CHAPTER 3

DETERMINATION OF METHYL PARATHION IN PLANT MATERIALS AND COMMERCIAL FORMULATIONS

3.1 Introduction

In this chapter, application of the proposed FIA spectrophotometric method for the determination of methyl parathion in real samples was attempted. The real samples were vegetables and some commercial formulations from Chiang Mai markets. A comparative determination of methyl parathion in the same samples was also carried out in order to validate the recommended method. A polarographic procedure was also tested for determining this pesticide residue in the above samples.

Polarographic methods have been reported for determining some organophosphorus pesticides for examples Smyth *et al.*(6) have proposed an indirect method for the simultaneous electroanalytical determination of parathion and paraoxon based on the different hydrolysis rates in alkaline media; measurements are made after 25 min. Mendez *et al.*(63) have also proposed polarographic procedures for the simultaneous determination of both pesticides, based on the different hydrolysis rates exhibited by these pesticides in the presence of Pd(II). Both of these workers have reported only the polarographic behavior of the pesticides, but they have not applied the method to the determination of these pesticides in real samples. Koen *et al.* (32) have reported the application of polarographic determination to column liquid chromatography and applied this method for residue analysis in plant materials. In the present work a polarographic procedure was proposed for the determination of methyl parathion in vegetable samples and commercial formulations without sample separation prior to analysis.

3.2 Experimental

3.2.1 Apparatus and Instruments

1) Optimized flow injection system.
2) UV-VIS Spectrophotometry, model U-2000 Spectrophotometer (Hitachi, Japan).
3) Cecil 1000 series (Cecil Instruments, England).
4) Polarographic analyzer, model 746 VA (Metrohm, Switzerland) in conjunction with a three-electrode cell system having a Ag/AgCl reference electrode, a platinum counter electrode and dropping mercury electrode (DME) as a working electrode.

3.2.2 Chemicals

1) Methyl parathion, AR grade (Dr. Ehrenstorfer, Germany).
2) Sodium hydroxide pellets (EKA Nobel, Sweden).
3) Hydroxylamine hydrochloride, AR grade (Ajax Chemicals, Australia).
4) Ethanol, absolute (E. Merck, Germany).
5) Sodium nitrite, AR grade (Fluka, Switzerland).
6) Sulfamic acid, AR grade (E. Merck, Germany).
7) Guaiacol, (o-Methoxyphenol), AR grade (Fluka, Switzerland).
8) Ethylene diamine tetraacetic acid, AR grade (E. Merck, Germany).
9) Zinc dust, AR grade (Carlo Erba, Italy).

10) Hexane, commercial grade.
11) Sodium acetate, AR grade (E. Merck, Germany).
12) Acetic acid, gracial (E. Merck, Germany).

3.3 Sample Preparation

Vegetable Samples: Various vegetable samples from Ton Payom Market were sliced and homogenized in an electric mixer. An amount of 50 g of each homogenized sample was shaken with two 100-ml portions of n-hexane. The n-hexane extract was then evaporated to dryness and the residue was dissolved in 50% ethanol(33). The volume was made up to 25 ml with 50% ethanol and aliquots were used for the determination of methyl parathion by the FIA method, spectrophotometric and polarographic methods.

To check the recovery, a known amount of methyl parathion was added to the homogenized sample and analyzed by these three methods. Table 3.6 shows the recovery by the FIA method in comparison to spectrophotometric and polarographic methods.

Commercial Formulations: 25 ml of 10 ppm methyl parathion from 50%w/v E.C. commercial formulations (Super pyret, Folez, Karlavin, Kabidon, Fonotox, Panadon, Folidol-E605 M50) was prepared in 50% ethanol solution.

3.4 Analysis of Methyl Parathion in Vegetable Samples and Commercial Formulations

3.4.1 FIA Method

The developed FIA method was applied to the determination of methyl parathion in vegetable samples and commercial formulations. The peak heights of each sample were compared with standard calibration curve as presented in Figure 2.22. The results were given in Table 3.1 and 3.2.

Table 3.1 Application of the FIA method for the determination of methyl parathion in various vegetable samples.

