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DETERMINATION OF MALATHION AND GLUFOSINATE-AMMONIUM IN PALM OIL MILL EFFLUENT USING HIGH PERFOMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

The analysis of palm oil mill effluent (POME) is carried for determination of organophosphorus pesticide (OPPs) concentration namely malathion and glufosinate-ammonium. The sample pesticide is taken at three palm oil mill effluent pond which is Felda Palm Industries Sdn Bhd - KKS Lepar Hilir, Kilang Sawit Felda Chini 2 and Kilang Sawit Felda Chini 3. As to confirm the aim of pesticide that discharge from the production mill, the analysis is carried by using High-Perfomance Liquid Chromatography (HPLC). The HPLC is used for the separation and detection of the malathion and glufosinate-ammonium in the palm oil mill effluent sample. It is expected that there is malathion and glufosinate-ammonium in the POME. As the analyte presence in the sample, the concentration of it is obtained by plotting calibration curve. The area obtain by the POME chromatogram was compared with standard analyte as to get the individual concentration. As the result yield, all of the palm oil mill sampling site show the presence of the malathion and glufosinate-ammonium. But then, the identification of the concentration was due to the value of LOD and LOQ.

ABSTRAK

Analisis sisa kumbahan kilang pemprosesan kelapa sawit (POME) akan dilakukan untuk menentukan kepekatan racun organofosforus (OPs) yang bernama malathion dan glufosinate-ammonium. Sample racun akan di ambil di tiga kawasan kilang pemprosesan kelapa sawit iaitu Felda Palm Industries Sdn Bhd - KKS Lepar Hilir, Kilang Sawit Felda Chini 2 dan Kilang Sawit Felda Chini 3. Untuk mengesahkan racun yang dikeluarkan oleh kilang pemprosesan, analisis tersebut dijalankan menggunakan Cecair Kromatografi Prestasi Tinggi (HPLC). HPLC ini digunakan untuk pemisahan dan penentuan malathion dan glufosinate-ammonium dalam POME. Dengan kehadiran analit dalam sampel, kepekatannya ditentukan dengan memplotkan lengkungan kalibrasi. Luas yang diperoleh dari kromatogram POME akan dibandingkan dengan ukuran dasar analit demi mendapatkan kepekatan masing-masing. Setelah keputusan diperoleh, kesemua kilang pemprosesan kelapa sawit menunjukkan kehadiran malathion dan glufosinate-ammonium. Tetapi, penentuan kepekatan racun berdasarkan nilai LOD dan LOQ

TABLE OF CONTENTS

		Page
SUPERVISOR'S DECLARATION		**ii**
STUDENT'S DECLARATION		**iii**
ACKNOWLEDGEMENTS		**iv**
ABSTRACT		**v**
ABSTRAK		**vi**
TABLE OF CONTENTS		**vii**
LIST OF TABLES		**ix**
LIST OF FIGURES		**xi**
LIST OF ABBREVIATIONS		**xiii**
CHAPTER 1	**INTRODUCTION**	
1.1	Background of Study	1
1.2	Problem Statements	2
1.3	Objective of Study	3
1.4	Scope of Study	3
CHAPTER 2	**LITERATURE REVIEW**	
2.1	Introduction	4
2.2	Glufosinate-ammonium	4
2.3	Malathion	5
2.4	Instrumental method	6
2.5	Palm oil mill effluent	8
CHAPTER 3	**METHODOLOGY**	
3.1	Chemicals and material	11

