SYNTHESIS AND CHARACTERIZATION OF PHENYL FUNCTIONALIZED MAGNETIC NANOPARTICLES AS SORBENT IN MICRO-SOLID PHASE EXTRACTION OF POLYCYCLIC AROMATIC HYROCARBONS AND ORGANOPHOSPHORUS PESTICIDES

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by

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LIST OF ABBREVIATIONS

ACE	Acenaphthene
ACY	Acenaphthylene
AFME	Agarose film microextraction
ANT	Anthracene
APTES	3-aminopropyltriethoxysilane
AZM	Azinphos-methyl
BaA	Benzo[a]anthracene
BaP	Benzo[a]pyrene
BbF	Benzo[b]fluoranthene
BET	Brunauer–Emmett–Teller
BghiP	Benzo[ghi]perylene
Bio	Biosorption
BkF	Benzo[k]fluoranthene
C_{18}	Octadecasilyl-bonded silica
CE	Capillary electrophoresis
CHR	Chrysene
CLP	Chlorpyrifos
CLT	Chlorothalonil
CRL	Co-ral
DahA	Dibenz[a,h]anthracene
DI	Direct immersion
DLLME	Dispersive liquid–liquid microextraction
DSF	Disolfonate

DSPE	Dispersive solid phase extraction
DSM	Demeton-s-methyl
DSME	Dispersive micro solid phase extraction
DZN	Diazinon
ECD	Electron captured detector
EDS	Endosulfan
EFs	Enrichment factors
EPA	Environmental Protection Agency
EU	European Union
ETN	Ethion
FESEM	Field emission scanning electron microscope
FID	Flame ion detector
FLA	Fluoranthene
FLD	Fluorescence detector
FLU	Fluorene
FNF	Fonofos
FNN	Fenthion
FNP	Fenamiphos
FNT	Fenitrohion
FPD	Flame photometric detector
FTN	Fenitrohion
GC	Gas chromatography
HF	Hollow-fiber
HPLC	High performance liquid chromatography-
HS	Headspace

IcdP	Indeno[1,2,3-cd] pyrene
ICP	Iscocarbonphenphos
I.D.	Internal diameter
IL	Ionic liquid
ISP	Isofenphos
LC	Liquid chromatography
LDS	Low-density solvent
LLE	Liquid–liquid extraction
LOD	Limit of detection
LOQ	Limit of quantification
LPME	Liquid-phase microextraction
MAE	Microwave assisted extraction
mCNTs	Magnetic carbon nanotubes
MDMSPE	Micro-dispersive magnetic solid phase extraction
MEPS	Microextraction in packed syringe
$MgSO_4$	Magnesium sulphate
MLN	Malathion
MNPs	Magnetic nanoparticles
MPT	Methyl-parathion
MRLs	Maximum residue limits
MSPE	Magnetic solid phase extraction
MTN	Methidathion
MWCNTs	Multiwalled carbon nanotubes
NAP	Naphthalene
NaCl	Sodium chloride

Na_2SO_4	Sodium sulphate
OPPs	Organophosphorus pesticides
PAHs	Polycyclic aromatic hydrocarbons
PFP	Profenphose
PMS	Phenyl-functionalized magnetic sorbent
POP	Persistent organic pollutant
PSL	Phosalone
PSM	Phsomet
PNP	Phenamiphos
PRE	Phorate
PXM	Phoxim
PYR	Pyrene
QNP	Quinalphos
RSD	Relative standard deviation
SBSE	Stir bar sorptive extraction
SDME	Single drop microextraction
SID	Salt induced demulsification
SIM	Selective ion monitoring
SLP	Sulfotep
SMT	Sumithion
SPE	Solid phase extraction
SPME	Solid phase microextraction
STP	Sulfotep
TCM	Tolclofos-methyl
TEM	Transmission electron microscopy

TEOS	Tetraethyl orthosilicate
TEPS	Triethoxphenylsilane
THF	Tetrahydrofuran
UAE	Ultrasound-assisted extraction
UADLLME	Ultrasound-assisted dispersive liquid-liquid microextraction
VA-d-µ-SPE	Vortex-assisted dispersive micro solid phase extraction
VAE	Vortex-assisted emulsification
VALLME	Vortex-assisted liquid-liquid phase microextraction
VSM	Vibrating sample magnetometer
XRD	X-Ray diffraction

LIST OF SYMBOLS

eV	Electronvolt
K_{ow}	1-octanol/water partitioning coefficients
Log P	Partition coefficient
m/z	Mass-to-charge ratio
mL min^{-1}	Milliliter per minute
MΩ	Mega ohms
n	Number of observations or replicates
ng L^{-1}	Nanogram per liter
OH	Hydroxyl
r^2	Coefficient of determination
rpm	Revolution per minute
w/v	Weight per volume

SINTESIS DAN PENCIRIAN NANOZARAH MAGNET BERFUNGSIKAN FENIL SEBAGAI PENGERAP DALAM PENGEKSTRAKAN MIKRO FASA PEPEJAL BAGI SEBATIAN HIDROKARBON AROMATIK POLISIKLIK DAN RACUN PEROSAK ORGANOFOSFORUS

