CHAPTER 4

RESULTS AND DISCUSSION

4.1. Peroxidase like activity of Iron oxide NPs

Peroxidase like activity of the γ-Fe_2O_3, Fe_3O_4 and mixed iron oxide NPs were examined based on the catalytic oxidation of peroxidase substrate (TMB) in the presence of H_2O_2. The results showed that TMB solutions in the absence of H_2O_2 and nanozymes exhibited as colorless. However, the color was changed from colorless to blue color after adding H_2O_2 and/or each mixed iron oxide NPs in the TMB solution. These observations proved that γ-Fe_2O_3, Fe_3O_4 and mixed iron oxide NPs possess peroxidase like activity and was capable of catalyzing the oxidation of TMB in the presence of H_2O_2 (Figure 4.1).

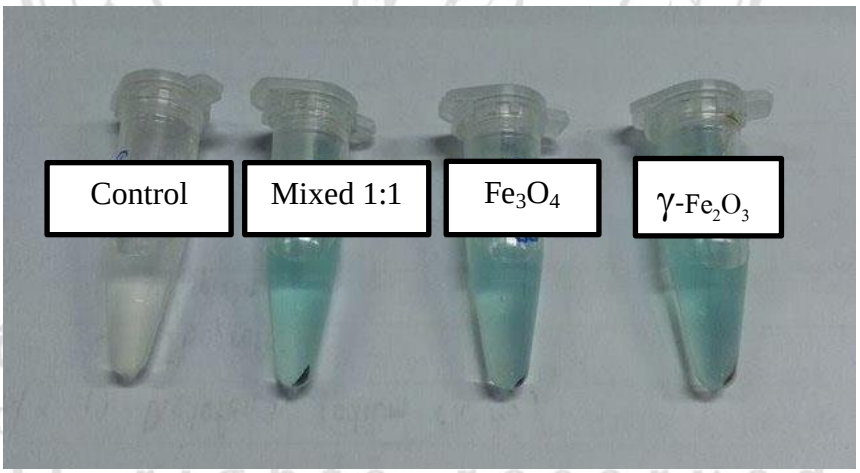

Figure 4.1 The color formation of oxidized TMB

The UV-Vis spectra of oxidized TMB (Figure 4.2) indicated that oxidized TMB obtained with the presence of each iron oxide NPs and H_2O_2 exhibited three absorption peaks. It was found that this result was similar to the absorption peaks of TMB- H_2O_2 – HRP reaction system, proving that all iron oxide NPs catalyzed the oxidation reaction of TMB in the presence of H_2O_2 [30].

It was also observed that the absorption peak at 654 nm was credited to the charge transfer complex (3, 3′, 5, 5′-tetramethylbenzidine diimine (TMBDI)) consequent of one electron oxidation of TMB and it was also similar to previous study [32]. In addition, Figure 4.2 also indicated that the higher absorbance, the higher peroxidase-like activity is showed no significant difference between the peroxidase-like activity of Fe_3O_4 and mixed iron oxides NPs whilst that of γ-Fe_2O_3 is lower.

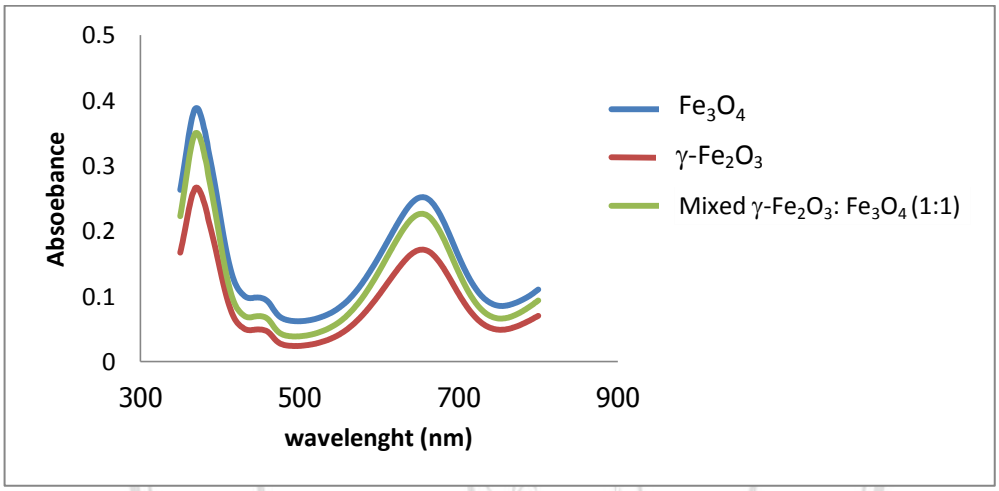

Figure 4.2 UV-Vis spectra of oxidized TMB obtained from the oxidation reaction with presence of each iron oxide NPs