3.2	Equipments	11
3.3	Chromatographic conditions	12
3.4	Preparation of working standards for analysis pesticide	13
	3.4.1 Preparation of standard from solid.	14
	3.4.2 Preparation of standard from liquid.	14
	3.4.3 Preparation of ammonium-acetate	14
3.5	Collection of palm oil mill effluent	15
CHAPTER 4	**RESULTS AND DISCUSSION**	
4.1	HPLC parameter optimization	17
	4.1.1 Malathion detection	19
	4.1.2 Glufosinate-ammonium detection	20
4.2	Method validation	21
4.2.1	Limit of detection	21
4.2.2	Limit of quantification	22
4.3	Real sample analysis	22
	4.3.1 Lepar sampling site	22
	4.3.2 Chini 2 sampling site	23
	4.3.3 Chini 3 sampling site	24
CHAPTER 5	**CONCLUSION AND SUGGESTION**	
5.1	Conclusion	25
5.2	Suggestions	25
REFERENCES		**27**

LIST OF TABLES

Table No.		Page
2.1	Properties of pesticide	6
2.2	Comparison of HPLC usage	8
2.3	Content of POME	10
3.1	Properties of HPLC	12
3.2	Composition of mobile phase for malathion	13
3.3	Composition of mobile phase for glufosinate-ammonium	13
4.1	Isocratic mode	17
4.2	Gradient elution for malathion	18
4.3	Gradient elution for glufosinate-ammonium	18
4.4	Data for malathion at different sampling point	19
4.5	Data for glufosinate-ammonium at different sampling point	20
4.6	Summary of the calibration curve	21
4.7	Correlation coefficient and LOD of each pesticide studied.	22
4.8	Correlation coefficient and LOQ of each pesticide studied	22

LIST OF FIGURES

Figure No.		Page
2.1	Structure of glufosinate-ammonium	5
2.2	Structure of malathion	5
4.1	Calibration curve for malathion	19
4.2	Calibration curve for glufosinate-ammonium	20

LIST OF ABBREVIATIONS

IMS	Ion mobility spectrometry
PP	Polypropylene
OP	Organophosphorus
HPLC	High performance liquid chromatography
POME	Palm oil mill effluent
GC	Gas chromatography
LOD	Limit of detection
LOQ	Limit of quantification

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND OF STUDY

In recent years, the public has become more concerned about the extensive use of herbicides and their effects on the environment on a global scale. (Songa et al., 2009). Among the herbicides used, glyphosate and glufosinate are two important examples and are broad-spectrum, nonselective herbicides for control of long grasses and broad-leaved weeds (Songa et al., 2009). The United Nations Food and Agricultural Organization (FAO) has set maximum residue limits (MRLs) for residues of glyphosate and glufosinate on most crops at 0.1–5 and 0.05 mg kg –1, respectively (Songa et al, 2009)

Currently, glyphosate is in the list of the United States national primary drinking water contaminants with a maximum contaminant level goal (MCLG) of 0.7 mg L−1 (Guo et al., 2005). In many countries, the glyphosate is known as tradename Roundup. From Guo et al, (2005) since glyphosate is used for different application and has been widely spread around the world indicating potential toxicological risk to humans, it is necessary to develop rapid, easy and sensitive method monitor glyphosate residue in the environment. However, in comparison to all herbicides used in agriculture, glyphosate is probably the most difficult to analyze (Coutinho et al., 2007)

The organophosphorus compound malathion is extensively used as insecticide and acaricide in agricultural, veterinary, medical and public health practices.(Hazarika et al., 2003). Despite its high toxicity, malathion is still extensively used throughout the

al., 2009). Some studies have reported that if they are ingested over a period of time, they may affect the central nervous system, resulting in respiratory, myocardial, and neuromuscular malfunctions, which can even lead to death (Songa et al., 2009).

Organophosphorus insecticides like malathion are considered to be hazardous and have been known to potentially cause adverse effects on human health by inhibition of acetylcholinesterase activity in the body (Mohamed et al., 2010).

If impurities present in the sample interact with the analyte to change the instrumental response or themselves produce an instrumental response, then a calibration curve based on pure analyte samples will give an incorrect determination. (Songa et al., 2009)

1.3 OBJECTIVES OF STUDY

The objective of this study is to identify the concentration of glufosinate-ammonium and malathion in different sampling site of palm oil mill effluent (POME) using High Perfomance Liquid Chromatography (HPLC).