ABSTRAK

Suatu pengerap magnet berfungsikan fenil (PMS) telah disediakan dan digunakan dalam kaedah pengekstrakan mikro yang dicadangkan bagi analisis sebatian hidrokarbon aromatik polisiklik (PAHs) dan racun perosak organofosforus (OPPs) yang terpilih. Satu teknik pengemulsian dibantu vortex bergabung dengan penyahemulsian garam teraruh dan pengekstrakan mikro fasa pepejal serakan magnet (VAE-SID-MDMSPE) berasaskan PMS telah dibangunkan bagi penentuan sebatian PAHs yang terpilih iaitu, fluorena (FLU), anthacena (ANT), fluoranthena (FLA) dan pirena (PYR) di dalam sampel air sekitaran, jus tebu dan teh. Di bawah keadaan VAE-SID-MDMSPE dan GC-MS optimum, kaedah ini menunjukkan kelinearan yang baik ($R^2 \geq 0.9931$) bagi semua sebatian PAHs bagi julat kepekatan 0.01-100 µg L^{-1} dengan had pengesanan rendah dan had kuantitif, masing-masing adalah 0.003-0.016 µg L^{-1} dan 0.01-0.054 µg L^{-1}. VAE-SID-MDMSPE menunjukkan kelebihan daripada segi keringkasan, cepat (5 min proses pengekstrakan), faktor pengkayaan yang tinggi (EFs, 61-239) dan penggunaan pelarut organik yang sedikit (200 µL), keperluan pengerapan yang sedikit (15 mg) dan membuktikan kejayaan kaedah pengekstrakan dengan pengembalian relatif yang tinggi dalam julat 85.3-109.1% dengan sisihan piawai relatif (RSDs) dari 1.0-8.5 % (n=5). PMS juga digunakan sebagai pengerap dalam erapanbio berdasarkan pengekstrakan mikro serakan cecair-cecair serakan bergabung dengan pengekstrakan mikro-fasa pepejal serakan magnet (Bio-DLLME-MDMSPE) bagi pengekstrakan sebatian OPPs terpilih iaitu, sulfotep (STP), diazinon (DZN), tolklofos-metil (TCM), chlorpirifos (CLP), isofenfos (ISP) and etion (ETN) dalam sampel kubis. Di bawah keadaan optimum, Bio-DLLME-MDMSPE menunjukkan kelinearan yang baik ($R^2 \geq 0.9953$) dengan had pengesanan yang baik dan had kuantitatif, masing-masing adalah 0.075-0.15 μg L^{-1} dan 0.25-0.5 μg L^{-1} kesemua analit OPPs. Kaedah ini memberikan faktor pengkayaan yang tinggi (EFs, 46-516) dengan pengembalian relatif yang cemerlang dalam julat 85.8-107.9 % bagi semua analit OPPs di dalam sampel kubis**.** Kaedah pengekstrakan mikro bergabung dengan bahan PMS yang digabungkan menunjukkan sebagai ringkas, cepat dan cekap dengan cirian yang lebih baik berbanding teknik pengekstrakan yang selalunya digunakan dan sesuai digunakan bagi pengesanan PAHs di dalam sampel air, jus tebu dan teh serta OPPs di dalam sampel kubis.

SYNTHESIS AND CHARACTERIZATION OF PHENYL FUNCTIONALIZED MAGNETIC NANOPARTICLES AS SORBENT IN MICRO-SOLID PHASE EXTRACTION OF POLYCYCLIC AROMATIC HYDROCARBONS AND ORGANOPHOSPHORUS PESTICIDES

ABSTRACT

A phenyl-functionalized magnetic sorbent (PMS) was prepared and employed in the proposed microextraction methods for the analysis of selected polycyclic aromatic hydrocarbons (PAHs) and organophosphorus pesticides (OPPs) compounds. A vortex-assisted emulsification combined with salt-induced demulsification and magnetic dispersive micro-solid phase extraction (VAE-SID-MDMSPE) based on PMS was developed for the determination of selected PAHs compound namely, fluorene (FLU), anthracene (ANT), fluoranthene (FLA) and pyrene (PYR) in environmental water, sugarcane juice and tea samples. Under optimized VAE-SID-MDMSPE and GC-MS conditions, the method revealed good linearity ($R^2 \geq 0.9931$) for all PAHs compounds over a range of 0.01-100 µg L^{-1} with low limits of detection and limits of quantification, 0.003-0.016 µg L^{-1} and 0.01-0.054 µg L^{-1}, respectively. VAE-SID-MDMSPE exhibited superior advantages in terms of simplicity, rapid (5 min extraction process), high enrichment factors (EFs, 61-239), and low usage of organic solvent (200 µL), low sorbent consumption (15 mg) and proved a successful extraction method with high relative recoveries in the range of 85.3-109.1% with relative standard deviations (RSDs) of peak area varied from 1.0-8.5 % (*n*=5). PMS was also employed as sorbent in a biosorption based dispersive liquid-liquid microextraction combined with magnetic dispersive micro-solid phase extraction (Bio-DLLME-MDMSPE) for the extraction and preconcentration of selected OPPs compounds namely, sulfotep (STP), diazinon

(DZN), tolclofos-methyl (TCM), chlorpyrifos (CLP), isofenphos (ISP) and ethion (ETN) in cabbage samples. Under the optimized conditions, Bio-DLLME-MDMSPE showed good linearity range ($R^2 \geq 0.9953$) with good limits of detection and limits of quantification in the range of 0.075-0.15 µg L^{-1} and 0.25-0.5 µg L^{-1}, respectively for all OPPs analyte. The method provided high enrichment factors (EFs, 46-516) with excellent relative recoveries in the range of 85.8-107.9 % for all OPPs analytes in cabbage samples. The developed microextraction methods incorporated with PMS material have demonstrated to be simple, rapid and efficient with characteristics that are superior over the commonly used extraction methods and suitable for the detection of PAHs in water, sugarcane juice and tea samples as well as OPPs in cabbage samples.

CHAPTER 1

INTRODUCTION

1.1 Background of study

Throughout the past few decades, abundance of different organic chemicals has been synthesized for numerous applications to be utilized as pesticides, herbicides, detergents, insulating materials, etc. Polycyclic aromatic hydrocarbons (PAHs) and organophosphorus pesticides (OPPs) are examples of persistent organic pollutants (POPs) which may cause long-term effects on humans and wildlife even at trace levels (Arslan-Alaton and Olmez-Hanci, 2013). Hence, more studies regarding the effect of organic pollutants and pesticides have been carried out by various researchers.

PAHs is a large class of persistent organic compounds which are particularly composed from incomplete combustion of natural materials throughout industrial process and other anthropogenic activities including automobile traffic, cooking, processing of coal and tobacco smoking (Goda *et al.*, 2014). The stability of PAHs in the environment are influenced by their chemical structures, chemical configuration and physical-chemical properties. Most PAHs compounds are capable to be transported over long range before they come back to the earth with rainfall (Goda *et al.*, 2014). Thus, PAHs causes a serious problem to human health through different routes of exposure such as food, air, water and soil (Wu *et al.*, 2012). Nevertheless, contamination of PAHs in food is the main sources of their exposure to human.

Formerly reported work stated that PAHs compounds cause the boom of cancer cell in human (Arslan-Alaton and Olmez-Hanci, 2013).