Moreover, the peroxidase-like activity of the mixed iron oxide NPs at different ratios was examined as shown in Figure 4.3. It was found that the ratio 1:1 revealed the highest absorption (Figure 4.3), suggesting highest activity.

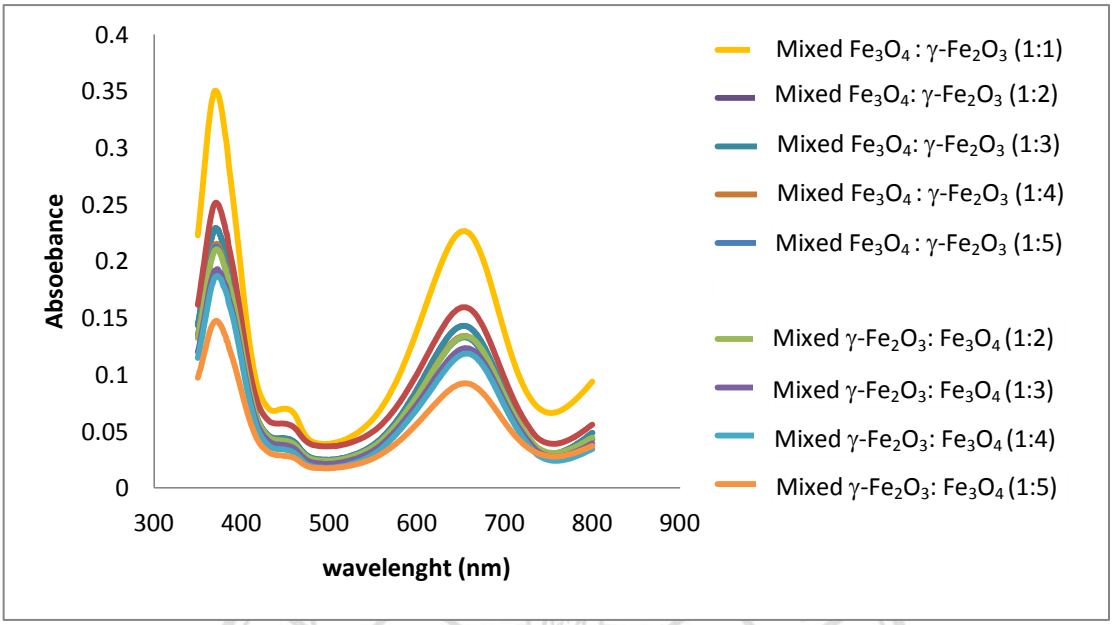

Figure 4.3 UV-VIS spectra of oxidized TMB obtained from the different ratios of mixed iron oxide NPs

The common association of the two iron oxides in nature are found to be oxidation products of irons. In general, Fe_3O_4 could be easily transformed to γ-Fe_2O_3 by the further oxidation under anoxic conditions. Consequently, γ-Fe_2O_3 is the common impurity of Fe_3O_4 as follows. Fe_3O_4 is topotactically oxidized by protons in water to create magnetic (γ-Fe_2O_3), $Fe_3O_4 + 2H^+ \longrightarrow \gamma\text{-}Fe_2O_3 + Fe^{2+} + H_2O$ [13]. Therefore, it is more practical to study and apply the peroxidase-like activity of mixed iron NPs in the detection of OPs.

4.2. Catalytic performance of mixed nanozyme under the optimum condition

4.2.1 Effect of pH

The effect of pH values for the catalytic activity of mixed iron oxide NPs in the presence of TMB and H_2O_2 in a pH range of 3.0- 6.0 at 653 nm were investigated. Figure 4.4 show the maximum absorbance of oxidized TMB at pH 3.0. It dramatically decreased at pH 4.0 and was constant from pH 4.0 to pH 6.0. This might be resulted from the decomposition H_2O_2 into H_2O and O_2 at high pH, which caused the decreased oxidative activity of iron oxides towards TMB [39]. This reaction was more robust in an acidic solution. Therefore, pH 3.0 was selected as an optimal pH for the OPs detection.

