

# CHAPTER I

## INTRODUCTION

### 1.1 Problem Definition

Lately, sample preparation steps for the determination of different pollutants in natural water have become vital. Because of the advancement in today's analytical instruments that offer excellent performance, sample preparation step becomes critical to obtain good method sensitivity and low detection limit. The ideal aims of all of extraction techniques are to increase the concentration of the analyte and simultaneously reducing the concentration of other matrix compounds. These steps are usually termed "sample enrichment" and "sample cleanup", respectively. There are others expected requirements such as the procedure must be environmental friendly, low cost, require less time consumption, compatibility to many instruments, reliable and simplicity, to minimize the loss of analyte.

The most classical extraction technique is liquid-liquid extraction (LLE). This technique uses organic solvent to extract analyte from aqueous sample. The basis is the partition of organic analytes between organic aqueous and aqueous phases. Because it is well established, the technique is widely used in laboratory worldwide and is commonly accepted as standard method by international agency such as the U.S. Environmental Protection Agency (EPA). The main drawback of this technique is the consumption of large quantity of organic solvent, and therefore increases high purchasing cost as well as waste treatment. Organic solvents are toxic to the chemists and the environment as well.

Alternative to LLE is solid-phase extraction (SPE), which uses a solid adsorbent to extract analyte from samples. The principle of SPE is based on the sorption of analytes on a sorbent. Aqueous sample is passed through the SPE column, and the analytes are trapped on the sorbent and are later eluted with small volume of suitable organic solvent. One advantage of this technique over LLE is the reduction of

organic solvents. However, applications of SPE are still not widely accepted due to the limitation of adsorbents, breakthrough volume problems, disposal cost and high sorbent cost.

Membrane extraction techniques are alternatives to overcome the disadvantages of LLE and SPE using by impairing selectivity and analyte enrichment. One of the recent popular membrane techniques reported in literature is hollow fiber-liquid phase microextraction (HF-LPME). It is a technique, as its name implies, that uses hollow fiber membrane to extract the analyte from sample matrix. Due to the fiber size, the technique employs very small quantity of organic solvent and can be used simultaneously for sample enrichment and cleanup in one step. The technique provides very high enrichment power, especially in the fiber module; low-cost, simple and fast.

Thailand is an agricultural country in Southeast Asia where uses of herbicides are common. Among these, glyphosate tops the import pesticide list and its application in the field has been increasing from 8,000 tons in 1998 to 24,000 tons in 2003 (1). Glyphosate is a non-selective systemic herbicide that controls weeds by inhibiting the synthesis of amino acids necessary for protein formation in plants (2). The main breakdown product of glyphosate is aminomethylphosphonic acid (AMPA), which can be further broken down by soil microorganism (3). According to the literature, glyphosate has relatively low toxicity toward birds, mammals, and fish. The maximum contaminant level (MCL) of glyphosate in drinking water was limited by the EPA at 0.7 milligrams per liter (4). Because of the similarity in the structures of glyphosate and AMPA, the two compounds have similar toxicological profiles (5). Their toxicological evaluations estimated by acceptable daily intake (ADI) for humans are 0-0.3 mg/kg. Therefore, monitoring of glyphosate and AMPA residues in the environment is important for the well being of the general population.

From the analytical perspective, analyzing glyphosate and its metabolite AMPA is challenging due to their high polarity, non-volatility, and lack of detectable chromophore. Common analytical method for the analysis of glyphosate and AMPA is high performance liquid chromatography with post-column derivatization which is compatible to HF-LPME and widely to use.

## 1.2 Literature Reviews

There are several published literatures on chromatographic methods and sample preparation for the analysis of glyphosate and AMPA. Discussions of each analytical method are as followed.