The growing numbers of utility of pesticides in agricultural industry have prompted severe hazard to human health and the environment. Pesticide may be described as any substance or mixture of materials supposed for mitigating, repelling, destroying, or stopping any pest. It is a poison design specifically to kill unwanted plants and insects. Pesticides are labeled according to their capabilities such as fungicides to kill mold or fungus, herbicides to kill weeds and insecticides to kill bugs (Fenik *et al*., 2011). Besides that, they are also categorized based on their physical state, target organisms, chemical structure and chemical families such organophosphorus pesticides, organochlorines pesticides and carbamates pesticides.

Pesticides are used extensively during cultivation, harvesting and storage in order to protect the crops from pest, bacteria and to provide food quality assurance (Nan e*t al*., 2015). Furthermore, they are used to avoid many dreadful diseases. In order to increase the production yield of crops; pesticides were applied in many stages of cultivation to provide protection against pest-eating crops. Nevertheless, improper use of pesticides can be dreadful to human health. Therefore, a good agricultural practice is very important to minimize the possible risk to human health and the environment.

Due to adverse effect of POPs to the ecology and human health, stringent constitutional controls are enforced to monitor the manufacture, utilization and emission of POPs. It is necessary to determine and monitor POPs contamination level in environmental matrices to ensure that they are within the safe limits. Therefore, in order to identify and determine POPs in complex matrices, a selection of an appropriate analytical technique and a robust sampling design is very crucial. There has been a great challenge face by the scientist to identify and determine POPs as they are usually found at a trace level amount and due to matrix interferences (Arslan-Alaton and Olmez-Hanci, 2013). Hence, sample preparation procedure is required to reduce or eliminate complex matric interferences, pre-concentrate the targeted analytes and to improve sensitivity of the developed analytical methods (Ali *et al.*, 2015).

Over the past decades, numerous procedures have been developed for the extraction and preconcentration of organic compounds. The most commonly procedures used are liquid-liquid extraction (LLE) (Taylor *et al.*, 1995), solid-phase extraction (SPE) (Kayali-Sayadi *et al.*, 1998), solid-phase microextraction (SPME) (Fisichella *et al.*, 2015) and stir bar sorptive extraction (SBSE) (Blasco *et al.*, 2004; Bourdat-Deschamps *et al.*, 2007; Gomes *et al.*, 2011; Hu *et al.*, 2013; Xiao *et al.*, 2016). Although, LLE has been an effective sample preparation technique, it is exhaustive, labor-intensive and utilize huge amount of hazardous organic solvents (Farajzadeh and Feriduni, 2016). Even though SPE has minimized the limitations of LLE, in term of simplicity, high selectivity and recovery, low consumption of organic solvents (Fotouhi et al., 2017), it also suffer from limitations such as large secondary wastes, a lengthy step, solvent loss and a need for complex gear. Nowadays, modern trend in analytical chemistry are heading to the simplicity and minimality of sample pretreatment as well as reducing the consumption of hazardous organic solvent (Blasco *et al.*, 2004).

In this study, a phenyl-functionalized magnetic sorbent (PMS) was synthesized using a simple method and used as a sorbent material for the extraction and preconcentration of PAHs and OPPs. The previously successful reported works on functionalization of magnetic nanoparticles with thiol and urea, have led for further study on the functionalization of magnetic nanoparticle with triethoxyphenylsilane to form PMS for the extraction of PAHs and OPPs (Salehi *et al*., 2015). To the extent of our knowledge, this is the first employment of PMS in proposed microextraction systems for PAHs and OPPs determination. Factors affecting the extraction performance based on PMS on the developed microextraction methods were comprehensively studied and evaluated.

1.2 Problem statement

Regardless of the advances in highly efficient analytical instrumentations, some compounds are still beyond from the detection thresholds of these instrumentation. Desired analytes are usually subjected to hindrance by the profoundly complex matrices (e.g., food, environmental and biological). Besides that, most of the analytical tools cannot deal with the sample matrices directly. Thus, sample pretreatment is crucial in the overall chemical analyses. It is essential to concentrate and isolate the targeted analyte of interest from the matrices to ensure the suitability of the analyte for a separation and detection system.

In order to overcome the limitations of conventional methods, a magnetic solid phase extraction (MSPE) is introduced as a refined type of SPE. MSPE is an extraction technique that utilized magnetic materials as sorbents. An alternative of

MSPE termed magnetic dispersive micro-solid phase extraction (MDMSPE) has been reported (Cheng *et al*., 2014). The selection of adsorbent material is very critical as it largely determine the selectivity and sensitivity of the method (Han *et al*., 2012). Compared to the traditional SPE sorbents (florisil, C_{18}, silica, etc.) which usually co-extract compounds that interfere with analysis due to non-particular interface interactions (Cheng *et al.*, 2014), magnetic materials had simplify and improve the SPE extraction technique. The magnetic materials are directly dispersed in sample solutions, which can enhance the extraction efficiency by broadening the proximity between analytes and the sorbents (Bai *et al.*, 2010). They can be easily recovered with the help of external magnet force and eliminates the tedious filtration or centrifugation procedure (Cheng *et al*., 2014). Despite their superior characteristic, bare magnetic materials are easily agglomerated and oxidized when exposed to air (Al-rashdi, 2016). Therefore, it is necessary to modify the surface of magnetic materials to achieve selective and sensitive extraction system.

In this present study, a phenyl silane-coupling agent has been selected to be functionalized to the surface of the magnetic sorbent due to its unique structure which is benzene ring and Si-O. The presence of benzene rings in the functionalized magnetic sorbent is most likely will form a hydrophobic and π-π interaction with the targeted analytes (Guo et al., 2015; Stevenson et al., 2017). PMS material with high affinity, high selectivity, and high stability were synthesized and developed as extraction sorbent for PAHs and OPPs. PMS offers many benefits such as high selectivity towards analytes of interest and compatibility for complex matrices, high extraction efficiency, rapid separation after extraction, simple preparation and surface modification of the material, exceptional dispersibility in water sample and good reusability. The application of PMS in vortex-assisted emulsification combined with salt-induced demulsification (VAE-SID-MDMSPE) and biosorption based dispersive liquid-liquid microextraction combined with magnetic dispersive micro-solid phase extraction (Bio-DLLME-MDMSPE) methods are expected to enhance the feasibility and capability of microextraction systems for the detection of PAHs and OPPs.

