CHAPTER ONE

1.0 INTRODUCTION

1.1 Pesticides

An estimate of 1 billion people went hungry in 2010, and with the ever increasing world population, there is need for 70% increase in global food production by the year 2050 (IUPAC, 2012). The increase in world population which has led to drastic increase in demand for food supply has also led to immeasurable rise in the application of chemical pesticides and fertilizers (Aulakh et al., 2005). To increase agricultural production and meet the growing demand for food, pesticides are used for control of pest and vector of plant diseases. (Araoud et al., 2007). Pesticides are also use in non-agricultural activities to control and eradicate carriers of vector borne diseases, such as malaria, yellow fever, typhoid fever and dengue, which are major public health concerns (Cabras, 2003; Chai, Tan, & Kumari, 2008b; Maharaj, 2010; Rose, 2001; WHO, 1995).

Pesticides refer to all natural and synthetic chemicals that are used to prevent, destroy, repel or fight crop pest and vector of plant diseases (Cabras, 2003). The Food and Agricultural Organization (FAO), comprehensively defined pesticides (FAO, 2003), as

> "*any substances or mixture of substances that is intended for preventing, destroying, attracting, repelling, or controlling any pest including unwanted species of plants or animals during production, storage, transport, distribution and processing of food agricultural commodities, or animal feeds or which may be administered to animals for the control of ectoparasites"*

Pesticides are mostly organic compounds with different functional groups, forming various types of isomeric compounds (Aulakh et al., 2005). They differ in their substitution groups, degree of ionization, octanol/water coefficients, polarity, volatility and their solubility. The production and applications of pesticides in agriculture and non-agricultural purposes have led to steady increase in food production, high food quality and reduced incidence of illness due to insect-borne diseases. However their continuous use has negative impact on the environment and their presence in soil, air, water and food pose a potential health risk due to their biocide activity (Araoud et al., 2007; Aulakh et al., 2005; Beceiro-González et al., 2012; Chai & Tan, 2010; Cooper & Dobson, 2007; Lambropoulou & Albanis, 2007a).

Therefore, pesticides must be used efficiently and effectively in order to strike a balance between their expected benefits and the possible risk to human health. This will enable their economic viability and environmental sustainability (Tadeo et al., 2012). Pesticides are widely used to control pests of fruits and vegetables, which are an important part of a healthy diet (Garrido Frenich et al., 2012; Tuzimski, 2012). Use of pesticides in fruits and vegetables plays a beneficial role in the provision of large quantities and high quality, low-cost supply of fruits and vegetables (Bidari et al., 2011).

1.1.1 Importance of Food Analysis

A nutritious balanced diet plays a vital role in human health and well-being. Fresh fruits and vegetables therefore, constitute an essential part of a balanced diet, due to the presence of significant amounts of essential nutrients, minerals, vitamins and antioxidant compounds (Lee, C. Y. & Smith, 2000; Lewis & Ruud, 2004; Sharma et al., 2010). Different classes of pesticides have been used effectively to control pest and disease of fruit and vegetables, but

they may penetrate into the tissue and remain as residues. The analysis of fruit and vegetable samples for qualitative and quantitative evaluation of pesticides is an important quality control procedure, put in place to ensure their quality and safety for human consumption. Hence, their concentration must always remain minimal in fruit and vegetable samples (Tan & Abdulra'uf, 2012).

To ensure that the residues on fruits and vegetables are below levels which are not harmful to the consumers and ensure their quality, the joint Food and Agricultural Organization of the United Nations (FAO) and World Health Organization (WHO) Food Standard established the Codex Alimentarius Commission that set the maximum residue limit (MRL) for pesticides and other contaminants in fruits and vegetables. Several other countries also set default MRLs, through various monitoring agencies such as the United State Environmental Protection Agency (USEPA), United States' Food and Drug Administration or regional organizations such as the European Union (EU) Commission and Parliament, and the MRLs may vary by countries.

The monitoring of pesticide residues in fruits and vegetables is undertaken to ensure their quality, due to the increasing awareness of the health effects of their accumulation in the body. Therefore, there is need for fast, effective and efficient analytical methods, which allow the simultaneous determination of multiclass and multi-residue pesticides, with high selectivity and sensitivity, low cost, high sample throughput, less tedious and allows proper quantification of the residues (Garrido Frenich et al., 2012; Guo, X. et al., 2013). The analysis of fruit and vegetable samples for the presence of pesticide residues, belonging to different classes, with a broad range of physicochemical characteristics, which are also

present at trace levels embedded in complex matrices, is not a simple task and poses a special problem for analytical and environmental chemists (Kataoka, Lord, & Pawliszyn, 2000; Lambropoulou & Albanis, 2007a).

The purpose of any analytical method is to evaluate and obtain information about the nutritional value, quality of the product and to monitor the presence of pesticide residues and other food contaminants. Due to the complex nature of the fruit and vegetable samples, they cannot be handled directly by analytical instruments (Kataoka et al., 2000), and hence the need for sample preparation prior to the instrumental analysis.

1.1.2 Use of Pesticide

Pesticides are unavoidable inputs in agriculture and public health that are produced in large quantities, since the end of World War II (Barbash, 2006; Sharma et al., 2010) and their worth have been demonstrated through increase in global agricultural production, eradication of insect borne and epidemic diseases and conservation of the ecosystem (Ecobichon, 2001a). The use of pesticides increased significantly in the late 1940s, and thus the immediate benefit of its uses overshadowed its toxicity. The most widely used pesticides in agriculture are insecticides on insects, herbicides on weeds, rodenticides on rodents and fungicides to control fungi, mold and mildew. The USEPA estimated (Table 1.1) that in 2007, about 5.2 billion pounds of pesticides are used worldwide with US accounting for 22% of the total use and 80% of this is used for agricultural purposes. Herbicides are used in high quantities compared to other pesticides and the US accounted for 25% of the global herbicide use, 10% of the insecticide use, 14% of the of fungicide

use, and more than 25% of other classes of pesticides use (EPA, 2011a, 2011b; Grube, Donaldson, & Kiely, 2011).

Table 1.1: World and US Pesticide Consumption: Volume of Active Ingredient

Year/Type	World Market Million lbs.	US Market Million lbs.	US % of World Market
1993	4,500	1,081	24
1994/1995	5,710	1,222	21
1996/1997	5,684	1,231	22
1998	5,650	1,206	22
1999	5,650	1,244	23
2000	5,341	1,234	23
2001	5,046	1,203	23
2006	5,197	1,127	22
2007	5212	1,133	22

(Aspellin, 1997; Aspellin & Grube, 1999; Donaldson, Kiely, & Grube, 2002; Grube et al., 2011; Kiely, Donaldson, & Grube, 2004)

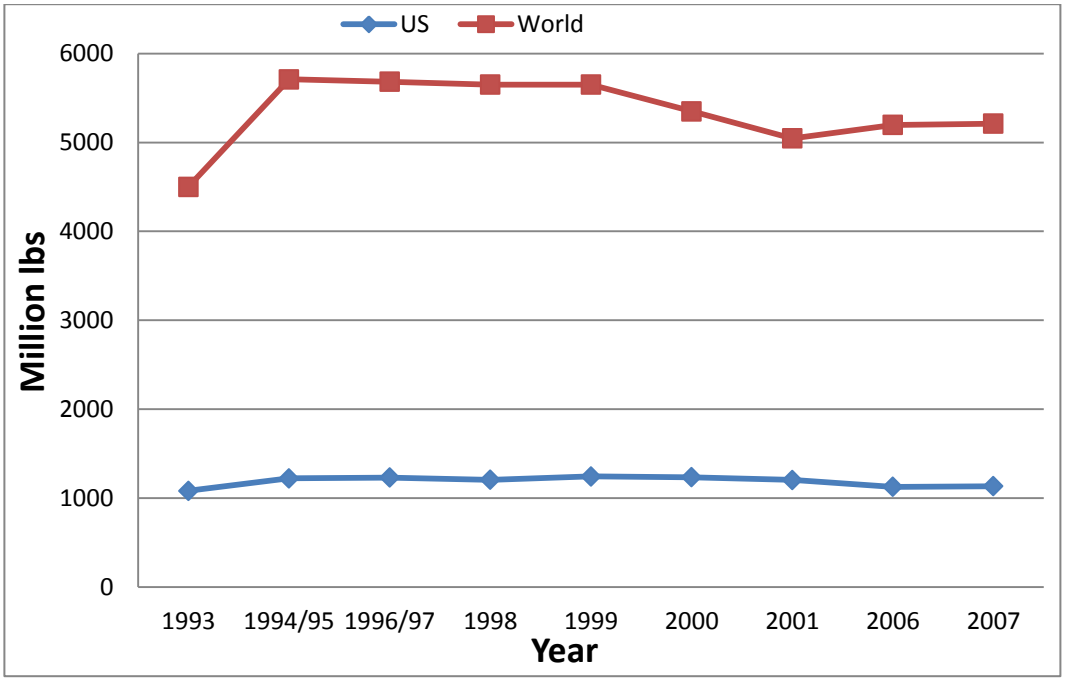


Fig 1.1: World and US Pesticide Consumption: Volume of Active Ingredients. (Aspellin & Grube, 1999; Donaldson, Kiely, & Grube, 2002; Grube et al., 2011; Kiely, Donaldson, & Grube, 2004)

Table 1.2: World and US Use of Pesticides by Type: 2007

Pesticide class	US	% of a.i	World	% of a.i.	US % of World Use
	Million pound of a.i		Million pound of a.i		
Herbicides[1]	531	47	2,096	40	25
Insecticides	93	8	892	17	10
Fungicides	70	6	518	10	14
Others[2]	439	39	1705	33	26
Total	1,133	100	5,211	100	22

[1] include herbicides and plant growth regulators

[2] other= rodenticides, fumigants, nematocides, molluscicides and other chemicals

a.i = active ingredients. (Grube et al., 2011)

1.1.3 Benefits of Pesticide Use

In many parts of the world, especially in the developing and under-developed countries, excessive loss of farm produce to insects and other pests has been reported to lead to starvation and famine (Costa, 2008). Postharvest loss of crop contributed to hunger and malnutrition which has killed more than 15 million children (Cooper & Dobson, 2007). The use of pesticides seems to have allowed the production of inexpensive, affordable, and low cost food, especially fruits and vegetables, which are vital in the protection against cancer and heart diseases, due to the presence of antioxidants in them (Lee, C. Y. & Smith, 2000; Lewis & Ruud, 2004).

Although the most obvious use of pesticides in agriculture is in the improved yield of crops, there are some salient features which provide subtle or incremental benefits, distributed over a large area. Cooper and Dobson (2007) adopted the effect and benefits model for analyzing the numerous potential benefits of pesticides use. The other benefits include increase in revenue for farmers, due to reduced labour costs, reduced fossil fuel use of farm machines, reduce the production of highly toxic alkaloids like mycotoxins and increase shelf life of fruits and vegetables. Thus higher yields of farm produce reduce pressure to cultivate un-cropped land which is beneficial for the environment, thereby conserving the natural ecosystem (Bruns, 2003; McNeely Jeffrey & Scherr Sara, 2003).

1.1.4 Risk of Pesticide Use

Pesticides are considered as one of the most dangerous contaminants in the environment, because of their persistency, biotransformation in the food chain, bioaccumulation in animals and mobility in the environment and their possible risks to human health (Andreu & Picó, 2012; Araoud et al., 2007; Bagheri et al., 2012). Due to non-selectivity of pesticides for the target specie, they always cause an adverse effect on non-target organisms (Costa, 2008). Pesticides affect normal and basic metabolic activities in the human system. Pesticides are meant to be poisonous and pose a hazard with their production, transport and applications, while their normal use often leads to the contamination of the environment. Pesticides alter the electrophysiological properties of the nerve cell membrane and its associated enzymes, disrupting the kinetics of essential mineral ions flowing in the membrane. They interfere with the sodium channel in the axonal membrane and cause imbalance in the ratio of sodium and potassium surrounding the nerve fibers (Costa, 2008; Kamrin, 2000; Smith, 2004). This results in nerve motor unrest and increased frequency of continuous transmission in the nerves and abnormal susceptibility to external stimuli.

Organochlorine pesticides (OCP), are known endocrine disruptors, and interfere in the synthesis, secretion, transport and other actions of natural hormones in the body. Organophosphorus and carbamates pesticides are acetylcholinesterase inhibitors, and inhibit the activities of the enzymes that regulate neurotransmission by hydrolyzing acetylcholine which accumulates at the synaptic junction, and they also form a covalent bond at the active serine site, thereby deactivating acetylcholinesterase which often leads to behaviourial change. OPP also cause delayed neuropathy. (Costa, 2008; Ehrich & Jortner, 2010; Stenersen, 2004; Thompson, C. M. & Richardson, 2004; Wilson, 2010). Pyrethroid

pesticides are relatively less toxic, their use in enclosed and poorly ventilated spaces has been observed to affect the sensory, motor and central nervous system and disrupt the voltage-gated sodium channels, by binding to the α-subunit of the sodium channel and slow the rate of activation and deactivation of the sodium channel (Costa, 2008; Ecobichon, 2001b).

Pesticide exposure can be as a result of occupational, accidental and pesticide residues in food and can be contacted through skin, mouth, lung and eyes (Mahmoud & Loutfy, 2012). Acute exposure to pesticides can lead to a wide range of chronic effect: including blood disorder, reproductive effect, birth defect, benign or malignant tumor, endocrine disruption, nerve disorder and genetic change, while symptoms such as nausea, vomiting, diarrhea, severe headache, dizziness, tonic and clonic convulsion, muscle fasciculation, joint swelling, leg and back pain, hypertension, drowsiness, increased sweating, abdominal pain, anorexia dyspnea, skin dryness and nail discolouration. They also cause memory loss, hyper-susceptibility to external stimuli, tremor, flaccid paralysis, emotional liability, restlessness, loss of coordination and in some cases enlargement of the liver (Costa, 2008; Ecobichon, 2001b; Lotti, 2010; Mahmoud & Loutfy, 2012; Yu, M.-H., 2001). Some are carcinogenic and have been used for suicidal purposes.

Pesticide use has been estimated to cause deaths of about 220 thousand people, with 3 million poisoning reported and 750 thousand cases of chronic illness, most especially in the developing countries (Atreya et al., 2011; WHO, 2006). Exposure of people to pesticides also reduces their productivity due to declining health condition, economic loss due to

payments for health services and change in social behavior due to loss of household income as a result of ill-health (McIntyre et al., 2006).

1.1.5 Pesticide Economics

The United States is the single largest market for pesticides (Tables 1.3 and 1.4). The US represents more than 30% of the total world market expenditure between 1993 and 2007 (Aspellin, 1997; Aspellin & Grube, 1999; Donaldson et al., 2002; Grube et al., 2011; Kiely et al., 2004). The world pesticide market expenditure was estimated at over USD35 and USD39 billion in 2006 and 2007 respectively, with US share of over USD11 and USD12 billion in the same year (Grube et al., 2011). Pesticides sale in the world has been relatively stable since 1994. The volume of active ingredient was about 3.9 billion pound in 1993 and increased to about 5.7 billion pound in 1994 and has been relatively stable at about 5.2 billion pounds in 2007 (Fig 1.2). In some Asian countries (Table 1.5), China accounts for the use of more than half (52 %) of the active ingredient with an expenditure of 5.67 billion USD.

Table 1.3: World and US Pesticide Market Expenditure

Year/Type	World Market Million USD	US Market Million USD	US % of World Market
1993	25,289	8,484	33
1994/1995	37,696	11,316	30
1996/1997	37,048	11,897	32
1998	33,503	11,416	34
1999	33,593	11,155	33
2000	32,769	11,165	34
2001	31,756	11,090	35
2006	35,813	11,784	33
2007	39,443	12.456	32

(Aspellin, 1997**;** Aspellin & Grube, 1999**;** Donaldson, Kiely, & Grube, 2002**;** Grube et al., 2011**;** Kiely, Donaldson, & Grube, 2004)

Table 1.4: World and US Pesticide Market Expenditure: 2007

Pesticide class	US Million USD	%	World Million USD	%	US % of World Use
Herbicides[1]	5,856	47	15,512	39	38
Insecticides	4,337	35	11,158	28	39
Fungicides	1,375	11	9,216	23	15
Others[2]	886	7	3,557	9	25
Total	12,454	100	39,443	100	32

[1] include herbicides and plant growth regulators
[2] other= rodenticides, fumigants, nematocides, molluscicides and other chemicals
a.i = active ingredients. (Grube et al., 2011)

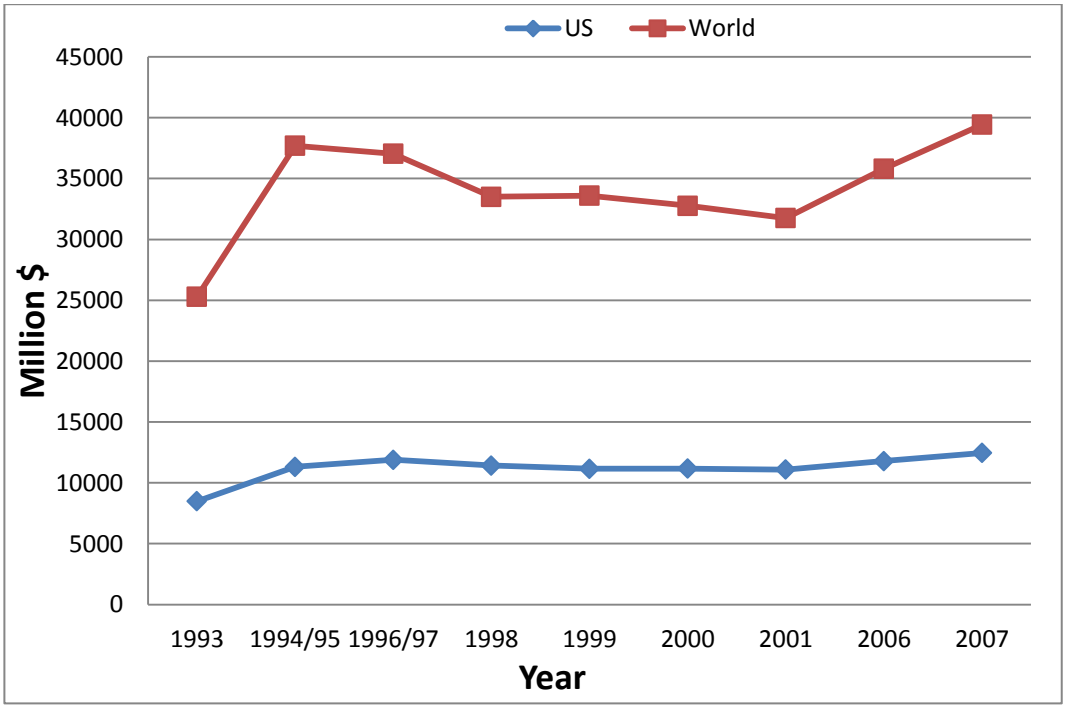


Fig 1.2: World and US Pesticide Market Expenditure. (Aspellin, 1997; Aspellin & Grube, 1999; Donaldson, Kiely, & Grube, 2002; Grube et al., 2011; Kiely, Donaldson, & Grube, 2004)

Table 1.5: Pesticide Use in some Asian Countries

S/No.	Country	Ton a.i.	Ton product	USD '000 value
1	Bangladesh	3635	22100	75000
2	Cambodia	42	198	226
3	China	258000	1000000	5670000
4	DPR Korea	3000	12000	60000
5	India	41020	164080	820400
6	Rep. of Korea	26610	100000	842638
7	Lao PDR	10	40	200
8	Malaysia	51065	204260	85020
9	Myanmar	758	3030	15095
10	Nepal	145	580	2100
11	Pakistan	32500	129589	172300
12	Philippines	7934	31735	158675
13	Sri Lanka	1696	6329	49000
14	Thailand	49108	132509	253537
15	Vietnam	24473	50000	159000

(Abhilash & Singh, 2009)

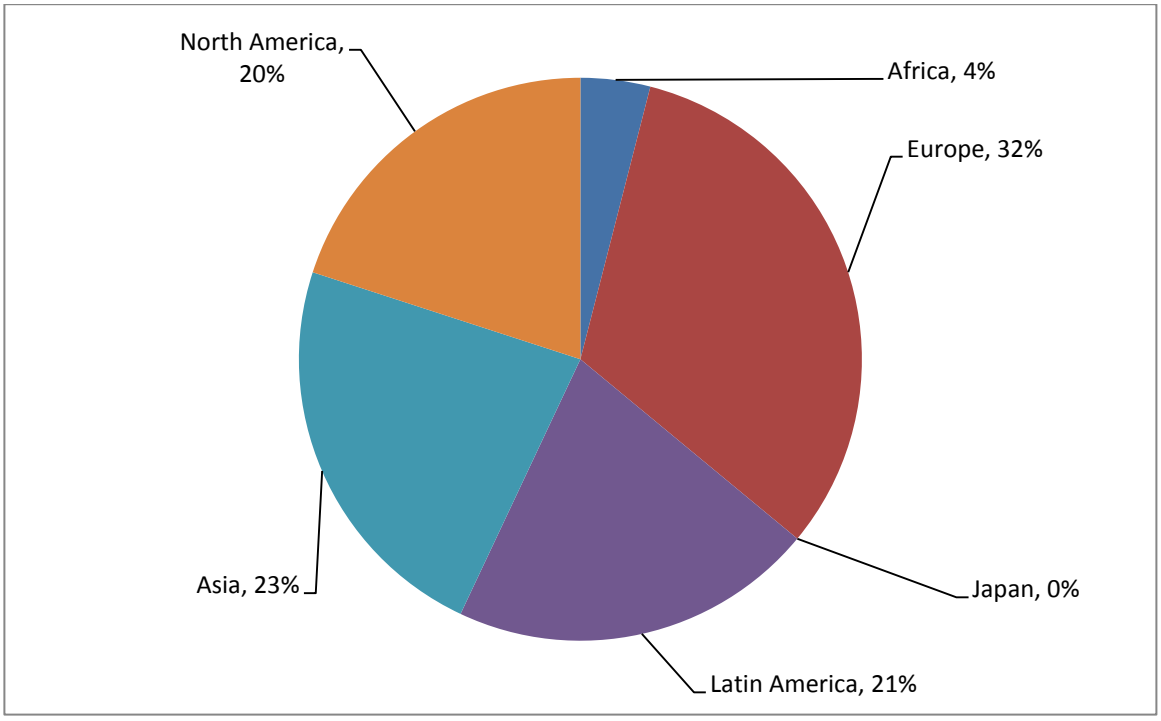


Fig 1.3: World Market Share of Pesticides by Region: 2008. (Mahmoud & Loutfy, 2012)

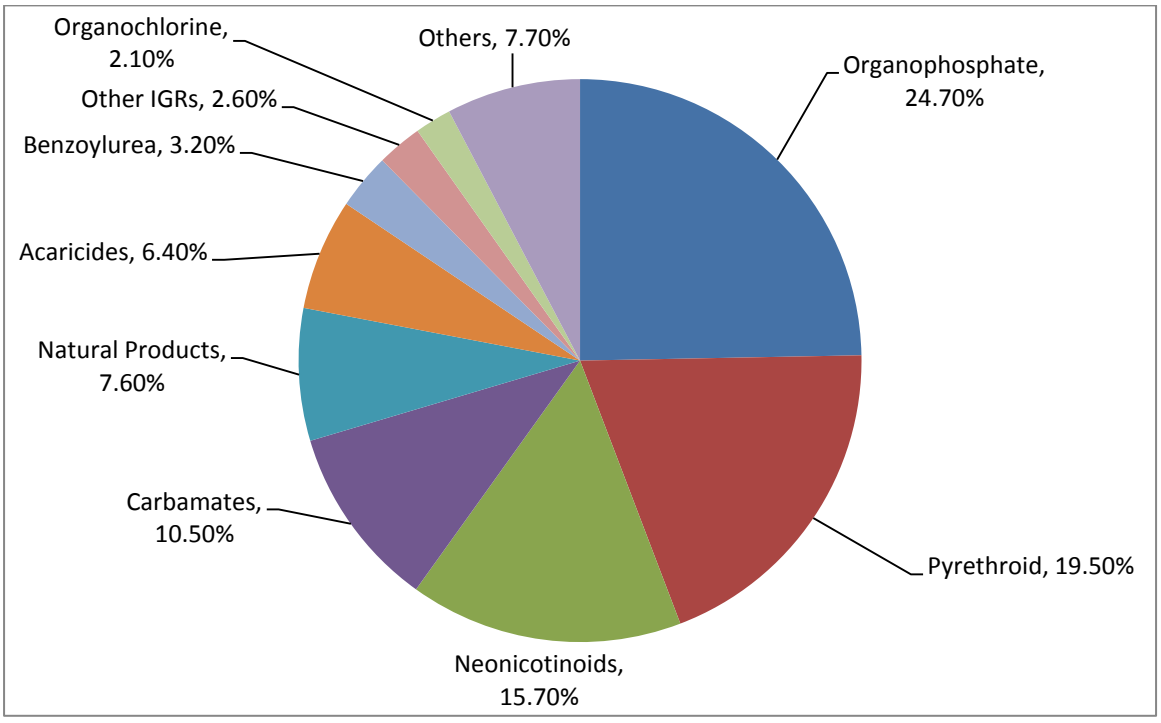


Fig 1.4: Major Classes of Pesticides and their Global Market Share: 2004. (Nauen, 2006)

The pesticide market sales and expenditure is affected by certain factors such as climate, economics and trend in agriculture and government policies. The relative increase in types of pesticide used despite a drastic reduction in amount used is attributed to the development of more potent pesticides, greater awareness and more efficient use of pesticides. Organophosphorus pesticides accounted for the highest percentage of global use, because it is less volatile and less persistent in the environment.

1.1.6 Pesticide Residues and Legislation

The production, sale and use of pesticides require strict rules and regulations to ensure their safe use and to protect human health and the environment (Costa, 2008; Ecobichon, 2001b; Yu, S. J., 2008). The pesticide legislations are aimed at increasing the level of protection to human health, animal welfare and the environment (Stark, 2011), the efficacy of pesticide products for their proposed use and to protect the economic interest of a country in international trade (Vapnek, Pagotto, & Kwoka, 2007). Over the years, there has been a greater public awareness about the presence of pesticide residues in food, drinking water and the environment. Therefore, there is a need to pay greater attention in order to regulate the use of pesticides.

There have been international and national legal frameworks guiding the trade and use of pesticides. The Food and Agricultural Organization of the United Nations (FAO), designed an International Code of Conduct on the Distribution and Use of Pesticides (FAO, 2003), to provide universal standards on the conduct of all stakeholders involved in the pesticide industry. Some of these legal frameworks are subjected to constant review and redesigning. The design of international regulatory framework involve FAO, the United Nation

Environmental Programme (UNEP), the World Health Organization (WHO), and the International Labour Organization (ILO), some of which are legally binding and some are not, but they implicate pesticides management (Vapnek et al., 2007).

The regulatory instruments that are legally binding on member nations include:

(i) Rotterdam Convention on the Prior Informed Consent Procedure for Certain Hazardous Pesticides and Industrial Chemicals in International Trade

(ii) Stockholm Convention on Persistent Organic Pollutants

(iii) Basel Convention on the Transboundary Movement of Hazardous Wastes and their Disposal

(iv) Montreal Protocol on Substances that Deplete the Ozone

(v) ILO Convention on Safety and Health in Agriculture

(vi) ILO Convention Concerning Safety in the Use of Chemicals at Work

The regulatory instruments that are voluntary and are not legally binding on member nations include:

(i) FAO International Code of Conduct on the Distribution and Use of Pesticides

(ii) FAO Guidelines to International Code of Conduct

(iii) Standards of the Codex Committee on Pesticide Residue

(iv) UN/ECOSOC Globally Harmonized System of Classification and Labelling of Chemicals

The United States Environmental Protection Agency (EPA), the Food and Drug Administration (FDA) and the US Department of Agriculture (USDA) are saddled with the responsibility of regulating the use and sales of pesticides in the United States. The US pesticide legislations include the Federal Insecticide Act, the Federal Food and Drug Protection Act and the Food Quality and Protection Act (Costa, 2008; Yu, S. J., 2008). In most European Union (EU) Countries, there are number of directives and regulations governing the use of pesticides, and these include Council Directives 79/117/EEC, 91/414/EEC, 2000/60/EC, 2006/42/EC and 2009/128/EC, Regulations (EC) No 396/2005 and (EC) No 1107/2009, Commission Regulations (EU) No 899/2012 (Ballantyne & Marrs, 2004; EU, 2005, 2009a, 2009c, 2012; Hillocks, 2012; Stark, 2011). Countries in the developing nations also have legislations that are varied in detail. In Malaysia, the use, sale and distribution of pesticides is regulated by the Pesticide Act 49 enacted in 1974 (Abdulahi, 2003) and amended in 2006 (Act 1974, 2006).

The regulatory control of pesticides involve registration and the enforcement of legislation, which include the provision of scientific evidence on the effective control of the target pest, the classification of pesticides for specific use, residue data, ecotoxicology and environmental fate and behavior, human toxicology and occupational medical data, proposed usage data and the specification of the active material (Ballantyne & Marrs, 2004; Matthews et al., 2011; Waxman, 1998). The legislation also involves the assignment of maximum residue levels (MRLs), which can be estimated from the toxicological characteristics such as the acceptable daily intakes (ADI) (Van Eck, 2004) and the median lethal dose (LD_{50}).

The regulatory status also indicates if the active ingredient is a General Used Pesticide (GUP) or as Restricted Use Pesticide (RUP) according to EPA guidelines (Kamrin, 2000). The various legislations and directives were proposed in order to check the overuse and misuse of pesticides in the environment and curb the adverse effects of the pesticides on human health and to reduce environmental pollution.

1.1.7 Pesticide Classifications

Pesticides are be classified according to target organisms (Table 1.6), chemical structures (Table 1.7), mode of action (Table 1.8), their environmental persistency and pathway of movement into the target organisms (Ballantyne & Marrs, 2004; Fenik, Tankiewicz, & Biziuk, 2011; Lydy et al., 2004; Matsumura, 2010; Novak & Lampman, 2010; PPDB, 2009; Waxman, 1998; Yu, S. J., 2008). The WHO classified pesticides by hazard, that refers to the oral and dermal acute toxicity which is experimentally-derived by the value of LD_{50} for oral and dermal exposures (Tables 1.9) (WHO, 2010).

Table 1.6: Classification of Pesticides According to Target Organisms

Pesticide Type	Target Organism
Insecticide	Insects
Fungicides	Fungi
Herbicides	Plants
Molluscicides	Slugs, Snails
Rodenticides	Rodents
Acaricides	Mites
Nematicides	Nematode worms
Avicides	Birds
Bactericides	Bacteria
Piscicide	Fish
Predacide	Vertebrate
Miticides	Mites

Table 1.7: Classification According to Chemical Structure

Pesticide Type	Description
Organophosphates	Contain central phosphorus atom
Organochlorines	Contain carbon, chlorine and hydrogen
Carbamates	Esters of carbamic acid
Pyrethroids	Analogs of natural pyrethrins
Triazines and Triazoles	Contains single ring with 3 nitrogen atom
Ureas	Contains central carbamide functional group
Neonicotinoids	Analogs of nicotine
Avermectin	Micro-organism derived
Phenylpyrazole	Contains ring with 2 nitrogen atom

Table 1.8: Classification According to their Mode of Action

Mode of Action	Pesticide Type
Acetylcholinesterase inhibitor	Organophosphates, carbamates
GABA-gated chloride channel antagonist	Organochlorines phenylpyrazole
Sodium channel modulator	Pyrethroids, semicarbazones
Nicotinic acetylcholine receptor agonist	Neonicotinoids
Glutamate-gated chloride channel activator	Avermectins
Chitin synthesis inhibitor	Benzoylureas
Octopamine receptor agonist	Formamidines
Endocrine disruptor	organochlorides

Table 1.9: WHO Recommended Classification by Hazard

		Rat LOD_{50} (mg/kg body weight)			
		Oral		Dermal	
Class	Hazard Level	Solid	Liquid	Solid	Liquid
Ia	Extreme	5 or less	20 or less	10 or less	40 or less
Ib	High	5 – 50	20 – 200	10 – 100	40 – 400
II	Moderate	50 – 500	200 – 2000	100 – 1000	400 – 4000
III	Slight	Over 500	Over 2000	Over 1000	Over 4000
IV	Unlikely on normal use	Over 2000	Over 3000	Over 4000	Over 6000

(WHO, 2010)

1.2 Pesticides: General Properties and Environmental Fate

The biotic and abiotic environmental processes influenced by the physicochemical properties of pesticides as well as their environmental biological processes, determine pesticides persistency and mobility in the environment, the relocation from area of application (Magga et al., 2012; Rice, Horgan, & Rittenhouse, 2010; Ulrich, Dietrich, & Fohrer, 2013) and their transport, partitioning and fate (Seiber, 2002), Their chemical structures determine the stability in terms of resistance to photolysis, chemical hydrolysis and microbial degradation (Carlile, 2006). The knowledge of their properties (physical and chemical) is also essential in the choice of extraction technique and method development in residue analysis. The following general properties are discussed:

1. Water solubility
2. Vapour pressure/Henry's law
3. Octanol/water partition coefficient
4. Sorption and desorption
5. Toxicity and Mode of Action
6. Bioconcentration factor
7. Degradation

1.2.1 Water Solubility

The solubility of pesticide in water describes the amount of pesticides in milligram that will dissolve in a liter of water and is usually given in milligram/liter (mg/L or ppm) measured at room temperature (20 to 25 ^{0}C) (Jenkins & Thomson, 1999). The knowledge of pesticide solubility in water can be used to trace its environmental distribution between water, soil, air and organisms, degradation pathway (Carlile, 2006; Linde, 1994; Yu, S. J., 2008),

partitioning ability between solid and liquid phases in the environment (Bowman & Sans, 1979) and its persistence on the plant surface (Thorbek & Hyder, 2006). The solubility of pesticides is determined by factors such as its polarity, the presence hydrogen bonding, pH, molecular weight and temperature (Kerle, Jenkins, & Vogue, 2007; Linde, 1994).

The pesticide mobility from soil to groundwater depends on its solubility (Åkesson et al., 2013), and is an indication of its ability to be leached into ground and surface water or precipitated on soil surface (Deeb & Goodarzi, 2010). Thus, the higher the solubility of pesticide in water, the more their run off by leaching, the less their accumulation in the environment and they can easily be degraded by hydrolysis (Jenkins & Thomson, 1999; Kerle et al., 2007; Zacharia, 2011).

1.2.2 Vapour Pressure and Henry's Law Constant

The vapour pressure of a substance is a measure of its volatility in pure state from water or moist soil (Åkesson et al., 2013; Jenkins & Thomson, 1999; Kerle et al., 2007; Zacharia, 2011), which is described by Henry's law constant. Henry's law constant measures the pesticide volatility as a function of its vapour pressure and its solubility in water and is defined as the ratio of concentration of pesticide in air to its concentration in solvents, estimates by the vapour pressure, temperature, molecular weight and solubility (Åkesson et al., 2013; Kerle et al., 2007; Linde, 1994). The higher the vapour pressure and the Henry's law constant of a pesticide, the higher its volatilization and therefore, the higher its tendency to be distributed and disperse over a wide area in the environment, while pesticides with low vapour pressure and Henry's law constant tend to be more accumulated in the environment and have high leaching potential (Carlile, 2006; Linde, 1994).

1.2.3 Octanol/Water Partition Coefficient

This is the indication of distribution and solubility of substance at equilibrium between organic solvent (octanol; relatively non-polar) and aqueous solvent (water; polar) (Åkesson et al., 2013). It is a measure of dissolved mass substances at equilibrium between equal volumes of n-octanol and water. It is the ratio of the concentration of pesticide in n-octanol to its concentration in water (Hristovski, Westerhoff, & Posner, 2011). It is used to predict the environmental fate and transport of pesticide and their partitioning between organic and aqueous phases (Rice et al., 2010; Schuurmann et al., 2007). Partition coefficient is dependent on polarity, solubility, molecular weight and density of the pesticide (Carlile, 2006; Linde, 1994).

The octanol/water partition coefficient is used to estimate and predict pesticide characteristics, such as lipophilicity, structure-activity relationship, distribution between the environmental compartment, bioaccumulation and bioconcentration factor (Altinok, Capkin, & Boran, 2011; Carlile, 2006; Hristovski et al., 2011; Rice et al., 2010; Seiber, 2002; Wasik, Miller, & Tewari, 1983). Pesticides with higher coefficients are partitioned in organic phase, while those with lower partition coefficient can be easily leached due to their high solubility in water. It is the difference in the free energy of solvation of solute in organic phase and the free energy of solvation in aqueous phase (De Fina et al., 2002) and is given by the equation:

$$K_{ow} = \frac{Pesticide\ concentration\ in\ octanol}{Pesticide\ concentration\ in\ water}$$

$$pK_{ow} = -log_{10}K_{ow}$$

1.2.4 Sorption and Desorption of Pesticides

This refers to the attraction and retention of pesticides on the surface of a solid or liquid substrate. It describes the attractive force between pesticide and soil particle and other solid environmental substrates (Gao et al., 2012; Kerle et al., 2007; Rao & Alley, 1993). Chemisorption (chemical sorption) occurs, when the pesticide molecules are retained on the surface through formation of chemical bond, while physisorption (physical sorption) is the retention of the pesticide molecules on the soil surface through the formation of weak intermolecular attraction (Van der Waal forces). The interactions of pesticides with soil and other environmental substrates are greatly affected by sorption and desorption processes (Arias-Estévez et al., 2008), and also on the rate of other physicochemical properties such as biodegradation, hydrolysis, volatilization and photochemical oxidation (Magga et al., 2012).

Sorption can either be adsorption on the surface of substrate or absorption into the interior of the sorbent matrix such as organic matter (Rao & Alley, 1993). Adsorption of pesticides to soil or other solid substrate is due to the attraction between the charged pesticide molecules and the charged soil or solid particles (Gao et al., 2012). Pesticides molecules that are strongly sorbed into the soil particle are not likely to leach, are less available for plant uptake and degradation and are more persistence in the environment (Jenkins & Thomson, 1999; Kerle et al., 2007). Pesticides molecules that are weakly sorbed will leach depending on their solubility in water. Sorption of pesticides on soil particles are influence by factors such organic carbon content, polarity, solubility, soil surface area and size, chemical function, octanol/water partition coefficient, organic matter in solution, salinity,

pH (for weakly acidic and basic pesticides), and soil moisture and texture (Gao et al., 2012; Kerle et al., 2007; Linde, 1994; Magga et al., 2012; Rao & Alley, 1993).

Adsorption of pesticides to soil particles is quantified using distribution coefficient, K_d (or adsorption partition coefficient), which is the ratio of concentration of pesticide sorbed onto soil particles to the concentration of pesticide in solution, i. e. the ratio of sorbed-phase concentration (µg/g) to the solution phase concentration ((µg/mL) at equilibrium. The distribution coefficient depends on the soil characteristic and therefore the need to adjust its value by the percentage of organic carbon content of the soil, this gives sorption coefficients (K_{oc}) which describes the affinity of pesticides to soil organic carbon content and is independent on soil type (Åkesson et al., 2013; Gao et al., 2012; Jenkins & Thomson, 1999; Kerle et al., 2007; Linde, 1994; Rao & Alley, 1993).

$$Distribution\ Coefficient, K_d = \frac{Sorbed-phase\ concentratio}{Solution-phase\ concentration}$$

$$Sorption\ Coefficient, K_{oc} = \frac{Distribution\ coefficient\ (K_d)}{Organic\ Carbon\ (OC)}\ x\ 100$$

Adsorption determines the mobility and bioavailability of pesticides in the environment. The higher the value of sorption coefficient, the more the pesticides sorbed onto the soil particle and the less its mobility. The sorption potential of pesticides are determined using sorption isotherm, with the assumption that the isotherms (Freundlich and Langmuir) are linear and reversible (Gao et al., 2012; Rao & Alley, 1993).

1.2.5 Toxicity and Mode of Action

Toxicity is the tendency of pesticide to produce adverse or harmful effect due to chronic or acute exposures which may range from slight to several symptoms (Watson, 2004; Waxman, 1998). Toxicity is directly proportional to exposure and exposure is a function of time and dose. Hazard which is the harmful effect of pesticide is a function of toxicity and exposure (Rozman, Doull, & Hayes, 2012; Waxman, 1998). The scientific study of the qualitative and quantitative adverse and harmful effect of pesticides and other chemicals on human, plants and animals is called toxicology (Rozman et al., 2012; Whitford et al., 2007).

Pesticide mode of action can be defined as a series of processes starting with the exposure and interaction of pesticide with organisms to biological response which result in harmful or adverse effect giving a set of physiological and biological signs (Borgert et al., 2004; Lydy et al., 2004; McCarty & Borgert, 2006). Toxicity of pesticides are performed with experimental animals physiology, exposed to various levels of pesticides active ingredient on long term study (Bermúdez-Saldaña et al., 2005; Séralini et al., 2012; Whitford et al., 2007) and is usually estimated using a dose-response and exposure relationship which is expressed in dose per unit weight lethal (mg/kg) to 50% of the population (LD_{50}) of the animals, or the concentration of the pesticides in an external media that will kill half of the test population (LC_{50}) under certain conditions (Carlile, 2006; Watson, 2004; Yu, S. J., 2008). The smaller the values of LD_{50} and LC_{50} the more toxic the pesticides, since it shows that small amount of the pesticides can kill half of the test animals.

The toxicology tests embrace all the circumstances of exposure of human to pesticides (Walker, 1998), and includes tests for hazard identification in animals such as: genetic toxicity, acute toxicity, short-term toxicity, chronic toxicity, immunotoxicity, reproductive toxicity and neurotoxicity (Carlile, 2006; Renwick, 2002; Watson, 2004; Yu, S. J., 2008). The tools developed to evaluate and analyze the toxic effects of pesticide include pesticide risk indicators (PRI) (Surgan, Condon, & Cox, 2010), toxic unit (TU) (Lydy et al., 2004), and pesticide toxicity indicator (PTI) (Belden et al., 2007).

1.2.6 Bioconcentration Factor

Bioconcentration of pesticide is the increase in the concentration that is present inside and/or on the surface an organism in relation with the concentration present in an external medium such as soil or water (Paraíba, 2007; Paraíba & Kataguiri, 2008). The bioconcentration factor describes the extent to which pesticide will accumulate in organisms (Fujikawa et al., 2009; Linde, 1994). It is a numeric value which evaluates the bioconcentration of pesticide, and expresses the partition of pesticide between organisms and the external medium (Paraíba & Kataguiri, 2008).

It is also described as the ratio of concentration of a chemical in an organism to the concentration in water at steady state (Fujikawa et al., 2009; Holland & Sinclair, 2004; Linde, 1994; Paraíba, 2007; Paraíba & Kataguiri, 2008). The higher the bioconcentration factor values, the higher the level of accumulation in lipid membrane (Fujikawa et al., 2009), and thus the measurement of bioconcentration factor values is required to allow for the estimation of the daily pesticide intake through the consumption of fruits and vegetables (Paraíba, 2007). The bioconcentration factors depend on the pesticides' solubility, polarity,

metabolism, lipid content and the habitats, and have a direct proportion to the pesticide octanol/water partition and adsorption coefficients (Linde, 1994).

1.2.7 Degradation

This refers to chemical processes through which pesticide molecules are broken down into smaller unit, which may be less toxic compared to the parent molecule (Yu, S. J., 2008; Zacharia, 2011). The chemical reactions involved in pesticides degradation could include abiotic reaction, such as photodegradation which occur in the presence of sunlight and biotic reaction (biodegradation), which occurs under enzymatic control in the presence of microorganisms. Both chemical and microbial degradation could lead to any of the following chemical reactions; oxidation, reduction, hydrolysis, isomerization, elimination, conjugation, rearrangement and dechlorination of pesticide molecules (Bansal, 2012; Chamberlain et al., 2012; Chambers, Meek, & Chambers, 2010b; Holland & Sinclair, 2004; Seiber, 2002; Yu, S. J., 2008). The rate of degradation which depends on the nature of pesticides and other factors, such as organic matter and clay contents, soil pH, temperature, pesticide reactivity and effluent irrigation (Bansal, 2012), is expressed as the half-life of the pesticide molecule in soils. The type of degradation process of a pesticide largely depends on the physico-chemical properties of the pesticide and the environmental conditions.

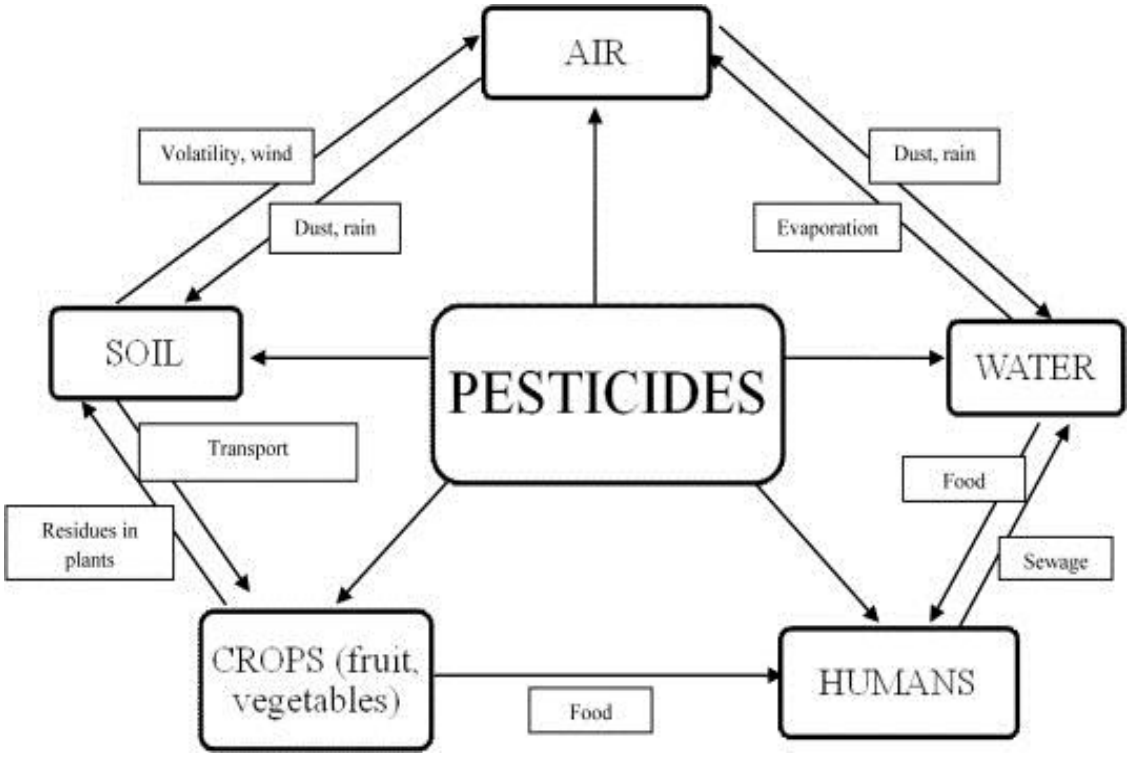


Fig 1.5: Pesticide Movement in Nature (Fenik et al., 2011)

1.3 Pesticide Classification according to Chemical Structure

1.3.1 Organophosphorus Pesticides (OPPs)

1.3.1.1 Structure of Organophosphorus Pesticides (OPPs)

Organophosphorus pesticides are esters and organic acid halides of phosphoric and phosphonic acids, with all the H atoms replaced by organic moieties and are the most widely used pesticides. Their structure is made up of a central phosphorous atom bonded to several side chains. They include organophosphate, organophosphonates, organophosphinates, organophosphoramidates, organophosphorothioates, organophosphorodithioates, organophosphonodithioates, organophosphonothioates, and organophosphoroamidothioates depending on the substituent atoms as shown in Figures 1.6 and 1.7 (Chambers, Meek, & Chambers, 2010a; Kamrin, 2000; Thompson, C. M. & Richardson, 2004). They are highly toxic and were by-products of chemical warfare

research in the development of nerves gas agent during World War II, such as sarin, soman and tabun (Yu, S. J., 2008).

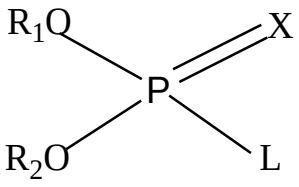


Fig 1.6: General Structure of OPPs (Chambers et al., 2010a; Costa, 2008; Kamrin, 2000)

where L is a very active and most variable leaving group, which can exhibit varieties of structures ranging from aliphatic or cyclic hydrocarbon to aromatic and heterocyclic structures. R_1 and R_2 are less reactive and are mostly alkoxy groups, but can also be alkyl, aryl, alkylthio, or alkylamino groups, while X is oxygen or sulphur atom (Chambers et al., 2010a; Costa, 2008).

OPPs vary in the groups attached to the central phosphorus atom through the sigma bonds, such as —— OR, ——SR,—— CR and ——NR in a variety of combinations. Organophosphates (phosphorus acid derivatives) are compounds in which the phosphorus atom is surrounded by four oxygen atoms, while in phophonates (phosphonic acid derivatives) contains three oxygen atoms and one carbon atom surrounding the phosphorus atom and phophinate has two oxygen atom and two carbon atoms bonded to the central phosphorous atoms. One or more of the oxygen atoms attached to the central phosphorous atom could be replaced by sulfur and/or nitrogen.

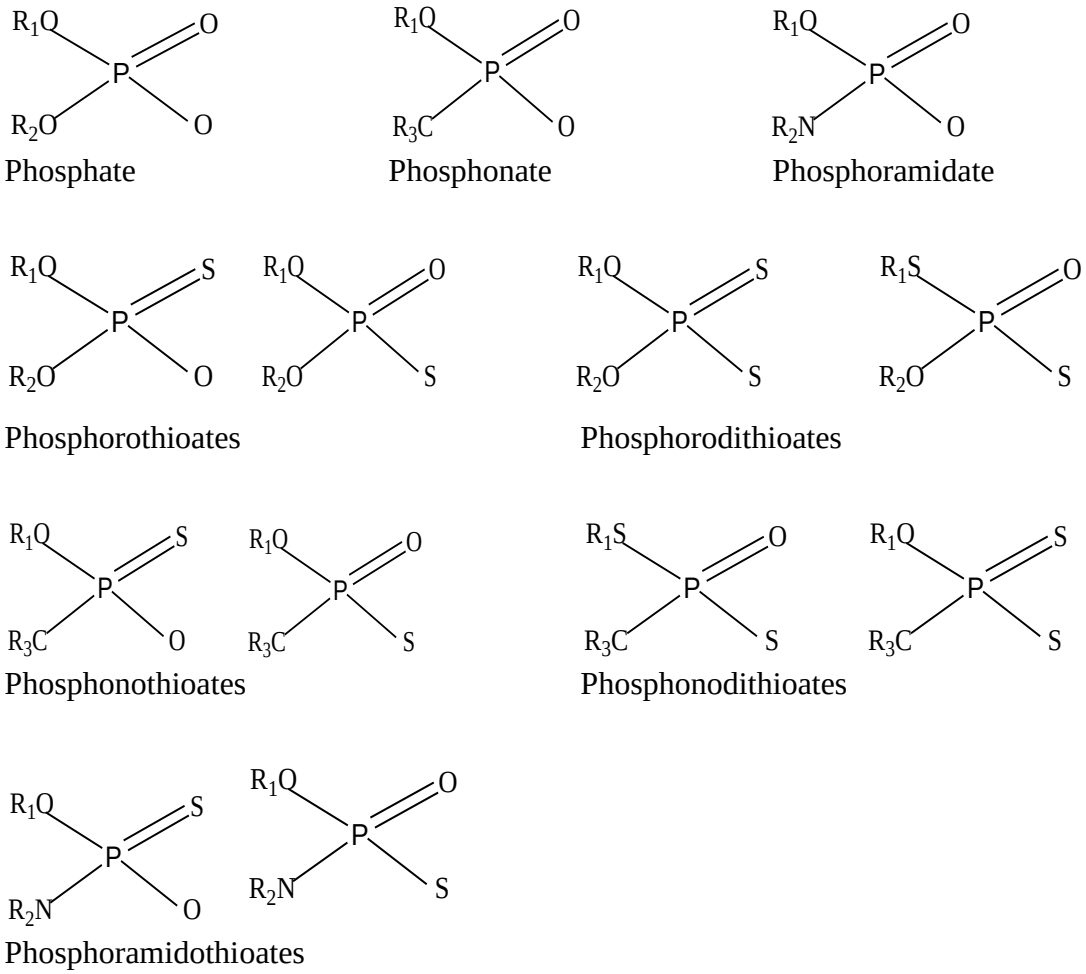


Fig 1.7: Structures of Major Classes of OPPs (Chambers et al., 2010a; Yu, S. J., 2008)

OPPs are synthesized by the reaction of elemental phosphorous with sulphur to produce P_2S_5 or by direct chlorination to yield PCl_5. The P_2S_5 and PCl_5 produced by these reactions are then converted to several intermediates through which most OPPs are synthesized. OPPs are stable in cool, dry and anhydrous conditions, but can be altered when exposed to light, heat and/or water and may undergoes hydrolysis, oxidation and rearrangement reactions (Chambers et al., 2010a).

1.3.1.2 Toxicology and Mode of Action of OPPs

OPPs are generally acutely toxic and they poison insects and mammals. Their toxicity depends on the nature of the leaving group attached to the phosphorus atom. The most toxic OPPs have oral LD_{50} in the range of 1–30 mg/kg, while the moderately toxic group has LD_{50} between 30–50 mg/kg and the less toxic group has LD_{50} between 60 and 1300 mg/kg.

Their mode of action is through the irreversible inhibition of acetylcholinesterase enzyme which causes interference in the nerve endings of the central nervous system. The leaving group is displaced by nucleophilic attack of the active site of serine when OPP phosphorylates the acetylcholinesterase enzyme (AChE). OPPs with P═S undergo oxidative desulphuration by phosphorylating a hydroxyl group on serine in the active site of the enzyme to their corresponding and highly polarized P═O analogues. The reaction which results in the formation of a transient intermediate complex is partially hydrolyzed with the loss of the leaving group, resulting in the formation of a stable and largely unreactive enzyme. The reaction can be carried out by chemical, biological and/or environmental agents and it speeds up the breakdown of acetylcholine produced in the nerve endings. Acute poisoning could cause respiratory failure, cardiac arrest which could result in death (Costa, 2008; Ecobichon, 2001b; Kamrin, 2000; Thompson, C. M. & Richardson, 2004; Waxman, 1998; Yu, S. J., 2008).

1.3.2 Organochlorine Pesticides (OCPs)

1.3.2.1 Structure of Organochlorine Pesticides (OCPs)

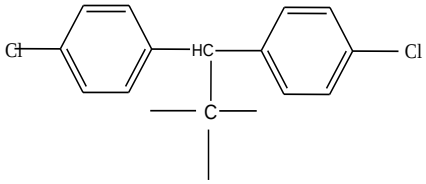
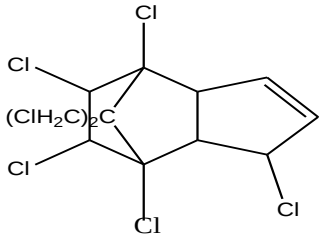
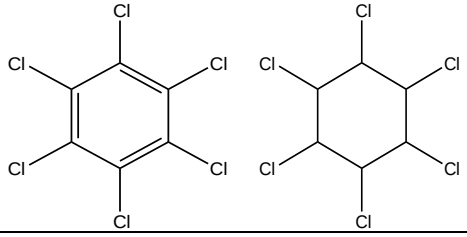
The organochlorine pesticides are hydrocarbon compounds which contain carbon, chlorine and hydrogen atoms with diverse group of agents. They include the chlorinated ethane derivatives and their analogues, though lacking a common structure are characterized by one or more chlorine atoms. OCPs are divided into three distinct chemical classes: dicholorodiphenylethanes, cyclodienes and chlorinated benzenes and cyclohexanes and related caged structures as shown in Table 1.10. Members of each group share similar or identical composition, but may have different stereo-structures and shapes and also differ in toxicities. OCPs also include fabricated chemicals such as polychlorinated biphenyls, dioxin and dibenzofurans which are by-products of several industrial processes. Their different chemical structures and properties lead to their broad range of uses. Most of the OCPs have been banned for use in some countries, but are still in use in the developing countries because of the effectiveness and low cost (Costa, 2008; Ecobichon, 2001b; Gallagher, De Souza, & Regan, 2004; Kamrin, 2000; Yu, S. J., 2008).

1.3.2.2 Toxicology and Mode of Action of OCPs

OCPs are stimulants of the nervous system which are absorbed orally, by inhalation and by dermal exposure. After exposure to the OCPs, some of the absorbed doses are stored in the fat tissues as an unaltered parent compounds. They interfere with fluxes of the cations in the nervous system and affect the nerve fibers along the length of the fiber. They increase neuronal irritability by disturbing the transmission of nerve impulse and disrupt sodium/potassium balance surrounding the nerve fibers. They are also known or suspected to be endocrine disrupting compounds, which interfere with the anabolic and catabolic

activities of the natural hormones, responsible for the maintenance of homeostatics, reproductive development and behaviour. Acute exposure to a high dose of OCPs have been found to cause motor unrest, spontaneous and uncontrolled movement of the body and hypersensitivity to external stimuli, but are rapidly reversible when the concentration falls below some threshold levels which varies depending on the structure of the OCPs (Costa, 2008; Ecobichon, 2001b; Waxman, 1998).

Table 1.10: Structural Classification of Organochlorine Pesticides

Dichlorodiphenylethanes		DDT Dicofol Perthane Methoxychlor Methlochlor
Cyclodienes		Aldrin Dieldrin Heptachlor Chlordane Endosulfan
Chlorinated benzenes Cyclohexanes		HCB, HCH, Lindane (α-BHC)

(Ecobichon, 2001b)

1.3.3 Pyrethroid Pesticides (PPs)

1.3.3.1 Structure of Pyrethroid Pesticides (PPs)

Pyrethroid pesticides (PPs) are synthetic materials which originate from the naturally occurring pyrethrins. Pyrethrins are extracts of the dried heads of flowers of *Chrysanthemum cinerariaefolium,* which has about 50% of active insecticidal ingredients. Natural pyrethrins consist of six ketoalcoholic esters of chrysanthemic and pyrethric acids, namely pyrethrins I & II, jasmolin I & II and cinerin I & II (Costa, 2008; Kaneko, 2010; Waxman, 1998; Yu, S. J., 2008). The instability of the natural pyrtethrins in daylight led to the development of pyrethroid, the synthetic analogs (Costa, 2008). PPs are classified into types I and II compounds. The type I pyrethroid pesticides are produced by esters lacking α-cyano substituent and are made up of esters of chrysanthemic acid and alcohols with a furan ring and terminal side chain substituent and is unstable in the presence of light, air and elevated temperatures. Type II are made up of 3-phenoxybenzyl alcohol derivatives in the alcohol substituent, with the α-cyano substituent, and they are stable to light, air and temperature, with high insecticidal activities (Costa, 2008; Kaneko, 2010).

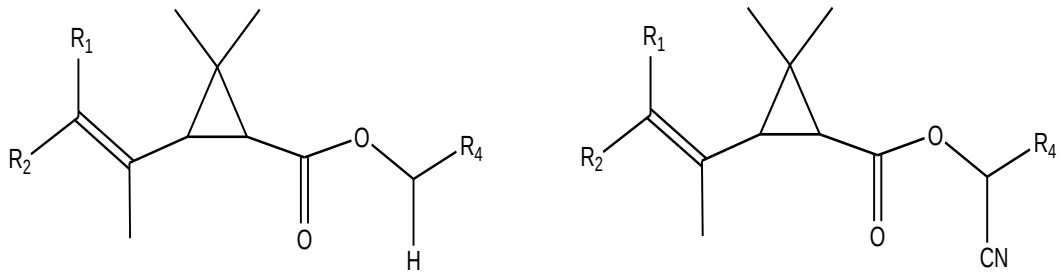


Fig 1.8: General Structures of (a) Type I and (b) Type II Pyrethroids

1.3.3.2 Toxicology and Mode of Action of PPs

Pyrethroid pesticides causes dermal and respiratory allergies, and have similar mode of action in insects and mammals, but mammals are relatively resistant due to their faster metabolic activities, higher body temperature and lower sensitivity of the target sites. PPs interfere with the balance of sodium ions in the nerve ending, disrupt the voltage-gated sodium channels, by binding to the α-subunit of the sodium channel thereby slowing down the rate of activation and inactivation of the channel (i.e. causing delay in the closing of the sodium channel) and rendering it hypersensitive (Costa, 2008; Kamrin, 2000; Ray, 2004; Waxman, 1998). Acute toxicity of PPs consists of two types. Type I syndrome includes sudden change in behavior, startle response and body tremor, and is produced by PPs with their esters lacking α-cyano substituents, while type II PPs with their esters consisting of the α-cyano substituents, induces slow depolarization of the nerve membrane and reduces the action potential amplitude and causes intense salivation, coarse tremor which can lead to chronic seizure (Yu, S. J., 2008).

1.3.4 Carbamate Pesticides (CPs)

1.3.4.1 Structure of Carbamate Pesticides (CPs)

Carbamate pesticides (CPs) are esters of carbamic acids in which the 3 replaceable H atoms (1 attached to C and 2 attached to N) of carbamic acid are displaced by aliphatic, aromatic or heterocyclic radicals to become carbamate pesticides (Yu, S. J., 2008). They are analogs of the drug physostigmine, a methyl carbamate alkaloid extracted from the plant *Physostigma venenosum*, called Calabar bean, which grows naturally in West Africa (Ecobichon, 2001b; Kamrin, 2000). They are colourless, odourless crystalline compounds, which are relatively stable to air, light and heat during storage. They are non-persistent

environmental pollutants and are more selective with less toxicity on mammals. They have several of chemical structures, which are all derivatives of carbamic acid and can be divided into three subclasses (Costa, 2008)

1. Methyl carbamates with aromatic radicals (e.g. carbaryl)
2. Methyl carbamates and dimethylcarbamates with heterocyclic radicals (e.g. carbofuran)
3. Methyl carbamates of oximes with a linear structure (e.g. aldicarb)

Their general and common structure is as shown in Fig 1.9.

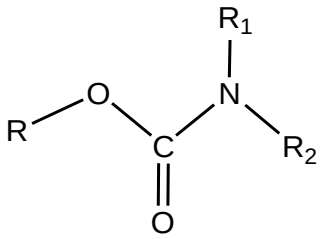


Fig 1.9: General Structure of Carbamate Pesticides (Kamrin, 2000)

where R is an alcohol, oxime, phenyl ring or heterocyclic group, R_1 and R_2 are either hydrogen or a methyl group. The carbamates in which the 2 H-atoms attached to the nitrogen are replaced have been found to be less toxic. Thus, in the manufacturing of the methyl carbamates, the second H-atom is not replaced because the monoalkyl substituted is more toxic than the N-disubstituted compounds (Yu, S. J., 2008).

1.3.4.2 Toxicology and Mode of Action of CPs

CPs have different degrees of toxicities, ranging from moderate, to high and extremely high toxicity and are open to different biotransformation reactions which are enzyme catalyzed, in which the reaction stages involve hydrolysis and oxidation (Costa, 2008). They have broad spectrum of biological activity and relatively short half-life (Ni, Qiu, & Kokot,

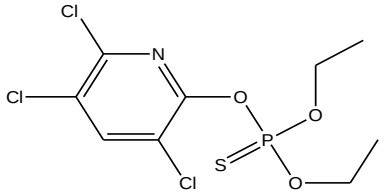
2005). They inhibit acetylcholinesterase by a reversible carbamylation of the serine hydroxyl group in the active site of the acetylcholine (neurotransmitter) at the parasympathetic neuroeffector junction, leading to the persistent amount of acetylcholine on the cholinergic postsynaptic receptor (Kamrin, 2000; Knaak et al., 2008; Waxman, 1998; Zhang, X. et al., 2010).

1.4 Pesticides Selected for this Study

For the purpose of this research work, six organophosphorus (ethoprophos, quinalphos, diazinone, parathion methyl, fenitrothion and chlorpyrifos), three organochlorine (α-endosulfan, β-endosulfan and chlorothalonil), three pyrethroid (bifenthrin, permethrin and fenpropathrin) and two carbamate (fenobucarb and thiobencarb) pesticides were selected. All the selected pesticides are widely used by farmers on fruits and vegetables.