Sample	Set no.	Methyl parathion added		Net signal (mV)	Methyl parathion found (ppm)	Recovery (%)
		µg	ppm			
Cabbage	1	0.00	0.00	0	0.00	-
	2	1.00	2.00	18	1.66	83.0
	3	2.00	4.00	42	3.37	84.3
	4	4.00	8.00	91	6.87	85.9
Lettuce	1	0.00	0.00	0	0.00	-
	2	1.00	2.00	18	1.66	83.0
	3	2.00	4.00	43	3.44	86.1
	4	4.00	8.00	90	6.80	85.0
Chinese kale	1	0.00	0.00	0	0.00	-
	2	1.00	2.00	17	1.58	79.0
	3	2.00	4.00	40	3.23	80.7
	4	4.00	8.00	88	6.66	83.2
Cowpea	1	0.00	0.00	0	0.00	-
	2	1.00	2.00	17	1.58	79.0
	3	2.00	4.00	41	3.30	82.5
	4	4.00	8.00	90	6.80	85.0

Table 3.2 Application of the FIA method for the determination of methyl parathion in different commercial formulations.

Commercial formulation	Net signal (mV)	Methyl parathion found	
		ppm[a]	Percentage[b]
Super pyret	161	11.87	59.4
Kabidon	153	11.30	56.5
Karlavin	159	11.78	58.9
Panadon	155	11.44	57.2
Folez	151	11.16	55.8
Fonotox	95	7.16	35.8
Folidol	147	10.87	54.4

[a] Theoretical methyl parathion concentration = 10.00 ppm

[b] Percentage methyl parathion found in the commercial formulation. Label declaration = 50%w/v.

3.4.2 Spectrophotometric Method(33)

In order to validate the FIA method for methyl parathion determination, a comparative batch spectrophotometric determination of this pesticide was carried out.

1) Calibration curve

An aliquot of standard solution containing 12.5, 25, 50, 100 and 200 μg of methyl parathion was pipetted and transferred into each test tube. To the test tube 1-g of zinc dust and 2ml of 5-M hydrochloric acid were added. The contents were vigorously boiled for 2 min and then the rate of heating was reduced so that the boiling continued gently for a further 10 min. After 10 min the mixture was cooled and filtered into a 25-ml volumetric flask. The test tube was washed thoroughly with deionized distilled water and the washings were added to the same volumetric flask. To this 1 ml of sodium nitrite solution was added and left for 10 min with occasional shaking for the complete diazotization. During diazotization the acidity was adjusted by adding five drops of 5 M hydrochloric acid. The excess of sodium nitrite was removed by the addition of 1ml of sulfamic acid and the metal ions were masked with 1 ml of EDTA solution. Finally 2 ml of guaiacol or o-methoxyphenol was added and the solution was left for 3 min to complete the reaction. The solution was then made alkaline with an excess of sodium hydroxide solution (about 10 ml of 5 M sodium hydroxide) to produce an azo dye. The absorbance of this dye was measured at 470 nm against a reagent blank. The results are shown in Table 3.3 and Figure 3.1. A correlation coefficient of the calibration curve as shown in Figure 3.2 is 0.9998 with the calibration equation:

$$Y = 0.077X - 0.023$$

where Y is absorbance

X is concentration of methyl parathion in ppm

Table 3.3 Absorbance for calibration curve

Methyl parathion (ppm)	**Absorbance**
1	0.052
2	0.131
4	0.291
8	0.594

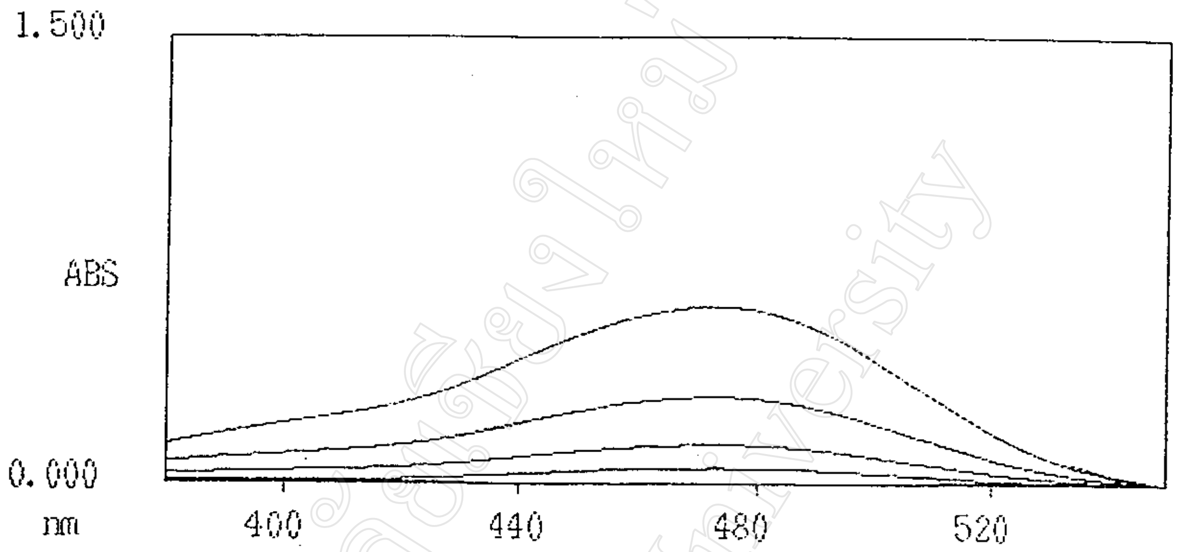


Figure 3.1 Absorption spectra of various concentrations of azo dye against a reagent blank.

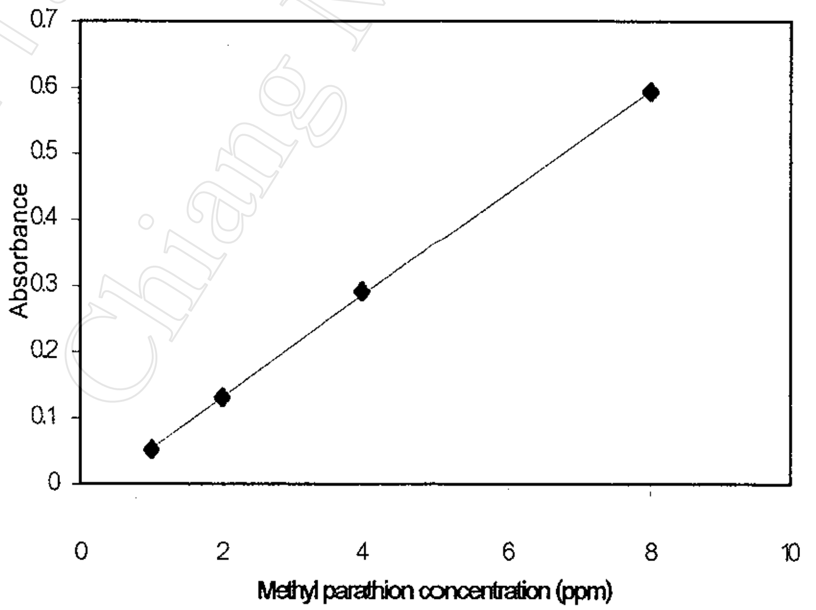


Figure 3.2 The calibration curve of spectrophotometric method for methyl parathion determination.

2) Application of the method for the determination of methyl parathion in vegetable samples and commercial formulations

10 ml of each sample was transferred into dry test tube and analyzed by this method. The amount of methyl parathion found in each vegetable sample was calculated by using the standard addition curve (Figure 3.3). The results obtained are shown in Table 3.4 and 3.5.

Table 3.4 Application of the spectrophotometric method for the determination of methyl parathion in various vegetable samples.

