4. DISCUSSION AND CONCLUSIONS

4.1 Discussion

Analysis of carbaryl in treated vegetables in this work was conducted by RP-HPLC employing 50 % (v/v) acetonitrile/water as mobile phase. This kind of pesticide was extracted from the vegetable matrices by using solid phase extraction accomplished on an octadecyl cartridge, where carbaryl was eluted with acetonitrile/water.

In order to know the amounts of carbaryl found in vegetable samples on each day during the analysis, it was necessary to construct a calibration curve for each concentration range. Table 3.1 lists peak heights and peak areas obtained at various concentrations of carbaryl. As shown in Fig.3.1, a straight line was obtained for the concentration range from 0.04 μg/ml to 2 μg/ml; the straight line has the linear equation of $C = 0.1967 + 7.15 \times 10^{-5}$ where C represents the concentration of carbaryl standard in μg/ml and R is the peak area response in microvolt×second. In order to estimate how well the experimental points fit a straight line, the calculation of the product-moment correlation coefficient was performed. This statistics is often referred to simply as the correlation coefficient because in quantitative sciences it is by far the most commonly used type of correlation coefficient. The value of r is given by [31]

$$r = \frac{\sum_i \{(x_i - \bar{x}) \times (y_i - \bar{y})\}}{\sqrt{\{[\sum_i (x_i - \bar{x})^2] \times [\sum_i (y_i - \bar{y})^2]\}}} \quad \text{---------- (4.1)}$$

where x_i = the value of variable x for i = 1, 2, 3,..., n

$\bar{x}$ = mean value of x

y_i = the value of variable y for i = 1, 2, 3,..., n

$\bar{y}$ = mean value of y

Σ = summation

Close study of this equation shows that r can take values in the range $-1 \leq r \leq 1$. An r value of -1 describes perfect negative correlation, i.e. all the experimental points lie on a straight line of negative slope. Similarly, when r=+1 it is the perfectly positive correlation, all the points lying exactly on a straight line of positive slope. When there is no correlation between x and y, the value of r is zero. In analytical practice, calibration curves giving numerical r values of greater than 0.99, and r values of less than about 0.90 are relatively uncommon [31]. It can be seen that the calibration curve for carbaryl standard from 0.04-2 ppm yielded the correlation coefficient, r, equal to 0.9998 which is relatively very good indeed.

The calibration curves for the other two ranges of concentration, i.e., 1-5μg/ml and 5-30μg/ml, as derived from data in Tables 3.2 and 3.3 as shown in Fig. 3.2 and Fig. 3.3, respectively, were found to be similar to the preceding one. The correlation coefficients found were 0.9998 and 0.9994, respectively.

The chromatograms of standard carbaryl at 0.4 μg/ml, 2 μg/ml and 10 μg/ml obtained with the μBondaPak C_{18} column employing 50% (v/v) acetonitrile/water as mobile phase are shown in Figs. 3.4-3.6. As the detector response is proportional to the analyte concentration, the carbaryl peaks in these chromatograms have different peak areas. However, all these carbaryl peaks were found to have the retention time at 4.80 ±0.05 minutes.

The investigation of optimization in HPLC attempted in this work also involved the determination of the optimum wavelength of the UV absorption. Wavelengths in the range of 200-300 nm were examined for the UV absorption of carbaryl. The results shown in Tables 3.4 and 3.5 and Figs.3.7 and 3.8, revealed that the highest UV absorption for carbaryl of both concentrations (0.2 and 5 μg/ml) was at 220 nm of which the UV absorption was more than ten times greater than the second highest UV absorption at 280 nm.

On the basis of the quantitative analysis obtained by constructing calibration curves, the linearity response of UV detector was examined for the carbaryl pesticide. The results of these are shown in Table 3.6 and Fig. 3.9. It was found that the linearity cut-off of the detector for carbaryl was at 120 μg/ml corresponding to 2.4 μg per injection. It should be noted here, however, that the detector linearity has not been a prime problem in this research project because it was found that the amounts of carbaryl in the vegetables samples were in the range of ND-0.43 μg per injection. The results of the investigation on the detection limit and the lower limit of determination of carbaryl given in Table 3.7 and Fig 3.10 indicate that the minimum detectable amount of carbaryl under the investigated

conditions was 0.8 ppb while the lower limit of determination was 1.4 ppb.

The efficiency of a chromatographic column as a separation device improves as the number of theoretical plates increases. Thus, the number of theoretical plates, N, is used as a measure of column efficiency. A second term, the height equivalent to a theoretical plate, H, also serves this purpose. The relationship between these two parameters is N = L/H, where L is the length of the column packing.