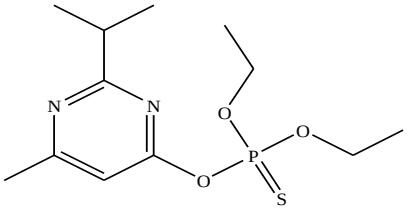
1.4.1 General Properties of the Selected Pesticides

Table 1.11: General Properties of Chlopyrifos

Properties	Descriptions
General Name	Chlropyrifos
Pesticide Type	Insecticide
Pesticide Class	Organophosphate
Chemical Formula	$C_9H_{11}Cl_3NO_3PS$
Structural formula	
IUPAC Name	O,O-diethyl O-3,5,6-trichlor-2-pyridylphosphorothio
Molecular Mass (g/mol)	350.89
Physical State	White to brown coloured crystal
Bulk Density (g/mL)	1.51
Water Solubility at 20 ^{0}C (mg/L)	1.05
Melting Point (^{0}C)	41.5
Boiling Point (^{0}C)	Decompose before boiling
Octanol/Water Partition Coeffi-cient at pH 7, Log K_{03} at 20 ^{0}C	4.7
Vapour Pressure at 25 ^{0}C (mPa)	1.43
Henry's Law Constant	2.8 x 10^{-04}
Sorption Coefficient, K_{oc}	8151
Bioconcentration Factor (BCF)	1374
Oral LD_{50} –Mammals (mg/kg)	64
Dermal LD_{50} –Mammals (mg/kg body weight)	>1250
Degradation Point (^{0}C)	170
Acceptable Daily Intake, ADI (mg/kg bw/day)	0.01
Mode of Action	Non-systemic with contact and stomach action. Acetylcholinesterase inhibitor
WHO classification	II, Moderately hazardous

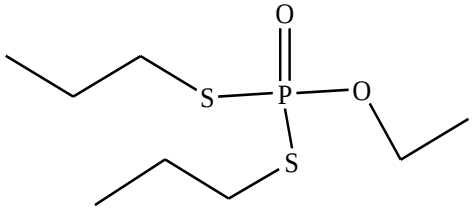
(PPDB, 2009)

Table 1.12: General Properties of Diazinon

Properties	Description
General Name	Diazinon
Pesticide Type	Insecticide, Acaricide
Pesticide Class	Organophosphate
Chemical Formula	$C_{12}H_{21}N_2O_3PS$
Structural formula	
IUPAC Name	O,O-diethyl O-2-isopropyl-6-methylpyrimidin-4-yl Phosphorothioate
Molecular Mass (g/mol)	304.5
Physical State	Colourless to dark brown liquid
Bulk Density (g/mL)	1.11
Water Solubility at 20 ^{0}C (mg/L)	60
Melting Point (^{0}C)	-
Boiling Point (^{0}C)	Decompose before boiling
Octanol/Water Partition Coefficient. at pH 7, Log K_{ow} at 20 ^{0}C	3.69
Vapour Pressure at 25 ^{0}C (mPa)	11.97
Henry's Law Constant	6.10 x 10^{-02}
Sorption Coefficient, K_{oc}	609
Bioconcentration Factor (BCF)	500
Oral LD_{50} –Mammals (mg/kg)	1139
Dermal LD_{50} –Mammals (mg/kg body weight)	>2000
Degradation Point (^{0}C)	140
Acceptable Daily Intake, ADI (mg/kg bw/day)	0.0002
Mode of Action	Non-systemic with respiratory, contact and stomach action, Acetylcholinesterase inhibitor
WHO Classification	II, Moderately hazardous

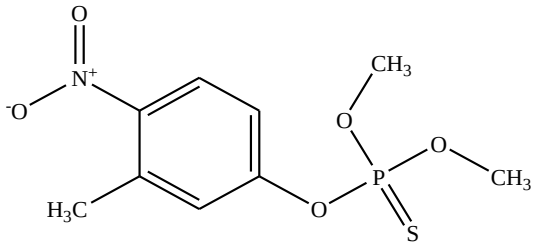
(PPDB, 2009)

Table 1.13: General Properties of Ethoprop

Properties	Description
General Name	Ethoprop
Pesticide Type	Insecticide, Nematocides
Pesticide Class	Organophosphate
Chemical Formula	$C_8H_{19}O_2PS$
Structural formula	
IUPAC Name	O-ethyl S,S-dipropyl phosphorodithioate
Molecular Mass (g/mol)	242.3
Physical State	Pale yellow liquid
Bulk Density (g/mL)	1.09
Water Solubility at 20 ^{0}C (mg/L)	1300
Melting Point (^{0}C)	70
Boiling Point (^{0}C)	244.3
Octanol/Water Partition Coefficient. at pH 7, Log K_{ow} at 20 ^{0}C	2.99
Vapour Pressure at 25 ^{0}C (mPa)	78
Henry's Law Constant	6.10 x 10^{-06}
Sorption Coefficient, K_{oc}	70
Bioconcentration Factor (BCF)	225
Oral LD_{50} –Mammals (mg/kg)	39.9
Dermal LD_{50} –Mammals (mg/kg body weight)	7.9
Degradation Point (^{0}C)	244.3
Acceptable Daily Intake, ADI (mg/kg bw/day)	0.0004
Mode of Action	Non-systemic with contact action, Acetylcholinesterase inhibitor
WHO classification	Ia, Extremely hazardous

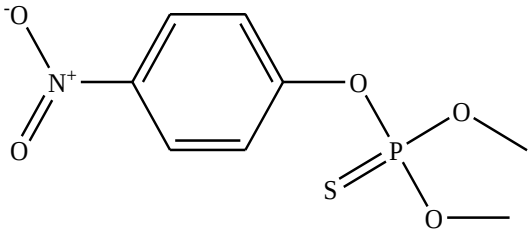
(PPDB, 2009)

Table 1.14: General Properties of Fenitrothion

Properties	Description
General Name	Fenitrothion
Pesticide Type	Insecticide
Pesticide Class	Organophosphate
Chemical Formula	$C_9H_{12}NO_5PS$
Structural formula	
IUPAC Name	O,O-diethyl O-4-nitro-m-tolyl phosphorothioate
Molecular Mass (g/mol)	277.23
Physical State	Yellow brown liquid
Bulk Density (g/mL)	1.33
Water Solubility at 20 ^{0}C (mg/L)	19
Melting Point (^{0}C)	1
Boiling Point (^{0}C)	Decomposes before boiling
Octanol/Water Partition Coefficient at pH 7, Log K_{ow} at 20 ^{0}C	3.32
Vapour Pressure at 25 ^{0}C (mPa)	0.676
Henry's Law Constant	3.00 x 10^{-06}
Sorption Coefficient, K_{oc}	2000
Bioconcentration Factor (BCF)	29
Oral LD_{50} –Mammals (mg/kg)	330
Dermal LD_{50} –Mammals (mg/kg body weight)	890
Degradation Point (^{0}C)	210
Acceptable Daily Intake, ADI (mg/kg bw/day)	0.005
Mode of Action	Non-systemic, broad spectrum with contact and stomach action. Acetylcholinesterase inhibitor
WHO classification	II, Moderately hazardous

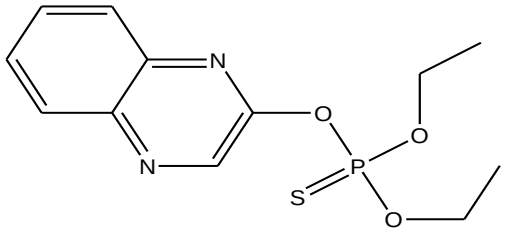
(PPDB, 2009)

Table 1.15: General Properties of Parathion-methyl

Properties	Description
General Name	Parathion-methyl
Pesticide Type	Insecticide
Pesticide Class	Organophosphate
Chemical Formula	$C_8H_{10}NO_5PS$
Structural formula	
IUPAC Name	O,O-dimethyl O-4-nitrophenyl phosphorothioate
Molecular Mass (g/mol)	263.21
Physical State	Colourless crystals
Bulk Density (g/mL)	1.36
Water Solubility at 20 ^{0}C (mg/L)	55
Melting Point (^{0}C)	35.5
Boiling Point (^{0}C)	-
Octanol/Water Partition Coefficient. at pH 7, Log K_{ow} at 20 ^{0}C	3
Vapour Pressure at 25 ^{0}C (mPa)	0.2
Henry's Law Constant	2.30 x 10^{-06}
Sorption Coefficient, K_{oc}	240
Bioconcentration Factor (BCF)	71
Oral LD_{50} –Mammals (mg/kg)	330
Dermal LD_{50} –Mammals (mg/kg body weight)	890
Degradation Point (^{0}C)	210
Acceptable Daily Intake, ADI (mg/kg bw/day)	0.005
Mode of Action	Non-systemic, broad spectrum with contact and stomach action. Acetylcholinesterase inhibitor
WHO classification	II, Moderately hazardous

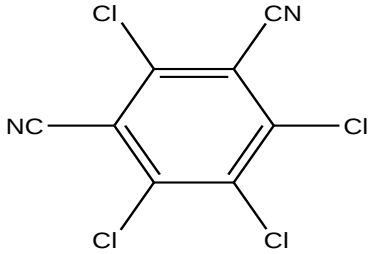
(PPDB, 2009)

Table 1.16: General Properties of Quinalphos

Properties	Description
General Name	Quinalphos
Pesticide Type	Insecticide, Acaricide
Pesticide Class	Organophosphate
Chemical Formula	$C_{12}H_{15}N_2O_3PS$
Structural formula	
IUPAC Name	O,O-diethyl O-quinoxalin-2-yl phosphorothioate
Molecular Mass (g/mol)	298.3
Physical State	Colourless crystals
Bulk Density (g/mL)	1.235
Water Solubility at 20 ^{0}C (mg/L)	17.8
Melting Point (^{0}C)	31.5
Boiling Point (^{0}C)	-
Octanol/Water Partition Coefficient. at pH 7, Log K_{ow} at 20 ^{0}C	4.44
Vapour Pressure at 25 ^{0}C (mPa)	0.346
Henry's Law Constant	2.38 x 10^{-06}
Sorption Coefficient, K_{oc}	1465
Bioconcentration Factor (BCF)	-
Oral LD_{50} –Mammals (mg/kg)	71
Dermal LD_{50} –Mammals (mg/kg body weight)	1750
Degradation Point (^{0}C)	-
Acceptable Daily Intake, ADI (mg/kg bw/day)	-
Mode of Action	Contact and stomach action. Acetylcholinesterase inhibitor
WHO classification	II, Moderately hazardous

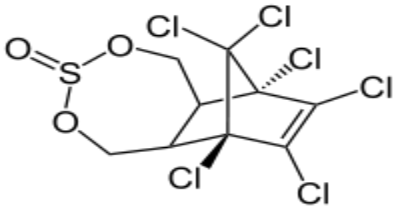
(PPDB, 2009)

Table 1.17: General Properties of Chlorothalonil

Properties	Description
General Name	Chlorothalonil
Pesticide Type	Fungicide
Pesticide Class	Organochlorine
Chemical Formula	$C_8Cl_4N_2$
Structural formula	
IUPAC Name	Tetrachloroisophthalonitrile
Molecular Mass (g/mol)	265.91
Physical State	White crystals
Bulk Density (g/mL)	1.74
Water Solubility at 20 ^{0}C (mg/L)	0.81
Melting Point (^{0}C)	252.1
Boiling Point (^{0}C)	350
Octanol/Water Partition Coefficient. at pH 7, Log K_{ow} at 20 ^{0}C	2.94
Vapour Pressure at 25 ^{0}C (mPa)	0.076
Henry's Law Constant	1.36 x 10^{-05}
Sorption Coefficient, K_{oc}	850
Bioconcentration Factor (BCF)	100
Oral LD_{50} –Mammals (mg/kg)	>5000
Dermal LD_{50} –Mammals (mg/kg body weight)	>2000
Degradation Point (^{0}C)	-
Acceptable Daily Intake, ADI (mg/kg bw/day)	0.015
Mode of Action	Non-systemic, broad spectrum, foliar action with some protectant properties
WHO classification	IV, Unlikely to present an acute hazard

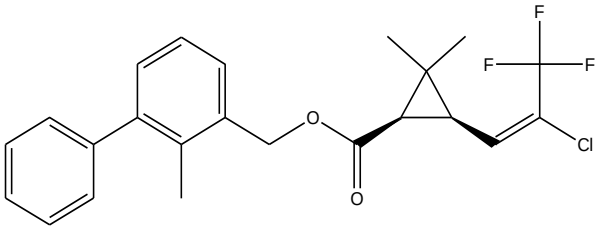
(PPDB, 2009)

Table 1.18: General Properties of α- and β-Endosulfan

Properties	Description
General Name	Endosulfan
Pesticide Type	Insecticide, Acaricide
Pesticide Class	Organochlorine
Chemical Formula	$C_9H_6Cl_6O_3S$
Structural formula	
IUPAC Name	6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide
Molecular Mass (g/mol)	406.93
Physical State	Colourless to brown coloured crystals
Bulk Density (g/mL)	1.8
Water Solubility at 20 ^{0}C (mg/L)	0.32
Melting Point (^{0}C)	80
Boiling Point (^{0}C)	-
Octanol/Water Partition Coefficient. at pH 7, Log K_{ow} at 20 ^{0}C	4.75
Vapour Pressure at 25 ^{0}C (mPa)	1.48
Henry's Law Constant	3.29 x 10^{-04}
Sorption Coefficient, K_{oc}	11500
Bioconcentration Factor (BCF)	2755
Oral LD_{50} –Mammals (mg/kg)	38
Dermal LD_{50} –Mammals (mg/kg body weight)	>500
Degradation Point (^{0}C)	-
Acceptable Daily Intake, ADI (mg/kg bw/day)	0.006
Mode of Action	Non-systemic with contact and stomach actions, acts non-competitive GABA antagonist
WHO classification	II, Moderately hazardous

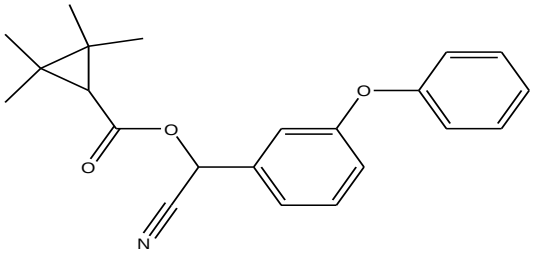
(PPDB, 2009)

Table 1.19: General Properties of Bifenthrin

Properties	Description
General Name	Bifenthrin
Pesticide Type	Insecticide, Acaricide
Pesticide Class	Pyrethroid
Chemical Formula	$C_{23}H_{22}ClF_3O_2$
Structural formula	
IUPAC Name	2-methyl-3-phenylbenzyl (1RS)-cis-3-(2-chloro-3,3,3-trifluorocyclopropanecarboxylate
Molecular Mass (g/mol)	422.88
Physical State	Off-white waxy solid
Bulk Density (g/mL)	1.26
Water Solubility at 20 ^{0}C (mg/L)	0.001
Melting Point (^{0}C)	79.6
Boiling Point (^{0}C)	Decomposes before boiling
Octanol/Water Partition Coefficient. at pH 7, Log K_{ow} at 20 ^{0}C	6.6
Vapour Pressure at 25 ^{0}C (mPa)	0.0178
Henry's Law Constant	4.10 x 10^{-02}
Sorption Coefficient, K_{oc}	236610
Bioconcentration Factor (BCF)	1703
Oral LD_{50} –Mammals (mg/kg)	54.5
Dermal LD_{50} –Mammals (mg/kg body weight)	>2000
Degradation Point (^{0}C)	280
Acceptable Daily Intake, ADI (mg/kg bw/day)	0.015
Mode of Action	Contact and stomach action with some residual effect
WHO classification	II, Moderately hazardous

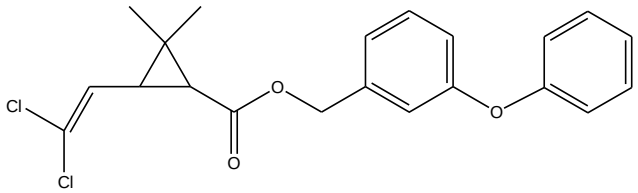
(PPDB, 2009)

Table 1.20: General Properties of Fenpropathrin

Properties	Description
General Name	Fenpropathrin
Pesticide Type	Insecticide, Acaricide
Pesticide Class	Pyrethroid
Chemical Formula	$C_{23}H_{23}NO_3$
Structural formula	
IUPAC Name	(RS)-α-cyano-3-phenoxybenzyl-2,2,3,3-tetramethyl cyclopropanecarboxylate
Molecular Mass (g/mol)	349.42
Physical State	Yellow-brown solid
Bulk Density (g/mL)	1.15
Water Solubility at 20 ^{0}C (mg/L)	0.33
Melting Point (^{0}C)	47.5
Boiling Point (^{0}C)	-
Octanol/Water Partition Coefficient. at pH 7, Log K_{ow} at 20 ^{0}C	6.04
Vapour Pressure at 25 ^{0}C (mPa)	0.76
Henry's Law Constant	3.20 x 10^{-04}
Sorption Coefficient, K_{oc}	5000
Bioconcentration Factor (BCF)	1100
Oral LD_{50} –Mammals (mg/kg)	870
Dermal LD_{50} –Mammals (mg/kg body weight)	870
Degradation Point (^{0}C)	-
Acceptable Daily Intake, ADI (mg/kg bw/day)	0.03
Mode of Action	Non-systemic with contact and stomach action. Sodium channel modulator
WHO classification	II, Moderately hazardous

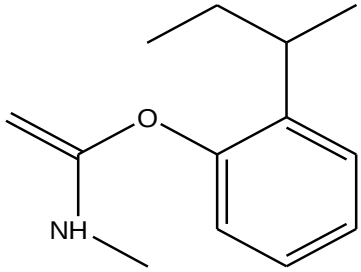
(PPDB, 2009)

Table 1.21: General Properties of Permethrin

Properties	Description
General Name	Permethrin
Pesticide Type	Insecticide,
Pesticide Class	Pyrethroid
Chemical Formula	$C_{21}H_{20}Cl_2O_3$
Structural formula	
IUPAC Name	3-phenoxybenzyl (1RS,3RS:1RS,3SR)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate
Molecular Mass (g/mol)	391.3
Physical State	Colourless crystalline solid to brown viscous liquid
Bulk Density (g/mL)	1.29
Water Solubility at 20 ^{0}C (mg/L)	0.2
Melting Point (^{0}C)	34.5
Boiling Point (^{0}C)	200
Octanol/Water Partition Coefficient. at pH 7, Log K_{ow} at 20 ^{0}C	6.1
Vapour Pressure at 25 ^{0}C (mPa)	0.002
Henry's Law Constant	7.76×10^{-05}
Sorption Coefficient, K_{oc}	100000
Bioconcentration Factor (BCF)	300
Oral LD_{50} –Mammals (mg/kg)	>430
Dermal LD_{50} –Mammals (mg/kg body weight)	>2000
Degradation Point (^{0}C)	-
Acceptable Daily Intake, ADI (mg/kg bw/day)	0.05
Mode of Action	Broad spectrum with contact and stomach action. Slight repellant effect. Sodium channel modulator
WHO classification	II, Moderately hazardous

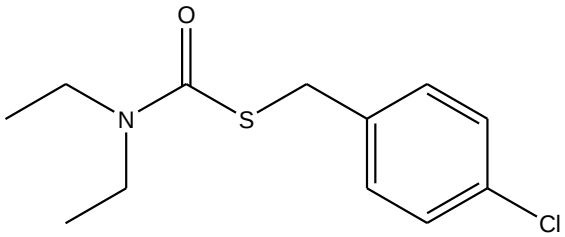
(PPDB, 2009)

Table 1.22: General Properties of Fenobucarb

Properties	Description
General Name	Fenobucarb
Pesticide Type	Insecticide,
Pesticide Class	Carbamate
Chemical Formula	$C_{12}H_{17}NO_2$
Structural formula	
IUPAC Name	(RS)-2-sec-butylphenyl methylcarbamate
Molecular Mass (g/mol)	207.27.24
Physical State	Oily yellow liquid which may solidify at lower
Bulk Density (g/mL)	1.04
Water Solubility at 20 ^{0}C (mg/L)	420
Melting Point (^{0}C)	31.5
Boiling Point (^{0}C)	Decomposes before boiling
Octanol/Water Partition Coefficient. at pH 7, Log K_{ow} at 20 ^{0}C	2.78
Vapour Pressure at 25 ^{0}C (mPa)	48
Henry's Law Constant	9.73 x 10^{-06}
Sorption Coefficient, K_{oc}	1068
Bioconcentration Factor (BCF)	-
Oral LD_{50} –Mammals (mg/kg)	>620
Dermal LD_{50} –Mammals (mg/kg body weight)	>5000
Degradation Point (^{0}C)	-
Acceptable Daily Intake, ADI (mg/kg bw/day)	-
Mode of Action	Contact action, with long residual effects Acetylcholinesterase inhibitor
WHO classification	II, Moderately hazardous

(PPDB, 2009)

Table 1.23: General Properties of Thiobencarb

Properties	Description
General Name	Thiobencarb
Pesticide Type	Insecticide,
Pesticide Class	Thiocarbamate
Chemical Formula	$C_{12}H_{16}ClNOS$
Structural formula	
IUPAC Name	S-4-chlorobenzene diethyl(thiocarbamate)
Molecular Mass (g/mol)	257.8
Physical State	Colourless viscous liquid
Bulk Density (g/mL)	1.16
Water Solubility at 20 ^{0}C (mg/L)	16.7
Melting Point (^{0}C)	-
Boiling Point (^{0}C)	326.6
Octanol/Water Partition Coefficient. at pH 7, Log K_{ow} at 20 ^{0}C	4.23
Vapour Pressure at 25 ^{0}C (mPa)	2.39
Henry's Law Constant	1.51 x 10^{-05}
Sorption Coefficient, K_{oc}	-
Bioconcentration Factor (BCF)	302
Oral LD_{50} –Mammals (mg/kg)	>560
Dermal LD_{50} –Mammals (mg/kg body weight)	>5000
Degradation Point (^{0}C)	-
Acceptable Daily Intake, ADI (mg/kg bw/day)	0.01
Mode of Action	Selective. Acetylcholinesterase inhibitor – inhibition of lipid synthesis
WHO classification	II, Moderately hazardous

(PPDB, 2009)

1.5 Scope and Objectives of the Study

Pesticides are artificially synthesized compounds produced to fight the pest and diseases of plant to increase and improve agricultural products. Although their use have tremendously increased agricultural production in many parts of the world, its uses have been of concern due to their toxicity and effects on human health. This study will determine the trace levels of multi-residue pesticides in fruits and vegetables to ascertain that the pesticide contaminants are kept at minimum level below the maximum residue levels (MRLs) to be considered as safe for human consumption.

The overall objective of this study is to develop and validate an analytical method for the extraction and chromatographic analysis of multi-residue pesticides in fruits and vegetables. This study will among other things develop and validate an effective, efficient, sensitive and selective solid phase microextraction method and chromatographic analysis for the qualitative and quantitative analysis of pesticide residues to ensure they are below the maximum residue levels (MRLs) proposed by various International Regulatory Agencies, such as European Union, USEPA and joint WHO/FAO Codex Alimentarius Commission on Food Standard. The study will also compare the univariate method and multivariate experimental design for qualitative and quantitative analysis of multiclass and multi-residue pesticides using SPME-GC-MS.

CHAPTER TWO

2.0 REVIEW OF MICROEXTRACTION TECHNIQUES FOR THE ANALYSIS OF PESTICIDE RESIDUES IN FRUITS AND VEGETABLES

2.1 Microextraction Techniques

The extraction and subsequent analysis of pesticide residues and other contaminants in fruit and vegetable samples is becoming increasingly important due to the health hazards caused by their accumulation in human tissues (Tan & Abdulra'uf, 2012). The consumption of fresh fruits and vegetables provides the body with some important nutrients and minerals that are beneficial and play an important role in human health and well-being (Lee, C. Y. & Smith, 2000; Sharma et al., 2010; Tan & Abdulra'uf, 2012). Thus, the need to satisfy the drastic increase in demand for fruits and vegetables by a growing population has led to the increase in the use of pesticides. Although the use of pesticides has helped to increase food production, there is need to strike balance between the expected benefit and the possible risks to human health (Araoud et al., 2007). Their concentration must always be at minimum level and below the maximum residue limits. Therefore there is an urgent need for quality control monitoring of the use of such pesticides on fruits and vegetables for safety purposes.

Analytical study is undertaken in order to obtain information on the quality and quantity of contaminants present in the sample. It involves several dependent steps: sampling, sample preparation, separation, quantification and data analysis (Pawliszyn, Pawliszyn, & Pawliszyn, 1997). Sample preparation is a very important step and indeed the bottleneck of analytical methodologies, in the analysis of fruits and vegetables for the presence of pesticide residues in fruit and vegetable samples.

The preliminary steps in any instrumental analysis are sampling and sample preparation with the later involving various sample pretreatment method (Ahmed, 2001; Omeroglu et al., 2012). This further involves the selective isolation of the target analytes from the sample matrix, which are present at trace concentration (usually µg.kg or less). This helps in the elimination of any interference and also reduces the volume of extracts (Nerín et al., 2009). The nature of sample matrix and the physico-chemical properties of analytes to be investigated determines the choice of separation and detection method to be employed (Jain & Verma, 2011; Kloskowski et al., 2007). The current trend of microextraction techniques is aimed at a reliable and accurate analysis of contaminants from complex samples. It is focused on the reduction of sampling time, cost and solvent volume, with the coupling of the sampling step to the analytical instruments (Beltran, López, & Hernández, 2000; Kataoka et al., 2000).

The traditional sample preparation methods: liquid-liquid extraction (LLE), solid phase extraction (SPE), accelerated solvent extraction (ASE), matrix solid phase dispersion (MSPD) (Adou, Bontoyan, & Sweeney, 2001; Ahmed, 2001; Albero, Sánchez-Brunete, & Tadeo, 2005; Buszewski, B. & Szultka, 2012; De Koning, Janssen, & Brinkman, 2009; Rial Otero, Cancho Grande, & Simal Gándara, 2003; Štajnbaher & Zupančič-Kralj, 2003; Tan & Chai, 2011; Turner, 2006), requires tedious and time consuming matrix pretreatment steps and uses large volumes of sample and toxic solvents which imposes environmental pollution and health hazards with high operation cost (Kataoka et al., 2000). Therefore in order to reduce the sources of error, there is need to reduce the number of matrix pretreatment steps. Microextraction techniques are recently developed sample preparation methods which are effective and efficient ways to save time, reduce solvent

use and increase sample throughput (Kataoka, 2010). The current trend of sample preparation techniques is focused on the simplifications, miniaturization, and combination of different steps, such as extraction, concentration, isolation of analytes, clean-up and instrumental analysis in one single step.

Over the years, different researchers have developed microextraction techniques, to corroborate the recent advances in the development of highly sensitive and efficient analytical instrumentations, such as gas chromatography (GC), liquid chromatography (LC) and capillary electrophoresis (CE) which are compatible with the microextraction techniques, and coupled to different detectors (such as electron capture detector, flame ionization detection, nitrogen phosphorous detector, mass spectrometry, diode array detector, ultraviolet detector, *etc.*), (Jin et al., 2012). Prior sample preparation is necessary in order to extract, isolate and concentrate the analytes of interest from the complex fruits and vegetables matrices, which contain high molecular mass compounds.

The low cost, easy to understand operation procedure and ease of hyphenation of the microextraction techniques to analytical instruments has drastically reduced errors, due to contamination and sample losses. The introduction of solid phase microextraction, by Pawliszyn and his co-workers in 1990 (Arthur et al., 1992a; Arthur et al., 1992b; Arthur & Pawliszyn, 1990), opened the floodgate of interest in the development of microextraction techniques. In this chapter, a review of liquid phase microextraction (LPME) and stir bar sorptive extraction (SBSE), are discussed, while solid phase microextraction (SPME) will be discussed in the subsequent chapter.

2.2 Liquid Phase Microextraction (LPME)

LPME, also called solvent microextraction techniques or liquid-liquid microextraction (LLME), is a miniaturized liquid-liquid extraction (Ridgway, Lalljie, & Smith, 2007), which has helped to drastically reduce the amount of solvent used in extraction (Theis et al., 2001). It was developed in order to overcome some problems inherent in solid phase microextraction, such as low recommended operating temperature of coated fiber, swelling of the fiber in organic solvents, fiber breakage due to its fragility, stripping of the coating and possible bending of the needles (Nerín et al., 2009; Sarafraz-Yazdi & Amiri, 2010).

It is a rapid and less-expensive sample preparation technique performed between microliter volume of water-immiscible solvent called the acceptor phase and an aqueous sample called the donor phase, containing the analytes of interest (Sarafraz-Yazdi & Amiri, 2010). The acceptor phase can either be immersed directly into the sample matrix or suspended above the sample for headspace extraction (Kataoka, 2010). The technique can broadly be classified into three major categories (Han & Row, 2012) and their difference is the way the extraction solvent is supported and in contact with the sample matrix (Kokosa, 2013).

1. Single Drop Microextraction (SDME)
2. Hollow Fiber Liquid Phase Microextraction (HF-LPME)
3. Dispersive Liquid-Liquid Microextraction (DLLME)

2.2.1 Factors Affecting LPME

The LPME method for the extraction and chromatographic analysis of pesticide residues in food samples requires the optimization of certain factors that are related to the donor and the acceptor phases. The following factors are important in improving the extraction

efficiency and must be carefully optimized; type of organic solvent, volume of donor and acceptor phase solutions, agitation, salt addition (ionic strength), pH, extraction time and extraction temperature (Han & Row, 2012; Jeannot, Przyjazny, & Kokosa, 2010; Kokosa, Przyjazny, & Jeannot, 2009; Lambropoulou & Albanis, 2007a).

The first factor is the selection of an organic solvent or mixture of organic solvents and dispersive solvents in case of dispersive liquid-liquid microextraction. The organic or dispersive solvents should be more than 99.9 % pure, less volatile, with high boiling point and density, in order to prevent solvent evaporation. The solvents should also be water immiscible for extraction of analytes from aqueous samples and should be compatible with chromatographic instruments (Asensio-Ramos et al., 2011; Barahona et al., 2010; Jeannot et al., 2010). Similar to SPME, LPME is a non-exhaustive microextraction technique, therefore, the need to select an appropriate extraction/dispersive solvent volume (usually between 0.9 and 1.6 µL), to prevent shrinking. Large volume of solvent may be difficult to manipulate, when stirring the sample matrix and may easily be dislodged from the syringe.

Stirring of the sample matrix increases the extraction efficiency and decreases the time required to attain equilibrium, by increasing the interfacial contact area and diffusion distance. It also leads to an increase in mass transfer, by exposing the target analytes to the extraction solvent and increases the partition coefficient of analyte between the sample matrix and the extraction solvent, and must also be carefully optimized to avoid the dislodgement of the microdrop solvent. Stirring can be achieved through magnetic stirring, vortex and mechanical vibration or syringe movement (Jeannot et al., 2010; Lambropoulou & Albanis, 2007a),

Salt addition, which implies alteration of the ionic strength (salting out effect) of the sample matrix, also enhances extraction efficiency and decreases the time taken to attain extraction thermodynamic equilibrium especially for the moderately polar and low molecular weight analytes. It has been found to have little effect on non-polar and water insoluble analytes. The salt addition is also used to adjust the pH of the sample matrix, which helps to suppress ionization of target analytes, reduces analyte solubility and enhances their extractability. Salt addition to sample matrix should be used at near, but not saturated concentration, since the undissolved salt particles may dislodge the solvent drop in direct immersion mode. The variation of pH helps to promote the formation of the molecular state of the target analytes (Barahona et al., 2010; Basheer et al., 2007; Jain & Verma, 2011; Jeannot et al., 2010; Lambropoulou & Albanis, 2007a; Pezo, Salafranca, & Nerín, 2007; Sarafraz-Yazdi & Amiri, 2010).

The optimization of extraction temperature is important especially in the headspace mode, because temperature has a greater effect on the solvent/headspace and the headspace/sample matrix interfaces than the solvent/sample matrix interface. The increase in extraction temperature increase the extraction efficiency especially for non-polar analytes, by increasing the headspace concentration, but the solubility of moderately polar analytes increases at higher temperature and may decrease extraction efficiency. A higher extraction temperature may affect the stability of the microdrop in SDME technique and high solvent lost in DLLME and HF-LPME, and thus compromise must be found especially in multi-residue analysis and also minimizing extraction time to reduce the effect of temperature on the stability of solvent drop (Jain & Verma, 2011; Jeannot et al., 2010; Lambropoulou & Albanis, 2007a).

The sample and headspace volume needs to be optimized as it has been observed that large sample volume can be counter-productive. The nature and composition of sample matrix will also determine the extraction mode (headspace or direct immersion) to be employed. Thus, the sample and headspace volume should be maintained constantly at minimal level. In the headspace mode, the optimized volume should be at a level that will allow the microdrop to be suspended over the sample matrix, while in the direct immersion mode, the volume should maintain at a level which ensures that the sample matrix is not in contact with the septum (Barahona et al., 2010; Basheer et al., 2007; Jain & Verma, 2011; Jeannot et al., 2010; Lambropoulou & Albanis, 2007a). The presence of air bubbles should also be avoided in the syringe to avoid errors in analysis. LPMEs are equilibrium extraction techniques, therefore the extraction time must also be carefully optimized.

2.3 Solvent Drop Microextraction (SDME)

SDME technique is based on the suspension of a single microdrop of water-immiscible organic solvents (typically 0.5 – 3 μL) from the tip of a microsyringe GC injection needle in aqueous solution (Bedendo & Carasek, 2010; Jain & Verma, 2011; Kataoka, 2010; Kokosa, 2013; Kokosa et al., 2009; Pezo et al., 2007; Sarafraz-Yazdi & Amiri, 2010), thereby reducing drastically the volume of organic solvent used. The transfer of target analytes from the sample matrix to the extraction solvent is limited by slow diffusion rate and the analytes are distributed between the microdrop of the solvent at the tip of the microsyringe and the sample solution (Asensio-Ramos et al., 2011; Jeannot et al., 2010). The solvent droplet which extracts the analyte by passive diffusion is retracted back into the syringe and injected directly into the analytical instruments (GC, LC or CE). The evaporation and reconstitution of analytes before injection is eliminated and the technique

provides highly enriched extracts of the analytes (Kataoka, 2010; Rezaee, Yamini, & Faraji, 2010).

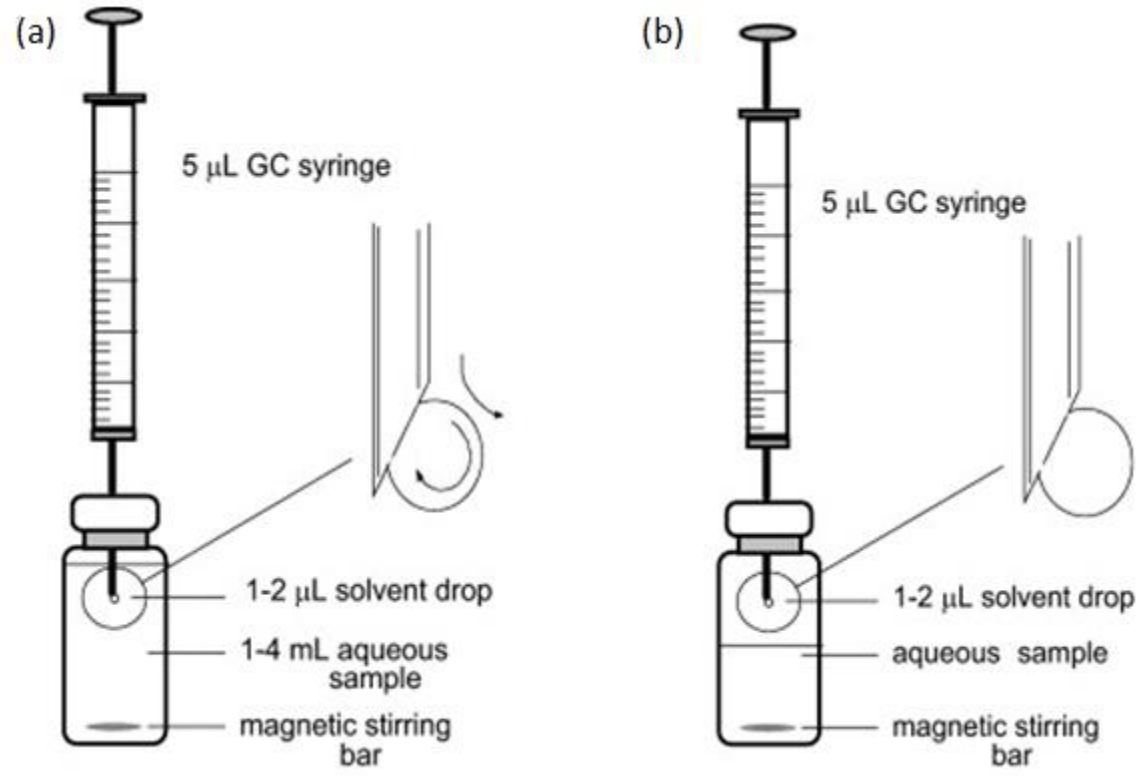


Fig 2.1; Solvent Drop Microextraction in (a) Direct Immersion (DI) and (b) Headspace (HS) Modes (Jain & Verma, 2011)

The SDME (Fig 2.1), can be carried out in direct immersion (DI) mode, in which the droplet is suspended directly inside the sample matrix, most suitable for the extraction of medium polar or non-polar analytes. The headspace mode (HS) involves the suspension of the solvent drop in the headspace of the sample matrix and is suitable for the extraction of volatile or semi-volatile analytes (Jain & Verma, 2011; Jeannot et al., 2010; Ridgway et al., 2007; Tankiewicz, Fenik, & Biziuk, 2011).

The SDME technique was first reported by Liu and Dasgupta, using 1.3 µL of chloroform suspended in a large aqueous solution containing methylene blue active substance (Liu, H. & Dasgupta, 1996a, 1996b, 1996c) and later by Jeannot and Cantwell for the extraction of 4-methylacetophenone in aqueous solution using 8 µL of n-octane suspended at the end of Teflon rod (Jeannot & Cantwell, 1996, 1997). He and Lee also reported the extraction of 1,2,3-tricholorobenzene and pentachlorobenzene in aqueous solution using 5 µL of toluene as the extraction solvent suspended at the tip of conventional microsyringe in dynamic and static SDME modes (He, Y. & Lee, 1997). The SDME technique was found to be simple, flexible, and less expensive and can easily be automated to chromatographic instruments. Its coupling to GC, LC and CE has been widely reported and extensively reviewed, while it has also been successfully coupled to ICP-MS, ET-AAS, FI-AAS, MALDI-MS, UV-VS and MS (ALOthman et al., 2012; Andruch et al., 2012; Dehghani Mohammad Abadi et al., 2012; Jain & Verma, 2011; Jin et al., 2012; Pakade & Tewary, 2010; Xu, Basheer, & Lee, 2007).

The use of Teflon rod as extraction solvent holder, as reported earlier implies that extraction from sample matrix and subsequent injection of concentrated analytes into analytical instruments are performed separately with different apparatus (Psillakis & Kalogerakis, 2002; Sarafraz-Yazdi & Amiri, 2010). This limitation was overcome by the introduction of a microsyringe as the solvent holder (Jeannot & Cantwell, 1996, 1997), and the organic solvent can easily be withdrawn after extraction and injected directly into the chromatographic system (Xu et al., 2007). The instability of the extraction solvents in the DI mode at high stirring rate and long extraction time (Jain & Verma, 2011), is a limitation

which can be overcome by the optimization of all factors as described above including automation into analytical instruments (Jeannot et al., 2010).

2.3.1 Theory of SDME

There are two sampling modes used in SDME. The DI-SDME consists of two-phase system, while the HS-SDME consists of three-phase system. Theoretically, in the two-phase system, the initial concentration of analyte and the concentration in the organic solvent and sample at equilibrium will remain the same (Ouyang, Zhao, & Pawliszyn, 2005; Psillakis & Kalogerakis, 2002). The mass transfer of analytes into the extraction solvent is a thermodynamic process, where maximum extraction is achieved at equilibrium and the mass balance equation at equilibrium can be defined as:

$$C_T = C_o + C_s \tag{2.1}$$

where C_T, C_o and C_s are the total amount of the analyte in the system, the equilibrium concentrations of analyte in the extraction solvent and in the sample respectively. The equilibrium concentration of the analyte in the extraction solvent can be expressed as (Jeannot & Cantwell, 1996, 1997; Jeannot et al., 2010):

$$C_O = KC_S = \frac{KC_s^o}{1+KV_o/V_s} \tag{2.2}$$

where the equilibrium constant, C_s^o is the initial concentration of analyte in the sample matrix and V_o and V_s are the volumes of extraction solvent and sample respectively.

The equilibrium distribution isotherm is the ratio of equilibrium concentration of the analyte in extraction solvent to its concentration in the sample matrix, and is expressed as:

$$K = \frac{C_0}{C_s} \tag{2.3}$$

In three phase system, which contains two interphases (headspace/sample matrix and headspace/extraction solvent), the mass balance equation can be defined as:

$$C_T = C_O + C_s + C_h \tag{2.4}$$

where C_h is the concentration of analyte in the headspace at equilibrium and the equilibrium concentration of analyte can now be expressed as:

$$C_O = KC_s = \frac{KC_s^o}{1+(K_{hs}V_h/V_s) + (K_{os}V_0/V_s)} \tag{2.5}$$

where K_{hs} is the headspace/sample distribution constant and K_{os} is the extraction solvent/sample equilibrium constant. When the headspace volume (V_h) or K_{hs} is small, Eq. (2.5) is reduced to Eq. (2.2).

The general kinetic model, which can be fitted to experimental data can be expressed as the function of initial and equilibrium concentrations of analyte in the extraction solvent and time (Psillakis & Kalogerakis, 2002), and can be expressed as:

$$C_s^o = C_o(1 - e^{-kt}) \tag{2.6}$$

As it can be observed from Eq. (2.6), the rate constant (k) increases with the increase in the interfacial contact area and is dependent on the volume of the extraction solvent, headspace volume, nature and composition of the sample matrix, the mass transfer coefficient and the

distribution coefficients of the extraction solvent (Jeannot et al., 2010; Ouyang et al., 2005).

2.3.2 Applications of SDME in Pesticide Residues Analysis in Fruits and Vegetables

SDME technique has been used successfully for the extraction and analysis of pesticide residues in fruit and vegetable samples. Although few studies have been published and reported in the literature. Table 2.1 shows the applications of SDME in the extraction of difference classes of pesticide residues in fruit and vegetable samples.

A method was developed for the analysis of multiclass pesticides in tomato sample. The method performance obtained by internal standard calibration curve in blank spiked tomatoes gave limit of detection (LOD) between 0.6 and 30 µg/kg, linearity from 5 to 500 µg/kg, with correlation coefficient greater than 0.975. The matrix effect was also investigated by comparing the recoveries of the target analytes in spiked tomato and courgette samples, and the average recoveries range from 29.9 to 58.3 % (RSD = 6.3 – 12 %, where RSD is the relative standard deviation) and 28 to 98.5% (RSD = 5.1 – 8.9 %), respectively. It was found that there were higher relative peak areas in matrix-matched donor solution obtained from tomato, and a positive matrix effect was observed for all the investigated pesticides (Amvrazi & Tsiropoulos, 2009a).

A method for the determination of six triazole fungicides in water and grape juice was also proposed. The proposed method was based on DI-SDME in a narrow-bore glass tube and subsequent desorption into GC-FID (FID, flame ionization detector). The analytical performance of the method determined in grape juice sample showed wide linear range

between 0.001 and 10 µg/mL with RSD >0.994. The LOD and LOQ ranged from 0.006 to 0.112 µg/mL and 0.010 to 0.375 µg/mL, respectively. The recoveries were between 71 and 99 % with RSD of 1 – 7 % and EF between 141 and 214. The sample matrix was found to have no effect on the extraction efficiency and thus no dilution was performed. The developed method was compared to other extraction methods such as QuEChERS (quick easy cheap effective rugged safe), SPE-DLLME (SPE, solid phase extraction), and SPE, and the SDME was found to have comparable or better extraction efficiency (Farajzadeh, Djozan, & Khorram, 2011).

A multivariate method for the analysis of 21 multiclass pesticide residues in apple and grape samples was also developed and validated. The method developed was validate using grape and apple sample, and the matrix-matched calibration curve constructed showed linearity between the limit of quantitation (LOQ) and 50(LOQ) µg/mL with correlation coefficient greater than 0.97. The LODs ranged from 0.0004 to 0.2 and 0.0003 to 0.2 µg/mL for apple and grape, respectively, while the LOQ was between 0.001 and 0.66 µg/mL for both samples. The recoveries were between 65 and 91 % in apple and 69 and 110 % in grape with RSD of 4 – 17% and enrichment factor (EF) was between 11 and 328. The multivariate experimental factorial design employed allows for the determination of optimal conditions and the effect of their interactions on the extraction efficiency, and all the parameters can be simultaneously optimized (Amvrazi & Tsiropoulos, 2009b).

The enrichment factors (EF) of two types of DI-SDME: static-SDME and cycle-flow SDME procedures, for the analysis of organophosphorus pesticides in fruit juice (apple, pear, and orange) samples were compared. The static-SDME showed a better EF (23–109)

than the cycle-flow SDME (2–15), the static-SDME coupled to GC-FID system was then used for method validation. Salt addition was found to reduce extraction efficiency and was not added for validation studies. The analytical figures of merit: LOD, linear range, RSD, average recoveries, and correlation coefficient were 0.21 – 0.56 ng/mL, 0.5 – 50 ng/mL, ≥0.9995, 77 – 113 %, and 0.6 – 13.4 %, respectively. This study showed that SDME analysis of pesticide residues in juice sample does not require any preliminary sample preparation when the dilution factor is carefully optimized (Xiao et al., 2006).

Organophosphorus pesticides (OPPs) were determined in orange juice sample using DI-SDME-GC-FID procedure. The method showed recoveries ranging from 73 to 10 8% with RSD of 4.6 – 14.1 % and LOD between 0.98 and 2.2 µg/L. Good linearity with correlation coefficient greater than 0.98 were obtained for all the investigated pesticides (Zhao et al., 2006).

The analytical performance of SDME was studied and compared with acetone partition extraction in the chromatographic analysis of 12 multiclass pesticides in tomato. The method was validated using a previously developed method (Amvrazi & Tsiropoulos, 2009a), under ISO 17025 norms and SANCO Guide, and compared with modified acetone-partition extraction procedure. The analytical method validated using matrix-matched internal standard calibration curve gave LOD from 0.1 to 116 µg/kg, LOQ between 0.5 and 382 µg/kg, and enrichment factor (EF) ranging from 0.7 to 812. The recoveries were between 65 and 91% with RSD of 6.9 – 17.8% and linearity ranges from 5 to 5000 µg/kg with correlation coefficient greater than 0.985. The method showed a

negative matrix effect for most of the pesticide investigated and is a more selective method compare to acetone-partition extraction (Amvrazi, Papadi-Psyllou, & Tsiropoulos, 2010).

The DI-SDME technique was used to investigate the presence of organochlorine pesticides (OCPs) in vegetable samples (cabbage, cauliflower, and Chinese cabbage). The analytical characteristics determined under the optimized conditions show linearity ranging from 0.05 to 50 ng/mL with correlation coefficient greater than 0.993 and the LOD was between 0.05 and 0.2 ng/mL. The relative recoveries were found in the range of 74 – 95 % (RSD = 10.4 – 18.5%), 72 – 87 % (RSD = 8.7 – 18.1 %), and 65–10 0% (RSD = 9.9–16.3 %) for cabbage, cauliflower, and Chinese cabbage, respectively. It was observed that the proposed method gave good extraction efficiency and low LOD. The method precision obtained indicated that the proposed method could be used for the analysis of OCPs from vegetable samples (Zhang, M. et al., 2008).

The extraction efficiency of HS-SDME, SPME and SPE were compared in the analysis of OPPs and OCPs in strawberry and cucumber samples. The SDME method validation gave linearity in the range of 1 – 100 µg/mL ($R^2 \geq 0.987$), mean recoveries ranged from 75 – 95 % (RSD = 4.7 – 3.6 %), while the LOQ and LOD ranged from 0.006 to 3 µg/mL and 0.001 to 1 µg/mL respectively. The addition of salt to the donor solution was observed to reduce extraction efficiency, which is due to the reduction in diffusion of analytes to the extraction solvent. It was concluded that HS-SDME enables extraction of analytes from more complex matrices, simpler to perform, free from carry-over effect, and time effective, but HS-SPME was found to be more efficient with better linearity, LOD, LOQ, and precision (Kin & Huat, 2009).

A method was also developed for the extraction of seven strobilurin and six oxazole fungicides in fruit (peach, peach and grape, pineapple and carrot) and juice (musts and canned fruit) samples. The SDME was compared with ultrasound-assisted emulsification microextraction (USAEME) technique by using EU Commission Decision (2002/657/EC) as a guideline for method validation. Both methods gave similar average recoveries (SDME = 79 – 117 %, RSD = 2.5 – 9.9 %, and USAEME = 80 – 119 %, RSD = 2.1 – 10 %), which shows that matric effect has little effect and was corrected using method of standard addition for quantification. The SDME method gave LOD in the range of 0.01 – 0.31 ng/mL, while linearity ranged from 0.06 to 300 ng/mL. The EF ranged from 81 to 1602 for SDME and 142 to 1141 for USAEME. The USAEME was found to be advantageous for the extraction of target analytes when considering the equilibrium time (4 min) and extraction temperature (room temperature). The two techniques provided high and comparable selectivity, reproducibility, and EF (Viñas et al., 2010).

A solvent drop microextraction method called floated organic drop microextraction (FDME) was developed for the extraction of two carbamate and three benzoylurea insecticides in peach juice and subsequently analyzed using high performance liquid chromatography (HPLC) coupled to UV-Visible detector. The method performance estimated at the optimal extraction conditions (extraction solvent, 1-dodecanol; solvent volume, 8 µL; extraction time and temperature; 25 min and 40 ^{0}C; salt addition, 30 g/L of NaCl; stirring rate, 420 rpm; and pH 4), gave linearity range of 0.01 – 10 µg/mL, with correlation coefficient of 0.999. The recoveries for all the investigated analytes range from 88.49 to 101.86 % with RSD of 1.99 – 3.47 % and LOD between 0.005 and 0.01 mg/L. The method developed was found to have limitation in the selection of organic solvent due

to the co-elution of the solvent with analytes and causing the overlapping of the solvent peak with analyte peak, but was overcome by the use of solvent with suitable melting point (Zhou, J. et al., 2009).

A modified SDME method was developed for the analysis of OCP in strawberry, strawberry jam and soil. The recovery and the method validation was carried out at the optimum conditions (extraction time, 45 min; agitation rate, 300 rpm; extraction solvent, n-hexane,; solvent volume, 5 µL) gave the recovery, RSD, linearity and LOD between 70 – 98 %, 0.3 – 14 %, 0.5 – 50 µg/kg and 0.001 – 0.11 µg/kg respectively. The addition of a silicon ring in the microsyringe needle helped to improve the stability of the large volume solvent drop (Fernandes et al., 2011).

Table 2.1: SDME for the Analysis of Pesticide Residues in Fruits and Vegetables

Pesticides Class	Matrix	Org. solv., vol (µL)	Ext. time (min)	Ext. temp. (^{0}C)	NaCl (%)	pH	St. Rate (rpm)	LOD	LOQ	Rel Rec (%)	RSD (%)	LR	EF	Detector	References
13 MCPs	Tomato	$ArCH_3$, 1.6	25	n.r	n.r	n.r	350	0.6-30 µg/kg	n.r	29-58	6.3-12	5-500 µg/kg	n.r	GC-NPD	(Amvrazi & Tsiropoulos, 2009a)
21 MCPs	Apple, grape	$ArCH_3$, 1.6	25	n.r	n.r	4	250	2-200 µg/kg	10-660 µg/kg	65-100	4-17	n.r	11-328	GC-MS	(Amvrazi & Tsiropoulos, 2009b)
6 TFs	Grape juice	HeOH/Hex 30	21	n.r	1	n.a	n.r	2-112 µg/L	8-375 µg/L	71-106	2.9-4.5	0.01-10 µg/L	141-214	GC-FID	(Farajzadeh et al., 2011)
12 MCPs	Tomato	$ArCH_3$, 1.6	30	n.r	n.r	4	250	0.1-116 µg/kg	0.5-382 µg/kg	65-91	6.9-17.9	5-5000 µg/kg	0.7-812	GC-NPD	(Amvrazi et al., 2010)
9 OCPs	Cabbage, cauliflower	p-$Ar(CH_3)_2$ /AcO, 1.0	30	n.r	n.r	n.r	400	0.05-0.2 ng/mL	n.r	65-100	8.7-18.1	0.05-50 ng/mL	n.r	GC-MS	(Zhang, M. et al., 2008)
6 OPPs	Apple, pear orange juices	$ArCH_3$, 1.5	20	R.T	n.a	5-6	600	0.21-0.56 ng/mL	n.r	77-113	0.6-13.4	0.5-50 ng/mL	23-109	GC-FPD	(Xiao et al., 2006)
7 OPPs	Orange juice	$ArCH_3$, 10	15	n.r	n.r	n.r	400	0.98-2.2 µg/L	n.r	73-108	4.6-14.1	10-500 µg/L	n.r	GC-FID	(Zhao et al., 2006)
5 OPPs, 3 OCPs	Cucumber strawberry	$ArCH_3$, 1.5	15	R.T	n.a	n.r	800	1-1000 µg/L	6-3000 µg/L	71-95	4.7-13.6	1-100 µg/mL	n.r	GC-ECD	(Kin & Huat, 2009)
7 SFs, 6 OFs	5 fruits, 2 juices	$C_8H_{16}O$/ $C_{11}H_{22}O$,10	30	50	10	5	n.r	0.01-0.31 ng/mL	n.r	79-117	2.5-9.9	0.06-300 ng/mL	81-1602	GC-MS	(Viñas et al., 2010)

Table 2.1: SDME for the Analysis of Pesticide Residues in Fruits and Vegetables (cont'd)

Pesticides Class	Matrix	Org. Solv. vol (µL)	Ext. time (min)	Ext. temp. (^{0}C)	NaCl (%)	pH	St. Rate (rpm)	LOD	LOQ	Rel. Rec. (%)	RSD (%)	LR	EF	Detector	References
2 CPs, 3 BUPs	Peach juice	1- $C_{12}H_{24}O$, 8	25	40	3	4	420	0.005-0.01 µg/ml	n.r	88-101	1.99-3.47	0.01-10 µg/mL	n.r	HPLC-UV	(Zhou, J. et al., 2009)
14 OCPs	Strawberry, jam	n-C_6H_{14}, 5	45	n.r	0.5	n.r	300	0.001-0.11 µg/kg	n.r	59-94	0.3-4.8	0.5-50 µg/kg	n.r	GC-MS	(Fernandes et al., 2012)

N.B. org. solv., organic solvent; vol, volume; ext. time, extraction time; ext. temp., extraction temperature; st. rate, stirring rate; rel. rec., relative recovery; RSD, relative standard deviation; LR. Linear range; EF, enrichment factor; MCPs, multiclass pesticides; TFs, triazole fungicides; OCPs. organochlorine pesticides; OPPs, organophosphorus pesticides; SFs, strobilurin fungicides; CPs, carbamate pesticides; OFs, oxazole fungicides; BUPs, benzoylurea pesticides; $ArCH_3$, toluene; HeOH, hexanol; n-C_6H_{14}, n-hexane; p-$Ar(CH_3)_2$, *p*-xylene; AcO, acetone; $C_8H_{16}O$, octanone; $C_{11}H_{22}O$, undecanone; 1- $C_{12}H_{24}O$, 1-dodecanone; n.r, not reported; n.a, not adjusted; R.T, room temperature; GC-NPD, gas chromatography nitrogen phosphorus detector; MS, mass spectrometry; FID, flame ionization detector; FPD, flame photometric detector; ECD, electron capture detector; HPLC, high performance liquid chromatography; UV, ultraviolet

2.4 Hollow Fiber Liquid Phase Microextraction (HF-LPME)

Hollow fiber liquid phase microextraction, also called liquid-liquid-liquid microextraction (LLLME), is described as a multi-phased microextraction system, and was developed due to the limitation of the stability of the organic solvent inherent in SDME. The extraction technique is based on the principle of a supported liquid membrane (SLM), involving the filling of both the wall pores and the lumen of a semi-permeable polypropylene hollow fiber (HF) with organic solvent (Lee, J. et al., 2008; Pedersen-Bjergaard & Rasmussen, 1999, 2008; Pedersen-Bjergaard, Rasmussen, & Grønhaug Halvorsen, 2000). It makes use of a polymeric membrane which forms a barrier between the solvent and the sample and acts as a support for the small volume of extraction solvents (Hyötyläinen & Riekkola, 2008). The HF-LPME can be carried out in either the static or dynamic mode of the HF attached to a syringe. In the static mode, the acceptor phase is introduced in the lumen followed by the immersion of the fiber into an aqueous sample, while in the dynamic mode, the HF is attached to a syringe connected to a programmable pump (Asensio-Ramos et al., 2011; Sarafraz-Yazdi & Amiri, 2010), which has also been developed for multiple extraction for up to eight sample vials simultaneously by the use of multiple channel syringe pump. This allows for the continuous pumping of fresh solvent through the HF lumen (Pezo et al., 2007; Salafranca, Pezo, & Nerín, 2009). The dynamic mode has been found to give a shorter extraction time, if all factors are well optimized (Ridgway et al., 2007).

The HF-LPME can be carried out in two or three phases (Fig 2.2). In the two-phase mode, the aqueous sample (donor) makes contact directly with the organic solvents (acceptor) through water immiscible solvent immobilized in the membrane pores of HF, by a repeated pushing and pulling of the microsyringe, and the mass transfer of analyte is driven by the diffusion of the analytes from the sample matrix into the organic solvent. In three-phase mode, the HF pores are prefilled with organic solvent which provided supported liquid membrane and analytes are extracted from the aqueous sample matrix into the organic solvent in the pores of the HF then to another aqueous solution present inside the lumen of the HF. The solvent used must be compatible with the membrane, strongly immobilized into the pores of the HF, have low viscosity for better diffusion coefficients through the SLM and have high partition coefficients, so as to ensure that the pores in the wall of the membrane is completely filled by the organic solvent, for efficient extraction of analytes (Bello-López et al., 2012; Ghambarian, Yamini, & Esrafili, 2012; Lambropoulou, Konstantinou, & Albanis, 2007; Lee, J. et al., 2008; Pedersen-Bjergaard & Rasmussen, 2008; Sarafraz-Yazdi & Amiri, 2010).

HF-LPME technique can also be carried out in the headspace mode, but the use of direct immersion mode has been widely used for efficient concentration of the analytes. The limitation of this technique is the issue of carry–over, therefore a new membrane should be used for each extraction (Krylov et al., 2011). The HF-LPME membranes have tendency to accommodate large volumes of organic solvents, with the pore acts as filter that prevents interferences caused by the presence of large molecular weight molecules in the sample matrix.

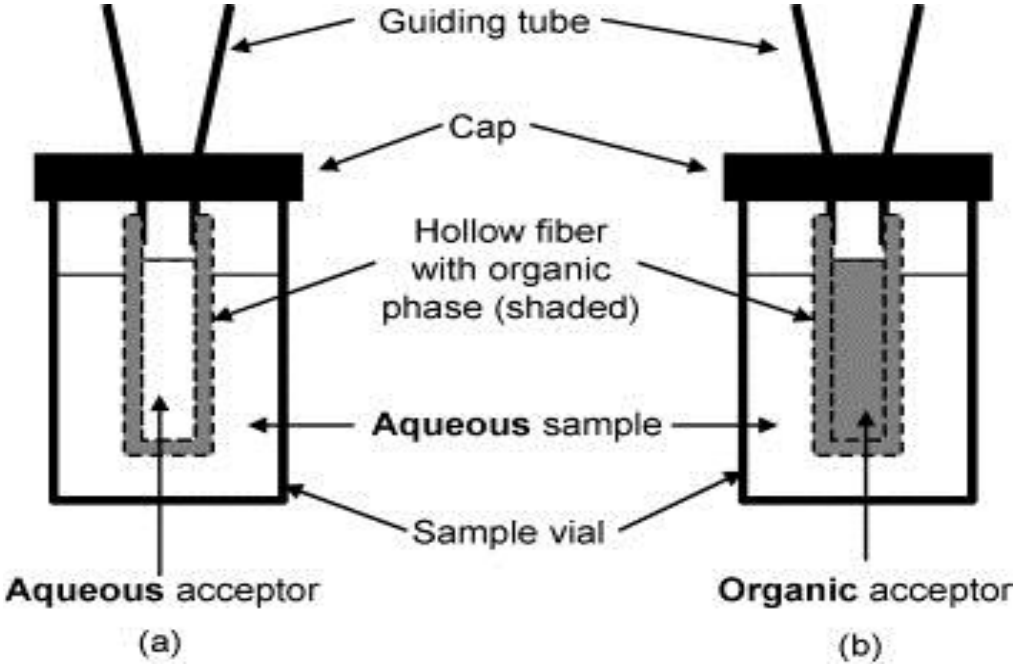


Fig 2.2: Hollow Fiber-Liquid Phase Microextraction in (a) Three Phase and (b) Two-Phase Systems (Lee, J. et al., 2008; Pedersen-Bjergaard & Rasmussen, 2008)

2.4.1 Theory of HL-LPME

In the HF-LPME technique, extraction of analytes from the sample matrix is driven by concentration differences of the analytes between the acceptor solvent in the HF membrane and the analytes in the aqueous sample. The mass transfer of analytes is a thermodynamic process which reaches its maximum at equilibrium. The HF-LPME consists of series of reversible reactions and the extraction process for a two-phase system can be illustrated by an expression which represents the partition of analytes between the aqueous and the organic phases (Ho, Pedersen-Bjergaard, & Rasmussen, 2002; Pedersen-Bjergaard & Rasmussen, 1999, 2008; Shen & Hian, 2002).

$$A_{sample} \longleftrightarrow A_{acceptor} \qquad (2.7)$$

where A is the target analyte at equilibrium, the distribution ratio of the analyte can be defined as:

$$K_{O/S} = C_s/C_{aq,s} \quad (2.8)$$

where $C_{aq,s}$ and C_s are the equilibrium concentration of analyte A, in organic solvent phase and the sample respectively. The amount of analyte in the system will remain the same, and thus the initial amount of analyte (n_i) is equal to the sum of the individual amount of analyte present in the acceptor (n_o) and the sample (n_s) phases during the extraction process (Ho et al., 2002; Ouyang & Pawliszyn, 2006a), and can be expressed as:

$$n_i = n_s + n_o \quad (2.9)$$

The variation of the analyte concentration in the acceptor phase with respect to time $C_a\,(t)$ which gives the kinetic of extraction can be expressed as:

$$C_a\,(t) = C_{eq,a}\,(1 - e^{-kt}) \quad (2.10)$$

where $C_{eq,a}$ is the analyte concentration at equilibrium in the acceptor phase and k is the rate constant. At equilibrium, Eq. (2.9) can be written as a function of concentration and volume of the organic solvents and sample (Ho et al., 2002):

$$C_i V_s = C_{eq,s} V_s + C_{eq,o} V_o \quad (2.11)$$

where C_i, $C_{eq,o}$ are the initial and equilibrium concentration of analyte in the sample respectively, while $C_{eq,o}$ is the equilibrium concentration of analyte in the organic solvent, and V_s and V_o are the volumes of aqueous sample and organic solvent (sum of the volume of organic solvent in the pores of the porous wall and lumen of the HF) respectively.

The amount of analytes extracted can then be expressed (Ho et al., 2002; Pawliszyn, 1997; Pedersen-Bjergaard & Rasmussen, 2008) as:

$$n_{aq,o} = \frac{K_{o/s}V_o C_i V_s}{K_{o/s}V_o + V_s} \quad (2.12)$$

then the recovery (R) and the enrichment factor (EF) of the analyte can be calculated using the following equations:

$$R = \frac{K_{o/s}V_0}{K_{o/s}V_o + V_s}\ x\ 100\% \quad (2.13)$$

$$EF = \frac{C_0}{C_i} = \frac{V_s R}{100 V_o} \quad (2.14)$$

Eq. (2.14) showed that the recovery is dependent on the partition coefficients, the volumes of the sample and the organic solvent and thus, high EF can be obtained at low V_s/V_o ratio (Lambropoulou & Albanis, 2007a).

For a three-phase system, analyte is extracted from the aqueous sample solution to the acceptor solution through the organic supported liquid membrane, immobilized into the pores of the HF, and this can be illustrated by the equation (Ghambarian et al., 2012; Ho et al., 2002; Pedersen-Bjergaard & Rasmussen, 1999, 2008; Psillakis & Kalogerakis, 2002):

$$A_{sample} \longleftrightarrow A_{org\ solv} \longleftrightarrow A_{acceptor} \quad (2.15)$$

At equilibrium, the recovery can be calculated by considering the distribution ratio of the analyte between the organic solvent phase and the sample in one hand and between the acceptor phase and the organic solvent phase on the other hand (Ghambarian et al., 2012; Ho et al., 2002; Pedersen-Bjergaard & Rasmussen, 2008):

$$K_{o/s} = \frac{C_{eq,o}}{C_{eq,s}} \quad (2.16)$$

$$K_{a/o} = \frac{C_{eq,a}}{C_{eq,o}} \quad (2.17)$$

where $K_{o/s,}$ $K_{a/o}$ are the distribution ratios between the organic solvent and the sample and between the acceptor phase and the organic phase respectively, while $C_{eq,o}$, $C_{eq,s}$ and $C_{eq,a}$ are the equilibrium concentrations of the analyte in the organic phase, sample matrix and the acceptor phase respectively. The partition between the acceptor phase and the sample matrix can also be expressed as:

$$K_{a/s} = \frac{C_{aq,a}}{C_{aq,s}} = K_{o/s}/K_{a/o} \quad (2.18)$$

The initial amount of analyte present is equal to the sum of the individual amount of analyte present in all the three phases during the extraction process and can be expressed as (Ho et al., 2002):

$$n_i = n_s + n_o + n_a \quad (2.19)$$

where $n_i,\ n_s,\ n_o\ and\ n_a$ are the amounts of analyte present initially, in the sample matrix, in the organic solvent and in the acceptor phase respectively. At equilibrium Eq. (2.19) becomes:

$$C_i V_s = C_{eq,s} V_s + C_{eq,o} V_o + C_{eq,a} V_a \quad (2.20)$$

where V_a, is the volume of the acceptor solution and other parameters are as defined earlier. The amount of analyte extracted into the acceptor phase at equilibrium can then be estimated using (Ho et al., 2002; Pawliszyn, 1997):

$$n_{aq,a} = \frac{K_{a/s} V_0 C_i V_s}{K_{a/s} V_a + K_{o/s} V_o + V_s} \quad (2.21)$$

The recovery (R) and the enrichment factor of the analyte can then be estimated using the following equations (Ho et al., 2002; Pedersen-Bjergaard & Rasmussen, 2008):

$$R = \frac{K_{a/s}V_a}{K_{a/s}V_a + K_{o/s}V_o + V_s} x\ 100\% \quad (2.22)$$

$$EF = \frac{C_a}{C_i} = \frac{V_s R}{100 V_a} \quad (2.23)$$

It can be observed from Eq. (2.23) that the recoveries in 3-phase HF-LPME are dependent on the distribution constants between the organic solvent phase and the sample and between the acceptor phase and the organic solvent phase, and on the volume of the sample, the organic and the acceptor phases (Pedersen-Bjergaard & Rasmussen, 2008).

2.4.2 Applications of HF-LPME in Pesticide Residues Analysis in Fruits and Vegetables

The use of HF-LPME for the analysis of pesticide residues from water samples has been extensively reported, where nonpolar organic solvents were used as the acceptor phase (Lambropoulou & Albanis, 2007a). Only a limited number of studies have been conducted on the analysis of pesticide residues from food samples, as shown in Table 2.2.

HF-LPME was first applied to pesticides analysis in grapes, and the method was called pressurized hot water extraction-microporous membrane liquid–liquid extraction (PHWE-MMLLE) coupled to GC/MS. The MMLLE was used as a trapping step after PHWE and the water from the PHWE is then directed to the donor side of the membrane unit where the analytes are extracted onto the acceptor solution. The MMLLE was thus used to clean and concentrate the extract before on-line transfer to the GC. Analytical performance

yields an average recovery in the range of 9 – 26 % (RSD = 1 – 6 %), the low recovery was attributed to the presence of microporous membrane. The EFs ranged from 24 to 75, indicating an efficient partitioning between the target analytes and the acceptor phase. The LOQ ranged from 0.3 to 1.8 µg/kg, with linearity between 0.015 and 3 mg/kg and correlation coefficient greater than 0.973. It was observed that the chromatographic behavior of the on-line PHWE-MMLLE-GC/MS was significantly better compared with liquid–solid and ultrasonic extractions with off-line analysis, indicating the selectivity of the developed method (Lüthje et al., 2005).

The HF-SLM method was developed for simultaneous determination of 23 multiclass pesticide residues in vegetable (cucumber, tomato, and pepper) samples. The quantification performed by matrix matched calibration yields LOD and LOQ ranging from 0.06 to 2.7 µg/kg and 0.2 to 9.0 µg/kg, respectively. The method linearity was between 10 and 200 µg/kg with correlation coefficient greater than 0.9910. The sample matrix was found to have no influence on the extraction efficiency and the donor samples were not diluted with water. The developed procedure was found to yield good analytical performance, which allows determination of pesticides below MRLs (Romero-González et al., 2006).

A HF-LPME method called liquid–liquid–solid microextraction (LLSME) coupled to HPLC, based on porous membrane protected molecularly imprinted polymer (MIP) coated SPME silica fiber (solid phase) for the extraction of triazine pesticides from sludge water, milk, urine, and water melon was developed. The MIP-coated fiber (SPME) was used as the acceptor phase protected by HF filled with toluene and extraction of the analyte was

performed in direct immersion mode for 30 min at a stirring rate of 1000 rpm and was desorbed into HPLC system (HPLC, high performance liquid chromatography). The method performance for watermelon gave good recovery in the range of 74 – 103% with RSD of 1.2 – 9.5%. The developed method was compared with MIP-SPME and HF-LPME (using *n*-octanol as the acceptor phase) in terms of linearity, correlation coefficient, LOD, and the result shows that the developed method (LLSME) has the best performance. The linearities ranged from 0.02 to 10 µg/L ($R^2 \geq 0.9956$), 0.5 to 10 µg/L ($R^2 \geq 0.9949$), and 0.5 to 100 µg/L ($R^2 \geq 0.9994$) for LLSME, MIP-SPME and HF-LPME respectively. The LOD for LLSME (0.006 – 0.02 µg/L) were lower than those of MIP-SPME (0.18 – 0.30 µg/L) and HF-LPME (0.08–0.20 µg/L). The RSDs for the target analytes was in the range of 2.3 – 8.5 %, 1.5 – 8.7 %, and 2.5 – 7.8 % for LLSME, MIP-SPME, and HF-LPME respectively. The method was found to be highly selective, effective, and suitable for the extraction of pesticide residues in complex sample matrices (Hu, Y. et al., 2009).

A simple and low-cost method based on the simultaneous application of HF-LPME-GC-ECD was developed for the analysis of OCPs in river water, tomato, and strawberry samples. The LODs were in the range of 0.5 – 1.15 and 1.53 – 12.79 µg/kg for strawberry and tomato respectively, while the LOQs were between 1.69 to 3.85 µg/kg and 5.49 to 42.53 µg/kg for strawberry and tomato respectively. The method linearity and relative recovery (RSD) were in the range of 2 – 230 µg/kg ($R^2 > 0.992$) and 59 – 123 % (RSD = 5.0 – 15%) respectively for tomato and strawberry samples. The developed method was compared with MMLLE and was found to more efficient (Bedendo & Carasek, 2010).

The analysis of postharvest diazole fungicides (thiabendazole, carbendazim, and imazalil) residues from orange juice sample using HF-LPME was also reported. The method was coupled to CE-DAD (CE-DAD, capillary electrophoresis-diode array detector) for method development but LC-MS was used for estimation of analytical method performance, due to poor LOD of the CE-DAD. The developed method yields linearity between 0.1 and 10 µg/L, with correlation coefficient greater than 0.998. The recoveries ranged from 17 to 33 % (RSD = 8.6 – 14.8). The LOD was between 0.05 and 0.1 µg/L, while the LOQ ranged from 0.17 to 0.33 µg/L, which were found to be consistent with the MRL permitted for pesticides in drinking water. Although the recoveries obtained were not quantitative enough, the method selectivity was found to be suitable for the estimation of LOD in drinking water and matrix effect from the orange juice sample, which might involve losses of analytes bonded to the solid materials in the juice, but analysis was carried out and high recoveries were obtained for the analysis of real sample (Barahona et al., 2010).

A hollow fiber microporous membrane liquid-liquid extraction (HF-MMLLE) method was developed based on the principle of LPME, for the extraction of 18 multiclass pesticides in industrial and fresh orange samples. Under the optimal conditions, the method linearity was found in the range 0.01 – 10 mg/L with correlation coefficient greater than 0.98. The method LOD and LOQ ranged from 0.003 to 0.35 mg/L and 0.010 to 1.6 mg/L respectively. The relative recovery was between 62 to 121 % with RSD less than 7.4 %. The good LOD and LOQ values, which was found to be similar to those obtained for methods based on HF-LPME, was as a result of the excellent sample clean-up promoted by

the porous membrane, which shows the suitability of the developed method for pesticide analysis in the sample (Bedendo, Jardim, & Carasek, 2012).

Two pesticides carbendazim and thiabendazole were analyzed in apple juice using a HF-LPME method coupled to high performance liquid chromatography with fluorescence detector (HPLC-FD). The performance of the developed method was estimated in apple juice spiked at 3 different concentration levels according to the established procedure, and yielded good linearity (2.5 – 500 µg/L) with linear regression greater than 0.999. The LODs were 0.8 µg/L in carbendazim and 1.5 µg/L in thiabendazole, while the recovery (RSD) and EF ranged from 86.3 – 106 % (3.3 – 8.5 %) and 106 – 114 respectively. The developed method was found to render good sensitivity which was attributed to the fluorescence detection and the analytical performance was observed to be satisfactory (Liu, Z. et al., 2012b).

A method based on HF-LPME coupled to GC-ECD was developed for the determination of OPPs (chlorpyrifos and profenofos) in vegetable samples. The validation of the developed HF-LPME method was performed under the optimized conditions and the correlation coefficient was greater than 0.99. The LOD and LOQ ranged from 99 to 128 µg/L and 331 to 427 µg/L respectively and the relative recovery (RSD) was between 60.8 and 88 % (0.54 – 8 %). Matrix effect was attributed to the selectivity of the HF because of the pores in its wall, which act as a filter in the complex sample, since large molecules, which can also be soluble in the organic solvent were not co-extracted (Sanagi et al., 2010).