1.4 SCOPES OF STUDY

The scope of this study basically is to identify the presence of malathion and glufosinate-ammonium in POME. This is carried by analyze the POME using HPLC and compare it to the malathion and glufosinate-ammonium standard. Once the existence of malathion and glufosinate-ammonium is identify, the concentration of glufosinate-ammonium, and malathion in the palm oil mill effluent is determine. This concentration was obtained by comparing the chromatogram of real sample with malathion and glufosinate-ammonium standard.

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

Organophosphorus compounds are extensively used as pesticides. Organophosphorus (OP) compounds are derivatives of phosphorus that have at least one organic (alkyl or aryl) group attached to the phosphorus atom either directly or indirectly by means of another element (e.g. oxygen, sulfur or nitrogen) (Quin, L.D., 2000). OP compounds are in many cases highly toxic, and some of these toxic OP compounds have importance as pesticides (Koskela, H. 2010). The majority of organophosphorus insecticides, except phosphates and phosphorothiolates, give rise to only slight inhibition of acetylcholinesterase by themselves, unless they are activated. (Błasiak et al., 1999).

2.2 GLUFOSINATE-AMMONIUM

Glufosinate ammonium, the active ingredient of the new non-selective herbicide BASTA is a phosphinic acid analogue of glutamic acid. (Ebert et al., 1990). The chemical structure of GLA [ammonium-DL-homoalanin-4-yl(methyl)phosphinate (IUPAC),CAS no. 77182-82-2] , the physico-chemical properties are as follows: mol wt, 198.2;appearance, white crystalline powder; sp. gr., 1.4(20 °C); m.p., 215 °C (pyrolysis); vapour pressure, 3.5 x 10-6 mm Hg (20 °C); solubility (water), 1370 g/litre (20 °C). The effectiveness of glufosinate-ammonium in controlling broad-spectrum weeds, especially Galium aparine (cleavers), in glufosinate-resistant winter oilseed rape has been reported (Merkel et al., 2004)

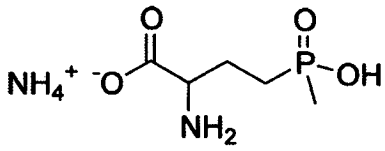

Figure 2.1: Structure of glufosinate-ammonium

2.3 MALATHION

Malathion is a widely used organophosphorus insecticide because of its relatively low toxicity to mammals and high selectivity towards insects compared to other organophosphorus insecticides.(Błasiak et al., 1999). Historically, two extraction methods have been used for pesticide residue analyses in fruits and vegetables: the Luke method, involving acetone extraction followed by partitioning with a mixture of dichloromethane and light petroleum, and a method involving ethyl acetate extraction in the presence of sodium sulfate (Na_2SO_4) as a drying agent (Hunter et al., 2010)

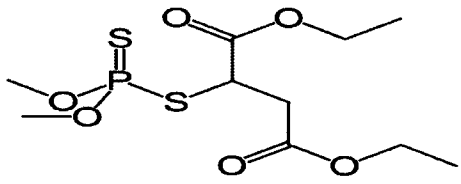

Figure 2.2: Structure of malathion

Table 2.1: Properties of pesticide

	Glufosinate-ammonium	Malathion
Tradename	Cythion, Malathion ULV Concentrate.	Basta, Buster and Liberty.
Molecular formula	$C_5H_{15}N_2O_4P$	$C_{10}H_{19}O_6PS_2$
Molar mass	198.2 g/mol	330.358021 g/mol
Melting point	205 °C	2.9 °C
Boiling point	433.91 °C	351.17 °C
Log K_{ow}	-4.49	2.36
Water Solubility	1e+006 mg/L	78.45 mg/L

2.4 INSTRUMENTAL METHOD

Ion mobility spectrometry (IMS) has been proven to be one of the best methods for the detection of trace level of organophosphorus compounds (Jafari, M.T, 2006). Ion mobility spectrometry (IMS) is an instrumental method in which two independent principles are combined to provide high speed response to trace levels of chemicals as gas or vapor species. In IMS, sample vapors are converted to ions at atmospheric pressure and those ions are characterized by their gas phase mobilities in weak electric fields. Early instruments for IMS exhibited picogram detection limits without sample preconcentration and generated strong interest in the technique. Organophosphorus compounds have high proton affinities and therefore readily produce positive ions in the reaction region of the ion mobility spectrometer, even in the presence of several other constituents present in ambient air (Jafari, M.T. 2006).