In general, H decreases as the efficiency of a column becomes greater. That is, as H becomes smaller, the number of equilibrations that occur in a given length of column becomes larger. It is important to note that H and N are retained as efficiency parameters in the rate theory. It should be appreciated, however, that a plate as a physical entity does not exist in a column. Thus, the plate and the plate height should be viewed as criteria for column efficiency. In this work, the calculated N and HETP values for the C_{18}-μBondaPak are given in Tables 3.8 and 3.9 and Fig. 3.11 illustrates terms used in the calculation of column efficiency. It can be seen that the average theoretical plate was 4265 and the HETP was 0.070 mm. In addition, the results of the optimal chromatographic conditions are tabulated in Table 3.10.

The air temperatures during the sample collections in Ban Sop Pao, Lamphun Province and in Ban Pa Sao, Chiang Mai Province were recorded in an attempt to find some criteria to compare the degradation of carbaryl at the two study sites. The temperature range was 24.5-31.6 °C in Ban Sop Pao, Lamphun

Province and that in Ban Pa Sao, Chiang Mai Province, was 29.0-34.0 °C. The average temperatures in these two sites was 28.4 and 31.6 °C, respectively.

One of the most significant aspects in quantitative analysis is to state the reliability of results which is expressed in terms of accuracy and precision. For accuracy, the recovery studies were carried out at two different fortification levels, namely 1.0 and 5.0 ppm, for both kinds of vegetables, as shown in Tables 3.13 and 3.14. The recovery rates obtained from these two concentration levels were found to be different. The recovery ranged from 91.4 to 97.1% and the average percent recovery was found to be 93.5% while the coefficient of variation was 1.8% at 1.0 ppm concentration level for both kinds of vegetable whereas at 5.0 ppm, the percent recoveries were found to have varied between 80.3 and 89.2% with the average value of 84.2% and with coefficient of variation of 2.5%. Two explanations could be given to these results. Firstly, the SPE column capacity probably became a limiting factor for retaining additional analyte. If other compounds in the sample were retained by the SPE column, it would decrease the capacity of retaining carbaryl. Secondly, the elution volume could have some contributing effect, e.g. the elution volume might not be adequate to elute the retaining substances [32]. The precision or reproducibility of results is described in terms of relative standard deviation (RSD or Cv%), as shown in Tables 3.15, 3.16 and Fig. 3.12. It was satisfactorily found that the RSD of carbaryl injected into the HPLC was 0.4% and 1.1% for 2 μg/ml and 5 μg/ml of carbaryl concentration, respectively.

By combining data from Tables 3.13-3.18, the coefficients of variation (Cv) for HPLC injection, recovery test and sample analysis were found to range from

0.8 to 9.0%, as shown in Table 4.1. These appear to be good repeatible values for a residue analysis method, especially considering these values were obtained from samples analyzed on different days. Of course, the best coefficients of variation were from direct injection which was expected since there were fewer steps for analysis, when compared with recovery test and sample analysis. Most of the errors could have arisen from the sampling method in general because it is quite difficult to obtain an exact mixture replica of the authentic product and to maintain this in homogeneity. In the sample analysis, including the sampling method, extraction of the sample and HPLC injection, the possibility to obtain a greater value of Cv existed as several experimental steps were involved.

Table 4.1 Coefficients of variation of the carbaryl analysis at different experimental steps.

Experimental steps	Cv%
HPLC Operation	0.8
Recovery Test	2.2
Sample Analysis	9.0

The carbaryl analyte was extracted from chopped vegetables which had been sprayed with the carbaryl pesticide as described in Section 2.14. Due to the unstable nature, it is very difficult for carbaryl to penetrate into the tissues of the plant. Most pesticide stays on the surface of plants. This is one reason why it was not necessary to use a blender to blend the vegetable to small pieces in this work. The other reason was due to the interference of chlorophyll in analysis. If the

pesticide was extracted from small pieces of vegetable, the extraction would involve more chlorophyll rather unnecessarily because seperation of carbaryl from chlorophyll is generally a very difficult task.