A two-phase HF-LPME coupled to GC-MS method was developed for the separation and chromatographic determination of 4 triazole fungicides (penconazole, hexaconazole, diclobutrazole and diniconazole) in grape juice and other environmental water samples. The extraction conditions which include 4 µL of extraction solvent (acetone) containing methidathion as internal standard, extraction time of 20 min, at a stirring rate of 720 rpm with no pH and ionic strength adjustment showed good method linearity ranging from 1 to 5000 µg/L with $R^2 \geq 0.997$. The recovery was satisfactory with acceptable RSD in the range of 83–114 % and 6 – 9 % respectively with EF of 134 – 240. The LOD were found between 0.3 and 0.8 µg/L. with little matrix effect. The developed method was compared with other microextraction techniques and was found to have comparable or better analytical performance in terms of linearity, recoveries, EF, LODs and RSD (Sarafraz-Yazdi, Assadi, & Wan Ibrahim, 2012).

The HF-LPME technique was used for sample pretreatment to enrich seven multiclass pesticide residues from cucumber sample. The optimized conditions include chloroform as the organic solvent, stirring speed of 300 rpm, and extraction time of 20 min at room temperature. The method validated using the optimal extraction conditions gave linearity between 0.05 and 500 µg/kg, enrichment factor of 100 to 147, recovery ranging from 63 – 147 % with RSD less than 20% and the LOD and LOQ ranged from 0.01– 0.31 µg/kg and 0.05 – 1 µg/kg respectively. The influence of matrix effect was evaluated and the result showed some effect on the extraction efficiency, thus matrix matched standard curves were used for quantitation (Wang, J. et al., 2012).

A method for the extraction and determination of carbamate pesticide residues (carbaryl, propoxur, pirimicarb, metolcarb, carbofuran, isoprocarb, bendiocarb and fenobucarb) in vegetable sample was proposed based on electrokinetic flow analysis (EFA) coupled on-line with HF-LLLME with UV detector. The method gave linear concentration from 0.0033 to 1 µg/mL, with correlation coefficient of 0.999. The recovery and LOD for carbaryl was found to be 89 – 108 % and 2 µg/kg respectively (Fu, G.-N. et al., 2009), which is comparable to other method such as SPME-HPLC (Gou et al., 2000) and LPME-HPLC (Hylton & Mitra, 2007).

Table 2.2: HF-LPME for the Analysis of Pesticide Residues in Fruits and Vegetables

Pesticides Class	Matrix	Org. Solv., vol	Acc. solv., vol. (µL)	Ext. time (min)	Ext. temp. (^{0}C)	NaCl (%)	pH	St. rate (rpm)	LOD	LOQ	Rel. rec. (%)	RSD (%)	LR	EF	Detector	Reference
6 MCPs	Grape	$ArCH_3$, 3 mL	n.r	40	120	n.r	n.r	n.r	n.r	0.3-1.8 µg/kg	9-28	1-6	15-300 µg/kg	24-75	GC-MS	(Lüthje et al., 2005)
23 MCPs	Cucumber, tomato, pepper	DHE/ TOPO, n.r	MeOH/ HCL, n.r	60	n.r	15	4	40	0.06-2.7 µg/kg	0.2-9.0 µg/kg	n.r	n.r	10-100 µg/kg	n.r	LC-MS	(Romero-González et al., 2006)
9 THs	Watermelon	$ArCH_3$, n.r	MIP fiber	30	n.r	n.r	n.r	n.r	6-20 µg/mL	n.r	74-103	1.2-9.5	20-10^3 µg/mL	n.r	HPLC-UV	(Hu, Y. et al., 2009)
13 OCPs	Tomato, strawberry	OcOH, 20 µL	$ArCH_3$/ C_6H_{14}, 30	59	60	2.91g	2-4	n.a	0.5-12.79 µg/kg	1.69-42 µg/kg	59-123	5-15	2-230 µg/kg	n.r	GC-ECD	(Bedendo & Carasek, 2010)
3 DFs	Orange juice	2-$C_8H_{16}O$ 20 µL	HCL	30	n.r	n.r	8-12	1000	0.05-0.1 µg/L	0.17-0.33 µg/L	17-33.7	8.6-14.8	0.1-10 µg/L	n.r	CE, LC-MS	(Barahona et al., 2010)
18 MCPs	Orange	n.r	$ArCH_3$/ EtAc, 400 µL	35	R.T	$(NH_4)_2SO_4$	7	n.r	3-35 µg/L	n.r	62-121	<7.6	0.01-10 mg/L	n.r	LC-MS	(Bedendo et al., 2012)
7 MCPs	Cucumber	$CHCl_3$ 32 µL	n.r	20	R.T	n.r	n.a	300	0.01-0.31 µg/kg	0.05-1 µg/kg	63-119	<20	0.05-500	101-147	UHPLC-MS/MS	(Wang, J. et al., 2012)

Table 2.2: HF-LPME for the Analysis of Pesticide Residues in Fruits and Vegetables (cont'd)

Pesticides Class	Matrix	Org. Solv., vol	Acc. solv., vol. (µL)	Ext. time (min)	Ext. temp. (^{0}C)	NaCl (%)	pH	St. rate (rpm)	LOD	LOQ	Rel. rec. (%)	RSD (%)	LR	EF	Detector	Reference
4 TFs	Grape juice	$ArCH_3$, 4 µL	n.r	20	n.r	n.a	n.a	720	0.3-0.8 µg/L	n.r	99-101	6-9	1-5000 µg/L	134-240	GC-MS	(Sarafraz-Yazdi et al., 2012)
2 BIFs	Apple juice	OcOH /HCl	n.r	40	n.r	n.r	7.5	800	0.8-1.5 µg/L	n.r	83-106	3.3-8.5	2.5-500 µg/L	106-114	HPLC	(Liu, Z. et al., 2012b)
2 OPPs	tomato, cabbage, water convolvulus	$C_{11}H_{24}$ 3 µL	n.r	n.r	n.r	n.r	n.r	n.r	99-128 µg/L	331-427, µg/L	60-88	0.54-8	n.r	n.r	GC-ECD	(Sanagi et al., 2010)

N.B: acc. sol., acceptor solution; DFs, diazole fungicides; THs, triazine herbicides; DHE, diethylether; TOPO, trioctylphospine oxide; OcOH, octanol; MIP, molecularly imprinted polymer; BIFs, benzylimidazole fungicides; others see Table 2.1

2.5 Dispersive Liquid-Liquid Microextraction (DLLME)

DLLME is a recently developed microextraction technique and was developed for the analysis of polyaromatic hydrocarbon in water samples, using tetrachloroethylene and acetone as the extraction and the dispersive solvents respectively (Rezaee et al., 2006), and also for the analysis of organophosphorus pesticide residues in water sample using acetone and chlorobenzene as the dispersive and extraction solvents respectively (Berijani et al., 2006). It makes use of small volume of a mixture of extraction and dispersive solvents with high miscibility thereby preventing the dislodgement of the organic solvent drop inherent in SDME. A cloudy solution is formed when an appropriate mixture of high-density water-immiscible extraction and dispersive solvents are injected rapidly into an aqueous solution of the sample matrix (Kocúrová et al., 2012; Rezaee et al., 2006; Rezaee et al., 2010), containing the analytes of interest. Its limitation lies in it manual procedure and centrifugation which is time consuming. Automation based on sequential injection system has been used to overcome the drawback (Andruch et al., 2013b).

The target analytes are then enriched into the extraction solvents, which are dispersed into the bulk aqueous solution when the mixture is centrifuged, thus making DLLME a two-step microextraction technique. After centrifuging, a sedimented phase of the extraction solvent accumulates at the bottom of the extraction vessel and can be injected into analytical instruments (Andruch et al., 2013a, 2013b), with or without further treatment (clean-up) (Fig 2.3). The selection of the type and volume of dispersive solvent is as important as that of the extraction solvent, because, it helps the extraction solvent to form fine droplets in the sample matrices and ensures high enrichment factor (Zgoła-

Grześkowiak Agnieszka & Grześkowiak, 2011). The nature of the fine droplet has been found to enhance extraction efficiency, because of the abundant surface contact between the droplet and the analytes, thus the mass transfer of analytes into the extraction solvent is speeded up (Rezaee et al., 2010; Sarafraz-Yazdi & Amiri, 2010), making the extraction process time independent, but depends on the rate of centrifugation.

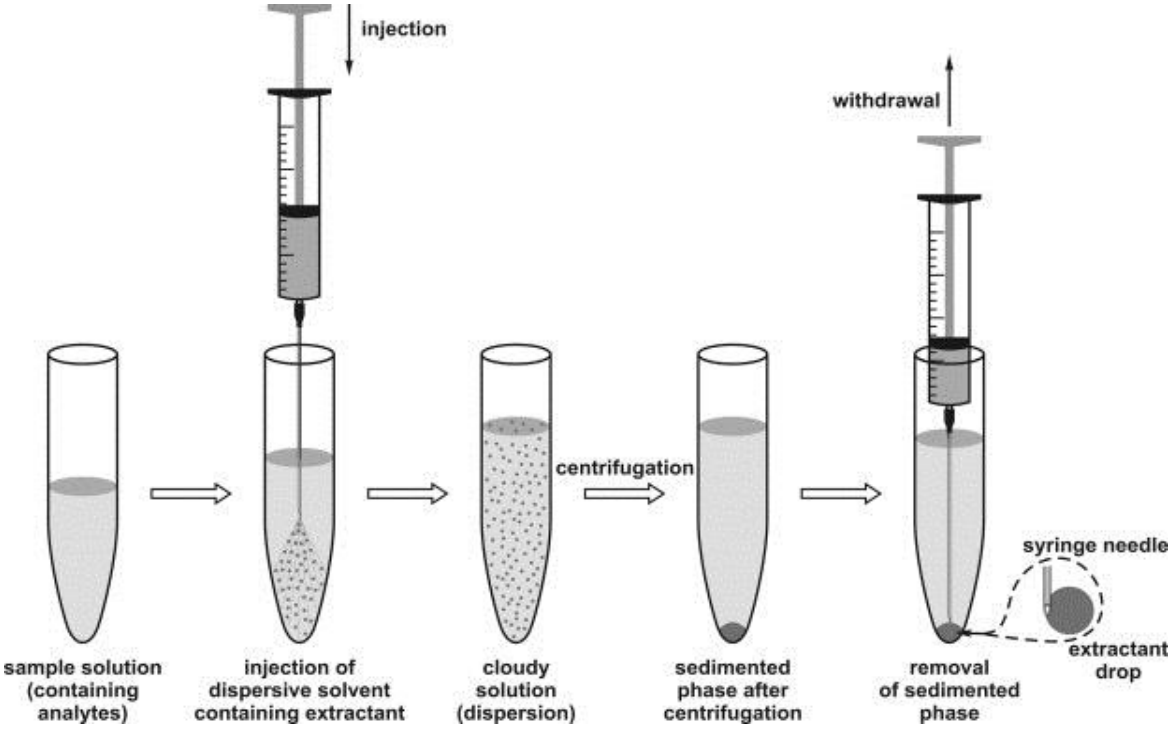


Fig 2.3: Steps in Dispersive Liquid-Liquid Microextraction (Sarafraz-Yazdi & Amiri, 2010; Zgoła-Grześkowiak Agnieszka & Grześkowiak, 2011)

2.5.1 Theory of DLLME

In DLLME, the enrichment factor (EF) is defined as the ratio between the concentration of analyte in the sedimented phase (C_{sed}) and the initial concentration of analyte (C_o) in the sample (Rezaee et al., 2006; Rezaee et al., 2010), and can be represented by the following equation:

$$EF = \frac{C_{sed}}{C_o} \tag{2.24}$$

The C_{sed} can be obtained from a suitable calibration curve of direct injection of the target analyte.

The extraction recovery (ER), defined as the percentage of the ratio of amount of analyte extracted into the sedimented phase (n_{sed}) to the total/initial amount of analyte (n_o) present in the sample matrix, can be expressed as:

$$ER = \frac{n_{sed}}{n_0} x\ 100 = \frac{C_{sed}V_{sed}}{C_oV_{aq}} \tag{2.25}$$

$$ER = \left(\frac{V_{sed}}{V_{aq0}}\right) EF\ x\ 100 \tag{2.26}$$

where V_{sed} and V_{aq} are the volumes of sedimented phase and sample solution respectively. Eq. (2.26) shows that the recovery of the analyte is dependent on the volumes of sedimented and aqueous phases and on the enrichment factor.

2.5.2 Applications of DLLME in Pesticide Residues Analysis in Fruits and Vegetables

The applications of LPME in the analysis of different classes of pesticide residues in fruits and vegetables using the DLLME technique has been extensively described in the published literatures as shown in Table 2.3.

Zang and his co-workers employed DLLME for the analysis of captan, folpet, and captafol in apple sample. Under the optimized condition, the method linearity ranged from 10 to 100 µg/kg ($R^2 \geq 0.9982$), LOD ranged from 3 to 8 µg/kg, with recovery and EF between 93 and 107% (RSD = 4.6 – 6.4 %) and 824 – 912 respectively. Salt addition was found to have no significant effect on the recoveries but slightly decrease the EF and was not added for method validation (Zang et al., 2008).

An extraction method was optimized using DLLME coupled to multidimensional GC/MS for trace analysis of 24 multiclass residual pesticides in apple sample. Under the optimized conditions, the linearity ranged from 0.04 to 0.188 mg/kg ($R^2 \geq 0.9950$), and the LOD and LOQ ranged from 0.06 to 2.20 and 0.2 to 7.3 µg/L, respectively, while the EF and recovery (RSD) were between 35 and 101 and 60 and 105 % (1 – 20 %), respectively (Cunha, Fernandes, & Oliveira, 2009).

A liquid phase microextraction method based on DLLME coupled to HPLC fluorescence detection for the analysis of pesticide (carbaryl and triazophos) residues in water and fruit juice (apple, grape, and peach) samples was developed. The analytical performance of the

method evaluated under this best extraction conditions yield linearity ranging from 0.1 to 1000 ng/mL ($R2 \geq 0{,}991$), the LOD ranged from 12.3 to 16.0 pg/mL. The recovery varied from 86.3 to 105.3 % (RSD = 1.11 – 9.6 7%) and the EF was in the range of 87.5 to 275.6. The developed method was compared to other LPME techniques (Xiong & Hu, 2008; Zhang, J. & Lee, 2006), and it showed lower RSD, LOD, and much wider linear range, while the extraction was very short and does not require any special approach and instrument in the pretreatment step (Fu, L. et al., 2009).

A room temperature ionic liquid based DLLME method for the extraction of trace amount of eight pesticide residues from banana. The linearity of the method ranged from 0.043 to 6.83 mg/L with correlation coefficient greater than 0.994. The recovery determined at three spiked levels was between 53 and 97% (RSD= 2.6 – 8.7%), except for thiophanate and carbofuran (53 – 63%). The LOD ranged from 0.320 to 4.66 µg/kg, which is below the harmonized EU MRLs established for bananas (Ravelo-Pérez et al., 2009a).

The same authors also used room temperature ionic liquids (1-hexyl-3-methylimidazolium hexafluorophosphate, $[C_6MIm][PF_6]$) for the extraction of eight multiclass pesticides in table grape and plum samples. Using the previously optimized conditions for banana samples (Ravelo-Pérez et al., 2009a), the mean recovery was in the range of 72 – 100 % (1.4 – 9.1 %) and 66 – 105 % (1.9 – 8.5 %) for table grape and plum respectively, except for thiophanate and carbofuran (64 – 75 %). The linearity ranged from 0.01 to 6.83 mg/L and the LODs in table grape and plum ranged between 0.651–5.44 and 0.902–6.33 µg/kg respectively. The target analytes found in the samples were at the levels that do not present

any threat for the consumer, since they were below the MRLs established by the EU (Ravelo-Pérez et al., 2009b). The use of ionic liquid as the extraction solvent reduces the exposure to dangerous toxic solvents especially chlorinated solvents used by most authors. The other advantages of IL include high viscosity, high thermal stability, negligible vapour pressure, solubility in water and other organic solvents, inflammable and dual natural polarity and more environmental friendly (Buszewski, Bogusław & Studzińska, 2008; Zgoła-Grześkowiak Agnieszka & Grześkowiak, 2011).

An ultrasonic-based DLLME method for the extraction of trace level of imidacloprid in tomato samples was reported. Under optimal condition, the linearity ranged from 6 to 100 µg/L (R^2 = 0.9980), the average recovery was between 87.6 and 110 % (RSD < 4.5 %), and the EF was 375-fold. The LOD was 0.45 mg/kg for the target analyte, which indicated that the proposed method could be used for the analysis of imidacloprid in tomato, with good sensitivity and accuracy (Qiao et al., 2010).

An LPME technique based on ultrasonic-assisted DLLME for simultaneous determination of cypermethrin and permethrin residues in pear juice using GC-FID was also reported. The EF for cypermethrin was 344-fold while that of permethrin was 351-fold. Linearity was observed in the range of 0.009 – 15.2 µg/kg ($R^2 \geq 0.9993$). The LOD was between 3.1 and 2.2 µg/kg, the recovery determined at three spiked levels ranged from 92.1 to 107.1 % (RSD < 4 %). The salt addition was found to have no effect on the recovery and EF, but caused the precipitation of the pear juice matrix, while the pH of the donor sample was

kept at 4.5. The ultrasound system was found to accelerate the formation of a fine cloudy solution, which increased the efficiency and reduced extraction time (Du et al., 2010).

A rapid and sensitive method was developed based on DLLME coupled with sweeping micellar-electrokinetic chromatography (sMEKC), for the analysis of six carbamate pesticides in apple. The method validation estimated using a six-point calibration curve gave linear range of 6 – 500 ng/g ($R^2 \geq 0.9952$), with EF and recovery ranging from 491 to 1834 and 85 to 113% (RSD = 4.3 – 7.4 %), respectively, and the LOD between 2 and 3 ng/g. The EF of the DLLME-sMEKC when compared with that of DLLME (74 – 151) and sMEKC (7.1 – 10.9), and it showed that the DLLME-sMEKC provided about 500 to 800 fold sensitivity enhancement without obvious loss of resolution (Zhang, S. et al., 2010).

The use of a new 1,3-dibutylimidazolium hexafluorophosphate ([BBIm][PF_6]) ionic liquid, as an extraction solvent for DLLME method for preconcentration of organophosphorus pesticides in water and pear juice samples was developed, optimizing the effect of different parameter on the extraction efficiency. The linearity of the method ranged from 5 to 1000 µg/L ($R^2 \geq 0.9988$). The LOD was 0.01 – 0.05 µg/L, the recoveries were between 78.6 and 86.8 %, and the RSD at three spiked levels ranged from 1.1 to 2.7 %, while the EF was over 300 fold. The recovery value (92.7 – 109.1 %) obtained in real pear sample shows that the sample matrix had little effect on the proposed method (He, L. et al., 2010).

A DLLME method was developed based on solidification of a floating organic droplet combined with LC-DAD for the simultaneous analysis of diethofencarb and pyrimethanil

in apple pulp and peel. The method linearity ranged from 8 to 800 µg/kg with correlation coefficients greater than 0.9916, LODs were 1.4 and 1.6 µg/kg for pyrimethanil and diethofencarb respectively. The recovery ranged between 83 and 101.3 % (RSD = 4.8 – 8.3 %), which demonstrated that the developed method was not significantly affected by matrix effect (Zhou, Y. et al., 2011). The proposed method was observed to give comparable results when compared with HS-SPME-GC-MS method (Navalón et al., 2002) in terms of LOD and extraction time.

An LPME method was introduced based on the use of dispersive SPE (DSPE) clean-up followed by DLLME for the extraction of neonicotinoid insecticides in vegetable (cucumber and tomato) samples prior to HPLC-DAD analysis. The method involved the use of multi-walled carbon nanotubes (MWCNTs) as DSPE sorbent for the removal of color-interfering substances from the samples. The linearity of the method ranged from 5 to 300 ng/g, with the correlation coefficients ranging from 0.9989 to 0.998 and the EF between 110 and 243. The LOD was from 0.5 to 1.0 ng/g while the recovery ranged between 84.6 and 97.5 %, with RSD from 3.7 to 6.2 %. It was observed that the combination of the DSPE procedure helped to achieve better sample cleanup, which was possible by the use of MWCNT-primary secondary amine dual sorbent (Wu, Q. et al., 2011).

A DLLME method coupled to MEKC was proposed for the extraction and preconcentration of 12 carbamate pesticides in fruit juice samples. The proposed method gave linearity in the range between 4 and 1000 µg/L with correlation coefficient greater

than 0.991, while the recovery, LOD, and LOQ ranged from 78 to 105 % (RSD = 3.6 – 8.9 %), 1 to 7 µg/L, and 6 to 24 µg/L, respectively. Three agitation modes (vortex, manual, and mechanical) were compared and no significant difference was observed on the recovery, but mechanical shaking gave the best reproducible results and was selected (Moreno-González et al., 2011). The method was found to provide similar sensitivity compared to other methods such as SPME-HPLC-UV method (Yang et al., 2008) and SPE-HPLC-UV (Liu, X. S. et al., 2009), used for the analysis of carbamates pesticides in fruit juice.

The residual level of multiclass pesticides (polychlorinated biphenyl (PCB), organochlorine pesticide (OCP), and pyrethroid pesticides (PP) were compared in peach juice, pulp, and peels using DLLME based on solidification of floating organic droplet method coupled to GC-ECD. Using the optimal conditions, the linearity ranged from 10 to 2000 ng/L in juice sample and 1 – 20 µg/kg in pulp and peel samples with correlation coefficient greater than 0.99. The relative recoveries in juice, pulp, and peels were in the range of 79 – 102 % (RSD = 3.2 – 7.6 %), 73 – 106 % (RSD = 2.6 – 11.8 %), and 81 – 106 % (RSD = 3.2 – 7.8 %) respectively. The LODs and LOQs ranged respectively from 2.8 to 18.5 ng/L and 9.3 to 53.8 ng/L in juice, and 0.23 to 1.75 µg/kg and 0.76 and 5.77 µg/kg in both pulp and peel samples, while the EF ranged from 409 to 1089. The result of the blank juice, peel and pulp analyzed indicated that the pyrethroid residues did not penetrate into the pulp and juice but were deposited on the peels of the fruits (Matsadiq et al., 2011).

An ultrasound-assisted solvent extraction-DLLME method for the extraction of 13 organophosphorus pesticides from tomato samples was developed and validated. The method validated under the optimized conditions showed good linearity that ranged from 0.5 to 1000 µg/kg with correlation coefficient greater than 0.9917. The LOD ranged from 0.1 to 0.5 µg/kg and the repeatability estimated in terms of RSD was between 7 and 10% (Bidari et al., 2011). The method was compared to HS-SPME (Lambropoulou & Albanis, 2003) and other extraction methods in term of LOD, linearity, volume of extraction solvent, RSD, and sample amount, and they were found to be comparable or lower than some of the methods .

The use of DLLME for the determination of six fungicides in fruit samples (pear, grape, apple, and strawberry) using GC-ECD was examined. The method validation estimated under the optimized conditions gave linearity in the range of 0.5 – 40 µg/kg with correlation coefficient greater than 0.9902. The EF ranged from 685 to 820 while the average recovery was between 81.3 and 98.4 %, with RSD ranging from 3.1 to 7.8 %, and the LOD of the developed method were in the range of 0.02 – 0.12 µg/kg. The method performance was found to fit the requirements for the determination of selected fungicides in real fruit samples (Huo et al., 2011).

A study for the development of a simple and sensitive analytical method for the determination of 7 pyrethroid residues in fruits juices (apple, orange, kiwi, passion fruit, pomegranate and guava) was conducted based on DLLME technique, with special attention given to method optimization to maximize efficiency and allow good ruggedness.

Validation study was carried out using the optimum conditions and the linearity evaluated at nine concentration levels ranged from 2 to 15000 µg/L with correlation coefficient higher than 0.995. The precision expressed as RSD was between 0.89 and 3.61 %. The relative recoveries obtained from two levels of spiked concentration were in the range of 84.5 and 98.3 %., while the LOD and LOQ were between 2 – 5 µg/L and 5 – 10 µg/L respectively. The matrix effect determined by comparing the slopes of calibration curves of the analytes in aqueous solution and representative sample showed no significant difference between the two sample solutions. The developed method was compared with other microextraction techniques for the analysis of pyrethroid pesticides in fruit juice samples, such as SPME-GC/MS (Cortés Aguado et al., 2008), UA-DLLME-GC-FID (Du et al., 2010) and DLLME-GC-GC/MS (Cunha et al., 2009), and were found to have similar analytical performance. but the SPME-GC/MS method was better compared to other methods (Boonchiangma, Ngeontae, & Srijaranai, 2012).

A new sample preparation method combining Quick, Easy, Cheap, Efficient, Rugged and Safe (QuEChERS) and DLLME procedures was developed for the determination of 13 multiclass pesticide residues in tomato using HPLC coupled to DAD. The optimization of the sample pH in DLLME was carried out using univariate analysis, while a full factorial central composite design (CCD) was used for the optimization of amount of primary secondary amine (PSA) and graphitized carbon black (GCB), extraction solvent volume and ionic strength. The verification of the analytical performance carried out using a matrix-matched calibration under the optimized conditions gave linearity range of 0.010 to 1.5 mg/kg, with correlation coefficient greater than 0.998. The mean recovery ranged

between 86 and 116 % with RSD estimated using the retention time and chromatographic peak area were lower than 5.4 and 17.4 % for retention time and peak area respectively. The LOD and LOQ ranged from 0.0017 to 0.045 mg/kg and 0.0058 to 0.15 mg/kg (Melo et al., 2012c). The results of analytical performance obtained show that the developed method were acceptable according to the EU SANCO Guideline (EU, 2009b).

A simple rapid and environmental friendly method based on DLLME was developed for the preconcentration of triazole (penconazole, hexaconazole, diniconazole, tebuconazole and triticonazole) pesticides in aqueous and grape samples. The analytical parameters determined to estimate the performance of the developed method gave linear range of 2 – 5000 µg/L with correlation coefficient greater than 0.995. The LOD and LOQ ranged between 0.3 to 5.0 µg/L and 0.9 to 16.7 µg/L respectively while the EF ranged from 263 – 380 and the mean recoveries varied between 74 – 90 with RSD of 3.2 – 5 % (Farajzadeh, Djozan, & Khorram, 2012). The developed method does not require centrifugation (reducing the extraction time), made use of non-toxic extraction solvents instead of toxic chlorinated solvents used by some researcher and was found to be more efficient than the conventional DLLME.

A method was developed and validated based on acetonitrile extraction followed by DLLME using GC-MS, for the monitoring and analysis of 30 multiclass and multi-residue pesticides in greenhouse tomato using GC-MS. The method combines modified QuEChERS method with DLLME. The tomato sampling was performed in accordance to the EU directives (EU, 2002). The method validation, which was estimated using the

SANCO guideline (SANCO, 2009) gave wide range of linearity from 0.010 to 6 mg/kg with correlation coefficient equals or greater than 0.9954. The LOD and LOQ ranged from 2.7 x 10^{-3} to 2.5 x 10^{-1} mg/kg and 8.9 x 10^{-3} to 8.4 x 10^{-1} which was in agreement to the EU MRLs. The recovery for all investigated pesticides ranged between70 and 110 % with RSD of between 1 and 20 %. The validated analytical method was used to analyze greenhouse tomato and six pesticides out of the 30 investigated were detected and the results showed that pesticide residues had higher persistence in fruits planted in the greenhouse than those planted in the field (Melo et al., 2012b).

Another study which involves the combination of QuEChERS with DLLME coupled to GC-MS was carried out for the determination of 19 multiclass pesticide residues in orange samples. The method which was validated in accordance to SANCO guideline (SANCO, 2009), using matrix-matched calibration yield linearity in the range of 0.1 to 3000 ng/g ($R^2 \geq 0.963$) and 1 to 3000 ng/g ($R^2 \geq 0.963$) in unpeeled and peeled oranges respectively. The LOD and LOQ in unpeeled tomato ranges from 0.07 to 14 ng/g and 0.22 to 47 ng/g respectively while they were respectively in the range of 0.01 to 0.52 ng/g and 0.02 to 1.7 ng/g in peeled orange samples. The recoveries showed good yield and ranged from 63 to 120 % with RSD lower than 20 %. Eight of the investigated pesticides were detected in the orange samples. The result showed that combining QuEChERS with DLLME is simple and inexpensive and could allow low detection limits for pesticides in a large number of samples (Andraščíková, Hrouzková, & Cunha, 2013).

The combination of solid phase extraction (SFE) and DLLME was also explored for the analysis of three benzimidazoles (carbendazim, thiabendazole and thiophanate-methyl) pesticides in tomato samples. The calibration curve of the proposed method was linear over the concentration range of 0.5 to 200 ng/g, with the correlation coefficient greater than 0.99. The LOD ranged between 0.18 and 0.55 ng/g, while the recovery and RSD were between 72 to 89.5 % and 4.2 to 5.3 % respectively, indicating good method performance (Han et al., 2013).

Some carbamate pesticides were determined in watermelon and tomato samples using DLLME combined with HPLC. The efficiency of the developed method was studied and the performance was estimated in terms of recovery and enrichment factor. The recovery was between 76.2 to 94.5 % with RSD less than 7.6 %. The linear range were between 10 and 1000 ng/g with correlation coefficient greater than 0.9984, while the LOD ranged between 0.5 and 1.5 ng/g. The analytical performance indicated the feasibility of the proposed method for the determination of carbamate pesticides in watermelon and tomato samples and offers short time extraction and high enrichment factor. (Liu, Z. et al., 2012a).

An ionic liquid based DLLME combined with HPLC method was employed for the analysis and determination of seven fungicides in fruit juices. The analytical performance determined under the optimal IL-DLLME conditions gave linearity in the range of 0.02 – 2 mg/L with correlation coefficient greater than 0.9902. The average recoveriy for all the studied analytes ranged from 66.2 to 92.9 % and the RSD varied between 2.2 and 11.6 %.

The LOD estimated based on the lowest extractable concentrations ranged from 3.1 to 10.2 µg/L (Wang, S. et al., 2012). The developed method was found to yield about 0.48 to 0.76 fold chromatographic peak area compared to a DLLME technique in which chlorobenzene and acetone were used as the extraction and the dispersive solvents respectively (Zang et al., 2008).

An ionic liquid-based vortex-assisted DLLME was employed for the analysis of OPPs (isocarbophos, phtalofos, troazophos, phoxim and profenofos) in apple and pear samples. The method validation evaluated using a matrix-matched calibration gave linearity in the range of 2 – 100 µg/kg with correlation coefficient between 0.9967 and 0.9983. The LOD ranged from 0.061 – 0.73 µg/kg, with relative recovery of the spiked samples ranging from 69.8 to 109 % with RSD = 2.1 – 7 %. The salting out effect was found to increase the solubility of the ionic liquid in the sample matrix which decreases the amount of analytes extracted and thus salt was not added. (Zhang, L. et al., 2012).

Table 2.3: DLLME for Analysis of Pesticide Residues in Fruits and Vegetable

Pesticides class	Matrix	Org. solv., vol (µL)	Disp. sol., vol(mL)	Ext. time (min)	Ext. temp. (^{0}C)	NaCl (%)	pH	Centrif (rpm)/ time (min)	LOD (µg/kg)	LOQ (µg/kg)	Rel. rec. (%)	RSD (%)	LR (µg/kg)	EF	Detector	Reference
3 DCFs	Apple	ArCl	AcO, 1	3	n.r	n.a	n.r	5000/5	3-8	n.r	93-107	4.6-6.4	10-100	824-912	GC-ECD	(Zang et al., 2008)
24 MCPs	Apple	CCl_4, 100	AcO, 0.4	1	n.r	n.a	n.r	5000/2	0.06-2.20	0.2-7.3	86-105	1-20	0.04-0.18	35-101	MD-GC-MS	(Cunha et al., 2009)
OP, CP	Grape, apple, peach juices	$C_2H_2Cl_4$, 15	AcO, 1	5 s	R.T	n.a	n.r	5000/2	12.3-16 pg/mL	n.r	86-105	1.11-9.67	0.1-1000 pg/mL	87.5-275.6	HPLC-FID	(Fu, L. et al., 2009)
8 MCPs	Banana	RTIL, 88 mg	MeOH, 0.714	1 s	R.T	25	2.7	4000/20	0.32-4.66	n.r	53-97	2.3-8.7	0.043-6.83 mg/L	n.r	HPLC-DAD	(Ravelo-Pérez et al., 2009a)
8 MCPs	Grape, plum	RTIL, 88 mg	MeOH, 0.714	1 s	R.T	25	2-7	4000/20	0.65-6.33	n.r	66-105	1.4-9.1	0.01-6.83 mg/L	n.r	HPLC-DAD	(Ravelo-Pérez et al., 2009b)
NI	Tomato	$C_2H_2Cl_4$, 30	USD	6	n.r	n.a	n.r	4000/5	45	n.r	87-100	<4.5	6 -100 µg/L	375	LC-UV	(Qiao et al., 2010)
2 PPs	Pear juice	$C_2H_2Cl_4$, 30	MeOH, 3.5	2.5	n.r	n.a	4.5	4000/5	2.2-3.1	n.r	92-107	<4	0.009-15.2	344	GC-FID	(Du et al., 2010)
6 CPs	Apple	$CHCl_3$, 60	AcO, 6	1	n.r	n.a	n.r	5000/5	2-3	n.r	85-113	4.3-7.4	6-500	491-1834	MEKC-DAD	(Zhang, S. et al., 2010)
4 OPPs	Pear juice	IL, 50	MeOH, 6	n.r	R.T	n.a	6.3	4000/5	0.01-0.05 µg/L	n.r	78-86	1.1-1.7	5-1000 µg/L	300	HPLC-UV	(He, L. et al., 2010)
CP, PF	Apple	$C_{11}H_{23}OH$ 10	AcO, 0.4	3	n.r	28	n.r	4000/2	1.4-1.6	n.r	83-101	4.8-8.3	8-800	n.r	LC-DAD	(Zhou, Y. et al., 2011)

Table 2.3: DLLME for Analysis of Pesticide Residues in Fruits and Vegetable (continued)

Pesticides class	Matrix	Org. solv., vol (µL)	Disp. sol., vol(mL)	Ext. time (min)	Ext. temp. (^{0}C)	NaCl (%)	pH	Centrif (rpm)/ time(min)	LOD (µg/kg)	LOQ (µg/kg)	Rel. rec. (%)	RSD (%)	LR (µg/kg)	EF	Detector	Reference
4 NI	Cucumber, tomato	CCl_4, 200	ACN, 2.5	0.5	n.r	8	n.r	n.r	0.5-1.0	n.r	84-97	3.7-6.2	5-300	110-243	HPLC-DAD	(Wu, Q. et al., 2011)
12 CPs	Banana, pineapple, tomato	$CHCl_3$, 800	MeOH, 1.5	5	n.r	n.a	7.5	5000/2	1-7 µg/L	6-24 µg/L	78-105	3.6-8.9	4-1000	n.r	MEKC-DAD	(Moreno-González et al., 2011)
14 MCPs	Apple	$C_{12}H_{25}OH$, 8	AcO, 0.4	2	60	n.a	n.a	4000/2	2.8-18.5 ng/L	9.3-53.8 ng/L	79-102	3.2-7.6	10-2000 ng/L	409-1089	GC-ECD	(Matsadiq et al., 2011)
13 OPPs	Tomato	ArCl, 60	AcO, 0.4	n.r	n.r	n.a	n.r	5000/4	0.1-0.5	n.r	n.r	7-10	0.5-1000	n.r	GC-FPD	(Bidari et al., 2011)
6 DCFs	Pear, grape, apple, strawberry	$C_2H_2Cl_4$, 14	ACN, 0.8	0.5	n.r	1	n.r	3200/5	0.02-0.12	n.r	81-98	3.1-7.8	0.5-40	685-820	GC-ECD	(Huo et al., 2011)
6 PPs	7 fruit juices	$CHCl_3$, 300	MeOH, 1.25	0.5	n.r	n.r	n.r	4000/5	2-5 µg/L	5-10 µg/L	84-94	1.3-2.9	2-1500 µg/L	62-84	HPLC-UV	(Boonchiangma et al., 2012)
13 MCPs	Tomato	$CHCl_3$, 400	ACN, 1	n.r	n.r	10	1	5000/4	1.7-45	5.8-150	86-116	5.8-17.4	10-1500	n.r	HPLC-DAD	(Melo et al., 2012c)
5 TFs	Grape juice	C_6H_{14}/ $C_6H_{12}O$, 45	ACN, 0.75	0.5	n.r	10	n.a	n.r	0.3-5 µg/L	0.9-16.7 µg/L	74-99	3.2-5	2-5000 µg/L	263-380	GC-MS	(Farajzadeh et al., 2012)
30 MCPs	Tomato	CCl_4, 100	ACN, 1	1	n.r	10	n.a	5000/5	2.7-250	8.9-840	70-110	1-25	10-6000	n.r	GC-MS	(Melo et al., 2012b)

Table 2.3: DLLME for Analysis of Pesticide Residues in Fruits and Vegetable (continued)

Pesticides class	Matrix	Org. solv., vol (µL)	Disp. sol., vol(mL)	Ext. time (min)	Ext. temp. (^{0}C)	NaCl (%)	pH	Centrif (rpm)/ time(min)	LOD (µg/kg)	LOQ (µg/kg)	Rel. rec. (%)	RSD (%)	LR (µg/kg)	EF	Detector	Reference
19 MCPs	Orange	CCl_4, 50	ACN, 2	1	n.r	n.r	n.r	3250/5	0.01-14	0.02-47	63-120	<20	0.1-3000	n.r	GC-MS	(Andraščíková et al., 2013)
3 BIPs	Tomato	CH_2Cl_2, 60	ACN, 1	5 s	n.r	n.r	n.r	3500/5	0.18-0.55	n.r	72-89.5	4.2-5.3	5-200	147-161	HPLC-UV	(Han et al., 2013)
5 CPs	Watermelon, tomato	$CHCl_3$, 40	ACN, 1	n.r	n.r	5	n.r	4000/5	0.5-15	n.r	76.2-94.5	<9.6	10-1000	80-177	HPLC-DAD	(Liu, Z. et al., 2012a)
7 SFs	Apple, grape	HMIM PF_6, 60	MeOH. 0.5	n.r	n.r	n.r	n.r	4000/5	3.1-10.3 µg/L	n.r	66.2-92.9	2.2-11.6	2-200 µg/L	n.r	HPLC-DAD	(Wang, S. et al., 2012)
6 OPPs	Apple pear	C_8MIM PF_6, 50	MeOH, 1	1	n.r	n.a	6-7	4000/5	0.061-0.73	n.r	69.8-109.1	<7	2-100	307.7	HPLC-UV	(Zhang, L. et al., 2012)

N.B: Disp. sol, dispersive solution; centrif, centrifugation rate; DCFs, dithiocarboximide fungicides; NI, neonicotinoid insecticides; PPs, pyrethroid pesticides; PF, pyrimidine fungicide; BIPs, benzimidazole pesticides USD, ultrasound dispersion; RTIL, room temperature ionic liquid; IL, ionic liquid; MEKC-DAD, micellar electro-kinetic chromatography-diode array detector; others are as in Table 2.1

2.6 Stir Bar Sorptive Extraction (SBSE)

SBSE is a microextraction technique similar to SPME but with a greater extraction capacity. It helps to overcome the small volume of the coated SPME fibers for a better enrichment factor and it delivers better sorptive-phase mass and higher surface area as a result of larger volume of the PDMS (Baltussen, Cramers, & Sandra, 2002; Beceiro-González et al., 2012; Ridgway et al., 2007). In the SBSE technique, a 10 to 40 mm long magnetic stir bar coated with thick layer (about 50–300 µL) of polydimethylsiloxane (PDMS) liquid phase as the extracting phase (Baltussen et al., 1999; Kataoka, 2010; Tankiewicz et al., 2011).

The mechanisms of SBE are similar to those of SPME but differ in the design of extraction system, with SBSE having higher enrichment factor, which is determined by the amount of extractive phase. The extracted analyte are adsorbed on the PDMS coated rod, by stirring the sample solution with the rod for a given time. The rod is removed from the sample and the adsorbed analyte can be desorbed thermally into GC system, which provides high chromatographic resolution and better sensitivity or by means of liquid solvent into LC system for improved and better selectivity (Baltussen et al., 2002; Hyötyläinen & Riekkola, 2008; Kawaguchi et al., 2006; Prieto et al., 2010).

The major limitation of SBSE technique is the polarity of PDMS (non-polar liquid), which implies that it is best used for low polar analytes as the recovery will be low for highly polar analytes (Kawaguchi et al., 2006; Sánchez-Rojas, Bosch-Ojeda, & Cano-Pavón, 2009), which is as a result of weak hydrophobic interactions, longer desorption time, due

to the large volume of the PDMS, and it also requires reconstitution of the extracted analytes on the stir bar, since it cannot be injected directly into the split/splitless injector port of the GC (Hyötyläinen & Riekkola, 2008; Nogueira, 2012; Prieto et al., 2010). The tedious reconstitution step can lead to loss of analytes and introduction of contaminants, but this has been eliminated by the use of thermal desorption unit (TDU) online to GC system (Blasco, Font, & Picó, 2002).

The polarity of PDMS has been addressed by employing in-situ derivatization (Chen, Y. et al., 2008) or the use of other phases such as restricted access materials (RAMs), carbon adsorbent, ionic liquid, dual phase materials, porous monolith, molecularly imprinted polymers (MIPs), microporous membrane and sol-gel prepared coatings (Bicchi et al., 2005; Bicchi et al., 2007; Chen, Y. et al., 2008; Fontanals, Marcé, & Borrull, 2007; Hu, C. et al., 2013; Hu, Y. et al., 2010; Lambert et al., 2005; Liu, W. et al., 2005; Liu, W., Wang, & Guan, 2004; Martín-Esteban, 2013; Montes et al., 2009; Sánchez-Rojas et al., 2009; Turiel & Martín-Esteban, 2010; Wan Ibrahim et al., 2011; Zhu et al., 2006), has helped to extend the technique for the analysis of a wide range of analytes from complex matrices.

The SBSE techniques can also be carried out in both direct immersion (DI) and headspace (HS) mode (Prieto et al., 2010; Turner, 2006), depending on the complexity of the sample matrix (Fig. 3.4). Like SPME sorptive extraction depends on the partition coefficients of the analyte between the coated stir bar and the sample matrix. The partition coefficients have been correlated with the octanol/water distribution coefficient of the target analyte, which is an indication of the efficiency of SBSE to extract a given analyte (David &

Sandra, 2007; Kawaguchi et al., 2006; Prieto et al., 2010; Urbanowicz, Zabiegała, & Namieśnik, 2011).

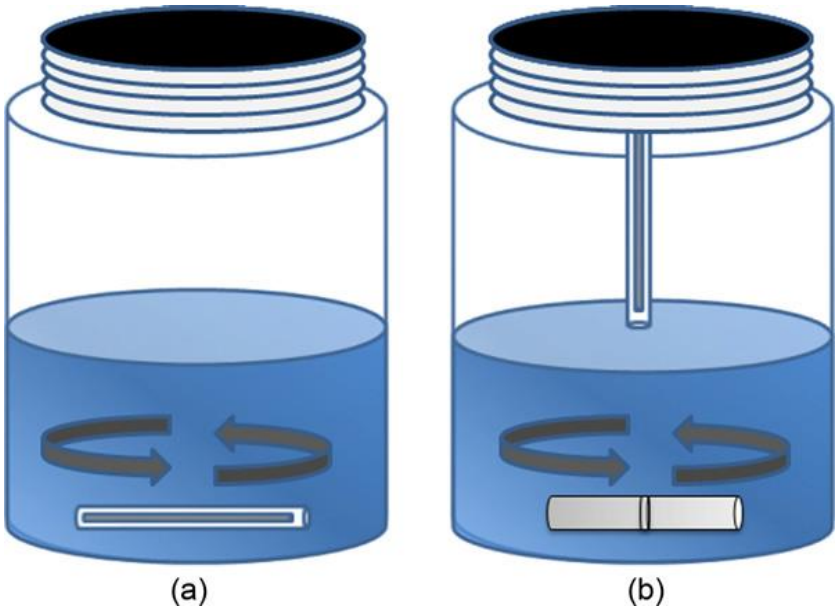


Fig 2.4: Schematic Diagram of the Extraction Modes in SBSE (a) DI and (b) HS (Prieto et al., 2010)

2.6.1 Factors affecting SBSE

In the development of SBSE method, the same factors as in SPME needed to be optimized for efficient extraction, high recovery and enrichment factor and low detection limits. These factors include the type, volume and size of the stir bar coating, extraction time, pH, salt addition, stirring rate, extraction temperature, sample volume and addition of organic solvents (Hyötyläinen & Riekkola, 2008; Prieto et al., 2010; Tankiewicz et al., 2011).

The volume of stir bar coatings affects the efficiency of SBSE technique. The higher the volume of stir bar coatings the higher the sensitivity for more polar compounds with low octanol/water partition coefficients, but the volume was found to have no significance difference for low polar or non-polar analytes with high octanol/water partition coefficients values (León et al., 2003; Prieto et al., 2010; Prieto et al., 2008). (Sánchez-Rojas et al., 2009).

SBSE is an equilibrium extraction technique, therefore, the extraction time must be fully controlled and optimized. The extraction time, studied to obtain an equilibrium extraction, can be determined at difference stages of method development, but its optimization is better conducted after other variables have been fixed and optimized (Prieto et al., 2010), since equilibrium extraction yield better sensitivity and precision. In order to minimize extraction and analysis time, SBSE can be conducted under non-equilibrium conditions, but this could lead to low precision and sensitivity (David & Sandra, 2007; Guan et al., 2008; León et al., 2003; Popp et al., 2005; Prieto et al., 2008).

The pH and ionic strength of sample matrix is important in method development. The pH should be adjusted in analytes with acidic or basic properties, in order to obtain the analytes in a non-ionic form. The pH adjustment should be between 3 and 9, to avoid degradation of the coatings on the stir bar (Portugal, Pinto, & Nogueira, 2008; Portugal et al., 2010; Prieto et al., 2010). The addition of inert salts such as NaCl modify the ionic strength of the sample matrix, and improve extraction by decreasing the solubility of polar analytes (Blasco et al., 2002; Giordano et al., 2009; Ochiai et al., 2006), while it was

observed to reduce extraction efficiency of non-polar analytes (Guan et al., 2008), as a result of increase in viscosity of the sample matrix (Quintana et al., 2007), occupation of the coated stir bar surface by the salt (Portugal et al., 2008), and ion-pairing interactions between the non-polar analytes and the inert salts (Prieto et al., 2010).

The agitation of sample matrix increases the diffusion of analytes, by decreasing the thickness of boundary layer between the analytes in the sample matrix and the coated stir bar. The agitation rate must be carefully optimized, because higher stirring rate (> 750rpm) may lead to formation of air bubbles formation and cause the stripping of the coatings on the stir bar, due to its contact and friction against the bottom of the sample vial (Liu, W. et al., 2005). Higher stirring rate (≥1000 rpm), can be achieved when using coatings that are stronger than PDMS (Portugal et al., 2008; Portugal et al., 2010), without stripping of the coating on the stir bar.

Extraction at elevated temperature increases the partitioning of analytes between the coated stir bar and the sample matrix, which increase extraction efficiency, thereby decreasing the equilibrium time (Liu, W. et al., 2005). Although temperature has dual effects, increase in temperature also decreases the sorption distribution coefficients and the life time of the coated stir bar (Liu, W. et al., 2005; Prieto et al., 2010). Therefore, compromise must be made between extraction efficiency and life time. Thus, an adequate and optimal extraction temperature must be selected based on the nature of the target analytes and the nature of sample matrix.

Finally, the extraction efficiency depends on sample volume which is related to the phase ratio. Higher sample volume increases chromatographic response for non-polar analytes, while it has no significance effect on the extraction of polar analytes (Prieto et al., 2010). The presence of organic solvent such as methanol and acetonitrile in the sample matrix helps to minimize loss of analytes due to the adsorption on the walls of sample vials (Ochiai et al., 2005). Organic solvent addition can also decrease extraction efficiency by increasing the solubility of more polar analytes. The volume of organic solvent must be minimized (about 5 %), at higher percentage, and depending on the nature of sample matrix, it reduces the affinity of the coated stir bar for the target analytes (Serôdio & Nogueira, 2005).

2.6.2 Theory of SBSE

In SBSE, the extraction equilibrium depends on the phase ratio (β), and the volume of the coating on the stir bar (PDMS). This can be represented by the distribution coefficient of the analyte between PDMS and the sample matrix, using Eq. (2.27) which can be used in combination with the mass-balance equation Eq.(2.28), (Prieto et al., 2010) to calculate the extraction recovery (R%), under full equilibrium conditions (David & Sandra, 2007; Kawaguchi et al., 2006), provided all factors have been fully optimized.

$$\mathrm{K}_{PDMS/w} = \frac{C_{PDMS}}{C_s} = \frac{m_{PDMS}V_s}{m_w V_{PDMS}} = \frac{m_{PDMS}\beta}{m_w} \qquad (2.27)$$

$$m_{w,o} = m_{PDMS} + m_w \qquad (2.28)$$

combining Eq. (2.27) with Eq. (2.28), yields Eq. (2.29)

$$R = \frac{m_{PDMS}}{m_{w,o}} = \frac{K_{PDMS/w}}{K_{PDMS/w}+\beta} \qquad (2.29)$$

where C_{PDMS} is the concentration of analyte in the PDMS coated stir bar, C_S is the concentration of analyte in the sample matrix, $m_{w,o}$, is the initial mass of the target analyte in the sample matrix, m_{PDMS} and m_w are the masses of the target analyte in the coated stir bar and in the sample matrix respectively, while β is the phase ratio, which is defined as the ratio of volume of the stir bar coating and the volume of sample solution. It can be observed from Eq. (2.29), that increase in the volume of stir bar coatings increases the phase ratio and improves the extraction efficiency.

The variation of the concentration ($C_{PDMD}(t)$), of the target analytes in the stir bar coatings as a function extraction time t, gives the kinetic of extraction and can be expressed as (Prieto et al., 2010):

$$C_{PDMS}(t) = C_{w,o} \times \frac{k_1}{k_2}(1 - e^{-k_2 t}) \quad (2.30)$$

where $C_{w,o}$ is the initial concentration of analyte is the sample matrix and k_1 and k_2 are the uptake and elimination rate constant respectively.

2.6.3 Applications of SBSE in Pesticide Residues Analysis in Fruits and Vegetables

The use of SBSE technique has been employed by many researchers in the extraction and subsequent chromatographic analysis of different classes of pesticide residues in fruit and vegetable samples (Table 2.4), although the method was originally developed for the extraction of contaminants in water samples.

The SBSE was first used for analysis of 10 pesticide residues in orange by Blasco and his coworkers. The method was compared with matrix solid-phase dispersion (MSPD), in terms of recoveries, relative standard deviation and limits of detection. The extraction of polar analytes was poor and thus was not used in method validation. The quantification was performed using a standard addition calibration in a matrix matched sample solution. The linearity was between 0.001 – 6 mg/kg (R^2 >0.995) and 0.008 – 10 mg/kg (R^2 >0.998) for SBSE and MSDP respectively and the LOQs respectively ranged from 0.001 – 0.05 mg/kg and from 0.008 – 0.12 mg/kg for SBSE and MSPD. The recovery was very low in SBSE and ranged from 8 – 84 % with RSD between 4 and 16 % with MSPD having better recovery and ranged from 47 – 96 % (RSD = 1 – 15 %). The MSPD was found to have better extraction efficiency for the target analyte, although SBSE involve the use of small solvent and have high sensitivity, it has low enrichment factor for polar compounds (Blasco et al., 2002).

An SBSE method coupled to thermal desorption retention time locked (RTL) capillary GC-MS has been employed for the screening of 17 multiclass pesticides (MCPs) in lettuce, pear, grape and baby food samples. The performance of the developed method was estimated at the optimal extraction conditions and gave linearity ranging from 5 to 200 µg/L with correlation coefficient greater than 0.992, while recoveries ranged from 43–75 % with RSD between 4.6 and 8.8 % in lettuce. The low recoveries were attributed to the degradation of the target analytes during sample enrichment or in the TDU injection system (Sandra, Tienpont, & David, 2003).

The SBSE approach has been used for the extraction of 5 fungicides (bitertanol, flutiafol, tridimefon, tebuconazole, carboxin and pyrimethanil) residues at low µg/kg levels in grape samples. The developed SBSE method was also compared with solid-phase extraction (SPE) technique. The mean recovery ranged from 15 to 100 % with RSD from 10 to 19 % in SBSE while it ranged from 60 to 100 % and 7 to 17 % respectively for SPE. The LOQ was 10 µg/kg for the investigated analytes. The linearity which was estimated at the LOQ and 100 times the LOQ gave correlation coefficient that is greater than 0.995 for SBSE and greater than 0.994 for SPE. The better analytical performance showed by SPE compared to SBSE was showed to be as result of the equilibrium nature of the adsorption of the coated stir bar , while the SPE is a non-equilibrium adsorption (Juan-García et al., 2004).

The optimization of a multi-residue screening of 85 pesticides of various classes (OPPs, CPs, OCPs and PPs) in vegetables (tomato, cucumber, green soybean and spinach), fruit (grape) and green tea was developed based on SBSE coupled to TD-RTL-GC-MS operating in scanned mode. The developed method was validated using a standard addition and matrix match calibration methods in order to compensate for the effect of sample matrix. The method linearity ranged from 4 – 100 µg/kg, with correlation coefficient greater than 0.99 for most of the investigated pesticides. The LODs were between 0.63 and 26 µg/kg (Ochiai et al., 2005).

The extraction and quantitative determination of 12 OPPs in cucumber and potato samples using a sol-gel prepared hydroxyl terminated PDMS coating, coupled to GC-TDS (thermal desorption system) was reported. The performance of the developed method was estimated

by analyzing standard solution to give a linear range of 0.25 to 50 ng/g with correlation coefficient greater than 0.99. The LODs ranged from 0.007 to 0.15 ng/g and 0.0056 to 0.098 ng/g in cucumber and potato respectively (Liu, W. et al., 2005).

A study which compared SBSE and SPE for the determination of 8 pesticide residues in strawberry, lettuce and tomato samples using MEKC coupled to diode array detector (DAD) was also reported. The method linearity was between 1 and 100 mg/kg with correlation coefficient higher than 0.996. The recoveries were between 12 and 47 % with RSD between 3 and 17 %, while the LOQ was 1 mg/kg for all the studied pesticides. The SBSE was found to better than SPE in terms of linearity, repeatability and cleaner chromatogram, but gave lower recoveries and high LOQ which was found to be too high to meet the MRLs of the investigated pesticides in the sample analyzed. In this study, SPE was found to provide higher extraction efficiency, robustness, better sensitivity and rapid extraction than the SBSE (Juan-García, Picó, & Font, 2005).

An organochlorine pesticide residues and chlorobenzene was determined in 5 fruit and vegetable samples using accelerated solvent extraction (ASE) combined with SBSE and SPME coupled to GC/MS. The evaluation of method performance of the developed ASE-SBSE method gave LOD ranging from 0.5 to 30 µg/kg and RSD between 5 and 25 % (Wennrich, Popp, & Breuste, 2001).

The SBSE coupled to RTL-GC/MS and automated mass spectral deconvolution was employed for the identification of pesticide residues in 28 fruit and vegetable samples. The chromatograms obtained were evaluated in MSD Chemstation by the calculated command using a homebuilt quantitation database, which contained 150 pesticides. The method identified 52 pesticides in 16 samples and the contaminated samples were further examined by comparing the quality of evaluation done by the quantitation database (QD), RTL-RS (result screener) and mass spectral deconvolution software (AMDIS). The AMDIS software was found to support most of the decision making process and perform best in the identification and discovering of positive hits and proved to be more reliable and can efficiently reduce time taken for data analysis (Kende et al., 2006).

A novel poly(phthalazine ether sulfone ketone) (PPESK) stir bar coatings prepared by immersion precipitation technique was reported for the extraction of 4 OCPs in seawater and 8 OPPs in grape and peach samples. The new coatings showed better extraction efficiency, higher thermal stability (290 ^{0}C), long life time and ability to extract analytes of varying degree of polarities. The method validation estimated using a 6-point calibration curve gave linearity between 20 to 1000 ng/L with R^2 greater than 0.99. The LOD were in the range of 0.17 to 2.25 ng/L and 2.47 to 10.3 ng/L in grape and peach juices respectively. The PPESK coated stir bar was found to have a porous homogenous surface structure and a sponge-like sub-layer which gave it a better adsorption mechanism and exhibited better selectivity and higher extraction capability (Guan et al., 2008).

A method was developed for the extraction of oxazole fungicide residues in wines and juices (grape, peach, strawberry and multifruit), based on the comparison of SBSE and membrane-assisted solvent extraction (MASE), coupled to ultra-performance liquid chromatography (UPLC). The method performance was estimated using the least-square linear regression analysis of the chromatogram peak area versus analyte concentration at 6 concentration levels using external standard calibration method. The validation parameters include linearity (0.5 – 250 ng/mL), correlation coefficient (R^2= 0.999), LOD (0.05 – 2.5 ng/mL), LOQ (0.15 – 8 ng/mL), recovery (95 – 113 %) and RSD (5.3 – 7.9 %). The SBSE method showed a better extraction efficiency in terms of sensitivity, repeatability and analyte recovery than the MASE (Viñas et al., 2008).

A stir bar microextraction procedure was developed for the extraction of seven strobilurin fungicides in fruit (apple, pear, grape, orange, lemon, peach and plum) samples using LC-DAD. The matrix effect was estimated by comparing the slope of calibration curve of aqueous solution spiked with 70 ng/g of internal standard and that of the standard addition method and showed no significance difference. Thus, the use of internal standard helps to compensate for the matrix effect. The calibration parameters obtained include linearity which ranges from 0.01 to 5 µg/mL with correlation coefficient greater than 0.996. The LOD and LOQ ranged from 0.3 to 2.0 ng/g and 0.9 to 6.7 ng/g respectively, while the mean recovery ranged from 88 to 101 % with RSD between 2 and 9 %. The analytical performance obtained for the developed method make it a useful, reliable and sensitive method for routine analysis of strobilurin in fruit samples and the use of internal standard

for quantification helps to compensate for matrix effect and avoids the use of tedious standard addition method (Campillo et al., 2010).

The performance of coupling the SBSE technique with TDU-GC/MS analysis with the statistical variance component model (VCM) was evaluated for the determination of 13 multiclass pesticide residues in fruit-based soft drink. The VCM statistical procedure was used to account for the contribution of matrix- and time-induced deviations to the uncertainty. The limit yielding of recovery of each analyte was evaluated, and was found to range from 38 to 113 % and 17.5 to 103 % in real sample matrix and sample diluted with methanol (1:10) respectively with detection limit ranging from 7 to 68 ng/L. The Cochran test was used to check the heteroscedasticity of the experimental measurement at fixed matrix and various calibration concentrations and compared with data set measured at fixed concentration and varied amount of sample matrix. The Cochran test showed non-constancy of the measurement variances with the concentration level and the heteroscedasticity resulting in the use of different matrices was evident. It was concluded that the dispersion of the experimental data could be as a result of instrumental uncertainties, handling of solutions and change of the sample matrix, and that matrix effect could be corrected using labeled or unlabeled internal standard. Thus the VCM allowed the calculation of an inter-matrix detection limit and of an inter-matrix confidence interval of a discriminated concentration value (Lavagnini, Urbani, & Magno, 2011).

Table 2.4: SBSE for Analysis of Pesticide Residues in Fruits and Vegetable

Pesticides class	Matrix	Org. solv., vol (mL)	SB Phase	Ext. time (min)	Ext. temp. (^{0}C)	NaCl (%)	pH	St. rate (rpm)	LOD (µg/kg)	LOQ (µg/kg)	Rel. rec. (%)	RSD (%)	LR (µg/kg)	Detector	Reference
10 MCPs	Orange	MeOH, 5	PDMS	120	n.r	5	n.r	900	n.r	1-50	8-84	4-16	1-6000	LC-MS	(Blasco et al., 2002)
17 MCPs	Lettuce, pear grape	MeOH, 30	PDMS	60	n.r	n.r	n.r	1000	n.r	n.r	43-75	4.6-8.8	5-200 µg/L	TD-GC-MS	(Sandra et al., 2003)
5 MCPs	Grape	H_2O, 25	PDMS	120	n.r	30	n.r	900	n.r	10	15-100	10-19	10-1000	LC-MS	(Juan-García et al., 2004)
85 MCPs	5 fruits and vegetables	MeOH, 100	PDMS	60	R.T	n.r	n.r	1000	0.63-26	n.r	n.r	n.r	4-100	TD-GC-MS	(Ochiai et al., 2005)
12 OPPs	Cucumber, potato	AcO, 15	PDMS-OH	30	30	30	n.r	600	0.007-0.15	n.r	n.r	n.r	0.25-50	TD-GC-TSD	(Liu, W. et al., 2005)
8 MCPs	Strawberry, lettuce, tomato	AcO/H_2O, 10	PDMS	240	n.r	40	n.a	900	1 mg/kg	n.r	12-47	3-17	1-100 mg/kg	MEKC-DAD	(Juan-García et al., 2005)
11 MCPs	5 fruits and vegetables	AcO/H_2O, 10	PDMS	60	n.r	n.r	n.r	850	0.5-30	n.r	n.r	5-25	n.r	TD-GC-MS	(Wennrich et al., 2001)
52 MCPs	28 fruits and vegetables	MeOH/ H_2O, 10	PDMS	60	n.r	n.r	n.r	1000	n.r	n.r	n.r	n.r	n.r	n.r	(Kende et al., 2006)

Table 2.4: SBSE for Analysis of Pesticide Residues in Fruits and Vegetable (continued)

Pesticides class	Matrix	Org. solv., vol (mL)	SB Phase	Ext. time (min)	Ext. temp. (^{0}C)	NaCl (%)	pH	St. rate (rpm)	LOD (µg/kg)	LOQ (µg/kg)	Rel. rec. (%)	RSD (%)	LR (µg/kg)	Detector	Reference
8 OPPs	Grape, peach	H_2O, n.r	PPESK	30	40	n.a	n.r	600	0.17-10.3 ng/L	n.r		1.6 -20	20-1000 ng/L	TD-GC-MS	(Guan et al., 2008)
6 OF	Grape, peach, strawberry, multifruit juices	AcO/ AcOH, 5	PDMS	30	60	40	5	1700	0.05-2.5 ng/mL	0.15-8 ng/mL	95-113	5.3-7.9	0.5-250 ng/mL	UPLC	(Viñas et al., 2008)
7 SFs	7 fruits	EtOH, 2	PDMS	20	45	5	n.r	2000	0.3-2.0	0.9-6.7	88-101	2-9	0.01-5 µg/mL	HPLC-DAD	(Campillo et al., 2010)
11 MCPs	Fruit-base soft drinks	MeOH, 5	PDMS	360	30	n.r	n.r	1400	7-68 ng/L	n.r	38-113	n.r	n.r	TD-GC-MS	(Lavagnini et al., 2011)

N.B: TD, thermal desorption; OFs oxazole fungicides; EtOH, ethanol; PDMS, polydimethylsiloxane; PPESK, poly(phthalazine ether sulfone ketone); PDMS-OH, hydroxyl terminated-PDMS; UPLC, ultra-performance liquid chromatography; others are as used in Table 2.

2.7 Interface to Analytical Instrumentation

The automation of the microextraction techniques described in this review has gone a long way in increasing the efficiency and accuracy of the extraction procedures and subsequent instrumental analysis, by preventing loss of sample and introduction of other contaminants. All the techniques except SBSE have been conveniently interfaced to chromatographic analytical instruments. At present SPME offers the best technique because of its solvent-less nature, since other microextraction techniques make use of water-immiscible solvents, and the GC technique is the most preferred analytical instrument and has been used in most of the published work in microextraction analysis. The techniques have also been successfully interfaced with HPLC and CE, but only few papers have been reported (Pedersen-Bjergaard et al., 2000; Xu et al., 2007). The GC analysis provides higher sensitivity, selectivity and better detection limits than LC in pesticide analysis, while the CE provides a faster alternative to the chromatographic techniques but with higher detection limit (Lambropoulou & Albanis, 2007a).

2.8 Limitations of Microextraction Techniques

The use of microextraction techniques is emerging as a very reliable sample preparation method, while employing little or no solvent. The advantages over the traditional method include their simplicity of operation, rapid sampling, low cost, high recovery and EF, and being environmentally friendly. The major limitations of the technique include low volume and instability of the microdrop solvent, possible loss of organic solvent in SDME, the presence of porous membrane, use of a large amount of solvent for analyte elution in HF-LPME. The difficulty of the automation, use of large volume of dispersive solvent, which

usually decreases the partitioning between the analyte and the organic solvent, and solvent dissolution are the major drawbacks in DLLME, while in SBSE, the major limitations include the need to rinse, dry and reconstitute the analyte, and the polarity of the commercially available PDMS coating.

The use of more selective, efficient, and versatile extraction procedure and increasing interest in overcoming the aforementioned limitations and trend towards automation will provide better integration of sampling and instrumental analysis, which can be used for a wide range of analytes. The limitations still remain when the techniques are used in the analysis of pesticides in food matrices, thus dilution of sample with water or other solvents, which help to reduce matric effects by increasing diffusion of analytes into the extraction solvent, should be carefully optimized. Although most of the microextraction applications employed GC, organic solvents are used for LC. Recent development has been geared toward the use of HPLC for thermally labile and nonvolatile pesticides that are not amenable to GC analysis.

Future trend is aimed at the use of less-toxic solvents such as ionic liquids, and supramolecular solvents as the microdrop solvents in LPME and the use of other materials such as restricted access materials (RAMs), carbon adsorbent, molecularly imprinted polymers (MIPs), ionic liquids (ILs), microporous monolith, sol-gel prepared coatings and dual phase material in SBSE. The application of microextraction techniques can be applied to a wide range of pesticide residues ranging from polar, nonpolar, volatile, to semivolatile, provided all factors are carefully optimized.

The microextraction techniques discussed in this chapter have been compared by various authors and were found to have similar analytical performance in food analysis. All microextraction techniques have shown to be very effective and efficient with good analytical figures of merit, in the analysis of pesticide residues and other contaminants from environmental samples (Hu, Y. et al., 2009; Zhou, Y. et al., 2011), but their direct comparison is a difficult task (Nerín et al., 2009; Tankiewicz et al., 2011).

CHAPTER THREE

3.0 REVIEW OF SOLID PHASE MICROEXTRACTION TECHNIQUE FOR THE ANALYSIS OF PESTICIDE RESIDUES IN FRUITS AND VEGETABLES

3.1 Solid Phase Microextraction (SPME)

Solid phase microextraction is a solvent-free sample preparation technique which was developed by Pawliszyn and his co-workers in 1990 (Arthur & Pawliszyn, 1990). The technique was developed to eliminate the use of toxic solvent and to address the need to facilitate a rapid effective, efficient and field compatible sample preparation method (Lord & Pawliszyn, 2000; Pawliszyn et al., 1997; Risticevic et al., 2009). It helps to save preparation time, reduces overall cost of analysis and offers a benefit of short sample preparation steps, small sample volume and enrichment of analytes from solid, liquid or gaseous samples. Its application for the analysis of pesticide residues in fruit and vegetable samples has been examined (Bagheri et al., 2012; Chai & Tan, 2010; Filho, dos Santos, & Pereira, 2010), optimized, automated (Arthur et al., 1992a; Melo et al., 2012a) and reviewed (Aulakh et al., 2005; Beltran et al., 2000; Kataoka et al., 2000; Lambropoulou & Albanis, 2007b).

It is a simple and effective sorption (adsorption/absorption) and desorption technique, which can easily be automated. It combines sampling, isolation, concentration and enrichment and sample introduction into analytical instruments in a single and uninterrupted sampling step, which results in high throughput analysis (Ouyang & Pawliszyn, 2008; Pawliszyn, 1997; Picó et al., 2007; Risticevic et al., 2009). SPME was

developed to overcome the problems associated with the solvent-based, time consuming conventional techniques, which are multistep and usually requires a large amount of samples and solvents that can cause environmental pollution and be hazardous to human health.

SPME is a very attractive alternative technique in sample preparation that results in high throughput analysis, and remarkable analytical characteristics, including linearity, reproducibility, repeatability, low and improved limits of detection and quantitation, high selectivity, sensitivity and versatility with minimum matrix interferences (De Fátima Alpendurada, 2000; Fytianos et al., 2007; Pawliszyn, 1997; Risticevic et al., 2009). It is widely used for the analysis of volatile or semi-volatile organic compounds (Pawliszyn, 1999), when coupled to gas chromatography with variety of detection methods, and for analysis of thermally labile, polar and nonvolatile compounds (Lambropoulou & Albanis, 2007b), when coupled to liquid chromatography or capillary electrophoresis.

The SPME process involves two basic steps: the partitioning of analytes between the coating and the sample matrix, and the desorption of the extracted analytes into the analytical instruments, thermally into GC or with organic mobile phase into liquid chromatography (LC) (Pawliszyn et al., 1997; Risticevic et al., 2009), without any need for clean-up (Arthur & Pawliszyn, 1990). The extraction and sorption of analytes from the matrix begins with the exposure of the coated fiber to the vapour phase above the sample matrix or by inserting the fiber into the sample matrix. The analytes are transferred to the fiber based on the mass transfer process and follows the second law of thermodynamics

(Nerín et al., 2009), when exposed for a period of time and extraction is considered to be completed when equilibrium is attained.