Sample	Set no.	Methyl parathion added		Absorbance	Methyl parathion found (ppm)	Recovery (%)
		µg	ppm			
Cabbage	1	0.00	0.00	0.009	0.02	-
	2	20.00	0.80	0.036	0.69	83.8
	3	40.00	1.60	0.070	1.21	74.4
	4	80.00	3.20	0.156	2.27	70.3
Lettuce	1	0.00	0.00	0.014	0.05	-
	2	20.00	0.80	0.023	0.71	82.5
	3	40.00	1.60	0.067	1.17	70.0
	4	80.00	3.2	0.017	2.39	73.1
Chinese kale	1	0.00	0.00	0.014	0.06	-
	2	20.00	0.80	0.024	0.71	81.3
	3	40.00	1.60	0.072	1.23	73.1
	4	80.00	3.20	0.163	2.42	73.8
Cowpea	1	0.00	0.00	0.016	0.09	-
	2	20.00	0.80	0.022	0.69	75.0
	3	40.00	1.60	0.076	1.29	75.0
	4	80.00	3.20	0.163	2.42	72.8

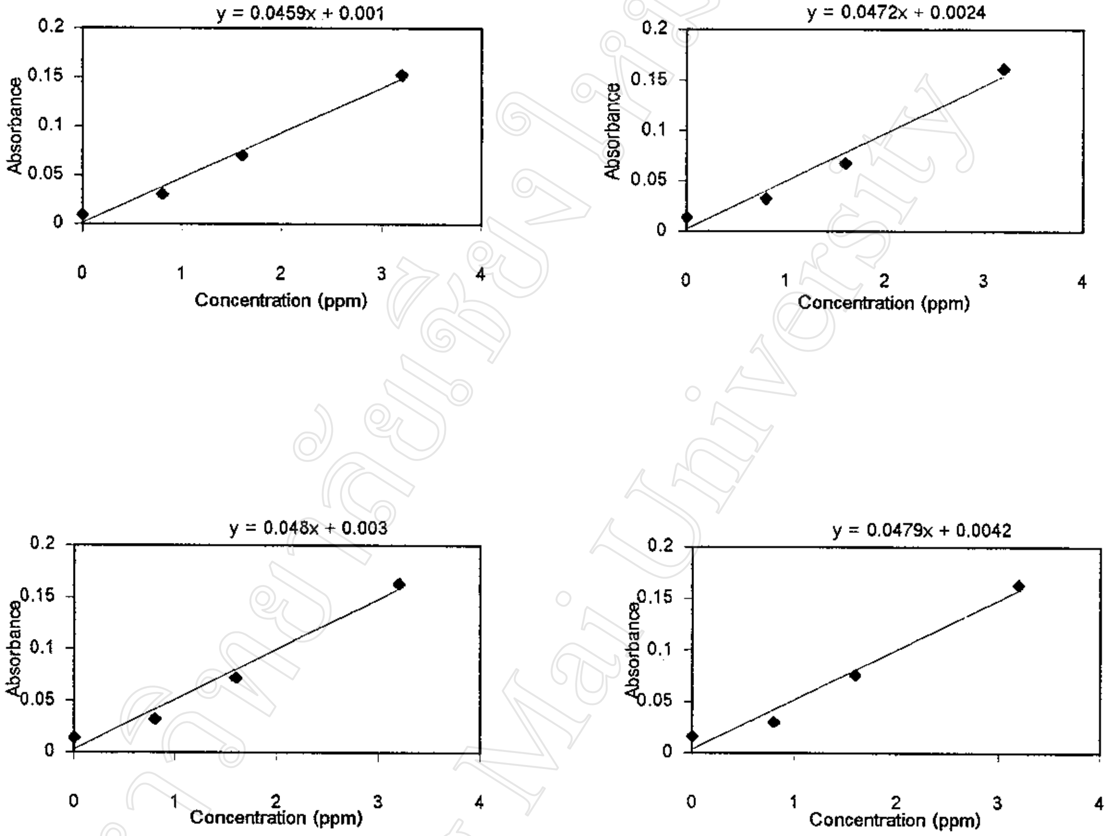


Figure 3.3 Standard addition curves for methyl parathion determination in Cabbage (a), Lettuce (b), Chinese kale (c), and Cowpea (d), using spectrophotometric method.

Table 3.5 Application of the spectrophotometric method for the determination of methyl parathion in different commercial formulations.