The amounts of carbaryl found in vegetables are shown in Tables 3.17 and 3.18. As might be expected, no carbaryl was detected in the samples collected before spraying. The highest amount of carbaryl found was from the samples collected on the day directly after spraying. For kale samples, the carbaryl amounts were discovered to have dropped drastically one day after spraying (Fig.3.13). The edible rape samples yielded a similar pattern but to a lesser extent (Fig.3.14). Gradual decrease could be seen three days after spraying for kale samples whereas such a decrease was observed 8 days after spraying for the edible rape samples. Factors which could influence the amounts of carbaryl could be due to watering the crops and rainfall since the solubility of carbaryl in water is 40 ppm at 30 °C [2]. However, when considering the results of the kale samples analysis on the last day analysis, the coefficient of variation was found to be higher than that obtained earlier which probably resulted from the fact that lower concentrations could contribute greater errors in the precision and accuracy during the analysis.

Attempts were made to establish a relationship between the amount of carbaryl remaining and time. The only linear relationship was obtained when a plot of ln[carbaryl] versus time was made, as shown in Fig.4.1, which can be seen as a first order plot. The analysis on degradation rate of carbaryl was hence performed by using the first order rate equation as follows [33- 35] :

$$\ln (N/N_o) = - \lambda t$$

where N is the amount of carbaryl at time t,

N_o is the amount of carbaryl at the zero time.

Since $t_{1/2} = 0.693/\lambda$,

$$\ln (N/N_o) = - (0.693t/t_{1/2})$$

or $$t_{1/2} = -0.693t/ (\ln(N/N_o))$$

Having done the calculation according to the above expression, the average half-life for carbaryl degradation was found to be 1.84 days, suggesting that this kind of pesticide could quickly decompose in the environment and thus belong to the class of short-lived pesticides [3]. However, research findings during the last few years have shown that carbaryl has possible chronic toxic effects. In fact, the United States Department of Agriculture has recently changed the status of carbaryl to a higher risk category because of its possible chronic toxic effects and its wide distribution [4].

The maximum residue limits (MRL) for carbaryl in cabbage and other vegetables in Germany since 1989 is 3.0 mg/kg, which is still lower than the amounts of this pesticide found in the vegetable samples during 5 days after application. In Thailand, the MRL for carbaryl is 10.0 mg/kg. If a 60 kg man went to one of the sampling sites and took 1 kg of the vegetable on the day directly after spraying without washing to consume within 1 day, he would consume the amount of carbaryl of about 35 mg/60 kg weight, corresponding to 0.58 mg/kg/day; this is a very high amount when compared with 0.01 mg/kg body weight of the ADI value. According to the property of carbaryl, it was responsible