1.3 Objectives of study

The aim of the study is to develop microextraction methods based on PMS material. The particular objectives of this work are to: -

i. synthesize and characterize phenyl functionalized magnetic sorbent (PMS).
ii. develop a vortex-assisted emulsification combined with salt-induced demulsification (VAE-SID-MDMSPE) for the analysis of PAHs in aqueous matrices.
iii. develop a biosorption based dispersive liquid-liquid microextraction combined with magnetic dispersive micro-solid phase extraction (Bio-DLLME-MDMSPE) for the extraction of OPPs in cabbage samples.

1.4 Scope of Study

This study focuses on the development of new microextraction methods based on PMS, namely VAE-SID-MDMSPE and Bio-DLLME-MDMSPE for the determination of PAHs and OPPs in water, juice and vegetable matrices. Four selected PAHs compound namely fluorene (FLU), anthracene (ANT), fluoranthene (FLA) and pyrene (PYR) were used in this study. The selected OPPs are sulfotep (STP), diazinon (DZN), tolclofos-methyl (TCM), chlorpyrifos (CLP), isofenphos (ISP) and ethion (ETN). The extraction capability of the developed microextraction methods was determined using gas chromatography mass-spectrometry (GC-MS) system and gas chromatography flame ionization detector (GC-FID) for PAHs and OPPs, respectively. Factors influencing the extraction efficiency were optimized thoroughly prior to method validation and application to real samples. The capability of the developed microextraction methods were tested on real samples such as lake water, pond water, river water, paddy field water, sugarcane juices, tea and cabbage samples.

1.5 Significance of study

In order to overcome major limitations in the conventional LLE and SPE methods such as high consumption of solvent and sorbent material, time consuming and tedious operation, new microextraction methods that are efficient, rapid, environmentally friendly and cost-effective are desirable. Magnetic based microextraction system has been introduced to shorten the extraction time and simplify the conventional SPE by hastening the mass transfer of analytes from sample matrices to the magnetic sorbent. The developed VAE-SID-MDMSPE and Bio-DLLME-MDMSPE operation in extraction providing attractive and creative way of combining different microscale sample preparation method. The developed microextraction systems have ‘green chemistry’ nature due to its microscale format (mg amount of sorbent and μL of solvent required for the extraction). More importantly, the procedure for conducting VAE-SID-MDMSPE and Bio-DLLME-MDMSPE is simple and involved unsophisticated devices (vortex and ultrasonication system), generally found in a typical analytical laboratory.

1.6 Outline of the thesis

This thesis composes of five chapters. Chapter 1 provides in detail the research background, problem statement, objectives, scope as well as significance of the study. Chapter 2 compiles the fundamentals of the selected organic contaminants studied and their properties, introduction to conventional and microextraction methods and potential microextraction methods of PAHs and OPPs and finally the applications of magnetic sorbents in sample preparation. Chapter 3 elaborates the research methodology of each objective independently while Chapters 4 describes in specific the study associated to the objective of the study as mentioned in Section 1.3.

Chapter 4 is divided into three parts, corresponding to each specific objective. The first part describes the results for synthesis and characterization of PMS material. The second part reports the results and discussion on optimization study, method validation and application of in VAE-SID-MDMSPE towards the determination of selected PAHs in aqueous matrices. The third part investigates the application of PMS materials in Bio-DLLME-MSDMSPE for determination of selected OPPs in cabbage samples.

The performance of VAE-SID-MDMSPE was verified for the analysis of four target PAHs analyte namely, fluorene (FLU), anthracene (ANT), fluoranthene (FLA), and pyrene (PYR) in environmental water, sugarcane juice and tea samples. Several significant VAE-SID-MDMSPE parameters such as, type and volume of emulsification solvent, extraction time, type of desorption solvent, desorption time, type and amount of salt-induced demulsified and sample volume were investigated. The performance of Bio-DLLME-MDMSPE was optimized and validated for the determination of selected six OPPs analytes, namely, sulfotep (STP), diazinon (DZN), tolclofos-methyl (TCM), chlorpyrifos (CLP), isofenphos (ISP) and ethion (ETN) in cabbage samples. Important Bio-DLLME-MDMSPE parameters such as addition of biosurfactant, amount of biosurfactant, type and volume of disperser solvent, mass of sorbent, extraction time, type and volume of desorption solvent, desorption time, salt addition and sample volume were thoroughly investigated.

Lastly, Chapter 5 summarizes the major findings of the study and directions for future studies. This chapter covers the overall results obtained including the optimization study, validation data and analytical characteristics of the developed microextraction methods. Future directions are discussed for potential further studies.

CHAPTER 2

LITERATURE REVIEW

2.1 Overview

This chapter compiles and discusses the fundamentals of the selected polycyclic aromatic hydrocarbons (PAHs) and organophosphorus pesticides (OPPs) with its analytical determination, development of conventional and microextraction methods and applications of magnetic sorbent in sample preparation.

2.2 Organic pollutants

Over decades, variety of organic chemicals has been manufactured for different purposes such use as insecticides, herbicides, detergents and insulating materials. However, many of them are persistent in the environment which they stay pristine for long spans as they withstand chemical, photolytic and biological degradation (Wong *et al*., 2005). Polycyclic aromatic hydrocarbons (PAHs) and organophosphorus pesticides (OPPs) are classified as persistent organic pollutants (POPs) due to their physical and chemical characteristics such as highly toxic even at low levels, ability to transport at a long range through air and water, hydrophobicity and lipophilicity which enable them to bio-accumulate in fatty tissue of an organism, especially on a species at the top of the food cycle (Jacob and Cherian, 2013; Tang, 2013; Xu *et al*., 2013).

Nowadays, POPs contamination had been a public concern due to their adverse effects to the environment and human health. POPs are spread globally and they may act as hormone disruptors and causing neurologic disorders, suppressing the immune system and increasing the risk of cancer (El-shahawi *et al.*, 2010; Xu *et al.*, 2013). Therefore, there is a concern of special environmental relevance in regard to the investigation and monitoring of these compounds. However, direct analysis of organic compounds by analytical instrumentation is mostly difficult due to the existence of matrix hindrances and traces amount of the analytes. Hence, sample pretreatment process is crucial to separate the analyte from the sample and to preconcentrate the targeted analyte, to enhance the sensitivity and capability of the detection systems.

