APPENDICES

Appendix A

EPA Pesticide Toxicity Classes [35]

Toxicity Class	Toxicity Rating	Signal Word on Label
I	Highly toxic	Danger poison
II	Moderately toxic	Warning
III	Slightly toxic	Caution
IV	Practically non-toxic	Caution

Appendix B

Limit of Determination and Limit of Quantification

A definition of limit of detection (LOD) is based on the concentration, which give signal equal to the blank signal plus three standard deviations of the blank. LOD was calculated from the calibration curve by means of the blank signal, which can be used as an estimation of the calculated intercept, plus three standard deviations of the blank. It can be used as an estimation of the calculated value from the regression line. The limit of detection was calculated by using Linear Least Squares' Line procedure.

$$Y = a + bx \quad (1)$$

Where Y = Instrument signals

x = concentrations

a = intercept

b = slope of the straight line

$$Y_L = Y_B + k\, S_B \quad (2)$$

Where Y_L = lowest detectable instruments signals

Y_B = Y intercept, a

k = constant depending on definition such as

k = 1, 5, 3 of 10 according to IUPAC, in calculation

of limit of detection, k = 3 was used in this work.

S_B = blank signal standard deviation

$S_{y/x}$ can be calculated from the equation

$$S_{y/x} = \{\Sigma [Yi\, \hat{Y}i\,]^2 / (n-2)\}^{1/2} \quad (3)$$

Where, Yi = response value from instrument corresponding to the individual x value

$\hat{Y}i$ = value of y on the instrument corresponding to the individual x value

n = number of points on the calibration line

From equation (1) and (2)

$$Y_L = a + 3\ S_{y/x} \quad (4)$$

$$Y_L = a + b\ C_L \quad (5)$$

Thus,

$$a + 3\ S_{y/x} = a + b\ C_L$$

$$C_L = 3\ S_{y/x} / b \quad (6)$$

Limit of quantification (LQO) is the lowest amount of analyte which can be quantified with an acceptable statistical significance. According to the IUPAC definition, LOQ is the amount or concentration resulting in an analyte singnal (measured value) Q_L.

$$Q_L = 10\ S_{y/x} / b \quad (7)$$

The values lower than LOD was called non- detected (ND) whereas the values higher than LOQ were acceptable values.

Limit of detection and limit of quantification calculated using equation (6) and (7) are shown in Table A.

Linear regression $y = 30.518x + 0.3946$, $R^2 = 0.9969$

Table A. Calculation from linear regression equation at different concentrations of carboxin

Concentration (mg/L)	**Yi**	$\hat{Y}i$	**[Yi -** $\hat{Y}i$ **]**	**[Yi -** $\hat{Y}i$ **]^2**
0.1	3.49	3.4464	0.0436	0.0019
0.2	6.25	6.4982	-0.2482	0.0616
0.4	12.82	12.6018	0.2182	0.0476
0.6	18.86	18.7054	0.1546	0.0239
0.8	24.64	24.8090	-0.169	0.0286
				$\Sigma [Yi - \hat{Y}i]^2 = 0.1636$

By using equation (3) and (6)

$$S_{y/x} = \{\Sigma [Yi\ \hat{Y}i]^2/(n-2)\}^{1/2}$$

$$S_{y/x} = \{0.1636/3\}^{1/2}$$

$$= 0.2335$$

$$C_L = 3\ S_{y/x}/b$$

$$= 0.02295$$

Limit of detection for carboxin is 0.02.

$$Q_L = 10\ S_{y/x}/b$$

$$= 0.07651$$

Limit of quantification for carboxin is 0.08

Appendix C

Confirmation method by GC-MS [38, 39]

Gas chromatography mass spectrometry is the single most important tool for the qualitative identification and quantitative measurement of individual components in complex mixtures. GC can separate volatile and semi volatile compounds with great resolution, but it cannot identify them. Once the GC separates the constituents of the original sample, the individual components enter the mass spectrometer through an interface between the GC and the MS. MS can provide detailed structural information on most compounds such that they can be exactly identified, but it cannot readily separate them. The mass spectrometer receives the compounds as they exit the GC analytical column. The mass spectrometry splits the compounds into ion fragments. These fragments are then separated according to molecular weight. Since the majority of organic compounds have unique fragmentation patterns, the mass spectrometer can distinguish between the different parent compounds that were in the original sample. Quantitation can be based on peak areas from mass chromatograms or from selected ion monitoring.