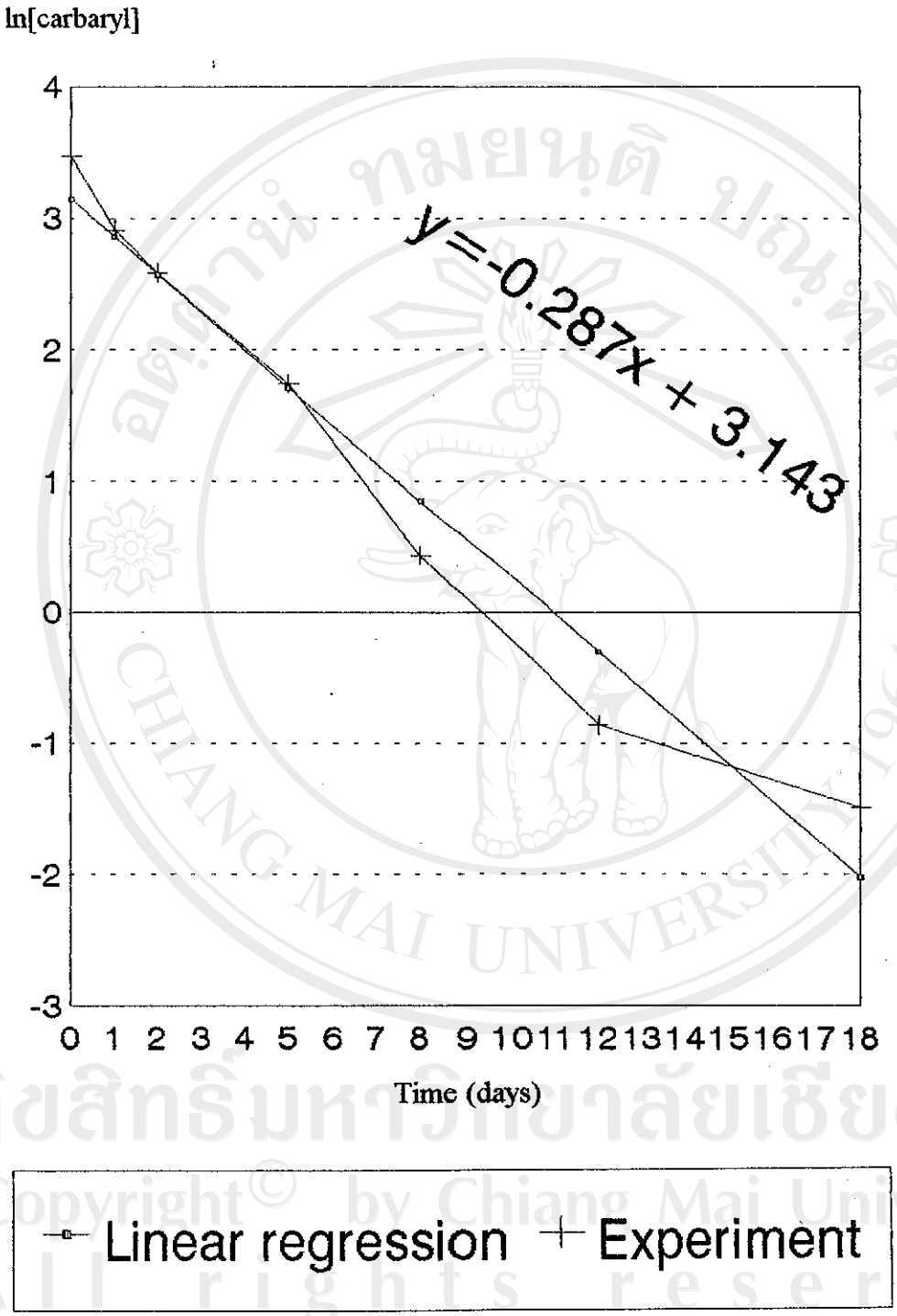

Fig.4.1 First order plot between ln[carbaryl] and time.

for a number of cases of poisoning, but no deaths have been reported yet. However, this would depend on the original concentration consumed also. Illness as a result of carbaryl poisoning involved abdominal pain, vomiting, headache, and blurred vision. There was one case study of accidental exposure to carbaryl dust and vapor to seven workers . The symptoms were nausea, dizziness and headache which had an average urinary 1-naphthol concentration of 13.2 mg/liter and with a range of 2-31 mg/liter. Seven other exposed employees were asymptomatic, with higher urinary 1-naphthol levels averaged to be 22.4 mg/liter with a range of 10 - 42 mg/liter [6].

In other cases of carbaryl poisoning described by the World Health Organization , spontaneous recovery was complete within several hours of exposure [6].

Normally, farmers will harvest their crops 3-4 days after application of the pesticide. If they still water their crops 1 day or 2 days after spraying, the concentration of carbaryl found 3 days after spraying should be decreased rapidly from their original concentration. On the other hand, if farmers do not do any watering on their crops after application, the amounts of carbaryl found in the crops harvested 2-5 days after spraying will be very high when compared with the established value of MRL. In such a hypothetical situation, the consumer would face the higher risk in terms of possible chronic toxic effects.

Moreover, the author also attempted an investigation to study the effect of washing and non-washing of spiked vegetables in a laboratory of the Chemistry

Department of Chiang Mai University. After washing, it was found that the amounts of carbaryl still left in vegetables were about 10 ppm whereas the carbaryl concentration of the unwashed vegetable sample was 104 ppm. This simply means that only 90 % of this pesticide could be removed with water.

There has been a report of a case study by the Toxic Substances Subdivision, Division of Agriculture, revealing that washing guava with potassium permanganate solution could wash out 82.36 % of carbaryl while washing with the solution of sodium chloride could remove only 64.60 % of carbaryl. Washing guava with detergent left 40% of carbaryl in the fruit investigated [36]. Table 4.2 lists various methods of decreasing the amount of carbaryl in guava.

For the fruits and vegetables sold on markets, most of them could have some pesticide remaining. It is therefore highly recommended for consumers to decrease the amount of the pesticides remaining as much as possible.

It should be noted that the percentage of carbaryl sprayed on the surface of the vegetables did not account for 100 % application. On the day directly after application, the amounts of carbaryl found on the soil surface was 0.18 mg/kg [37] while 2 days and 4 days after carbaryl spraying (with the farmers watering their crops and rain fall in the night time) the amounts of carbaryl found were higher which were equal to 0.35 and 0.65 mg/kg, respectively.

Table 4.2 Methods of removing carbaryl remaining in guava and their efficiency [36].

