3. RESULTS

3.1 Results of Construction of Calibration Curves

Table 3.1 Peak heights and peak areas obtained in the concentration range from 0.04 to 2 μg/ml using 50% acetonitrile/water (v/v) as mobile phase.

Concentration (μg/ml)	Peak Height (microvolt×second)	Peak Area (microvolt×second)
0.04	668	11704
0.08	1730	21859
0.2	4197	53732
0.4	8360	107877
0.8	16575	214686
2.0	43169	559569

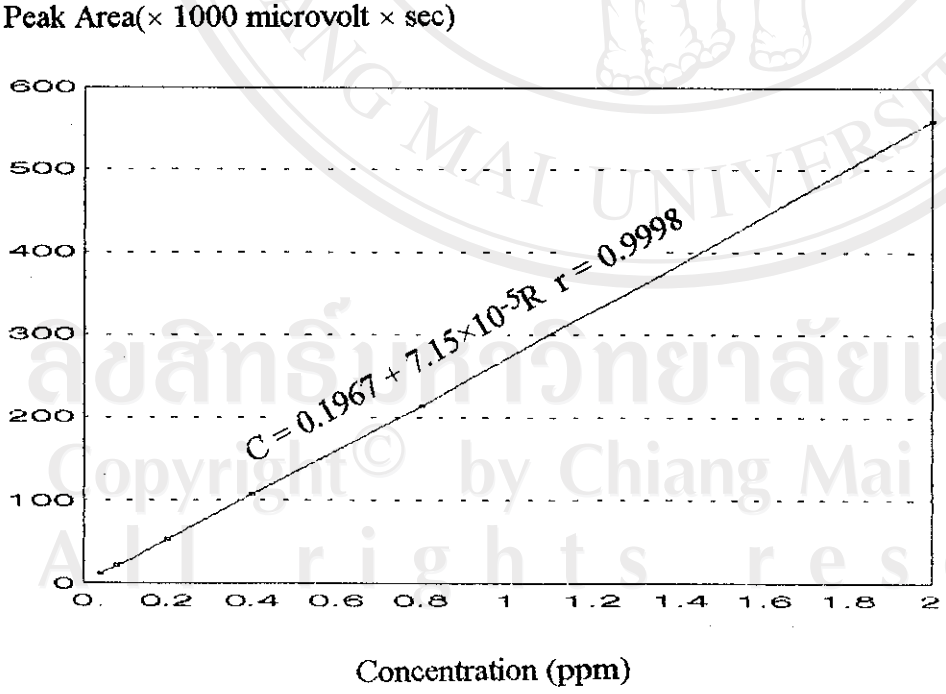

Fig.3.1 Calibration curve of carbaryl standard from 0.04 to 2 ppm.

Table 3.2 Peak heights and peak areas obtained in the concentration range from 1 μg/ml to 5 μg/ml using 50% acetonitrile/water (v/v) as mobile phase.

Concentration (μg/ml)	Peak Height (microvolt×second)	Peak Area (microvolt×second)
1	26463	344075
2	49734	646662
3	73276	955114
4	97346	1.28×10^6
5	122559	1.59×10^6

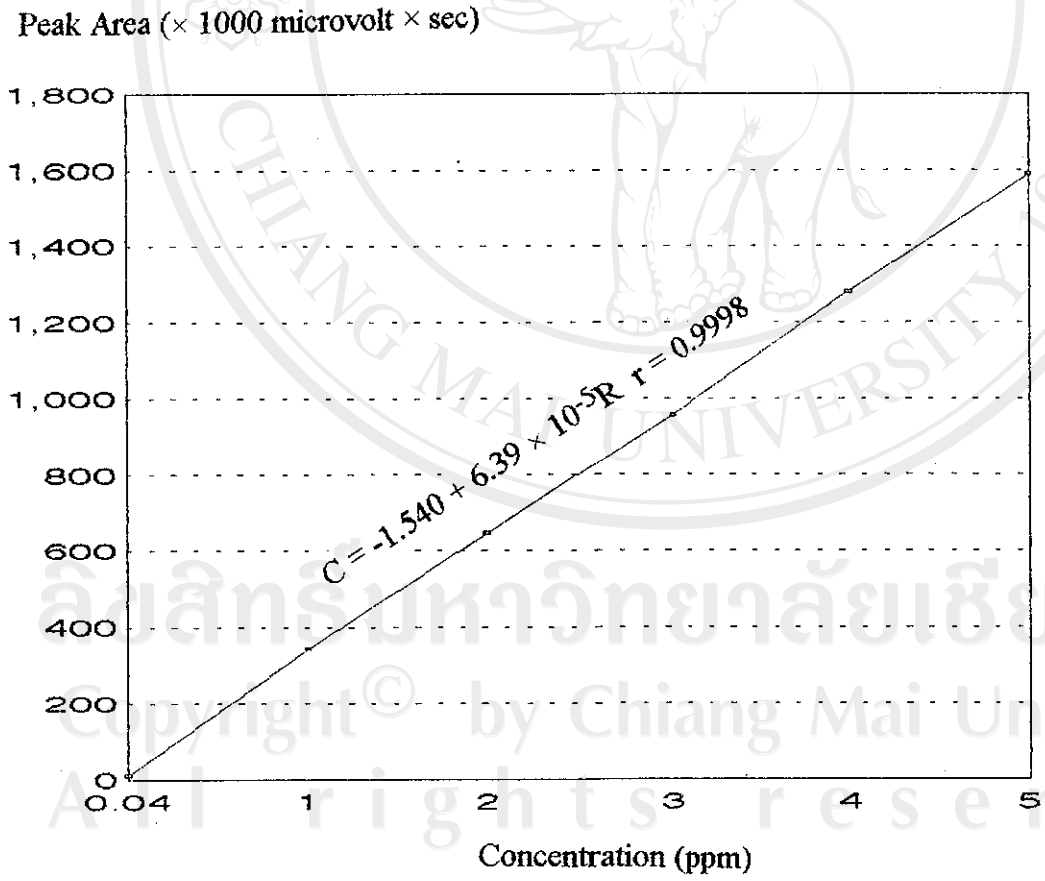

Fig.3.2 Calibration curve of carbaryl standard from 1 to 5 ppm.

Table 3.3 Peak heights and peak areas obtained in the concentration range from 5 μg/ml to 30 μg/ml using 50% acetonitrile/water (v/v) as mobile phase.