Commercial formulation	Absorbance	Methyl parathion found	
		ppm[a]	Percentage[b]
Super pyret	0.311	4.34	54.2
Kabidon	0.301	4.21	52.6
Karlavin	0.302	4.22	52.8
Panadon	0.304	4.25	53.1
Folez	0.290	4.06	50.8
Fonotox	0.170	2.50	31.2
Folidol	0.290	4.06	50.8

[a] Theoretical methyl parathion concentration = 4.00 ppm

[b] Percentage methyl parathion found in the commercial formulation. Label declaration = 50%w/v.

By using spectrophotometric method, methyl parathion concentrations in various vegetable samples and commercial formulations were found to be 0.02-0.09 ppm and 31.2-54.2 %, respectively.

3.4.3 Polarographic Method

In order to validate the FIA method for methyl parathion determination, a comparative polarographic determination of this pesticide was carried out.

1) Calibration curve

A set of standard solutions containing methyl parathion in the range of 0.1-3.2 ppm were pipetted in 20 ml final volume of 50%EtOH/H_2O(v/v)-0.1M HAc-0.1M NaAc medium. The oxygen is eliminated by bubbling N_2 through the solution for 300 sec. The corresponding differential pulse polarogram

(DPP) is recorded between –200mV and –550 mV with a pulse amplitude of –50 mV and a scan rate of 15 mV/s. The results are shown in Table 3.4 and Figure 3.3 with the reduction peak at -384 mV. The correlation coefficients (r) of the calibration curves as shown in Figure 3.4 are 0.9999 in the range of 0.099-0.392 ppm and 0.9984 in the range of 0.392-2.759 ppm with the calibration equation:

$Y = 41.522X$ and $Y = 30.987X + 5.438$, respectively.

where Y is current in -nA

X is methyl parathion concentration in ppm

Table 3.6 Variation of peak intensity of various concentrations of methyl parathion

Methyl parathion (ppm)	Current (-nA)
0.10	4.00
0.20	8.23
0.39	16.30
0.77	29.80
1.48	52.88
2.76	90.14

