DETERMINATION OF GLUFOSINATE-AMMONIUM AND MALATHION IN RESIDUE OIL FROM PALM PRESSED FIBER USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

MUHAMMAD HAZIQ BIN RUSSLI

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Faculty of Industrial Sciences & Technology
UNIVERSITI MALAYSIA PAHANG

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ABSTRACT

DETERMINATION OF GLUFOSINATE AMMONIUM AND MALATHION IN RESIDUE OIL FROM PALM PRESSED FIBER USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

A study on the determination of organophosphorus pesticides (OPPs), namely glufosinate-ammonium and malathion in residue from palm pressed fiber (PPF). Three PPF samples were taken from three different areas, FELDA Lepar, FELDA Chini 1 and FELDA Chini 2.The extraction of oil residue in PPF was carried out using supercritical fluid extraction (SFE), using supercritical carbon dioxide (CO_2) at 3000 psi in isothermal in 60°C for 60 minutes. Residue then were diluted uisng acetonitrile. Chromatogram of peak analyte was determine using Waters Alliance high performance liquid chromatography (HPLC) Dissolution System Series liquid chromatography interfaced to a 2998 Photodiode Array Detector. Determination was first done using standard malathion and glufosinate-ammonium of 10, 15 and 20 ppm run on a iscoratic elution mode for 30 minutes. Optimization method later was done using water and acetonitrile as mobile phase and run for 16 minutes for malathion and 20 minutes for glufosinate-ammonium. Malathion peak is retained at 8.0 minutes while glufosinate-ammonium at 16.0 minutes. Calibration curve of the standards were plot with R^2 values of 0.9085 for malathion and 0.9249 for glufosinate-ammonium. The detection limits (LOD) were 10.3 mg/L for malathion and 14.1 mg/L for glufosinate-ammonium. Later, determination of malathion and glufosinate-ammonium were done on real samples. For malathion determination, sample from Lepar showed to have concentraion of 19.46 ppm, Chini 1 (14.34 ppm) and Chini 2 (9.72 ppm). Determination of glufosinate-ammonium showed sample from Lepar to have concentraiton of 12.64 ppm, Chini 1 (17.72 ppm) and Chini 2 (39.64 ppm).

Satu kajian untuk menentukan kehadiran racun perosak jenis organophosphorus (OPPs), iaitu glufosinate-ammonium dan malathion di dalam mendakan minyak daripada fiber kelapa sawit tertekan (PPF) dijalankan. Tiga PPF sampel diambil dari tiga tempat berlainan, FELDA Lepar, FELDA Chini 1 dan FELDA Chini 2. Proses pengekstrakan mendakan minyak dilakukan dengan menggunakan supercritical fluid extraction (SFE), dengan menggunakan supercritical karbon dioksida (CO_2) pada tekanan 3000 psi didalam suhu tetap 60°C selama 60 minit. Mendakan minyak kemudian dilarutkan menggunakan acetonitirle. Kromatogram puncak komponen sasaran dikenal pasti dengan menggunakan Waters Alliance high performance liquid chromatography (HPLC) Dissolution System Series liquid chromatography yang disambungkan kepada 2998 Photodiode Array Detector. Proses dimulakan dengan menggunakan sampel malathion dan glufosinate standard berkepekatan 10, 15 dan 20 ppm, pada mod aliran isokratik selama 30 minit untuk mendapatkan proses yang optimum. Kemudian proses optimum dijalankan dengan menggunakan air dan acetonitrile sebagai mobile phase selam 16 minit untuk malathion dan 20 minit untuk glufosinate-ammonium. Puncak malathion dikenal pasti muncul pada minit ke 8 dan glufosinate-ammonium pada minit ke 16. Lengkungan penentu ukuran kemudian diplotkan dengan nilai R^2 adalah 0.9085 untuk malathion dan 0.9249 untuk glufosinate-ammonium. Had penentuan (LOD) pula adalah 10.3 mg/L untuk malathion dan 14.1 mg/L untuk glufosinate-ammonium. Kemudian, kehadiran malathion dan glufosniate-ammonium dieknal pasti pada sampel sebenar. Unutk malathion, sampel Lepar mencatatkan kepekatan 19.46 ppm, Chini 1 (14.34 ppm) dan Chini 2 (9.72 ppm). Untuk glufosinate-ammonium pula, sampel dari Lepar mencatatkan kepekatan 12.64 ppm, Chini 1 (17.72 ppm) dan Chini 2 (39.64 ppm).