Concentration (μg/ml)	Peak Height (microvolt×second)	Peak Area (microvolt×second)
5	110741	1.44×10^6
10	209234	2.75×10^6
15	318264	4.15×10^6
20	421593	5.49×10^6
25	526263	6.87×10^6
30	617422	8.23×10^6

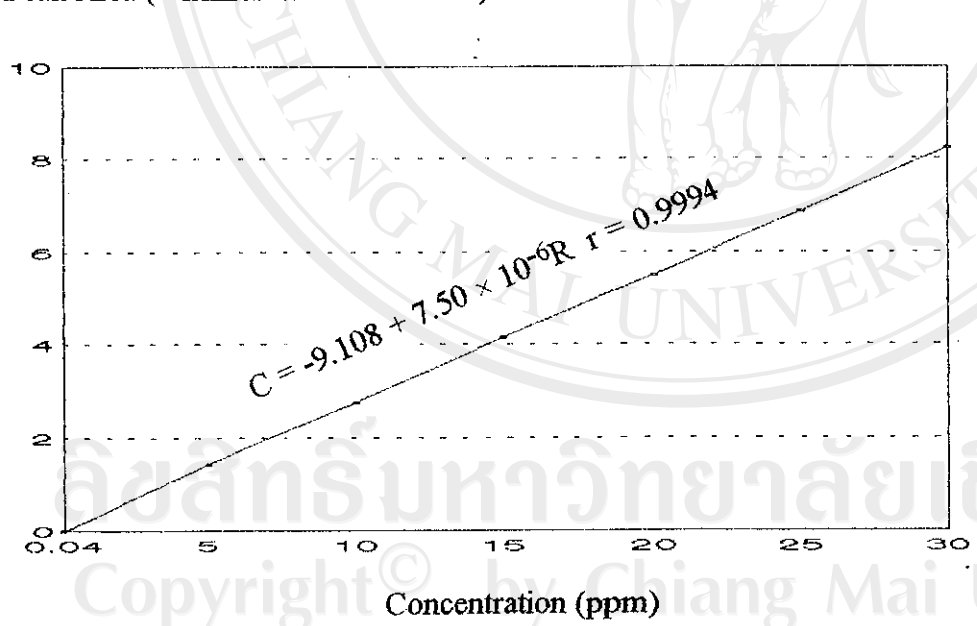

Fig.3.3 Calibration curve of carbaryl standard from 5 to 30 ppm.

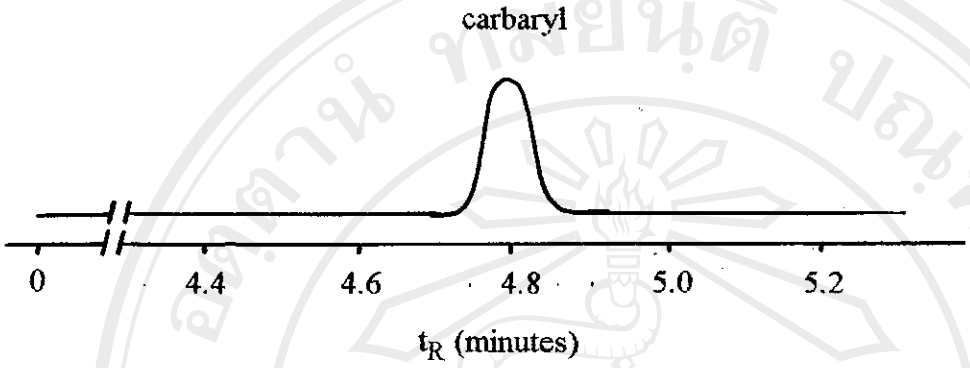

Fig 3.4 Chromatogram of standard carbaryl at concentration 0.4 μg/ml obtained with the μBondaPak C_{18} column employing 50% (v/v) acetonitrile/water as mobile phase.

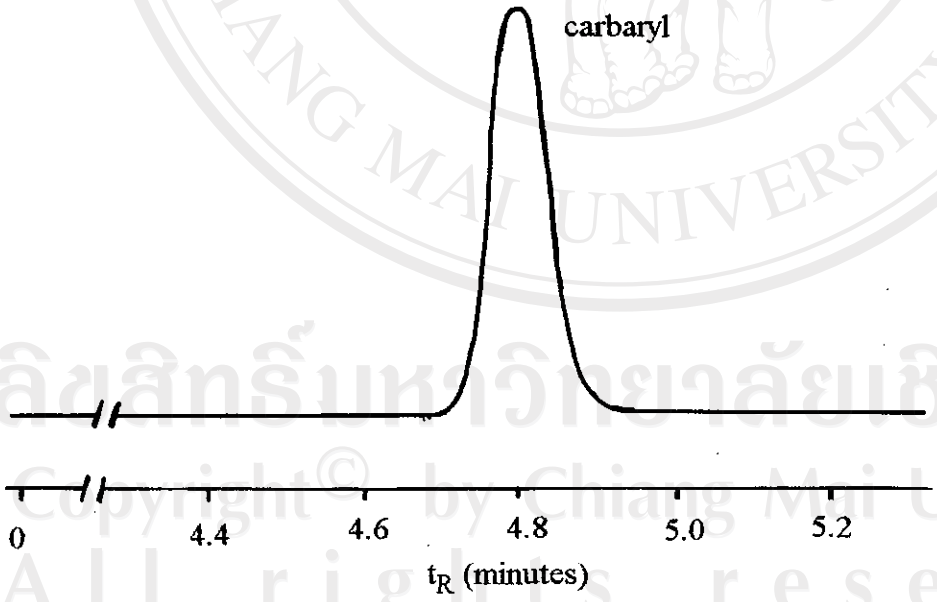

Fig. 3.5 Chromatogram of standard carbaryl at concentration 2 μg/ml obtained with the μBondaPak C_{18} column employing 50% (v/v) acetonitrile/water as mobile phase.

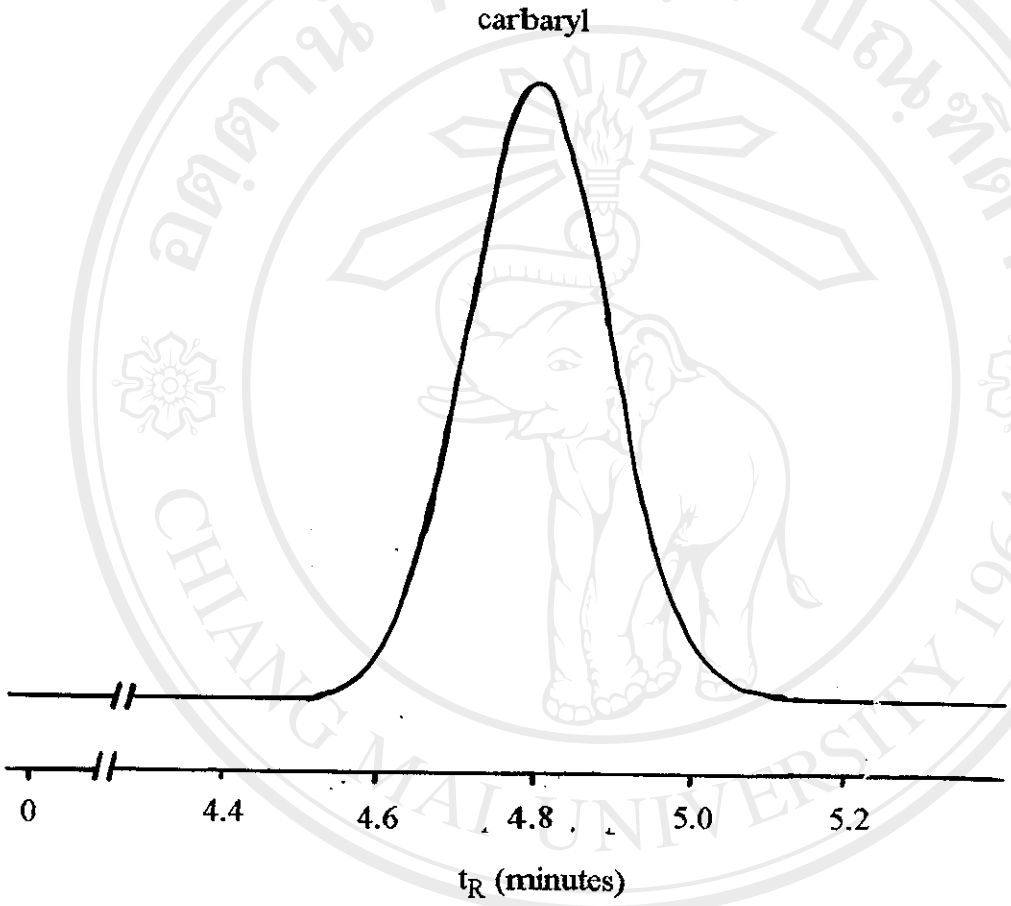

Fig. 3.6 Chromatogram of standard carbaryl at concentration 10 μg/ml obtained with the μBondaPak C_{18} column employing 50% (v/v) acetonitrile/water as mobile phase.