2.2.1 Polycyclic aromatic hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) are a set of chemical mixture that are constitute of two or more fused aromatic rings. There are abundant of PAH compounds, each distinct in the orientation of substituents on the general ring system and the number and position of aromatic rings (Eisler, 1987). PAHs are classified into light PAHs and heavy PAHs based on the number of fused aromatic ring in their structures (Goda *et al.*, 2014). PAHs that contain four or less fused aromatic aromatic rings are categorized as light PAHs whereas heavy PAHs are those with more than four fused rings (Farhadian *et al.*, 2010). Unsubstituted lower molecular weight PAHs made up of 2 or 3 rings, display significant severe toxicity and other deleterious effects to some organisms, but are noncarcinogenic; the higher molecular weight PAHs, consists 4 to 7 rings, are significantly less toxic, but some of the compounds are teratogenic, mutagenic or carcinogenic to the diversity of organisms such as mammals, aquatic life, amphibians and birds (Eisler, 1987).

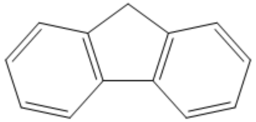
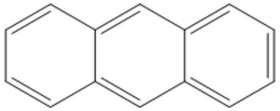
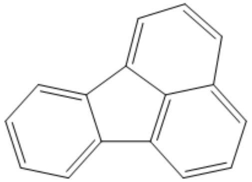
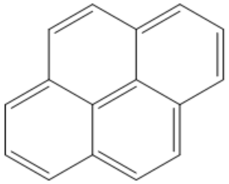
PAHs are mainly generated during incomplete combustion or pyrolysis of carbon-containing organic substances, fossil fuels, domestic burning, industrial operations and other human activities (Food Safety Authority of Ireland, 2015; Guo and Lee, 2011). Other than that, PAHs are formed by natural processes such as volcano eruptions and forest fires (Srogi, 2007). They are ubiquitous in the environmental compartments such as atmosphere, water and soil. The production of PAHs leads to food contamination causing dangerous effects to human health. PAHs are possibly formed in foods during domestic food preparation and industrial processing and such as frying, smoking, roasting, barbecuing, drying, baking or grilling (Food Safety Authority of Ireland, 2015).

PAHs are generally have relatively low water solubility but highly lipophilic, low vapour pressure and high melting and boiling points (Srogi, 2007). These compounds tend to move through the food cycle and can be transmit over long ranges (Katsoyiannis and Breivik, 2014; Ting *et al.*, 2017). Regardless of PAHs high lipid solubility, they exhibit low tendency to bio-magnify in food cycles, probably due to their rapid metabolism (Bansal and Kim, 2015). Although they exist in minute concentrations in the environment and food samples, many studies have shown that it could lead to cancer (Menezes *et al.*, 2013; Shi *et al.*, 2016).

The US Environmental Protection Agency (US EPA), Agency of Toxic substance and Disease register (ATS-DR), International Agency for Research on

Cancer (IARC), and European Union have named 16 unsubstituted PAHs as priority pollutants and they are commonly used to characterize the PAH content (Bansal and Kim, 2015; Singh *et al*., 2016). Benzo(a)pyrene (BaP) is frequently used as a marker for total PAHs exposure in industry, food as well as in the environment due to its highest volatility and lowest boiling point (Srogi, 2007). However, in 2008, the European Food Safety Authority (EFSA) has governed that there was unreliability in BaP as a dependable marker for the occurrence of PAHs in food (Bansal and Kim, 2015; Purcaro *et al*., 2013). Nevertheless, up until now, the administrative regulation for PAH levels have been lacking due to the complexity of the matrices (Bansal and Kim, 2015). Even though, lighter PAHs have weaker mutagenic and carcinogenic properties, they are the most abundant in the urban atmosphere and reactive toward other pollutants to form more toxic derivatives (Srogi, 2007). Besides that, there is only few info regarding the study of lighter PAHs compared to heavier PAHs. Thus, in this study, four PAHs were selected as model analytes, such as fluorene (FLU), anthracene (ANT), fluoranthene (FLA) and pyrene (PYR). Chemical structures and characteristics of selected PAHs are described in Table 2.1.

Table 2.1: Chemical structures and characteristics of selected PAHs compound.

Common name	Empirical formula	Molecular mass (g mol-1)	Log K_{ow}	Water solubility (mg L^{-1})	Boiling point (°C)	Chemical structure
Fluorene (FLU)	$C_{13}H_{10}$	166.22	4.12	1.992	295	
Anthracene (ANT)	$C_{14}H_{10}$	178.23	4.56	0.022	340	
Fluoranthene (FLA)	$C_{13}H_{10}$	202.25	5.64	0.265	375	
Pyrene (PYR)	$C_{13}H_{10}$	202.25	5.58	0.135	404	

2.2.2 Organophosphorus pesticides

Pesticides are widely utilized to restrain pests and obviate infections in harvest like vegetables, cereals and fruits. Organophosphorus pesticides (OPPs) are widely employed for agricultural processes due to their high efficiency in increasing agricultural productivity by controlling pests and diseases. However, due to the extensive consumption of OPPs, they have become the most common and persistent contaminants of products of agriculture. Moreover, the slow deterioration and improper utilization of OPPs causes in excess of these compound in the environment which lead to soil contamination. Majority of OPPs are persist in the environment and may enter the food chains through various ways resulted in potential risk to human health (Eleršek and Filipi, 2006). They alter the enzyme acetyl-cholinesterase used to control the nerve function for animals and humans as well as insects (Sogorb and Vilanova, 2002). The European Union had established the maximum residue levels in vegetables and fruits ranging from 0.01 to 3 mg kg^{-1} (Xu *et al.*, 2014). The detection of OPPs in agricultural products is necessary to eliminate and reduced the potential risks to human health. Thus, it is critical to establish an effective analytical method which is high in sensitivity, selectivity and simplicity for the analysis of trace OPPs in variety of samples to guarantee public welfare and international trade. In this current study, six OPPs were chosen namely sulfotep (SLP), diazinon (DZN), tolclofos-methyl (TCM), chlorpyrifos (CLP), isofenphos (ISP) and ethion (ETN). The chemical structures and characteristics of selected OPPs are shown in Table 2.2. From the table, these OPPs composed of similar general structure but indifferent in terms of their chemical and physical properties.