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LIST OF ABBREVIATIONS

AChE	Acetylcholinesterase
CNS	Central nervous system
CO_2	Carbon dioxide
CPO	Crude palm oil
EPA	Environmental Protection Agency
GC	Gas chromatography
HPLC	High performance liquid chromatography
LC	Liquid chromatography
LSC	Liquid solid chromatography
NMR	Nuclear magnetic resonance
OPPs	Organophosphorus pesticides
PDA	Photodiode array detector
PPF	Palm pressed fiber
SFE	Supercritical fluid extraction
UV	Ultraviolet

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND OF STUDY

Malaysia is the world's largest exporter of palm oil, accounting for about 61.1% or 10.62 million tons of the total exports of 17.37 million tons based on 2001 statistics (Hai, T.C., 2002). In the early 1970s 56,674 hectares of land was planted with oil palm, increased to the latest data at 2002, about 3.6 million hectares are used to plant oil palm trees (Abdullah, R., 2003; Teoh, C.H., 2002). The increasing areas of plantation plant contribute to the increasing requirement of pesticides. Wibawa, W. et al. stated that in 2004, the herbicide usage in Malaysia was 67.49% of the total pesticides used and in 2005, more than 15.6 million liter was used in oil palm alone.

Pesticides consists of insecticide, fungicide, herbicide, organophosphorus pesticides (OPPs). Herbicides are known to increase specific plant disesase (Altman and Campbell, 1977; Hornby et al., 1998; Mekwatanakarn and Sivasithamparam, 1987), and several are reported to influence micronutrient availability (Evans et al., 2007; Huber et al., 2004, 2005). Some 1000-1500 new chemicals are manufactured each year with perhaps 60,000 chemicals in daily use (OECD, 1981). Glyphosate and glufosinate are two of the world's most widely used non-selective OPPs herbicides. Although they are not considered to present a major risk to human health, recent evidence suggest that exposure may cause neurological disorders

(Barbosa, E.R., et al., 2001). Glufosinate can affect the nervous system. Glutamate is an 'excitatory' neurotransmitter in the brain, and it appears to affect some of the processes in the nervous system that normally involve glutamate.

The usage of pesticide is to eliminate the non-desired plants, thus stimulate the growth of the palm oil plant, can be resulting of the presence of pesticide in the plant, or in residue oil. The residue oil is in the palm pressed fiber (PPF), a byproduct of palm oil milling. It usually used to generate electricity supply for mill and small housing estates around the mill (Basiroh and Simeh, 2005). Malaysia produce about 8.56 million tons of PPF per year (Hussain et al., 2003) at about 5-6% residue oil content, it such a waste to burnt it to produce electricity. Some of the contents in the residue oil are highly valuable which are carotenes, tocols, sterols, squalene and phospholipids.

Many attempts have been made to recover the residue oil from PPF including solvent extraction and supercritical fluid extraction (SFE) (Choo, et al., 1996; Lau, et al., 2006;de Franca and Meireles, 2000). Application of supercritical carbon dioxide (SC-CO_2) extraction has advantages over the solvent extraction method as it uses non-hazardous and non-inflammable CO_2 when compared to highly flammable petroleum-based solvent such as hexane or acetone (Lau, H.L.N., et al., 2007). SC-CO_2 extraction has been used to concentrate minor constituents from various oilseed and other products. These include the isolation of tocopherols from soybean and canola oil deodorizer distillates (Mendes, et al., 2002), sterols and tocopherols from olive oil (Ibanez, et al., 2002), squalene from PPF and palm leaves (Britigh, et al., 1995; Choo, et al., 1996; de Franca, et al., 2000).

