage samples has been done by comparing the retention time of the suspected peak in the sample chromatogram with the retention of standard compounds.

In the extraction step prior to analysis, it was found that the extraction of pyrethroid insecticides are rather simple compared to the organophousphate and carbamate insecticides because they formed a deposit on the surface of the leaf after the treatment. The methods include sampling procedure, extraction, clean-up (the filter was used as a clean up to remove interferences in the sample extract before determination using GC-ECD) and determination of cypermethrin and fenvalerate insecticide residues by GC-ECD. For extraction step, a method from Department of Agriculture, Ministry of Agriculture and Cooperatives using acetone and methylenechloride as solvent for extraction has been modified to solvent mixture of acetone, *n*-hexane and ethyl acetate to reduce expenses, toxicity and to improve extraction efficiency. Acetone is miscible in plant materials, is the usual approach of most multiresidue methods. However, acetone will also extract many interfering compounds from the sample matrix. Ethyl acetate is not completely miscible with water, no extra partition steps after extraction are needed remove water. Addition of sodium chloride as an ionic strength modifier was necessary to improve recovery of less hydrophobic compounds. The extracted samples were filtered through anhydrous

sodium sulfate to remove moisture content. Accuracy of the extraction method was calculated through the recovery of each insecticide. The recoveries using cypermethrin and fenvalerate insecticides fortified cabbage samples at levels of 0.5 and 3.0 mg/L are summarized in Table 3.4. The fortification levels used in this study were selected because they covered the ranges of cypermethrin and fenvalerate levels set by the MRLs. Results shown that recoveries for the acetone, *n*-hexane and ethyl acetate (1:1:1, v/v/v) solvent system, ranging from 93-103% for cypermethrin and 83-91% for fenvalerate. Percent recoveries of extraction depends on the ratio of the solvent to sample volume and the extractant.

A validation of the method was performed in terms of percent recovery, detection limit, linearity and precision of the method. All of the parameters were needed as a part of an evaluation of the instrument efficiency. Satisfactory precision values in terms of %RSD of retention time was in the range of 0.0134-0.0152%. The linearity ranges for cypermethrin and fenvalerate were studied in the ranges of 0.3-3.0 mg/L. Table 3.6 and 3.7 shown the linear concentration ranges investigated and linearity curves are presented in Figure 3.4 and 3.5. The correlation coefficients of these insecticides were 0.9929 and 0.9982 respectively. The detection limit values were 0.064 mg/L for cypermethrin and 0.061 mg/L for fenvalerate.

The sample study in this work was cabbage available in the Muang Mai market of Chiang Mai province. The level of cypermethrin and fenvalerate insecticides in cabbage samples depends on the concentration of nature precursor and insecticide residues in cabbage samples, including the condition of reaction. In this experiment, cypermethrin and fenvalerate residues were detected in cabbage samples resulted in no potential risk to consumers because level of residues were lower than the MRLs

established by Thailand legislation. The amounts of cypermethrin and fenvalerate found in cabbage samples were in the range from 0.086 to 0.221 mg/kg and 0.093 to 0.201 mg/kg respectively. There was no great potential risk for human health and an average man could take this contaminated cabbage without harmful effect to him. Low levels of cypermethrin and fenvalerate residues were detected in the cabbage sample resulted in no potential risk for human heath after cabbage consumption. It can be suggested that the consumers who seek to reduce their exposure to insecticides can do by choosing food which low level residues.

In the desorption experimemts, cypermethrin and fenvalerate insecticide residues in the cabbage samples were determined directly after spraying at 350 mg/L and 100 mg/L. A comparision of rate of desorption at 28 °C and 90 °C for cypermethrin and fenvalerate (Table 4.1 and 4.2) shown that rate of desorption of cypermethrin increased with increasing temperature, whereas the rate of desorption of fenvalerate,on the other hand, decreased with increasing temperature. Washing the cabbage, after applying the insecticides, with water and with washing liquid solution, resulted in the maximum amounts of 11% and 12% of cypermethrin desorbed at 28 °C, 13% and 22% of cypermethrin desorbed at 90 °C, 24% and 20% of fenvalerate desorbed at 28 °C, and 21% and 18% of fenvalerate desorbed at 90 °C, respectively. The data indicated that washing by washing liquid solution was more effective than that by water for the elimination of insecticide residues. Moreover, the rate of desorption of cabbage for cypermethrin and fenvalerate respectively were 3.0×10^{-4} and 2.0×10^{-4} $mg.kg^{-1}.h^{-1/2}$ in water and 4.0×10^{-4} and 3.0×10^{-4} $mg.kg^{-1}.h^{-1/2}$ in washing liquid solution at 28 °C. The rate of desorption of cabbage for cypermethrin and fenvalerate were respectively

8.0×10^{-4} and 6.0×10^{-4} $mg.kg^{-1}.h^{-1/2}$ in water and 1.2×10^{-3} and 3.0×10^{-5} $mg.kg^{-1}.h^{-1/2}$ in washing liquid solution at 90 °C. Desorption rates were also calculate from the graphs plotted between square root of time ($h^{1/2}$) and desorbed concentration. It is highly recommended for the consumers to decrease the amount of insecticides remaining as much as possible such as thoroughly washing vegetables

Table 4.1 Desorption study of cypermethrin and fenvalerate insecticides at room temperature (28 °C)