Table 2.2 Chemical structures and characteristics of selected OPPs compound.

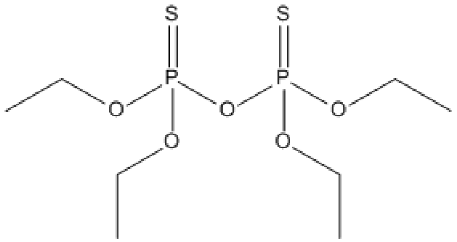
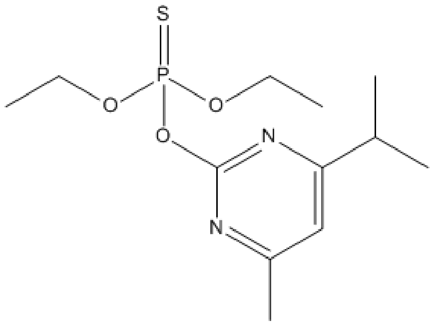
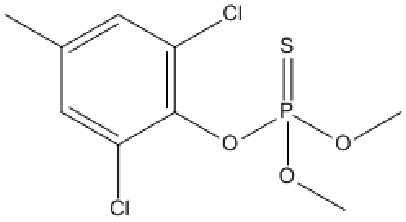
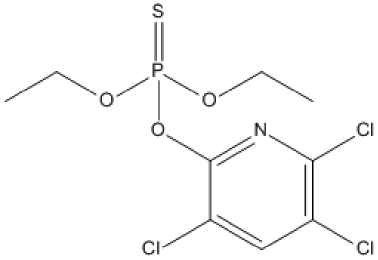
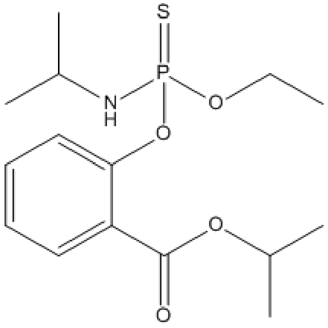
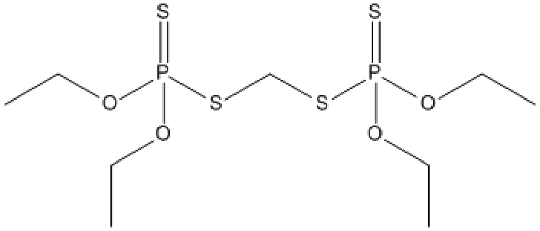
Common name	Empirical formula	Molecular mass (g mol $^{-1}$)	log *P*	Water solubility (mg L^{-1})	Boiling point (°C)	Chemical structures
Sulfotep (SLP)	$C_8H_{20}O_5P_2S_2$	322.3	3.99	10	136 to 139	
Diazinon (DZN)	$C_{12}H_{21}N_2O_3PS$	304.3	3.69	60	315.9	
Tolclofos - methyl (TCM)	$C_9H_{11}C_{l2}O_3PS$	301.1	4.56	0.708	338.5	

Table 2.3 Chemical structures and characteristics of selected OPPs. (Continued)

Common name	Empirical formula	Molecular mass (g mol $^{-1}$)	log *P*	Water solubility (mg L^{-1})	Boiling point (°C)	Chemical structures
Chlopyrifos (CLP)	$C_9H_{11}C_{13}NO_3PS$	350.62	4.70	1.05	349	
Isofenphos (ISP)	$C_{15}H_{24}NO_4PS$	345.3	4.04	24	345	
Ethion (ETN)	$C_9H_{22}O_4P_2S_4$	384.4	5.07	2	384	

2.3 Extraction methods for chemical analysis

Analysis process consists of five steps namely, sampling, sample preparation, separation, detection as well as data analysis which are extremely important to achieve reliable data. The main aspects in achieving a successful analysis from complex matrices are the quality of sampling and sample preparation as they covered more than 80% of analysis time (Vas and Vékey, 2004). Hence, the preferred sample preparation technique has huge impacts towards the accuracy of the analysis. This chapter gives some insight on the extraction techniques, i.e. from conventional to microextraction techniques

2.3.1 Liquid-liquid extraction

Liquid–liquid extraction (LLE) in Figure 2.1 is a classical separation technique in analytical chemistry which take advantages of the dissimilar solubility of the analyte in the sample solution and in immiscible organic phase, to selectively extract the analyte into one solvent leaving the rest of the matrix in the other. LLE has been broadly utilized as sample pretreatment for metal cations (Khoutoul *et al.*, 2016), biological (Jouyban *et. al.*, 2016), beeswax (Yáñez *et al.*, 2013) and aqueous samples (Farajzadeh and Feriduni, 2016). Nevertheless, there are major limitations of conventional LLE; it needs huge amounts of organic solvent that is costly and hazardous to the environment and it is tedious due to evaporating step of large volume of organic solvent.

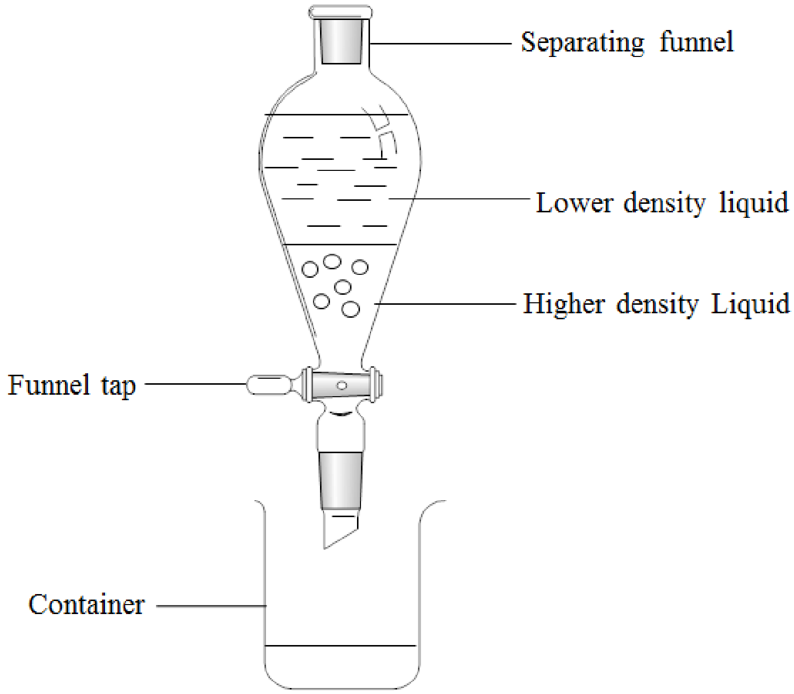


Figure 2.1 Schematic of liquid-liquid extraction (LLE).