### 1.2.1 Literature Reviews of Chromatographic Methods

#### 1.2.1.1 Gas Chromatography

Glyphosate and AMPA require pre-derivatization in order to render them less polar and sufficiently becoming volatile derivatives for analysis by gas chromatography (GC). Many reaction schemes have been reported through out the years. Deyrup et al. (6) used a mixture of fluorinated alcohol such as trifluoroethanol (TFE) with perfluorinated anhydride such as trifluoroacetic anhydride (TFAA) to derivatize glyphosate and AMPA. The derivatization reacted under anhydrous conditions using TFE-TFAA (1:2) and heating at 90-100 °C for 60 minutes which were tedious and time consuming. In 1994, Alferness and Iwata (6) interestingly selected certain alcohols to vary chromatographic retention and improving method detection ability by eliminating potential interferences. They presented a derivatization method that involved the direct addition of an aqueous extract or water sample to a mixture of TFAA and 2,2,3,3,4,4,4-heptafluoro-1-butanol to derivatize glyphosate and AMPA. The resulting derivatives despite their high molecular masses were thermally stable and exhibited rapid chromatographic elution. This method utilized the convenience of water sample evaporation unlike the procedure of Deyrup et al. that required complete absence of water from the reaction mixture. The method of a mixture of TFAA and 2,2,3,3,4,4,4-heptafluoro-1-butanol was also reported in the literatures (7,8,9). However, high temperature reaction and time consuming were still the disadvantages.

The convenient method for the analysis of glyphosate and AMPA via N-isopropoxycarbonylmethyl ester derivatives was reported (10). The method required diazomethane reagent that is highly toxic, carcinogenic and explosive and should not be selected for routine analysis.

Flame photometric detection (FPD) and nitrogen phosphorous detection (NPD) seemed to be the preferable choices for detection after the derivatization (6). Both detection methods were good prospects and as such they have gained much popularity in the analysis of both analytes. Mass spectrometry detection of glyphosate and AMPA was also employed (11). In 2000, Elisabet and Lennart (11) developed a method involving ligand-exchange, anion-exchange, derivatization and quantification by gas chromatography-mass spectrometry (GC-MS). For both compounds, the limit of quantification in ground water was 0.1 µg/L and the limit of quantification in soil is 0.006 µg/g.

#### **1.2.1.2 High-performance Liquid Chromatography**

Because of the compatibility of aqueous sample with reversed phase chromatographic separation system together with the possibility of derivatization in aqueous solution made high performance liquid chromatography (HPLC) a preferred technique over GC. The derivatization procedure is required to produce chromophore or fluorophore of glyphosate and its metabolite. The sensitivity and selectivity of HPLC analysis can be enhanced by derivatization methods using either pre- or post-column modes.

It should be noted that the EPA standard method is focused on direct-aqueous injection and post-column derivatization (12). Water samples are directly injected into a cation-exchange column and separated. After elution from the analytical column, the analytes are reacted with calcium hypochlorite solution followed by *o*-phthalaldehyde-2-

mercaptoethanol complex to give a fluorophore, which is detected by a fluorometer with excitation wavelength at 340 nm and detection of emission measured at above 455 nm. The method detection limit (MDL) of the EPA method is 6.00 µg/L in reagent water. The method can be analyzed only glyphosate but not AMPA.

Similar methods to the EPA's were reported (13,14). A new chemical, Thiofluor, was substituted for 2-mercaptoethanol, the OPA reagent, Thiofluor can form a more stable and longer-lasting fluorophore. The method used sodium hypochlorite rather than calcium hypochlorite which has a potential to cause plugging of the post-column reactor. This method can analyze both glyphosate and AMPA. It is widely used in laboratory nowadays because of its good sensitivity, reproducibility, low-cost and commercially available.

For pre-column derivatization, 9-fluorenylmethyl chloroformate (FMOC-Cl) which has been shown to be highly reactive toward amines and is commercially available is commonly used. The glyphosate-FMOC derivative is formed under alkaline conditions. The procedure involves a pre-column derivatization step yielding highly fluorescent derivatives of the analyte, which can be detected by fluorescence at 315 nm. In 1996, J.V. Sancho et al. (15) proposed a rapid determination of glyphosate and AMPA in water samples. After derivatization with FMOC-Cl in 0.5-mL water sample, large volume injection into a short C<sub>18</sub> separation column to separate reagent excess from the analyte was employed, followed by a separation by an amino column. Regardless of the high recovery and low relative standard deviations obtained, the method is tedious and expensive due to the cost of the separation column.

R.J.Vreeken et al. (16) developed a high performance liquid chromatography-electrospray ionization mass spectrometry (HPLC-ESIMS) for the analysis of glyphosate and AMPA. The procedure used

FMOC-Cl derivatization followed by separation by a C<sub>18</sub> column. Mass spectrometry using full-scan negative mode was performed. The HPLC-MS method reported the detection limits as low as 0.03 µg/L.