1.2 OBJECTIVE

The objective of this study is:

1. To analysis the presence of OPPs in real sample from different area.
2. To conduct real sample analysis of OPPs using supercritical fluid extraction (SFE) using supercritical CO_2.
3. To optimize the parameter (mobile phase) for the determination of OPPs using high performance liquid chromatography (HPLC).

1.3 PROBLEM STATEMENT

Previous experiments use Soxhlet extraction, which is a versatile classical sample preparation technique prescribed in many standard analytical methods. Soxhlet extraction is considered to be a time consuming, tedious and uses a lot of extraction solvent.

1.4 SCOPE OF STUDY

Scope of study focus on determination of glufosinate ammonium and malathion in residue oil from palm pressed fiber (PPF). In this study, samples from different area was used to compare the presence of selected OPPs. Samples were extracted using SFE by supercritical carbon dioxide (CO_2).

Extracted analytes then were analyzed using high performance liquid chromatography (HPLC). Optimization of extraction parameter will not be carried out since this study is more towards to environmental analysis.

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION TO THE PALM OIL

Oil palm refers to the palm, *Elais guineensis Jacq.* (Hatrley, 1988). Oil palm produces two types of oil, palm oil from the fibrous mesocarp and palm kernel oil, a lauric oil from the palm kernel. The development of the oil palm industry in Malaysia is attributed to Frechman, Henri Fauconnier and his association with Hallet. In 1911, Fauconnier visited Hallet's oil palm development in Sumatra and had purchased some oil palm seeds and these were planted at his Rantau Panjang Estate in Selangor (Tate, 1996). With seedlings obtained from the 1911 and 1912 importation, Fauconnier established the first commercial oil palm planting at Tennamaram Estate, to replace an unsuccessful planting of coffee bushes (Tate, 1996).

Gray (1969) and Hacharan Singh (1976) had analyses of palm oil industry in Malaysia, and classified the development of the industry in Peninsular Malaysia into three distinct phases. Starting with the experimental phase for the late 1800s early 1900 to 1916 while the plantation development phase commenced in 1917 with Tennamaram Estate until about 1960. To reduce the dependence of the national economy on natural rubber, the Government decides to expand on oil palm, by the recommendation of the World Bank Mission in 1955. The palm oil industry has since undergone two further phases, from 1970 with the expansion of large scale planting in Sabah and Sarawak and from around 1955 when Malaysian extended their upstream operations off-shore, particularly to Indonesia where there is adequate supply of workers and availability of land for plantation development and cost of production is lower than in Malaysia.

2.1.1 Oil Palm

An oil palm fruit bunch consists of two main parts, the stalk and the fruit lets. The fruit lets are made up of pericarp (mesocarp and endocarp) and the nut. Two types of oils are produced from the fruit, namely palm oil from the oil cells in mesocarp and palm kernel oil from seed (kernel) of the nut. These oils are extracted and recovered separately in palm oil mill and kernel crushing plant. In general a palm oil mills produce the following products and by-products from the fresh fruit bunch: 20% crude palm oil (CPO), 23% empty bunch, 5-7% kernel depending on type of fruit and 15% fiber. After CPO is extracted from the fruit by screw press, this fiber is referred to as palm pressed fiber (PPF) and contains 5-6% residue oil, which is burnt, along the with the valuable oil to provide steam and power for the mill. PPF is highly valuable with high level of phytonutrients content like carotenoids, tocopherols and tocotrienol. The extraction can be done using hexane of food grade as solvent.