Normally, high-performance liquid chromatography has been used in combination with fluorescence and UV/VIS detection after derivatization, although in a few cases glyphosate has been determined directly by ion chromatography with UV detection or suppressed conductivity detection, but with limited sensitivity. (Songa et al.,

2009). The detection of the glyphosate derivatives in LC and GC exhibited high sensitivity and selectivity; however, these derivatization procedures are quite complicated and require special equipment. (Coutinho et al., 2007). For compounds that are anions in the mobile phase, such as glyphosate, this offers the possibility of a weak anion-exchange mechanism in addition to hydrophilic interactions (Coutinho et al., 2007)

Ion-exchange with LC–MS–MS was popular for the determination of glyphosate (Guo et al., 2005). However, it is one of serious disadvantages of ion exchange that this method provides no direct information on events occurring at the surfaces of the stationary phase, because the ion-exchange equilibrium is always determined by the balance between the solute interaction and the eluent interaction with active sites of a resin. (Okada, T. 1998). Ion-rexchange with LC–MS–MS was now popular for the determination of glyphosate .(Guo et al., 2005).

High-performance liquid chromatography will be used in the study. The sample standard of each analyte with different concentration will be analyse using the HPLC in order to get the calibration curve. Once the extraction using the CSM-LPME done, the analysis of real sample will be done and the result yield will be compared with the calibration curve.

Table 2.2: Comparison of HPLC usage.

References	Peng et al., 2009	Schuster, R., 1997	Tang T et al., 2010	Lucio et al., 2004
Title of journal	Determination of trace amounts of organophosphorous pesticides in water using high performance liquid chromatography.	Analysis of Pesticides in Salad Samples and Spices using HPLC.	Determination of triazole fungicides in environmental water samples by high-performance liquid chromatography.	High-performance liquid chromatographic determination of pesticides in tomatoes.
Type of HPLC	Shimadzu LC-20AT	Agilent 1100 series	Assemble from Modular Component	The HPLC system consisted of a Waters
Mode	-	Gradient elution	-	-
Mobile Phase	Methanol + water (90:10)	Water + ACN (95:5)	MeOH + water (75:25)	ACN + NH4OH (35:65)
Flowrate	0.6mL/min	0.5mL/min	1mL/min	0.7mL/min
Column	C18	Hypersil BDS	C18	RP-18 column
Detector	UV	UV	-	UV

2.5 PALM OIL MILL EFFLUENT

The sampling of the effluent was taken at Lepar and Chini palm oil mill. Those three palm oil mill spared a pond for discharge from the operation plant and used for treatment before it release to the river nearby. Regard of the huge pond, the sample taken on the surface of the freshly discharge effluent pond. These effluent streams are normally disposed of in drainage channels or stored in evaporation ponds or worse still discharged in arable lands to possibly avert the cost of treatment (Chris et al., 2010)

The sample should be preserved first in order to retain its existed compound and not degraded by the action of microbiology organism and bacteria. The preservation process should be done by adding pH 2 nitric acid at temperature 4 °C into the sample.

Stalikas and Konidari reported that phosphorus-containing amino acid–herbicides tend to be adsorbed on glass surfaces. To avoid unintentional adsorption, polypropylene (PP) bottles were used for storing the standard solutions. But, due to non-preserve the sample, the extracted sample was not reliable as the action of bacteria has taken place. Chemical degradation in water is a function of both pH and temperature. Malathion's half-life in double-deionized water was greatest at pH 4 and decreased rapidly with either increasing or decreasing pH. The researchers concluded that elimination reactions predominate at high temperatures and carboxyl ester hydrolysis reactions predominate at lower temperatures. The half-life of malathion in water was estimated as 1.65 days at pH 8.16 and 17.4 days at pH 6.0.(Wang, T. 1991).