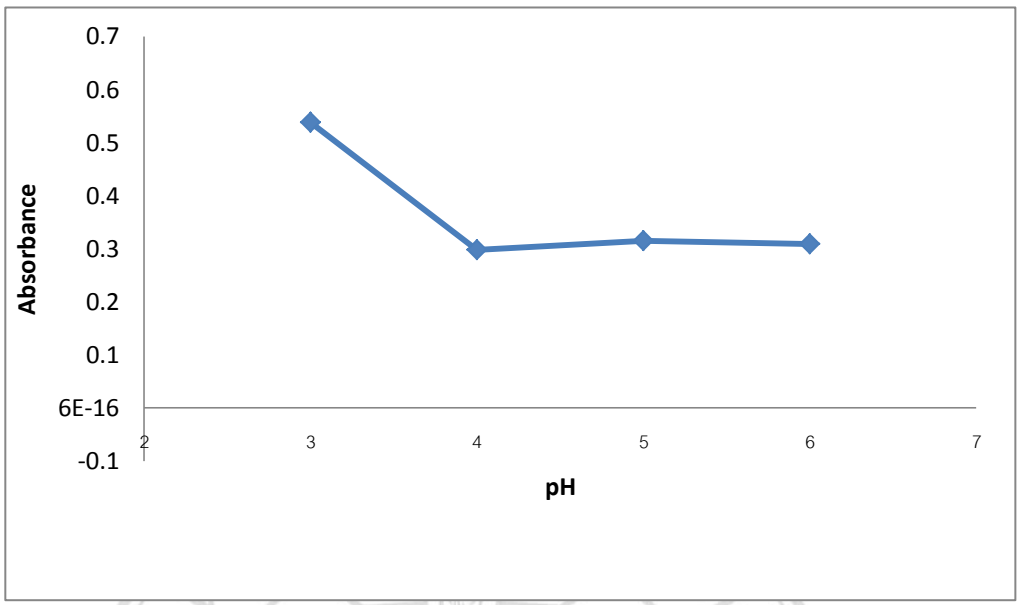

Figure 4.4 The absorbance of the catalytic reaction of iron oxide NPs undertaken in different pH

4.2.2. Effect of the concentration of mixed iron oxide NPs

As revealed in Figure 4.5, the catalytic activity of mixed iron oxide NPs increased with increasing concentration of mixed iron oxide NPs (0 - 200 µg/mL) and slightly increased since 250 µg/mL. Therefore, the concentration of mixed iron oxide NPs at 250 µg/mL was chosen as optimal concentration for this assay.

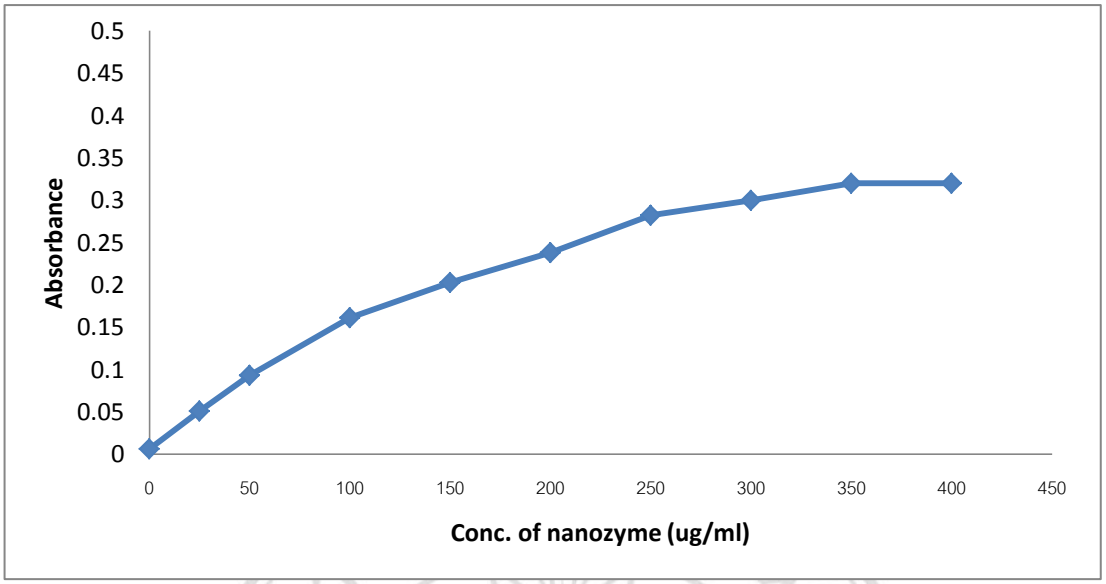

Figure 4.5 Concentration dependence of peroxidase-like activity of mixed iron oxide NPs