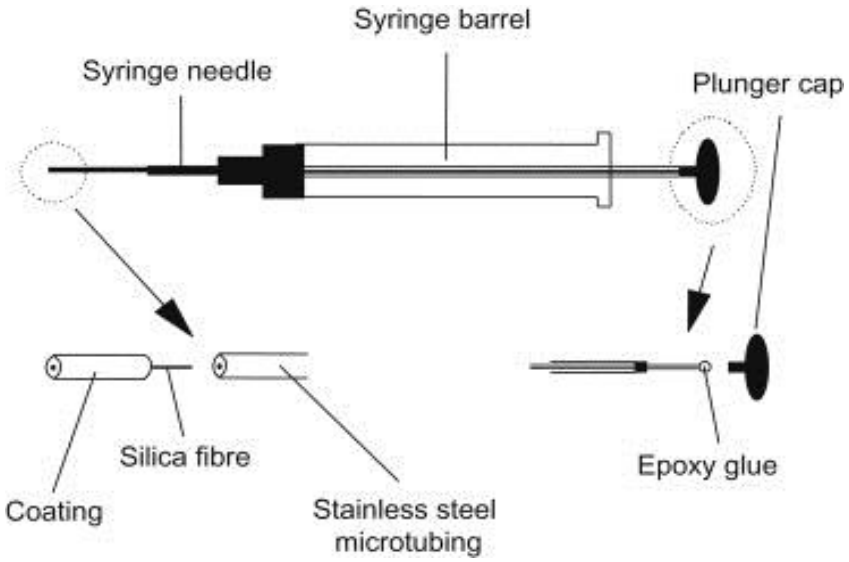


Fig 3.1: Custom-made SPME on Hamilton 7000 Series Syringe (Lord & Pawliszyn, 2000)

The SPME fiber assembly (Fig 3.1), is made of chemically inert fused-silica optical fiber, stable flex, or metal alloys (Shirey, 2012), coated on the outside with a thin-film of sorbent (Arthur et al., 1992a) as the extraction phase containing a polymeric organic compounds or a mixture of polymers (Kataoka et al., 2000; Pawliszyn et al., 1997), that are permanently attached to a stainless steel rod. The SPME fiber shown in Fig. 3.2, which is mounted on a SPME fiber holder shown in Fig 3.3 consists of a spring loaded plunger, a stainless steel barrel and an adjustable depth gauge with a hollow septum-piercing needle house in a modified syringe (Simplício & Vilas Boas, 1999).

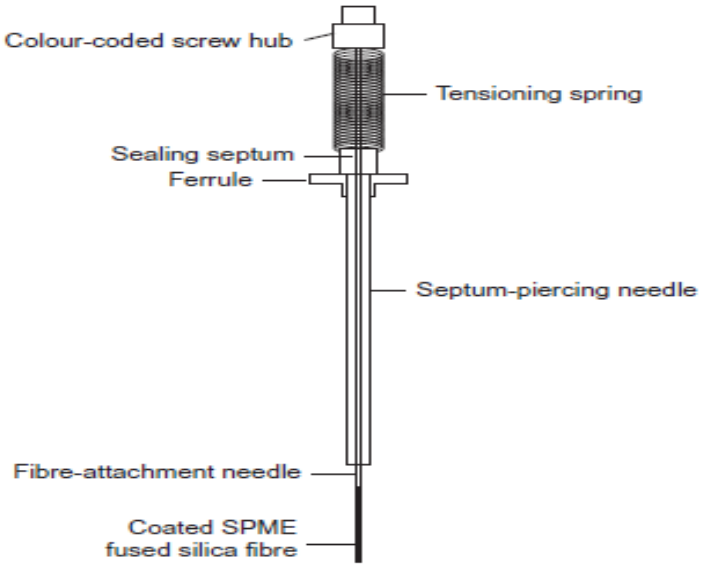


Fig 3.2: View of the SPME Manual Fiber Assembly (Lord & Pawliszyn, 2000; Pawliszyn, 1997; Shirey, 2012)

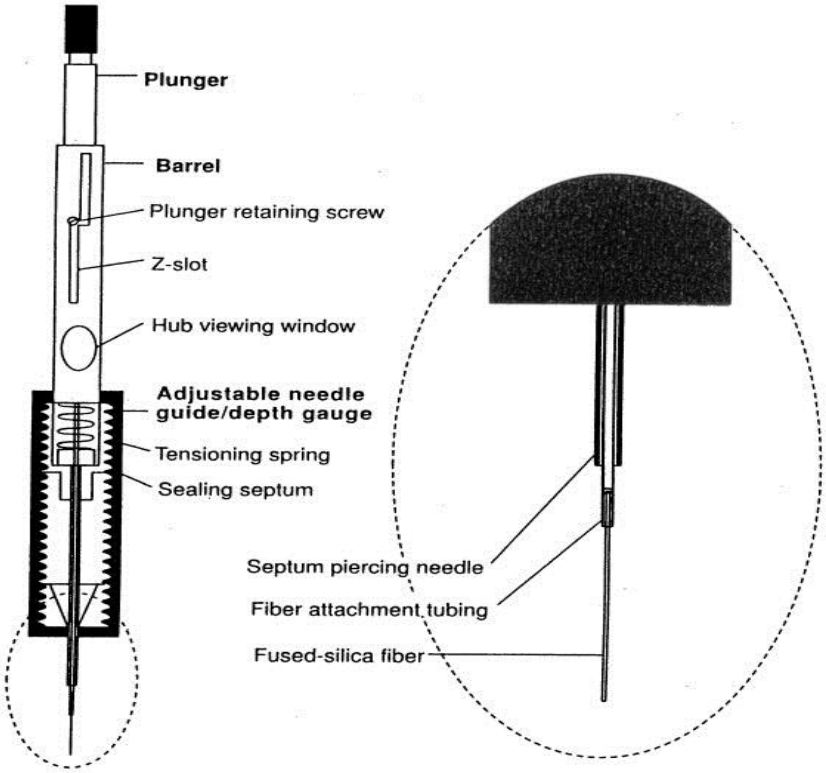


Fig 3.3: View of the SPME Manual Fiber Holder (Shirey, 2012; Zhang, Z., Yang, & Pawliszyn, 1994)

SPME is based on the partitioning of analyte and establishment of equilibrium between the analytes in the sample matrix and the stationary phase of the coated fused silica, which can either be liquid or solid particles suspended in liquid polymer or combination of both (Arthur et al., 1992a; Lambropoulou & Albanis, 2007b; Shirey, 2012). The attainment of equilibrium depends on the partition coefficient (Aulakh et al., 2005), which reflects the chemical composition of the extraction phase and hence, its selectivity towards a given analyte.

3.2 Theory of SPME

The theory of SPME as described by Pawliszyn and his coworkers (Arthur et al., 1992a; Arthur & Pawliszyn, 1990; Pawliszyn, 2012a; Zhang, Z. & Pawliszyn, 1993), showed that there is partition of analytes between the SPME coated fiber and the sample matrix, then a linear relationship exists between the amount of analyte extracted by the fiber and the initial concentration of the analyte present in the sample matrix. This will enable the partitioning process to achieve quantitative extraction. Thus, the amount of analyte extracted at equilibrium can be determined using the thermodynamic principle, which is based on the partition equilibrium. Solving the differential equations described by mass transfer conditions of the extraction system can be used to estimate the extraction time (Pawliszyn, 2012c).

3.2.1 Thermodynamic Theory

SPME extraction is a phase equilibrium process (Pawliszyn, 1997); direct immersion involves two phases (fiber coating and sample matrix) with one interface, while headspace extraction involves three phases (the fiber coating, headspace gas and the sample matrix) with two interfaces (Ai, 1997a, 1997b, 1998). The amount of analyte extracted from the sample matrix can be described using Nernst's partition law (Arthur & Pawliszyn, 1990). In an equilibrium situation, there exists a linear proportional relationship between the amount of analyte extracted (n) and the initial concentration of the analyte of the analyte present in the sample matrix (C_o), which is described by the relation $n \propto C_o$ for qualitative analysis (Ai, 1997b).

The partition coefficient or equilibrium constant is expressed as the concentration of analyte in the fiber coating (C_f), the concentration of analyte in the sample matrix (C_s) and the concentration of analyte in the headspace (C_h). The extraction process is completed when the concentration of the extracted analyte reaches equilibrium between the sample matrix and the fiber coating as described by Eq. (3.1) for one interface and Eq. (3.2) for two interfaces (Pawliszyn, 2012c):

$$C_o V_s = C_s^{\infty} V_s + C_f^{\infty} V_f \tag{3.1}$$

$$C_o V_s = C_s^{\infty} V_s + C_f^{\infty} V_f + C_h^{\infty} V_h \tag{3.2}$$

where $C_s^{\infty}, C_f^{\infty} and\ C_h^{\infty}$ are the equilibrium concentrations of the analyte in the sample matrix, fiber and the headspace respectively.

The distribution coefficient between the SPME fiber coating and the sample matrix interface can be defined as:

$$K_{fs} = \frac{C_f}{C_s} = \frac{(n/V_f)}{C_o - (n/V_s)} \tag{3.3}$$

while the distribution coefficients for the headspace/sample matrix and the fiber coating/headspace interfaces are defined as:

$$K_{hs} = \frac{C_h}{C_s} = \frac{(n/V_h)}{C_o - (n/V_s)} \tag{3.4}$$

$$K_{fh} = \frac{C_f}{C_h} = \frac{(n/V_f)}{C_o - (n/V_h)} \tag{3.5}$$

where V_s, V_f and V_f are the volumes of the sample matrix, fiber coating and headspace respectively. The amount of analytes extracted by the SPME fiber coating is then given as (Ai, 1998; Pawliszyn, 2012c):

$$n_f = \frac{K_{fs} V_f V_s C_o}{V_s + K_{fs} V_f + K_{hs} V_h} \tag{3.6}$$

$$K_{fs} = K_{hs} K_{fh} \tag{3.7}$$

Eq. (3.6) shows that the amount of analyte extracted is independent of the location of the fiber in the sample vial. The fiber can thus be placed either in the headspace or directly in the sample matrix, provided the volumes of the SPME fiber coating, headspace and sample are kept constant (Pawliszyn, 1997, 2012c).

The parameters $K_{fs}V_f, K_{hs}V_h$ and V_s represent the analyte capacity for the fiber coating, headspace and volume phases respectively. In the direct immersion mode containing two phases and one interface, Eq. (3.6) becomes (Bojko et al., 2012; Pawliszyn, 2012c):

$$n_f = \frac{K_{fs}V_f V_s C_o}{V_s + K_{fs}V_f} \tag{3.8}$$

In many cases, the SPME fiber coating/sample matrix distribution constant (K_{fs}) is relatively small with respect to the phase ratio of the fiber coating and sample matrix *($V_f << V_s$)*, and if the analyte has a very high affinity for the fiber coating, K_{fs} will be very large and thus $K_{fs}V_f >> V_s$ and Eq. (3.8) becomes:

$$n_f = K_{fs}V_f C_o \tag{3.9}$$

Eqs. (3.8 and 3.9) show that there is direct relationship between the amount of analyte extracted (n) and the initial concentration (C_o) of the analyte in the sample matrix and that the amount of analyte extracted by the fiber is independent of the sample volume, provided $K_{fs}V_f >> V_s$ and quantitative analysis can be achieved (Pawliszyn et al., 1997). The distribution constant can be calculated from chromatographic parameters and the theoretical models showed that, there is diffusion of analyte from the sample to the fiber coating, which does not exist in solution (Prosen & Zupančič-Kralj, 1999).

3.2.2 Kinetic Theory

The speed of an SPME process is described by the kinetics of extraction. SPME extraction rate is determined by mass transfer and diffusive transport of analytes from the sample matrix to the fiber coating (Zhang, Z. & Pawliszyn, 1993), or the evaporation of the analyte from the condensed phase into the headspace (Ai, 1997a). The theory of mass transfer of analytes to the SPME fiber coatings is a fast process in gas phase (Ai, 1997a), and is based on Fick's second law of thermodynamics, which describes mass balance in a dynamic system (Pawliszyn, 1997). In headspace SPME, when diffusion of analyte from the fiber surface to its inner layers is a slow process, it can be considered as the rate determining step, whereas, if the evaporation of the analyte from the sample matrix to its headspace becomes the rate determining step, the mass transfer at the headspace/fiber coating interface is considered as relatively fast process (Ai, 1997a). The rate of extraction is inversely proportional to the diffusion coefficient of the analyte in the sample matrix and directly proportional to the square of the fiber thickness.

The rate of SPME extraction can be calculated using a dynamic model, which involve solving the mathematical equation of the second-order differential equation, resulting in simple analytical solution and the model is based on the steady state situation in which the rate of mass transfer between sample solution and headspace is equal to the rate of mass transfer between headspace and fiber coating (Ai, 1998).

$$n = n^{\infty}(1 - e^{-a_n t}) \tag{3.10}$$

where n and n^{∞} is the amount of analyte extracted by the fiber coating prior to partition equilibrium and at equilibrium respectively, t is the extraction time and a_n is a complex

parameter which determines the speed at which equilibrium could be attained. At the start, the concentrations of analytes in the fiber coating increase rapidly and then decrease with time until equilibrium is reached. Fibers coated with thicker films require a longer time to attain equilibrium (Kataoka et al., 2000; Prosen & Zupančič-Kralj, 1999), but more analytes will be extracted onto the fiber resulting in higher sensitivity. The extraction kinetics is important for the optimization of the parameters which affect the efficiency of the SPME extraction, such as extraction time and temperature, agitation and salt addition. It helps to identify its limitation and indicates strategies to increase the extraction speed (Pawliszyn, 1997). The modification of the kinetic theory is possible for the extraction in a fiber coating that contains a high reagent concentration.

3.3 SPME Methods

3.3.1 In-Tube SPME

The in-tube SPME is an extraction technique developed for coupling to HPLC or LC-MS (Kataoka et al., 2000), and it is based on an open tubular capillary column that is housed in a needle (Eisert & Pawliszyn, 1997a). It consists of a piece of fused-silica capillary column, which is coated internally by a thin film of stationary extraction phase or a capillary packed with an extracting phase dispersed on an inert supporting material (Lord & Pawliszyn, 2000). It can easily be automated with LC and can simultaneously perform continuous extraction, desorption and injection, where the extracted analyte is desorbed into the chromatograph by a moving stream of solvent or static desorption solvent.

In-tube SPME has two approaches depending on the capillary tube used for the extraction (Fig 3.4). Dynamic in-tube, contains an open-tubular fused-silica capillary column, and analytes are directly extracted and subsequently concentrated in the stationary phase of the column (Ouyang & Pawliszyn, 2006b). Static in-tube SPME technique involves the extraction of analyte through the static gas phase present in the needle. In this technique the extracting phase is not directly exposed to the sample, but is contained in a protective tubing and does not involve any flow of the sample through the extracting phase (Lord & Pawliszyn, 2000).

Analytes are continuously desorbed from the fiber by the mobile phase in a dynamic mode, while desorption takes place in a small volume of solvent inside the desorption chamber in the static mode (Blasco et al., 2003b). When the extraction process reaches equilibrium or a sufficient extraction is achieved, the extracted analytes are directly desorbed from the stationary phase by mobile phase flow or by aspirating a desorption solvent (Kataoka et al., 2000). The in-tube SPME has been applied to the determination of several pesticides in aqueous samples, but its use for the analysis of fruits and vegetable (Guo, F. Q. et al., 2006) samples is limited.

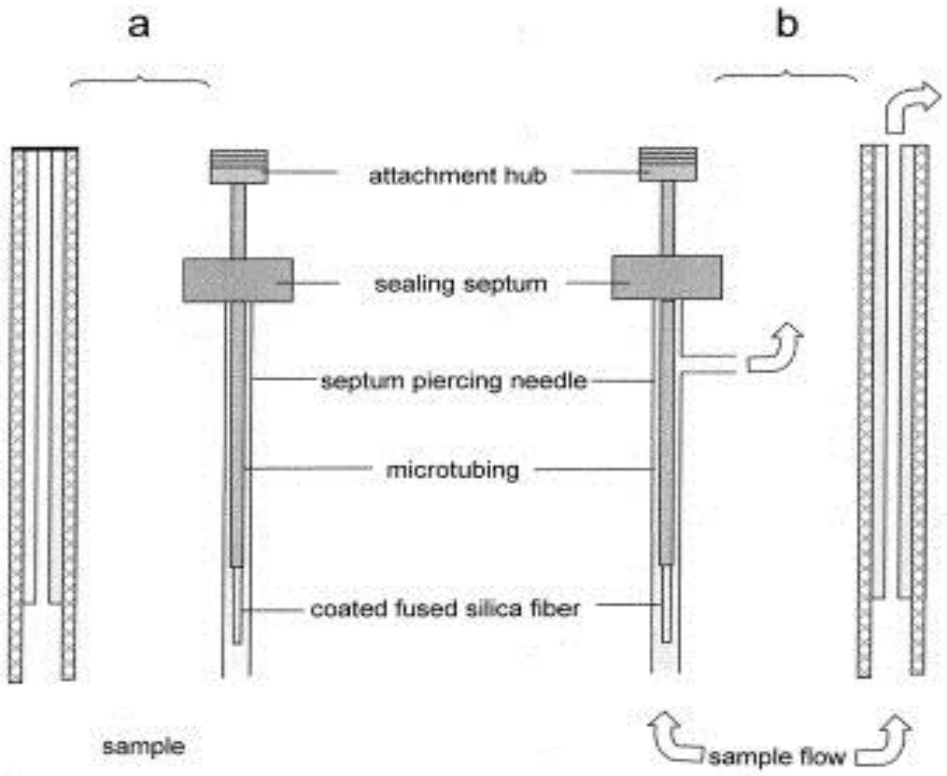


Fig 3.4: In-tube SPME in (a) Passive and (b) Dynamic Modes (Lord & Pawliszyn, 2000)

3.3.2 Fiber SPME

Fiber SPME is an extraction method in which the fiber is exposed to the analyte sample, contained in a vial sealed with a septum-type cap (Kataoka et al., 2000). The SPME needle is pierced through the septum and the fiber is exposed to the analyte sample, for a predetermined time (Lord & Pawliszyn, 2000), during which partitioning occurs between the sample matrix and the extracting stationary phase (Kleeberg et al., 2008). The extraction process is controlled by diffusion of analytes from surrounding solution into the fiber, through the boundary layer between the solution and the fiber, where equilibrium is established. There is maximum sensitivity at equilibrium where a proportional relationship is obtained, between the amount of analyte extracted by the fiber and its initial concentration in the sample matrix (Ai, 1997a, 1997b). For this reason, SPME analysis

does not require full equilibration for quantitative analysis (Kataoka et al., 2000). Although, the extraction can be considered to be completed, when distribution equilibrium of the analyte concentration is reached between the sample matrix and the fiber coating (Risticevic, Vuckovic, & Pawliszyn, 2010c), at this stage, the concentration of analyte extracted remains constant and does not change with time, within an experimental error.

3.4 Extraction Mode

There are three different modes of fiber SPME: direct immersion (DI-SPME), headspace (HS-SPME), and membrane protection (Kudlejova, Risticevic, & Vuckovic, 2012; Lord & Pawliszyn, 2000; Pawliszyn, 2012c), but DI-SPME, HS-SPME and solvent extraction prior to DI or HS SPME are widely used for the extraction of pesticide residues from fruits and vegetables. Fig 3.4 shows the difference between the HS-SPME and DI-SPME (Aulakh et al., 2005; Kataoka et al., 2000).

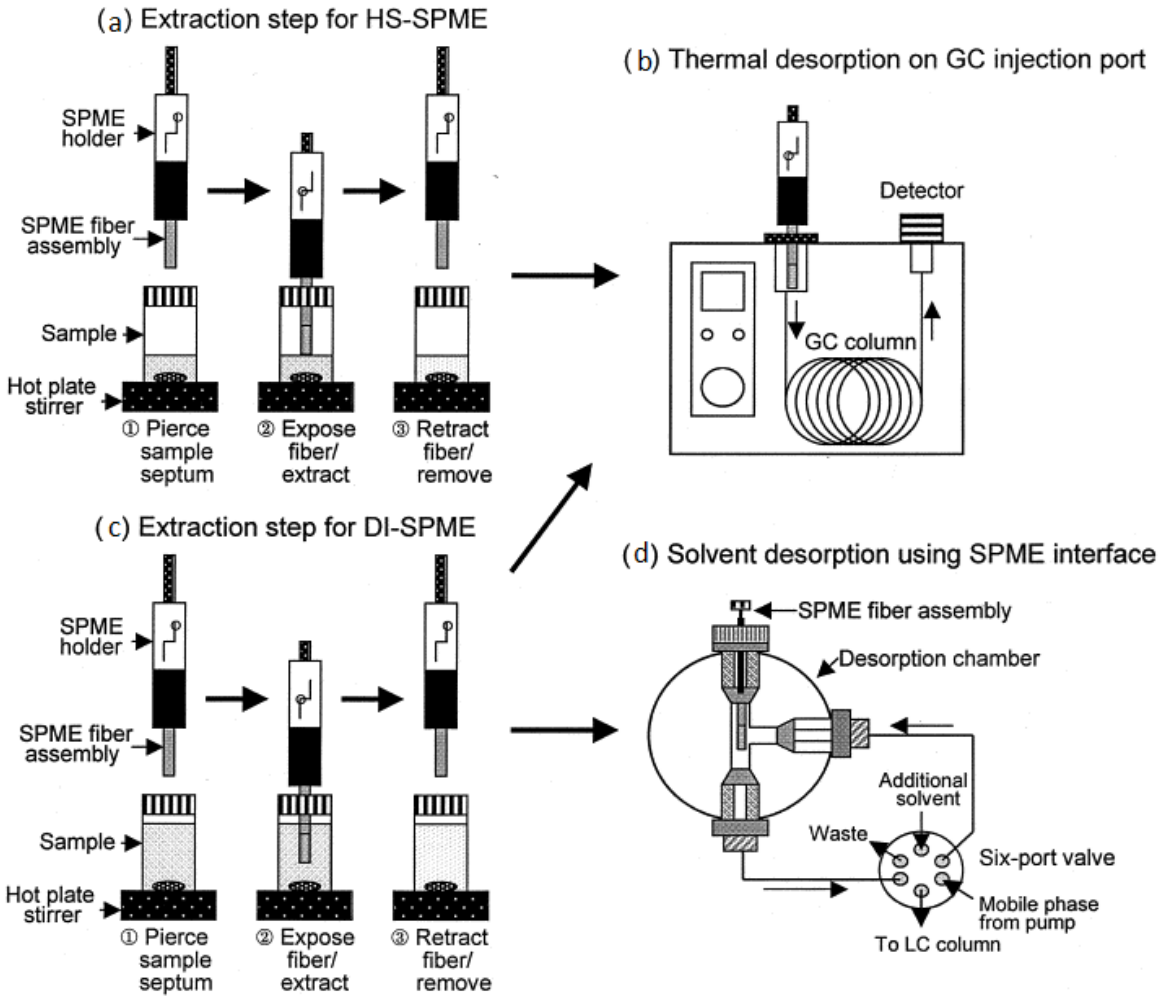


Fig 3.5: SPME Extraction Mode (a) HS-SPME, (b) DI Extraction, (c and d) Desorption Types (Kataoka et al., 2000)

In the headspace sampling mode, the analyte is transported through a layer of gas before reaching the coating. The HS-SPME involves the exposure of the fiber to the vapor phase above a liquid or solid sample (Kataoka et al., 2000), where the analyte are extracted from the gas phase equilibrated with the sample matrix (Pawliszyn, 1999). This method helps to protect the coated fiber from the effects of any non-volatile high molecular weight compounds in the sample matrix, which binds irreversibly to the coating and often cause interference in the extraction process (Risticevic et al., 2010b).

In the direct immersion mode, the coated fiber is inserted into the sample, where the transport of the analyte from the sample matrix into the extracting phase is achieved (Lord & Pawliszyn, 2000). The extraction in an aqueous sample is improved by agitation of the sample, and this is done in order to reduce the effects of fluid shielding, and reduce sample matrix diffusion layer (Beltran et al., 2000), thereby increasing the diffusion coefficients of analytes in the zone closer to the fiber (De Fátima Alpendurada, 2000). Effective agitation techniques, such as fast sample flow, rapid fiber or vial movement, stirring or sonication are employed to reduce the effect of boundary layer.

In HS-SPME there are three phases: the sample matrix, the headspace gas, and the SPME polymeric fiber coating (Ai, 1997a; Beltran et al., 2003)(30,33), with two interfaces (gas/polymer and sample matrix/gas), whereas only two phases are involved in DI-SPME, which are the coated fiber and the sample solution with a single interface. The membrane protected SPME is widely used for polluted and dirty aqueous sample, and for the extraction of low volatile analyte (Chen, Y. I., Su, & Jen, 2002)(54), and is also used to protect the fiber. It involves two processes occurring simultaneously: extraction of analytes from the aqueous sample matrix by the porous membrane materials and subsequent extraction of the analytes from the membrane through stripping phases (Pawliszyn, 1995).

The HS-SPME helps to shorten extraction time, due to the increased rate of diffusion of analytes in the gaseous phase than in the liquid phase, it also reduces matrix effects (Lambropoulou & Albanis, 2002; Lambropoulou et al., 2007). This would allow for various modifications of extraction conditions such as pH and salt addition without any

effect on the fiber efficiency. Besides the DI-SPME and HS-SPME some of the authors also combine other technique prior to the SPME extraction, such as microwave-assisted extraction (MAE-SPME) (Chen, Y. I. et al., 2002; Falqui-Cao et al., 2001; Sanusi, Guillet, & Montury, 2004), LPME (Hu, Y. et al., 2009) or solvent extraction prior to SPME (Cortés Aguado et al., 2008; Cortés Aguado et al., 2007).

The efficiency of extraction in the HS mode may be increased by increase in temperature of the sample matrix, this enhances the diffusion coefficient of the analytes and conversely reduces the distribution constant (De Fátima Alpendurada, 2000). This opposing effect of temperature, that is reduction in distribution constant was overcome by the development and optimization of circulating cooling (or cold fiber) HS-SPME (CC-HS-SPME) (Chai, X. et al., 2008; Jiang et al., 2012; Pawliszyn, 1997, 2012b), which increases mass transfer and simultaneously increase the distribution constants of the analyte (De Fátima Alpendurada, 2000; Risticevic et al., 2009). The CC-HS-SPME method allows for the simultaneous heating of the sample solution and cooling of the fiber coating leading to an increase in the efficiency of the extraction process.

There is a direct relationship between the amount of analyte extracted by the fiber and the concentration of analyte in the sample matrix ((Ai, 1997a, 1997b; Arthur et al., 1992a), and is independent of the fiber location (De Fátima Alpendurada, 2000). Therefore, the amount of analyte extracted into the coating from the sample solution at equilibrium is the same, for DI- and HS-SPME (Lord & Pawliszyn, 2000). This is due to the fact that equilibrium

concentration does not depend on the location of the fiber provided the sample vial and gaseous headspace volume are the same and remain constant.

Extraction of analytes from the sample matrix by the SPME fibers in fruits and vegetables analysis involves both mass transfer and diffusion. Any of the two processes can be the rate determining step (Arthur et al., 1992a), but in dynamic SPME, mass transfer is considered as the rate determining step either in HS-SPME or DI-SPME.

3.5 SPME Fiber Coatings for Fruit and Vegetable Analysis

The SPME fiber is a fiber coated with a liquid polymer, solid sorbent or a combination of both (Kumar et al., 2008). Several SPME fiber coatings have been developed for the extraction of various classes of pesticide residues in fruit and vegetable samples. The effectiveness and efficiency of the technique depend on the type, thickness and coating volume of the fiber (Lord & Pawliszyn, 2000; Wardencki, Michulec, & Curyło, 2004). The sensitivity is dependent on the distribution constant of the analytes partitioning between the sample matrix and the fiber coating. The distribution constant describes the properties of a fiber coating and its selectivity and specificity for the extraction of the pesticide residues against other complex components present in the fruit and vegetable matrices (Pawliszyn, 2012b). Therefore, appropriate fiber coatings are selected based on the nature, volatility and their affinities to the pesticides. Thicker fibers are used for volatile pesticides, while thin fibers are suitable for pesticides with higher boiling points.

3.5.1 SPME Fiber Core

The SPME fibers was first coated on fused silica core, which is an inert glass used in the production of capillary column, but due to its fragility it has been replaced by other cores. Stableflex fiber core was introduced to overcome the fragility nature of the fused silica core. It consists of 80 µm fused silica core coated with a 20 µm stable plastic polymer, which provides a protective cover. The coatings on the fused-silica fibers can be non-bonded, bonded, partially cross-linked, or highly cross-linked (Kataoka et al., 2000; Krutz, Senseman, & Sciumbato, 2003). Its disadvantage lays in the fact that only adsorbent type polymer such as PDMS/DVB and DVB/CAR/PDMS could be coated on the Stableflex core. The recently developed metal core polymer has a unique non-ferrous alloy with shape memory properties, which is highly inert. These properties make it more durable than fused silica core and with higher thermal stability than the Stableflex (Shirey, 2012).

3.5.2 SPME Fiber Coatings

There is need for proper selection of fiber coating in order to achieve an efficient extraction of target analyte from the complex sample matrix, since the extraction performance is highly dependent on the availability of appropriate fiber coating (Shirey, 2012). Several commercial SPME fibers are available with different fiber coatings, volume, thickness and phase mixtures. They are designed to be used by manual or automated sampling and are classified into four different categories based on the type of coatings, coating thickness, polarity and sorption type (adsorption/absorption) (Shirey, 2012).

There are seven commercially available SPME fibers, with two homogenous and five mixed phase coatings. This enables the use of SPME technique for the extraction of wide range of pesticide residues including non-polar and polar pesticides and volatile and non-volatile pesticides as well.

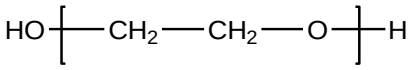
3.5.2.1 Commercial SPME Fibers

The homogenous polymer coatings extract via absorption with the analyte diffusing into the bulk of the coatings causing the analyte to interact with the polymeric coating material. The polydimethylsiloxane (PDMS) is a non-polar homogenous fiber, manufactured in 3 different film thicknesses: 7, 30 and 100 µm and in two forms: bonded and non-bonded (Mani, 1999). Due to its extraction properties (Seethapathy & Górecki, 2012), PDMS is the most commonly used SPME fiber coating for the extraction of pesticide residues from fruits and vegetable samples. The PDMS fiber is preferred for the extraction of non-polar pesticides. However it has widely been used for the extraction of more polar pesticides, after the extraction conditions have been optimized.

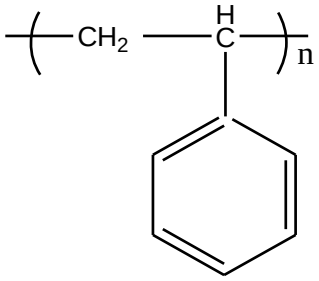
PDMS fiber is very rugged and cross linked, and can withstand high injector temperatures up to about 300^0C. PDMS was found to have low affinity for polar pesticides, thus polar SPME was developed. The polyacrylate (PA) fiber coating is also a homogenous fiber which is available in 85 µm thickness. It is a partially cross-linked solid crystalline coating and more polar. Its moderate polarity makes it suitable for the extraction of polar pesticides. The coating thickness reduces with use, which limit its thermal stability. Both

fibers are stable at a pH of 2 – 11, except the 100 µm PDMS which is stable over a pH range of 2 – 10 (Kataoka et al., 2000; Shirey, 2012).

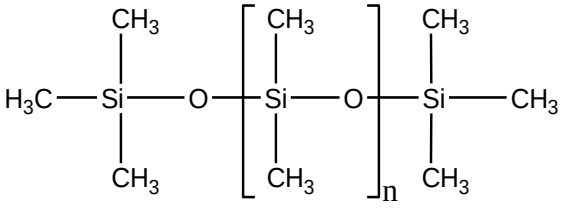
The mixed fiber coatings contain fibers that are coated with different porous particles embedded in partially cross-linked polymeric phases and contain a solid material suspended in a liquid polymer. The mixed coating: PDMS/divinylbenzene (DVB), DVB/PDMS/carboxen (CAR), carbowax (CW)/PDMS, CW/DVB and CW/template resin (TPR) extract by adsorption and extract through physical trapping of the analyte, and have complementary properties of each constituent polymeric coating. They are of lower mechanical stability than the homogenous coating, but have an increased retention capacity, high distribution coefficient, smaller diffusion coefficient and high selectivity (De Fátima Alpendurada, 2000; Mani, 1999), this is attributed to their mutually potentiating adsorption capacity. The extraction efficiency of the mixed coatings depend on their total surface area, porosity and pore size (Shirey, 2012). The mixed-phase coating are used for the extraction of polar, volatile and low molecular mass analytes (Risticevic et al., 2010b).



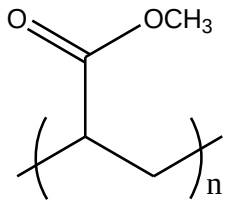
Polyethylene glycol



Divinylbenzene



Polydimethylsiloxane



Polyacrylate

Fig 3.6: Chemical Structure of Polymers used for Commercial SPME Coatings (Seethapathy & Górecki, 2012; Shirey, 2012)

There are different methods for the preparation of the SPME fiber coatings. There is the dipping technique which consists of placing a fiber in a concentrated organic solution of the polymerized material for a short time. The fiber is then removed and evaporated by drying and the deposited fiber is then cross-linked. The other method, electrodeposition, which is an extension of the previous method, involves the selective deposition of the coating materials on the surface of a metallic rod (Brondi & Lanças, 2005; Pawliszyn, 2012b). The difference in coating thickness which varies from fiber to fiber is a limitation of this fiber preparation method (Lord & Pawliszyn, 2000), and is overcome by simultaneously depositing the coating during the drawing of the fused-silica rod. Another method includes drawing the fiber by means of a fiber optic tower, which involves melting of a fused-silica rod and drawing it into a thin rod. The drawn fiber is then allowed to cool

at room temperature and then passed through an applicator containing a concentrated solution of the coating material in an organic solvent (Mani, 1999).

3.5.2.2 Sol-Gel Fibers

Sol-gel technology derived fibers are produced using an appropriate precursor compounds, which have high solubility in organic solvent (Kessler, 2005). It involves the removal of the protective polyimide layer of the fused-silica fiber by dipping in acetone, after which it is dipped in NaOH to clean the fused silica surface (Kumar et al., 2008; Zeng et al., 2008). The fused-silica is then dipped into a sol solution containing the appropriate precursor. The sol-gel preparation involves the use of a precursor, usually a metal alkoxide, $M(OR)_x$, a hydroxyl terminated sol-gel active polymer, solvents to disperse the precursors, an acidic or a basic catalyst and water (Kumar et al., 2008). The method is used to prepare thin coatings that could lead to increase in extraction efficiency (Pawliszyn, 2012b).

The sol-gel technology for the production of SPME fiber coatings was introduced to address the limitations observed in the commercial SPME fiber coatings. It is a simple and effective method for the synthesis of materials used for the SPME fiber coating. It provides a new approach for the efficient combination of organic compounds into inorganic polymeric structures in solution. Organic-inorganic materials are produced, under extraordinary mild thermal conditions, using an appropriately designed sol solution (Chong et al., 1997; Kumar et al., 2008). The sol-gel process is an acid-catalyzed reaction, which involves hydrolysis and alcohol or water condensation of the precursors and hydrolytic poly-condensation reactions of the hydrolysis and condensation products, carried out in a

sol solution. It provides a greater surface area and higher thermally stable SPME fiber coatings (Nerín et al., 2009). It does not lose its sensitivity and selectivity in organic solvents and can withstand high and low pH of the complex sample matrix contained in fruit and vegetable samples. The efficiency of the sol-gel fiber coatings is dependent on the reaction conditions; such as temperature, nature of the precursor, solvent concentrations and the type of catalyst. The most widely used catalyst is triflouroacetic acid (TFA) containing 5% of water.

Its advantage over the commercial SPME fiber coatings are better homogeneity and purity, low preparation temperature, particle size control, low cost, ability to achieve molecular level uniformity, strong bonding of the coating to the substrate (silica-gel) and enhanced stationary phase stability (Chong et al., 1997; Kumar et al., 2008). It has been shown that sol-gel fibers possess a significantly higher thermal stability (>320 °C) than the commercial SPME fibers, due to the strong chemical bonding between the sol-gel generated organic-inorganic composite coating and the silica surface (Chong et al., 1997). These properties allow the efficient desorption of less volatile and non-volatile analytes and prevent carry-over, thereby extending the range of pesticide residues that can be handled by its automation to the GC technique and a longer lifetime. It has also be found to have high reproducibility of performance (Lord & Bojko, 2012).

3.5.2.3 Molecularly Imprinted Polymer Fibers

Other fiber coatings used for pesticide residues analysis in fruit and vegetable samples include molecularly imprinted polymers (MIPs), which is prepared by polymerization of functional and cross-linking monomers in the presence of a high concentration of initiator compounds (Nerín et al., 2009). The cross-linking polymer such as ethylene glycol dimethyacrylate (EDMA) or trimethylolpropane trimethacrylate (TMP-TMA), is used to make the MIP hydrophobic in nature, while the functional monomer like methacrylic acid (MAA) or vinylpyridine (VP), provides weak ion exchange and hydrogen bonding acid and pyridine group (Anderson, 2006; Djozan & Ebrahimi, 2008; Djozan et al., 2010; Djozan, Mahkam, & Ebrahimi, 2009). The use of MIP helps to produce antibodies mimic with specificity to the desired analytes, with good selectivity, chemical stability, cost effective and fast equilibrium time (Pawliszyn, 2012b). The MIP coated fibers have high resistance to heat, mechanical stress with a longer use and storage life (Lord & Bojko, 2012).

3.6 SPME Method Development

There are different parameters that influence the partitioning of the analytes between the sample matrix and the SPME fiber. When considering the optimization of parameters for fruits and vegetables analysis, the complex nature of the sample should be taken into consideration (Risticevic et al., 2010b). The amount of analytes extracted from fruit and vegetable samples depend on the nature of the stationary phase (fiber) and on the properties of the sample matrix. The most important method used in the optimization of extraction parameters is the consideration of the thermodynamic and theoretical models

(Pawliszyn, 1995), in the selection of a particular procedure for the development of method for the determination of pesticides in fruits and vegetable samples.

The optimization of the SPME conditions for fruit and vegetable samples involve: selection of extraction mode, fiber coating, extraction time and temperature and desorption time and temperature, agitation, salt addition, pH, dilution and solvent addition (Kataoka et al., 2000; Kudlejova et al., 2012; Risticevic et al., 2010b; Wardencki et al., 2004). All these conditions are developed, optimized and validated for effective extraction and quantification of pesticide residues from the complex fruit and vegetable matrices. This is as a result of the fact that extraction step largely determined the figures of merit of an analytical methodology (Zhang, Z. et al., 1994), such as sensitivity, repeatability, reproducibility, precision and accuracy, limits of detection and quantification and linearity in pesticide analysis.

3.6.1 Fiber Coating

The sensitivity and efficiency of SPME technique in the extraction of pesticide residues from fruit and vegetable samples depend on the distribution constant between the stationary phase and the sample matrix. The distribution constant describes the fiber coating properties and its selectivity towards a given analyte in the presence of other matrix components (Kudlejova et al., 2012; Lord & Pawliszyn, 2000; Risticevic et al., 2010b). The fiber affinity to the analyte is based on the principle of "like dissolves like" (Kataoka et al., 2000). The efficiency of extraction depend on the type of fiber as well as the coating volume (Lord & Pawliszyn, 2000; Wardencki et al., 2004), although it takes a

longer time to attain equilibrium in thicker fiber coating, it allows the extraction of higher quantity of analytes.. Thicker fibers are used for volatile pesticides, while thin fibers are suitable for pesticides with high boiling points to ensure fast diffusion and quick release into the desorption chamber of the chromatography.

Proper selection of the fiber is necessary to achieve an efficient extraction of target analyte from the complex sample matrix. Different fibers are used to extract different classes of pesticides. Several fibers with different coatings and thickness are commercially available. The sol-gel technology has been used to prepare refined tuned coatings for specific applications (Cai et al., 2006; Cai et al., 2003; Chai, X. et al., 2008; Dong, Zeng, & Li, 2005; Farajzadeh & Hatami, 2004; Yu, J., Wu, & Xing, 2004; Zeng et al., 2008), and molecular imprinted polymer has also been used for the extraction of triazine herbicides (Djozan & Ebrahimi, 2008; Djozan et al., 2010; Djozan et al., 2009; Hu, X., Hu, & Li, 2007; Turiel, Tadeo, & Martin-Esteban, 2007) and thiabendazole (Barahona, Turiel, & Martín-Esteban, 2011) in food samples.

The sol-gel prepared fibers are stable in strong organic solvents as well as in acidic and basic solutions depending on the precursor used. The sol-gel technology provides higher surface area for the fiber (Dong et al., 2005), due to its porous-like characteristics. The fiber coated with polydimethylsiloxane (PDMS), a liquid coating polymer, is the most frequently used fiber for the extraction of non-polar pesticide residues from fruit and vegetable samples. This is due to its rugged and robust nature and its ability to withstand high injector temperatures, between 280 and 320^0C (De Fátima Alpendurada, 2000;

Kataoka et al., 2000), and also allows analysis of a wider linear range of concentrations (Simplício & Vilas Boas, 1999). It is a non-polar fiber which has been widely used to extract non-polar analytes, but can also be used for the extraction of polar analytes after extraction conditions, such as pH, salt addition and temperature are optimized. Polyacrylate (PA) fiber is a solid crystalline coating, however, it exhibits extraction capabilities inherent of a liquid coating absorption, and is suitable for the extraction of more polar pesticides (Berrada, Font, & Moltó, 2004; Navalón et al., 2002; Zambonin et al., 2004), and it has smaller diffusion coefficients compared to PDMS fiber coatings (De Fátima Alpendurada, 2000).

3.6.2 Extraction Modes

Selection of extraction mode depends on the nature and properties of the sample matrix and the volatility of the pesticides. Extraction of less volatile compounds is achieved by DI-SPME, while sampling of more volatile analytes from complex matrix is mostly done in the HS-SPME mode (Kataoka et al., 2000; Kudlejova et al., 2012). The HS-SPME also allow for the variation of sample matrix properties, without having any effect on the fiber. In DI-SPME matrix effects is reduced by dilution with distilled water (Fernández et al., 2001; Lambropoulou & Albanis, 2003; Simplício & Vilas Boas, 1999).

The extraction efficiency can also be improved by a previous extraction of the analyte into water or other solvent (Berrada et al., 2004; Blasco et al., 2003a; Cortés Aguado et al., 2008; Cortés Aguado et al., 2007; Falqui-Cao et al., 2001; Guillet, Fave, & Montury, 2009; Hu, R. et al., 1999; Sanusi et al., 2004; Wang et al., 2000), using focused microwave

assisted extraction (FMAE), followed by DI-SPME. This is done due to the complexity of matrix in fruit and vegetable samples, and using HS-SPME results in a higher peak area and efficient extraction of the analyte in this sample than in DI-SPME (Cai et al., 2006). It also reduces the extraction time and increase the fiber lifetime. SMPE in combination with GC has been found to have low affinity for the extraction of less volatile or thermally labile analytes (Kataoka et al., 2000), but its automation with LC has been used to overcome this setback.

3.6.3 Sample Volume

The optimization of sample volume is also an important factor that must be considered. The sample volume also determines the amount of analyte extracted. The effect of sample volume on the amount of analyte extracted is dependent on the distribution constant. The amount analytes with high distribution constant is dependent on sample volume, while analytes with low distribution constant are independent on sample volume. When the volume of sample is greater than the coating volume, the amount of analyte extracted is also independent on the volume of sample, above a critical volume (Kudlejova et al., 2012; Pawliszyn, 1997; Risticevic et al., 2010b).

3.6.4 Optimization of Sample Matrix Related Conditions

The extraction temperature affects the diffusion coefficients between the analytes and the fiber and the distribution constant of the analytes. An increase in the extraction temperature enhances the transport of analyte from the sample matrix and leads to an increase in the extraction rate (Kataoka et al., 2000; Kudlejova et al., 2012; Risticevic et al., 2010b), by

increasing the diffusion coefficient. The partitioning of the analytes into the coated fiber is an exothermic process. Therefore, increase in temperature causes a decrease in the distribution constant and decreases the amount of analyte extracted at equilibrium, but may be acceptable if the target limits of detection can be reached. Therefore optimal and adequate extraction temperature which depends on the sample matrix and the stationary phase used is always employed, to provide satisfactory sensitivity and a high rate of extraction. A consistent fiber exposure time is also important for good accuracy and precision. Pesticides with low diffusion coefficients have longer equilibration time (De Fátima Alpendurada, 2000) and a longer extraction time favours the extraction of higher amounts of analyte.

Temperature and equilibrium time are closely related, because increase in the extraction temperature decreases the distribution constant, and shorten the equilibrium time with less analyte being extracted at equilibrium. The equilibration time of the sample before the exposure of the fiber coating should also be optimized. In the optimization of extraction time, which is the time limiting step, the objective of the SPME analysis, such as high-throughput analysis, sensitivity, reproducibility, should be well defined (Risticevic et al., 2010b). The time required for SPME extraction is independent of the concentration of the analyte in the sample matrix, therefore, the extraction time profile can be optimized using any sample concentration (Kudlejova et al., 2012). Most researchers have employed equilibrium time, because it provides better sensitivity and high reproducibility.

Extraction efficiency can be improved by agitation (Eisert & Pawliszyn, 1997b; Kudlejova et al., 2012; Risticevic et al., 2010b), because it reduces the diffusion layer (Beltran et al., 2000), and improves the mass transfer from the sample matrix onto the fiber coating. Previous studies have shown that the rate of extraction increases with increased stirring, which decreases the equilibrium time. Although there is a maximum sensitivity at the equilibrium time, full equilibration is not necessary because an accurate and precise analysis can be achieved before equilibrium is reached (Ai, 1997a, 1997b). At the same time, faster agitation tends to be uncontrollable and the rotational speed might cause a change in equilibrium time, and a higher stirring rate can lead to magnetic flutter and air bubbles formation in the solution (Zeng et al., 2008), which can lead to poor precision and accuracy. There are different methods of agitation: magnetic stirring, fiber vibration, sonication and flow through cell extraction for the determination of pesticides, but studies have shown that there is no significant difference in the accuracy and precision of the different agitation methods (Eisert & Pawliszyn, 1997b, 1997c).

The addition of salt is also an important parameter, it has been found that saturating the sample matrix with salt helps to decrease the limit of detection by increasing the distribution constant (Arthur et al., 1992a). The partition coefficients depend on the matrix-analyte-fiber interactions (Rial Otero et al., 2002), since water soluble pesticides have low affinity for the coated fiber, the extraction is increased by reducing their solubility via the addition of salt which changes their ionic strength (Fernández et al., 2001). Salt addition improves analyte diffusion from the sample matrix to the fiber especially in the headspace mode (Kudlejova et al., 2012).

Recovery of water soluble and polar analyte is improved by salt addition (Zambonin, Cilenti, & Palmisano, 2002), but higher amount of salt decreases the extraction efficiency (Risticevic et al., 2010b), due to the fact that the sample matrix can be saturated, and the undissolved salts may occupy active sites in solid coatings, therefore restraining partition between the analyte and the fiber (Chai, X. et al., 2008). However when analyzing fruits and vegetables, it might be important to add salt to normalize the random salt concentration in natural matrices.

Sodium chloride has been widely used in SPME applications, resulting in higher sensitivities in some cases, while potassium sulphate (Dong et al., 2005), sodium hydrogen sulphate (Eisert & Pawliszyn, 1997a; Lambropoulou & Albanis, 2002) and other salts such as sodium hydrogen carbonate, potassium carbonate and ammonium sulphate have also been used (Kataoka et al., 2000; Risticevic et al., 2010b), and they are effective for the extraction of analytes onto the coated fiber due to their salting-out effects. The pH of the sample is also important in extraction techniques and the nature of pesticides present in the sample depends on the pH of the sample matrix. Therefore, addition of acidic solution to the sample when analyzing acidic analytes, and alkaline solution for basic analytes increases extraction efficiency (Kataoka et al., 2000). The sample solution can be controlled and maintained at a desired pH using buffer solution. The adjustment of sample pH helps to convert analytes into their neutral form (Kudlejova et al., 2012; Risticevic et al., 2010b).

Sample water content is another important parameter, because, pesticide residues in fruit and vegetable samples cannot be extracted directly onto the coated fiber, without blending or homogenizing, and this depends on the type of sample to be extracted. The coated fiber cannot be directly submerged in fruits and vegetables matrix, and therefore the need to homogenize fruit and vegetable samples before their extraction. Improved diffusion of the analytes from this complex matrix to the fiber can be achieved by addition of water and other organic solvent before SPME extraction (Menezes Filho, dos Santos, & de Paula Pereira, 2010), this helps to facilitate the diffusion of analytes from the complex sample matrix (Risticevic et al., 2010b). Addition of water (Pawliszyn, 1997; Simplício & Vilas Boas, 1999), helps to improve extraction and protect the coated fiber. The problem of matrix effect in analysis of fruits and vegetables is solved by obtaining an aqueous extract from the sample followed by SPME. This preparatory method has been used successfully for the extraction of pesticide residues by both HS-SPME and DI-SPME. In HS-SPME, the headspace volume affects extraction efficiency and should be carefully minimized (Prosen & Zupančič-Kralj, 1999), to avoid splashing of the solution into the coated fiber. The vial size and sample volume (Kataoka et al., 2000), with little or no headspace volume are also critical in DI-SPME and should be kept constant for a particular analysis.

3.6.5 Desorption Conditions

The coupling of SPME to gas chromatography has widely been employed by most researchers, because, the fiber SPME configuration is designed to suit automation to GC (Pawliszyn et al., 2012). A good optimization of parameter involves a selection of desorption conditions. Desorption time and temperature, and the position or depth of the

fiber coating in the GC injector should be optimized in thermal desorption of analytes. The desorption step should be done in the shortest time possible, therefore the desorption temperature (maintained within its maximum recommended operating value) should be higher than or equal to the boiling point of the least volatile analytes (Kataoka et al., 2000; Wardencki et al., 2004), and the initial temperature of the GC column should be kept low or even cryofocused (cooled), to avoid peak broadening (Prosen & Zupančič-Kralj, 1999), and ensure sharper peaks.

The efficient thermal desorption of analyte also depend on the volatility of the analytes and the thickness of the fiber coating (Kataoka et al., 2000). To ensure high linear flow rate, a narrow-bore insert is required (Lord & Pawliszyn, 2000), which ensures the efficient removal of the desorbed analytes. Split/splitless injector should be operated in the splitless mode, which ensures an efficient and complete transfer of analytes (Prosen & Zupančič-Kralj, 1999). A small volume and narrow liner (78.5mm x 6.5mm o.d x 0.75 mm i.d), with an internal diameter (i.d) closer to the outside diameter (o.d) of the SPME needle (Pawliszyn, 2012b; Pawliszyn et al., 2012) should be used to avoid tailing which occurred when large volume liner is employed. The following GC-MS parameters are very crucial and must be optimized: Injection port temperature (desorption temperature), column flow rate (carrier gas flow), ion source temperature and interface temperature (Kudlejova et al., 2012). Other detectors coupled to GC that have used for pesticide analysis in fruits and vegetables include nitrogen phosphorus detector (NPD), electron capture detector (ECD), flame ionization detection (FID) and flame photometric detector (FPD) (Jin et al., 2012).

The coupling of SPME to LC was designed for analytes which are not amenable to GC and for polar, poorly volatile and thermally labile analytes. Its use is very attractive, but it is lagging behind due to their long equilibration time, difficulty of interfacing, lack of commercially available interfacing options and automation and limited number of commercially available SPME fiber for LC application (Lord, 2007; Nerín et al., 2009; Pawliszyn et al., 2012). The mobile phase desorption solvents reduce the fiber coating life time, due to swelling by the organic solvent. When the SPME device is coupled to LC, solvent desorption is employed and this can be carried out in two different ways: dynamic desorption, in which the analytes are removed by a moving stream of mobile phase, used when the analytes are not strongly bonded to the fiber, while static desorption in which the fiber is soaked in the mobile phase or other strong solvents for a specified time, is used if the analytes are strongly bonded to the fiber (Aulakh et al., 2005; Kataoka et al., 2000). In both cases there is the need to use a minimum amount of solvent. The temperature and the linear flow rate around the fiber determine the time for complete and efficient desorption of the analytes from the coated fiber, and must be optimized for better recovery. The LC has also been coupled to different detectors such as mass spectrometry (MS), ultra-violet (UV), diode array detector (DAD) and fluorescence detector (FD) (Jin et al., 2012).

3.6.6 Calibration Methods (CM)

A calibration method is used to establish the relationship between the measured signal of an analytical instrument and known concentrations of the analytes. In order to determine the concentration of an unknown sample in chromatographic measurement, the peak area of the sample is determined by the calibration function. The sample amount as weighed out

and diluted or concentration steps is taken into account in order to be able to give the concentration in the original sample (Husbschmann, 2009). The use of a calibration curve for the analysis of pesticide residues in fruits and vegetables helps to solve the problem encountered in quantification. Although each calibration method has its merits and limitations (Ouyang, 2012).

The traditional calibration methods, such as external standard (calibration curve), internal standard, or isotopic dilution and standard addition, are efficient for quantification in the laboratory analysis of pesticide residue in fruit and vegetable samples using SPME technique. Other calibration methods: equilibrium, exhaustive and diffusion-based calibrations (Kudlejova et al., 2012; Ouyang, 2012; Ouyang & Pawliszyn, 2006b, 2008; Risticevic et al., 2010b) have been suitably used for on-site air sampling.

3.6.6.1 External Standard (ES)

An external standard involves the use of the substances to be determined in the preparation of several standard solutions of different concentration levels. A relationship between the peak responses and the target standard solution is obtained, by plotting the peak area determined against the concentrations at different calibration levels (Husbschmann, 2009), and by analyzing the sample with the same extraction conditions as the target analytes (Ouyang & Pawliszyn, 2008). The concentration of the target analyte in the sample can then be calculated using the calibration curve equation with a correlation coefficient greater or equal to 0.99. This method has widely been used in SPME in which a blank sample matrix is prepared to avoid matrix effect. It does not require an extensive sample

preparation, but there is a need to keep the sample compositions, sampling procedure and chromatographic conditions constant (Ouyang & Pawliszyn, 2006b, 2008). In the presence of matrix effect, which could interfere with the extraction process, matrix-match analyte free standards are employed when using external standard (Risticevic et al., 2010b).

3.6.6.2 Internal Standard (IS)

The internal standard involves the addition of one or more substances to the calibration solution and sample and is used as a fixed reference material and is kept constant in the standard solution (Husbschmann, 2009; Ouyang & Pawliszyn, 2008). The compound used is different from the analyte but should be well resolved in the chromatographic separation. The calibration curve is plotted by determining the ratio of peak area of the analyte to that of the internal standard for calibration solution that contains different concentration of analyte with a fixed concentration of the internal standard (Ouyang & Pawliszyn, 2006b, 2008). The ratio is then used for sample calibration. The use of an internal standard helps to compensate for volume error, variation in function of the instrument, matrix effect, losses of analytes during sampling and irreproducibility in parameters (Husbschmann, 2009; Ouyang & Pawliszyn, 2006b, 2008; Sánchez et al., 2008). Isotopic dilution using isotopic-labeled or deuterated compounds provides highest accuracy and precision for the internal standard quantification, but the compounds are not available for all analytes (Ouyang & Pawliszyn, 2008; Risticevic et al., 2010b).

3.6.6.3 Standard Addition (SA)

The use of the method of standard addition involves spiking the sample matrix with a known concentration of target analyte, containing an unknown concentration of the analyte in the mixture and then analyzed. A plot of the responses of target analyte at different concentration levels is developed (Ouyang & Pawliszyn, 2006b, 2008) and the original concentration in the unspiked sample is determined, by extrapolating the plot to zero response. The method of standard addition requires an extensive sample preparation but it helps to compensate for matrix effect, when it is not possible to acquire matrix-matched blank samples (Risticevic et al., 2010b).

3.7 Multivariate SPME Experimental Design

The univariate optimization of SPME technique involves optimizing each factor once at a time, in which factors are kept constant except the one being optimized and it involves many experiments (Miller & Miller, 2010). This does not allow the estimation of possible interaction between the studied factors. Experimental design helps to identify the significant factors that maximize the response of an experiment. It also helps to improve the yield or chromatographic separation by optimizing the significant factors using response surface methodology or central composite design. It saves time and requires few experimental runs and can be used for quantitative modeling of mathematical relationships between factors and response (Brereton, 2003, 2007). Its use is aimed to understand the effect of each factor and model the relationship between the factors and response with a minimal number of experiments carried out in an orderly and efficient manner (Massart et al., 1997).

The multivariate experimental design is carried out in two stages: First, the significance of each factor is estimated using the first-order experimental design such as full or fractional factorial design and Plackett-Burman (P-B) design, which are very important for preliminary studies and in identifying the possible interactions between the studied factors. The second stage involves approximation of a response function or the optimization of the significant factors identified in the first stage and it involves the use of second-order models such as response surface methodology and central composite design (Ferreira et al., 2004).

There are several factors affecting the SPME of pesticide residues in fruits and vegetables as mentioned earlier. However the use of univariate method requires large number of experiments, which is time consuming. A well planned experimental design which could simultaneously determine the effect of all factors and determine the optimal condition in few experimental runs is a more convenient approach (López et al., 2007).

The use of Plackett-Burman (P-B) design for the determination of the significant factors helps to screen out factors which has little or no effect on the extraction efficiency as measured by the peak area of a chromatogram and also helps to predict the behavior of other factors (Stalikas et al., 2009). It has 4n experiments, which avoids the complexity and limitations of full factorial design (Miller & Miller, 2010), and main effects are confounded with interaction terms, thus interaction is completely ignored and the main effects are calculated, while interaction effect can be studied later. The central composite

design (CCD) is used to determine the interaction effect and the optimal extraction conditions using a response surface methodology (RSM) (López et al., 2007)

3.8 Application of SPME in Pesticide Residues Analysis in Fruits and Vegetables

Since its introduction, extensive amount of research work has been done on the use of SPME for the extraction and subsequent chromatographic analysis of all classes of pesticides in fruit and vegetable samples. Therefore the following section focuses on the SPME modes (including the optimization of various parameters as discussed previously in this review), calibration methods used and chromatographic methods adopted by various workers for the extraction and subsequent analysis of different classes of pesticide residues in fruits and vegetables.

Non-polar organochlorine pesticides (OCPs) (*o,p´*-DDT, *p,p´*-DDT, *p,p´*-DDE and *p,p´*-DDD) and pyrethroid (PP) (fenpropathrin, beta-cyfluthrin, cyhalothrin and bifenthrin) residues were determined in celery cabbage, garlic and cabbage, using a 70μm polymethylphenylsiloxane (PMPS) coated fiber prepared by a sol-gel process. The home-made fiber showed better extraction efficiency for the pesticides, compared with the commercially available fibers (85 μm PA, 100 μm PDMS and 65 μm PDMS/DVB). This was attributed to its longer coating (1.5 cm), also to its porous nature, and the presence of phenyl group that enhances π-π interaction between the fiber and the selected pesticides.

The samples of cut-up vegetables (10 g) were mixed with 4μL of 10 μg/mL standard solution and were dried for 30 min, followed by addition of 20mL acetone and the mixture

was mixed ultrasonically for 20 min. The extract was transferred into a 100ml volumetric flask and was made up to the mark with ultrapure water at 1:5 (v/v). A 10mL aliquot of the solution was placed in a 15mL glass vial and was extracted by DI-SPME at room temperature for 20 min which showed higher extraction efficiency than the HS-SPME at 90 ^{0}C for 30min, stirred at 1000rpm. The extracted analyte was desorbed at 280 ^{0}C for 4 min into GC-electron capture detector (ECD) for analysis. Dilution factor was not considered due to the low solubility of pyrethroids in water.

The extraction and desorption temperature and time, extraction mode and fiber type were chosen after full optimization of the conditions. Addition of salt to the sample matrix was found to decrease extraction efficiency and thus was not used in the method validation step. Quantification was performed by external standardization in a blank vegetables spiked at 0.5 – 100 ng/g and analyzed in triplicate. The linear range was found between 0.5 to 100 ng/g with correlation coefficient (R^2) greater than 0.99 for all pesticides. The recovery of the pesticides spiked in various vegetables at 4 ng/g ranged from 42.9 % to 105.3 %, the relative standard deviations (RSD) ranged between 2.6 to 16.2 %, and the limit of detection (LOD) was between 0.13 and 1.45 ng/g (Zeng et al., 2008).

An HS-SPME-GC-ECD method for the multiresidue analysis of OPP (diazinon, malathion, chlorpyrifos, profenofos, and quinalphos) and OCP (α and β endosulfan and chlorothalonil) residues in tomato and guava was developed. The developed method involved the extraction of 1 g of homogenized sample of fruits and vegetables in 100 µL of 20 % methanol/acetone (1:1, v/v) mixture, and distilled water containing 10 % NaCl was

added to make a total mass of 5 g. The mixture was extracted with 100 µm PDMS fiber, which was selected due to its lower detection limit and higher sensitivity for all pesticides investigated. The extraction was carried out in the headspace mode under constant stirring (800 rpm) at 60 ^{0}C for a pre-equilibrium time of 30 min and desorbed at 240 ^{0}C for 6 min, after optimization of necessary parameters. Internal standard (1-chloro-4-flourobenzene), added to the sample mixture before SPME extraction, was used for quantification. The detection response of the fiber was found to be enhanced by addition of water up to a certain dilution factor and an optimum dilution factor, giving a recovery between 82 and 97 % with RSD ranging from 0.3 to 3.3 % (n=3). The linearity ranged from 0.5 to 5000 µg/L and the LOD was between 0.1 and 1 µg/L (Chai, Tan, & Asha, 2008a).

A method for the determination of 7 pyrethroid pesticides (PP) (bifenthrin, lambda-cyhalothrin, permethrin, cyfluthrin, cypermethrin fenvalerate and tau-fluvalinate) in water, fruit (tomato) and vegetable (strawberry) samples was proposed. The method developed was based on DI-SPME and subsequent desorption into the injection port of a GC-MS. The SPME procedure involved the extraction of 0.5 g of chopped samples of tomato and strawberry mixed with 2.5ml of distilled water and 3 ml of 20 % NaCl solution and was thoroughly mixed by shaking in an ultrasonic bath for 30 min. Extraction parameters were optimized, and the maximum peak area was observed at an extraction temperature of 40 ^{0} C, at an equilibrium time of 30 min in 5 mL vials, at an agitation speed and time of 120 rpm and 20s respectively, using 65 µm PDMS/DVB fiber. The fiber used show efficient absorption of the pesticide residues, and an increase in extraction efficiency using a mixture of hexane/acetone (1:1) as the extraction solvent. The extracted pesticide was

desorbed into the GC injection port with a mass spectrometry (MS) detector at 270 ^{0}C for 5 min (optimized time for complete desorption).

The linearity, RSD, LOD and limit of quantification (LOQ) values were determined by plotting a five point calibration curve (external standard) prepared in matrix by spiking blank chopped tomato samples with methanolic standard mixture of the pyrethroids and analyzed using the developed method by GC-MS in the selected ion monitoring (SIM) mode, by selecting one target ion and between 2 – 4 reference ions. The DI-SPME procedure showed a linear behaviour in the range of 0.01 – 0.1 mg/kg with R^2 values ranging from 0.976 and 0.999. The LOD were between 0.003 and 0.025 mg/kg and LOQ for all pesticides investigated was 0.05 mg/kg with RSD (n=3) determined at three different concentration levels: below 20% at 0.25 mg/kg and below 25 % at 0.1 and 0.05 mg/kg (Beltran et al., 2003).

Berrada, et al, investigated residues of 3 phenylurea pesticides (PUP); metobromuron, monolinuron and linuron residues, and their aniline homologous in vegetable samples, including carrot, onion and potato. Fifty grams of a previously cut vegetable sample was extracted in juice extraction, and the aqueous extract was made up to 50 ml with ultra-pure water. A 5 mL aliquot was extracted in the presence of 14 % NaCl (w/v) at pH = 4 for analysis of phenylurea and pH = 11 for both phenylurea and aniline metabolites. The mixture was extracted using 85 µm PA fiber at a room temperature for 60 min and desorbed at 300 ^{0}C for 5 min, and optimization of extraction variables using two GC detectors; nitrogen-phosphorous detector (NPD) and MS. The linearity ranges from 2.5 –

2500 µg/kg with correlation coefficient greater than 0.995 for both phenylurea, and their aniline metabolites. LOQ ranges from 0.8 to 2.2 µg/kg and average recovery of less than 10 % was obtained for the herbicides, with the MS detector showing lower LOQ and recoveries compared to NPD (Berrada et al., 2004).

A new fiber coating, vinyl crown ether (VCE), prepared by the sol gel process was used for pesticide analysis in fruit samples. The fiber was found to have higher extraction efficiency and sensitivity for organophosphorus pesticides (dichlorvos, phorate, diazinon, methyl parathion, fenitrothion, malathion, parathion and ethion), compared to the commercial fibers namely 85 µm PA and 65 µm PDMS/DVB. The extraction efficiency was attributed to the cavity structure and strong electronegative effect of the heteroatoms on the ring of the crown ether, as well as its porous three-dimensional network, making it to exhibit medium polarity, which provides a higher surface area and better selectivity for polar compounds. The extraction involved the optimization of several factors affecting the performance of SPME in fruit samples (apple, apple juice and tomato). All extractions were performed in a 25 mL vial containing 15 mL aliquot, obtained by fortifying a comminuted and homogenized fruit sample spiked with an appropriate pesticide standard solution and diluted with water containing 5 g NaCl. The HS- and DI-SPME mode was employed for 45 min at 70 ^{0}C, and 60 min at 30 ^{0}C respectively.

The extracted analytes were desorbed into a GC with an MS detector at 270 ^{0}C for 5 min. The HS-SPME showed good extraction efficiency and was selected for further method validation. The dilution of sample matrix with water, at a dilution ratio of 1:30, 1:50 and

1:70 for apple juice, apple and tomato respectively, reduces the matrix effect and improves recovery. The analytical methods was validated using standard addition, the LODs were 0.003 – 0.075 ng/g for apple juice, 0.032 – 0.09 ng/g for apple and 0.0042 – 0.076 ng/g for tomato. The relative recovery was between 55.3 – 106.4 % for sample matrix spiked at three different concentration levels with RSD between 3.3 – 10.1 % (n=3). The linearity range was found to be 0.1 – 0.5 ng/g for apple and apple juices and 0.1 – 100 ng/g for tomato with correlation coefficient greater than 0.993 in all samples analyzed (Cai et al., 2006).

An analytical procedure based on ultrasonic extraction of target analytes (carbaryl, diazinon, malathion, chlorpyrifos, profenofos, quinalphos, α and β endosulfan and chlorothalonil), from cucumber and tomato was developed. 1.0 g of homogenized vegetable samples, diluted with 5mL of distilled water followed by DI-SPME. The method involves immersing a 100 μm PDMS fiber into a solution of previously extracted sample at room temperature under magnetic stirring for 15 min. The extracted analytes was desorbed in split mode into GC- ECD injector for 7 min at 270 ^{0}C. Calibration plots were constructed by extraction of target analyte in aqueous solution spiked with standard solutions at 7 different concentration levels and a constant volume of internal standard. All optimized extraction conditions showed positive effects on the extraction efficiency up to the optimum values which were then selected for method validation. The SPME procedure showed a linear behavior between 0.001 – 200 mg/kg with R^2 values for all pesticides greater than 0.994. The LOQ ranges from 0.0005 – 0.01 mg/kg with LOQ between 0.001 and 0.05 mg/kg in water spiked with pesticides. Recoveries were between 53 and 75 % for

cucumber and 53 – 82 % in tomato with RSD (n = 3), below 10 % (1.27 – 8.93 %, for cucumber and 0.47 – 9.3 % for tomato) (Chai, M. K. et al., 2008b).

Chai, et al., evaluated the use of circulating cooling (CC)-SPME combined with GC-NPD, for the determination of 5 organophosphorus pesticides (OPPs) in tomato. The method involved simultaneous cooling of the fiber used in the headspace mode and heating the sample matrix. The method developed showed a better extraction efficiency in terms of sensitivity, linearity, and recovery better than the traditional HS-SPME. The procedure involved extraction of 5 g of spiked homogenized tomato sample, mixed with 10 mL of water containing 2 g of NaCl at 80 ^{0}C for 30 min in the headspace mode using CC-HS-SPME using activated carbon fiber (ACF), and desorbed at 270 ^{0}C for 2 min. The method was compared to the traditional HS-SPME based on the same parameters. The two methods showed good and acceptable linearity, recovery and detection limits. The CC-HS-SPME showed a wider linear range (1– 200ng/g), with correlation coefficient better than 0.987, and lower detection limit (0.2 – 0.5 ng/g). The mean recovery range from 82.5 to 90 % with RSD of 5.9 – 8.7 % (n=3), which is better than the results obtained by HS-SPME (lower than 9.2 %) (Chai, X. et al., 2008).

An SPME GC-MS method for the determination of 8 OPPs (phorate, diazinon, methyl parathion, fenitrothion, malathion, fenthion, ethyl parathion, and methidathion) in different fruit juices and wine was developed by Zambonin and his co-workers. The juice samples were centrifuged and diluted as required, while the wine sample was directly subjected to SPME, without dilution. The DI-SPME extraction was carried out at room temperature

under magnetic stirring for a non-equilibrium time of 30 min using 85 µm PA fiber. The analytes adsorbed on the fiber were desorbed at 250 ^{0}C for 5 min. Validation of the developed method was based on quantification with an external standard prepared by spiking the analytes in 10 mL of triply distilled water. The calibration curves gave good linearity for all investigated range (10 – 500 ng/mL) and correlation coefficient better than 0.992. The estimated LOD and LOQ ranged from 2 – 90 ng/mL and 7 – 297 ng/mL respectively in all the fruits investigated. The addition of salt and dilution with water showed a significant difference in extraction while change in pH (4-11) had no significant difference on the extraction efficiency (Zambonin et al., 2004).