Raw POME is a colloidal suspension containing 95–96 % water, 0.6–0.7 % oil and 4–5 % total solids including 2–4 % suspended solids that are mainly consisted of debris from palm fruit mesocarp generated from three main sources, namely sterilizer condensate, separator sludge and hydrocyclone wastewater. For a well-controlled conventional mill, about 0.9, 1.5 and 0.1 m^3 wastewater are generated from sterilizer condensate, separator sludge and hydrocyclone wastewater, respectively, for each tonne of crude palm oil produced. In the year 2004, more than 40 million tonnes of POME was generated from 372 mills in Malaysia. If the effluent is discharged untreated, it can certainly cause considerable environmental problems due to its high biochemical oxygen demand (25,000 mg/l), chemical oxygen demand (53,630 mg/l), oil and grease (8370 mg/l), total solids (43,635 mg/l) as well as suspended solids (19,020 mg/l). Therefore, the palm oil mill industry in Malaysia is identified as the one that produces the largest pollution load into the rivers throughout the country (Badroldin, N.A., 2010).

The discharge of POME to the pond may lead to generation of microb which effect the content of it, it is said that POME contains high concentrations of protein, carbohydrate, nitrogenous compounds, lipids and minerals that may be converted into useful materials using microbial processes. The discharge of untreated POME though creates adverse impact to the environment, the notion of nurturing POME and its derivatives as valuable resources should not be dismissed.

From environmental perspective, fresh POME is a hot and acidic brownish colloidal suspension, characterized by high amounts of total solids (40,500 mg/l), oil and grease (4000 mg/l), COD (50,000 mg/l) and BOD (25,000 mg/l) (Singh et al., 1999; Ma, 2000). POME has been identified as one of the major sources of aquatic pollution in Malaysia. The characteristic of a typical POME is shown below

Table 2.3: Content of POME

Parameter	Average	Metal	Average
pH	4.7	Phosphorus	180
Biochemical Oxygen Demand (BOD_5)	25000	Potassium	2270
Chemical Oxygen Demand (COD)	50000	Calcium	439
Total Solids	40500	Boron	7.6
Suspended Solids	18000	Iron	46.5
Total Volatile Solids	34000	Manganese	2.0
Ammonical Nitrogen	35	Copper	0.89
Total Nitrogen	750	Zinc	2.3

Source: LORESTANI, A.A.Z. (2004)

Palm Oil Mill Effluent (POME) is the largest palm oil industry by-product, it is a colloidal suspension containing 95–96 % water, 0.6–0.7 % of oil and grease and 4–5 % of total solids. It is a thick, brownish in color liquid with a discharged temperature of between 80 and 90 °C, being fairly acidic with a pH value in the range of 4.0–5.0 (Sethupath, S., 2004)

CHAPTER 3

METHODOLOGY

3.1 CHEMICALS AND MATERIALS

The reagent used for the experiment is malathion and glufosinate-ammonium. The stock solution was prepared by diluting malathion and glufosinate-ammonium with acetonitrile accordingly due to the different concentration. The sample prepare were stored in refrigerator under -18 °C when it is not in used.

The mobile phase for HPLC is acetonitrile HPLC grade, ultrapure water and ammonium acetate pH 4.2 that filtered before usage.

All the apparatus used in the experiment was washed using deionized water.

3.2 EQUIPMENTS

A burette 100 µL-1000 µL was used in order to transfer the solution for dilution process. The 1 µL- 10 µL was used for malathion regards its very small volume. All samples that undergoes dilution process was through vortex mixer to homogenized the solution.