2.3.2 Solid phase extraction

Solid phase extraction (SPE) in Figure 2.2 apply the affinity of analytes dissolved in a sample solution for a solid sorbent. The sorbent is the heart of SPE which regulate the effectiveness and the selectivity of the extraction (Pichon, 2000). Therefore, the selection of sorbents depends on the analytes characteristic and interactions between analyte and sorbents. SPE is an alternative method of LLE to pretreatment and preconcentration analytes, and it is successfully applied for the detection of pollutants in environment (Blackwell *et al*., 2004; Heuett *et al*., 2015; Li *et al*., 2007) and biological samples (Mei *et al*., 2011). However, most of conventional SPE methods need to utilize organic solvents to elute the analytes after all the samples was completely pass through the cartridges filled with sorbents. Thus, SPE method is exhaustive, relatively expensive, especially for large volumes of samples, time-consuming and labor intensive.

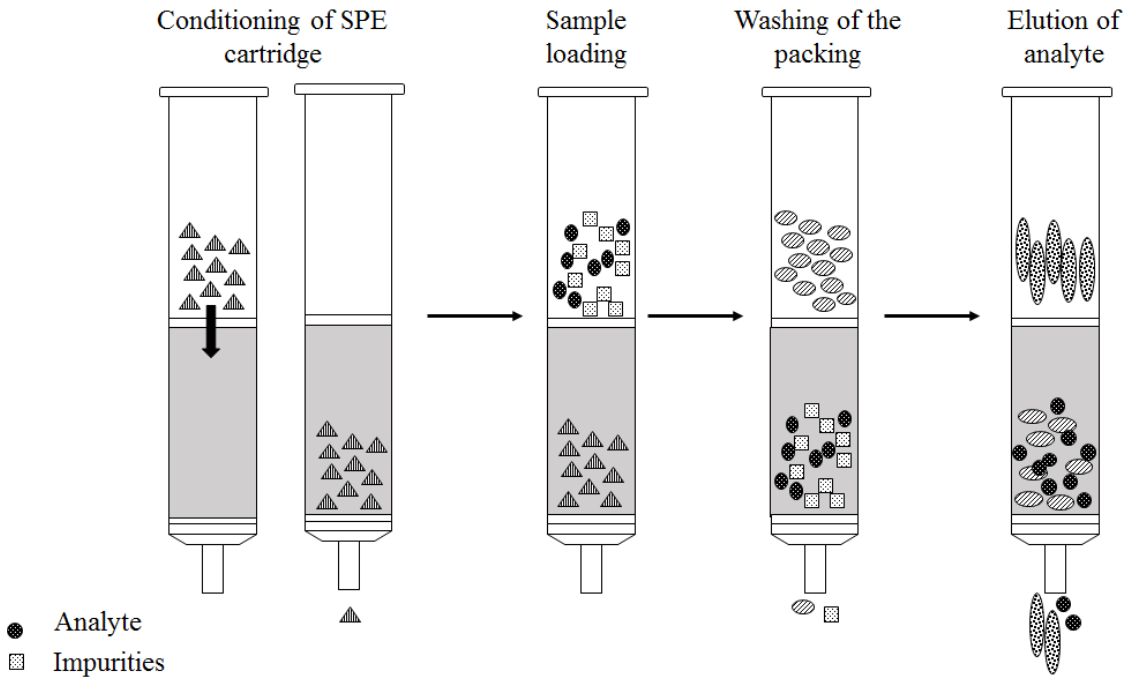


Figure 2.2 Schematic of solid phase extraction (SPE).

2.3.3 Supercritical fluid extraction

Supercritical fluid extraction (SFE) needs a supercritical fluid as extracting solvent to extract the desired analytes from the sample. Carbon dioxide (CO_2) is commonly employed as critical fluid due to its critical pressure of 72.8 atm and critical temperature of 31.2 °C (Rissato *et al.*, 2005). Moreover, CO_2 is nonflammable, odorless, non-toxic, tasteless, inert and cost effective. SFE has shorten the extraction time and it is environmentally friendly with high precision and selectivity compared to the conventional extraction techniques (Han *et al.*, 2015).

Nonetheless, this method is inappropriate for routine analysis due to the need for special gear which is costly.

2.3.4 Microwave assisted extraction

Microwave assisted extraction (MAE) is considered as a new sample pretreatment of liquid, semi-solid and solid matrices. MAE depend on the dielectric susceptibility of both solvent and matrices result from the rapid heating processes that happen when a microwave field is employed to a sample. MAE is beneficial due to its flexibility to cover variety types of sample and the selectivity can be maneuver by changing the solvent polarities. MAE has been widely used in food (Bouaid *et al*., 2000; Z. Wang *et al*., 2013) and biological analysis (Kumar *et al*., 2014).

2.4 Development of microextraction methods for chemical analysis

In order to overcome the problems in the classical extraction techniques such as large solvent consumption, labour-intensive operations, and high cost, new microextraction method based on minituarized methods were developed. They have received a tremendous attention from the researchers as they are more simple, efficient, economic and environmental friendly due to minimum usage of hazardous chemicals. In order to minimize the environmental pollution and towards the green analytical chemistry process, a miniaturization in the development of sample preparation methods is critical.

2.4.1 Sorbent based microextraction methods

2.4.1(a) Solid phase microextraction

Solid phase microextraction (SPME) device in Figure 2.3, consists of a syringe assembly which act as a holder for the fiber assembly and a fiber assembly which comprised of a needle that protects the polymeric fiber (Magdic *et al*., 1996). SPME is an extraction method of volatile and semi-volatile analyte from sample to the adsorptive polymeric fiber of SPME (extracting phase) (Menezes *et al*., 2015). The amount of adsorption on the extracting phase mostly relies on the thickness of the coating which decide the volume and surface area of the extracting phase. SPME is simple, fast and solventless alternative the classical extraction methods. However, SPME has some drawbacks which the process for preparation of the film for commercial devices is costly, suffer from carry-over effect and fragility of the fiber which it can break easily during sampling and injection (Merkle *et al*., 2015).