3.2 Results of Investigation for the Optimum Wavelength

Table 3.4 Absorbance values of 0.2 µg/ml carbaryl in terms of peak heights and peak areas at different wavelengths.

Wavelength (nm)	Peak Height (microvolt × sec)	Peak Area (microvolt × sec)
200	653	9997
210	1521	28337
220	2823	53844
230	576	10898
240	-	-
250	-	-
260	166	2773
270	219	4160
280	238	4522
290	184	3117
300	-	-

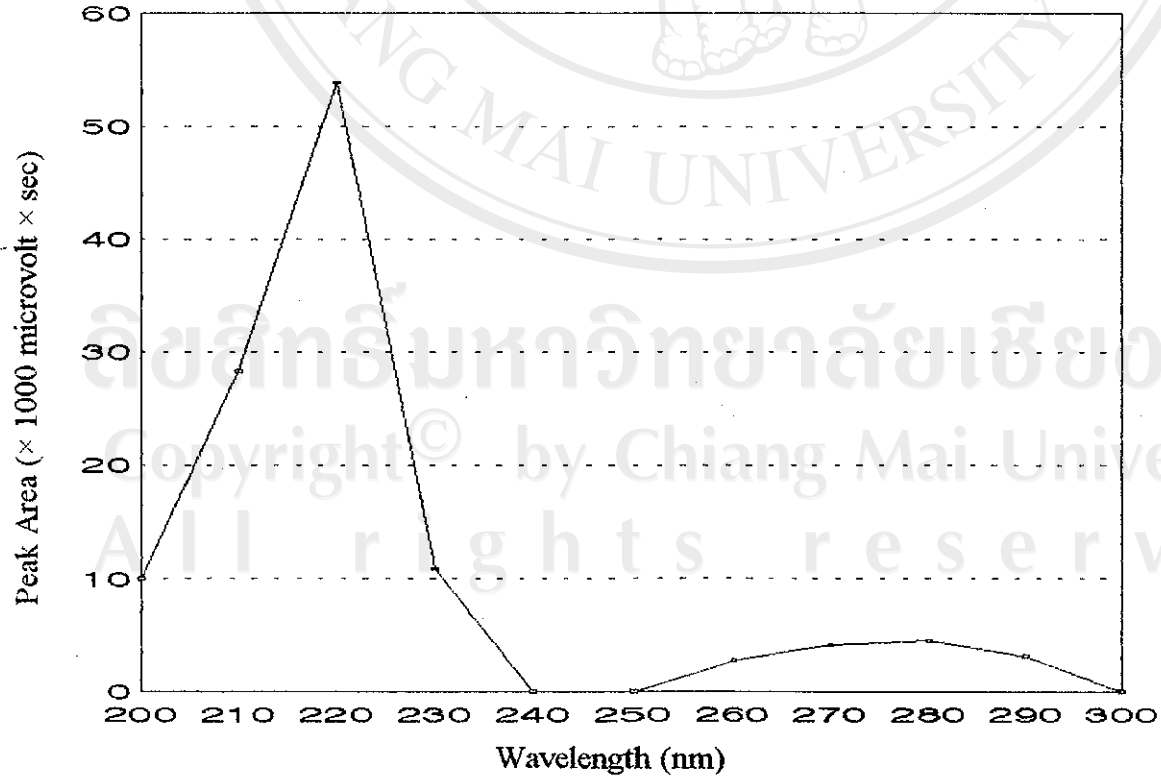

Fig.3.7 The UV spectrum of 0.2 µg/ml carbaryl.

Table 3.5 Absorbance values of 5 µg/ml carbaryl in terms of peak heights and peak areas at different wavelengths.

Wavelength (nm)	Peak Height (microvolt × sec)	Peak Area (microvolt × sec)
200	20181	364057
210	43302	849106
220	78298	1.61×10^6
230	14954	309876
240	1606	33530
250	2100	43032
260	3939	82066
270	5988	123860
280	6668	138021
290	4748	98608
300	1534	31571

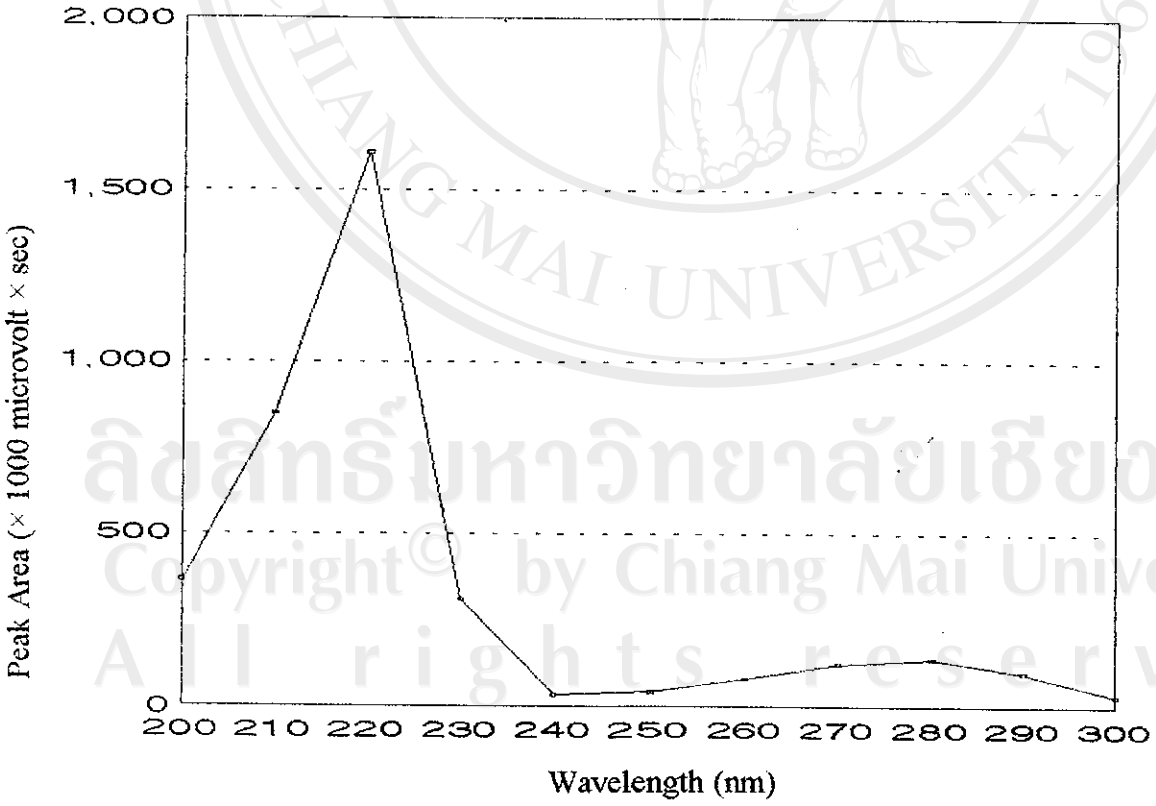

Fig.3.8 The UV spectrum of 5 µg/ml carbaryl.