4.2.4 Effect of the TMB concentrations

The effect of the TMB concentration on the color formation was investigated using different concentrations of TMB (0.1, 0.2, 0.3, 0.4 and 0.5 mg/mL). From Figure 4.6, it was observed that the peroxidase-like activity of mixed iron oxide NPs increased with increasing TMB concentrations, reached its maximum at 0.3 mg/mL, and then decreased to 0.5 mg/mL. Therefore, 0.3 mg/mL TMB solution was chosen for further study.

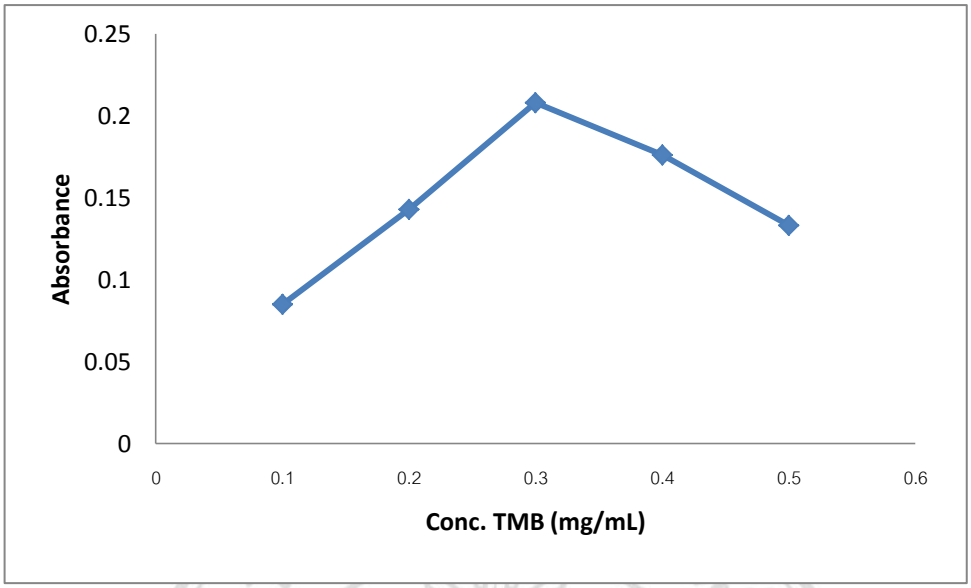

Figure 4.6 Effect of TMB concentration on color formation

4.3 Detection of OPs using optimum

Under the optimal conditions (pH 3.0, 250 µg/mL mixed iron oxide NPs, 1:1 mixed iron oxide NPs, 0.3 mg/mL TMB), an assay of OPs using catalyzed TMB color reaction was applied for the detection of OPs; dimethoate and profenefos in the real samples.

Figure 4.7 and 4.8 show the AChE inhibitory activity of both dimethoate and profenefos. It can be seen that the color intensity was decreased as the concentrations of the two OPs increased due to reduction of H_2O_2 product. It is expected that H_2O_2 is generated in the presence of both AChE and CHO in the ACh solution, leading to the oxidation of TMB over mixed iron oxide NPs; however, after OPs exposure, AChE activity was inhibited due to covalent bond formed between OPs and active site of AChE. As the result, less H_2O_2 was produced when there is inhibition by the OPs, resulting in decreased amount of oxidized TMB [8], [40]. As illustrated in both figures, The AChE enzyme inhibition was observed in the concentration range of OPs from 0.1 to 3.0 µM.