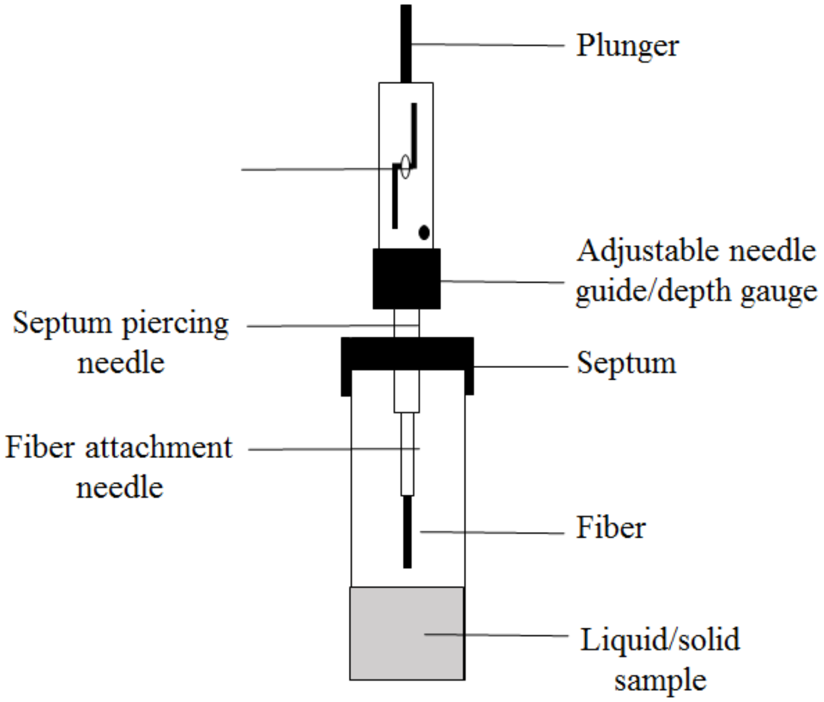


Figure 2.3 Schematic of solid phase microextraction (SPME).

2.4.1(b) Dispersive micro-solid phase extraction

Dispersive micro-solid phase extraction (D-µ-SPE) in Figure 2.4 is a diversion of dispersive solid phase extraction (DSPE) which utilized solid sorbent in the range of µg or mg to be dispersed in a sample solution for rapid interaction between the sorbent and targeted compound as well as reducing the time taken for sample pretreatment (Kocot *et al.*, 2013). This microextraction technique had simplify and subdue the limitation of conventional solid phase extraction (SPE) method, which is tedious, produce large of secondary wastes, and need for special equipment. D-µ-SPE have been successfully employed for the analysis of semi volatile compounds from plant tea (Cao *et al.*, 2014), cadmium and lead from water samples (Krawczyk *et al.*, 2016) and triazines from water samples (Jiménez-Soto *et al.*, 2012).

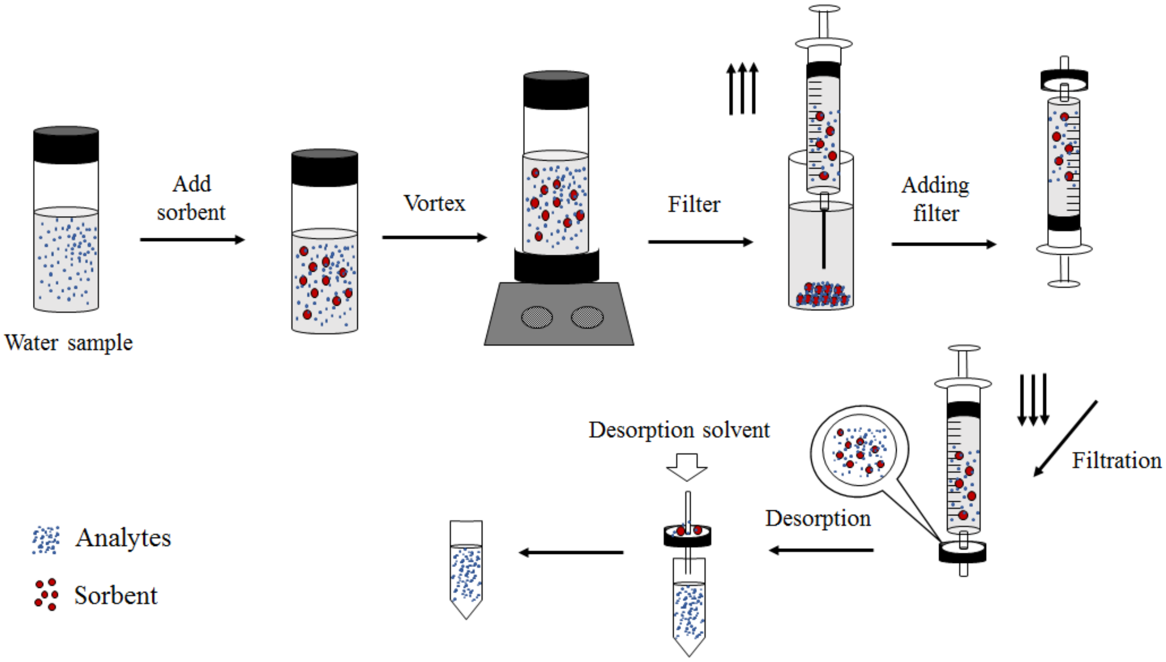


Figure 2.4 Schematic of dispersive micro-solid phase extraction (D-µ-SPE).

2.4.1(c) Magnetic dispersive micro-solid phase extraction

Magnetic dispersive micro-solid phase extraction (MDMSPE) in Figure 2.5, is variation of magnetic solid phase extraction (MSPE) method. MDMSPE utilized just a small amounts of solvent and milligrams of sorbent in the extraction compare to the common MSPE (Es'haghi *et al.*, 2016). MSPE and MDMSPE have been applied for the extraction of organic compounds and heavy metal (Es'haghi *et al.*, 2016) in different types of aqueous and food matrices (Bai *et al.*, 2010; Galán-Cano *et al.*, 2013; Rocío-bautista *et al.*, 2016; Zhao *et al.*, 2011; Zhou *et al.*, 2017).