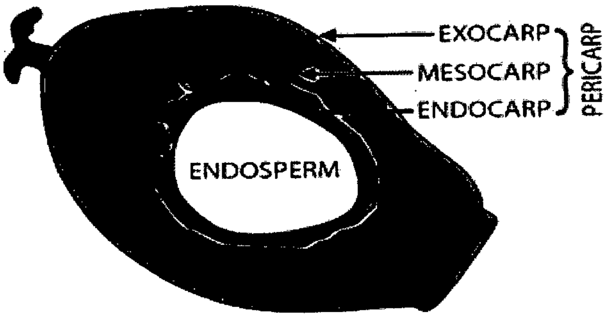

Figure 2.1: Parts in palm oil fruit.

Source: http://www.etawau.com

2.2 ORGANOPHOSPHATES

2.2.1 Organophosphorus Pesticides

OPPs are normally esters, amides or thiol derivatives of phosphoric, phosphonic, phosphorothioic or phosphonothioic acids. Most are only slightly soluble in water and have a high oil-to-water partition coefficient and low vapor pressure (International Programme on Chemical Safety Databank, 1986).

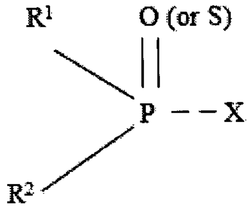

Figure 2.2: General chemical structure of OPPs with R^1 and R^2 as alkyl substitutions.

Source: International Programme on Chemical Safety Databank. 1986.

R^1 and R^2 are usually simple alkyl or alkyl groups, both of which may be bonded directly to phosphorus (in phosphinates), or linked via –O-, or –S- (in phosphates), or R^1 may be bonded directly and R^2, bonded via one of the above groups (phosphonates). In phosphoramidates, carbon is linked to phosphorus through an –NH group. The group X can be any one of a wide variety of substituted and branched aliphatic, aromatic, or heterocyclic groups linked to phosphorus via bond of some liability (usually –O- or –S-) and is referred to as the leaving group. The double-bonded atom may be oxygen or sulfur and related compounds would, for example be called phosphates or phosphorothioates (the nomenclature 'thiophosphate' or 'thionophosphate' is now less used).

In 1820, effects on human beings from the exposure to the OPPs can cause musacarinic, nicotinic and central nervous system (CNS) manifestations. Symptoms may develop rapidly or there may be a delay of several hours after exposure before they become evident. The delay tends to be a longer in the case of more lipophilic compounds, which also require metabolic activation. Symptoms may increase in severity for more than one day and may last for several days.

Continuous long-term exposure to high levels of OPPs may precipitate typical cholinergic symptoms, though most of the compounds do not accumulate extensively in the body.

As OPPs are commonly used on vegetation in Malaysia, such as acephate, diazinon, dichlorvos, dimethoate, fenitrothion, malathion, methamidophos, phenoate, prothiophos, triaziphos and tolcofos-methyl, numerous methods have been developed for the analysis of OPPs (Lee et al., 1991). Bio-assay, enzymatic methods, nuclear magnetic resonance (NMR), mass spectrometry, polarography and partition function are among applied technique for analysis of organophosphorus compounds (Thomas, 1974).

2.2.2 Glufosinate

The ammonium salt of glufosinate was first registered in the U.S. for use as an herbicide in 1993 by Hoescht Celanese (Caroline, C., 1996). Glufosinate inhibits the activity of an enzyme involved in the synthesis of the amino acid glutamine. This enzyme is called glutamine synthethase. Essentially, glufosinate acts enough like glutamate, the molecule used by this enzyme to make glutamine, that it blocks the enzyme's usual function. Glutamine synthetase is also involved in ammonia detoxification. Treatment with glufosinate leads to reduced glutamine and increased ammonia levels in plant tissues. This causes photosynthesis to stop, the plant to wither, and then die within a few days

(Horlein, G., 1994). Although most herbicides are not nerve poisons, glufosinate can affect the nervous system. Glutamate is an 'excitatory' neurotransmitter in the brain, and it appears to affect some of the processes in the nervous system that normally involve glutamate (U.S. EPA, 1985).