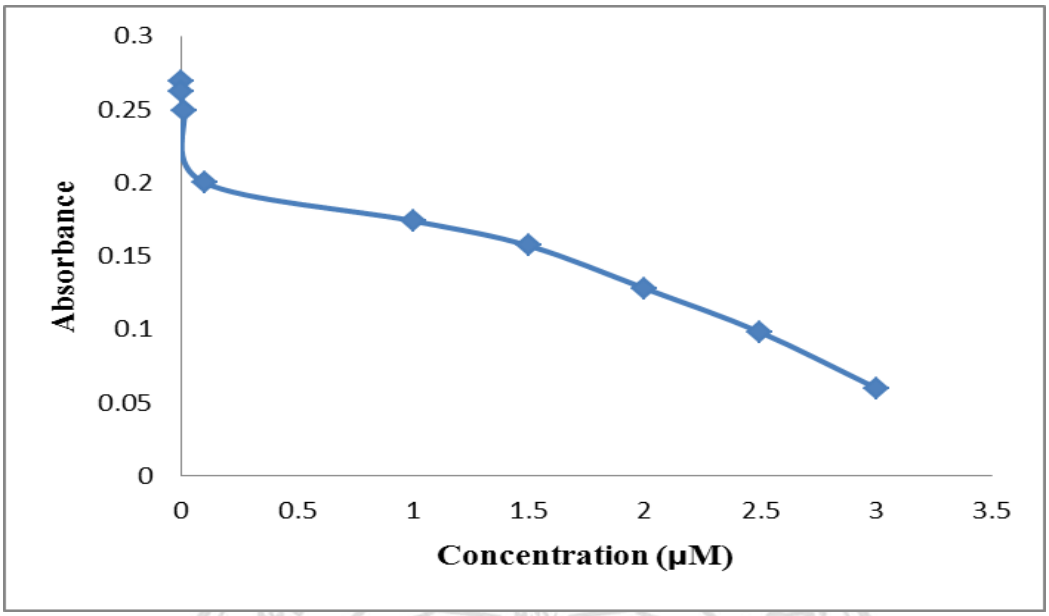

Figure 4.7 Dose-response curve for dimethoate detection using the mixed iron oxide NPs based colorimetric assay

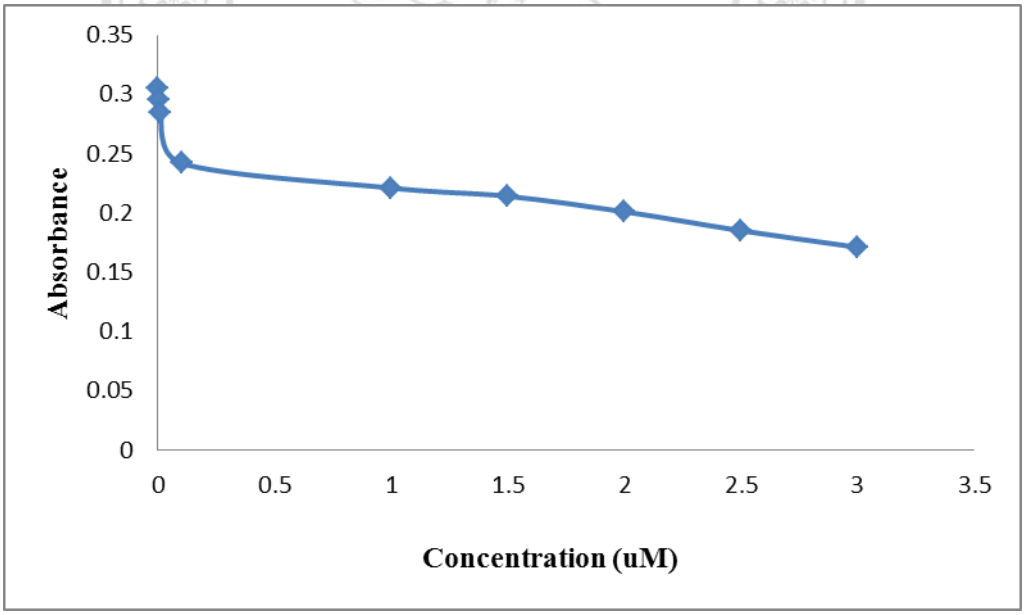

Figure 4.8 Dose-response curve for profenofos detection using the mixed iron oxide NPs based on colorimetric assay

Figures 4.9 and 4.10 show the standard calibration curves of both dimethoate and profenofos, which obtained from the graph (Figure 4.7 and 4.8, respectively). Table 4.1, shows that % recoveries were 98 ± 3 and 103 ± 2 for dimethoate and 102 ± 3 and 102 ± 2 for profenofos. RSDs were 2 and 3% for dimethoate and 3% for profenofos, indicating the satisfactory precision and high reproducibility and reliability of the proposed method. The % recoveries were good agreement with those obtained by Singh *et. al.*, (80-106 %)[41].