Blasco, et al., established an analytical procedure for the determination of postharvest and relatively polar fungicides viz, dichloran, flutriafol, *o*-phenylphenol, prochloraz, tolclofos methyl in fruits (cherries, oranges and peaches) using MRM with SPME-LC/IT-MS (MS/MS) with atmospheric pressure chemical ionization (APCI). The selectivity of LC/MS/MS was compared with LC/DAD and LC/MS. One gram of sample was homogenized in acetone/water mixture (5:1, v/v), by sonication for 15 min and then centrifuged at 3000 rpm for 20 min, filtered and the mixture evaporated. The resulting aqueous solution was extracted by DI-SPME in a 2 mL vial, adjusted to 1 mL containing 300 mg of NaCl with continuous stirring (1000 rpm) for 90 min using carbowax/template resin (CW/TPR) fiber coating. The extracted analytes were desorbed into an LC injection port operated in the static mode using a solvent containing a mixture of methanol/water (70/30, v/v %) for 10 min.

The method validation showed good linearity and sensitivity, with better LOQ (0.0005 – 0.01 mg/kg). The quantification determined using a six point calibration curve for fruit samples spiked with the analytes gave LOD of 8 µg/L for dichloran, 80 µg/L for flutriafol, *o*-phenylphenol and tolclofos methyl and 120 µg/L for prochloraz. The recovery was between 8 and 69 % with prochloraz showing the lowest (5-22 %) with RSD 0f 0.5–12 % (n = 3) and linearity range of between 0.0005 – 10 µg/mL ($R^2 > 0.995$). Pesticide residues were detected in 60 % of the fruits analyzed with tolchlfos methyl exceeding the MRLs. The extraction efficiency of CW/TPR, PA, and PDMS/DVB, 7, 30 and 100 µm PDMS fibers was evaluated. The 100 µm PDMS gave the best recoveries, and the PDMS/DVB fiber was found to exhibit slightly better extraction efficiencies for the analytes than CW/TPR, but the CW/TPR fiber was used for the experiment because the PDMS/DVB coating was stripped off after two analysis in the interface during the desorption in water/methanol mixture (Blasco et al., 2003a).

A HS-SPME and GC-ECD methods was developed for the analysis of 5 OPPs (diazinon, malathion, chlorpyrifos, profenofos, and quinalphos) and 3 OCPs (α and β endosulfan and chlorothalonil) in fruit (star fruit, strawberry and guava) and vegetable (cucumber, tomato) samples. 1 mL of the pesticides was spiked into 30 g of chopped fruit and vegetable samples drop wise and the mixture was kept at room temperature for 1 hr and 30 mL of distilled water added. The resulting mixture was homogenized and 5 g aliquot was placed in 15 mL clear glass vial extracted using 100 µm PDMS fiber in the headspace mode. The extraction conditions (60 ^{0}C, 30 min, stirring) were chosen after careful optimization of variables. Complete desorption was achieved at 240 ^{0}C for 15 min. Quantification was

carried out on a five point calibration using an internal standard and the analytes spiked into the sample matrix, using a dilution factor of 2 and 2 % (v/w) of organic solvent. The recovery which was increased by the addition of water and organic solvent has an average range of 70 – 99 %, with RSD less than 10 %, for all pesticides spiked in all the fruit and vegetable sample investigated. The calibration curve showed linearity between 1 to 400 mg/kg with correlation coefficient greater than 0.99 (Chai, Tan, & Asha, 2006).

Chai and Tan, performed the validation of the optimized HS-SPME parameters for the analysis of diazinon, malathion, chlorpyrifos, profenofos, quinalphos, α- and β-endosulfan and chlorothalonil in strawberry, guava, cucumber, tomato and pakchoi samples, using a previously developed method (Chai, M. K. et al., 2008a). Thermal desorption was carried at 240 ^{0}C for 10 min instead of 6 min used previously, this helped to overcome carry-over problem and the reduction in the injector port temperature. The results showed that desorption at 10 min gave a better extraction efficiency compared to 4 min desorption time. The average recovery obtained for each pesticide ranged from 71 and 98 % at three fortification levels with RSD less than 5% (n=3). Repeatability (0.3 – 3.7 %) and intermediate precision (0.8 – 2.5 %) was found to be satisfactory for all samples investigated. Quantification using internal standard yielded LOD (0.01 – 1 µg/L) and LOQ (0.05 – 5 µg/L) with linearity between 0.1 and 5000 µg/L and correlation coefficient greater than 0.99 (Chai & Tan, 2009).

A method was also developed to investigate the effects of washing fruit and vegetable samples with different solutions on the persistence of OPP and OCP residues. The

extraction procedure followed a previously developed method (Chai & Tan, 2009). The pesticides free sample (strawberry and cucumber) were soaked for 1 hr in tap water spiked with 2mL of standard stock solution of different concentrations (0.5 – 50 mg/L). The spiked samples were air-dried at room temperature and soaked in acetic acid, sodium carbonate, sodium chloride and water (5 and 10 % each), for 10 and 30 min. The dried samples were extracted by HS-SPME at 60 ^{0}C for 30 min with stirring at 800 rpm without pH adjustment and desorbed in GC-ECD at 240 ^{0}C for 10 min. The method linearity ranged from 0.1 to 5000 µg/L ($R^2 > 0.996$), with RSD < 4 %. The LOD was 0.01 mg/L and LOQ were between 0.05 to 5 mg/L, which is three orders of magnitude lower than the EU MRL (50 – 5000 mg/L). The results showed that washing the fruit and vegetables with various solvents of different pH was effective in reducing the residues of OPP and OCP on samples. Acetic acid was found to be the most effective solvent, and removed more than 70% of the pesticide residues. The percentage removal was shown to increase with increase in concentration of the solvents and treatment time, the amount of pesticide residues removed by various solvents was also found to be dependent on the solubility of the pesticides in water (Chai & Tan, 2010).

A vanguard-rearguard method was developed by Cortes-Aguado, et al., for the analysis of 70 multiclass pesticides (MCP), from cucumber, pepper and tomato samples. The method is based on binary analysis: the screening (vanguard) method is used to classify samples as negative and potentially non-negative (samples that contain pesticides higher than the MRLs), and a quantifying (rearguard) method applied to samples that are previously classified as potentially non-negative. The extraction step involves the pre-extraction of the

analytes with 10 mL of ethyl acetate using 4 g of the sample, followed by evaporation and the residue was re-dissolved in 10 mL of mixture of water/acetone (9:1 v/v) solution. 5µl of the IS solution was added to a 1 mL vial and the volume filled up with the water/acetone extract, was then extracted using 65µm PDMS/DVB fiber in DI-SPME mode at ambient temperature for 55 min. The extracted analytes were desorbed into a GC-MS for 9 min at 250 ^{0}C. Only 12 pesticides were confirmed and the procedure gave good extraction efficiency with recovery between 77 and 106 %, with RSD of 3 – 11 %. The LOD (0.0006 – 5 µg/kg) and LOQ (0.002 – 3 µg/kg) were found to be one-to-three orders of magnitude below conventional pesticide residues methods. All samples that were analyzed contained pesticide residues, but only the pepper sample was above the MRL (Cortés Aguado et al., 2007).

A method based on focused microwave assisted extraction (FMAE), using microwave energy (132 W) coupled to an SPME device was proposed for the analysis of dichlorvos in vegetable. The microwave energy was used to irradiate the sample in a 50 mL ground bottle containing 2 g of whole sample dissolved in 20 mL aqueous solution spiked with 300 µg/L of dichlorvos in 10 % ethylene glycol, and extracted in the headspace mode for 10 min at a pH=5. The extracted analytes was desorbed into a GC–ECD at 220 ^{0}C for 3 min. The addition of salts to the sample solution decreased the extraction efficiency and was not used for method validation. The method was validated by plotting a calibration curve, which gave a linear range of 5 – 75 µg/L with a correlation coefficient of 0.9985. Recovery (106.1 %) was good for all samples with RSD less than 8 % (5.5 – 7.9 %). The

investigated pesticide was detected in pakchoi at a concentration of 8.65 µg/L (Chen, Y. I. et al., 2002).

A method was developed for rapid screening of triazole pesticides, in wine and strawberry by optimizing SPME-GC-MS conditions. Fifty grams (50 g) of homogenized strawberry sample was centrifuged for 30 s and mixed with 40 mL tri-distilled water and centrifuged again, while the wine sample was filtered through a 0.45 µm Millex-HV filter and diluted 1:2, with an aqueous solution containing 0.2 g/mL of NaCl. The resulting aqueous solution of the strawberry sample was recovered and brought to 100 mL with an aqueous solution containing 0.2 g/L NaCl solution. An aliquot of 5 mL of solutions of wine and strawberry were transferred into a separate 7 mL clear vial for SPME extraction. The extraction of sample was carried out at 50 ^{0}C for a pre-equilibrium time of 45 min under magnetic stirring using the direct immersion mode, which was desorbed at 250 ^{0}C at 5 min. The method was validated and quantification was achieved by standard addition method. The RSD ranged between 2 – 11 % (n=5) and the detection limit estimated at a signal to noise ratio of 3 ranged from 30 to 100 mg/kg. The dynamic range of the method using the SIM mode was found to be linear over at least two concentration decades with the correlation coefficient better than 0.999 and an intercept not significantly different from zero. All pesticides investigated were detectable except propiconazole and appeared completely separated from interfering peaks (Zambonin et al., 2002).

A method in which 4 pesticide residues were extracted by DI-SPME using 100 µm PDMS fiber was proposed for the analysis of pesticide residues in biphasic water/plant tissue

mixture and analyzed by GC coupled to flame photometric detector (FPD). In the extraction procedures, plant tissues were not separated from the aqueous solution before extraction and the pesticides were distributed between the aqueous phase and plant tissue, and the SPME fiber was in equilibrium with the analytes in the aqueous phase. Optimum extraction conditions used were: room temperature, 90 min, agitation and pH=7. The water/plant tissue partition coefficient of the pesticides was also investigated in a series of samples containing 50 g of fresh vegetables and various amount of deionized water which were well mixed. An aliquot (5 g) of the resulting pastes were placed into 10 mL vials and extracted at the optimum conditions and desorbed at 270 ^{0}C for 5 min. Validation of the method gave LOD between 9 – 75 ng/g, with recovery of about 25.5 % and RSD ranges between 1.5 – 19.8 % (n=15). All 4 OPPs investigated showed good linearity up to 100 ppm. The experimental water/plant tissue partition coefficient of the pesticides was found to correlate remarkably with their octanol/water partition coefficients (Chen, W., Poon, & Lam, 1998).

A new sol-gel derived bisbenzo-16-crown-5 (B16C5) ether/hydroxyl-terminated silicone oil (HO-TSO) SPME coating coupled to GC flame photometric detector (FPD), was used for method development in the analysis of 10 OPP residues. The sample of orange juice was diluted in water (1:50), saturated with NaCl placed in a 25 mL vial and spiked with pesticide standard solutions, while 10 g of pakchoi was cut and homogenized with 100 mL of water (1:50), and 23 mL aliquot of each of the mixture was used for DI-SPME extraction. Extraction temperature of 55 ^{0}C and 20 ^{0}C was validated at 60 min under constant stirring for all samples, and desorbed at 270 ^{0}C for 5 min. The analysis was

quantified using an IS to construct a 6 point calibration plot. The linearity of the plot was between 1 – 500 ng/g with correlation coefficient greater than 0.99. The LOD ranges from 0.003 – 1 ng/g with relative recovery of 76.8 – 101.2 % and RSD between 2 – 9.2 %. Pesticide residues were found in orange (2.1 ng/g of triazophos and 10.2 ng/g of fenitrothion) and pakchoi (6.8 ng/g) (Yu, J. et al., 2004).

A vanguard-rearguard analytical method was also employed for the SPME determination of 54 multiclass pesticide (MCP) residues in orange, peach and pineapple juice samples. The method was found to be very simple, fast and reduces the average extraction time by 50 %, and minimized human errors. The procedure involved the pre-extraction of 1 mL homogenized samples with 1 ml ethyl acetate and centrifuged for 2 min. 0.5 mL aliquot of the ethyl acetate extract was evaporated in a vial by a soft stream of nitrogen, followed by addition of 1 mL of water/acetone (9:1 v/v) mixture, containing 0.2 mg/L of IS. The resulting mixture was extracted using 65 µm PDMS/DVB in the direct immersion mode at room temperature and desorbed at 250 ^{0}C for 9 min. Similar extraction conditions were used for screening (vanguard) and confirmation (rearguard) methods, except with an extraction time of 10 min and 55 min respectively. Validation of the confirmation/quantification (rearguard) method gave good recovery between 71 – 107 % with RSD of 2 – 17 %. The LOD and LOQ were calculated and varied between 0.01 – 16.7 µg/L and 0.1 – 50 µg/L respectively, with linearity from 0.01 up to 1 µg/L with coefficient higher than 0.99, for all samples (Cortés Aguado et al., 2008).

A radish sample was analyzed for the presence of pesticide residues of 12 OCPs, using a sol-gel derived calix[4]arene/hydroxyl-terminated silicone oil (C[4]/HO-TSO)-coated SPME fiber. The extraction was carried out in the headspace mode. Samples of 100 g of radish were comminuted and homogenized with 100 mL of water; the homogenate of 25 g was further diluted to 100 mL with water. All extractions were performed with a 12 mL amber vial, containing a 10 µL aliquot of the standard solution, 4 mL of radish matrix solution and 1.0 g K_2SO_4 and were carried out at 70 ^{0}C for 30 min with constant stirring at 600 rpm. The extracted analyte was desorbed at 270 ^{0}C for 2 min in a GC coupled to an ECD. Method validation which was determined by a calibration curve constructed using a method of standard addition gave a linearity of 1 – 10,000 ng/L with correlation greater than 0.992. Recoveries for all the analytes was between 79.85 and 119.3 % with RSD (n=5) of 7.61 – 13.1 %. The LOD was found to range from 0.185 and 21.7 ng/L. The higher extraction efficiency of the fiber used, compared to 100 µm PDMS, was attributed to π-π and hydrophobic interactions of the sol-gel prepared fiber (Dong et al., 2005).

The analysis of carbamate pesticides (CP); methiocarb, napropamide, fenoxycarb and bupirimate in strawberry using SPME/LC/diode array detector (DAD), was carried out with a 60 µm PDMS/DVB. An aliquot of 125 µL of mixed standard solution at 0.01 mg/mL, 50 and 100 µL at 0.05 mg/mL and 50, 100 and 200 µL at 0.25 mg/mL were added to 25 g of frozen strawberry respectively. The sample was defrosted, blended and transferred into a 50 mL tube; 20 mL of water was added and then centrifuged. The resultant supernatant was collected in a 50 mL volumetric flask and another 20 mL of water was added and centrifuged again. The final volume of the extract was adjusted to the

mark with water. An aliquot of 4 mL of the extracted solution was subjected to SPME for 45 min at room temperature with constant stirring of 1000 rpm. The extracted analytes were separated on a Pinnacle ODS Amine (5mmx 250 x 4.6 mm) column in a static mode with a mobile phase solvent containing 55/45 (v/v %) of acetonitrile/water mixture at a flow rate of 1 mL/min. The calibration curve showed linearity of 0.05 – 2 mg/kg and correlation coefficient greater than 0.99 was constructed for quantification using strawberry spiked with a standard solution of pesticides. A good repeatability with RSD between 2.92 and 9.25 % was obtained and LOD of 10 – 50 µg/kg. The better repeatability determined in this method was attributed to the characteristic detection precision of UV detectors like DAD and the observed performance showed that SPME/LC is a good complementary analytical tool to SPME/GC for pesticide residue analysis in food, especially for thermally-labile or non-volatile pesticides (Wang et al., 2000).

A sensitive and efficient SPME method for the determination of seven pyrethroid pesticides (PPs), including fenpropathrin, alpha-cyhalothrin, deltamethrin, fenvalerate, permethrin, τ-fluvalinate and bifenthrin in cucumber and watermelon samples using liquid chromatography combined with post-column photochemically induced fluorimetry derivatization and fluorescence detection (LC–PIF–FD), was developed and validated. 1 g of chopped, mixed and homogenized spiked sample (cucumber or watermelon) with standard solutions of pesticides mixture was diluted with Milli-Q water (1:10, w/v) in a 10 mL volumetric flask containing 2.5m L of ACN solution and 0.7 ml of 0.1 M K_2HPO_4/H_3PO_4 buffer (pH 3). An aliquot of 3 mL of this solution was used for DI-SPME extraction with 60 µm PDMS/DVB at 65 ^{0}C for 30 min with a stirring rate of 1100 rpm.

The extracted analytes were desorbed into a HPLC column by a mobile phase (acetonitrile/water, 80:20, v/v %) in a static mode with DAD, with simultaneous detection at a wavelength of 283 and 330 nm. Quantification was determined statistically according to the EURACHEM standard, and the LOQ obtained was between 1.3 and 5 µg/kg, with the LOD between 0.1 and 1.1 µg/kg. Recovery value calculated at 2 concentration levels ranged between 91 and 110 % with RSD (n = 6) of 2 – 9.4 % for both fruit samples. The calibration curves showed good linear relationship and ranged between 0.0013 and 1.5 mg/kg ($R^2 \geq 0.996$) (Vázquez, Mughari, & Galera, 2008).

The precision of different detectors (MS and MS/MS) was compared for SPME-GC analysis of 4 carbamate pesticide (CP) residues in apple and grape juices. Food samples were prepared by diluting 50 µL of liquid matrix, spiked at different concentration with the analytes, in 10 ml of distilled water containing 30 % of sodium chloride, placed in a 12 mL dark glass vial and extracted in the DI mode at room temperature for 30 min under magnetic stirring. The extracted analyte was desorbed at 250 ^{0}C for 15 min. A long desorption time was selected in order to purge any residue of the target analytes and to eliminate eventual interferences of co-extracted compounds. The precision of each detector was reported as relative standard deviation of three replicates. The RSD in the two detectors were comparable, but the MS detector gave a better precision (RSD = 3.4 – 17.6 %) than the MS/MS (RSD=2.2 – 36.2 %) for all analytes investigated in grape and apple juices, while the MS/MS yield better precision for myclobutanil (2.2 %) and acetochlor (3.1 %) than the MS in the white wine sample. The detectors gave good linearity ranging from 20 to 2000 µg/L, and the limit of detection was lower in the MS detector (0.1 – 3

µg/L) than in MS/MS detector (2 – 17 µg/L). The sensitivities of both detectors were found to be generally comparable, with the GC/MS having the better sensitivity (Natangelo, Tavazzi, & Benfenati, 2002).

An analytical method was proposed for the determination of OCP, OPP and organonitrogen pesticide (ONP) residues in grape fruits. Six SPME fiber coatings containing different percentages of activated charcoal and polyvinylchloride were evaluated for their extraction efficiency. The fiber containing 70:30 % of activated charcoal/polyvinyl chloride (AC/PVC), was found to have highest efficiency, and was used for method validation. The analytes were extracted in the DI-SPME mode at room temperature for 25 min in the presence of 0.5 g of NaCl at a constant stirring rate of 900 rpm, and were desorbed into GC-FID at 200 ^{0}C for 5 min. The method gave good linearity which ranges from 25 – 5000 ng/L, and a recovery of between 42 and 63% (RSD = 5.8 – 9). The LOD were found to be in the range 8 – 40 ng/L. The use of NaCl at higher concentrations was observed to decrease the extraction recoveries, because the NaCl crystals can occupy some of the active sites of the coated fiber The proposed fiber showed high extraction capacity, good stability and low cost of production (Farajzadeh & Hatami, 2004).

A method was described for the analysis of residues of OPP in pear and fruit juice. Samples of 20 g of fruit and juice were spiked at different concentration levels, with the pesticides stock solution. The fortified juice were diluted (1:100) and extracted by DI-SPME using 100 µm PDMS fiber for 25 min at room temperature and 1250 rpm, and then

desorbed into GC-FPD for 2 min at 250 ^{0}C. Linearity determined using a calibration curve for quantification was between 0.250 and 25 µg/L, with correlations greater than 0.998. The recovery was good for all pesticides and range from 75.9 – 102.6 % and RSD of 1.6 – 8.7 % at triplicate analysis. LOD and LOQ ranged from 0.004 to 0.014 µg/L and 0.016 to 0.070 µg/L respectively. The recoveries were found to improve by diluting samples of the fortified juice, and the recoveries observed were four times higher than the recoveries from the undiluted sample. The origin of interference was identified by separately adding sodium dodecyl sulfate and pectin to a standard solution of pesticides. It was shown that suspended and dissolved matters could interfere with the analysis by forming micelles and slow down the diffusion of analytes towards the fiber. The effect of pectin was reduced by addition of pectinase. The proposed method was found to be applicable for the routine analysis of pesticide residues in fruits and the dilution with water helps to reduce matrix effects (Simplício & Vilas Boas, 1999).

A new analytical method was developed for simultaneous determination of 14 MCP residues, in mango fruits using SPME/GC/MS. The sample (3 g) was weighed into a 20 mL vial, fortified with 50 µL of the pesticide standard solution and was allowed to rest for 10 min, followed by the addition of 10 mL of a 20:80 (v/v, %) isopropyl-alcohol/water mixture containing 5 % NaCl at pH 3. The resulting mixture was stirred at 1000 rpm and the upper layer transferred to a 10 mL volumetric flask, and the volume completed with the alcohol/water mixture. The resulting solution was then transferred to a sealed 10 mL headspace vial for the SPME extraction using a 85 µm PA fiber by direct immersion mode,

at 50 ^{0}C for 30 min, while stirring at 250 rpm. The fiber was placed in the GC injector for desorption for 5 min at 280 ^{0}C.

The method developed was validated using an external standard calibration constructed with nine point concentrations (1 – 500 ng/mL) and each analyzed in triplicate. The average relative recovery (n = 3) for the lowest concentration level ranged from 71.6 to 117.5 %, with relative standard deviations between 3.1 and 12.3 %. The addition of small portions of binary mixtures (water/ethanol, water/isopropanol and water/acetonitrile at 80:20 v/v %) was investigated in order to reduce the matrix effect. The mixture of water/isopropanol was found to efficiently extract nine pesticides with larger peak areas compared to other three solvents in pure water. Detection and quantification limits ranged from 1.0 to 3.3 μg/kg and from 3.33 to 33.33 μg/kg respectively. The method was found to be selective, sensitive and with good precision and mean recoveries and the residue levels below the MRLs values of the pesticides investigated (Menezes Filho et al., 2010).

A total of 150 samples of 21 types of fruits sold in Greek markets was collected and analyzed for the presence OPP residues. The fruit samples (5 g) were homogenized in 5 mL of water with a blender and 3 mL of the homogenate were transferred into an SPME vial containing 0.8 g of NaCl. The sample was then spiked with methanolic solutions of OPs (50 and 500 μg/mL) and placed in headspace vial. The resulting solution was subjected to headspace extraction using 85 μm PA fiber for 20 min. The analytes were desorbed by inserting the fiber into the heated injector port of the GC for 4 min at 230 ^{0}C. The method linearity ranged from 1.2 to 667 ng/mL, giving correlation coefficient values

of 0.998 – 0.999. The LOD and LOQ ranged from 0.03 to 3 ng/mL and 0.12 to 10 ng/mL respectively. The absolute recoveries were low and ranged from 0.3 to 3.1 % with RSD between 2.5 and 8 %. The effects of washing and peeling was also investigated and the results showed that 18 % of the pesticide residue can be removed by washing, while peeling was found to be more effective, and was able to remove about 85 % of the residues (Fytianos et al., 2006).

A method based on off-line SPME and capillary electrophoresis (CE)/MS has been described, for the determination of acidic pesticides (AP); o-phenylphenol, ioxynil, haloxyfop, acifluorfen and picloram in apple, orange, grape and tomato samples, using CW/TPR fiber. A 200 g of the fruit samples was chopped and a 5 g portion was homogenized with 0.5 mL of 0.1 M NaOH and 5 mL acetone by sonication for 15 min. The resulting supernatant was filtered and the acetone was evaporated at 50 ^{0}C under a stream of nitrogen. The resulting aqueous phase was placed in a 2 mL vial containing 250 mg of NaCl and the pH adjusted to 3. The extraction was carried out by direct immersion at a constant stirring rate of 1000 rpm for 120 min. The extracted analytes were desorbed from the fiber, by sonication for 15 min at a buffer temperature of 15 ^{0}C, with 100 µL of methanol and running buffer (ammonium formate–formic acid) of 0.5 mL. The recovery of the analytes ranged from 7 to 94 %, and RSD was between 3 and 13%. The method was found to be linear between 0.02 and 500 mg/kg with correlation coefficients ranging from 0.992 – 0.997 and the LOQ were from 0.02 to 5 mg/kg (Rodriguez, Mañes, & Picó, 2003).

An analytical method was also developed for the determination of pyrethroid pesticides, using focused microwave assisted extraction (FMAE) followed by SPME extraction. A 25 g sample of frozen strawberry was immersed in 50 % acetonitrile in a 20 mL beaker, spiked with a known amount of the mix standard solution, drop by drop, and kept overnight at room temperature. The mixture was introduced into a microwave heating tube and was irradiated at 30W. The cooled solution was collected and decanted. A 9 mL aliquot of the supernatant was transferred into a vial and a 100 µm PDMS fiber was immersed for 30 min at room temperature under constant stirring. The fiber was introduced into a GC/MS injector for desorption at 270 ^{0}C for 2 min. Calibration curve, constructed from blank strawberry spiked at different concentrations with standard solutions, showed a linear range between 1 and 250 µg/kg with correlation greater than 0.992 and coefficient below 15 %. The LOD and LOQ were found to be lower than 14 µg/kg and 40 µg/kg respectively. The validated method was compared to other analysis done on the same sample by two certified trading laboratories and the residues were found in the same order of magnitude, which were far below the MRLs (Sanusi et al., 2004).

A method which involved the use of microwave energy for a fast and controlled pre-extraction of 25 MCP residues from tomato was developed. The tomato sample matrix was prepared by spiking 200 g of frozen tomato with known aliquot of mixed standard solution, drop wise, and kept overnight at room temperature. The spiked tomatoes were introduced into a microwave heating tube with a 240 mL of acetonitrile/water (50:50, v/v %) mixture and were put into a microwave oven. The mixture was irradiated for 5 min at 30W. The sample temperature under microwave effect should not exceed 65 ^{0}C in order to minimize

the possible degradation of thermally labile pesticides, while potential vapour lost was prevented by using a condenser at the top of the extraction vessel. The solution was collected, cooled to room temperature and centrifuged into a brown bottle. Two 9 mL aliquots were removed for SPME analysis. The fibers used were a 100 µm PDMS(for water insoluble pesticides) and a 60 µm PDMS/DVB (for water soluble pesticides), and the analysis was carried out at room temperature with a stirring rate of 500 rpm, for 45 min and 30 min for water soluble and water insoluble pesticides respectively. The use of microwave assisted extraction was found to be effective in suppressing the matrix effects. It also showed high performance in terms of linearity, which ranges from 0.1 to 5000 µg/kg and 10 to 1000 µg/kg for water soluble and water insoluble pesticides respectively and good repeatability with relative standard deviations of 2.2 to15.7 %. The LOD (0.01–7.62 µg/kg) and LOQ (0.20 – 25.4 µg/kg) for all pesticides investigated were much lower than the MRLs (Guillet et al., 2009).

The screening of 13 different vegetables for the presence of OPP residues (diazinon, methyl parathion, fenitrothion, malathion and parathion), was described using HS–SPME coupled to GC/NPD. Fresh vegetable samples (5 g) were cut, diluted with water (1:1) and then homogenized in a speed blender. An aliquot of 3 mL of the homogenate was transferred with 0.8 g NaCl, into a 9 mL headspace vial, capped and shaken thoroughly with a Vortex mixer. The sample mixture was preheated for 15 min, then subjected to SPME extraction at 70 ^{0}C for 20 min using a 100 µm PDMS and then desorbed at 230 ^{0}C for 4 min. The linearity of the method was determined by plotting a calibration curve, and the results showed regression in the concentration range 2.31 – 662 ng/g, with correlation

coefficient between 0.998 and 0.999. The LOD was between 0.06 and 5 ng/g while the LOQ ranged from 0.21 to 13.3 ng/g. The RSD value was between 2.2 and 7.6 % with an absolute recovery of 0.3 – 3.1 %. The fiber performance was not affected by the sample matrix which prolonged fiber life time (Fytianos et al., 2007).

An analytical method was developed for the determination of carbamate pesticides (CP); pirimicarb, benfuracarb, carbofuran, carbosulfan and diethofencarb and phenylurea pesticide (PUP); monolinuron, diuron and monuron, residues in orange, strawberry, cherry and apple juices, using SPME coupled to LC/MS and LC/MS/MS. The sample was prepared by taking 7 mL of fresh fruit juice and centrifuged for 15 min, followed by filtration of the supernatant. An aliquot of 0.5 mL of the filtrate was diluted with water (1:1 v/v), in a 1.5 mL screw cap vial containing 30 % NaCl and was extracted with 50µm CW/TPR and 60µm PDMS/DVB at 250 ^{0}C for 90 min at a stirring rate of 1000 rpm. The extracted analytes were desorbed in the static mode for 5 min, with a mobile phase mixture of methanol/water (70:30, v/v %) at a flow rate of 0.5 ml/min. The recovery ranged from 25 to 80 % with RSD between 1 and 17 %. The LOQ was between 0.005 – 0.05 ng/g, while the LOD ranged from 0.001 to 0.005 ng/g (Sagratini et al., 2007).

The results obtained when focused microwave assisted extraction (FMAE) was used prior to SPME, for the analysis of carbamate pesticide (CP) residues in strawberry was presented. The method does not involve blending and centrifuging of the strawberry sample before extraction. A whole frozen strawberry (25 g) was weighed into a beaker, and spiked with a known amount of pesticide standard mixture drop by drop and kept at room

temperature overnight. The mixture was then transferred to a microwave heating tube, and heated for 7 min at 30 W. The mixture was cooled to room temperature and the supernatant was decanted into a brown bottle. A 4 mL aliquot of the extract was transferred into a vial containing 50 mg powdered Na_2PO_4 and was subjected to DI-SPME using 60 µm PDMS/DVB, and stirred at 1000 rpm for 45 min. The analytes were desorbed into an LC/DAD injection port with a mobile phase containing a mixture of acetonitrile/water/methanol (30:50:20, v/v %) at a flow rate of 1 mL/min with simultaneous detection at a wavelength of 205 and 240 nm. The regression coefficient were higher than 0.99 and linearity ranging from 0.05 to 1 mg/kg, with LOD and LOQ from 0.013 to 0.022 mg/kg and 0.044 to 0.074 mg/kg respectively and RSD values from 3 to 7.3 %. The use of FMAE as a sample pre-treatment step preceding SPME for pesticide analysis was found to be more efficient than the blending and homogenizing methods (Falqui-Cao et al., 2001).

An SPME method was described for the determination of 16 MCPs in strawberry. A sample of frozen strawberry was weighed (50 g) into a 150 mL beaker, spiked with different concentrations of pesticide standard mixture and mixed. A 25 g portion of the mixture was diluted with 40 mL of water in a Teflon tube and centrifuged for 5 min at 5200 rpm. The supernatant was collected into a 100 mL flask and was made up to the mark with distilled water. A 4 mL aliquot of the solution was transferred into a 5 mL septum cap vial and the SPME fiber (100 µm PDMS) was introduced and then extraction by direct immersion was carried out with stirring at 800 rpm, for 45 min at room temperature. The extracted analytes were desorbed into a GC/MS injector at 270 ^{0}C for 3 min. The

regression coefficient for 8 of the investigated pesticides were higher than 0.99, six of them were between 0.98 and 0.99, while others were between 0.95 and 0.97. The RSD for 13 pesticides were less than 13 %, while the other pesticides were greater than 20 %. The pesticides with higher RSD (up to 88.9 %) were found to be unstable and degraded within 7 days (Hu, R. et al., 1999).

The analysis of seven multiclass pesticides (7 MCPs) in tomato was investigated, using micellar electrokinetic chromatographic MEKC) analysis. The reversed electrode polarity stacking mode (REPSM) and SPME was used as on-line and off-line preconcentration procedures respectively. Five grams of homogenized tomato were ultrasound-assisted extracted with 5 mL of acetone for 5 min, and the extracts were evaporated to dryness at 45 ^{0}C. A 10 mL aliquot of the tomato sample (pH adjusted to 9.5 by addition of 1 M NaOH) was reconstructed with 10 mL water containing 3 g of NaCl. The resulting solution was placed in 16 mL screw cap vial and extracted in DI mode with 65 µm PDMS/DVB fiber at ambient temperature for 143 min with continuous stirring at 900 rpm. The analytes were desorbed from the fiber with 1 mL methanol by stirring for 13 min at 1000 rpm. The extract obtained from the SPME procedure was evaporated to dryness in a rotary evaporator at 40 ^{0}C, and reconstituted with a mixture of water/sodium tetraborate (1:3), the injection was carried out following the REPSM procedure into MEKC coupled to DAD. The recovery of the analyte ranged from 94 to 102 %, and the RSD was between 3 and 13 %. The method validated using a matrix match calibration curve was found to be linear between 0.5 and 2.5 mg/kg, LOD were between 0.134 – 0.476 mg/kg. The recovery was

independent of the concentrations for the different spiked assayed (Ravelo-Pérez et al., 2008).

The applicability of the HS-SPME was determined for the determination of 7 OPPs in strawberry and cherry juice using GC/FID and GC/MS for analysis. 200 g of the whole fruits were sliced and homogenized for 30 s, then centrifuged and diluted with water (1:1, v/v). The resulting solution was spiked with appropriate amounts of the standard solution (0.5 – 50 µg/L). An aliquot of 5 mL was transferred into a 10 mL amber vial followed by addition of 15 % Na_2SO_4. The mixture was extracted with 100 µm PDMS placed 1cm in the headspace of the sample kept at 75 ^{0}C for 45 min and agitated at 960 rpm. After extraction, the fiber was inserted into the hot injector of the GC system at 240 ^{0}C for 2 min. The method was linear for the concentrations studied with correlation coefficient between 0.986 and 0.999. The recovery ranged from 82 – 105 % with RSD of between 5.2 – 18.7 %, and LOD from 0.0025 to 0.050 µg/L. The responses of the two detectors were found to be similar for all pesticides and samples analyzed (Lambropoulou & Albanis, 2002).

An analytical method was proposed for the determination of carbamate pesticides (carbendazim and thiabendazole) in apple, based on SPME coupled to liquid chromatography. A sample of apple was blended and 25 g of the blended apple was transferred into a Teflon tube followed by addition of 20 mL of water. The tube was vortexed and centrifuged, the resultant supernatant was collected in a 50 mL volumetric flask and 10 mL of water added. The extraction procedure was repeated and the final

volume of the extract was made up to the mark with water. An aliquot of 4 mL of the extract was subjected to DI-SPME with 65 µm PDMS/DVB fiber for 35 min at room temperature and continuous stirring at 1100 rpm. The fiber content was desorbed into the hot injection port of an LC with fluorescence detector in static mode for 8 min, with a mobile phase of methanol/water (50:50, v/v %) mixture at a flow rate of 1.0 ml/min. The method was linear over the range 0.01 – 1 mg/kg, with detection limit between 0.003 and 0.005 mg/kg, quantification limit of 0.006 – 0.01 mg/kg, and correlation coefficient of 0.9995 and 0.9998. The recovery was between 91.5 and 96.1 % with RSD between 3.3 and 4.7 %. The sample pH was found to have no significant effect on the extraction efficiency of the analyzed pesticides (Hu, Y. et al., 2008).

Lambropoulou and Albanis, developed a HS-SPME method in combination with GC/MS for the extraction and analysis of OPP residues, using a 100 µm PDMS fiber. The sample preparation involved the mixing of 5 g of samples containing 15 % (w/v) of Na_2SO_4 with a known amount of water in a vial and equilibrated. The SPME fiber was then exposed to the headspace of the sample in the vial for 45 min at 75 ^{0}C, and agitated at 960 rpm. After extraction the fiber was inserted into the hot injector of the GC system performed in the splitless mode, at 240 ^{0}C for 5 min. The results obtained gave a higher response by addition of water and solvent. Quantification was performed by constructing a calibration curve for the samples spiked with the analytes and it showed linear response from 50 – 500 µg/kg with good correlation coefficient ($R^2 > 0.986$). LOD ranged between 6.32 and 12.7 µg/kg, and LOQ were between 17 and 35.7 µg/kg. The recovery was between 74 and 94 % and precision (RSD) ranged from 7.8 to 14.6 %. The developed method was found to be in

good agreement with the results obtained with other methods, and has better efficiency in terms of time and accuracy (Lambropoulou & Albanis, 2003).

Samples of grape, strawberry, tomato and ketchup was analyzed for residues of strobilurin fungicides (SF); pyrimethanil and kresoxim-methyl, using HS-SPME coupled to GC/MS. Samples were prepared by blending and spiking of the fruits with an appropriate standard in methanol and agitated for 60 min. The homogenates were then diluted with a buffer solution to adjust the pH to 7. An aliquot of 6 g of the diluted sample was transferred into a 14 mL vial followed by addition of NaCl and agitated. The mixture was extracted with 85 µm PA fiber at 100 ^{0}C for 25 min with stirring at 500 rpm. Desorption was carried out at 250 ^{0}C for 5 min. Quantification of the analytes concentration in the fruit samples were carried out by standard addition to avoid any matrix effect. The results gave good linearity which ranged from 12.50 to 250 ng/g, with correlation greater than 0.998. The recovery and RSD were 90.4 – 106.4 % and 7.4 – 15 % respectively. The LOD ranged from 1.8 to 3.1 ng/g, while the LOQ were between 5.5 and 9.4 ng/g. The extraction temperature of the developed method is most suitable for stable pesticides, as many pesticides undergo hydrolysis at this temperature (Navalón et al., 2002).

Degradation of four pesticides namely methyl parathion, parathion, diazinon and cypermethrin by dissolved ozone has been investigated. The effectiveness of pesticide oxidation in aqueous solution using a low level of dissolved ozone was determined using solid-phase micro-extraction (SPME) and GC–MS. The homogenized slurry sample was extracted twice with acetone followed by cleanup with dichloromethane for OP pesticides

and petroleum ether for cypermethrin. It was then evaporated and reconstituted in acetone before DI-SPME at 15 and 30 min extraction time and subsequent analysis with GC-MS at a desorption temperature and time of 250 ^{0}C for 3 min and 270 ^{0}C for 5 min for OPP and cypermethrin respectively. Dissolved ozone (1.4 mg/l) was effective in oxidizing 60 – 99 % of methyl-parathion, cypermethrin, parathion and diazinon in aqueous solution in 30 min and the degradation was mostly completed in the first 5 min. The removal efficiency of the pesticides was found to be highly depended on the dissolved ozone levels and temperature (Wu, J. et al., 2007).

A new sol gel hybrid coating, polydimethylsiloxane–2-hydroxymethyl-18-crown-6 (PDMS–2OHMe18C6) in-house was developed for use in solid phase microextraction (SPME). The three sol gel compositions produced were assessed for its extraction efficiency towards three selected OPP residues (diazinon, chlorpyrifos and profenofos) in strawberry, green apple and grape samples. All three compositions of the sol gel fiber showed superior extraction efficiencies compared to commercial 100 µm PDMS fibers. The composition showing the best extraction performance was used to obtain optimized SPME conditions: 75 ^{0}C extraction temperature, 10 min extraction time, 120 rpm stirring rate, desorption time of 5 min, desorption temperature of 250 ^{0}C and 1.5 % (w/v) of NaCl. The method detection limit (S/N = 3) of the OPP with the new sol–gel hybrid material ranged from 4.5 to 4.8 ng/g. The recovery of the new hybrid sol–gel SPME material ranged from 65 to 125 % with good precision of the method (% RSD) ranging from 0.3 to 7.4 % (Wan Ibrahim et al., 2010).

The efficiency of molecular imprinted polymer (MIP) fibers for the SPME extraction of triazine herbicides has also been developed and evaluated. A novel molecular imprinted SPME fiber coupled directly to HPLC for the determination of triazines (prometryn, atrazine, simetryn, tertbutylazin, ametryn, propazine and tertbutryn) in complicated samples such as soy bean, corn and lettuce was developed. The dry samples were grinded and sieved with a mesh gauge. Five gram of the grinded samples were spiked with 5 µL of 1 mg/L of triazines mixed standard solution and the extraction was performed in 3 mL solvent with a microwave oven at 60 ^{0}C for 30 min. The microwave extracted solutions were concentrated to remove the solvent and then dissolved with 10 mL of benzene. The resulting solutions (3 mL) were placed in a 5 mL glass vial and were extracted with the MIP coated fiber in DI mode for 30 min at a stirring rate of 1000 rpm. The extracted solution was desorbed into HPLC-UV by a mobile phase mixture of acetonitrile/water (50/50, v/v %) for 10 min and determined at detector wavelength of 225 nm. The extraction yield of six triazine analogues with the MIP coated fibers were found to be much higher than that of non-imprinted polymer, which was attributed to the presence of hydrogen bonding interaction between secondary amino groups in the triazines and the carboxylic groups in the MIP coating. The developed method yielded a good linear range of 0.1 – 2 µg/L with correlation coefficient greater than 0.99, and the LOD for the five triazines were in the range of 0.012 – 0.090 µg/L and recovery ranged between 75.5 and 113.4 % with RSD from 5.7 to 10.6 % (Hu, X. et al., 2007).

A monolithic SPME fiber was prepared based on atrazine-MIP for the analysis of triazine herbicide (TH), such as atrazine, simazine, propazine cyanazine, prometryn, terbutryn and

1,3,5 – triazine, from onions and rice. A weighed sample of onion was crushed with a juicer to produce juice and scum, and the liquid juice was spiked with two different amounts of the mixed triazines to give final concentrations of 100 and 500 ng/mL. The spiked sample was then stirred in an ultrasonic bath for 10 min and centrifuged, and 3 mL of the supernatant solution with pH adjusted to 7 by addition of phosphate buffer was transferred into a 4 mL vial containing 8 % NaCl. The solution was extracted in the DI mode by an MIP fiber, at room temperature for 25 min stirred at 500 rpm and desorbed into GC/MS at 250 ^{0}C for 1 min. The MIP polymer was shown to be thermally and chemically stable and its extraction efficiency increases with increase in the pH of the sample solution. The analytical performance yielded LOD in the range of 20 – 90 ng/mL, with average recovery between 90 to 96.4 % and RSD of 5.25 – 9.58 % for the onion sample. The linear range was between 50 – 9000 ng/mL with a correlation coefficient greater than 0.99 (Djozan & Ebrahimi, 2008).

An ametryn-MIP fiber coated on a home-made glass capillary and ametryn-MIP fiber coated on an anodized aluminum wire were also prepared, for the SPME extraction of TH (ametryn, prometryn, terbutryn atrazine, simazine, propazine, cyanazine and 1,3,5-triazine) from maize, onion and rice. The sample preparation, extraction and desorption conditions are the same as discussed in their earlier paper (Djozan & Ebrahimi, 2008), but with 6 % NaCl and extraction times of 25 min and 12 min for the MIP fiber coated on a home-made glass capillary and ametryn-MIP coated on an anodized aluminum respectively. The ametryn-MIP coated on an anodized aluminum was found to have higher efficiency compared to the MIP fiber coated on a home-made glass capillary, while the latter is better

than PA and PDMS fibers. The LOD values are in the range of 14 – 95 ng/mL and 9 – 85 ng/mL, with average recoveries of 85.2 – 95.2 % (RSD = 5.6 – 10.8 %) and 86.1 – 97.0 % (RSD = 3.2 – 7.4 %), the calibration curves were linear in the range of 50 – 10,000 ng/mL and 20–16,000 ng/mL for the MIP fiber coated on the home-made glass capillary and ametryn-MIP fiber coated on an anodized aluminum wire respectively. The correlation coefficient which was greater than 0.99, is the same for both fibers. The binding properties of the MIP fiber were also studied (Djozan et al., 2009), and the experimental data was found to fit well to bi-Langmuir isotherm, showing the existence of two types of binding sites on the prepared fiber (Djozan et al., 2010; Djozan et al., 2009).

An MIP monoliths coated on fused silica were prepared and evaluated for their use in supported liquid membrane (SLM)-protected MI-SPME of thiabendazole (TBZ) from orange juice. A volume of 1.7 mL of standard solution was added into 2 mL vial and was extracted by the home-made MIP fiber in the DI mode for 60 min stirred at 600 rpm. After the extraction, the fiber was washed in toluene to remove nonspecific interactions and then air dried for 5 min. The extracted analyte was desorbed with a mixture of methanol/acetic acid (95:5, v/v %) by agitation at 600 rpm for 30 min in a 0.4 mL vial insert. The resulting acidic extract was then diluted (1:4) with methanol/water mixture (70:30, v/v %) which was also used as the mobile phase and injected into a HPLC with simultaneous fluorescence (λ=305 and 345 nm) and UV (λ=290 nm) detections. The calibration curve showed good linearity in the range of 0.01 – 5 mg/L with a correlation coefficient greater than 0.995. The detection limit was 4 µg/L, and low recovery (6.9 and 7.0 %) with RSD of 7.6 and 6.6 % for low and high spiking levels respectively. Its limitation is the lack of

selective recognition of the target analyte in aqueous solution, and the lack of compatibility between the solvent required to desorb the analyte from the MIP and the mobile phase used. However, its use as an SPME fiber has helped to improve the results of the analytical methodology in the extraction of triazine and thiabendazole pesticides (Barahona et al., 2011).

An HS-SPME-GC-MS study for rapid screening of eleven OPPs was developed and fully validated in seven samples based on CODEX classification. The method adopted square root sampling approach to achieve statistically significant analysis. Each sample were pretreated according to the specific requirements for individual sample groups (Codex Alimentarius Commision, 2010). A paste (1 g) of the finely chopped sample was accurately weighed into a 20 mL glass vial, followed by addition of 100 µL of methanol/acetone (1:1, v/v) and 10 % (w/v) of NaCl solution to make up a total sample weight of 5 g. The mixture was vortexed for 1 min and warmed to 70 ^{0} for 10 min. The mixture was then subjected to SPME extraction at the same temperature by exposing a PDMS fiber to the sample headspace for 45 min. After extraction, the extracted analytes were desorbed into the injector port of GC for 7 min at 240 ^{0}C in splitless mode.

The analytical method validated using 7 fruit and vegetable samples gave calibration linearity which ranges from 0.01 to 2.5 mg/L, and the recovery ranged from 70 – 120 % with RSD of 0.5 – 10.9 %. The method sensitivity estimated in terms of LOD and LOQ ranged from 0.02 – 2.88 µg/L and 0.05 – 8.7 µg/L respectively. The method was compared

with a conventional LLE and was found to have better chromatographic separation with no matrix interference. (Sang et al., 2013).

A new method for the determination of 10 MCPs in lettuce was developed and validated using SPME coupled to HPLC/DAD. The analysis of sample involved mashing of 2.5 g of lettuce followed by addition of 10 µL of phosmet (IS) and the mixture sonicated for 10 min at 40 ^{0}C, with 1 mL of acetonitrile in an ultrasonic bath and then centrifuged. An aliquot of 2 mL of the resulting extract containing 0.7 g of NaCl at pH 8 was transferred into 4 mL Teflon-lined septum cap equipped with magnetic bar. The solution was subjected to DI-SPME using CW/TPR coated fiber for 30 min at room temperature stirred at 1000 rpm, and the extracted analytes were desorbed using mobile phase containing a mixture of methanol/acetone (90:10, v/v %) into HPLC-DAD equipped with a desorption chamber. The developed method was validated using an 8 point calibration curve gave a linear range of 0.8 – 25.6 mg/kg with correlation coefficient greater than 0.996. The limits of detection and quantitation calculated from the calibration curve parameters were between 0.37 – 1.53 mg/kg and 0.9 4 – 5.10 mg/kg respectively. The method was used to study the dissipation behavior of the folpet and fenhexamid and was not detected after 14 days which is in agreement with EU regulation (Melo et al., 2012a).

The structure of fiber SPME fiber coating was optimized for the analysis of triazole fungicides in water and grape sample. Uncontaminated grape samples collected were pretreated and crushed with a blender. A 9 g aliquot was weighed into 10 mL vial and fortified with pesticide standard. The sample solution was incubated for 5 min at 500 rpm

and 30 ^{0}C, followed by extraction with PDMS-modified fiber (prepared by coating a commercial PDMS/DVB fiber by a layer of PDMS) at the same temperature and stirring rate for 30 min. After extraction the fiber was rinsed in deionized water at 30 ^{0}C and stirred at 500 rpm for 50 s followed by desorption of the extracted analytes into injection port of GC/MS/MS for 7 min at 260 ^{0}C. The PDMS-modified fiber which was prepared in order to overcome the problem of fouling of coated fiber in DI mode showed improved repeatability and robustness. The result showed that the extraction efficiency of the PDMS-modified fiber only decrease by 8 – 14 % after 20 extraction, conditioning and desorption cycle, compared to the commercial PDMS fiber whose efficiency dropped by 83 – 89 % after the same number of cycle. The fiber was able to perform 130 DI extractions in complex matrix due to reduction in fouling of the fiber (Souza Silva & Pawliszyn, 2012).

A new SPME fiber coated with polypyrrole/sol gel (Ppy/sol gel) composite on a stainless wire was used to develop a method for the analysis of OPPs in water and vegetable samples. The cucumber and lettuce samples were washed, chopped and homogenized in a blender and an aliquot of 2 g was accurately weighed into a glass centrifugation tube and spiked with the analytes. The spiked sample was then diluted with 2 mL of water and mixed ultrasonically for 10 min. The diluted solution was placed in a water bath at 70 ^{0}C for 30 min and then centrifuged at 905 rpm for 15 min. The resulting supernatant was collected and diluted with water containing 0.2 g/mL of NaCl(1:2), and subjected to DI-SPME using the home-made fiber at 45 ^{0}C for 30 min stirred at 300 rpm and desorbed into GC-NPD. The method analytical figures of merit estimated using a 3 point standard addition calibration method gave linearity between 5 and 2000 ng/L with correlation

coefficient greater than 0.995. The average recovery ranged from 87 – 106 % with RSD of 3.5 to 9.8 %, while the LOQ was from 1.5 to 10 ng/L. The Ppy/sol-gel fiber showed a better extraction performance and longer life time (150 extractions) compared to other fibers, which was attributed to the presence of phenyl and hydrophilic groups which enhance π-π interaction, hydrogen bonding and dipole-dipole interaction with sol gel providing a porous structure with increased surface area (Saraji et al., 2013).

The pesticide residues contents of fruit juices (peach, orange and pineapple), were evaluated by employing SPME coupled to multi-dimensional gas chromatography mass spectrometry (MDGC-MS). The sample was prepared by spiking 1 mL aliquot of water in 5 mL vial, followed by exposing the PDMS/DVB fiber to the headspace of the sample and extracted for 30 min at 40 ^{0}C, and was dissolved into the MDGC-MS system at 250 ^{0}C for 5 min. The method was validated by comparing the figure of merits obtained using one-dimensional (1D) GC and two dimensional (2D) GC in terms of repeatability, relative standard deviation, limit of detection and linearity. The LODs ranged from 0.685 to 4.485 ng and 0.026 to 32.79 ng in 1D and 2D GC respectively. The RSD was found between 0.16 to 1.53 % and was linear over a range of 0.6 to 15 ng in the 1D GC. The analytical performance obtained using 1D GC was found to be satisfactory, but the 2D GC was employed in order to achieve better separation and selectivity of the target pesticides that were closely eluted. (del Castillo et al., 2012).

A high-throughput SPME method was developed based on a 96-well plate coupled to GC-MS for the monitoring and determination of 7 multiclass pesticides in cucumber sample.

The SPME extraction involved chopping and homogenizing a 15 g portion of cucumber, spiked with an appropriate amount of standard solution of target analytes, and left for 1 hr. The sample was diluted with 25 mL of double distilled water and then shaken for 30 min at 150 rpm. The sample was then centrifuged at 8000 rpm for 12 min, and 1 mL of the upper layer was transferred into a well on the 96-deep well plates. The home-made 96 fiber array was immersed into the well and stirred for 150 rpm for 40 min. After extraction, the extracted analytes were desorbed into another 96-well plate using 600 µL acetronitrile for 5 min, followed by evaporated of the solvent and refilled with 20 µL of n-octane containing the internal standard and an aliquot of 1 µL was injected into GC-MS system. The optimum extraction was used to validate the developed method, and the linearity was found between 25 and 1000 µg/kg with satisfactory correlation coefficient (0.99). The LOD and LOQ ranged from 8 – 60 µg/kg and 25 – 180 µg/kg respectively. The average recovery ranged from 80 – 111 % with RSD values between 6.5 to 15.4 %. The method was successfully applied to cucumber sample obtained in the market and the target pesticides were found to be present at concentration lower than the MRLs set by the European Commission (EU, 2005). The use of the reusable PDMS fiber was found to be more cost effective than the disposable SPE sorbent (Bagheri et al., 2012).

3.9 Advantages and Limitations of Solid Phase Microextraction (SPME)

The SPME extraction technique has also been applied for the extraction of pesticides and other contaminants from a wide range of matrices including environmental, industrial wastes, process monitoring, drugs, crime and forensics, food and water analysis. The technique is frequently selected for the qualitative and quantitative sample preparation

method for chromatographic analysis but also have its advantages and limitations, which must be carefully considered in its selections as sample preparation of choice.

3.9.1 Advantages of SPME

The numerous advantages of selecting the SPME technique for pesticide residues analysis are:- it does not include the use of toxic solvents, it has a short sample preparation time, it is compatible with analyte separation and detection with chromatographic instruments and is amenable to automation, it allows the extraction of polar, semi-polar and non-polar pesticides and other food contaminants from solid, liquid or gaseous sample matrices, it gives linear results for a wide range of analytes, it gives better consistency and highly quantifiable results from very low analyte concentrations, it allows for the use of small volumes of sample, the cost of analysis is relatively low, it has a small size, which makes it convenient for designing portable field sampling devices and it is rugged.

3.9.2 Limitations of SPME

The most important limitation is the fragility of the fiber, which has to be handled with care to avoid breakage. The quality of the needle also depends on the manufacturer and in some cases the performance is different from batch to batch. A new fiber has to be conditioned before use, but some bleeding of the fiber coating can sometimes occurs even after careful conditioning. The GC injector temperature should always be maintained below the maximum operating temperature of the coating as specified by the manufacturer (De Fátima Alpendurada, 2000; Prosen & Zupančič-Kralj, 1999). After desorbing the analytes into chromatographic instruments, some may be carried over by the fiber, and

therefore the need to run blank analysis with the fiber after each sampling is performed. The fiber may be permanently damaged due to the irreversible adsorption of high molecular weight compounds. Some of these limitations have been overcome by the use of headspace SPME and the introduction of new fiber coating such as the sol gel and MIP coated fibers.

Table 3. 1: SPME for the Analysis of Pesticide Residues in Fruits and Vegetables using GC

Pesticides class	Matric	Mode	Fiber	Ext. time (min)	Ext. temp. (^{0}C)	St. rate (rpm)	NaCl (%)	pH	Des. time (min)	Des. temp. (^{0}C)	LOQ	LOD	Rel. Rec. (RSD) (%)	LR	Detector	Reference
OCPs, PPs	C. cabbage garlic, cabbage	DI	60 µm PMPS	40	40	1000	n.r	n.r	4	280	n.r	0.13-1.45 ng/g	42-105 (2.6-16)	0.05-45 ng/g	GC-ECD	(Zeng et al., 2008)
OCPs, OPPs	Tomato, guava	HS	100 µm PDMS	30	60	n.r	10	n.r	6	240	n.r	0.1-1 µg/L	81-97 (0.3-3.3)	0.5-5000 µg/L	GC-ECD	(Chai, M. K. et al., 2008a)
PPs	Tomato, strawberry	DI	65 µm PDMS/DVB	30	40	120	20	n.r	5	270	3-25 µg/kg	50 µg/kg	n.r (20-25)	10-100 µg/kg	GC-MS	(Beltran et al., 2003)
PUPs	Carrot, onion, potato.	DI	85 µm PA	60	R.T	n.r	14	4	5	300	0.8-2.2 µg/kg	n.r	76-90 (4-8)	2.5-2500 µg/kg	GC-NPD	(Berrada et al., 2004)
OPPs	Apple, tomato, apple juice.	HS	85 µm B15/C5	45	75	n.r	30	n.r	5	270	n.r	3-76 µg/g	55.3-106 (3.3-10.1)	0.01-100 ng/g	GC-MS	(Cai et al., 2006)
OCPs, OPPs,CPs	Tomato, cucumber.	DI	100 µm PDMS	15	R.T	n.r	n.r	n.r	7	270	1-50 µg/g	0.5-10 µg/kg	53-82 (0.47-9.2)	0.005-200 µg/g	GC-ECD	(Chai, M. K. et al., 2008b)
OPPs	Tomato	CC-HS	ACF	30	80	500	20	n.r	2	270	1-50 ng/g	0.2-0.5 ng/g	82.5-90 (5.9-8.7	1-200 ng/g	GC-NPD	(Chai, X. et al., 2008)
OPPs	Orange, grape, lemon juices.	DI	85 µm PA	30	R.T	n.r	10	n.r	5	250	n.r	2-90 µg/L	5-98 (4-12)	10-500 µg/L	GC-MS	(Zambonin et al., 2004)
OPPs, OCPs	5 fruits and vegetables.	HS	100 µm PDMS	30	60	800	19	n.r	10	240	7-297 µg/L	0.01-1 µg/L	77.3-95.3 (1.1-4)	0.1-5000 µg/L	GC-ECD	(Chai & Tan, 2009)
OPPs	C. *coronarium*	DI	100 µm PDMS	90	R.T	n.r	n.r	7	5	250	n.r	4.7-75 ng/g	2.4-25.66 (1.5-19.8)	n.r	GC-FPD	(Chen, W. et al., 1998)
OPPs, OCPs	4 fruits and vegetables	HS	100 µm PDMS	60	R.T	n.r	n.r	n.r	15	240	n.r	n.r	70-99 (<10)	1-400 mg/kg	GC-ECD	(Chai et al., 2006)

Table 3.1 SPME for the Analysis of Pesticide Residues in Fruits and Vegetables using GC (continued)

Pesticides Class	Matric	Mode	Fiber	Ext. time (min)	Ext. temp. (^{0}C)	St. rate (rpm)	NaCl (%)	pH	Des. time (min)	Des. temp. (^{0}C)	LOQ	LOD	Rel. Rec. (RSD) (%)	LR	Detector	Reference
70 MCPs	Cucumber, pepper, tomato	DI	65 µm PDMS/DVB	10	R.T	n.r	n.r	n.r	9	250	0.002-3 ng/g	0.0006-5 ng/g	77-106 (3-11)	0.002-500 ng/g	GC-MS	(Cortés Aguado et al., 2007)
OPP	Strawberry, tomato, pakchoi	MAE – HS	100 µm PDMS	10	132W	n.r	n.r	5	3	220	n.r	1 µg/L	106 (5.5-7.9)	5-75 µg/L	GC-ECD	(Chen, Y. I. et al., 2002)
TFs	Strawberry	DI	85 µm PA	45	50	n.r	20	n.r	5	250	n.r	30-100 ng/kg	n.r (2-11)	n.r	GC-MS	(Zambonin et al., 2002)
OPPs	Orange juice, pakchoi	DI	B16/C5	65	20-50	n.r	n.r	n.r	5	270	n.r	0.003-1 ng/g	76.8-101 (2.3-9.2)	1-500 ng/g	GC-FPD	(Yu, J. et al., 2004)
54 MCPs	Orange, peach	DI	65 µm PDMS/DVB	55	R.T	n.r	5	n.r	9	250	0.1-50 µg/L	0.01-16.7 µg/L	72-107 (2-17)	0.01-1 µg/L	GC-MS^2	(Cortés Aguado et al., 2008)
OCPs	Radish	HS	C[4]/OH-TSO	30	70	600	25 K_2SO_4	n.r	2	270	n.r	1.48-174 ng/kg	78.9-119 (7.4-13.1)	1-10000 ng/L	GC-ECD	(Dong et al., 2005)
CPs	Apple, grape juice	DI	65 µm CW/DVB	30	R.T	n.r	30	n.r	15	250	n.r	0.1-10 µg/L	n.r (2.2-36.2)	20-2000 µg/L	GC-MS	(Natangelo et al., 2002)
OCPs, OPPs, ONPs	Grape	DI	AC/PVC	15	R.T	900	6.25	n.r	5	200	n.r	8-400 µg/L	42-63 (5.8-9)	25-5000 µg/L	GC-FID	(Farajzadeh & Hatami, 2004)
OPPs	Pear, apple	DI	100 µm PDMS	20	R.T	1250	n.r	n.r	2	250	16-17 ng/L	16-70 ng/L	50-102 (1.6-8.7)	0.250-25 µg/L	GC-FPD	(Simplício & Vilas Boas, 1999)
14 MCPs	Mango	DI	85 µm PA	30	50	250	5	3	5	280	3.3-33.3 ng/g	1-3 ng/g	71-117 (3.1-12.3)	3.33-1665 ng/g	GC-MS	(Menezes Filho et al., 2010)
OPPs	21 fruits	HS	85 µm PA	20	n.r	n.r	8	n.r	4	230	0.24-20 ng/g	0.07-6 ng/g	n.r (2.5-3)	1.2-667 ng/g	GC-NPD	(Fytianos et al., 2006)

Table 3.1: SPME for the Analysis of Pesticide Residues in Fruits and Vegetables using GC (continued)

Pesticides Class	Matric	Mode	Fiber	Ext. time (min)	Ext. temp. (^{0}C)	St. rate (rpm)	NaCl (%)	pH	Des. time (min)	Des. temp. (^{0}C)	LOQ	LOD	Rel. Rec. (RSD) (%)	LR	Detector	Reference.
25 MCPs	Tomato	DI	60 µm PDMS/DVD; 100 µm PDMS	30 45	R.T	500	n.r	n.r	2	250	0.2-25.4 µg/kg	0.01-7.62 µk/kg	n.r (2.2-5.7	0.1-1000 µg/kg	GC-MS	(Guillet et al., 2009)
OPPs	Strawberry, cherry juices	HS	100 µm PDMS	45	75	960	15 Na_2PO_4	3.5	2	240	n.r	5-25 µg/L	82-100 (5.2-18.7)	0.5-500 µg/L	GC-MS	(Lambropoulou & Albanis, 2002)
16 MCPs	Strawberry	DI	100 µm PDMS	45	R.T	800	n.r	n.r	3	270	n.r	1-50 µg/kg	n.r (3.8-88.9)	4-500 µg/kg	GC-MS	(Hu, R. et al., 1999)
OPPs	Strawberry, cherry	HS	100 µm PDMS	45	75	960	15 Na_2PO_4	3.5	5	240	17-35 µg/kg	6.3-12.7 µg/kg	74-94 (7.8-14.6)	50-500 µg/kg	GC-MS	(Lambropoulou & Albanis, 2003)
OPPs	13 vegetables	HS	100 µm PDMS	20	70	n.r	26	n.r	4	230	0.2-13.3 ng/g	0.06-5 ng/g	n.r (2.2-7.6)	2.31-662 ng/g	GC-NPD	(Fytianos et al., 2007)
SFs	4 fruits and vegetables	HS	85 µm PA	25	100	500	36	7	5	250	5.5-9.4 ng/g	1.8-3.1 ng/g	90-106 (7.8-14.6)	12.5-250 ng/g	GC-MS	(Navalón et al., 2002)
OPPs	4 fruits and vegetables	HS	PDMS/20H/ Me18C6	10	75	120	1.5	n.r	5	250	15.1-16.2 ng/g	4.5-8.8 ng/g	65-125 (0.3-7.4)	0.005-0.7 ng/g	GC-MS	(Wan Ibrahim et al., 2010)
THs	Onion	DI	MIP	25	40	500	8	7	1	250	n.r	20-88 ng/mL	90-96.4 (5.2-9.5)	0.05-9 ng/L	GC-MS	(Djozan & Ebrahimi, 2008)
THs	Onion	DI	MIP	20	40	500	6	7	1	250	n.r	10-90 ng/mL	85.2-95.2 (5.6-10.8)	0.05-10 ng/L	GC-MS	(Djozan et al., 2009)
THs	Onion	DI	MIP	12	n.r	600	6	7	1	250	n.r	9-85 ng/mL	86.1-97 (3.2-7.4)	0.02-16 ng/L	GC-MS	(Djozan et al., 2010)
OPPs	7 fruits and vegetables	HS	100 µm PDMS	45	70	n.r	10	n.r	7	240	0.05-8.7 µg/L	0.02-2.88 µg/L	70-120 (0.5-10.9)	0.01-2.5 mg/L	GC-MS	(Sang et al., 2013)

Table 3.1: SPME for the Analysis of Pesticide Residues in Fruits and Vegetables using GC (continued)

Pesticides Class	Matric	Mode	Fiber	Ext. time (min)	Ext. temp. (^{0}C)	St. rate (rpm)	NaCl (%)	pH	Des. time (min)	Des. temp. (^{0}C)	LOQ	LOD	Rel. Rec. (RSD) (%)	LR	Detector	Reference
OPPs	Cucumber, lettuce	DI	Ppy/sol-gel	30	45	800	20	n.r	3	280	n.r	1.5-10 ng/L	87-106 (3.5-9.8)	5-2000 ng/L	GC-NPD	(Saraji et al., 2013)
TFs	Grape	DI	100 µm PDMS-MF	30	R.T	500	n.r	n.r	7	270	n.r	n.r	n.r	n.r	GC-MS	(Souza Silva & Pawliszyn, 2012)
PPs	Strawberry	MAE-DI	100 µm PDMS	30	30W	n.r	n.r	n.r	2	270	0.9-13.8 µg/kg	2.84-41.3 µg/kg	n.r (1.2-14.2)	1-250 µg/kg	GC-MS	(Sanusi et al., 2004)
46 MCPs	Peach, orange, pineapple	HS	65 µm PDMS/DVB	30	40	n.r	n.r	n.r	5	250	n.r	0.026-32.79 ng	n.r	0.6-15 ng	MD-GC-MS	(del Castillo et al., 2012)
7 MCPs	Cucumber	DI	100 µm PDMS	40	n.r	150	n.a	n.r	n.r	260	25-180 µg/kg	8-60 µg/kg	80-111 (6.5-15.4)	25-1000 µg/kg	GC-MS	(Bagheri et al., 2012)

Table 3.2: SPME for the Analysis of Pesticide Residues in Fruits and Vegetables using LC

Pesticides class	Matric	Mode	Fiber	Ext. time (min)	Ext. temp. (^{0}C)	St. rate (rpm)	NaCl (%)	pH	Des. time (min)	LOQ	LOD	Rel. Rec. (RSD) (%)	LR	Detector	Reference
5 MCPs	Cherry, orange, peach	DI	50 µm CW/TPR	90	n.r	1000	30	n.r	10	0.5-10 ng/g	n.r	8-69 (8-12)	0.0005-10 mg/kg	LC-MS^2	(Blasco et al., 2003a)
CPs	Strawberry	DI	60 µm PDMS/DVB	45	25	1000	n.r	n.r	1	10-50 ng/g	n.r	n.r (2.9-9.2)	3-1500 ng/g	HPLC-DAD	(Wang et al., 2000)
PPs	Cucumber, watermelon	DI	60 µm PDMS/DVB	30	65	1100	n.r	3	5	0.2-1.1 ng/g	1.3-5 ng/g	91-110 (2-9.4)	1.3-1500 ng/g	HPLC-FD	(Vázquez et al., 2008)
Aps	4 fruits	DI	50 µm CW/TPR	120	n.r	1000	12.5	2	n.r	0.02-5 µg/kg	n.r	7-94 (3-13)	0.02-500 µg/kg	CE-MS	(Rodriguez et al., 2003)
CPs	Strawberry	MAE-DI	60 µm PDMS/DVB	45	30W	1000	50 mg	6	n.r	13-22 µg/kg	44-74 µg/kg	n.r (3-7.3)	50-1000 µg/kg	HPLC-DAD	(Falqui-Cao et al., 2001)
CPs	Apple	DI	60 µm PDMS/DVB	35	25	1100	n.r	n.r	8	3-5 µg/kg	6-10 µg/kg	91.5-96.1 (3.3-4.7	10-1000 µg/kg	HPLC-FD	(Hu, Y. et al., 2008)
TFs	Orange juice	DI	MIP	60	n.r	600	n.r	10	30	n.r	4 µg/L	n.r	0.01-5 mg/L	HPLC-FD	(Barahona et al., 2011)
TBZs	Lettuce	DI	MIP	30	60	1000	n.r	n.r	10	n.r	12-90 ng/L	75.5-113. (5.7-10.6)	0.1-2 µg/L	HPLC-UV	(Hu, X. et al., 2007)
10 MCPs	Lettuce	DI	50 µm CW/TPR	30	R.T	1000	17.6	8	10	0.94-5.10 mg/kg	0.37-1.53 mg/kg	n.r (n.r)	0.8-25.6 µg/kg	HPLC-UV	(Melo et al., 2012a)

N.B: Ext time, extraction time; Ext temp, extraction temperature; St. rate, stirring rate; Des time, desorption time; Des temp, desorption temperature; LOD, limit of detection; LOQ, limit of quantitation; Rel. Rec., relative recovery; LR, linearity rate; OPPs, organophosphorus pesticides; PPs, pyrethroid pesticides, OCPs, organochlorine pesticides; CPs, carbamate pesticides; PUPs, phenyl urea pesticides; MCPs, multiclass pesticides; THs, triazine herbicides, TFs, triazole fungicides; SFs, strobilurin fungicides; TBZ, thiabendazole; DI, direct immersion; HS, headspace; R.T, room temperature; n.r, not reported; MAE, matrix assisted extraction; n.a, not adjusted; GC-MS, gas chromatography; MS, mass spectrometry; ECD, electron capture detector; NPD, nitrogen phosphorous detector; FPD, flame photometric detector; FID. Flame ionization detector; LC, liquid chromatography; HPLC, high performance liquid chromatography; DAD, diode array detector; FD, fluorescence detector; UV, ultraviolet; CE, capillary electrophoresis

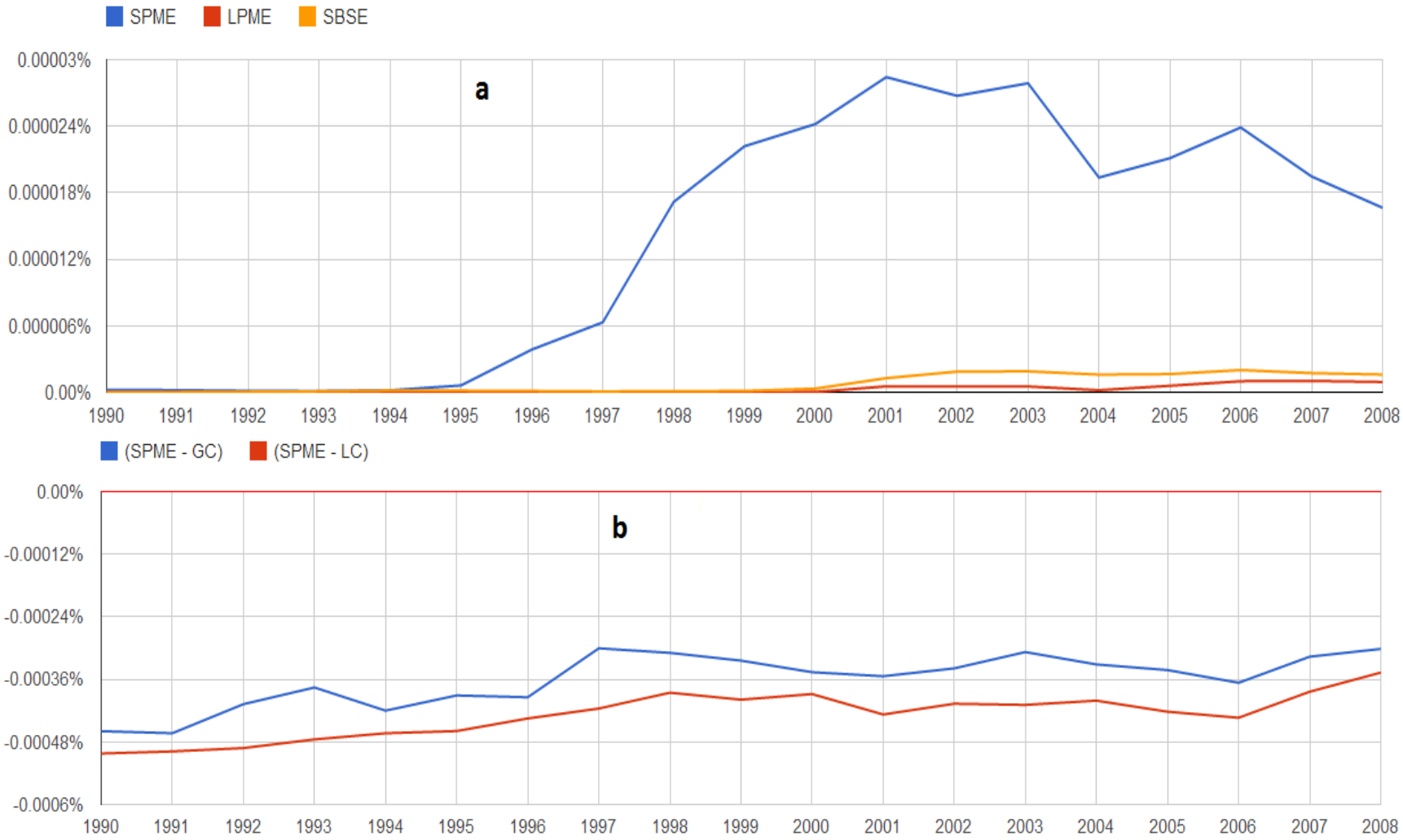


Fig 3.7: Comparison of the use of (a) Microextraction Techniques and (b) Chromatographic Techniques (Goodle Ngram, 2013)

CHAPTER FOUR

4.0 EXPERIMENTAL

4.1 Materials

4.2 Analytical Reagents and Standards

Pesticide standards of fenobucarb, ethoprop, diazinon, chlorothalonil, fenitrothion, methyl parathion, chlorpyrifos, thiobencarb, quinalphos, endosulfan I, endosulfan II, bifenthrin, fenpropathrin and permethrin at 100 µg/mL and 1-chloro-3-nitrolbenzene (1000 µg/mL) used as internal standard with more than 95 % purity, were purchased from AccuStandard Inc. New Haven CT, U.S.A. All solvents used were pesticide grade: methanol, acetone, acetonitrile, isopropanol and were purchased from Fisher Scientific, Loughborough, U.K. Sodium chloride, sodium sulphate, ammonium chloride were purchased from Merck. The pH buffer solutions 4, 6, 8 – 10 and 5 – 7 were purchased from Fisher Scientific and Sigma-Aldrich respectively. Millipore filtered (0.45 µm) deionized water was used for method development.