Nylon 6,6 was impregnated of aim solvent before undergoes filtration process. The filtration was aimed to filter all the unwanted dust as not to clogging the HPLC column.

3.3 CHROMATOGRAPHIC CONDITIONS

For determination of malathion and glufosinate-ammonium using HPLC, C18 (4.6 X 150mm, 5 micron) X-Bridge column was used. The temperature of the column was 26.6 °C. The mobile phase used for the detection was acetonitrile HPLC grade, ultrapure water, and ammonium acetate. For each of analyte, the active compound was detected at wavelength 254 nm. There are two method as two detection of analyte was concerned, for malathion method, the run time was 16 minutes while glufosinate-ammonium was 21 minutes. Each of method used gradient elution mode. The injection volume for both analyte was 20 µL. After done with all the separation, the column washed by flowing 95 % ACN and 5 % water at least 5 minute until the linear baseline was achieved.

TABLE 3.1: Properties of HPLC

Column	C18 (4.6 X 150 mm, 5 micron) X-Bridge column
Phase	Reverse phase
Detector	Waters 2998 Photodiode-Array Detector (PAD)
Wavelength	254 nm

The HPLC analysis was carried by injecting the sample to the instrument as to separate the compound with the basis of polarity. The column used was C18, a polar compound which any compound with the same polarity will retain longer on the column, this phenomena could be describe as 'like dissolve like'. Elution order or retention time was the term to explain the time of sample soon it injected up to it detected by the detector. The mode used to run the HPLC was gradient elution which allowed the mixing of mobile phase. The composition of mobile phase over a time for malathion show as Table 3.1.

TABLE 3.2: Composition of mobile phase for malathion.

Time (minutes)	% Water	% Acetonitrile
0	95	5
12	50	50
15	40	60

For the of glufosinate-ammonium, other method was used.

TABLE 3.3: Composition of mobile phase for glufosinate-ammonium.

Time (minutes)	% Acetonitrile	% Ammonium Acetate
0	5	95
2	20	80
5	35	65
8	45	55
12	65	35
16	45	55
20	30	70

3.4 PREPARATION OF WORKING STANDARD AND MOBILE PHASE FOR ANALYSIS OF PESTICIDE.

The aim of this preparation of standard regard the instrument require small amount of analyte. The given standard for individual analyte is in concentrated form that have to be dilute to lower concentration. In order to produce 1 ppm, 2 ppm, 3 ppm, 4 ppm and 5 ppm, the concentration of analyte should be gradually decrease from higher concentration to lower concentration.

3.4.1. Preparation of standard from solid.

Regard of the formula, 1000 ppm = 10 mg of solute ÷ 10 ml of solution, it used to produce 1000 ppm of sample standard for glufosinate-ammonium . As the equation refer, 10mg of sample standard which is vinclozolin, glyphosate and glufosinate was taken out by micro-spatula and put in weighing boat for measurement using analytical balance. After getting 10mg of sample, the sample was inserted into the 10ml volumetric flask and filled the methanol up to the mark. Once the dilution process done, sealed the volumetric flask using parafilm to avoid any evaporation of analyte. The sample standard was homogenized using vortex mixer as to dilute all solid. The sample standard was stored in its individual freezing point. For the next dilution which is 100 ppm, 25 ppm, 20 ppm, 15 ppm, 10 ppm, 7 ppm 5 ppm, 3ppm and 1 ppm. The equation used was m1v1 = m2v2.

3.4.2. Preparation of standard from liquid.

To prepare 1000 ppm of malathion sample standard, a calculation regards of density needed as the information given is just density plus it is in very concentrated liquid form. The malathion then is pipette using micropipette 1 µL-10 µL and put into 10 mL volumetric flask that contain deionized water and top up to the mark. After the dilution done, the volumetric flask is seal with parafilm to avoid any evaporation or leakage. The solution is then homogenize using vortex mixer. For another concentration which is 100 ppm, 25 ppm, 20 ppm, 15 ppm, 10 ppm, 7 ppm 5 ppm, 3ppm and 1 ppm the equation $m1v1 = m2v2$ was used for dilution ratio of stock solution and deionized water.