Whether or not glufosinate causes cancer is ambiguous. One of the studies submitted in support of its registration, a long-term feeding study in rats, showed an increase in the frequency of adrenal medullary tumors. (U.S. EPA, 1992). But still, the lack of publicly available studies about the ability of glufosinate-containing products to cause cancer cannot confirm this study.

There are some cases that glufosinate was found in the edible parts of spinach, radishes, wheat, and carrots that had been planted 120 days after treatment with a glufosinate-containing herbicides (U.S. EPA, 1988).

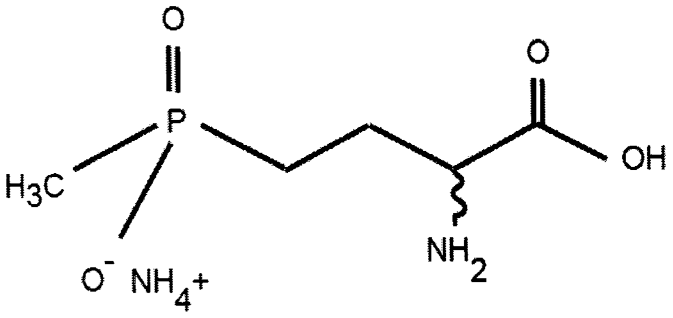

Figure 2.3: Chemical structure of glufosinate ammonium

Source: Journal of Pesticide Reform, 2003

IUPAC name : Ammonium (2*RS*)-2-amino-4-(methylphosphinato)butyric acid
CAS number : 77182-822
Molecular weight : 198.16 g mol^{-1}
Molecular formula : $C_5H_{15}N_2O_4P$
Log Kow : -5.34
Boiling point : 433.91°C
Melting point : 272.12°C
Vapor pressure : 1.58 e^{-1} mmHg at 25°C
Water solubility at 25°C : 1.0 e^{6} mg/L

2.2.3 Malathion

Malathion is an insecticide in the organophosphate chemical family. It is one of the oldest insecticides in that family and has been used since 1950 (Ware, G.W., 2000). Malathion is the most commonly used insecticide in the U.S., the U.S. Environmental Protection Agency (EPA) estimates that annual use of malathion is over 30 million pounds (Donaldson, D.T., and Grube, A., 2002). Malathion kills insects by converting inside animals into maloxon, a chemical relative that inhibits an important central nervous system enzyme called acetylcholinesterase (AChE) (U.S. EPA, 2000). AChE is involved with the transmission of nerve impulses. When this enzyme is inhibited, the transmission 'jams' resulting in restlessness, hyperexcitability, convulsions, paralysis, and death. All insecticides in the OPPs chemical family share a similar mode of action (Journal of Pesticide Reform, 2003).

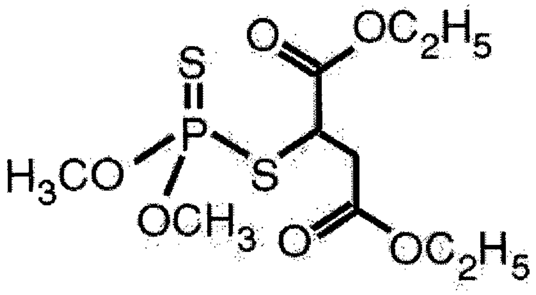

Figure 2.4: Chemical structure of malathion

Source: Journal of Pesticide Reform, 2003

IUPAC name : Diethyl [(dimethoxyphosphorothioyl)sulfanyl]butanedioate
CAS number : 121-75-5
Molecular weight : 330.35 g mol^{-1}
Molecular formula : $C_{10}H_9N_2O_4P$
Log Kow : 2.29
Boiling point : 433.91°C
Melting point : 272.12°C
Vapor pressure : 1.58 e^{-1} mmHg at 25°C
Water solubility at 25°C : 1.0 e^{6} mg/L

2.3 SUPERCRITICAL FLUID EXTRACTION

The unique solvent properties of supercritical fluids were first reported well over 100 years ago in 1879 by Hannay and Hogarth (Jonin, T.M., et al., 1986), who measured the solubility of several inorganic salts in supercritical ethanol. By 1980s and 1990s, supercritical fluids have been used in several industrial processes, including decaffeination of coffee (Brunner, G., et al., 2005) and tea, extraction of hop flavor for the beer industry and extraction of lipids and aromas from plant material. Other use including as solvents for supercritical chromatography (Jonin, T.M., et al., 1986).