4.3 Apparatus and Glassware

The following apparatus were used for sample processing: Food processor, weighing balance, ultrasonicator, sonicator. All glassware including the glass vials were cleaned thoroughly with detergent and bristle brush and then rinsed with tap water. The amber glass vials were further cleaned in a Branson sonicator and rinsed again first with tap water and then with distilled water and were dried in the oven for 2 hours at 120 ^{0}C. After drying for 2 hrs, they were removed from the oven and allowed to cool to room temperature, while being covered with aluminum foil and stored in a cupboard to prevent any accumulation of dust. The amber glass vials were rinsed with acetone and dried before use.

4.4 Equipment and Instrumentation

4.4.1 Materials for Solid Phase Microextraction

The SPME device autosampler holder and fibers (100 µm PDMS, 85 µm PA and 65 µm PDMS/DVB), sample vial (20 mL amber glass), and PTFE (white)/silicone (red) septa were purchased from Supelco, Bellenfonte, PA, USA. The autosampler fiber holder was mounted on the CTC CombiPAL Autosampler for automatic extraction and injection into the GC-MS.

4.4.2 Gas Chromatography – Mass Spectrometry (GC-MS) Analysis

The extraction of pesticides were carried out with CTC CombiPAL autosampler equipped with agitator and needle heater (for fiber conditioning and inter-extraction clean up) coupled to a GC-MS (Shimadzu QP2010Series) and operated in the split/splitless mode at an injection temperature of 270 ^{0}C. The separation of target analytes were achieved on a DB-5MS fused capillary column containing 5 % diphenyl and 95 % dimethylpolysiloxane (30 m x 0.25 mm i.d. x 0.25 µm film thickness). The injection port of the GC was equipped with a high-pressure Merlin Microseal septumless injection kit and a silanized narrow bore liner (78.5 x 6.5 mm o.d x 0.75 mm i.d). Helium (carrier gas) was set to a constant flow rate of 1.3 mL/min with linear velocity of 42 cm/sec. The GC column oven temperature program was set as follows. Initially set at 60 ^{0}C for 2 min, ramped at 30 ^{0}C/min to 180 ^{0}C, then ramped to 210 ^{0}C at 5 ^{0}C/min, and finally to 270 ^{0}C held for 5 min, for a total runtime of 24.50 min. The MS operation condition includes transfer line of 300 ^{0}C, ion source of 200 ^{0}C, electron ionization (EI) of 70 eV. The optimization of methods was done in scan mode while quantitation was done in selected ion monitoring (SIM) mode. A target ion (most abundance ion) and two other reference ions were monitored for the target analytes.

A number of GC-MS parameters were optimized to achieve better separation and sensitivity.

4.5 Experimental

4.5.1 Standard Stock Solution

A working standard mixture containing 10 µg/mL of each of the pesticides were prepared daily in methanol from the stock pesticides standard (100 µg/mL), by mixing aliquots of the individual standards and kept in the freezer at 4 ^{0}C before use. An aliquot of 50 µL of the working standard mixture was used to spike 5 mL of water to concentration of 100 ng/ml for method development. A 5 g of the sample matrix was also spiked with the working standard solution to concentration between 5 and 150 µg/kg used for method optimization and validation studies. A concentration range of 1 – 500 µg/kg was prepared directly in the matrix for the calibration purpose and method validation.

4.5.2 Sample Collection

All fruit and vegetable samples were collected from Malaysian hypermarkets and night markets for multi-residue and multiclass pesticide analysis. Fruit and vegetable samples used for method development, calibration and recovery studies were first analyzed to ensure the absence of the target pesticide residues (Cortés Aguado et al., 2008; Cortés Aguado et al., 2007; Melo et al., 2012a; Souza-Silva, Lopez-Avila, & Pawliszyn, 2013).

4.5.3 Sample Preparation

For solid phase microextraction method development, 100 g of pesticide free fruits and vegetables were accurately weighed, finely chopped and homogenized in a blender. A known aliquot of the homogenized sample was then weighed into a separate 20 mL amber glass vial containing the internal standard and diluted accurately with Milli-Q filtered deionized water containing 10 % of NaCl to make up a total mass of 5 g. The mixture was then spiked with the working standard solution at two concentration levels 50 µg/kg (low) and 100 µg/kg (high), then homogenized at 3000 rpm for 5 min and subjected to SPME procedure.

4.5.4 GC-MS Conditions

A number of GC-MS conditions were optimized before obtaining suitable instrumental conditions. The following parameters were optimized by spiking 5 ml of deionized water with 50 µL of the working standard solution: injection port temperature (desorption temperature), desorption time, ion source temperature, interface temperature (detector temperature) and column flow rate. The oven temperature programming is as described in Section 4.4.2. The optimized GC-MS parameters were initially set at default values. The optimization involves varying one factor at a time while keeping the others constant. The desorption temperature was first optimized and the optimized factor of one parameter was used for the subsequent factors until all the factors were fully optimized.

4.5.5 Solid Phase Microextraction Procedures

A preliminary SPME experiment was carried out to select the best fiber coatings. The efficiency of three commercially available coating materials with dissimilar polarities and thickness was compared. The three fibers were selected based on the nature and polarities of the selected pesticides, which belong to different classes (organophosphorus, organochlorine, carbamate and pyrethroid) with different polarities. Selection and optimization of the major SPME factors were investigated in a spiked aqueous sample solutions, which were analyzed in triplicate for each of the factor investigated.

The SPME fibers were conditioned in the GC/MS injection at 250 ^{0}C for 30 min (PDMS and PDMS/DVB) and 280 ^{0}C for 1 hr (PA), prior to their first use as recommended by the manufacturer. Optimization of parameters and analysis were performed in a 20 mL amber glass vial containing 5 mL of Milli-Q filtered deionized water containing 10 % of NaCl and spiked with 50 µL of the working standard solution to give a concentration of 0.1 µg/mL. . The vial containing the sample was shaken ultrasonically for 5 min, agitated and incubated for 5 min at 60 ^{0}C in the autosampler agitator, before the SPME fiber was exposed to the headspace of the sample. The fiber was then exposed to the headspace of the aqueous sample matrix in the vial sealed with PTFE/silicone septum and the extracted analytes were desorbed into GC-MS system using the optimized GC-MS conditions and operated in the SCAN mode. The investigated pesticides were identified by comparing the mass spectrum obtained for each analyte to that of the reference compound in GC-MS library using the US National Institute of Standard and Technology (NIST) and PESTANA. In case of co-elution, easy spectral identification and integration was achieved by using the deconvolusion feature of the GC-MS system.

For the selection of extraction time, the time was varied between 10 – 100 min. The optimization of extraction temperature was investigated by varying the sample temperature between 30 and 90 ^{0}C. Stirring rate was varied between 250 and 750 rpm, while the effect of type of salt and amount were varied using NaCl, Na_2SO_4 and NH_4Cl and the selected salt were then varied between 5 – 30 %. The sample pH was also varied between 4 –10, the dilution factor was between 1 to 5 for each fruit and vegetable samples, while desorption time and temperature were varied between 2 – 10 and 230 – 280 ^{0}C respectively, while ensuring that it does not exceed the recommended temperature for each fiber. The solvent addition was optimized using simplex lattice mixture design and three (3) organic solvents were investigated, they include acetone, methanol and acetonitrile.

4.5.6 Data and Statistical Analysis Software

The chromatographic peak area response of the univariate experiments were analyzed using Microsoft Excel® software. The multivariate experimental design was carried out to further optimize each factor based on the optimized conditions in the univariate experiment. The significance of each factor was investigated using the Plackett-Burman design, while full factorial central composite design (CCD) was used to determine the optimum conditions of the significant factor which was analyzed using the response surface methodology (RSM). The multivariate experiment and mixture design were developed using Minitab® 16 statistical software package.

4.6 Method Validation

The analytical figures of merit were validated using internal standard prepared in matrix-match calibration standard. The calibration curve of each pesticide was constructed using matrix sample spiked at eight different concentrations with the working standard solution. The concentration prepared ranged from 1 to 500 µg/kg, and the peak obtained for each analyte and the internal standard were integrated and the calibration curve was constructed by plotting the ratio of peak area of each analyte to the peak area of the internal standard as a function of concentration. Each concentration point was analyzed in triplicate in three different sample matrices. The precision, selectivity and sensitivity, limit of detection (LOD) and limit of quantitation (LOQ), the average recovery were determined.

CHAPTER FIVE

5.0 RESULTS AND DISCUSSION

5.1 Optimization of GC-MS Parameters

The GC-MS parameters were first investigated in order to obtain the required sensitivity. Different parameters affecting the performance of the GC-MS system as stated in Section 4.5.4, require optimization in order to give a better chromatographic separation. The working standard solution containing the 14 pesticides were spiked into an aqueous solution at a concentration of 0.1 µg/mL and used to optimize the performance of the GC-MS system and were run in triplicate. The averaged total chromatographic peak area values were used to construct a bar chart using Microsoft Excel and the optimum parameters were estimated from the graph. The GC-MS was operated in the split/splitless mode.

5.1.1 Injection Temperature

The injection temperature of the GC injection port should be high enough to achieve column efficiency, consistent with the stability of the analyte to avoid thermal decomposition or chemical reaction. Therefore, it must be high enough for rapid vaporization of the analytes (Husbschmann, 2009; McNair & Miller, 2009). An optimized injection temperature ensures high peak resolution with narrow band. In the present study, the optimal injection temperature was determined by analyzing an aqueous solution spiked with the working standard solution at 0.1 µg/mL containing the target analytes and desorbed at injection temperature between 240 and 280 ^{0}, while keeping other conditions constant.

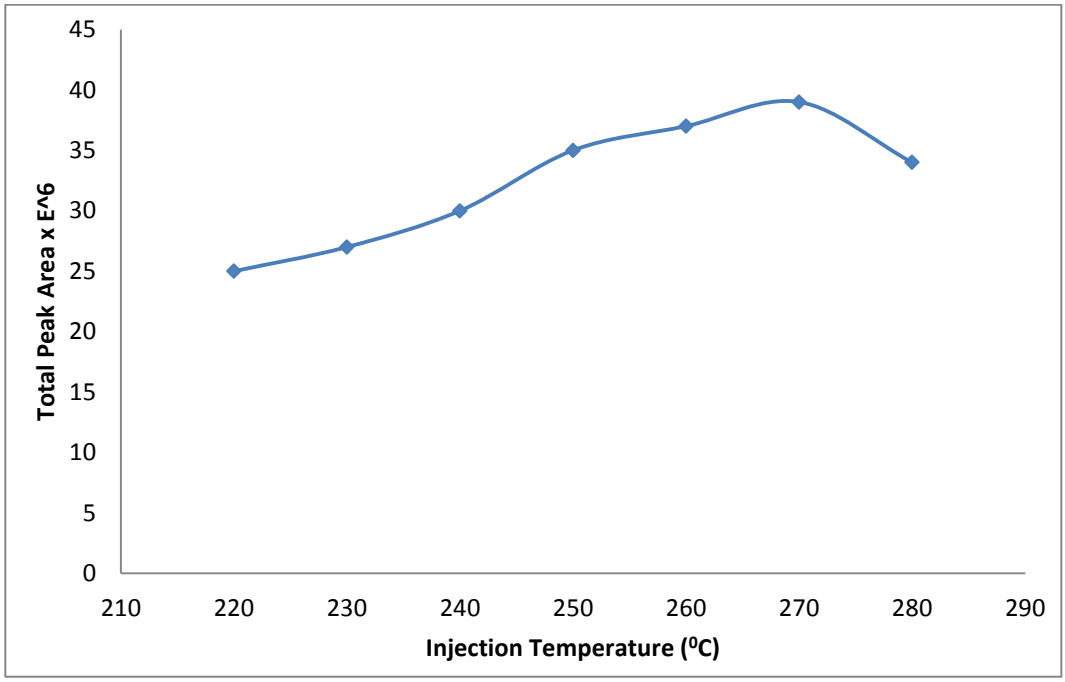


Fig 5.1: Optimization of Injection Temperature

Fig 5.1, shows the plot of the total chromatographic peak area of all the investigated analyte at different injection temperatures. It shows that the maximum sensitivity, as measured by the total peak area of the GC-MS chromatogram obtained was achieved at a temperature of 270 ^{0}C. It implies that there was complete desorption of the analyte at this temperature and thus 270 ^{0} C was selected for further study in order to eliminate carry-over effect and minimize residence time of analytes in the injection liner. The results obtained are in agreement with results reported in other studies with different pesticide residues in fruits and vegetables, such as pyrethroid (Beltran et al., 2003; Sanusi et al., 2004), organochlorine and organophosphorus (Cai et al., 2006; Chai, X. et al., 2008; Yu, J. et al., 2004) pesticide residues.

5.1.2 Interface Temperature

The interface temperature is a critical parameter for a better system performance. The optimization of the condition is important in order to prevent the condensation of the analytes, although it is a compromise between the speed and the sensitivity of analysis. The GC-MS interface temperature should be higher than the highest column temperature in the temperature programming. Thus, for this study, the interface temperature was investigated between 260 and 320 ^{0}C.

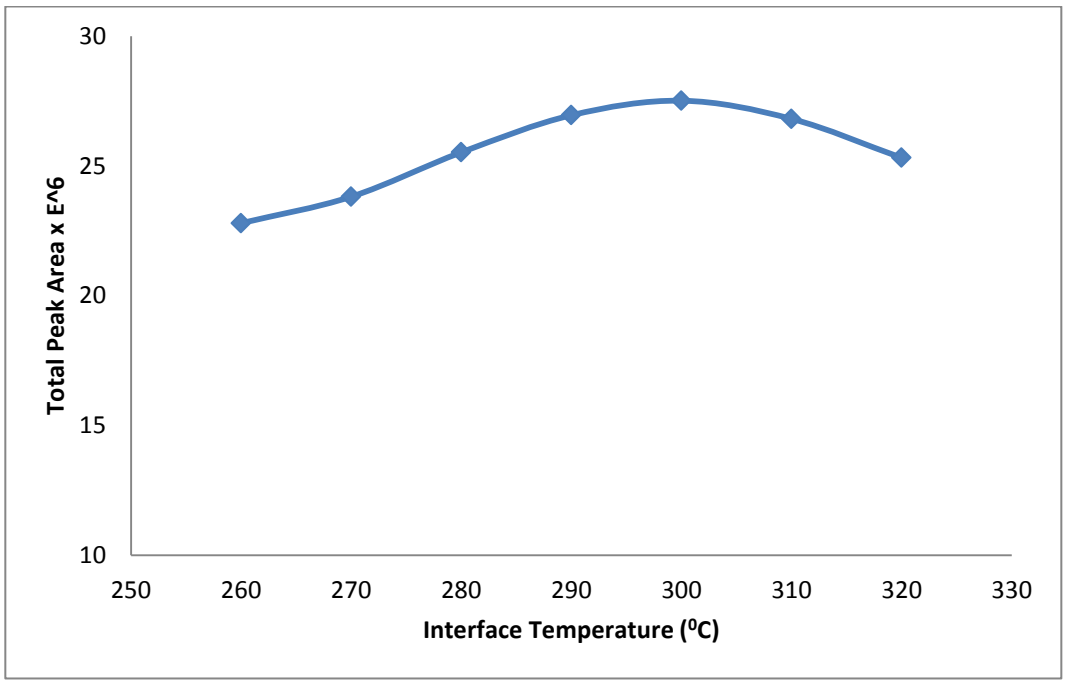


Fig 5.2.: Optimization of Interface Temperature

The plot of the total chromatographic peak area of the analytes and the GC-MS interface temperature (Fig 5.2), show the best interface temperature at 300 ^{0}C, and thus was selected for subsequent experiments. The ion source temperature is important because the analyte needs to be ionized for it to be attracted to the MS magnetic field. The ion source temperature was maintained at 200 ^{0}C. The analytes eluting from the GC column must pass

through the ion source which must be maintained at a constant and reproducible temperature.

5.1.3 Column Flow Rate of Carrier Gas

The column flow rate was investigated between 0.8 to 1.8 mL/min. The linear gas velocity of the column which is a measure of the column efficiency is dependent on the flow rate. The optimization of the flow rate is essential because chromatographic analysis is based on the comparison of retention times and the flow rate determines the elution time of each analyte. The increase in flow rate decreases the analysis time, and thus the separation capacity of the column will be better at the optimized column flow rate. This is as a result of the dependence of the height equivalent to a theoretical plate (HETP) and the number of theoretical plates on the column flow rate. A high column flow rate leads to rapid separation and peak broadening. Therefore the column flow rate was optimized in order to maximize the resolution of the chromatographic peaks.

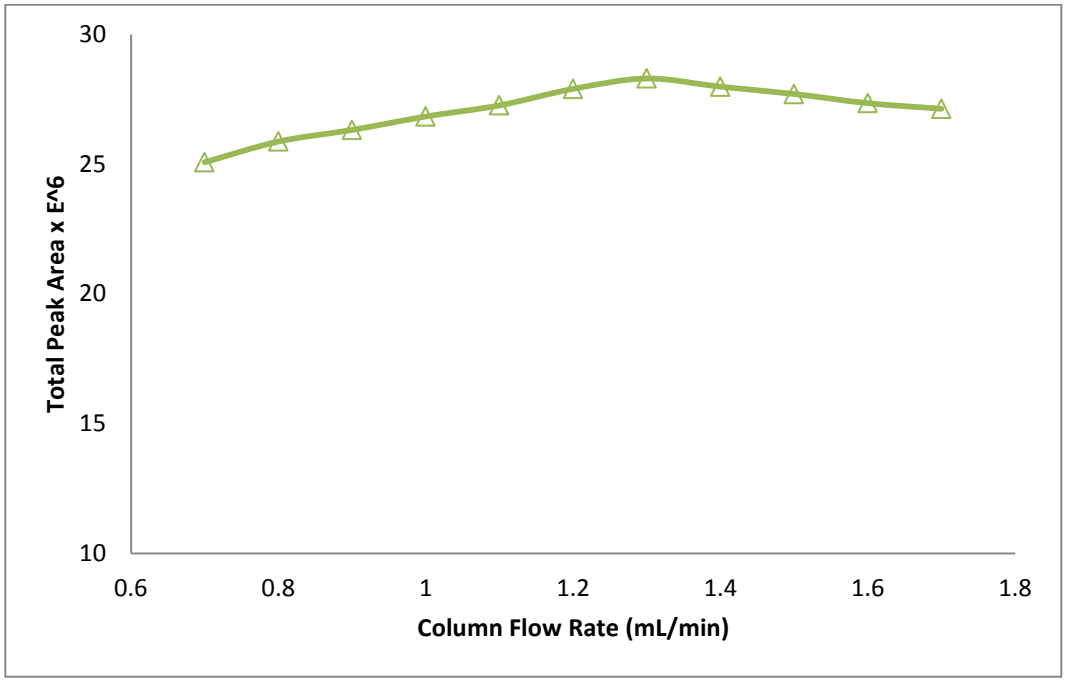


Fig 5.3: Optimization of the GC-MS Column Flow Rate

The effect of column flow rate on the total chromatographic peak area is as shown in Fig 5. It can be observed that the total peak area increases relatively with increase in the column flow. Although the retention time of each analyte varied slightly at the investigated flow rate, the optimal flow rate was found at 1.3 mL/min which gives the highest sensitivity in terms of chromatogram peak area and was selected for this study.

5.2 Optimization of Solid Phase Microextraction Technique

The development of SPME method is described in this section. The selection of fiber coating was conducted as the preliminary optimization step. The SPME extraction conditions were optimized using one factor at a time and also with design of experiment, where all factors where optimized at the same time. The headspace extraction mode was adopted for this study due to the volatility of the target analytes and also to prolong the fiber lifetime.

5.2.1 Selection of Fiber Coating Type

The pesticides selected for this study are of different physico-chemical properties. Therefore, there is a need to investigate the extraction efficiency and performance characteristics of three commercial SPME fiber coatings. The coating with the best extraction efficiency as shown by the total chromatographic peak area is then selected for subsequent analysis. Proper selection of fiber coating helps to achieve better extraction efficiency and improves selectivity.

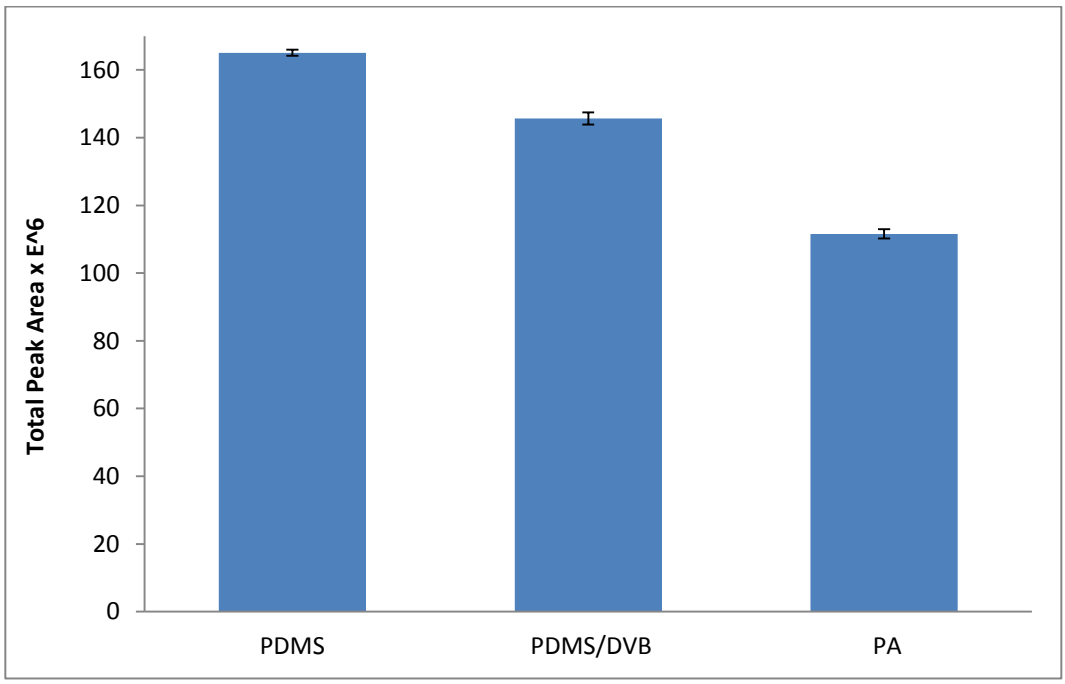


Fig 5.4: Optimization of Fiber Coating Type

The result as indicated in Fig 5.4, illustrated the extraction efficiency of the 3 investigated fibers. It showed that PDMS and PDMS/DVB were the most efficient fibers coating for the extraction of the multiclass pesticides under investigation, since they give the higher total chromatographic peak area compared to the PA. The two fibers have been shown in a previous study to be more efficient in the extraction of pesticide residues belonging to

different classes and having different physic-chemical properties as observed in Table 3.1 (pp. 200 – 204). Further experiments were carried out to determine the best fiber coating and the results are as represented in Fig 5.5.

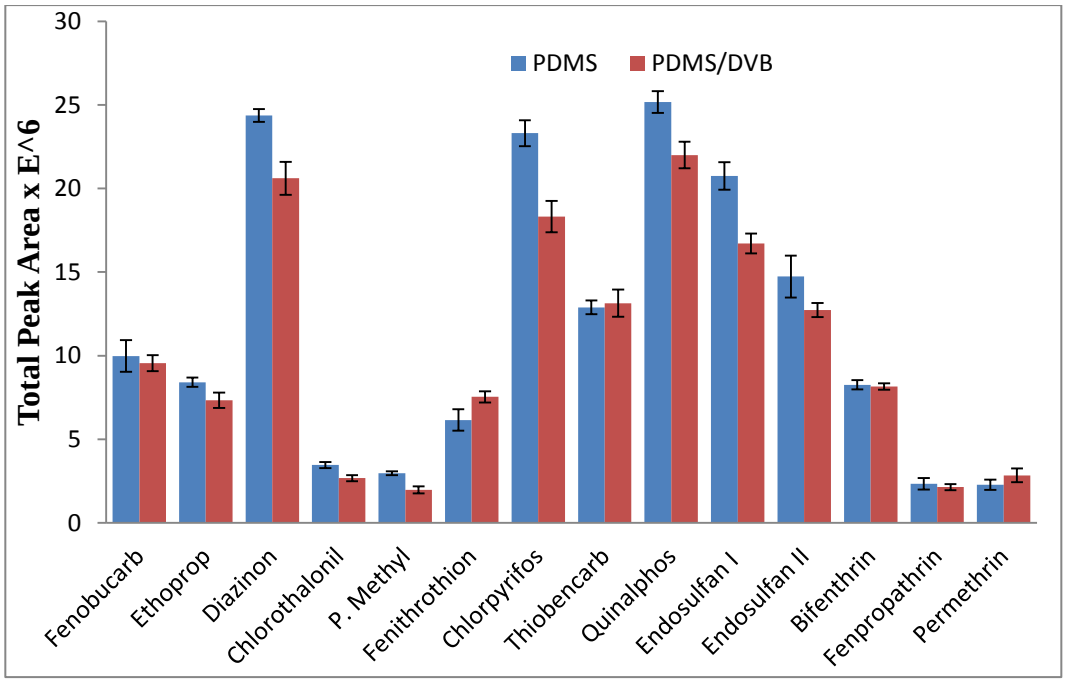


Fig 5.5: Performance Characteristics of PDMS and PDMS/DVB Fibers

It can be seen that the PMDS fiber coating gave the best extraction efficiency for the target analytes. The efficiency of PDMS for the extraction of multiclass pesticide residues have been shown in a previous study to have better performance characteristics for the extraction of wide range of pesticide residues in fruits and vegetables (Bagheri et al., 2012). The PDMS/DVB showed relatively better extraction efficiency for pyrethroid pesticides, but since PDMS showed a better efficiency for all the investigated analytes, it was selected for further method optimization and was used for real sample analysis.

5.2.2 Optimization of Extraction Time

It has been shown that the SPME extraction is an equilibrium process which depends on the partitioning coefficient between the analytes and the fiber coatings. The extraction time profile was investigated by analysis of an aqueous solution spiked with the working standard solution at an extraction temperature of 50 ^{0}C. The extraction time was optimized by varying the time between 10 and 100 min, this range was selected because a longer extraction time favours pesticides of low diffusion coefficients.

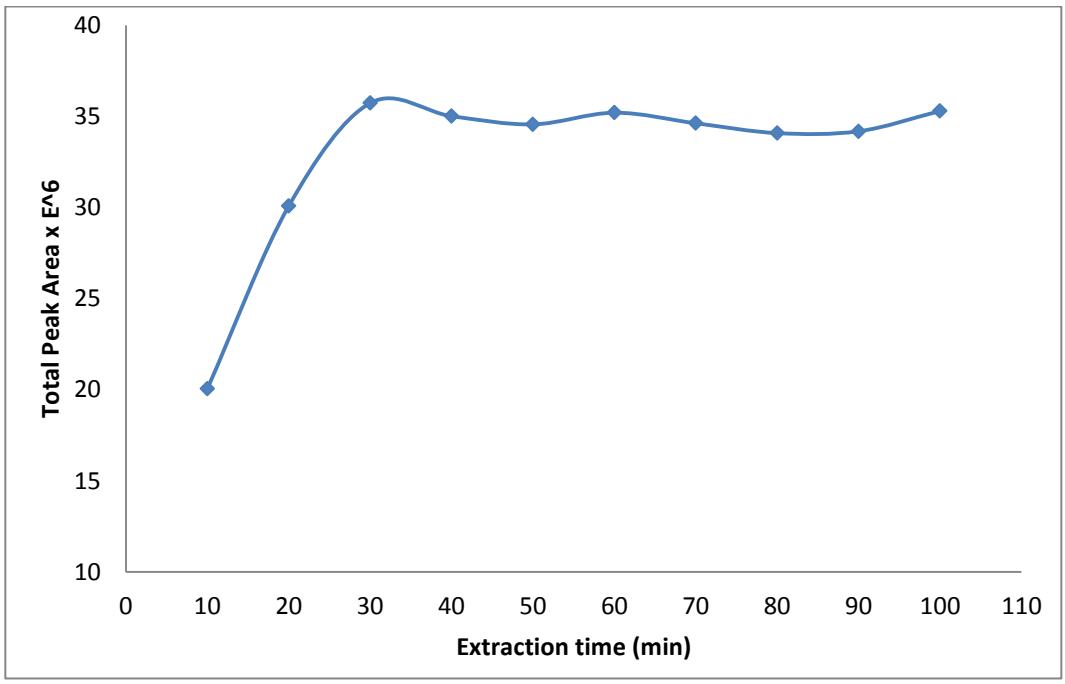


Fig 5.6: Optimization of Extraction Time

The extraction time profile presented graphically in Fig 5.6, shows that the extraction, which is a plot of total chromatographic peak area and the extraction time of the 14 pesticide residues in aqueous solution spiked with the standard mixture. It can be observed that an increase in extraction time increases the total peak area until 30 min, after which the peak area decreases with time, with no significant difference in the total peak area with

increase in time, this may be due to unavailable adsorption space or displacement of the already extracted analytes due to competition for the available adsorption site. Since the extraction efficiency is a compromise between the sensitivity and extraction efficiency, 30 min was selected for subsequent analysis. The time was selected to reduce the total time of analysis, since efficient extraction can also be achieved prior to equilibrium provided all other factors are constant (Ai, 1997a), and to avoid the condensation of water vapour on the fiber (Sang et al., 2013), when exposed to the sample matrix over a long period of time and to increase the fiber lifetime.

5.2.3 Optimization of Extraction Temperature

The diffusion coefficients of the analytes in the sample matrix onto the coated fiber and the distribution constant of analytes between the sample and fiber depend on the extraction temperature. Therefore an increase in extraction temperature, increases the diffusion coefficient and enhances the diffusion of analyte from the sample to the coated fiber and increase the extraction rate (Kataoka et al., 2000). Although, the partitioning of analytes into the coated fiber is an exothermic process, therefore temperature has dual effects on the amount extracted. Increase in temperature also decreases the distribution constant which can reduce the amount of analyte extracted at equilibrium. In order to maximize the amount extracted with respect to change in temperature, an optimal extraction temperature should be selected to achieve satisfactory sensitivity and faster extraction rate.

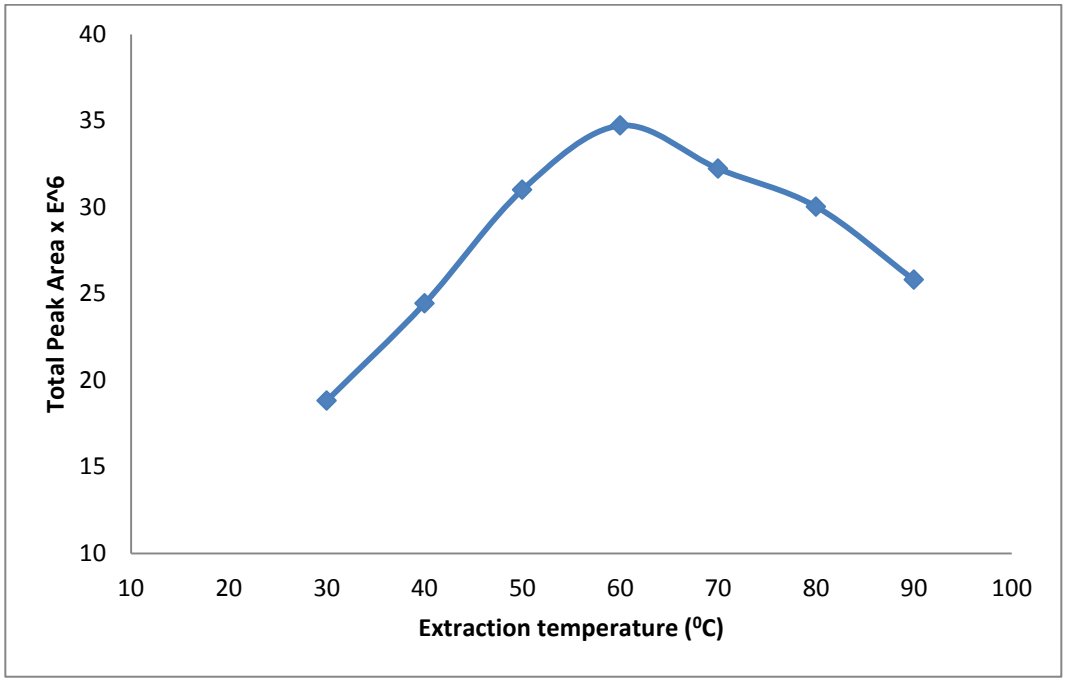


Fig 5.7: Optimization of Extraction Temperature

Fig 5.7, showed that amount of pesticides extracted increases with increase in the extraction temperature, and an optimal temperature is reached at 60 ^{0}C. The optimized temperature is also favourable because higher extraction temperature may lead to the decomposition of some pesticides by hydrolysis and can also lead to the vaporization of the aqueous sample solution, which can affect the extraction efficiency of the coated fiber. Thus, the reason why the amount extracted at elevated temperature decreased at 70 ^{0}C and above. Hence, the subsequent analysis were carried out at 60 ^{0}C.

5.2.4 Optimization of Stirring Rate

The efficiency of SPME technique can also be improved by agitation, because stirring the sample matrix will reduce the diffusion layer and improves the mass transfer of analytes from the matrix to the headspace and then to the coated fiber. Increase in stirring rate increases extraction rate and decreases the equilibrium time. Different methods of agitation

(magnetic stirring, sonication, fiber vibration, and flow through cell), have been proposed, but studies have shown that, there is no significant difference between accuracy and precision of the different agitation methods (Eisert & Pawliszyn, 1997b). For this study, vial agitation was achieved using a CTC CombiPAL autosampler equipped with agitator and the agitation rate was varied between 250 and 750 rpm. The range was used as specified by the manufacturer.

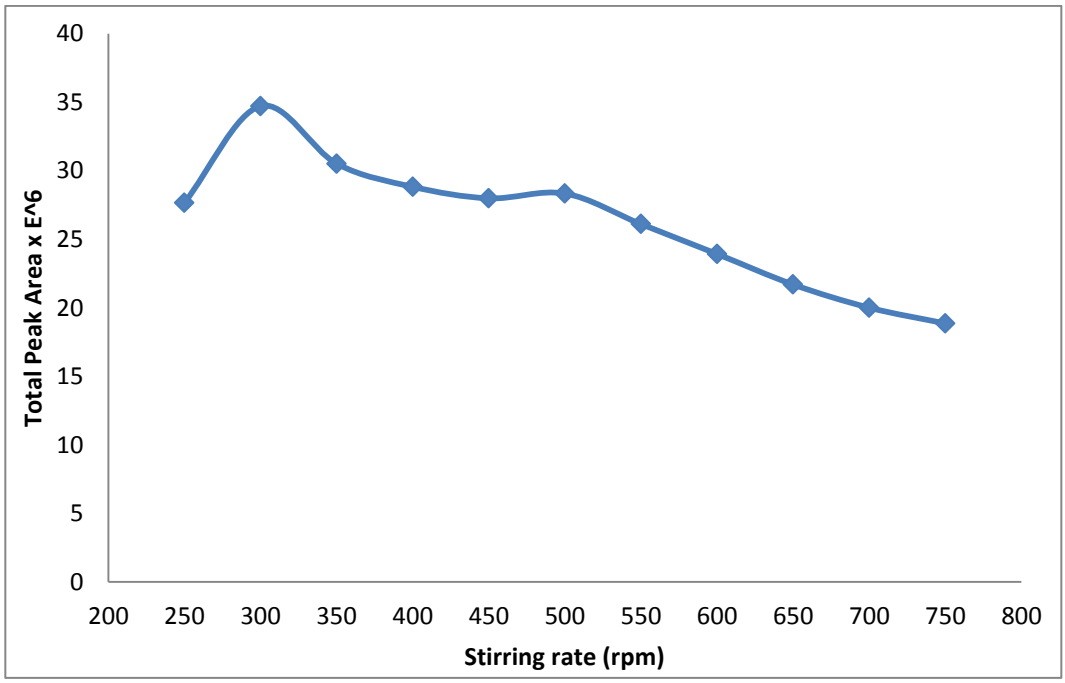


Fig 5.8: Optimization of the Stirring Rate

As can be observed from Fig 5.8, the amount extracted only increase between 250 rpm and 300 rpm after which further stirring leads to the decrease in the amount of pesticide extracted. It showed that a higher stirring rate can lead to the vibration of the fiber which could lead to displacement of extracted analytes. It has been observed that higher stirring rates can cause the formation of air bubbles which can reduce the extraction efficiency of the coated fiber (Zeng et al., 2008). Thus, an extraction rate of 300 rpm was selected for

subsequent experiment. The lower stirring rate which gives efficient extraction could also be due to the use of the agitator equipped with the autosampler (Menezes Filho et al., 2010), compared with the use of manual agitation using magnetic stirrer as reported by other authors (Chai, M. K. et al., 2008b).

5.2.5 Optimization of Salt Addition

The salting out effect can also be used to improve the extraction of pesticide residues from sample matrix, by saturating the sample matrix thereby increasing the analytes distribution constant. The addition of salt to sample matrix decreases the solubility of water-soluble pesticides, changes their ionic strength and also changes the physico-chemical properties of the pesticides (such as viscosity and surface tension) (Jeannot et al., 2010; Lambropoulou & Albanis, 2007a). Therefore, the need to determine the best salt to be added and the optimal amount required to enhance extraction efficiency, without any adverse effect on the amount extracted. For this purpose, three salts (NaCl, $(NH_4)_2SO_4$ and Na_2SO_4) were tested for their effect on the extraction of the 14 investigated pesticides at 5 % (v/v) for each salt.

The results as shown in Fig 5.9 indicates that NaCl enhances the extraction of the pesticides more than the other two salts, and was selected for further experiment. The amount of NaCl required to maximize the extraction of the pesticides was also investigated and the optimal amount was found at 10 % (v/v) as shown in Fig 5.10. Thus 10 % of NaCl was selected as the optimum concentration required for effective extraction of pesticides from the sample matrix.

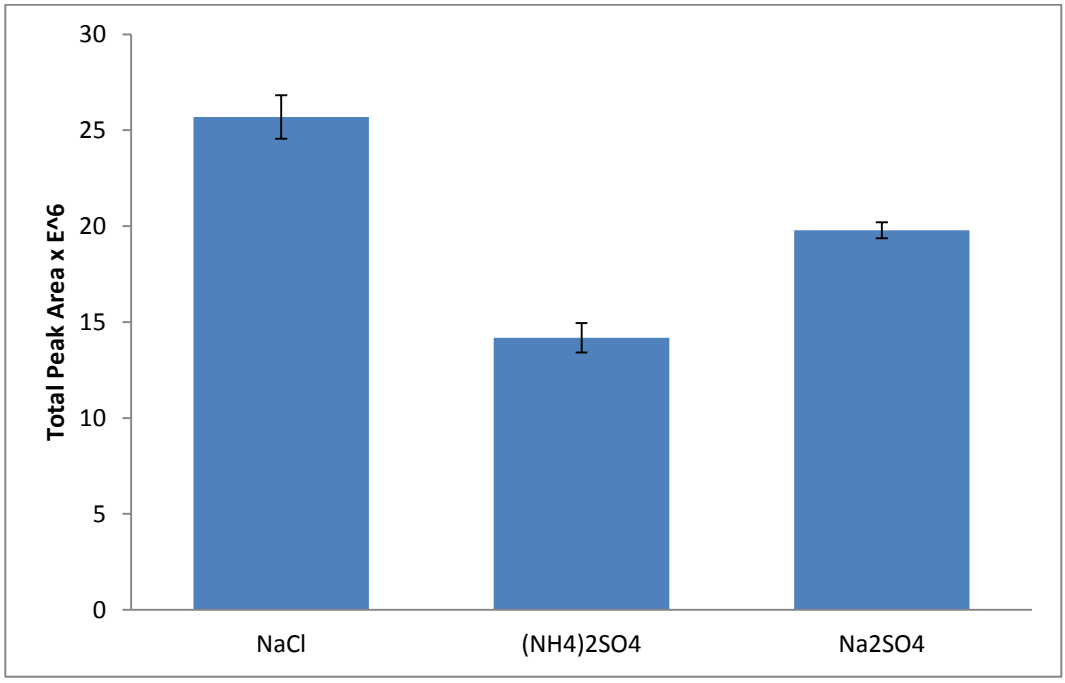


Fig 5.9: Effect of Types of Salt

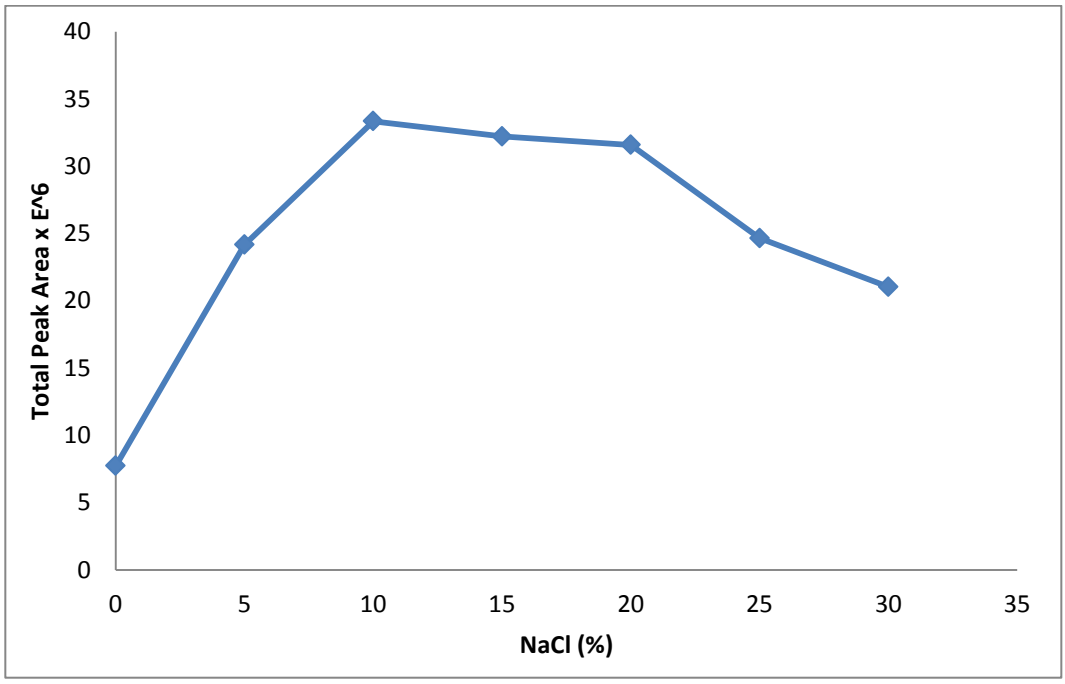


Fig 5.10: Optimization of Amount of NaCl (%)

The effect of NaCl concentration shows that salting out effect has a maximum sensitivity at equilibrium, after which further increase in salt concentration decreases the amount of pesticide extracted. This can be due to the saturation of the matrix by the salt and the reduction in the activity coefficient of the pesticides beyond the optimal condition.

5.2.6 Optimization of pH Value

The efficiency of SPME extraction is also improved by adjustment of sample pH, because, SPME involves the extraction of the dissociated and neutral species (Kudlejova et al., 2012; Risticevic et al., 2010b). The adjustment of sample pH also helps to transform the analytes into their molecular state and significantly improves the extraction efficiency.

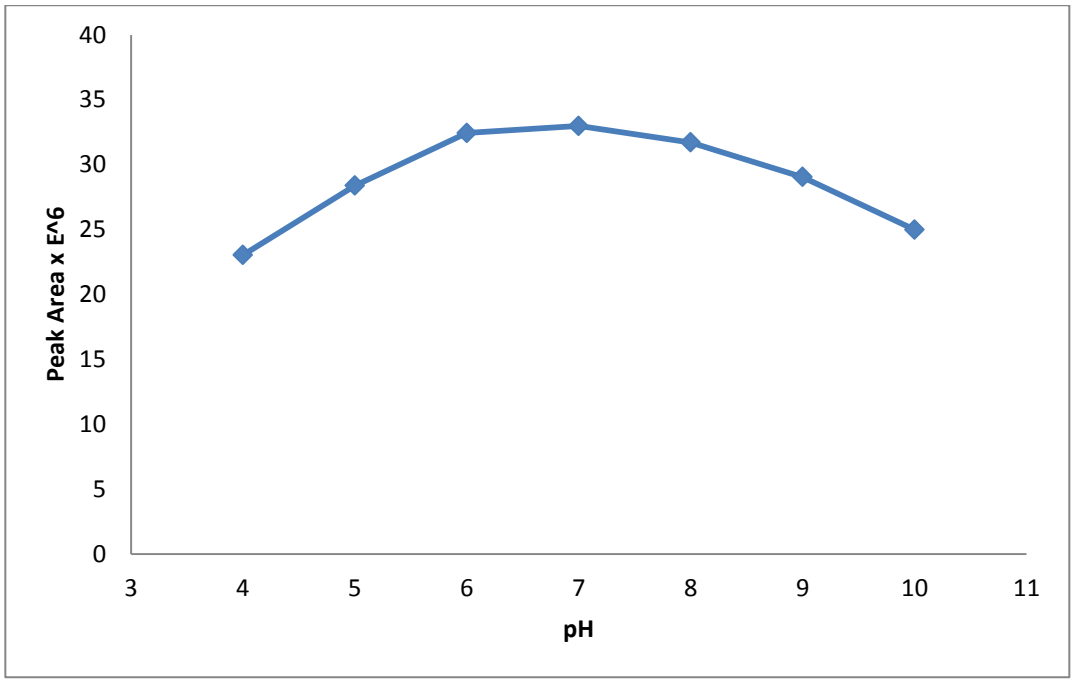


Fig 5.11: Optimization of pH Value

In this study, the sample pH was varied between pH 4 and pH 10 and was adjusted by addition of known amounts of pH buffer solutions into the sample matrix to maintain the desired pH values. The result (Fig 5.11) shows the effect of adjusting the pH of sample matrix and the optimum pH value was found at pH 7, indicating that the extraction efficiency of the investigated pesticides is enhanced in neutral medium. Although it was observed that, there was only a slight difference in the extraction efficiency at pH 6 and 7, pH 7 was selected for subsequent experiments.

5.2.7 Optimization of Desorption Time

The time taken to completely desorb the analytes extracted on the coated fiber is also very essential and must be optimized. This will give the highest chromatographic sensitivity and eliminate the carry-over effect. The desorption temperature has been optimized in Section 5.1.1, with optimal injection temperature set at 270 ^{0}C. Thus, desorption time was varied between 2 and 10 min, while keeping all other chromatographic and SPME conditions constant.

As shown in Fig 5.12, the optimal desorption time was found at 7 min, which implies that the SPME fiber should be left in the injection chamber of the GC for 7 min at 270 ^{0}C in order for the extracted pesticides to be completely desorbed into the injection chamber. Therefore desorption time of 7 min was selected for subsequent analysis.

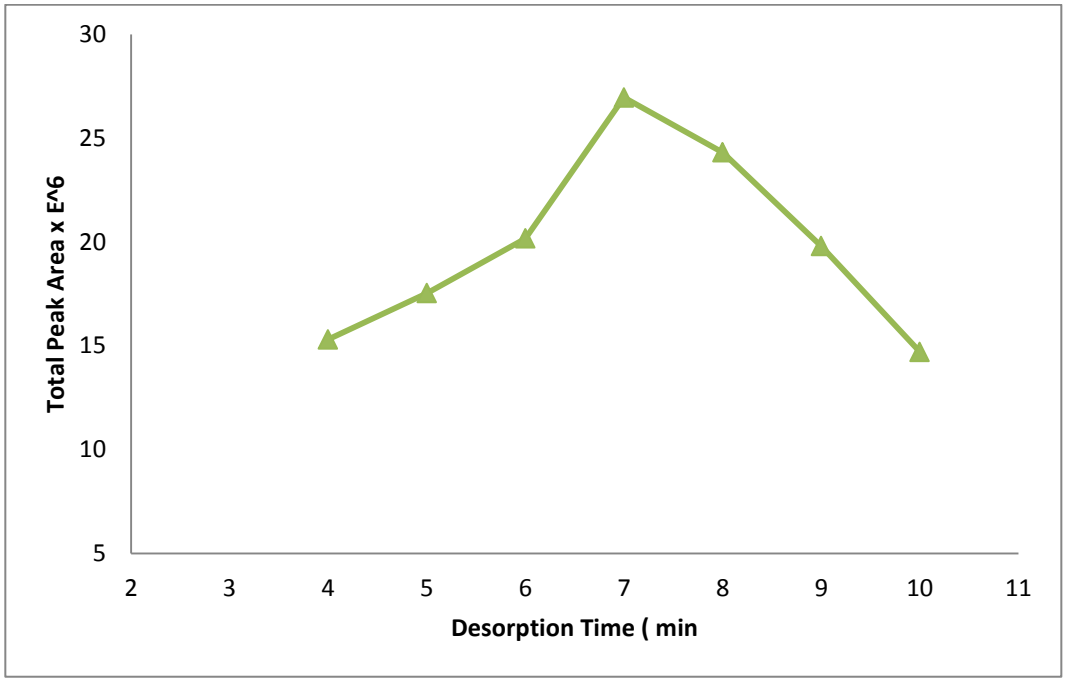
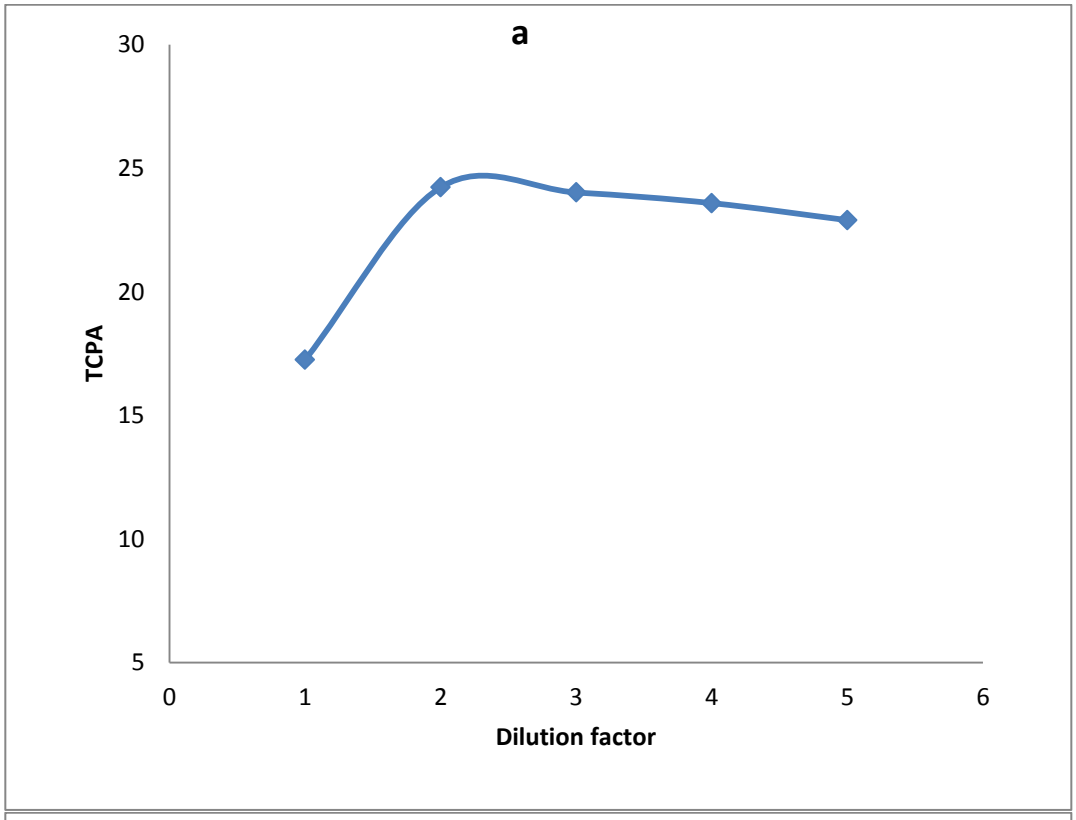
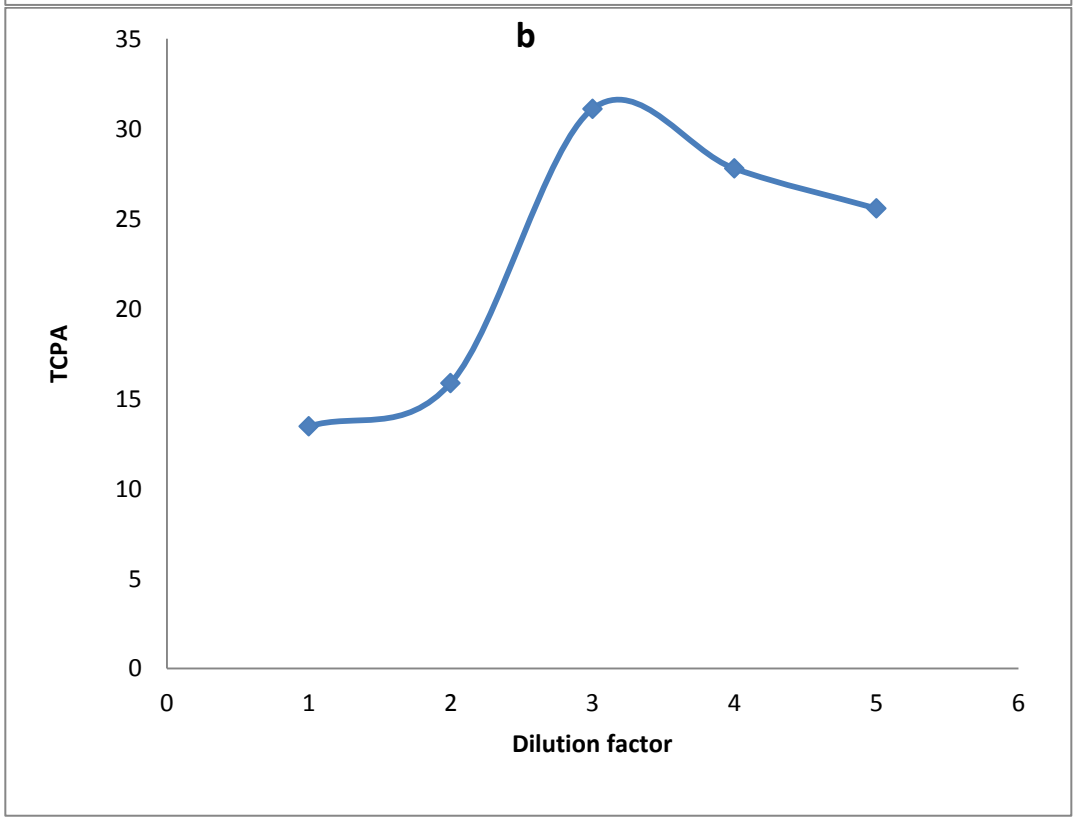


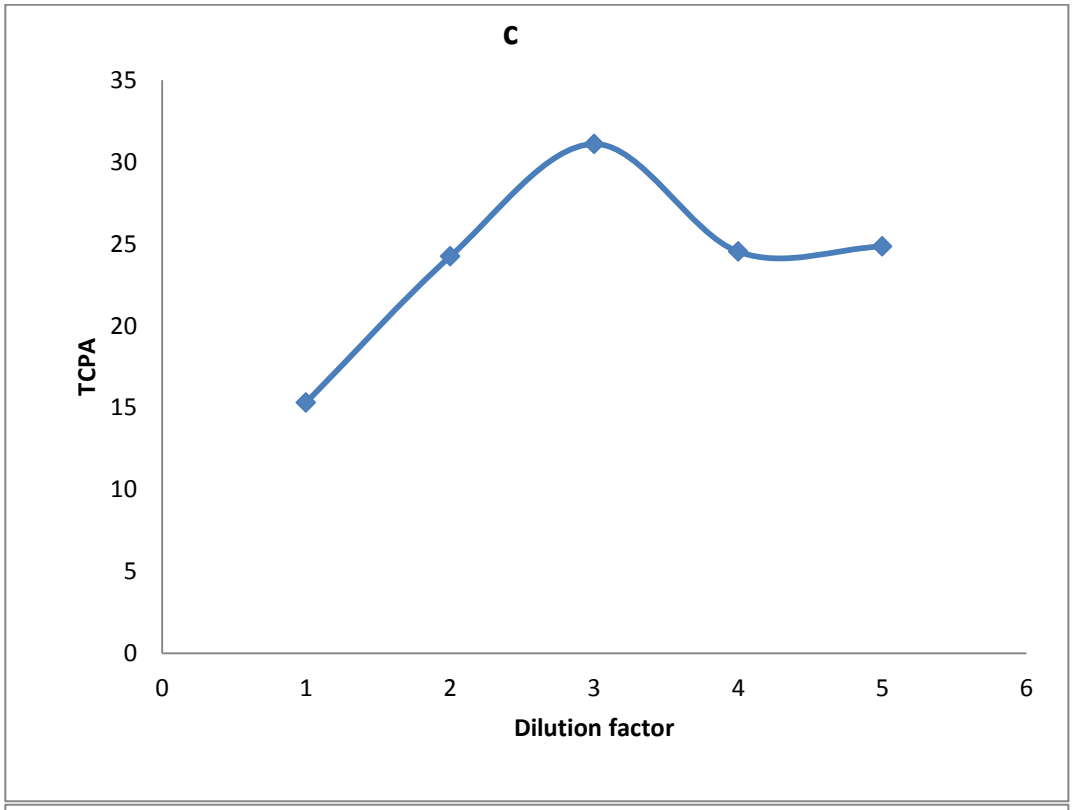
Fig 5.12: Optimization of Desorption Time

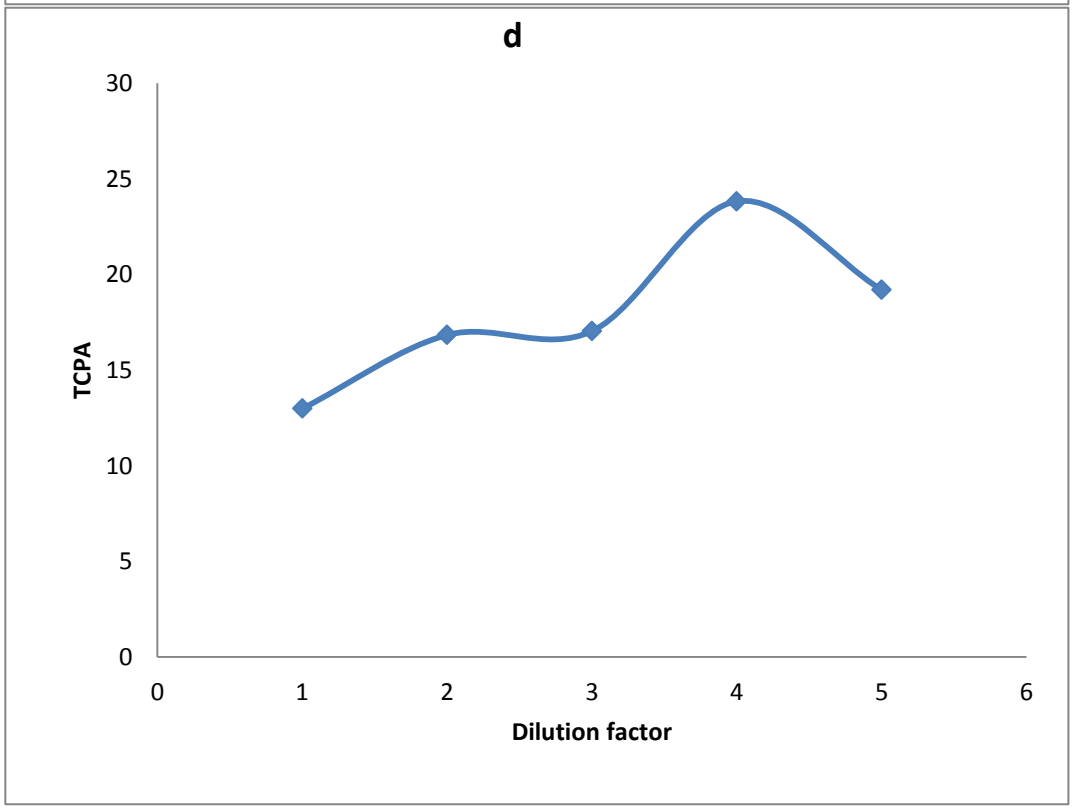
5.2.8 Optimization of Dilution Factor

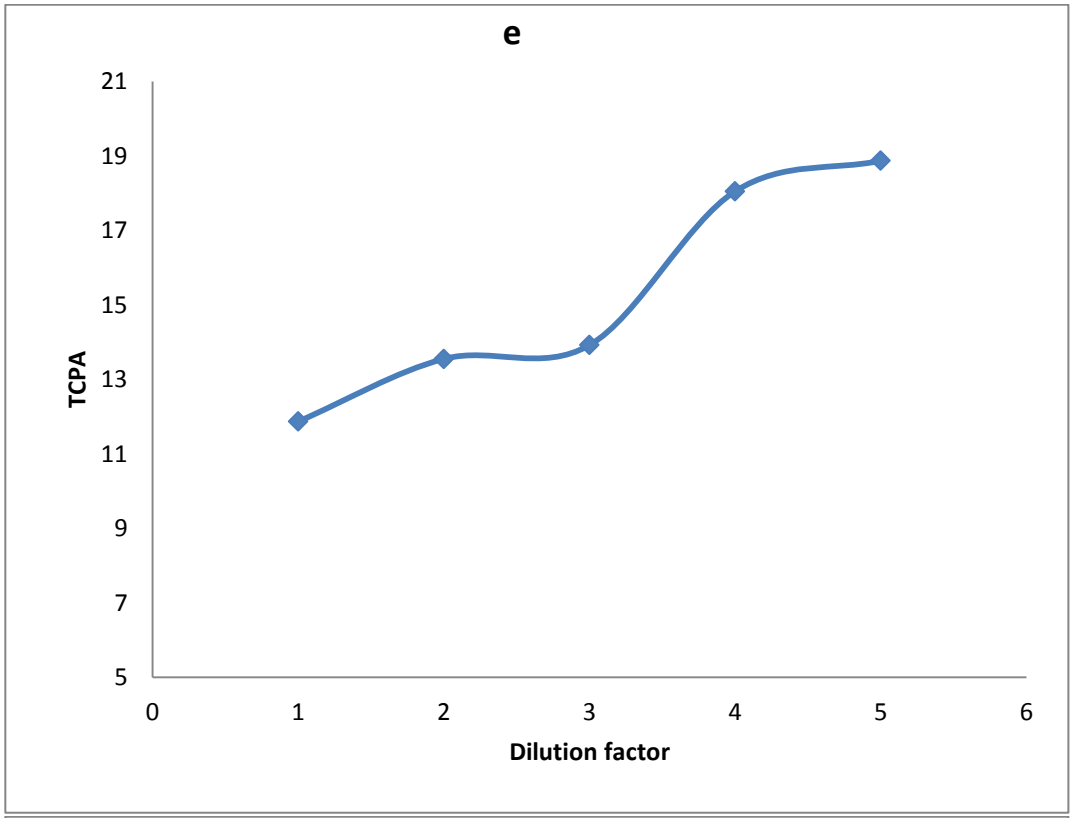
It has been shown that dilution of samples enhances extraction efficiency of pesticides from the sample matrix (Lambropoulou & Albanis, 2003; Simplício & Vilas Boas, 1999). However, the dilution ratio should be limited in order not to dilute the concentration of the pesticides in an aqueous sample. Addition of water to samples containing the pesticides has been found to increase the diffusion barrier of pesticides from the aqueous phase to the headspace (Lambropoulou & Albanis, 2003). Therefore, there is the need to investigate the ratio of water to the sample (w:v) that is required for maximum sensitivity and higher recovery of target analytes (Zambonin et al., 2004). The dilution will enhance the displacement of the pesticide bonded to the sample component and increases extraction efficiency. The optimum dilution ratio was investigated by adding different amounts of water to the sample, ranging from dilution factor of 1 to 5.