3.3 Results of Determination of the Detector Linearity

Table 3.6 Peak areas and corresponding amounts of carbaryl using 50 % acetonitrile / water (v/v) as mobile phase.

Concentraion of carbaryl (µg/ml)	Peak area (µv × sec)	Concentration of carbaryl (µg/ml)	Peak area (µv ×sec)
0.04	8591	10	2.75×10^6
0.08	18440	15	4.23×10^6
0.2	45390	20	5.15×10^6
0.4	108293	25	7.48×10^6
0.8	207892	30	8.18×10^6
1	342309	60	1.47×10^7
2	656983	120	2.78×10^7
3	1.02×10^6	240	4.68×10^7
4	1.32×10^6	480	6.61×10^7
5	1.46×10^6	960	7.79×10^7

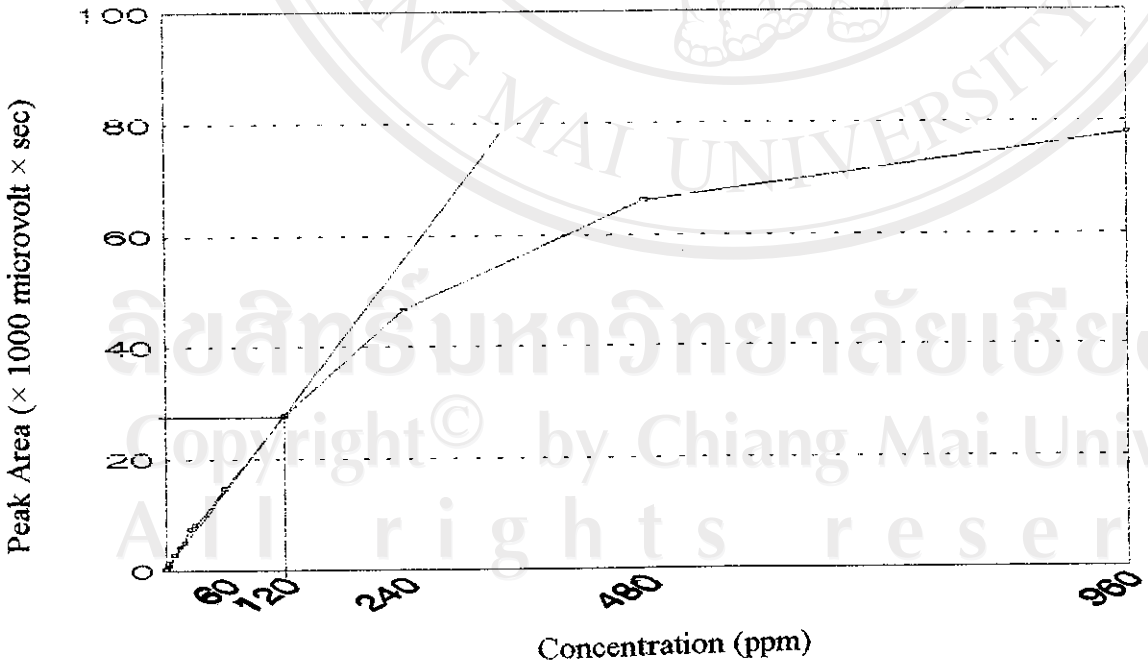

Fig.3.9 Linearity curve of the UV detector for HPLC determination of carbaryl.

3.4 Results of Determination of the Detection Limit and the Lower Limit of Determination

Table 3.7 Detection limit of carbaryl obtained with 50 % ACN/water as mobile phase with μBondapak C_{18} column employing UV detector.

Lower limit of determination(μg/kg)	Detection limit conc.(μg/ml)	amount (μg)
1.4	0.0008	1.6×10^{-5}

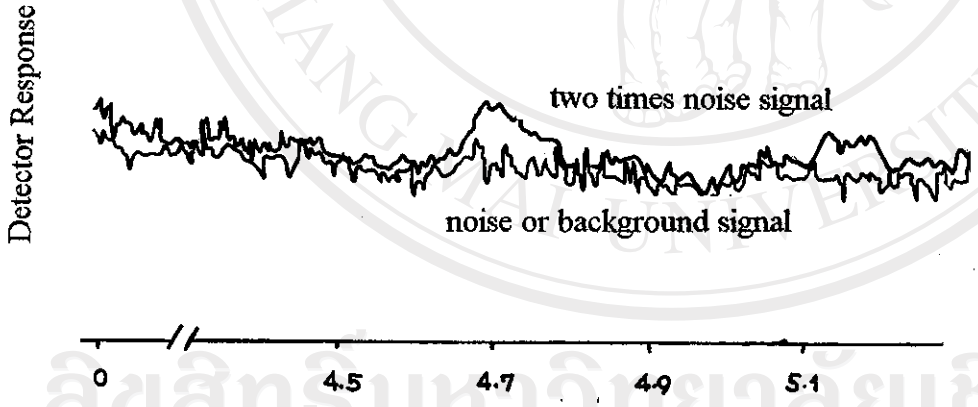

Fig. 3.10 Noise or background signal and lower limit of determination

.3.5 Results of Determination of Column Efficiency

Table 3.8 Chromatographic parameters obtained from 2 µg/ml of carbaryl at flow rate of 1.50 ml/min with 50 % ACN/water (v/v) as mobile phase.

No.	t_R (min)	$W_{1/2}$ (min)	N	HETP (mm)
1	4.62	0.166	4291	0.070
2	4.62	0.166	4291	0.070
3	4.62	0.166	4291	0.070
4	4.62	0.166	4291	0.070
5	4.62	0.166	4291	0.070
6	4.63	0.166	4258	0.070
7	4.62	0.167	4240	0.071
8	4.62	0.167	4240	0.071
9	4.63	0.167	4258	0.070
10	4.62	0.167	4258	0.071
Mean	4.62	0.166	4269	0.070
SD	4.00×10^{-3}	5.16×10^{-4}	24.0	4.0×10^{-4}
Cv%	0.09	0.3	0.6	0.7

Table 3.9 Chromatographic parameters obtained from 5 μg/ml of carbaryl at flow rate of 1.50 ml/min with 50 % ACN/water (v/v) as mobile phase.