A fluid is considered supercritical if it exists at conditions above its critical pressure and temperature. These critical values correspond to conditions in which condensation into a liquid or evaporation into a gas is no longer possible.

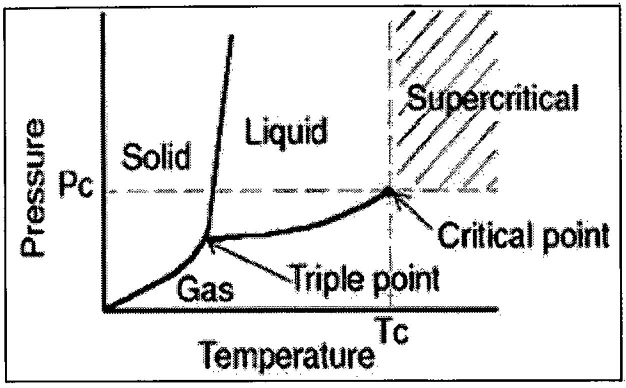

Figure 2.5 : Typical pressure-temperature projection of a phase diagram for pure material

Source : http://www.eolss.net/Sample-Chapters/C10/E5-10-04-08.pdf

The supercritical fluids most commonly used are CO_2, ethane, ethane, propane, ammonia and water. However, CO_2 is preferred because of its convenient critical temperature, cost, chemical stability, non-flammability and non-toxicity. Disposal of CO_2 is more environmentally friendly than for most other organic solvents typically used in extraction processes. It can be obtained in large quantities as a byproduct of several reactions, such as fermentation, combustion and ammonia synthesis. Another advantages of using supercritical CO_2 is that once the extract returns to standard conditions of pressure and temperature, the CO_2 returns to a gas phase and the extracted product precipitates since it is no longer soluble in the gas (Jonin, T.M., et al., 1986).

Recently, a different approach has been used to increase the extraction efficiency using pure CO_2. That is, raising the extraction temperature while using pure CO_2. At higher pressure and temperature, the solvent strength of the carbon dioxide is increased. This helps to increase the extraction recovery. However, when extraction temperatures are in the neighborhood of the vapor pressure of the compound of interest, the extraction recovery is significantly increased. In other word, the solubility of the analyte is influenced not only by the density of the CO_2, but also by the vapor pressure of the target analyte. And solubility of the compound of interest is not significantly influenced by the density of CO_2, compared to the vapor pressure of the analyte (Patel, S., 1999).

2.4 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

2.4.1 Introduction

High performance liquid chromatography (HPLC) is a technique that has arisen from the application to liquid chromatography (LC) of theories and instrumentation that were originally developed for gas chromatography (GC) (Sandie, L., and John, B., 1987). In the original method, an adsorbent for instance alumina or silica is packed into a column and is eluted with a suitable liquid. The separation of the solutes is possible if there are differences in their adsorption by the solid. This method is called adsorption chromatography or liquid solid chromatography (LSC) (Sandie, L., and John, B., 1987).

The efficiency could be improve if the particle size of stationary phase materials used in LC could be reduced. As HPLC has developed, the particle size of the stationary phase materials used in LC has become progressively smaller. The stationary phases used today are called microparticulate column packings and are commonly uniform, porous silica particles, with spherical or irregular shape, and nominal diameters of 10, 5 or 3 μm.

2.4.2 Instrumentation

There are five major HPLC components and their functions. It consists of pump, injector, column, detector and computer. A typical HPLC set-up uses an isocratic pump, a water/buffer and methanol eluent, a C_{18} column and a ultraviolet (UV) -detector. Injections in the range 5-100 μL is performed by an autoinjector and the peak area response is evaluated by an integrator.