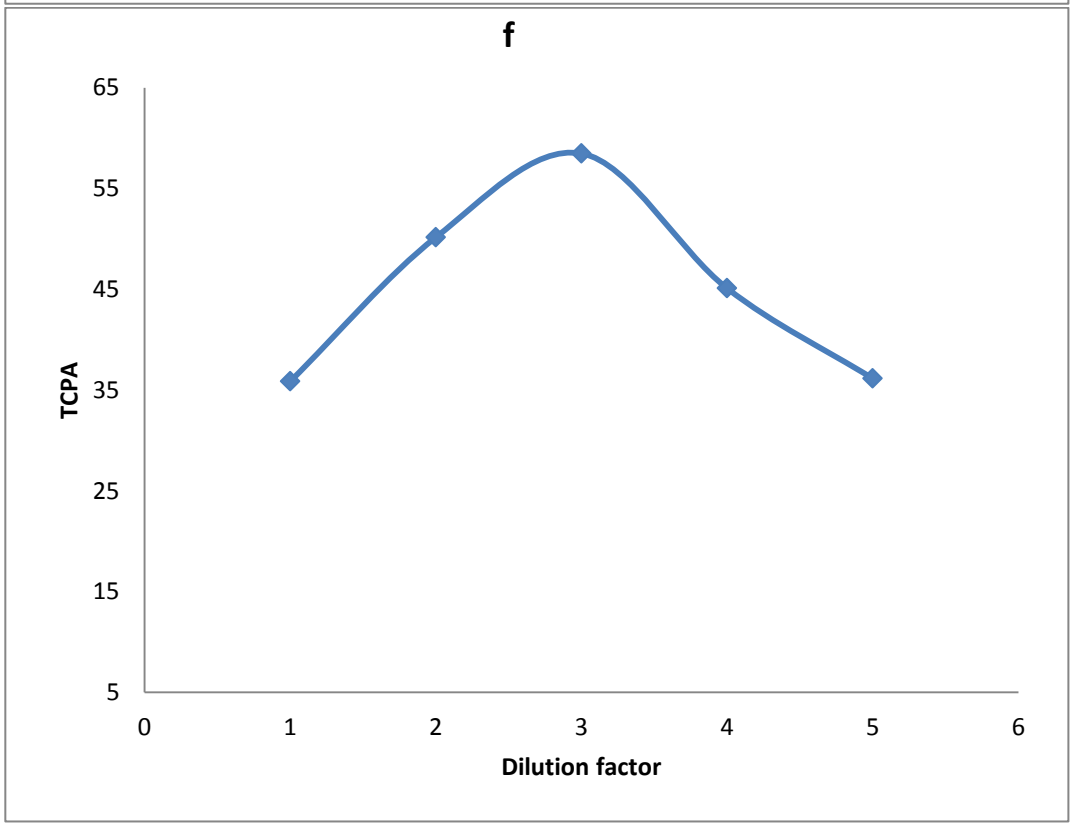

a
30
25
20
15
10
5
TCPA
0
1
2
3
4
5
6
Dilution factor


b
35
30
25
20
15
10
5
0
TCPA
0
1
2
3
4
5
6
Dilution factor


c
35
30
25
20
15
10
5
0
TCPA
0
1
2
3
4
5
6
Dilution factor


d
30
25
20
15
10
5
0
TCPA
0
1
2
3
4
5
6
Dilution factor


e
21
19
17
15
13
11
9
7
5
TCPA
0
1
2
3
4
5
6
Dilution factor


f
65
55
45
35
25
15
5
TCPA
0
1
2
3
4
5
6
Dilution factor

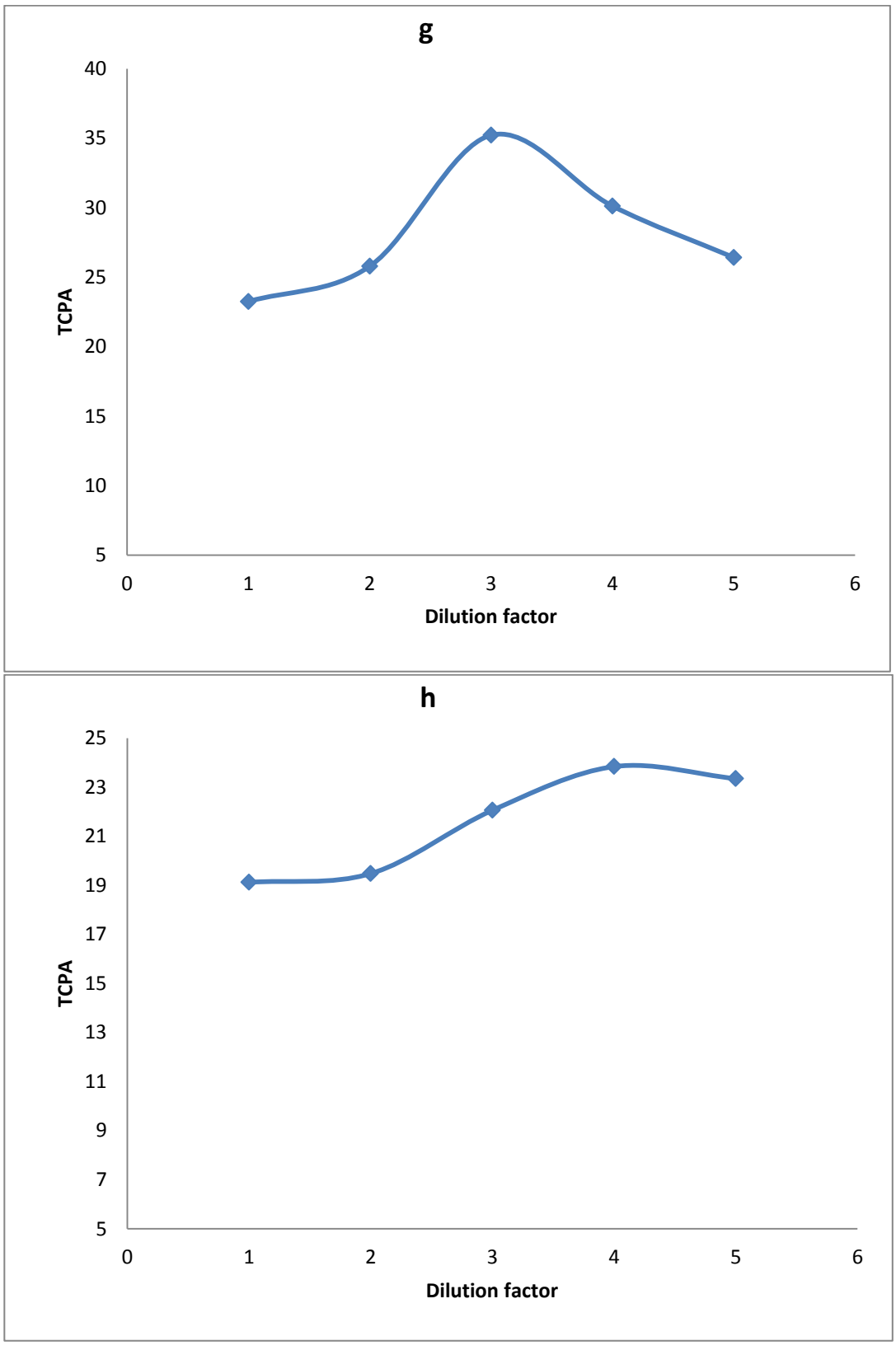


Fig 5.13 Optimization of Dilution Factor (a) Tomato (b) Grape (c) Pear (d) Cabbage (e) Broccoli (f) Apple (g) Cucumber (h) Lettuce

The addition of water to the sample matrix enhances the release of pesticide residues and reduces the effects of high molecular compounds present in the sample (e.g. pectin and sugar), which can also adsorb the analytes leading to the formation of micelles and results in the reduction of pesticide extracted (Lambropoulou & Albanis, 2003; Simplício & Vilas Boas, 1999). It also helps to increase the diffusion rate of pesticides and reduces poor recovery (Risticevic et al., 2010a). As shown in Fig 5.13 (a-h), the optimum dilution factor is as follows: (ratio, sample/water(w:v): tomato, 1:2; grape, 1:3: Pear, 1:3; Cabbage, 1:4; Broccoli, 1:5, Apple, 1:3, Cucumber, 1:3 and Lettuce, 1:4).

5.2.9 Selection and Optimization of Organic Solvent

The addition of organic solvents increases the extraction efficiency by increasing the release of analytes from the sample matrix to the headspace. The amount of organic solvents must be maximized and also be at the minimum in order to eliminate its negative effect on the distribution constants of the target analytes. The addition of organic solvents also helps to reduce the adsorption of target analytes to the sample vial wall (Ochiai et al., 2005).

Optimization of organic solvent was carried out using the design of experiment (DOE), by utilizing the simplex lattice design. The design was chosen because, it involves fewer experimental runs and spans the mixture space of solvents evenly (Brereton, 2003). It is assumed that the possible interactions of different mixture components can have both negative and positive effects on the extraction efficiency of the investigated pesticides. It is important to investigate the appropriate mixture of solvents that will result in effective extraction of pesticide residues from the sample matrix without compromising their

distribution constant. This was achieved by constructing a simplex lattice design matrix (Tab 5.1), consisting of three solvents using the Minitab® 16 Statistical Software package.

From the reviewed literatures, it has been observed that the use of different percentage of organic solvent enhances extraction efficiency depending on the classes of pesticides under investigation. In this study three solvents (methanol, acetone and acetonitrile) were selected due to the difference in the polarity, solubility and based on the available literature. The use of chlorinated solvents was not considered due to their health hazards, environmental pollution and cost of disposal.

Table 5.1: Simplex Lattice Design Matrix[(a)]

RunOrder	PtType	Blocks	MeOH	AcO	ACN
1	2	1	50.00	50.00	0.00
2	1	1	0.00	100.00	0.00
3	1	1	100.00	0.00	0.00
4	1	1	0.00	0.00	100.00
5	2	1	50.00	0.00	50.00
6	-1	1	66.67	16.67	16.67
7	-1	1	66.67	16.67	16.67
8	-1	1	16.67	16.67	66.67
9	2	1	50.00	50.00	0.00
10	1	1	0.00	100.00	0.00
11	0	1	33.33	33.33	33.33
12	-1	1	16.67	16.67	66.67
13	1	1	100.00	0.00	0.00
14	-1	1	16.67	66.67	16.67
15	2	1	0.00	50.00	50.00
16	1	1	0.00	0.00	100.00
17	0	1	33.33	33.33	33.33
18	2	1	50.00	0.00	50.00
19	2	1	0.00	50.00	50.00
20	-1	1	16.67	66.67	16.67

[a] Generated using Minitab Statistical Software®

The simplex lattice design with 10 experimental points was performed in triplicate randomly at all points and the experimental data was fitted to a quadratic polynomial model. The simplex design plot in amounts of solvent was constructed as shown in Fig 5.14. The overlay contour plot was also constructed with the total chromatographic peak area (TCPA) as response factor as shown in Fig 5.15. and Fig 5.16.

As shown in Figs 5.15 and 5.17, the optimum extraction as indicated by the higher chromatographic peak area (TCPA) was found between mixture of acetone and methanol, while the lowest TCPA was found between the mixture of methanol and acetonitrile. To determine the maximum desirability of the TCPA, the response optimizer was utilized, and it shows the main effect of each solvent on the TCPA (Fig 5.19).

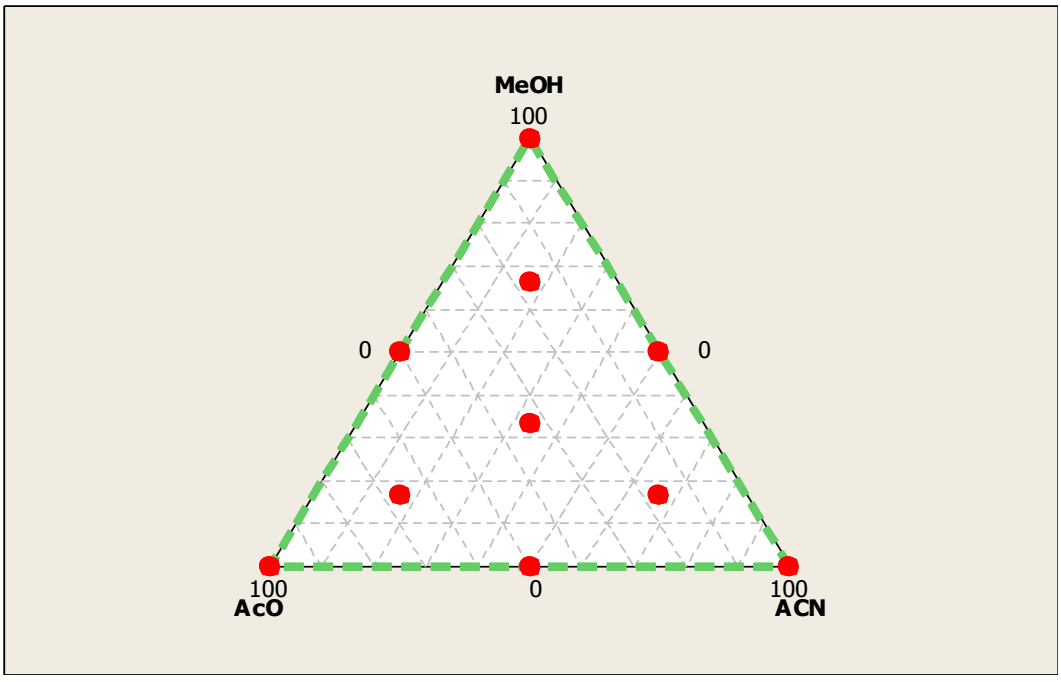


Fig 5.14: Simplex Design Plot in Amounts (Methanol, MeOH; Acetone, AcO; Acetonitrile, ACN)

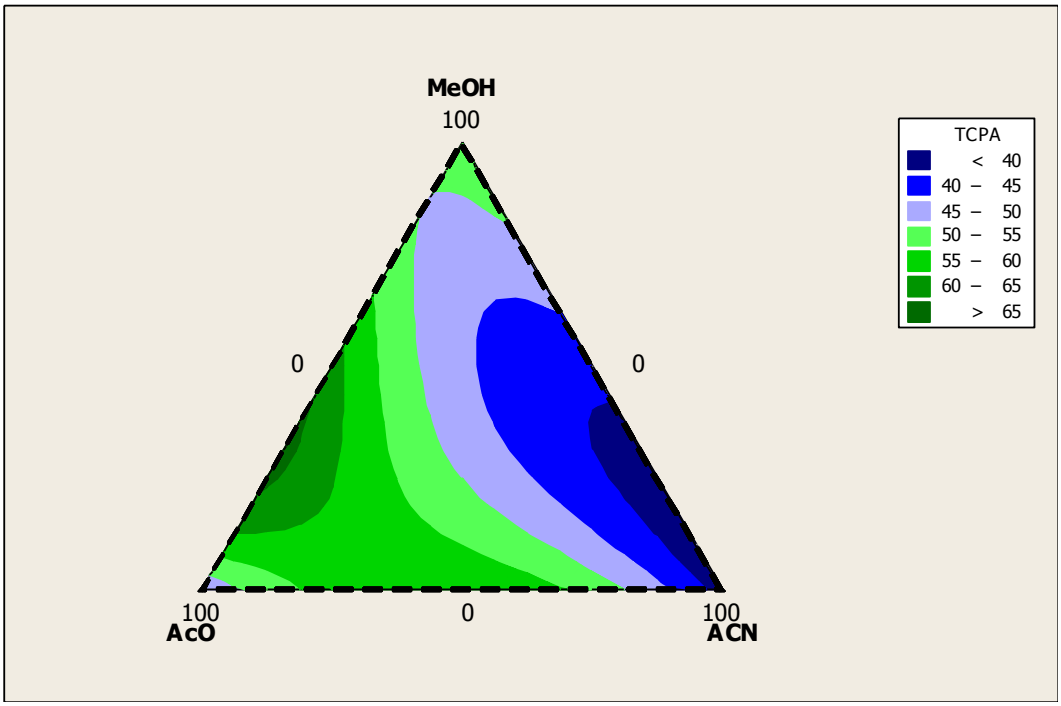


Fig 5.15: Mixture Contour Plot for TCPA

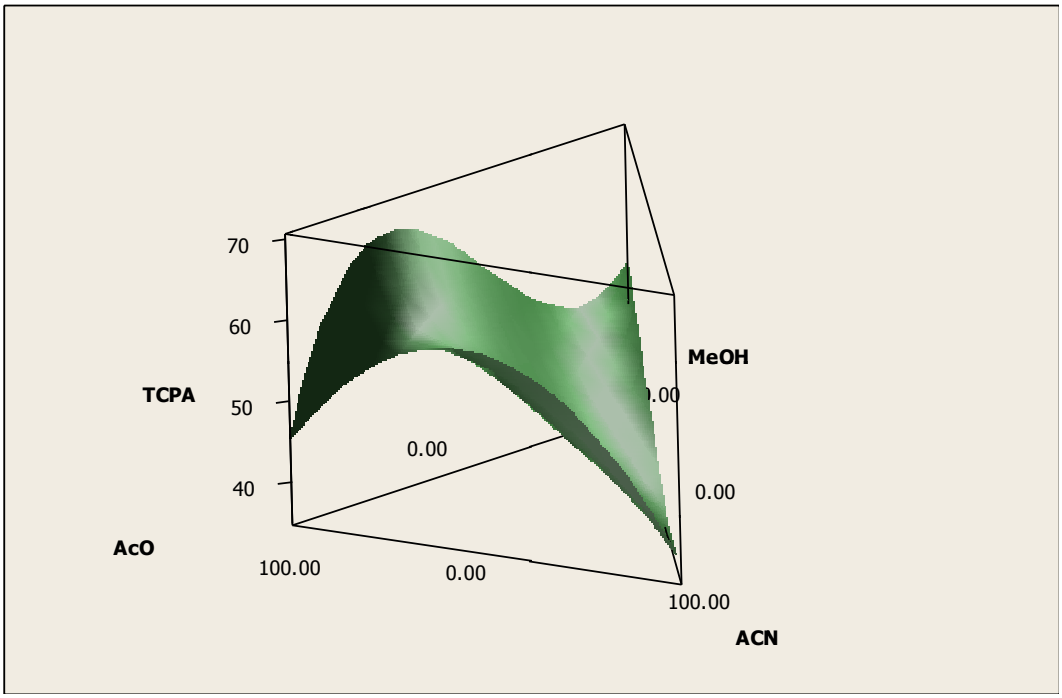


Fig 5.16: Mixture Surface Plot for TCPA

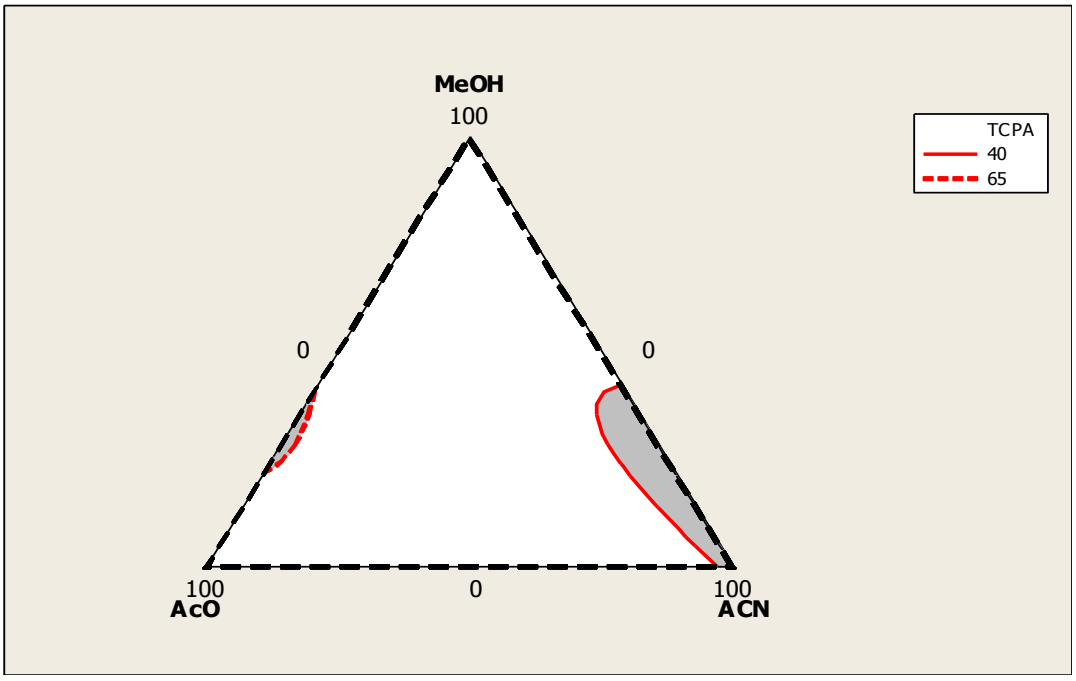


Fig 5.17: Contour Plot for TCPA

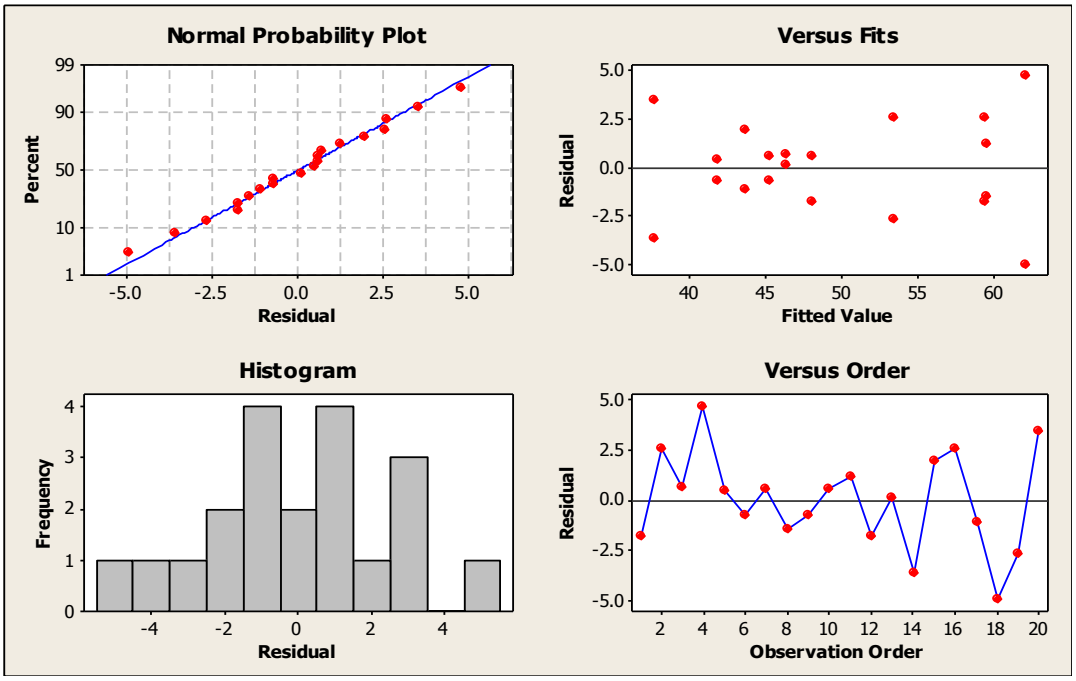


Fig 5.18: Residual Plot for TCPA in Mixture Design

As shown in the figure (Fig. 5.19) for the maximum desirability (0.99955) of component mixture, the optimal mixture consisting of approximately 21.32 % of methanol and 78.87 % of acetone, give the optimum extraction of the investigated pesticides. Therefore, further experiment was conducted using a mixture of methanol and acetone (21:79, v/v %). The result obtained is in agreement with the recent study (Sang et al., 2013), which showed that the use of binary solvents in SPME could accommodate a wide array of matrix characteristics. The residual plots (Fig 5.18) indicate that there is a non-variance constant and also show that the measured response is randomly distributed around the mean.

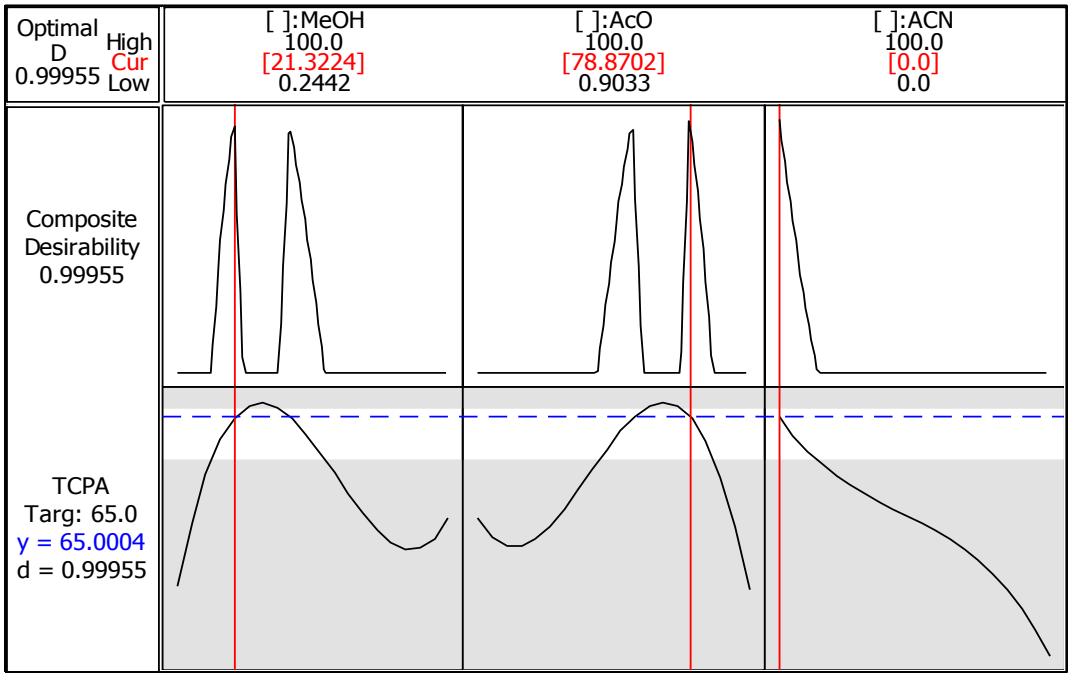


Fig 5.19: Response Optimizer for Maximum Component Desirability

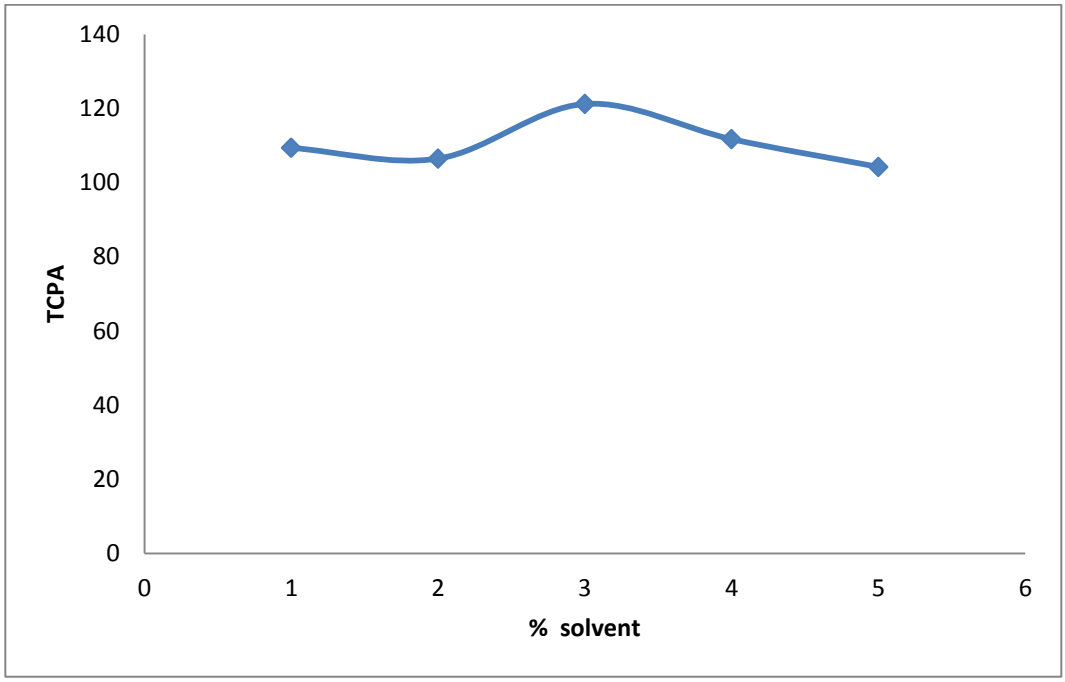


Fig 5.20: Optimization of the Percentage Solvent

The percentage of organic solvent in the sample matrix was also investigated. It has been shown that the presence of organic solvent can reduce the distribution constant of the analytes, therefore the addition of organic solvent should not exceed 5 % of the total sample weight or volume (Kudlejova et al., 2012), in order to achieve efficient extraction and improve selectivity. The solvent percentage was varied between 1 – 5 % and the result (Fig 5.20) shows that maximum chromatographic peak was observed at 3 % organic solvent and it was selected for further studies.

5.3 Multivariate Design of Experiment

The optimization of SPME condition investigated so far has been based on the univariate approach, in which one factor is optimized at a time, while keeping other factors constant. The univariate design has been found to yield satisfactory result, but it does not involve the estimation of effect of possible interaction of various factors (Kudlejova et al., 2012). The

estimation of the possible interaction of factors in the multivariate experimental design yields a true optimal value which may be different from the values obtained in the univariate experiments. Consequently, there is need to re-examine some important parameters (Kudlejova et al., 2012; Pawliszyn, 1997).

5.3.1 Preliminary Experiment with Full Factorial Design

A full factorial design was carried out by investigating the effects of three matrix related factors (extraction temperature, extraction time and salt addition), using 4 pesticide residues (fenobucarb, diazinon, chlorothalonil and chlorpyrifos). To study the effect of extraction temperature, time and salt addition (NaCl) on the extraction of pesticide residues, a factorial 2^3 randomized-block experimental design was applied. The factors and levels considered are as shown in Table 5.2, while response variables were peak areas of the selected pesticides. The design was executed in two blocks, each daily. This 2-block design allowed the elimination of sources of daily variability. The design matrix is shown in Table 5.3. The design allows the assessment of the main effect, block effect and interactions between the selected conditions (Table 5.4).

The experimental design model was confirmed using ANOVA assumptions for the response variables of each pesticide. The significance of the studied variables in the experimental design is shown in Fig. 5.21, in the form of a Pareto chart. The chart illustrates the influence each variable has on the response of the studied pesticides. This corresponds to the length of the bar, i.e. the length of the bar is proportional to the significance of the variables. The chart also shows the effect of the second- and third-order interactions among the variables. The results showed that temperature, time and salt

addition were significant for chlorothalonil and chlorpyrifos, while only temperature and salt addition were significant for fenobucarb and diazinon (Fig 5.21). This is due to their low polarity and high affinity to the PDMS fiber. The interactions of most of the factors were also significant except in chlorpyrifos, estimated from the significance value (p=0.05), of the interactions of various factors. The main effects and interactions of all the factors are significant for chlorothalonil, this is attributed to its low solubility in water.

Table 5.2: Factors and Levels of the Variables

Variables	Low	High
(A) Extraction temperature (^{0}C)	30	60
(B) Extraction time (min)	30	60
(C) Salt concentration (%, w/v)	5	10

Table 5.3: Factorial Design Matrix [a]

Run Order	Std Order	Block	Time (min)	Temp. (^{0}C)	Salt (%)
1	3	1	30	60	5
2	2	1	60	30	5
3	1	1	30	30	5
4	8	1	60	60	10
5	7	1	30	60	10
6	5	1	30	30	10
7	4	1	60	60	5
8	6	1	60	30	10
9	10	2	60	30	5
10	12	2	60	60	5
11	14	2	60	30	10
12	13	2	30	30	10
13	16	2	60	60	10
14	15	2	30	60	10
15	9	2	30	30	5
16	11	2	30	60	5

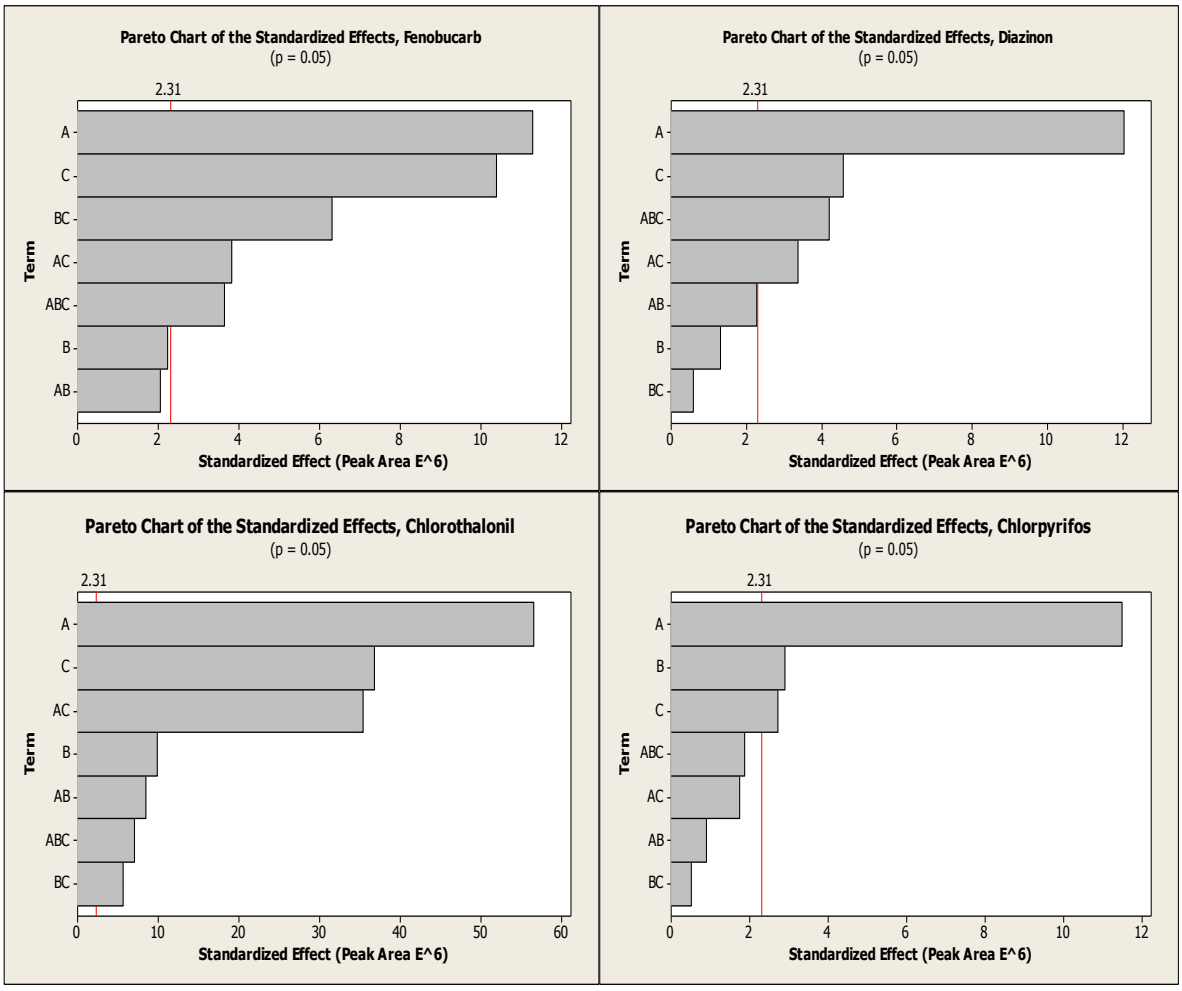
[a] Generated using Minitab statistical software

Table 5.4: Main Effect, Interactions between Factors for Pesticide Residues [a]

Pesticides	T	t	S	T, t	T, S	t, S	T,t,S
Fenobucarb	+	+	+	+	+	+	+
Diazinon	+	+	+	+	+	+	+
Cholorothalonil	+	+	+	+	+	+	+
Chlorpyrifos	+	+	+	+	+	+	+

[a] Factors: T, temperature; t, time; S, salt addition (NaCl); –, negative effect; +, positive effect

As can be observed from Fig 5.21; temperature showed the strongest positive effect for all the investigated pesticides and that increase in sampling time causes a significant increase in peak response at higher temperature. The addition of salt was also found to have positive effect, while extraction time showed a positive effect on all the pesticides investigated, but the effect was not significant for diazinon and fenobucarb. All investigated factors are significant for chlorothalonil (Fig 5.21). All the second-order interactions are significant on the response of chlrothalonil (all are significant), fenobucarb (temperature/time, not significant) and diazinon (time/salt addition, not significant), except in chlorpyrifos where none of the second order interactions are significant, but they all show positive effect on chromatographic response of all investigated pesticides. Third-order interaction is significant for all pesticides except chlorpyrifos, but also showed a positive effect. As shown in Table 5.4, a positive effect implies that the factors enhance extraction efficiency and gave better peak areas, while a negative effect showed that the interaction reduced extraction efficiency.



N.B: A Temperature; B, Time; C, salt effect. All factors and interactions beyond the red line are significant at p=0.05

Fig 5.21: Pareto Chart of Standardized Effects

The plots showed that more analytes were extracted at higher extraction temperature and in a shorter time and with a higher percentage of salt addition. The overall conditions found based on the peak area responses of individual analyte was observed to be similar in the sample matrix. The factors considered and their interactions at different levels were used to construct a calibration curve, which was used for the determination of the limits of detection and quantification (Miller & Miller, 2010), and were found below the MRL values for the sample analyzed. Consequently, a single factor was used for all the pesticides

at 60 ^{0}C for 30 min in the presence of 10 % of NaCl for the extraction of target pesticides in apple sample.

The analytical figure of merit of the developed method was validated under the best sampling conditions established above (30 min, 60 ^{0}C, 10 % NaCl), by determining the repeatability and recoveries at 10 µg/kg^{-1}and linearity at 0.5 to 50 µg.kg^{-1}. The external standard calibration curve was constructed by a-five point concentration level prepared in the sample matrix, each analyzed in triplicate, using the same sampling procedure and chromatographic condition as used for the sampling matrix. External standard calibration was employed due to lack of matrix effect on the extraction efficiency (Ouyang, 2012; Ouyang & Pawliszyn, 2008), this was achieved by carefully optimizing the dilution factor. The limits of quantification and detection values were estimated experimentally using a signal-to-noise ratio of 3 and 10 respectively. The precision expressed as the repeatability (%RSD) was estimated by three consecutive extraction of the selected pesticides from spiked apple sample. The method linearity ranged from 0.5 to 50 µg/kg^{-1}, with correlation coefficient greater than 0.99. The limit of detection ranged from 0.01 to 0.2 µg/kg^{-1} and limit of quantification were between 0.05 and 0.1 µg/kg^{-1}. The accuracy of the method was determined in terms of recovery experiments by extracting the selected pesticides in apple sample at two concentration levels. The relative recovery calculated by comparing the peak areas of spiked sample with that of standard solution at the same concentration and extraction conditions ranged from 80 – 105% with an RSD less than 15 % for all pesticides investigated (Table 5.5).

Table 5.5: Method Validation

Pesticides	Linearity ($\mu g \cdot kg^{-1}$)	LOD ($\mu g \cdot kg^{-1}$)	LOQ ($\mu g \cdot kg^{-1}$)	R^2	Recovery (%)	RSD (%)
1. Fenobucarb	1 – 50	0.2	1.0	0.998	105.5	13.7
2. Diazinon	0.5 – 50	0.01	0.5	0.999	89.48	1.7
3. Cholorothalonil	0.5 – 50	0.01	0.5	0.999	83.78	0.1
4. Chlorpyrifos	0.5 – 50	0.01	0.5	0.999	80.20	3.33

The HS-SPME procedure was routinely applied to apple samples purchased in the local Malaysian markets. In order to ascertain the applicability of the method, analyses were made in triplicate. A fiber blank was also carried out in order to check the carry-over effect, while calibration curves are prepared daily to ensure linearity in the working concentration range in order to avoid errors in quantification caused by possible instrumental fluctuation, which was found to be stable. The pesticide chlorpyrifos was detected at a concentration of 0.2 $\mu g/kg^{-1}$, which is 2 fold less than the MRL (0.5 mg/kg^{-1}) for apples (European Union (EU), 2005).

5.3.2 Plackett-Burman Design

Consequently, a Plackett-Burman (P-B) design matrix with a 2^{7-4} (resolution III) reduced factorial was generated for the screening of the most important factors affecting the SPME efficiency and recovery of pesticide residues from fruit and vegetable samples. It contains experimental runs of a multiple of four (4, 8 , 12, 16, etc.) and the factors are one less than the number of experiments (Brereton, 2007; López et al., 2007). It helps for the estimation of the significant factors affecting extraction efficiency. It does not yield the exact quantity, but provides valuable information on each variable with relatively few and reasonable experimental runs (Khodadoust & Hadjmohammadi, 2011; López et al., 2007). The factors and level of variables selected for P-D design are as shown in Table 5.6.

Table 5.6: Factors and Levels of Variables

Variables	Levels	
	Low (-)	High (+)
Extraction temperature (^{0}C)	30	60
Extraction time (min)	30	60
Salt addition (%, v/v)	5	10
Stirring rate (rpm)	300	600
pH	4	8
Desorption time (min)	5	10
Desorption temperature (^{0}C)	250	270

In this study, the Minitab® Statistical software was used to generate a 2^{7-4} Plackett-Burman design (Table 5.7), and was used to run the experiment for the determination of main effects of the factors under investigation. The P-B design consists of 12 runs, that were conducted in duplicate, to annul the effects of extraneous variables (Stalikas et al., 2009).

The factors and level of variables selected for the experimental design was selected to cover the range of optimal conditions that was estimated in using the univariate method and based on the available literature (Bordagaray, Garcia-Arrona, & Millán, 2011). The Minitab® Statistical Software was used to generate a Plackett-Burman design matrix used for the estimation of the significant factors. All other optimized factors (GC-MS parameters, fiber type, type and amount of organic solvent mixture) were used as estimated in the univariate method. The P-B design, the number of experimental runs is a multiple of four and has one experimental run more than the number of factors. The analysis of variance (ANOVA) test is employed to check the adequacy of the regression model in terms of lack of fit and to estimate the significance of the independent variables using the F-test (Bordagaray et al., 2011; Delgado-Moreno, Peña, & Mingorance, 2009). The

response is based on the total chromatographic peak areas (TCPA) of the investigated pesticides (Stalikas et al., 2009).

Table 5.7: Plackett-Burman Design Matrix[a]

Ext. temp (^{0}C)	Ext. time (min)	Salt add (%)	Stirring rate (rpm)	pH	Des. time (^{0}C)	Des. temp (^{0}C)
60	30	5	600	4	10	270
60	60	10	600	8	10	270
30	60	5	600	8	10	250
60	30	10	600	8	5	250
60	30	5	300	8	5	250
30	30	5	600	4	5	270
60	60	5	300	4	10	250
30	30	5	600	4	5	270
60	30	5	600	4	10	270
60	60	10	300	4	5	270
30	30	10	300	8	10	270
60	30	10	600	8	5	250
30	30	10	300	4	10	250
30	60	5	300	8	5	270
30	30	10	300	4	10	250
30	60	5	300	8	5	270
60	60	10	600	8	10	270
30	60	10	600	4	5	250
30	60	5	600	8	10	250
60	60	5	300	4	10	250
30	30	10	300	8	10	270
60	30	5	300	8	5	250
30	60	10	600	4	5	250
60	60	10	300	4	5	270

[a] Generated using Minitab® Statistical Software

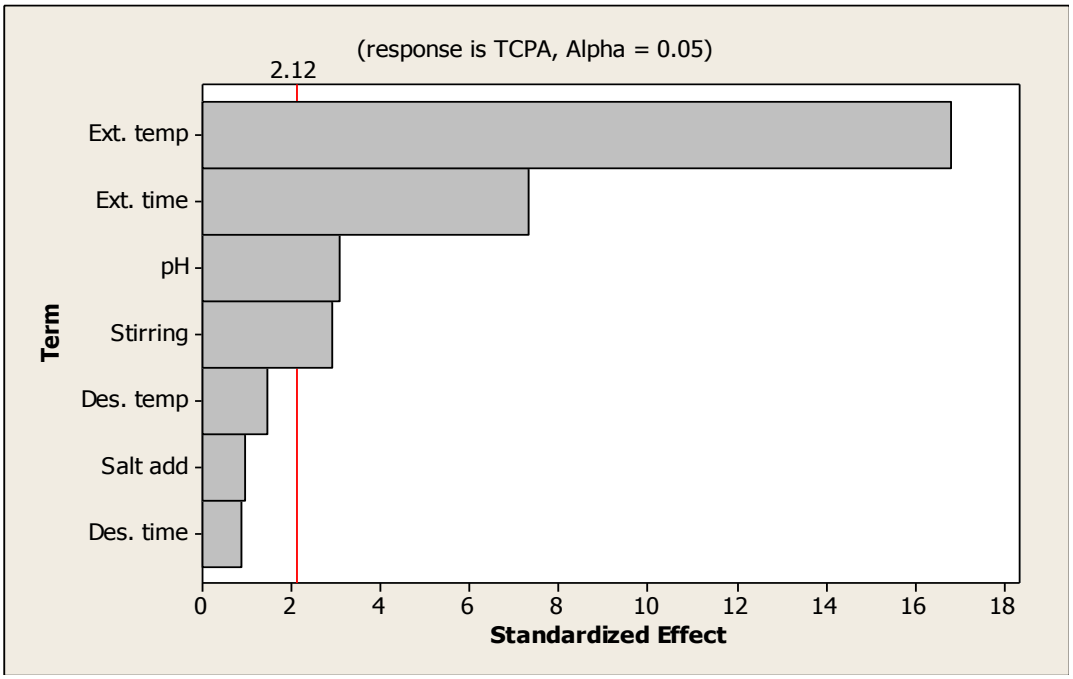


Fig 5.22: Pareto Chart of Standardized Main Effect

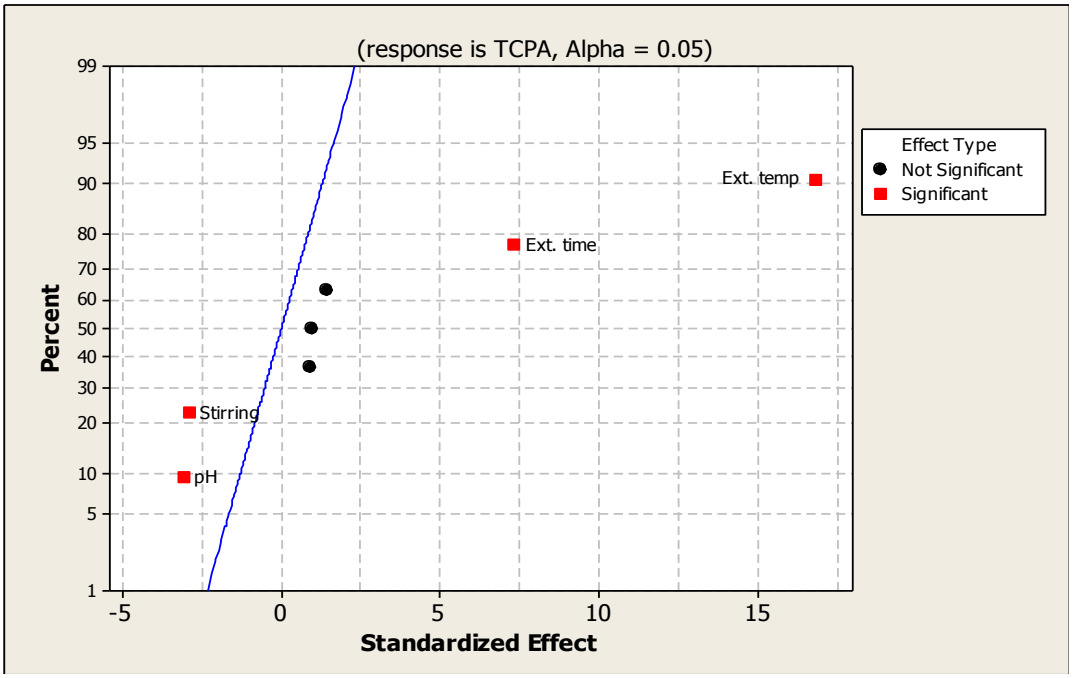


Fig 5.23: Normal Plot of Standardized Main Effect

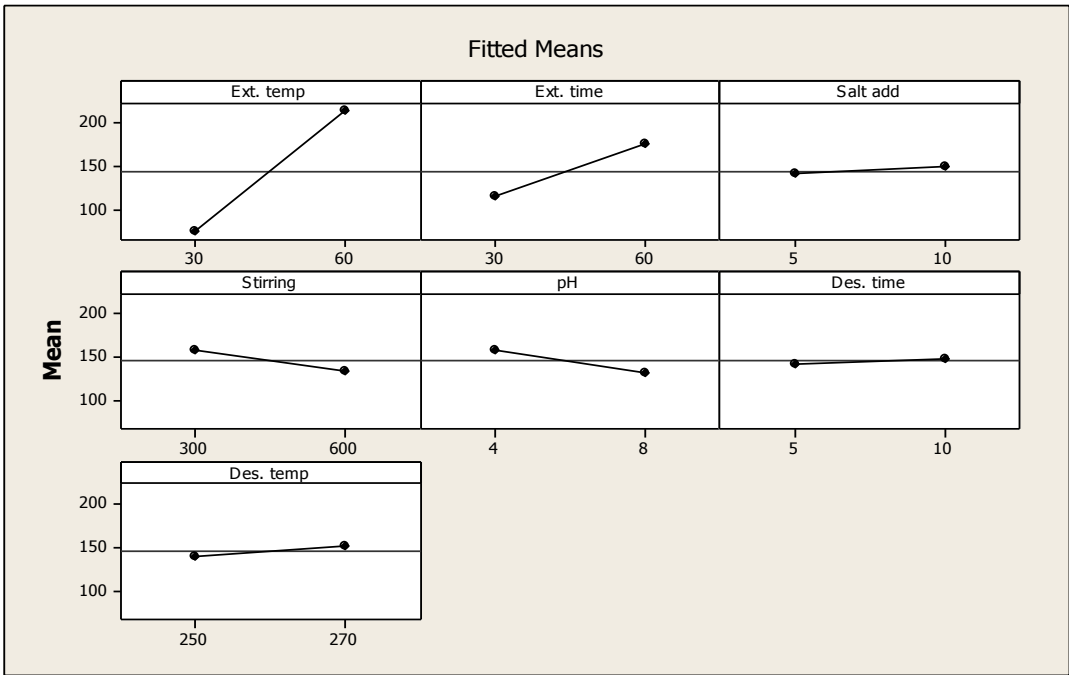


Fig 5.24: Main Effect Plot for TCPA in Plackett-Burman Design

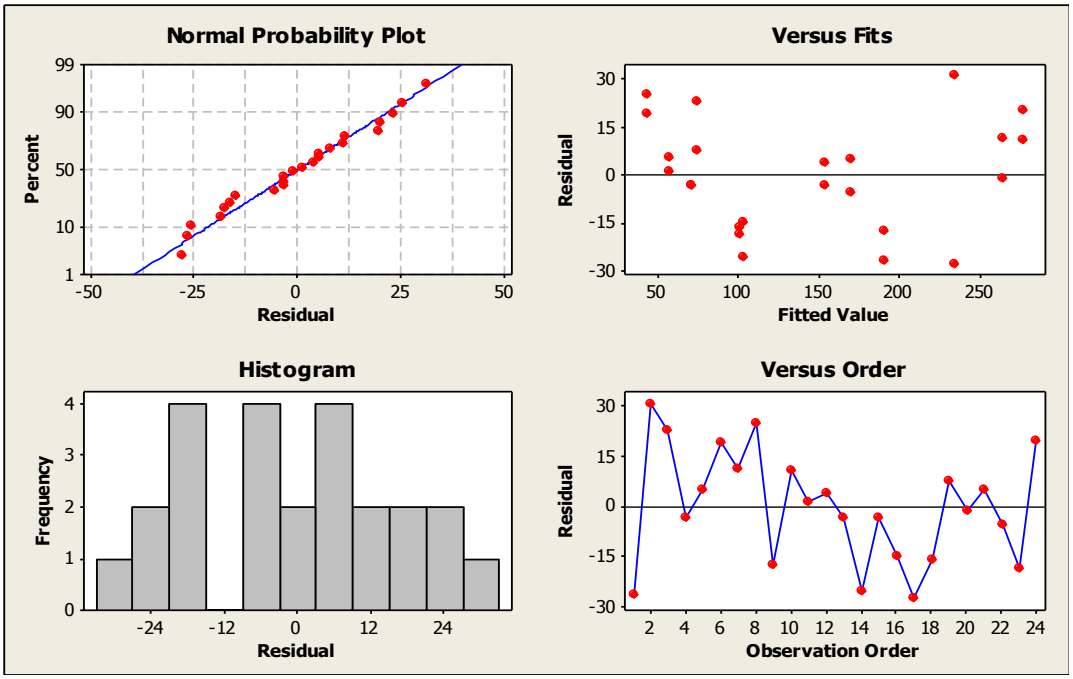


Fig 5.25: Residual Plot of TCPA in Plackett-Burman Design

The main effect of each factor was estimated using least square regression which indicates the significance in relation to the response (TCPA). In the Pareto chart (Fig 5.22), the length of the bar is proportional to the absolute value of the main effect (Khodadoust & Hadjmohammadi, 2011; López et al., 2007; Stalikas et al., 2009), while the vertical line indicates 95 % confidence level. The normal plot (Fig 5.23) shows the significance of each factor (estimated using ANOVA test) and the magnitude of various effects, while the residual plots (Fig 5.25) shows that the measurement deviation is randomly distributed around the mean. The main effect plot (Fig 5.24), as indicated by the slope of the plots, shows that when extraction temperature and extraction time increase from low value to high value, the extraction efficiency also increases, and the extraction efficiency increases with decrease in stirring rate and pH, while other factors such as salt addition, desorption time and desorption temperature show no significant effect. The extraction temperature is the most important factor followed by the extraction time. As can be observed from the normal plot (Fig 5.23) extraction temperature and time shows positive effects, while pH and stirring rate showed negative effect. Therefore, for the optimization step, all other factors were fixed, while extraction temperature, time, pH and stirring rate were considered for further optimization.

5.3.3 Optimization of Significant Factors

5.3.3.1 Central Composite Design

The screening experiment obtained by the use of Plackett-Burman design indicates that, desorption time, desorption temperature and salt addition do not affect extraction efficiency to a significant extent. Therefore, they were fixed according to the optimal value estimated using the univariate experiments (desorption time, 7 min; desorption temperature, 270 ^{0}C;

salt addition, 10 %). The extraction time, extraction temperature, salt addition and pH, which are the significant variables were further optimized by the use of second-order central composite design (CCD) utilizing a response surface methodology (RSM). The number of points in CCD contains a factorial run of 2^k, axial runs of 2k and C_o center point runs. Therefore the total experimental runs (N) of CCD is given by: $N = 2^k + 2k + C_o$, where k and C_o are the number of variables and the number of center points respectively (Stalikas et al., 2009; Stoyanov & Walmsley, 2006). In order to reduce the effect of uncontrolled variables, the CCD experiments were run in a random manner. The CCD design includes 16 cube points, 7 center points in cube, 8 axial points and 0 center point in axial with $\alpha = 2$ (selected to establish rotatability conditions) and a total of 31 randomized runs. The significant variables involved in the generation of CCD, their levels and the design matrix are shown in Tables 5.8 and 5.9 respectively.

Table 5.8: Factors and Levels used in CCD Design

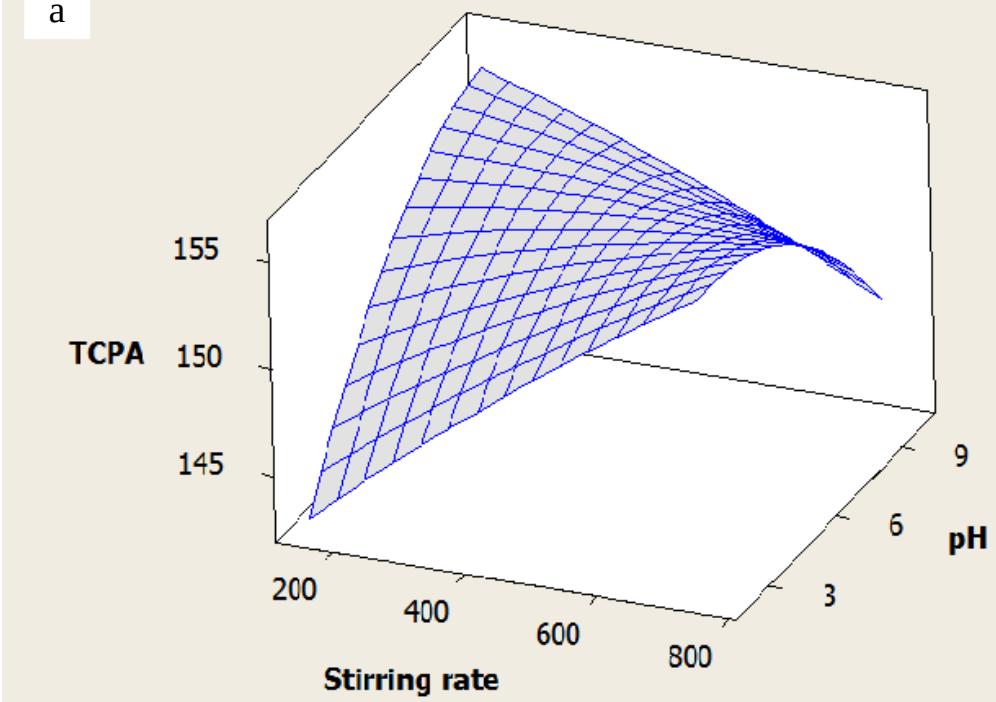
Variables	Level			Star points (α=2)	
	Low (–)	Central (0)	High (+)	–α	+α
Extraction temp. (^{0}C)	30	45	60	15	75
Extraction time (min)	30	45	60	15	75
pH	4	6	8	2	10
Stirring rate (rpm	300	450	600	150	750

Table 5.9: Central Composite Design (CCD) Matrix [a]

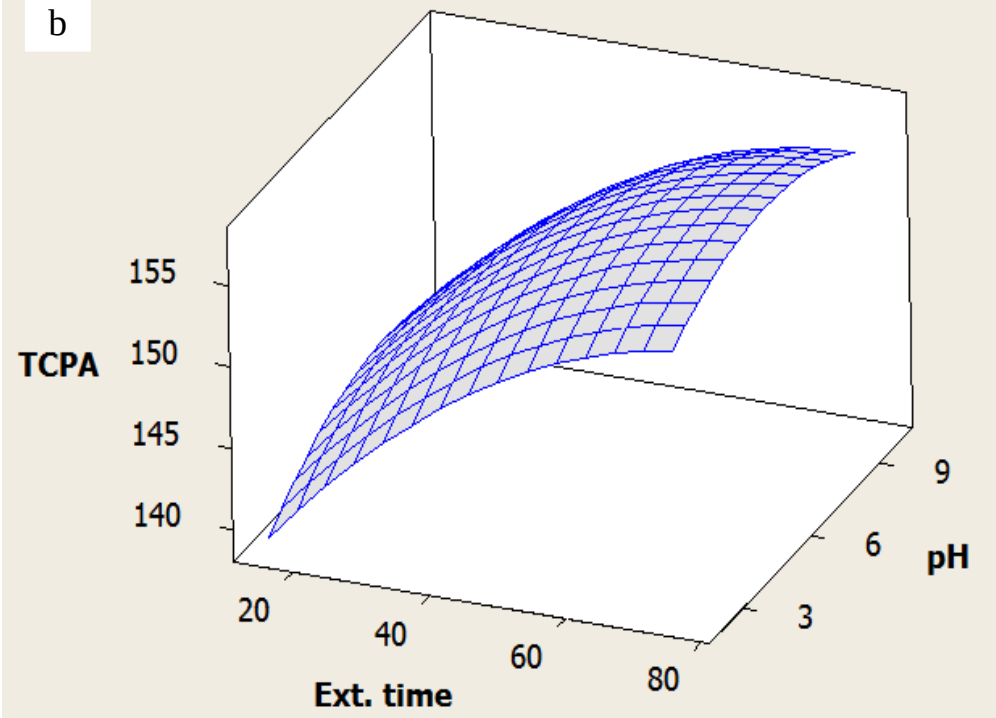
StdOrder	RunOrder	PtType	Blocks	A	B	C	D
10	1	1	1	60	30	300	8
6	2	1	1	60	30	600	4
8	3	1	1	60	60	600	4
13	4	1	1	30	30	600	8
18	5	-1	1	75	45	450	6
30	6	0	1	45	45	450	6
26	7	0	1	45	45	450	6
11	8	1	1	30	60	300	8
14	9	1	1	60	30	600	8
29	10	0	1	45	45	450	6
15	11	1	1	30	60	600	8
20	12	-1	1	45	75	450	6
24	13	-1	1	45	45	450	10
5	14	1	1	30	30	600	4
27	15	0	1	45	45	450	6
12	16	1	1	60	60	300	8
31	17	0	1	45	45	450	6
2	18	1	1	60	30	300	4
3	19	1	1	30	60	300	4
7	20	1	1	30	60	600	4
22	21	-1	1	45	45	750	6
9	22	1	1	30	30	300	8
25	23	0	1	45	45	450	6
4	24	1	1	60	60	300	4
16	25	1	1	60	60	600	8
17	26	-1	1	15	45	450	6
19	27	-1	1	45	15	450	6
23	28	-1	1	45	45	450	2
21	29	-1	1	45	45	150	6
28	30	0	1	45	45	450	6
1	31	1	1	30	30	300	4

[a] Generated using Minitab® Statistical Software

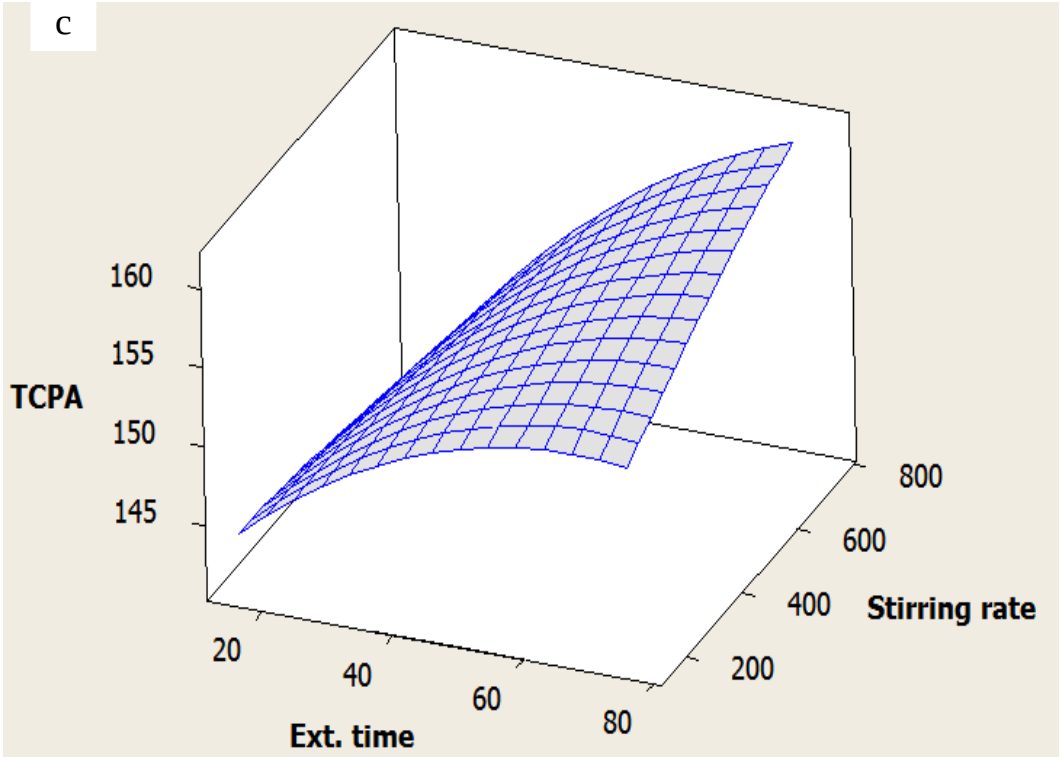
a


155
TCPA
150
145
200
400
600
800
Stirring rate
3
6
9
pH

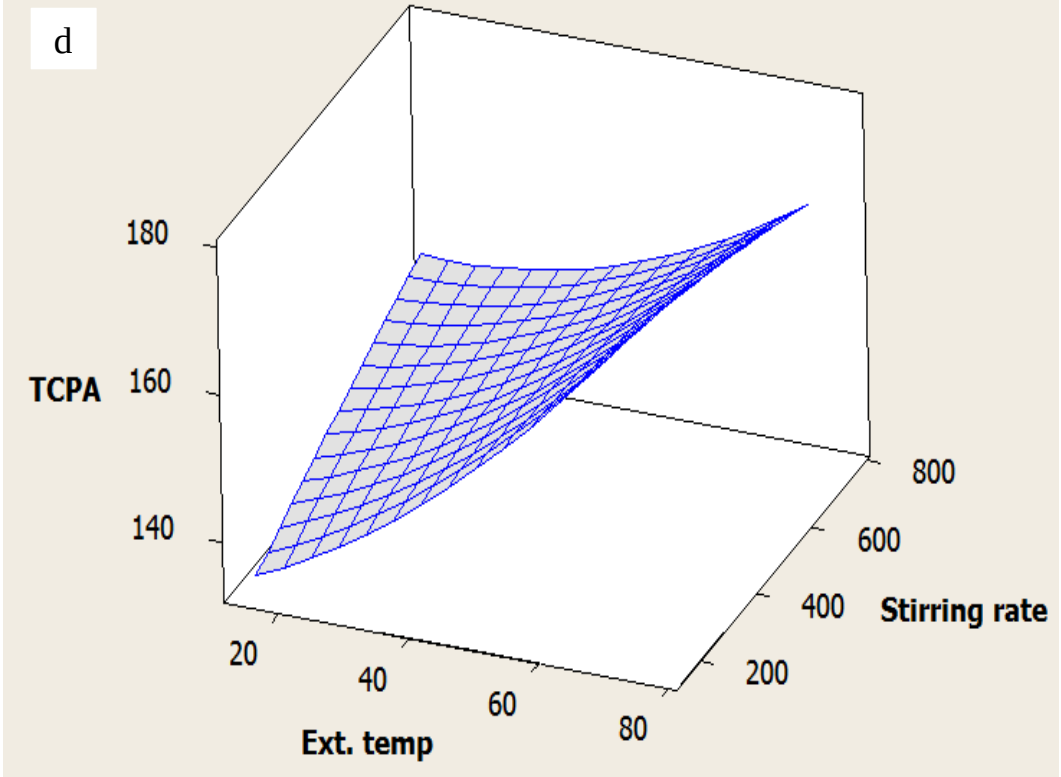
b


155
TCPA
150
145
140
20
40
60
80
Ext. time
3
6
9
pH

c


TCPA
160
155
150
145
20
40
60
80
Ext. time
200
400
600
800
Stirring rate

d


TCPA
180
160
140
20
40
60
80
Ext. temp
200
400
600
800
Stirring rate

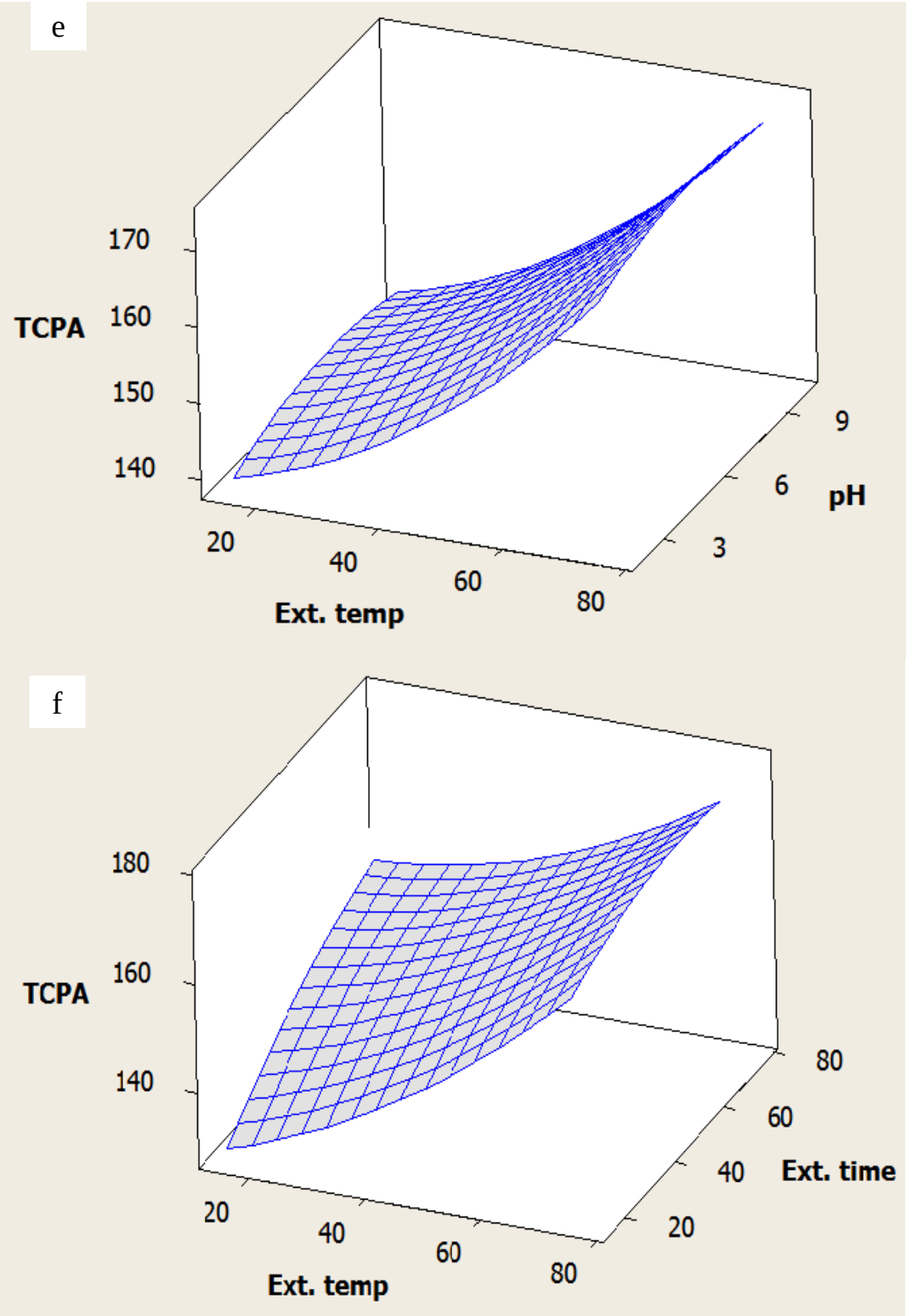


Fig 5.26: Desirability Response Surface Plot for TCPA (a) Stirring Rate vs. pH (b) Extraction Time vs. pH (c) Extraction Time vs. Stirring Rate (d) Extraction Temperature vs. Stirring Rate (e) Extraction Temperature vs. pH (f) Extraction Temperature vs. Extraction Time

The total chromatographic peak area (TCPA) corresponding to the 14 investigated pesticides for the experimental runs presented in Table 5.9, were used to obtain the response surface as shown in Fig 5.26. The desirability function was first fixed by assigning values of 0.0 (undesirable), 0.5 (medium desirability) and 1.0 (very desirable). The global desirability surface response in 3D plot was obtained for the optimized parameters as shown in Fig 5.26. The second order response is utilized because of its flexibility, the ability to give an approximation of the true value and the parameters can easily be estimated (Myers, Montgomery, & Anderson-Cook, 2009).