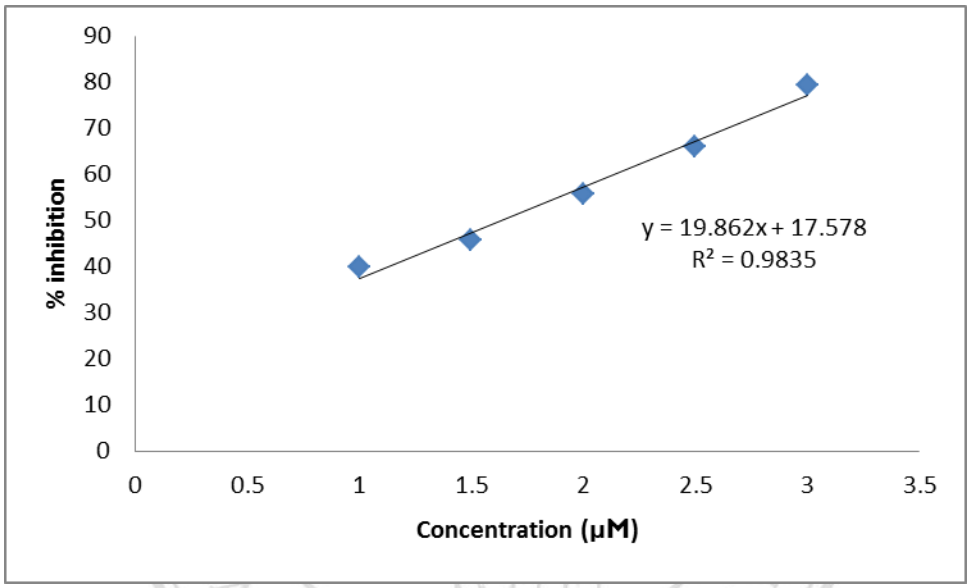

Figure 4.9 Standard calibration curve of dimethoate

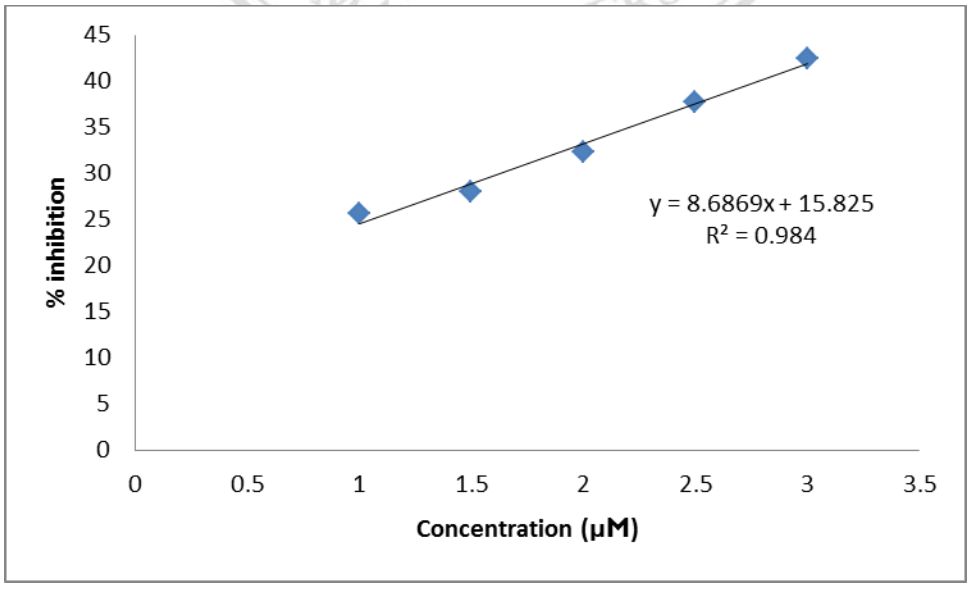

Figure 4.10 Standard calibration curve of profenofos

Table 4.1 % Recovery and RSD of the detection of OPs in spiked real sample using proposed colorimetric assay.