No.	t_R (min)	$W_{1/2}$ (min)	N	HETP (mm)
1	4.63	0.167	4258	0.070
2	4.63	0.167	4258	0.070
3	4.63	0.167	4258	0.070
4	4.63	0.167	4258	0.070
5	4.64	0.167	4277	0.070
6	4.63	0.167	4258	0.071
7	4.62	0.167	4240	0.070
8	4.62	0.166	4291	0.070
9	4.63	0.167	4258	0.070
10	4.63	0.167	4258	0.070
mean	4.63	0.167	4261	0.070
SD	5.68×10^{-3}	3.16×10^{-4}	14	3.2×10^{-4}
C_V %	0.1	0.2	0.3	0.5

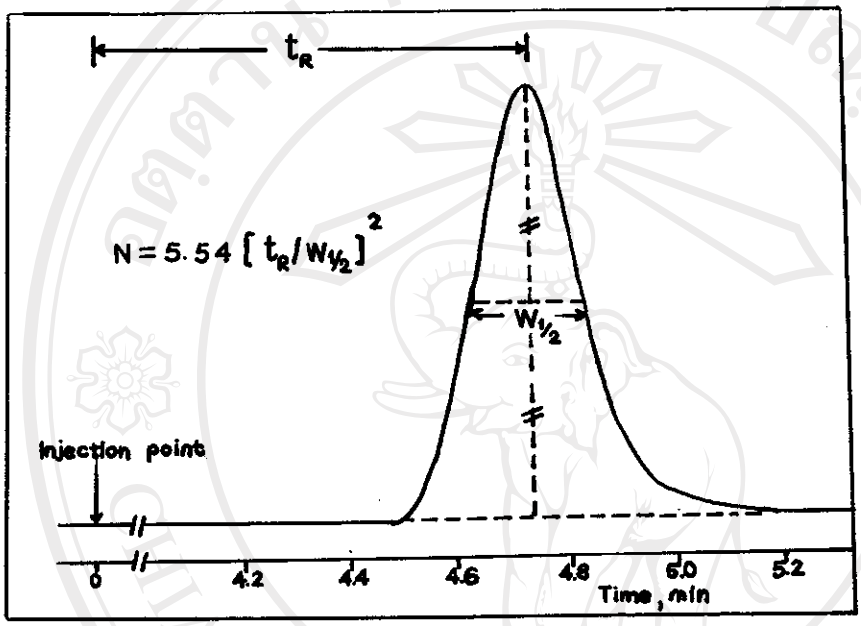

Fig. 3.11 Chromatogram of carbaryl showing calculation of the number of theoretical plates, N.

3.6 Results of HPLC Optimized Conditions.

Table 3.10 HPLC operating conditions for carbaryl determination

Injection volume	20 µl standard solution of carbaryl & samples
Solvent	Acetonitrile/water 50 % (v/v) with 0.5 mMole of ammonium acetate buffer
Column	µBondapak C_{18} 10 µm (3.9 mm x 300 mm) + guard pak C_{18} µBondapak precolumn inserts
Flushing time	30 minutes with 50 % (v/v) ACN / H_2O.
Run time	7.00 minutes
Pump Pressure	1260 psi (corresponding to solvent flowrate 1.50 ml/min)
UVdetector absorbance	220 nm

3.7 Results of the Air Temperature During Sample Collection.

Table 3.11 Air temperatures during sample collection in Ban Sop Pao, Lamphun Province.

Date	Time (A.M.)	Air temperature (°C)
25 Oct. 1993	10:00 - 11:00	31.6
26 Oct. 1993	11:30 - 12:30	28.0
27 Oct. 1993	9:30 - 10:30	27.6
28 Oct. 1993	9:30 - 10:30	28.5
29 Oct. 1993	10:00 - 11:00	29.0
30 Oct. 1993	9:35 - 10:30	24.6
3 Nov. 1993	10:00 - 11:00	29.5

Table 3.12 Air temperatures during sample collection in Ban Pa Sao, Chiang Mai Province

Date	Time (A.M.)	Air temperature (°C)
20 Nov. 1993	9:00 - 9:30	29.0
21 Nov. 1993	10:00 - 11:00	32.5
22 Nov. 1993	10:30 - 11:30	34.0
23 Nov. 1993	9:00 - 10:00	29.5
26 Nov. 1993	10:00 - 11:00	34.0
29 Nov. 1993	10:00 - 11:00	33.0
3 Dec. 1993	9:00 - 9:30	31.0
16 Dec. 1993	9:00 - 10:00	30.0

3.8 Results of the Accuracy and Precision

Table 3.13 Percentage recoveries of carbaryl at 1 µg/g and 5 µg/g in Kale (Brassica oleracea L.var. alboglabra Bail).

Vegetable	Conc.	Spiked level (µg)	Amount found (µg)	% recovery	Remarks
Kale	1 µg/g	2.00	1.89	94.5	mean: 93.3 S.D: 2.0 Cv: 2.4%
			1.89	94.6	
			1.88	94.0	
			1.86	92.9	
			1.86	93.2	
			1.86	92.8	
			1.89	94.4	
			1.89	94.6	
			1.89	94.7	
			1.87	93.4	
			1.80	90.0	
			1.81	90.6	
Kale	5 µg/g	10.0	8.36	83.6	mean: 82.3 S.D: 2.0 Cv: 2.4%
			8.52	85.2	
			8.39	83.9	
			8.44	84.4	
			8.49	84.9	
			8.24	81.4	
			8.04	80.4	
			8.05	80.5	
			8.03	80.3	
			8.08	80.8	
			8.07	80.7	
			8.06	80.6	

Table 3.14 Percentage recoveries of carbaryl at 1 µg/g and 5 µg/g in edible rape (Brassica chinensis L.var.oleifera Tsen et Lee.).

Vegetable	Conc.	Spiked level (µg)	Amount found (µg)	% recovery	Remarks
Edible rape	1 µg/g	2.00	1.83	91.4	
			1.88	93.9	
			1.90	95.0	
			1.85	92.6	
			1.83	91.4	
			1.89	94.4	
			1.87	93.5	mean:
			1.83	91.7	93.8
			1.89	94.7	S.D:
			1.90	95.1	1.8
			1.89	94.4	Cv:
			1.95	97.5	1.9%
Edible rape	5 µg/g	10.0	8.52	85.2	
			8.33	83.3	
			8.42	84.2	
			8.52	85.2	
			8.89	88.9	
			8.92	89.2	
			8.72	87.7	mean:
			8.92	89.2	86.0
			8.50	85.0	S.D:
			8.47	84.7	2.2
			8.70	87.0	Cv:
			8.32	83.2	2.6%

Table 3.15 The reproducibility of the HPLC employed at concentration of 2 µg/ml of carbaryl standard at 220 nm.

Run No.	Peak Height (microvolt × sec)	Peak Area (microvolt × sec)
1	63446	665992
2	63149	664803
3	63430	669227
4	63483	670167
5	63359	667423
6	63430	670793
7	63407	672270
8	63258	671658
9	63264	671898
10	64420	673990
mean value	63465	669822
SD	334	2794
C_V (%)	0.5	0.4

Table 3.16 The reproducibility of the HPLC employed at concentration of 5 µg/ml of carbaryl standard at 220 nm.

Run No.	Peak Height (microvolt × sec)	Peak Area (microvolt × sec)
1	153682	1.68×10^6
2	156685	1.66×10^6
3	157692	1.68×10^6
4	157535	1.68×10^6
5	157794	1.68×10^6
6	158027	1.68×10^6
7	159639	1.67×10^6
8	160167	1.66×10^6
9	160142	1.68×10^6
10	160244	1.70×10^6
mean value	158161	1.67×10^6
SD	1936	17776
C_V (%)	1.2	1.1