In 2002, Agilent Technology Inc. (17) published a liquid chromatography mass spectrometry method using an electrospray ionization source in positive mode. Both glyphosate and AMPA were derivatized with FMOC-Cl. The method combined simplicity and sensitivity of LC-MS approach with pre-column derivatization and provided adequate detection for a 10-50 ppb levels in a 100-mL water sample.

In 2004, T.V. Nedelkoska and G.K.-C.Low (18) described a robust and sensitive HPLC method for the determination of glyphosate in water and in plant materials after pre-column derivatization with FMOC-Cl followed by separation by single and coupled polymeric amine columns, respectively. The method detection limit was 0.16 µg/L for water samples and 0.3 mg/kg for grass samples.

A UV-vis detector was also used to quantify the derivatives of glyphosate and AMPA with *p*-toluenesulphonyl chloride. The sample was derivatized by *p*-toluenesulphonyl chloride under alkaline condition at pH 11. This mixture was heated in water bath at 50 °C for 5 minutes. The C<sub>18</sub> Hypersil ODS-5 column was selected for separation after the derivatization. An aliquot of the mixture was injected directly into the HPLC column (19).

### 1.2.2 Literature Reviews of Other Analytical Techniques

There were a few methods that were developed for the analyses of glyphosate and AMPA. In 2005, a strong anion-exchange resin, AMBERLITE®IRA-900, was used to preconcentrate both analytes followed by capillary electrophoresis

(CE). However, the detection limits (85 ng/mL for glyphosate and 60 ng/mL for AMPA) attained were unsatisfactory (20).

In 2002, a capillary electrophoresis-indirect fluorescence detection method was presented for the simultaneous determination of glufosinate, glyphosate and AMPA. The three analytes were separated by CE with a fluorescein solution which functioned as a background fluorophore for the indirect detection of these nonfluorescence species. The concentration limits of detection (S/N=3) are 2.5, 7.7, 15.9  $\mu\text{M}$  for glufosinate, glyphosate and AMPA, respectively. However, application of this method to glyphosate in groundwater is still problematic because glyphosate peak cannot be separated from the background signal (21).

In 1999, the first suppressed conductive ion chromatography was developed for the determination of glyphosate and AMPA with a simple, sensitive and, non-derivatized method. Zhu et al. was motivated to explore the technique of conductivity suppression to reduce the background signal (22). The detection limit obtained was 0.042  $\mu\text{g/mL}$ .

The coupling of ion chromatography with electrospray mass spectrometry was also presented in the same year. The usefulness of this method was tested on several polar and environmentally relevant micro pollutants such as glyphosate, AMPA. The chelating agents were ethylenediamine tetracetate (EDTA) and diacetonketogulonic acid (DAG). The detections of glyphosate, AMPA, DAG and EDTA in ground waters were achievable at 1  $\mu\text{g/L}$  (23).

### 1.2.3 Literature Reviews of Sample Preparation

The requirement of direct-aqueous injection of water sample in the EPA method made classical LLE inapplicable for sample preparation for glyphosate and AMPA. Due to the high polarity and water solubility, researchers turn their attention to the study of extraction materials. E. Mallat and D. Barcelo (24) purposed two steps of preconcentration. In the first step, a polymeric  $\text{C}_{18}$

cartridge, Lichrolut EN, was used to improve the percentage of extraction. Then, in the second step, an anion-exchange was performed. The selection of the two-step extraction is recommended for ionic analytes such as glyphosate and AMPA because the ion-exchange sorbent alone cannot effectively remove the organic interferences in sample.

On-line solid phase extractions were also performed for the convenient of automate methods. On-line SPE systems can be divided into two categories. The first group used anion-exchange cartridges such as PRP-X 100 poly (styrene-divinylbenzene)-trimethylammonium (25) before cation-exchange liquid chromatography and post-column derivatization. Another group applied Oasis SPE cartridges after sample derivatization by FMOC-Cl. It used to eliminate excess derivatizing reagent and increasing the selectivity for C<sub>18</sub> analytical column (26, 27).