Method	Efficiency (%decrease)
running water	55.48
soaking with water	68.81
washing from uncooked rice	71.20
vinegar	67.33
$Ca(OH)_2$ solution	81.81
$KMnO_4$ solution	82.36
NaCl solution	64.60
vegetable washing detergent	63.20

The confirmation method was achieved on the basis of comparison of the detector response at 2 different wavelengths, i.e. 220 and 270 nm, as shown in Fig. 3.16 and Table 3.19. The peak area ratios at these two different wavelengths (270/220) were all 0.076 for different concentrations of standard carbaryl (Cv% = 0.8%) whereas, the area ratios of 270/220 for samples were found to range from 0.076-0.080 and the average value was 0.078, giving the Cv% equal to 1.9%.

4.2 Conclusions

The aims of this research project, as stated in Section 1.5, have been to find the amounts of carbaryl in vegetables at different time intervals after the carbaryl application in order to observe the degradation rate or half life of this pesticide

under the natural conditions and to look into other parameters that could influence the quantity of such pesticide in analyzed samples. The standard operation procedure (SOP) for analysis of carbaryl in vegetables was also developed. Moreover, the amounts of carbaryl residues found in the vegetable samples were compared with the value of the maximum residue limit (MRL) and the acceptable daily intake (ADI).

The C_{18} μBondaPak column was selected for the quantitative analysis of carbaryl due to its chemical property in retaining the carbaryl analyte which is slightly non polar. The optimized HPLC conditions employed in this study can be summarized as follows.

Injection volume : 20 μl

Mobile phase : Acetonitrile/water 50% (v/v) with 0.5 mmol of ammonium acetate buffer.

Column : μBondaPak C_{18} 10 μm (3.9 mm. × 300 mm.) + guard pak C_{18} μBondaPak precolumn inserts.

Run time : 7.00 minutes

Mobile phase flowrate : 1.50 ml/min (corresponding to 1260 psi of the pump pressure)

UV detector absorbance : 220 nm.

Linearity range : 0.04-120 μg/ml

Detection limit : 0.8 ng/ml

Percent recoveries : 93.6 ± 1.9%, Cv% = 2.2% at 1 ppm.

84.2 ± 2.1%, Cv%= 2.5% at 5 ppm.

Several parameters related to the capacity of the HPLC method employed for carbaryl determinations in this study were determined. Such parameters include the detector linearity range, detection limit, repeatibility of several injections onto the HPLC column, number of theoretical plates and the heights equivalent to a theoretical plate (HETP). The last two parameters measure the column performance or column efficiency. All of the afore-mentioned data were needed as a part of an evaluation of the instrument efficiency.

Instead of using a blender, the vegetable samples in this study were chopped into small pieces prior to the extraction step in order to minimize the contamination by the plant pigments. Although some contaminants did exist in the samples analyzed, their peaks emerged far enough from the position of carbaryl in the chromatograms. Consequently, no trouble was encountered in doing peak identification and subsequent quantitation. However, such contaminants could possibly interfere with the component of interest during the extraction step and diminish the recovery percentage, especially at the higher concentrations. The further work on this line should therefore develop the process of removing the plant pigments from the sample to increase the percent recovery. Use of the aromatic sulfonic acid ($C_6H_5SO_3H$) as a sorbent may be a good approach to get rid of irrelevant components, especially plant pigments, for carbaryl analysis [38].

The average amounts of carbaryl found in vegetables were found to have varied with time starting from the day directly after spraying. None of the carbaryl was detected on the day before spraying while the highest amount of carbaryl detected was obtained on the day directly after application. The average

highest amount of carbaryl was 35.0 mg/kg (%Cv = 8.9%) a relatively very high figure when compared with the maximum residue limits (MRL) of 3.0 mg/kg in Germany and 10.0 mg/kg in Thailand whereas the ADI value for carbaryl was 0.01 mg/kg body weight. In considering a hypothetical situation in which a normal Thai man weighing about 60 kg consumed the vegetables on the day directly after spraying without any treatment, the amount of carbaryl consumed by him would be 0.58 mg/kg/day. This intake appeared to be lower than the MRL in both Thailand and Germany. However, in terms of the acceptable daily intake (ADI), this is quite an alarming figure since the ADI value as recommended by the WHO/FAO is only 0.01 mg/kg body weight. The man would face a great potential risk if he continued consuming the treated vegetables with residues remaining in comparable amounts. As for the degradation rate or half life of carbaryl when calculated by using the data from the second study site where there was no effect of watering and rainfall, it was about 1.84 days. However, the half life of carbaryl, like other pesticides, is generally independent to the original concentration. In conclusion, extraction of carbaryl using solid phase extraction combined with subsequent analysis by HPLC has been demonstrated to be a reliable analytical tool for carbaryl residue analysis.