Data	Cypermethrin		Fenvalerate	
	Water	Washing liquid	Water	Washing liquid
Initial concentration (mg/kg)	64.5	59.4	23.6	38.5
Maximum of amounts of insecticide desorbed (%)	11	12	24	20
Rate of desorption ($mg.kg^{-1}.h^{-1/2}$)	3.0×10^{-4}	4.0×10^{-4}	2.0×10^{-4}	3.0×10^{-4}

Table 4.2 Desorption study of cypermethrin and fenvalerate insecticides at 90 °C

Data	Cypermethrin		Fenvalerate	
	Water	Washing liquid	Water	Washing liquid
Initial concentration (mg/kg)	41.1	38.4	55.3	47.8
Maximum of amounts of insecticide desorbed (%)	13	22	21	18
Rate of desorption ($mg.kg^{-1}.h^{-1/2}$)	8.0×10^{-4}	1.2×10^{-3}	6.0×10^{-4}	3.0×10^{-5}

4.2 Conclusions

In this research, it can be concluded that GC-ECD provided good responses, sensitive, specific detector systems, ability to separate mixture of analytes on the column, fast analysis and high efficient.

For optimization the gas chromatographic conditions for cypermethrin and fenvalerate study, gas chromatograph Agilent 6890N series equipped with ECD, HP-5 capillary column, 5% phenyl methylsiloxane, 30 m. × 320 μm. × 0.25 μm., was used. Under the optimum conditions employed, the results shown that the cypermethrin has been four isomers which the retention times of four isomers were 28.186, 28.379, 28.529 and 28.603 min, respectively and the retention times of two isomers were 30.025 and 30.453 min for fenvalerate insecticide. The retention time and peak area precision expressed in terms of the relative standard deviation (RSD) were between 0.0137-0.0144% for cypermethrin and 0.0135-0.0152% for fenvalerate. The detection limits were investigated using five replicate injections of the lower concentration of insecticides, which are shown in Table 3.5. The detection limits for cypermethrin and fenvalerate were found to be 0.064 and 0.061 mg/L, respectively. Regarding linearity, linear calibration curves for cypermethirn and fenvalerate insecticides over seven calibration levels, from 0.3 to 3.0 mg/L were constructed. The calibration curves were linear over the whole concentration tested for insecticides with correlation coefficients (R^2) ranging between 0.9929 for cypermethrin and 0.9982 for fenvalerate. The ratio of solvent mixture of acetone, *n*-hexane and ethyl acetate at 1:1:1, v/v/v was appropriate.

In the analysis of cabbage samples were purchased from of Chiang Mai province, the accuracy of quantitative analysis for cypermethrin and fenvalerate in the cabbage

samples were improved using the spiked recovery method. The ten sources of the cabbage samples, two sources were found to contain cypermethrin in the range of 0.086-0.221 mg/kg and five sources were found to contain fenvalerate in the range of 0.093-0.201 mg/kg. According to the Thailand legislation, the MRLs of cypermathrin and fenvalerate for cabbages are 1 and 3 mg/kg respectively. Therefore, the values for cabbage samples were below than the MRLs. The use of cypermethrin and fenvalerate in cabbage samples may be due to its greater photostability and relatively low toxicity as compared with organochlorine and organophosephate insecticides. In order to minimize health risk as well as for enforcement activities, monitoring of insecticide residues is increasingly important and essential.

For desorption experiments were carrier out to evaluate the cypermethrin and fenvalerate insecticides residue levels in cabbage in the process of preparation by washing with water and washing liquid solution. In this experiments were repeated at two different temperatures, from which thermodynamic data were obtained. Figures 3.8-3.9 and Tables 4.1-4.2 shown that the rate of desorption increase with increase in temperature as indicated by the first order modeling curves. In case of fenvalerate insecticide, it was found that the rate of desorption decrease with increase in temperature. Results shown that washing by in water and in washing liquid solution for cooking are necessary to decrease the concentration of insecticide residues in cabbage. From the results, it should be recommended that cabbages should be washed with washing liquid solution before eating and even raw eating.

THE RELEVANCE OF THE RESEARCH WORK TO THAILAND

Pyrethroid insecticide is one of insecticide residues. They had been widely used in agriculture and it was effective in the control of pests and diseases. They become an important group of contaminants in the environment. In Thailand, pyrethroid insecticide had been used in the process of cabbage plantation and they might be residue in cabbage. In this work, focuses on cypermethrin and fenvalerate residues in cabbages will be studied. Within the Thai agricultural commodity and food standard, MRLs established for cypermethrin and fenvalerate in cabbages are 1 and 3 mg/kg, respectively. An analysis of pyrethroid insecticides in cabbage samples has been developed by first extracting cabbage samples with acetone, n-hexane and ethyl acetate and then both qualitatively and quantitatively analysed by GC employing the ECD system. The great advantages of ECD are its sensitivity, selectivity and ease to use.

The aims of this research to determine pyrethroid insecticide residues of cypermethrin and fenvalerate in cabbage using GC-ECD and study desorption of these insecticide residues from cabbage. The cabbage samples (*Brassica oleracea* L. cv. white headed cabbage cruciferae) were purchased from a local fresh market of Chiang Mai, Thailand. It was found that cypermethrin and fenvalerate residues were found in any cabbage samples resulted in no potential risk to consumers because level of residues were lower than the MRLs. For desoprtion study, it was found that rate of desorption of cypermethrin was higher than rate of desorption of fenvalerate.