Organophosphate	Added (μM)	Founded (μM)	Recovery (%)	RSD (%) (SD/Mean *100)
Dimethoate	2.0	1.90	95	3
		2.02	101	
		1.97	98	
		Mean = 98 ± 3		
	2.5	2.58	103	2
		2.53	101	
		2.62	105	
		Mean = 103 ± 2		
Profenofos	2.0	2.05	103	3
		1.08	104	
		1.98	99	
		Mean = 102 ± 3		
	2.5	2.54	102	2
		2.59	104	
		2.48	99	
		Mean = 102 ± 2		

4.4 Determination of OPs in the spiked samples by the GC-MS.

The GC-MS chromatograms of OPs standards were good separation as shown in Figure 4.11. The retention time of the pesticides were 4.97 and 8.73 min for dimethoate and profenofos, respectively. The chromatograms with and without adding OPs into the samples were established in Figure 4.12 and 4.13, respectively.

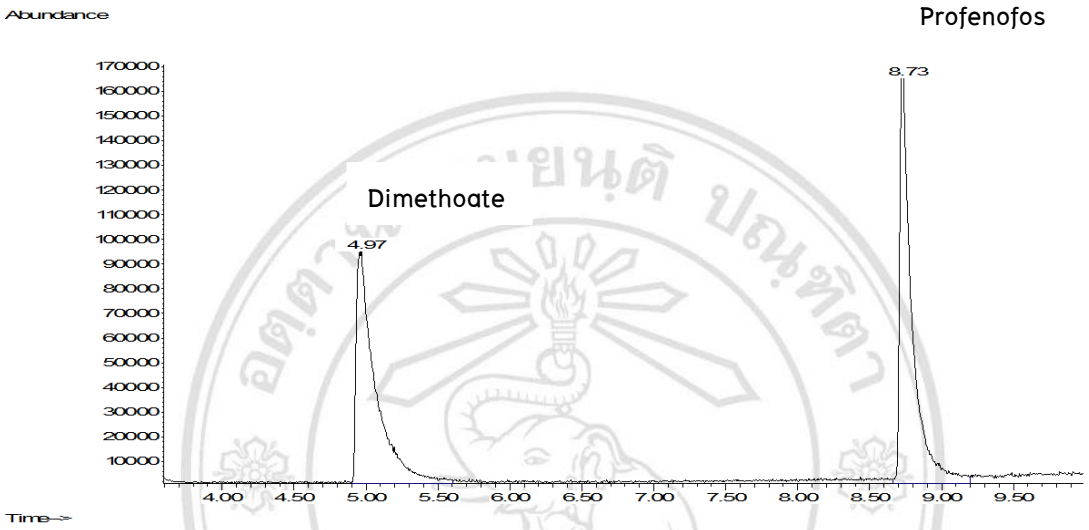

Figure 4.11 Typical chromatograms of the pesticide standards (1.0 µM)

Dimethoate and profenofos in spiked samples with 2 concentrations of each standard were determined by the GC-MS. RSD and % recoveries were calculated from the standard calibration curves as shown in Figure 4.12 and the typical chromatogram of the sample with no addition of OPs shown in Figure 4.13. No observable signal of OPs was found.