Chromatograms of standard carboxin and analyte carboxin found in the sample are shown in Figure C.1 and C.2.

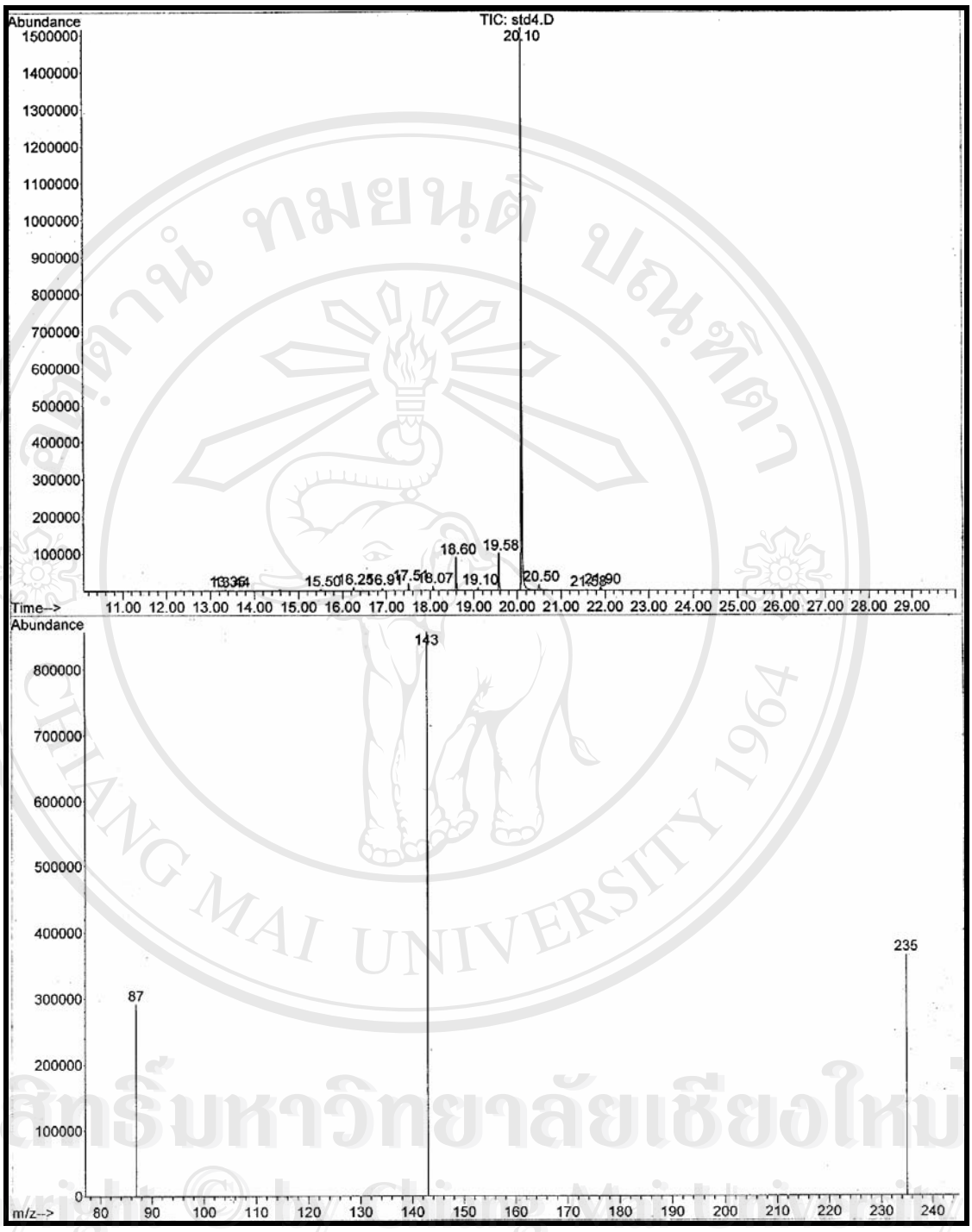

Figure C.1 MS chromatogram of the 1µg/ml of standard carboxin

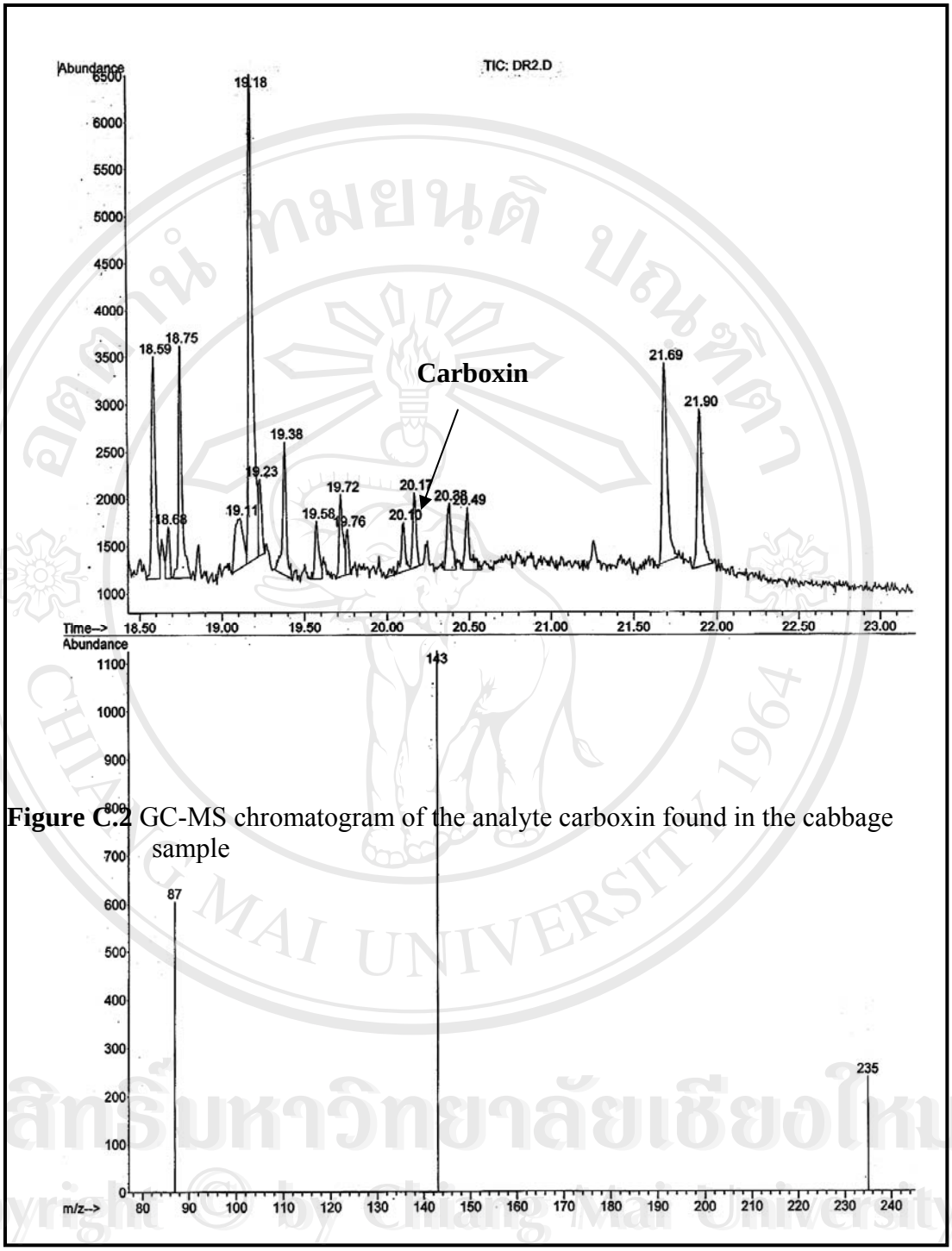

Figure C.2 GC-MS chromatogram of the analyte carboxin found in the cabbage sample

Figure C.2 GC-MS chromatogram of the analyte carboxin found in the cabbage sample

Appendix D

Calculation for the Concentration of the Analytes (Carboxin)

Vegetable sample = 10g

Final volume = 2.65 ml

Peak area (Y) = 11. 47

Regression equation, y = 68.448x + 0.6114

x = 0.16 mg/L

1 L of solution contains 0.16 mg of carboxin

1 ml "……" 0.16 µg of carboxin

2.65 ml "……." 0.420 µg of carboxin

In the extraction step, 0.5 ml was used from 5 ml of extracts.

So, 10 g of sample cabbage contained carboxin = 4.20 µg of carboxin

1000 g "……." = 4200 µg of carboxin

= 4200 µg/ kg of carboxin

= 4.20 mg/ kg of carboxin

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