Basically, a pump is used to propel the solvent or eluent. It considered the most important component in LC system and its basic parameter is pumping system. The systems are positive-pressure system or constant-pressure pump and positive-flow system or constant-flow pump (Yost, R.W. et al., 1980).

The primary advantages of most constant-pressure pumps are simplicity and freedom from pulsations, resulting in smooth baselines. But it also suffers from several disadvantages. Flow rates can be change if the solvent viscosity changes due to temperature change. This translates to component location and identification becomes inaccurate or impossible. In quantitative analysis, the UV and the refractive index detectors used most frequently in LC are concentration sensitive. Changes in flow result in changes the eluent/sample dilution ration, or in other words changes in concentration which show up as changes in peak area.

While the constant-flow systems are generally of two basic types: reciprocating and positive displacement (syringe) pumps. The basic advantages of such system are their inherent ability to repeat elution volume and area, regardless of viscosity changes or column blockage or settling occurrence, up to the pressure limit of the pump (Yost, R.W. et al., 1980).

It is obvious that these pumps deliver a series of 'pulses' of the mobile phase. Detector will be disturbed by the pulsations, especially at high sensitivities. To avoid this problem, several methods have been developed and most simple involves placing a large (often 50 feet) coil of narrow-bore tubing in the line between the pump and the column. It acts as absorbent, absorbing the energy of the pulsations as the pump strokes.

The primary consideration in injector design is the need to provide a low-volume, completely swept area to avoid sample diffusion and exponential dilution (Yost, R.W., et al., 1980). There are two systems, injection through a septum which is historically been the most frequently used and septumless syringe-injector valves.

Ideally, the septum injection is designed so it can be swept into the column without back-mixing and subsequent band spreading. Its design so that when the needle to close more rapidly, effectively sealing the system. When this injector is used, 50-60 injections are routinely made into ordinary silicone rubber septums, often at pressures as high as 2000 lb/sq.in. Most syringe used has the capacity of 10 microliters. Needle length or injection depth must be regulated according to the instrument since inserting the needle into the top of the column usually results in a plugged syringe and no injection of sample (Yost, R.W., et al., 1980).

CHAPTER 3

METHODOLOGY

3.1 INTRODUCTION

To achieve the objective of this study, various literature reviews had been done to improve the understanding of this study. Through the readings, there are many methodologies to be reconsidered, depend on the researcher capability to reproduce and the availability of such as reagents, materials and instruments. The result from this study cannot be the sole measurement of the achievement of this study though, as the result depends on various factors, considering some of its are out of researcher control.

From the literature reviews, the methodology of this study can be divided into two stages. The first stage is optimization of parameters and second stage is real sample analysis. The outcome result from the real sample analysis may not as it expected from the first stage. This may be due to different behavior of real sample form the standard sample when analysis takes place.

3.2 EXPERIMENTAL DESIGN

3.2.1 Reagent and Materials

Materials

Fresh palm pressed fiber (PPF) collected from a local palm oil mill (FELDA, Pahang, Malaysia). Freshly collected samples were dried at 60-70°C in oven for about 12 hours. The samples is checked and mix homogenously for every an hour to prevent samples burnt. The average water content before drying was 17%.

Reagents and solutions

Glufosinate-ammonium, and malathion analytical standards were purchased from Sigma Aldrich (Germany), methanol, acetonitrile, acetic acid, ammonia, water, and deionized water.

3.2.2 Instrumentations

HPLC analyses were run on a Waters Alliance HPLC Dissolution System series liquid chromatpgraphy (Waters Corporation, Milford, Massachusetts, USA) interfaced to a 2998 Photodiode Array Detector (PDA). The analytical column was packed with C_{18} stationary phase (150 mm x 4.6 mm I.D., 4.5 μm). The detection wavelength of the detector was set at 254 nm.