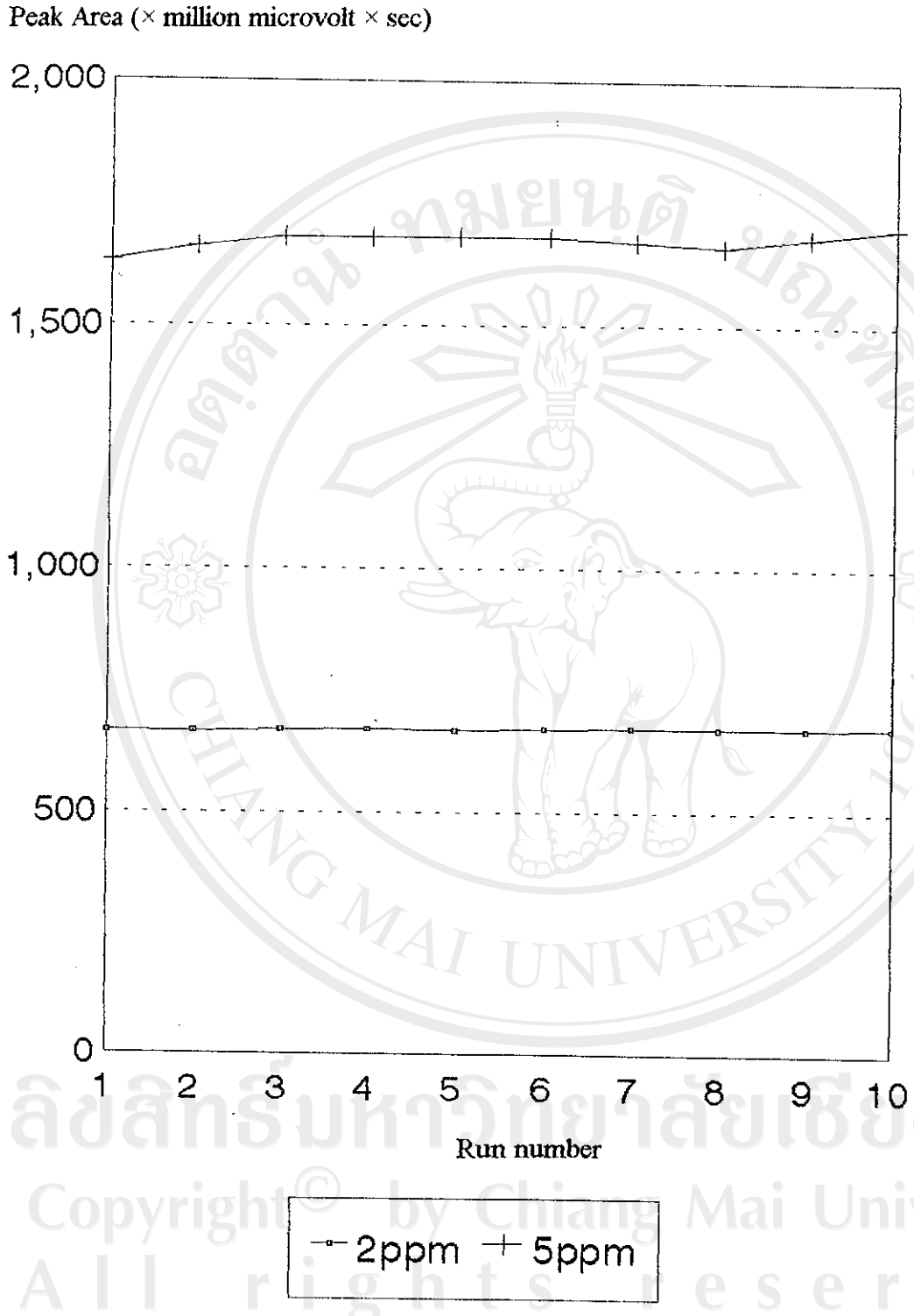

Fig.3.12 The reproducibility of the HPLC system with 2 and 5 μg/ml of carbaryl standards.

3.9 Results of the Amounts of Carbaryl Found in Vegetable Samples.

Table 3.17 The amounts of carbaryl found in kale at different time intervals (dosage of carbaryl :3 g/l).

Time interval	Amount (mg/kg) 1	2	3	4	5	6	7	8	9	10	No.	mean	SD	Cv %
before spraying	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	10	–	–	–
directly after spraying	30.5	30.1	36.2	34.5	40.4	24.0	32.2	30.0	30.4	29.2	10	37.8	4.4	13.8
1 day after spraying*	4.65	5.83	4.83	6.39	6.47	5.73	3.91	6.20	3.94	4.67	10	5.26	0.98	18.6
2 days after spraying	4.47	4.24	4.53	4.69	4.09	4.02	3.92	3.49	3.60	3.90	10	4.10	0.39	9.5
3 days after spraying**	1.68	1.63	1.62	1.79	1.62	1.78	1.49	1.61	1.79	1.44	10	1.64	0.12	7.3
4 days after spraying	0.774	0.799	0.663	0.789	0.711	0.837	0.689	0.763	0.768	0.727	10	0.752	0.054	7.2
8 days after spraying	0.390	0.400	0.333	0.432	0.422	0.376	0.250	0.249	0.325	0.249	10	0.342	0.072	21

* : watering the crop

** : rainfall in the night time

ND : not detected

Table 3.18 The amounts of carbaryl found in edible rape at different time intervals (dosage of carbaryl :3 g/l)

Time interval	Amount (mg/kg) 1	2	3	4	5	6	7	8	9	10	No.	mean	SD	Cv %
before spraying	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	10	–	–	–
directly after spraying	33.8	34.0	32.0	33.6	32.2	30.9	32.0	32.3	31.7	30.0	10	32.2	1.3	4.0
1 day after spraying	19.8	18.2	18.8	19.0	19.7	17.6	18.1	17.5	18.1	17.1	10	18.4	0.9	4.9
2 days after spraying	14.3	13.6	12.9	13.2	14.1	12.9	12.8	12.7	12.6	12.5	10	13.2	0.6	4.5
5 days after spraying	6.08	6.06	6.18	6.24	5.66	5.40	5.48	5.22	5.36	5.16	10	5.68	0.4	7.0
8 days after spraying	1.40	1.42	1.50	1.64	1.68	1.48	1.56	1.64	–	–	8	1.54	0.11	7.1
12 days after spraying	0.426	0.448	0.442	0.390	0.401	0.443	0.420	0.442	0.395	0.411	10	0.422	0.021	4.8
18 days after spraying	0.214	0.218	0.214	0.226	0.209	0.228	0.205	0.225	0.226	0.210	10	0.226	0.006	2.8
25 days after spraying	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	10	–	–	–