In 2000, P. Dzygiel and P. Wiczorek (28) used supported liquid membrane (SLM) for the extraction of glyphosate in water. Two circular polytetrafluoroethylene (PTFE) blocks sandwiched a porous PTFE membrane which was impregnated with Aliquat 336 (methyltrioctylammonium chloride) solution in di-n-hexyl ether. A 20-mL volume of sample solution was pumped through the donor channel, while the acceptor which was 1-mL sodium chloride solution was stagnant in 100-minute extraction time. After the extraction, the acceptor phase was transferred to CE analysis. However, the extraction efficiency was poor.

Another supported liquid membrane system for the extraction of glyphosate and AMPA was reported two years later (19). A polydifluoroethylene membrane supported by Aliquat 336 in dodecane modified with 4% dodecanol was connected with two separated compartments. The same experiment was repeated using a polypropylene hollow fiber module. Extraction time of the best concentration factors was 24 hours. The concentration factor of glyphosate and AMPA were very low but the experiment suggested that the hollow fiber membrane module was more effective than the flat sheet membrane.



The first application of supported liquid membrane technique was presented by M.V. Khrolenko et al. (29) in fruit juices. The circular PTFE membrane was used to extract glyphosate and AMPA from circulated donor to circulated acceptor. High recovery indicated that this is an effective extraction procedure for juices (orange, grapefruit, apple and, blackcurrant) in estimated 8-hour extraction time.

SLM with u-shaped hollow fiber membrane (HFM) techniques were reported. In 2004, D. Kou et al. (30) developed a technique to extract and preconcentrate haloacetic acids in water. Enrichment factors ranged from 300-3,000 were obtained. This technique also presented good enrichment factor when applied to the determination of triphenylphosphine in pharmaceutical samples (31) and the determination of drug residues such as salbutamol and terbutaline in urine samples (32).

In the recent years, a new matrix solid phase dispersion extraction was described for the quantitative extraction of glyphosate and AMPA from tomato. The extract was clean up by anion-exchange silica. Good recovery and reproducibility were obtained and the used of toxic solvents, which always incorporates in the extraction of plant materials was eliminated (33).

### **1.3 The Purpose of the Study**

The current EPA standard method for the analysis of glyphosate and its metabolite AMPA focuses on direct-aqueous injection and requires high injection volume up to 200  $\mu$ L. Due to the very low concentration of glyphosate and AMPA in the environment, current research has been concentrated on new sample preparation to obtain better method sensitivity and reducing the method detection limits. Alternative sample preparation techniques were reported. Among these, SPE has been the most successful. However, it suffers from breakthrough volume, requires large consumption of organic solvent, and incorporates high cartridges cost. On-line SPE is

possible but requires additional mechanical pump. Other published methods were inefficient or required specialized tasks.

Supported-liquid membrane (SLM) was successfully developed for glyphosate and AMPA. Flat sheet membrane was proof to be very effective for preconcentration of both glyphosate and AMPA. The flat sheet membrane module required peristaltic pump to circulate the donor and acceptor solutions. Flat sheet membranes are commonly reused and can suffer from carry-over effect. Moreover, extraction times of flat sheet module can be as long as 100 minutes to 24 hours.

Literature review revealed that there are several influencing parameters that had their own effects on the extraction efficiency. Examples of these parameters can be donor solution pH, acceptor solution concentration, and carrier concentration, etc. Membrane dimension and configuration had been reported to directly affect the extraction efficiency. Comparing to flat sheet membrane, hollow fiber membrane which have very large phase ratio, can be used to enhance the extraction efficiency of both glyphosate and AMPA.

The purpose of this research was to develop and optimized a hollow fiber supported-liquid membrane system for the extraction of the herbicides glyphosate and its metabolite AMPA from aqueous samples. This technique can be called hollow fiber-liquid phase microextraction (HF-LPME) since it represents a three-phase liquid membrane extraction technique of high efficiency. Because the cleanup and enrichment of glyphosate and AMPA can be performed in a one step, the procedure can eliminate large quantity of organic solvents, low cost, fast, reliable, and simple. The influences of several parameters were optimized. The method was fully validated and tested on real water samples. HPLC analyses with post-column derivatization were performed for analytes separation and detection.