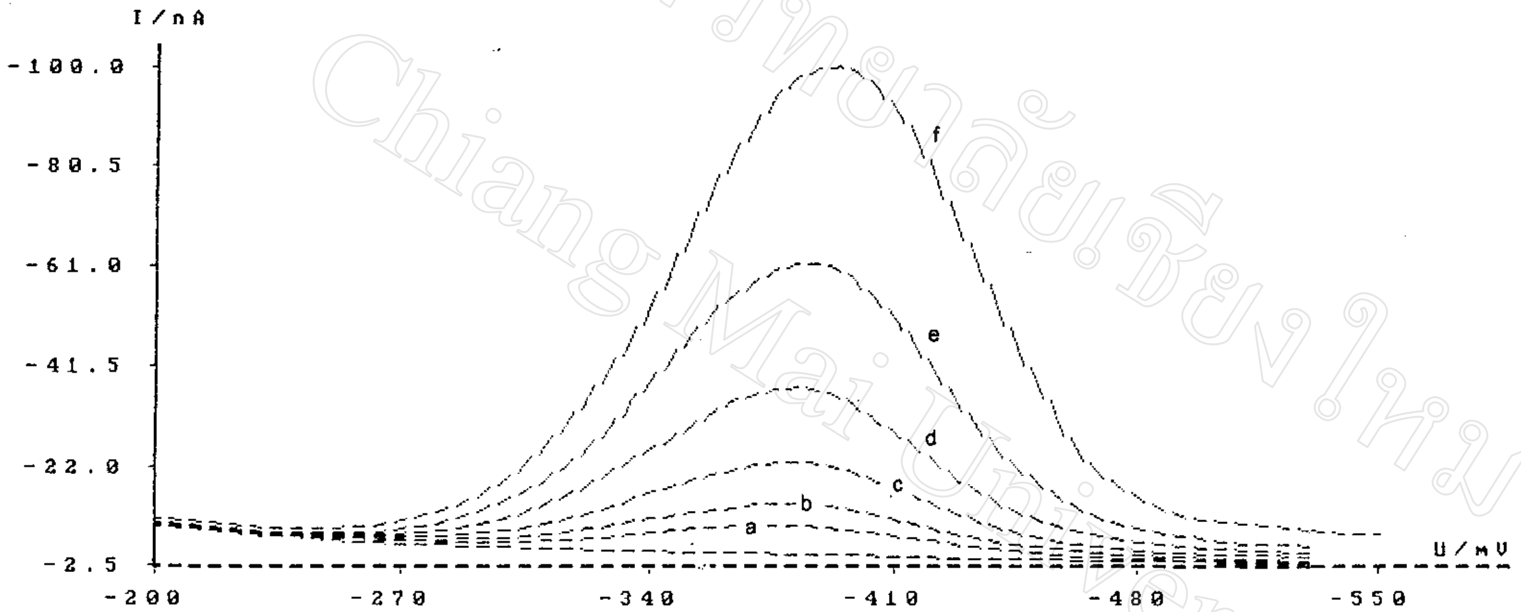
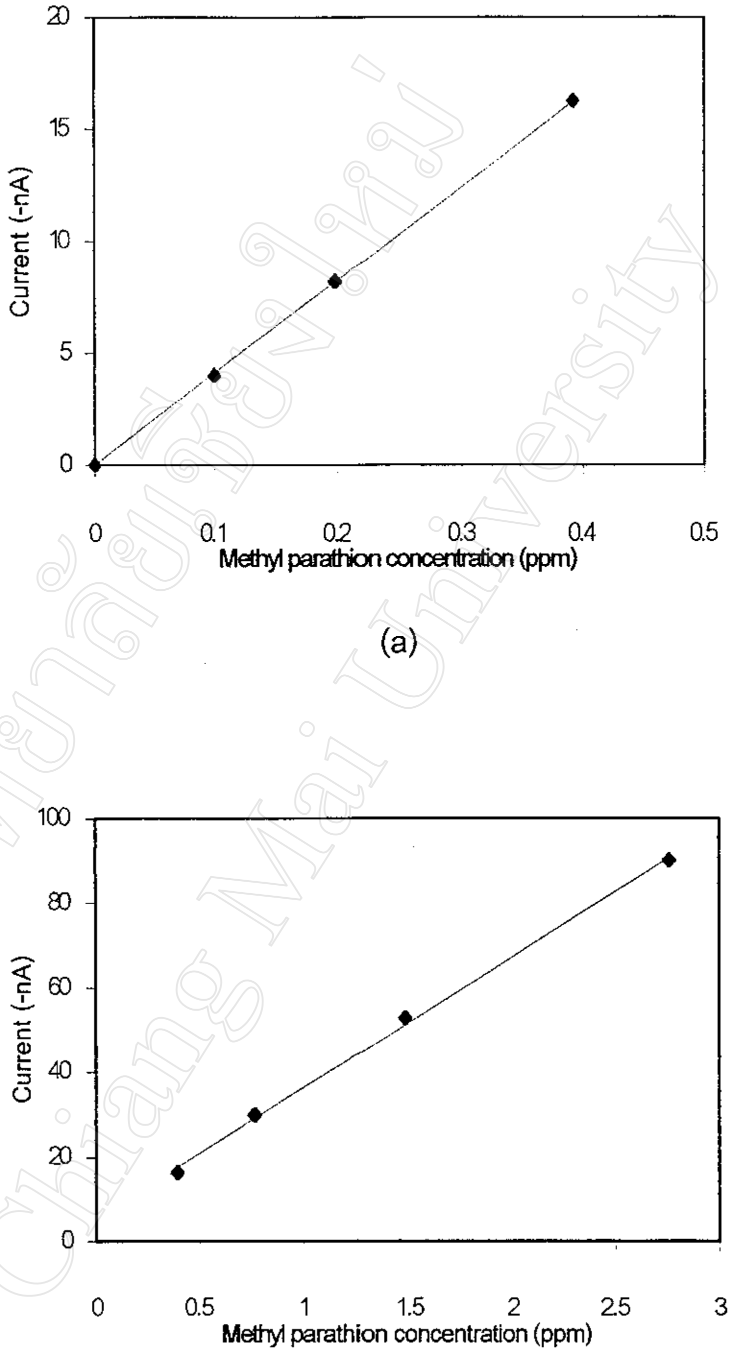


Figure 3.4 Polarogram of various concentration of methyl parathion; a=0.10, b=0.20, c=0.39, d=0.77, e=1.48, and f=2.76 ppm.