ND : not detected

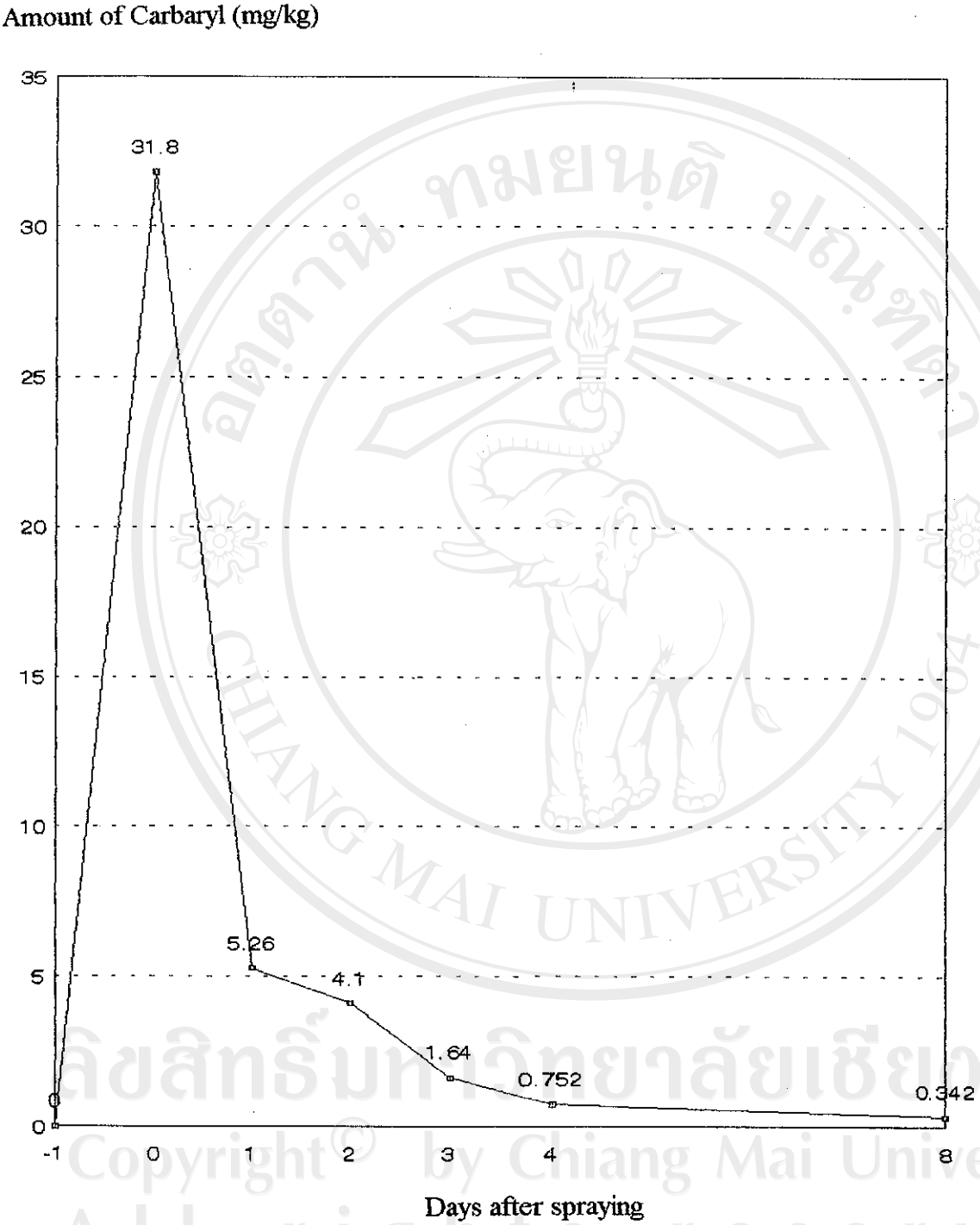

Fig.3.13 Amounts of carbaryl found in kale at different time intervals.

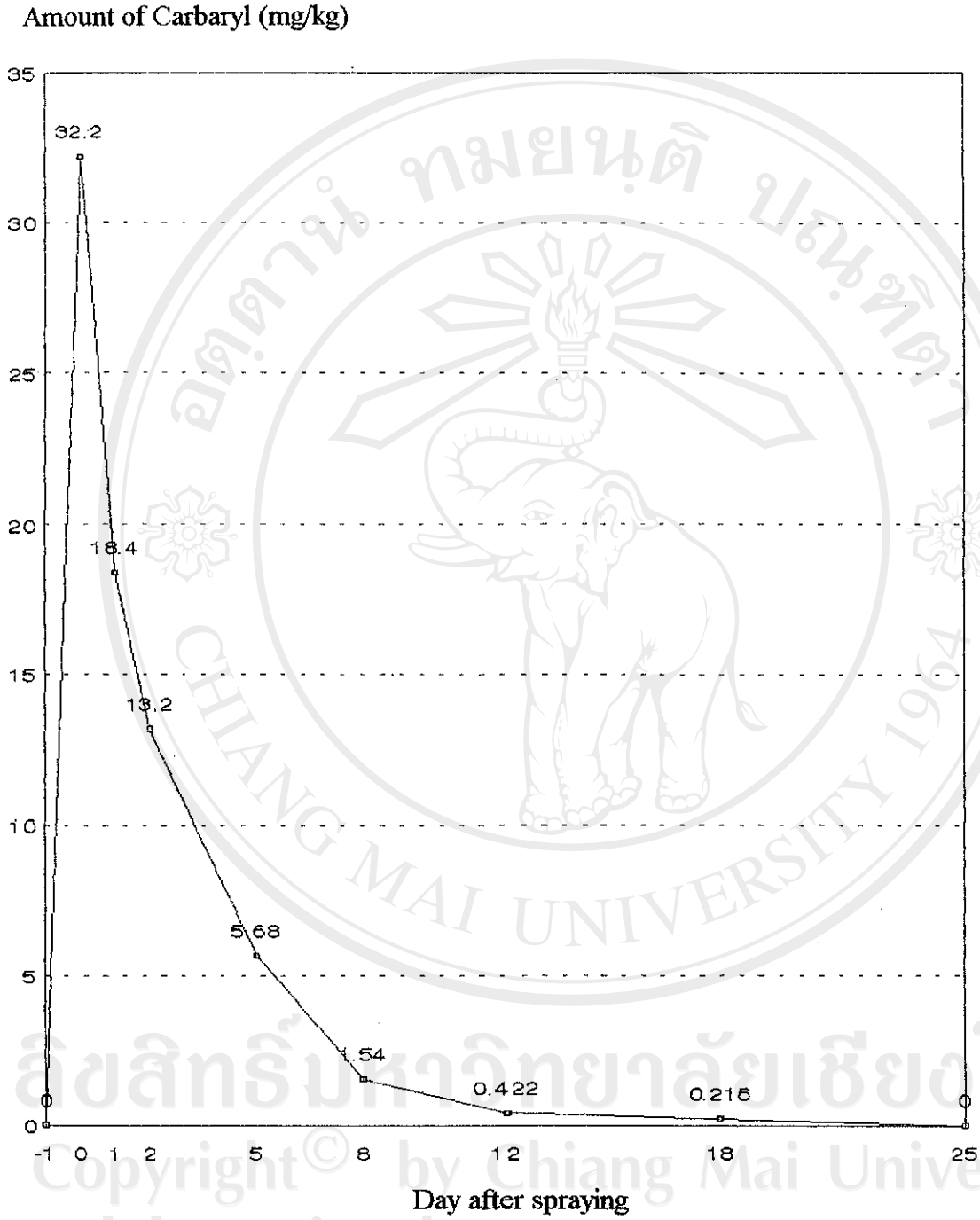

Fig.3.14 Amounts of carbaryl found in edible rape at different time intervals.