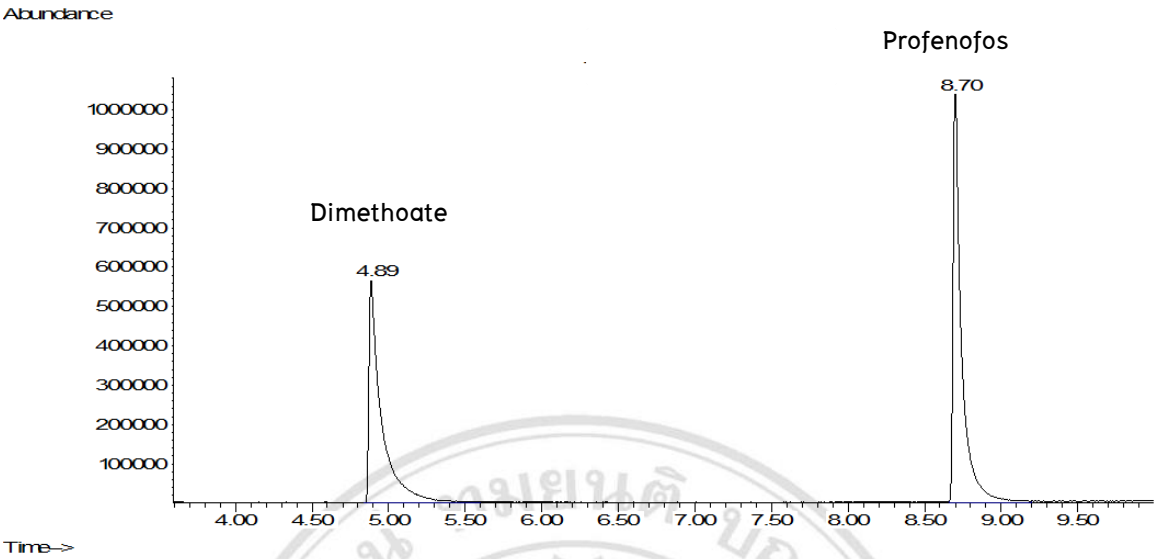

Figure 4.12 Typical chromatograms of the sample spiked with mixed pesticides standard at 2.0 µM.

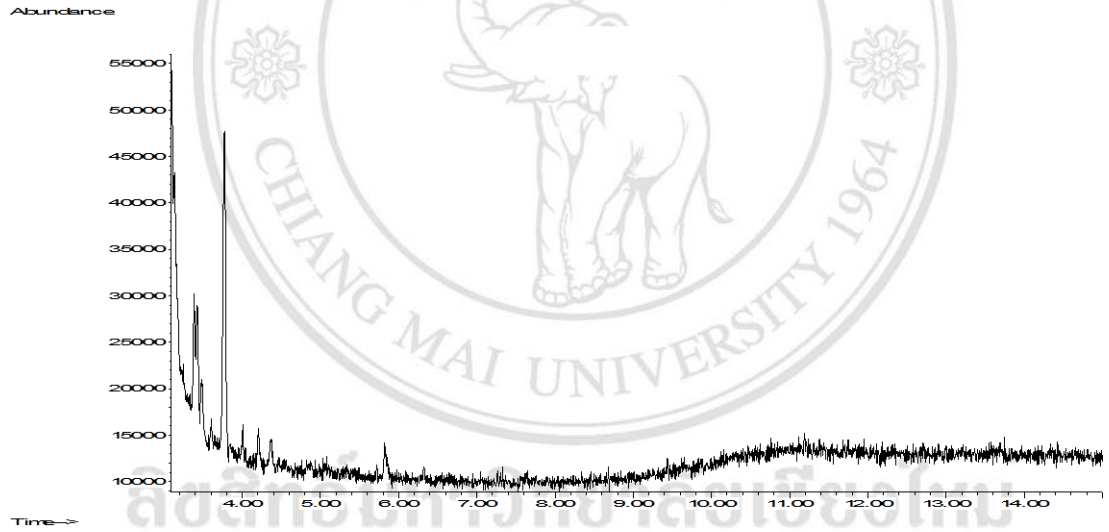

Figure 4.13 Typical chromatograms of the sample with no addition of OPs

Table 4.2, shows the data obtained from GC-MS. RSD was less than 2% for the detections of both pesticides, indicating the precision of the method. % recoveries for the detections of OPs were found in range of 99-105.

Table 4.2 Data for detection of OP standards in spiked real sample using GC-MS

Organophosphate	Added (µM)	Founded (µM)	Recovery (%)	RSD (%) (SD/Mean *100)
Dimethoate	2.0	2.09	105	1
		2.06	103	
			Mean = 104 ± 1	
	2.5	2.55	102	1
		2.53	101	
			Mean = 102 ± 1	
Profenofos	2.0	2.04	102	2
		1.97	99	
			Mean = 100 ± 2	
	2.5	2.48	99	1
		2.51	100	
			Mean = 99.6 ± 1	

In order to validate the proposed method, Table 4.3 shows that % recoveries of the detection from colorimetric assay has good agreement with GC-MS method. Therefore, our method has a potential to the detection of OPs in the real sample.

Table 4.3 Comparison of the proposed method for detection of OPs with the standard GC-MS method

Organophosphate	Standard spiked (µM)	% Recovery	
		Colorimetric method	GC-MS method
Dimethoate	2.0	98 ± 3	104 ± 1
	2.5	103 ± 2	102 ± 1
Profenofos	2.0	102 ± 3	100 ± 2
	2.5	102 ± 2	99.6 ± 1