3.4.3 Preparation of ammonium-acetate.

Ammonium acetate was prepared as to be a mobile phase for detection of glufosinate. It prepared by measuring approximately 0.3 g of ammonium-acetate using

analytical balance. 900 mL of HPLC grade water was inserted in 1 L beaker and 0.3 g of ammonium-acetate was dissolving into the water. Then, 0.5 mL of glacial acetic acid pH 4.2 was added into the beaker. The acetate buffer solution then was added 100 mL HPLC grade ACN and put into volumetric flask. As the solvent prepared, it undergoes degassing process by putting a magnetic bar and stir as to remove all gas before it used for HPLC mobile phase.

3.5 COLLECTION OF PALM OIL MILL EFFLUENT

The collection of palm oil mill effluent taken at Felda Palm Industries Sdn Bhd - KKS Lepar Hilir, Kilang Sawit Felda Chini 2 and Kilang Sawit Felda Chini 3. There are several pond on the palm oil processing, and each of them differ on its content of pesticide, microorganism, biological oxygen demand, chemical oxygen demand, dissolved oxygen and total suspended solid. The one that has taken was from the latest effluent which has been discharge from the operation mill. Soon the sample yield, it filtered as to separate the solid and any particulate matter. After the filtration done, it centrifuged as to vary the sample based on its density. The liquid part of it was taken and undergoes analysis using HPLC. After the filtration process done, the remaining effluent sample was preserved under as to maintain its freshness and not to degraded the composition of the sample. The effluent then was keep freezed at 4 °C.

The sample that has been analyze was palm oil mill effluent which taken from Felda Palm Industries Sdn Bhd - KKS Lepar Hilir, Kilang Sawit Felda Chini 2 and Kilang Sawit Felda Chini 3. Soon it taken from the effluent pond, it was filtered first to separate the colloidal suspension containing 95–96 % water, 0.6–0.7 % of oil and grease and 4–5 % of total solids.(Hojjat et al., 2009). As the extraction technique was carried, the sample should be preserved first in order to retain its existed compound and not degraded by the action of microbiology organism and bacteria. The preservation process should be done by adding pH 2 nitric acid into the sample as an option. But, the preservation of the sample done by stored the sample at -18 °C. Chemical degradation in water is a function of both pH and temperature. Malathion's half-life in double-

deionized water was greatest at pH 4 and decreased rapidly with either increasing or decreasing pH. The researchers concluded that elimination reactions predominate at high temperatures and carboxyl ester hydrolysis reactions predominate at lower temperatures. The half-life of malathion in water was estimated as 1.65 days at pH 8.16 and 17.4 days at pH 6.0.(Wang, T. 1991).

CHAPTER 4

RESULTS AND DISCUSSION

4.1 HPLC parameter optimization

The aim for the analysis is to detect the presence of malathion and glufosinate-ammonium in the sample and compare it to the standard. As in this experiment, the analysis was done to detect the pesticide in palm oil mill effluent and compare it to the standard. The analysis was carried by the aid of HPLC which not only provide the detection of sample, but the separation varies of compound. The standard given was 10 ppm, 15 ppm, 20 ppm and 25 ppm. By listed concentration, the calibration curve can be done thus the concentration of sample that lies between the calibration curve can be predicted.

Regard of the HPLC usage, the alteration of mobile phase was crucial to gave the faster elution of the sample. There are two mode of HPLC which isocratic mode and gradient elution mode, these modes was used through this analysis. At the very first run of the sample, the isocratic was performed as to identify when the aim analyte elute. Once the retention time was identified, the gradient elution mode was taken place which aim to alter the mobile phase composition for faster elution.

Table 4.1: Isocratic mode

	% Ultrapure water	% ACN	%Ammonium acetate
Malathion	95	5	0
Glufosinate-ammonium	0	5	95