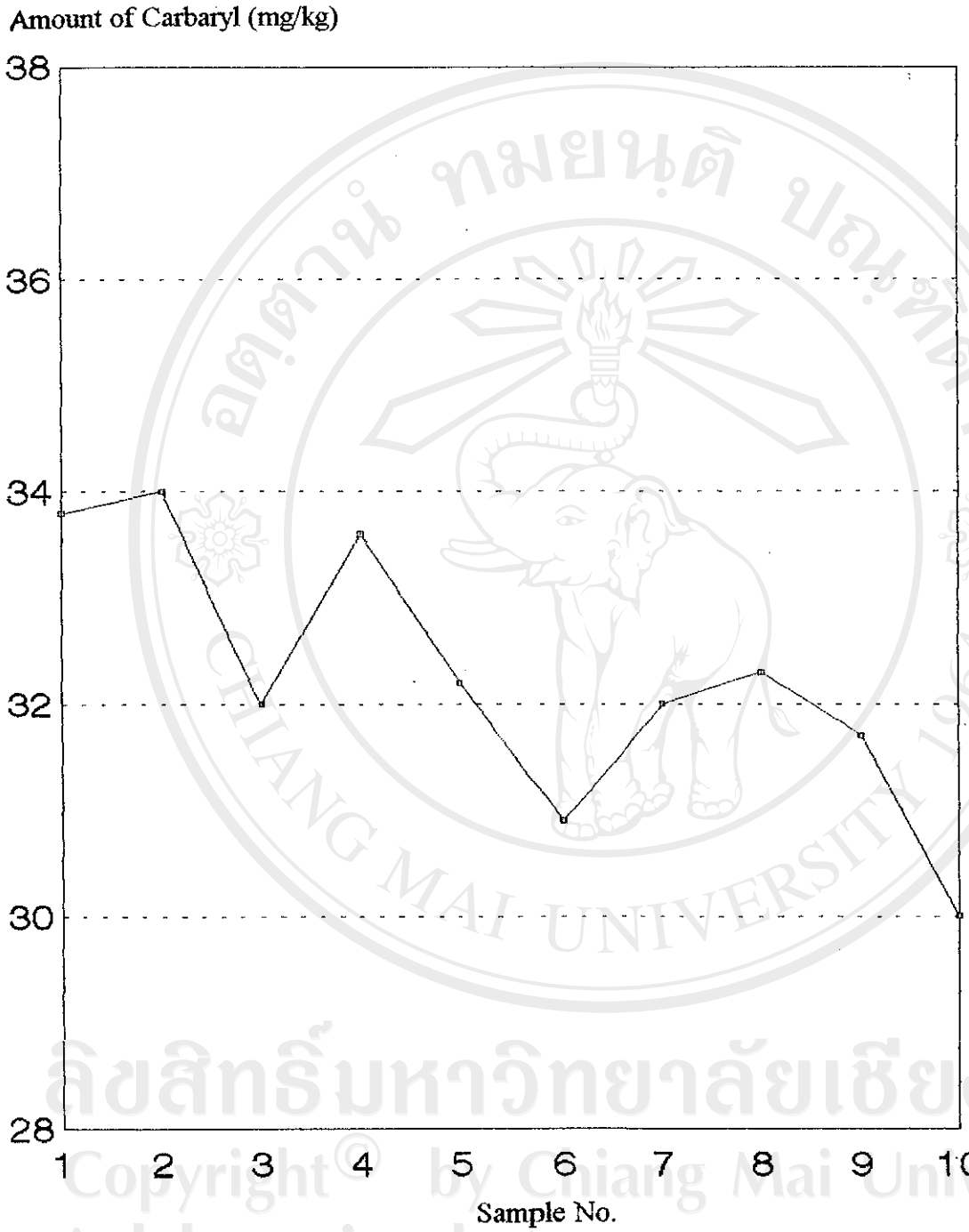

Fig.3.15 Variation of carbaryl amounts found in edible rape on the day directly after spraying.

3.10 Results of Peak Area Ratios at 270/220 nm.

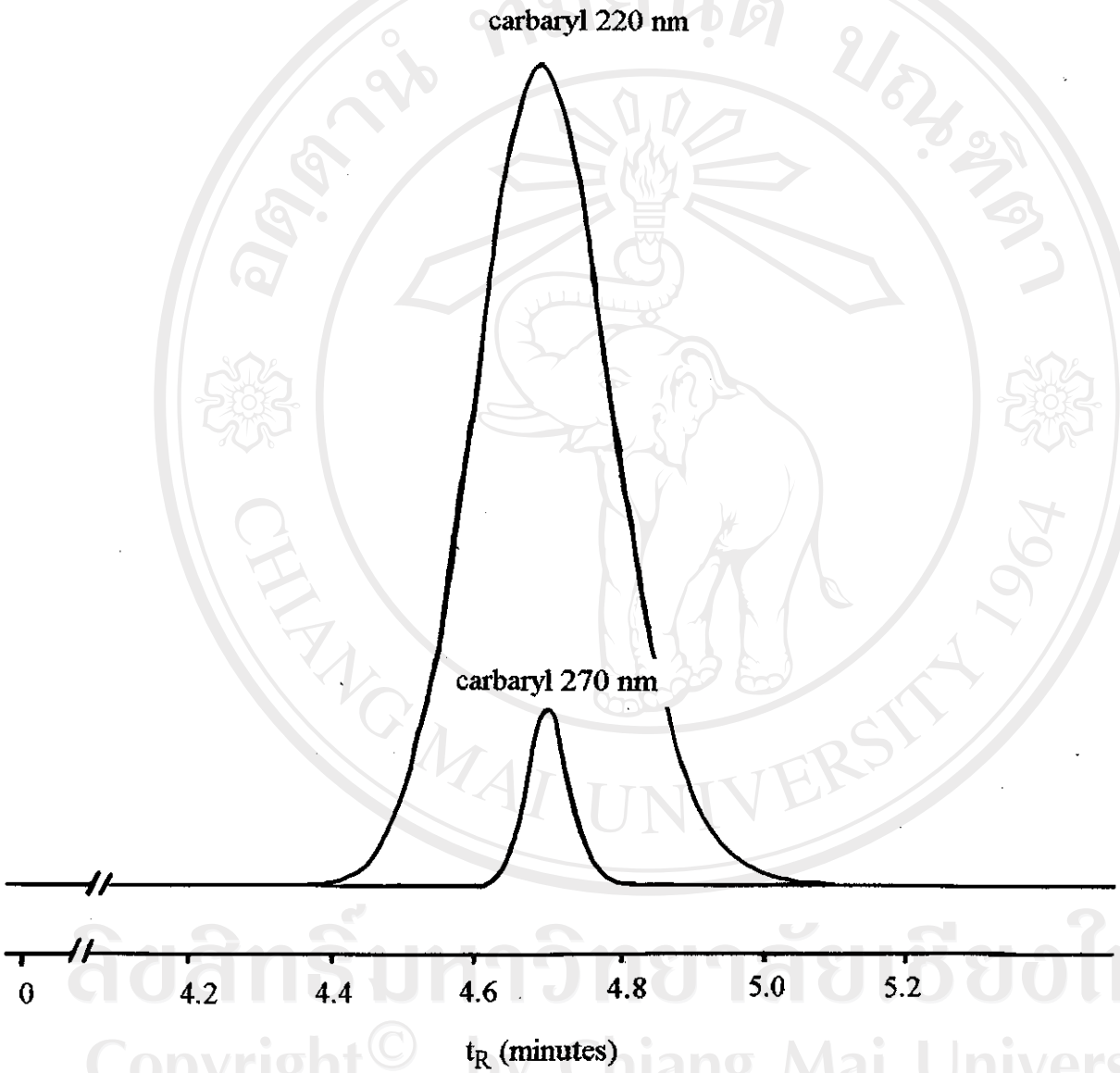

Fig. 3.16 Chromatogram of standard carbaryl at 220 nm and 270 nm obtained with the μBondaPak C_{18} column employing 50% (v/v) acetonitrile/water as mobile phase.

Table 3.19 Peak area ratios at two different wavelengths for confirmation of the analyte.

Standard	Ratio (270/220)	Sample No.	Ratio (270/220)
		1	0.079
0.8 ppm	0.076	2	0.076
		3	0.076
3 ppm	0.075	4	0.077
		5	0.077
15 ppm	0.076	6	0.079
		7	0.080
		8	0.077