(a)

(b)

Figure 3.5 Calibration curve of polarographic method for methyl parathion determination. Beer's law was obeyed in the range of 0.099-0.392 ppm (a), 0.392-2.759 ppm (b).

2) Application of the method for the determination of methyl parathion in vegetable samples.

5 ml of each sample was pipetted into a cell, containing 20 ml of medium and analyzed by DPP method. The results obtained are shown in Table 3.6 and 3.7.

Table 3.7 Application of the polarographic method for the determination of methyl parathion in various vegetable samples.

Sample	Set no.	Methyl parathion added		Current (-nA)	Methyl parathion found (ppm)	Recovery (%)
		µg	ppm			
Cabbage	1	0.00	0.00	1.96	0.13	-
	2	10.00	0.40	11.72	0.28	37.5
	3	2000	0.80	16.89	0.41	35.0
	4	40.00	1.60	33.45	0.90	48.1
Lettuce	1	0.00	0.00	0.33	0.02	-
	2	10.00	0.40	7.14	0.17	37.5
	3	20.00	0.80	15.77	0.38	45.0
	4	40.00	1.60	29.16	0.76	46.3
Chinese kale	1	0.00	0.00	0.07	0.02	-
	2	10.00	0.40	6.04	0.14	30.0
	3	20.00	0.80	14.61	0.30	35.0
	4	40.00	1.60	24.87	0.63	38.1
Cowpea	1	0.00	0.00	0.45	0.03	-
	2	10.00	0.40	8.21	0.20	42.5
	3	20.00	0.80	14.72	0.35	40.0
	4	40.00	1.60	30.04	0.79	47.5

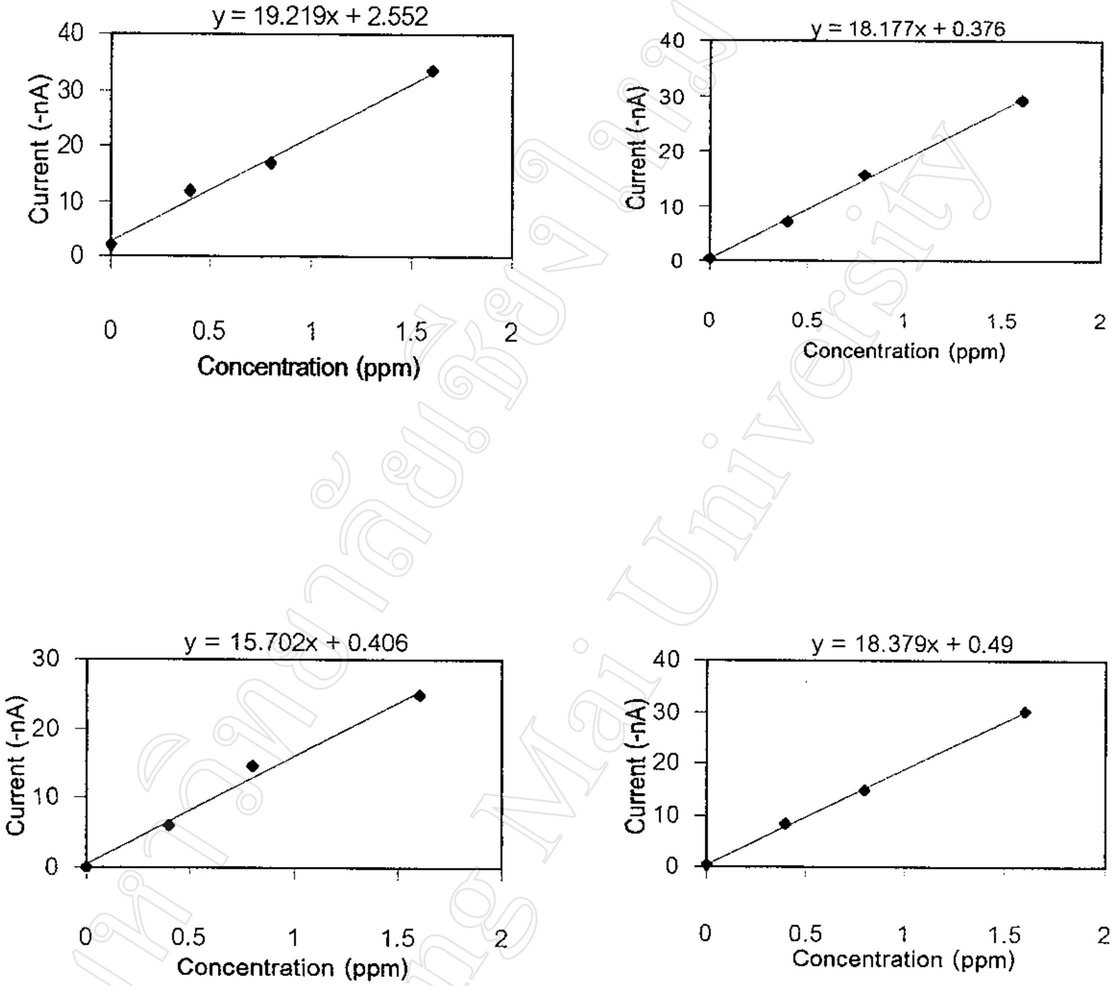


Figure 3.6 Standard addition curve for methyl parathion determination in Cabbage (a), Lettuce (b), Chinese kale (c), and Cowpea (d), using polarographic method.

Table 3.8 Application of the polarographic method for the determination of methyl parathion in different commercial formulations.

Commercial formulation	Current (-nA)	Methyl parathion found	
		ppm[a]	Percentage[b]
Super pyret	57.88	1.69	42.2
Kabidon	57.04	1.66	41.5
Karlavin	52.47	1.52	38.0
Panadon	55.38	1.61	40.2
Folez	57.95	1.70	42.5
Fonotox	34.45	0.94	23.5
Folidol	56.00	1.63	40.8

[a] Theoretical methyl parathion concentration = 2.00 ppm

[b] Percentage methyl parathion found in the commercial formulation. Label declaration = 50%w/v.

By using polarographic method, methyl parathion concentrations in various vegetable samples and commercial formulations were found to be 0.02-0.13 ppm and 23.5-42.5%, respectively.

3.4.4 Comparison of the Proposed FIA Method with Spectrophotometric and Polarographic Methods.

In order to validate the method, the recovery of the added pesticide was checked by adding a known amount of methyl parathion to the sample and analyzing by these three methods. Table 3.8 shows better recoveries by the proposed FIA method in comparison to spectrophotometric and polarographic methods.

Table 3.9 Comparison of the proposed FIA method with spectrophotometric and polarographic method for determination of methyl parathion in vegetable samples.

Sample	Set no.	Percentage recovery by		
		FIA method	Spectrophotrometric method	Polarographic method
Cabbage	1	-	-	-
	2	83.0	83.8	37.5
	3	84.3	74.4	35.0
	4	85.9	70.3	48.1
Lettuce	1	-	-	-
	2	83.0	82.5	37.5
	3	86.1	70.0	45.0
	4	85.0	73.1	46.3
Chinese kale	1	-	-	-
	2	79.0	81.3	30.0
	3	80.7	73.1	35.0
	4	83.2	73.8	38.1
Cowpea	1	-	-	-
	2	79.0	75.0	42.5
	3	82.5	75.0	40.0
	4	85.0	72.8	47.5

Comparison of the proposed FIA method with the other methods for methyl parathion determining in various commercial formulations is shown in Table 3.10.

Table 3.10 Comparison of the proposed FIA method with spectrophotometric and polarographic method for determination of methyl parathion in comercial formulations.

Commercial formulation	Methyl parathion found (%)[a]		
	FIA method	Spectrophotometric method	Polarographic method
Super pyret	59.4	54.2	42.3
Kabidon	56.5	52.6	41.5
Karlavin	58.9	52.8	38.0
Panadon	57.2	53.1	40.3
Folez	55.8	50.8	42.5
Fonotox	35.8	31.2	23.5
Folidol	54.4	50.8	40.8

[a] Percentage methyl parathion found in the commercial formulation. Label declaration = 50%w/v.