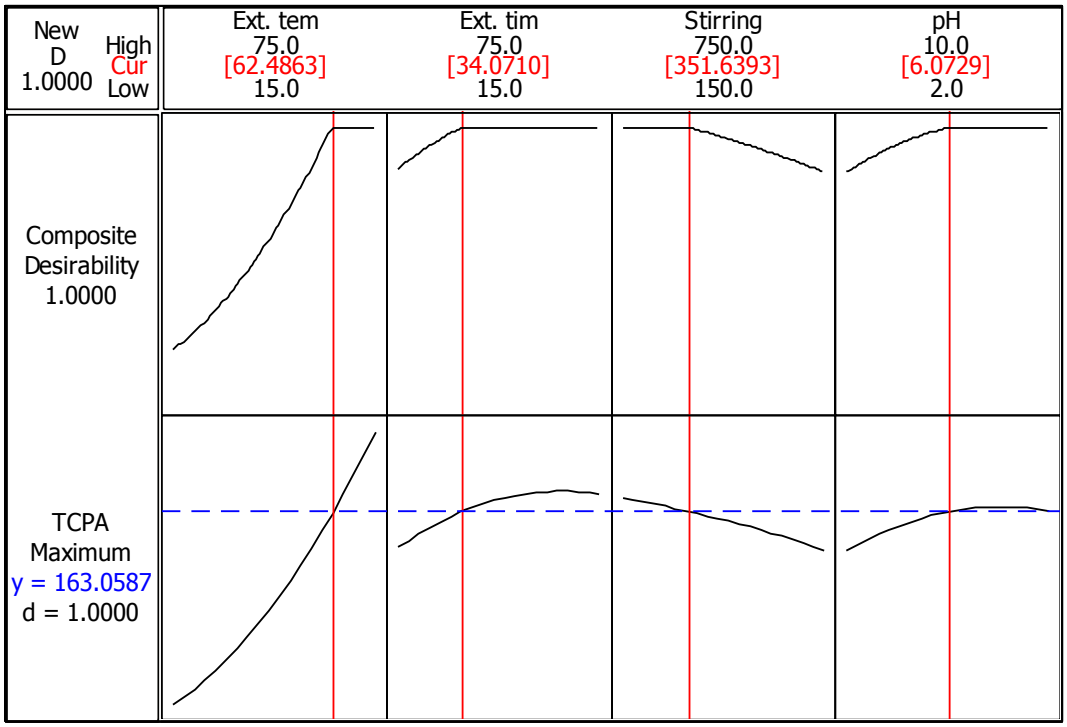


Fig 5.27: Response Optimizer for Optimized Parameters

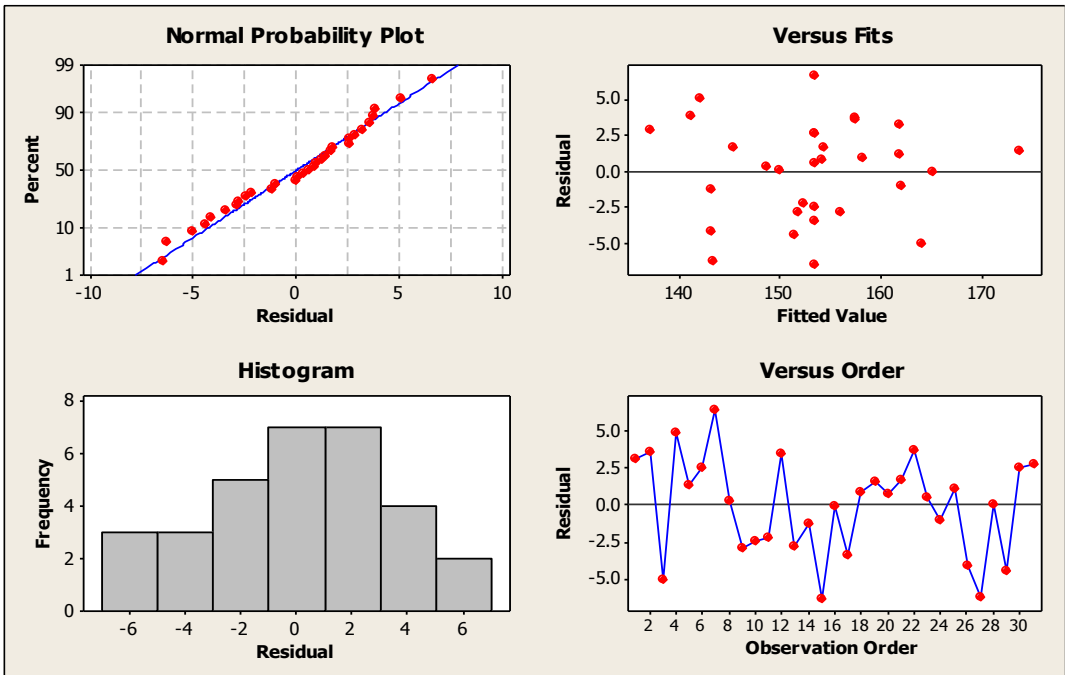


Fig 5.28: Residual Plot of TCPA for CCD Design

The surface plot (Fig 5.26) and response optimizer plot (Fig 5.27) are used to indicate the optimal conditions and the residual plots (Fig 5.28) shows that the measurement deviation is randomly distributed around the mean. It can be observed that the overall response desirability of the independent variables in the experimental domain was obtained at extraction temperature greater than or equal to 62 ^{0}C, while efficient extraction time can be obtained at 34 min or higher, while the stirring rate was at 351 rpm or lower and pH greater than or equal to 6. The result is in good agreement with the P-B design as represented by the main effect plot (Fig 5.24) where increase in extraction temperature and time increases extraction efficiency while extraction efficiency is increased with decreasing stirring rate and pH value. Consequently and taking the earlier results into consideration, the optimized extraction conditions obtained were as represented in Table 5.10. The chromatogram of the 14 investigated pesticides spiked in water sample and analyzed under the optimized

conditions is as shown in Fig 5.29. The chromatogram was integrated for each peak and the ion fragmentation obtained were compared with the NIST library (Figs 5.30 – 5.44).

Table 5.10: Optimized Extraction Conditions

Factors	Optimized condition
SPME fiber	PDMS
Extraction temperature (^{0}C)	65
Extraction time (min)	35
Salt addition (%, v/v)	10
Stirring rate (rpm)	350
pH	6
Desorption time (min)	7
Desorption temperature (^{0}C)	270

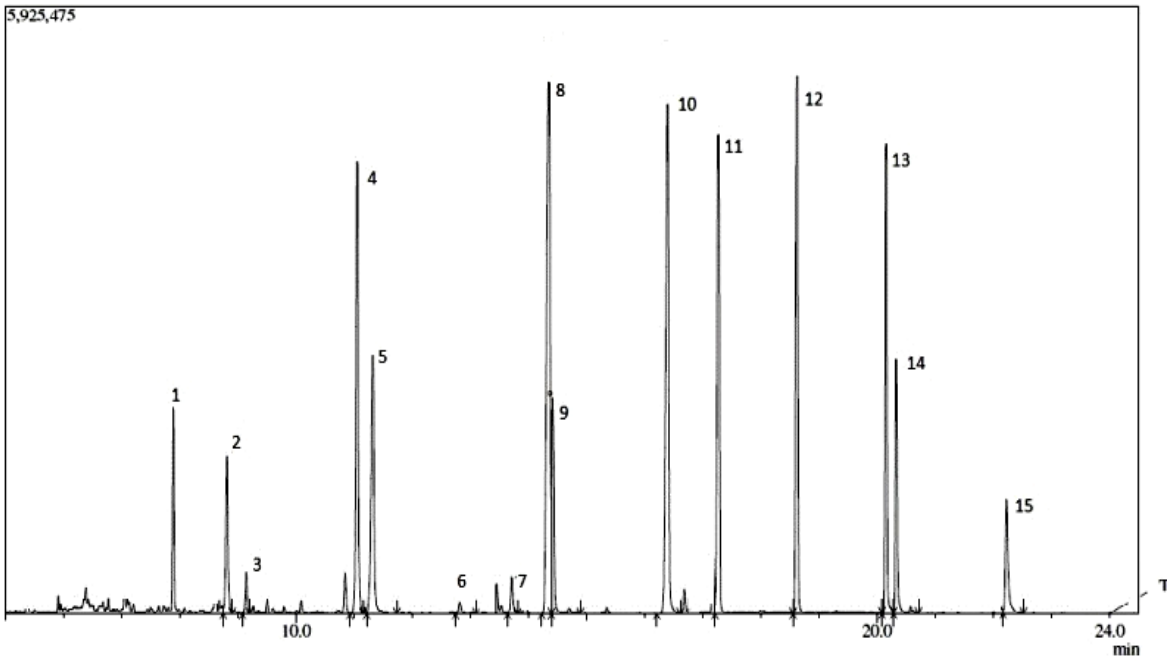


Fig 5.29: GC-MS Chromatogram of Aqueous Sample spiked at 50 µg/kg; 1. I.S (Internal Standard; 2. Fenobucarb;3. Ethoprophos; 4. Diaxinon; 5. Chlorothalonil; 6. Parathion Methyl; 7. Fenitrothion; 8. Chlropyrifos; 9. Thiobencarb; 10. Quinalphos; 11. Endosulfan I; 12. Endosulfan II; 13. Bifenthrin; 14. Fenpropathrin; 15. Permethrin

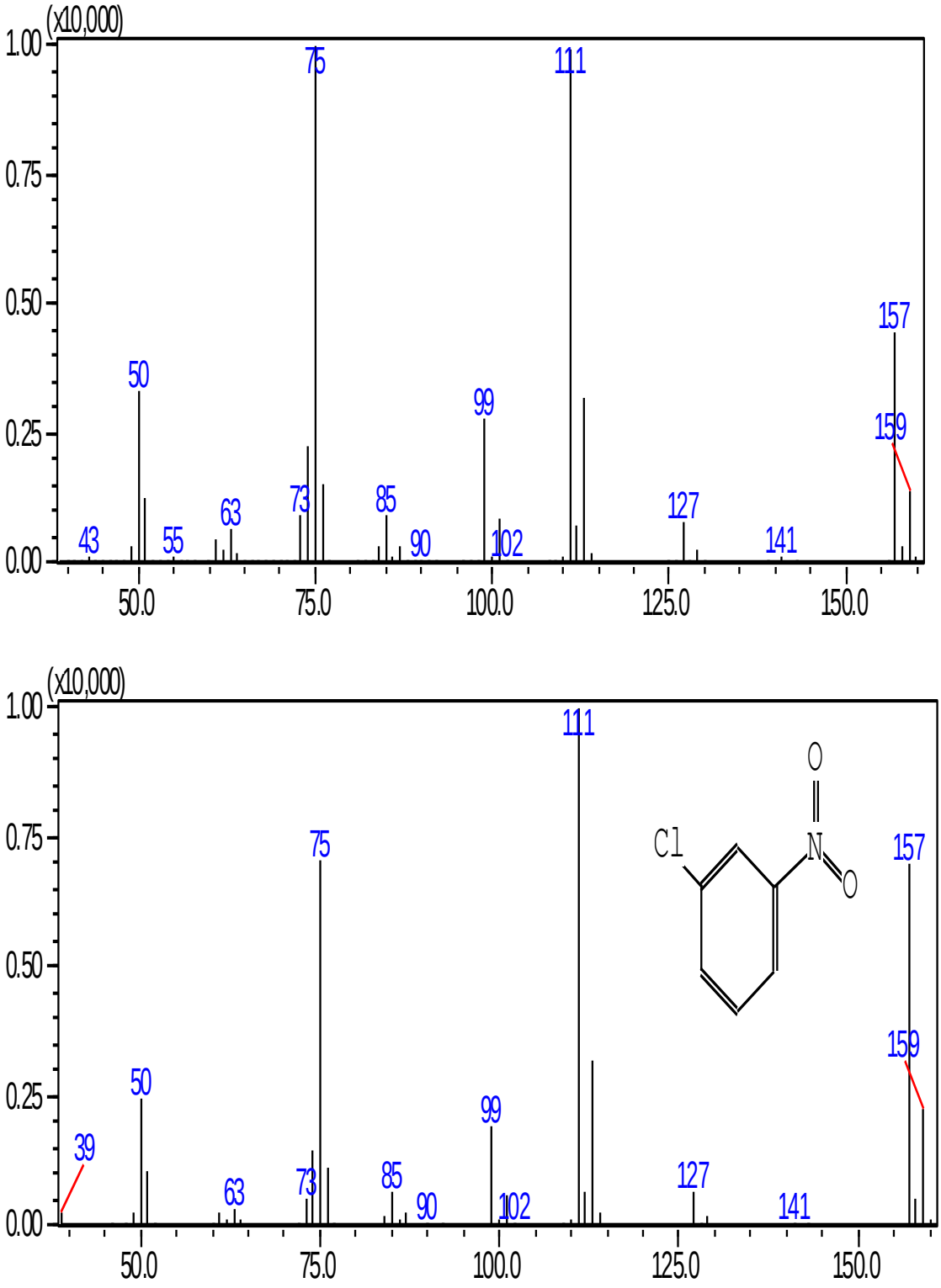


Fig 5.30: Ion fragmentation (top) and NIST Library Search (bottom) for 1-chloro-3-nitrobenzene (I.S) (Peak 1)

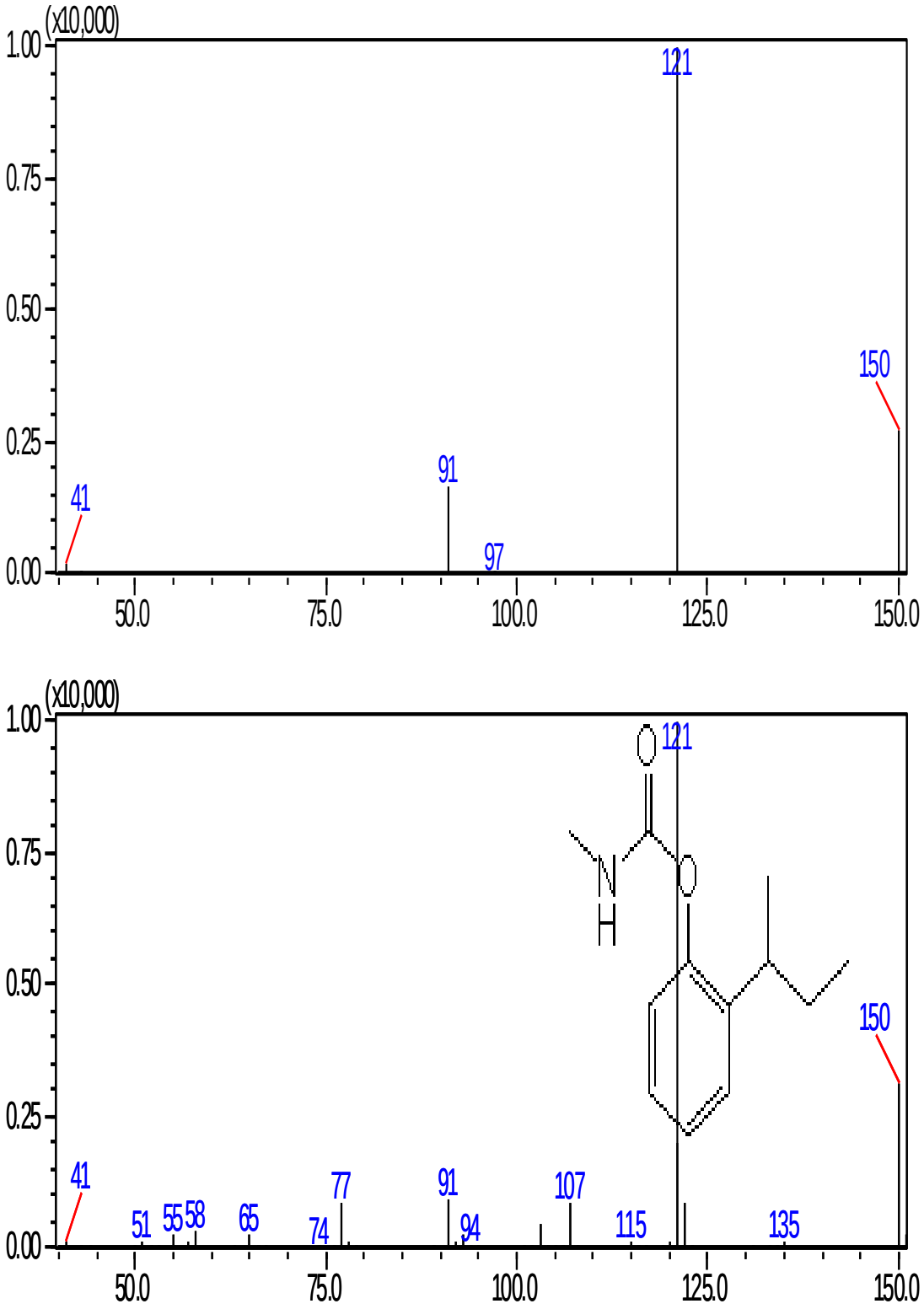


Fig 5.31: Ion fragmentation (top) and NIST Library Search (bottom) for Fenobucarb (Peak 2)

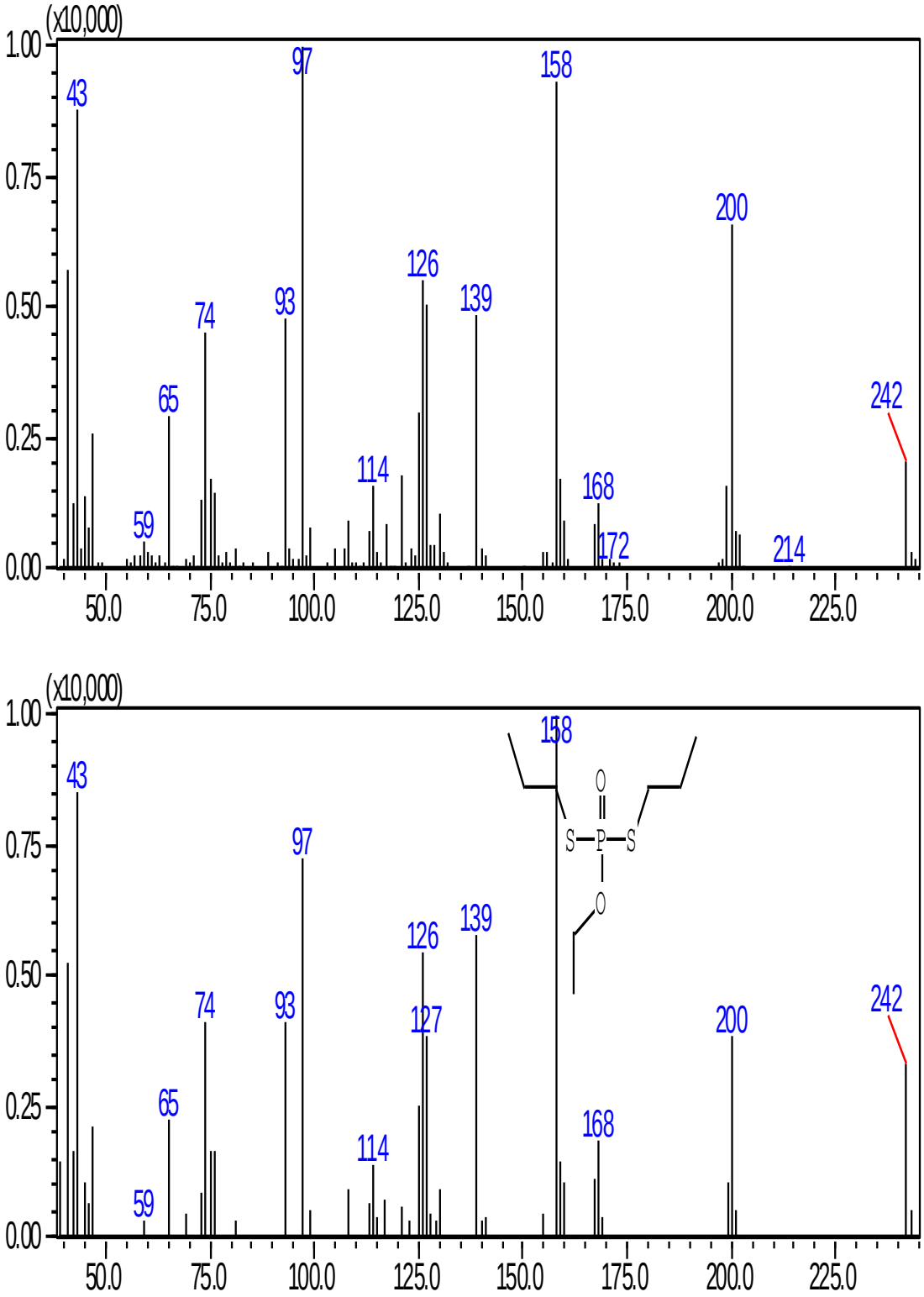


Fig 5.32: Ion fragmentation (top) and NIST Library Search (bottom) for Ethoprophos (Peak 3)

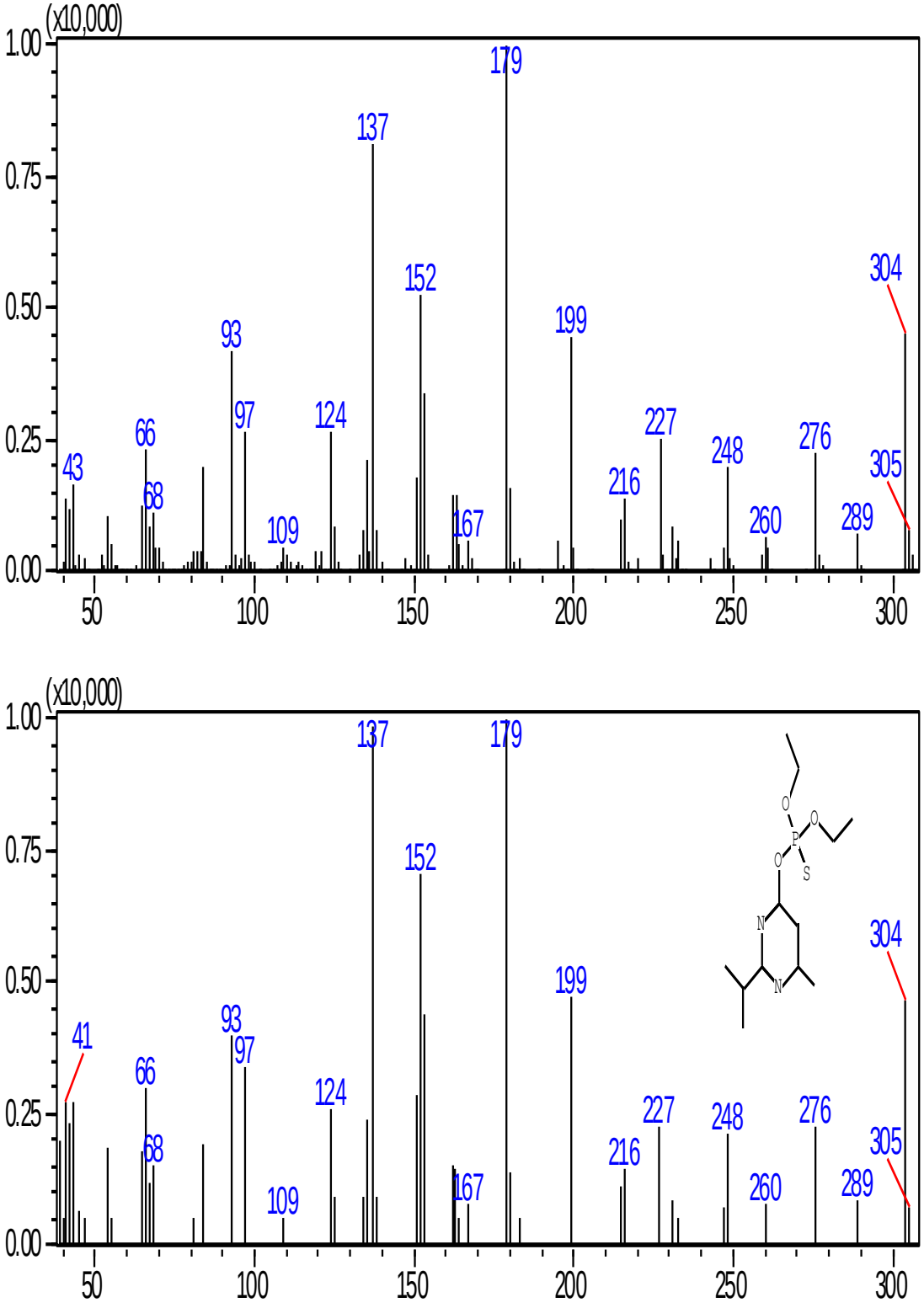


Fig 5.33: Ion fragmentation (top) and NIST Library Search (bottom) for Diazinone (Peak 4)

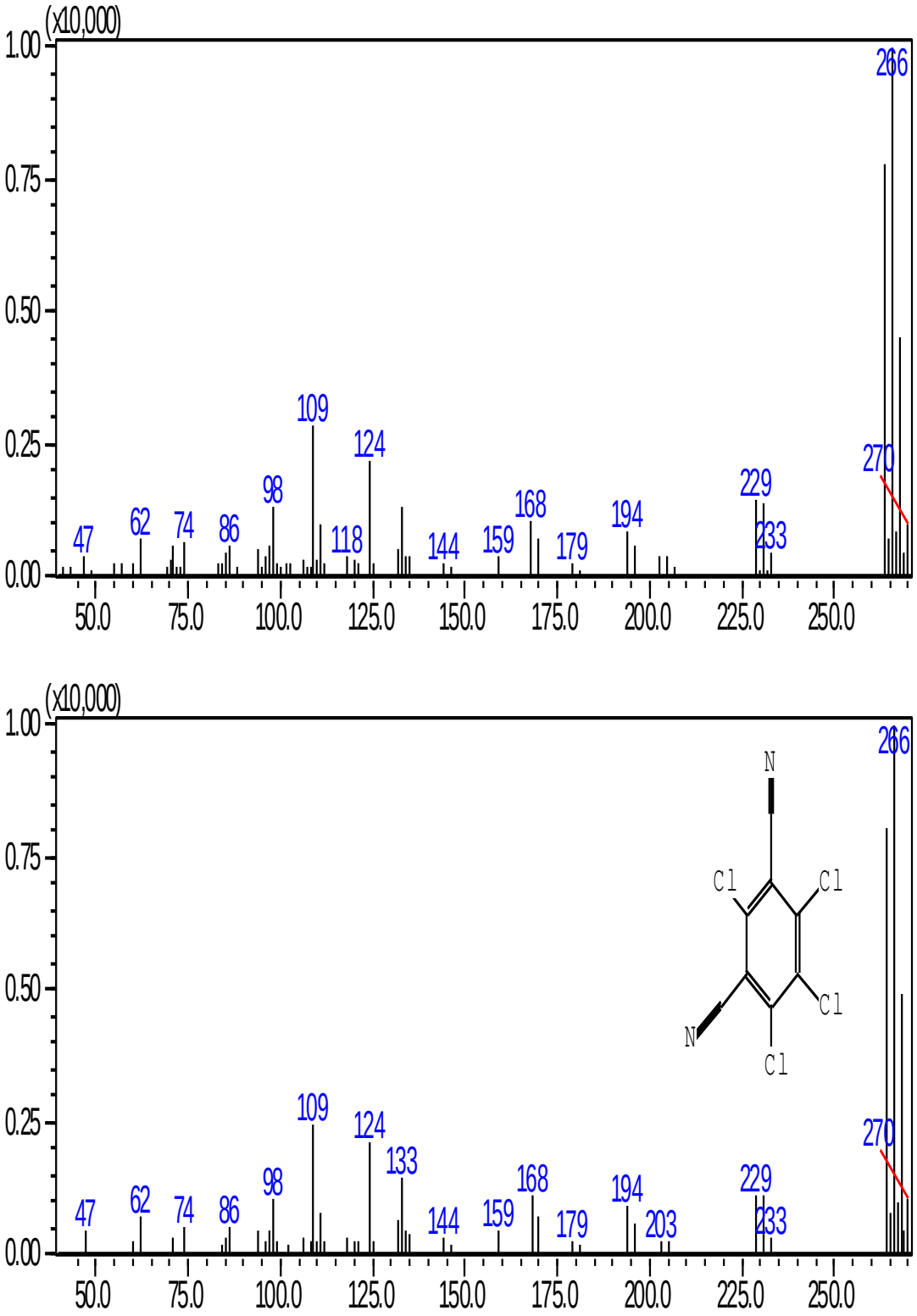


Fig 5.34: Ion fragmentation (top) and NIST Library Search (bottom) for Chlorothalonil (Peak 5)

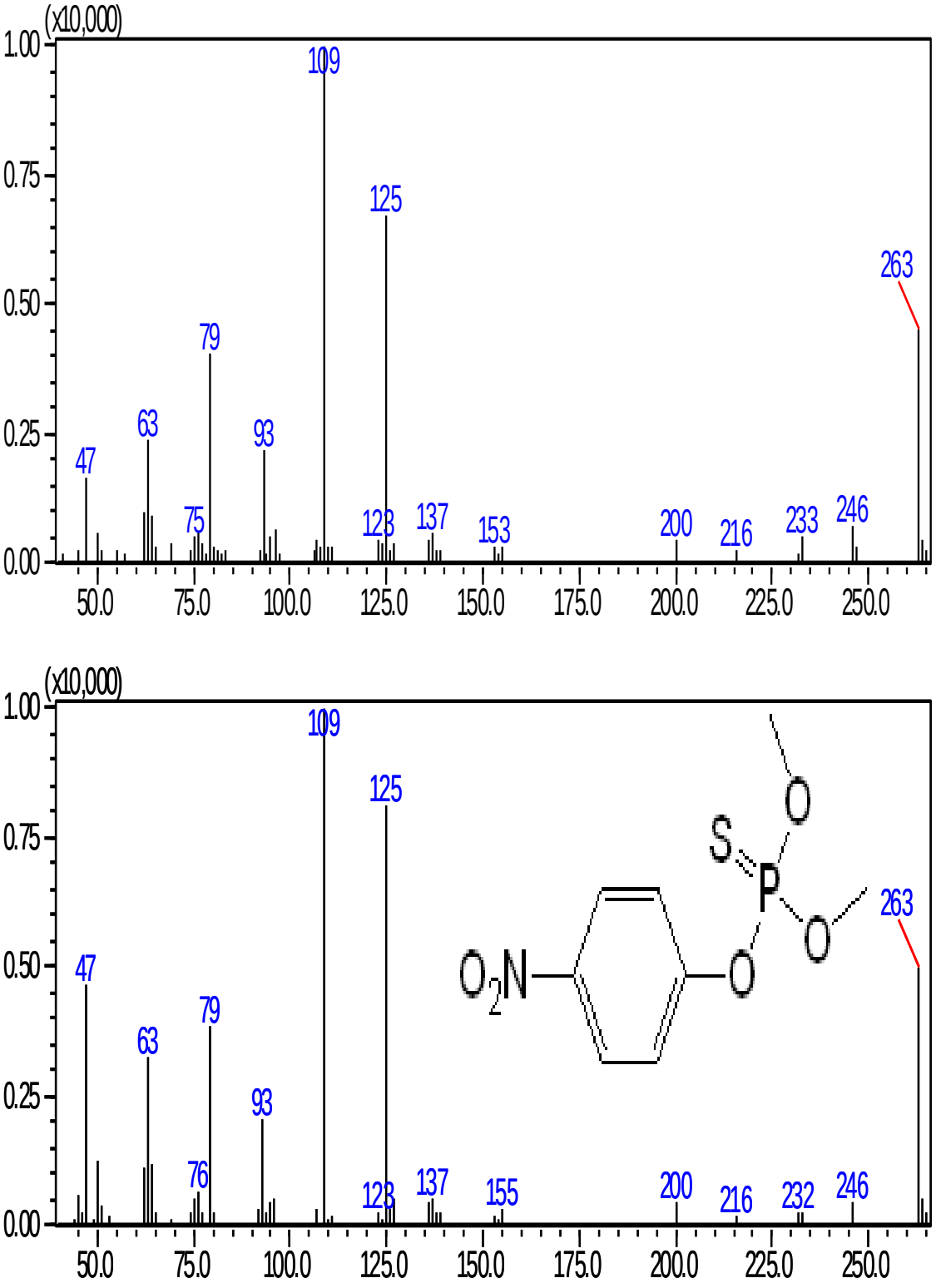


Fig 5.35: Ion fragmentation (top) and NIST Library Search (bottom) for Parathion methyl (Peak 6)

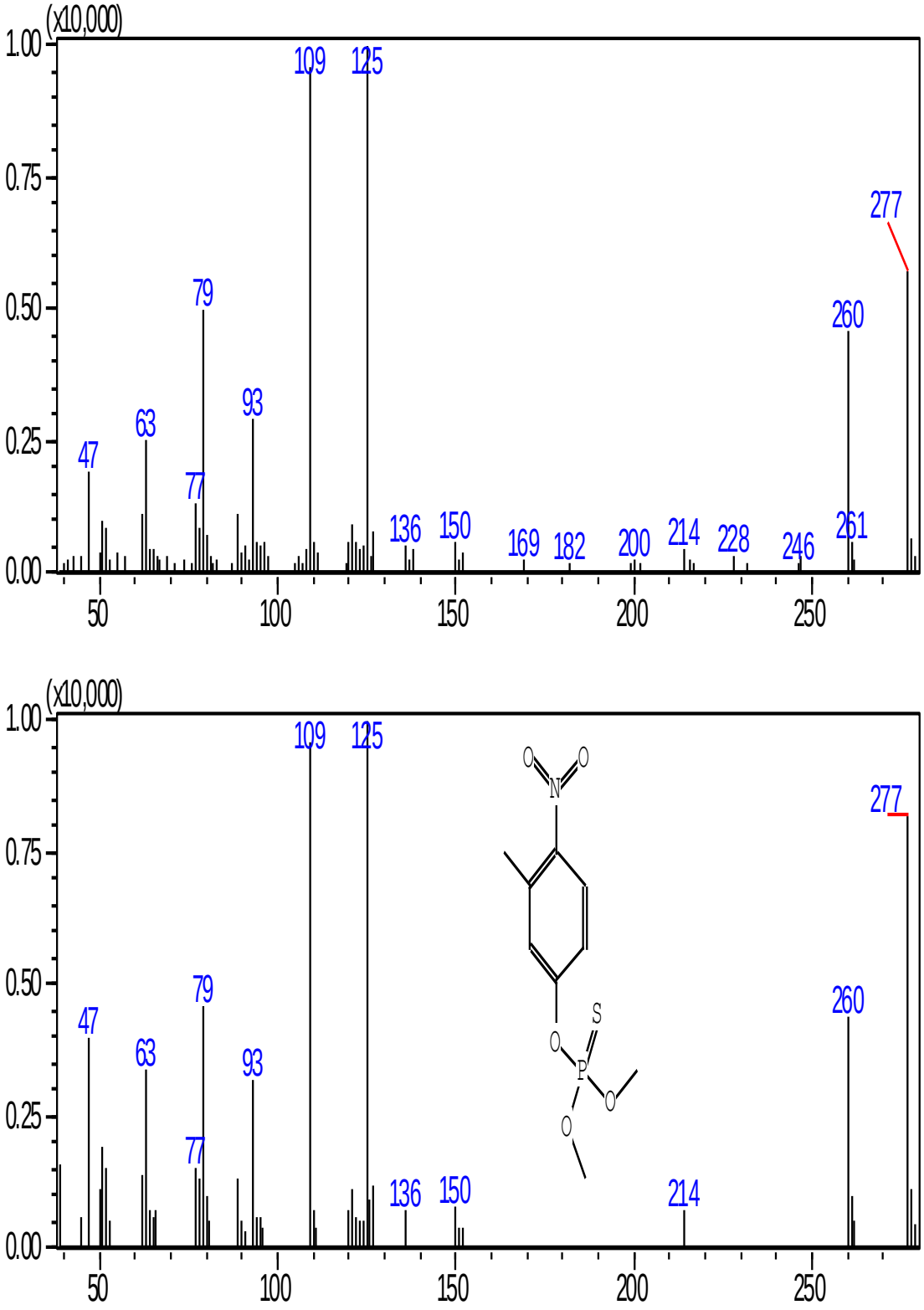


Fig 5.36: Ion fragmentation (top) and NIST Library Search (bottom) for Fenitrothion (Peak 7)

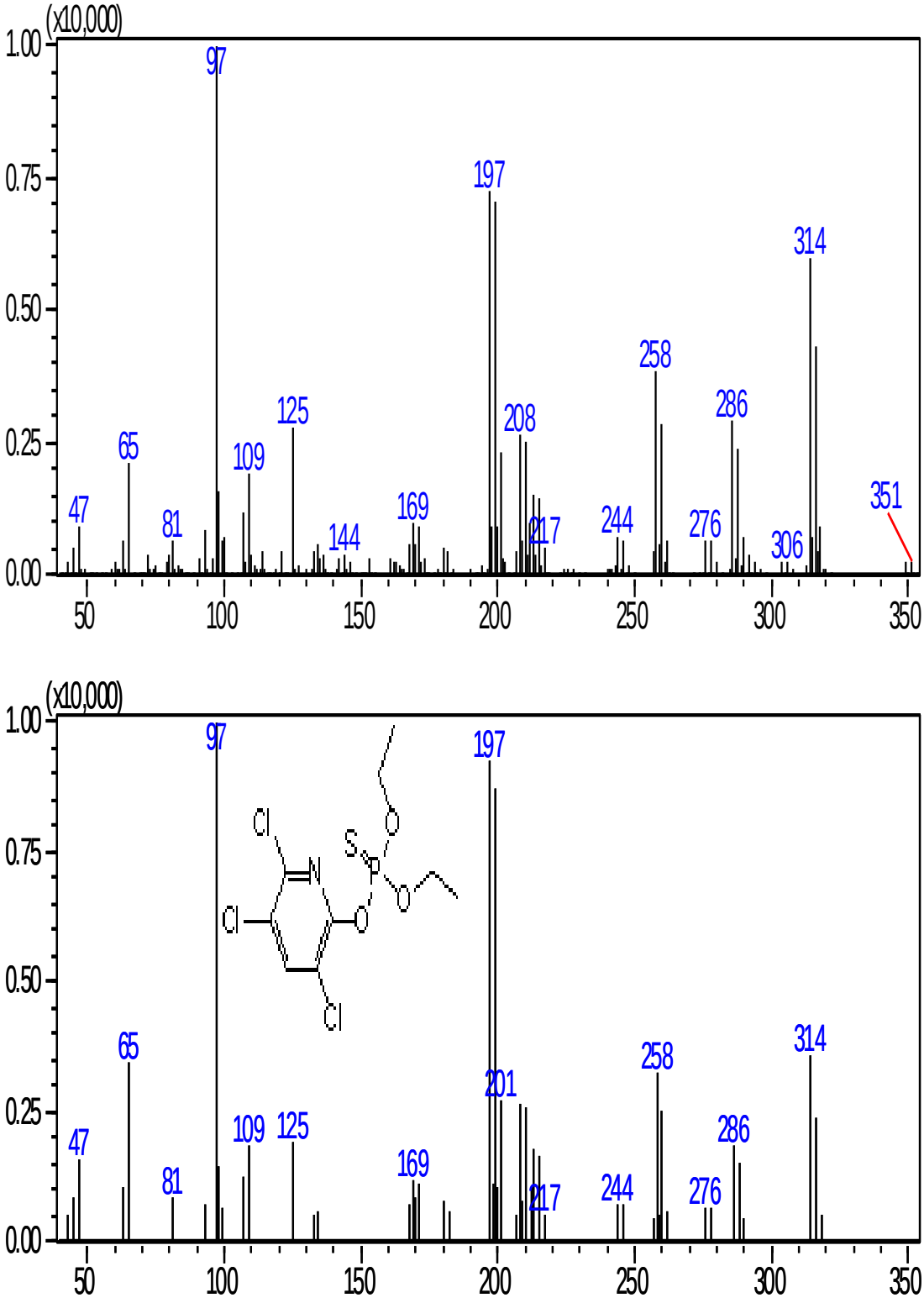


Fig 5.37: Ion fragmentation (top) and NIST Library Search (bottom) for Chlorpyrifos (Peak 8)

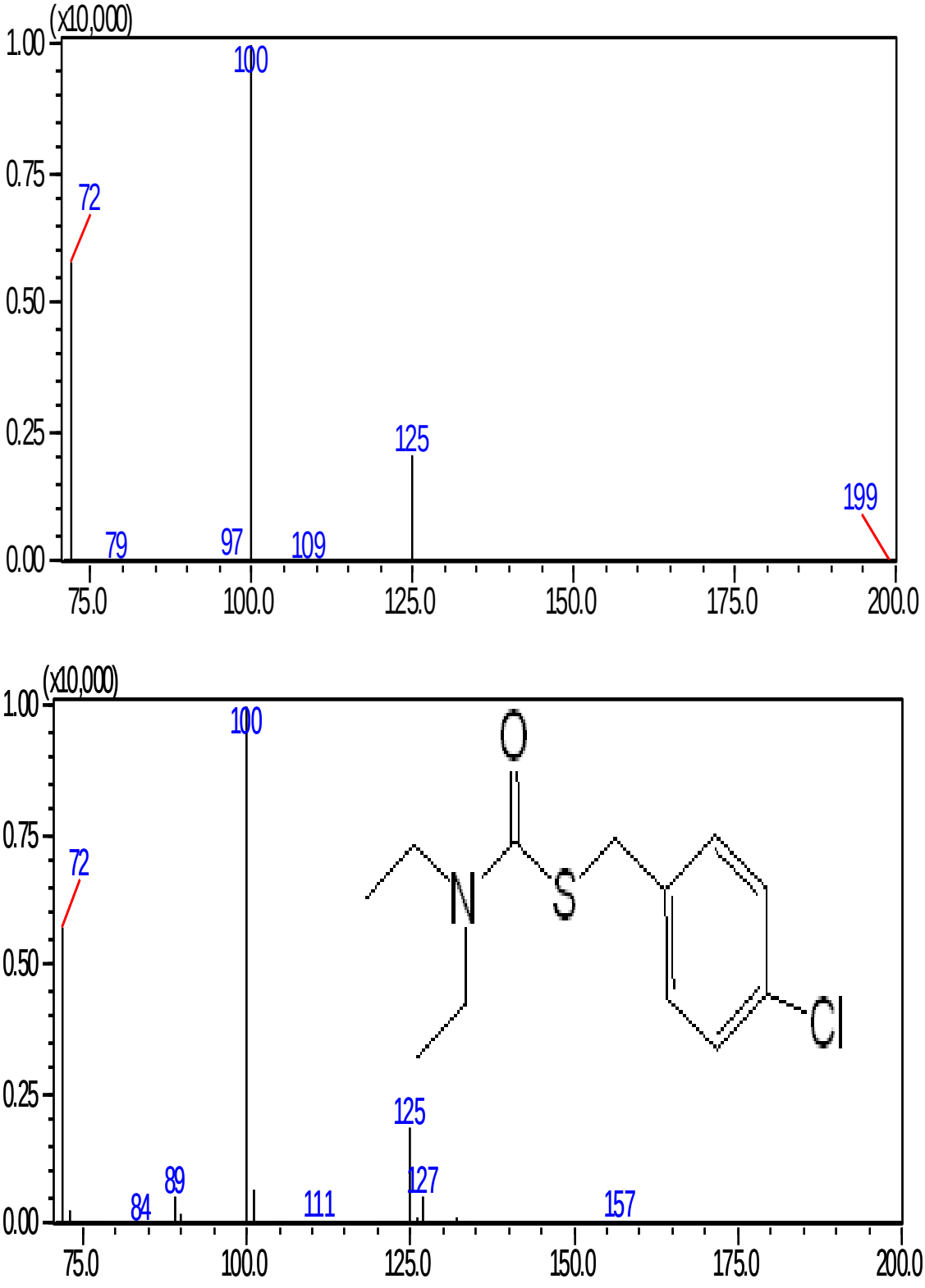


Fig 5.38: Ion fragmentation (top) and NIST Library Search (bottom) for Thiobencarb (Peak 9)

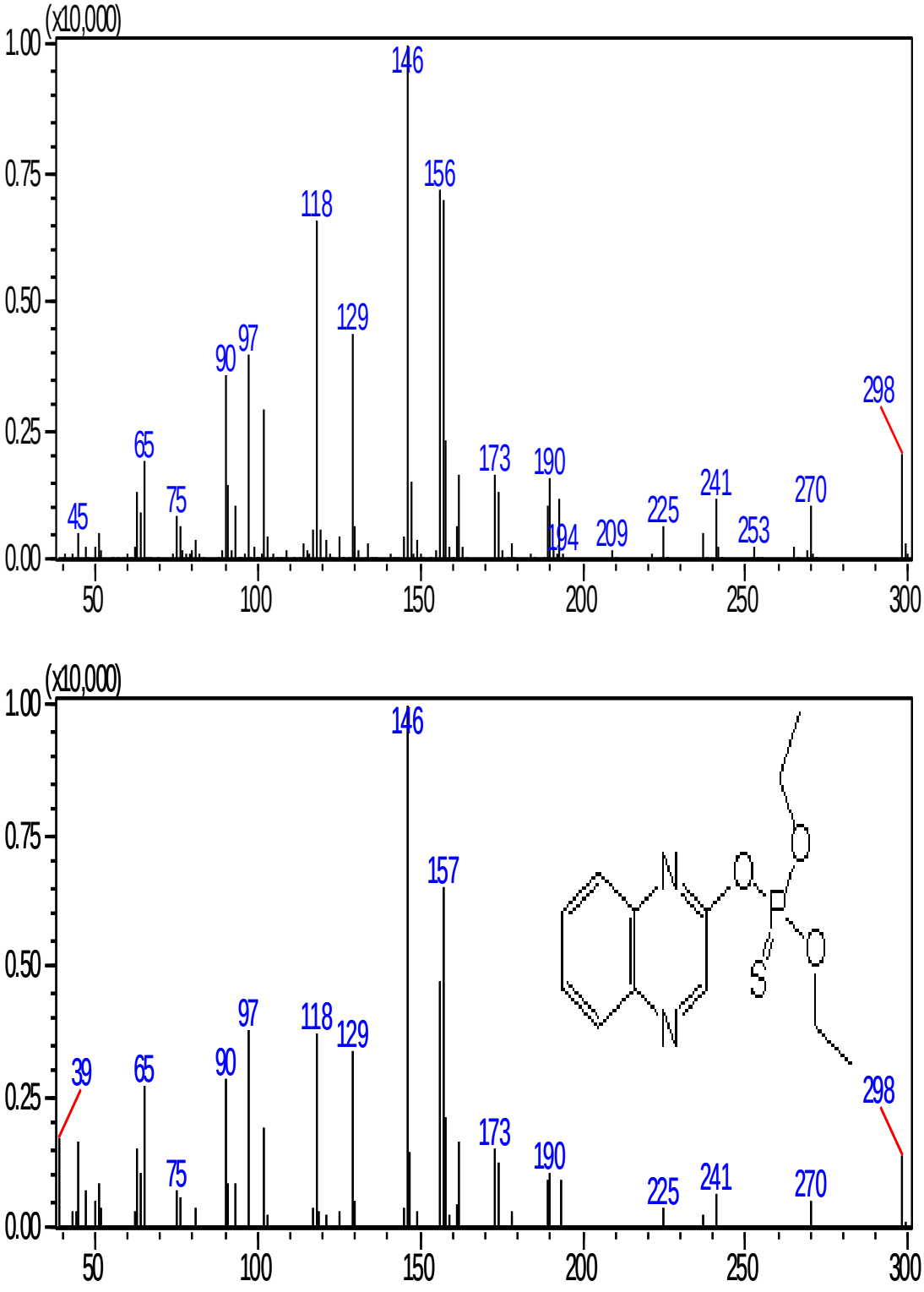


Fig 5.39: Ion fragmentation (top) and NIST Library Search (bottom) for Quinalphos (Peak 10)

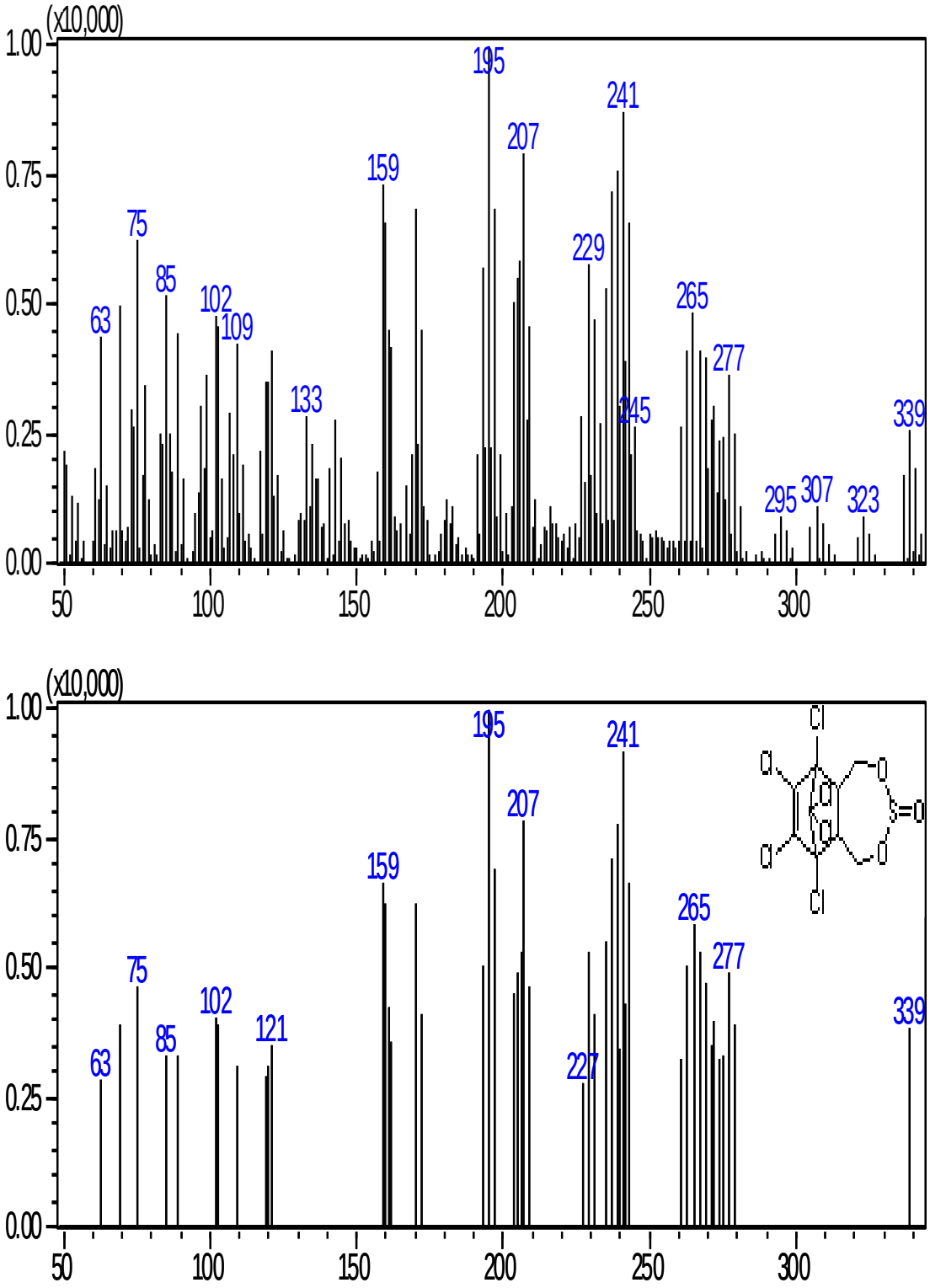


Fig 5.40: Ion fragmentation (top) and NIST Library Search (bottom) for Endosulfan I (Peak 11)

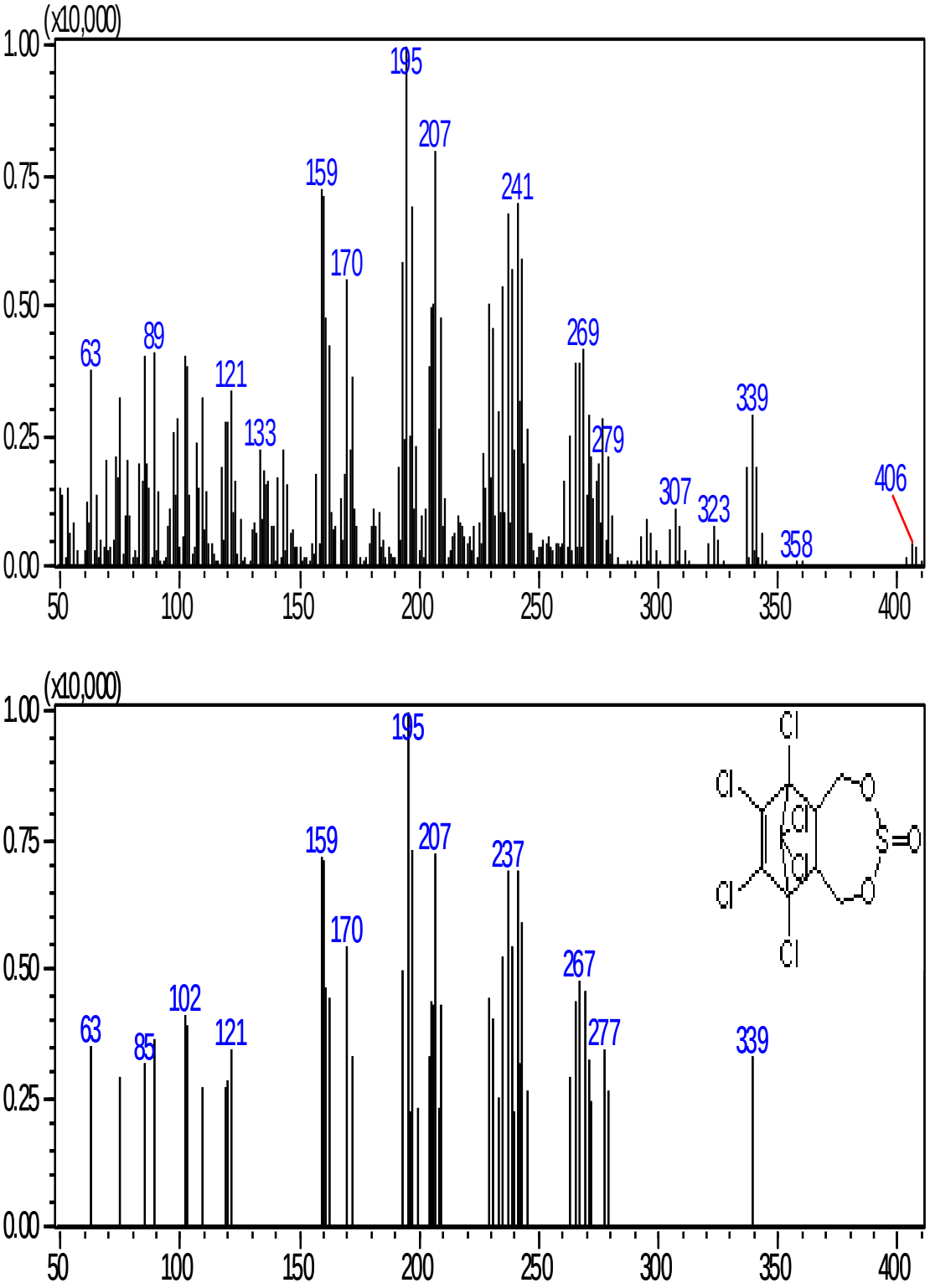


Fig 5.41: Ion fragmentation (top) and NIST Library Search (bottom) for Endosulfan II (Peak 12)

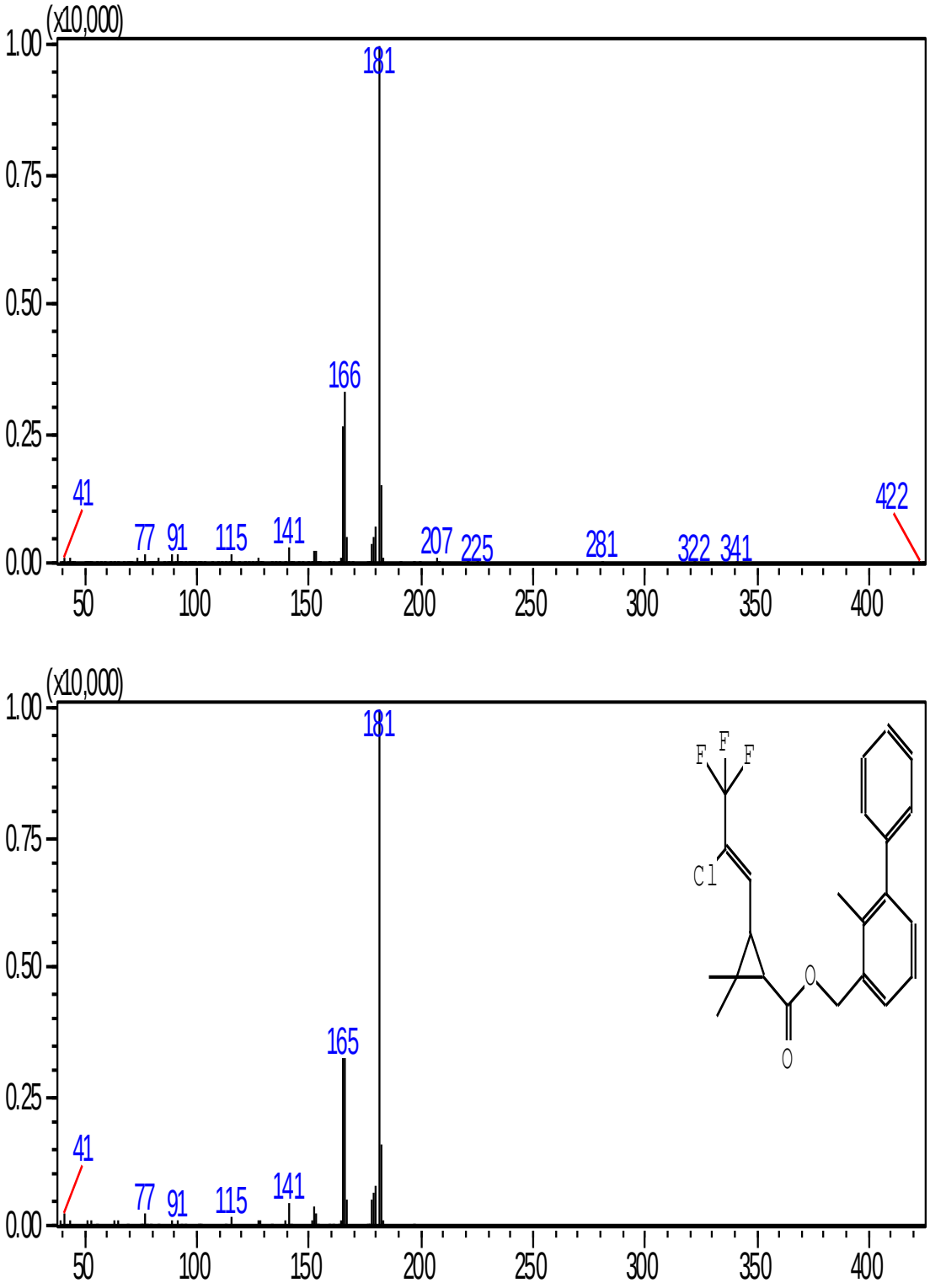


Fig 5.42: Ion fragmentation (top) and NIST Library Search (bottom) for Bifenthrin (Peak 13)

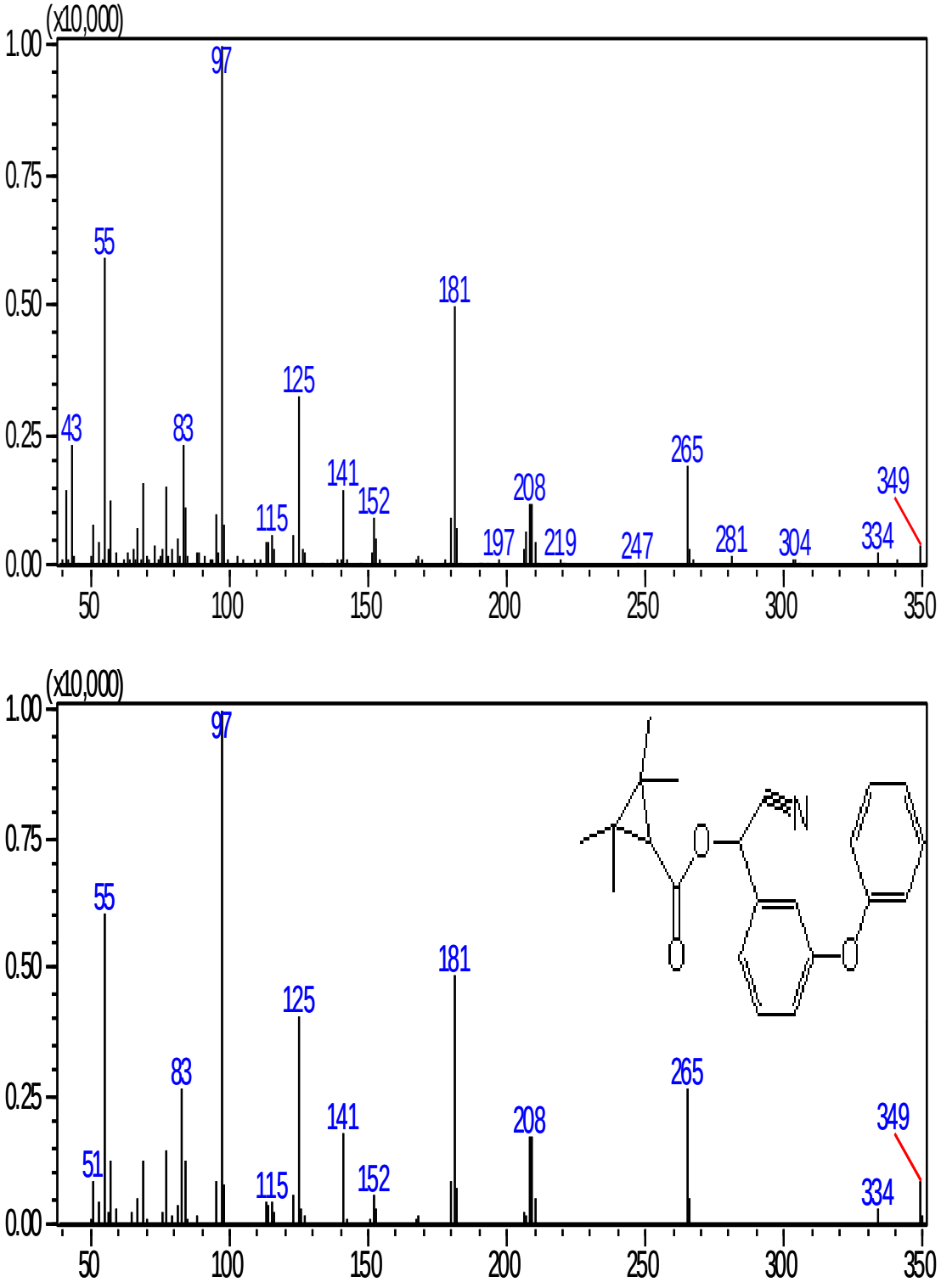


Fig 5.43: Ion fragmentation (top) and NIST Library Search (bottom) for Fenproprathrin (Peak 14)

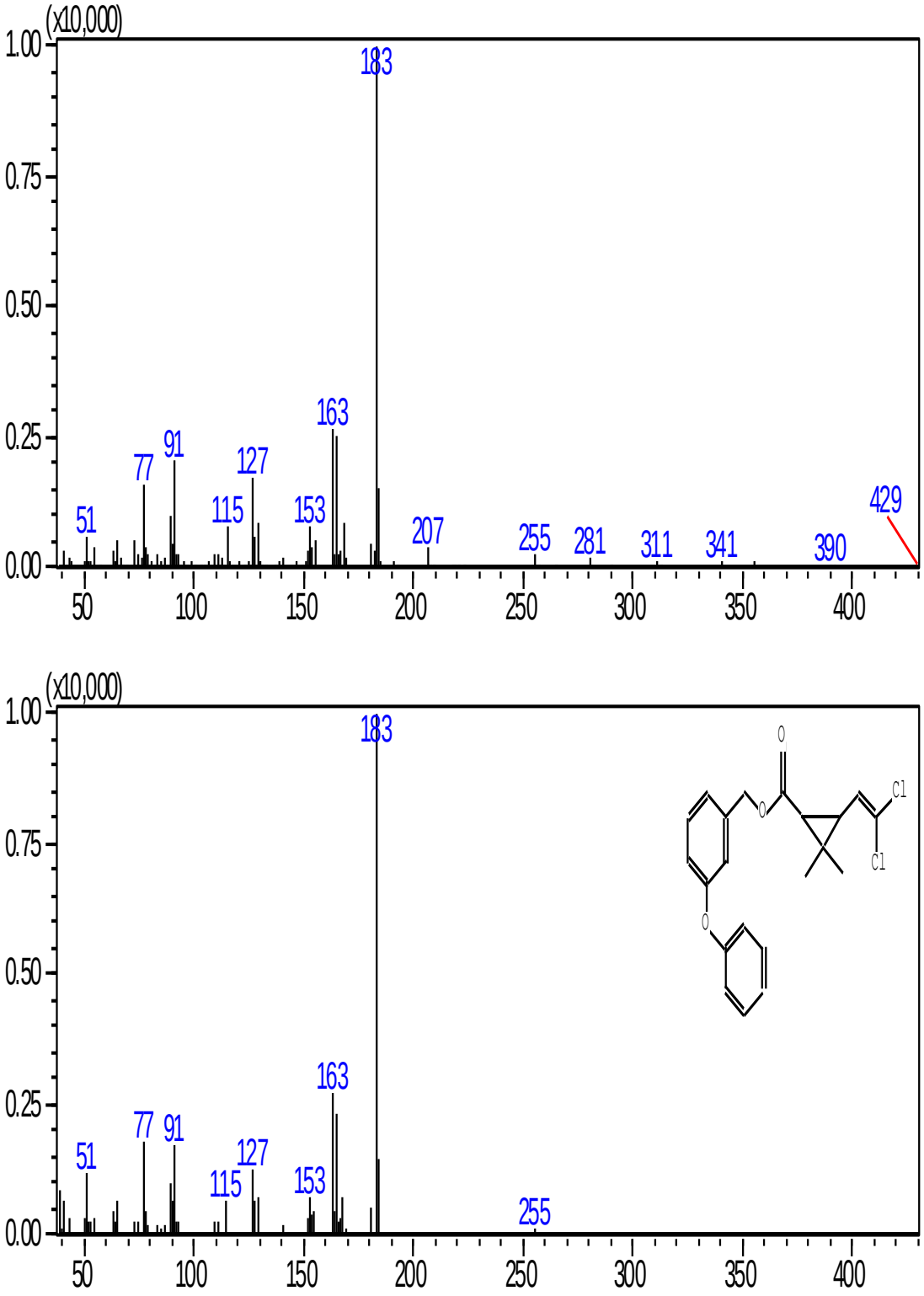


Fig 5.44: Ion fragmentation (top) and NIST Library Search (bottom) for Permethrin (Peak 15)

5.4 Method Validation

It is important to validate the developed method to know if it is suitable for its intended purpose. Validation has been observed to be a quality assurance step in method development (Thompson, M., Ellison, & Wood, 2002). In the present study, the figures of merit of analytical methodology of the developed method was validated in terms of linearity, accuracy, intra-day and inter-day precision, limit of detection (LOD) and limit of quantification (LOQ) using the optimized HS-SPME parameters as shown in Table 5.10. Although, validation of figures of merit of analytical methodology has been described to be a time consuming activity, it is very essential in order to ensure optimal utilization of analytical resources (Chan, 2008, 2011)

5.4.1 Linearity and Calibration Curve

The linearity of an analytical method is the ability to produce a measured value (chromatographic peak area) that a directly proportional to the concentration of the analyte in the sample matrices within a given range. The determination of linearity is used in connection with the formulation of the calibration curve. The range within which the measured response is directly proportional to the concentration of the analytes is called the linear range, which is the interval between the lower and upper calibration points of the spiked sample. In order to determine the linearity, 7 concentration levels of each pesticide was analyzed and the calibration curve was constructed.

A set of calibration curves was prepared with concentrations ranging from 1 – 500 µg/kg, using an internal standard calibration method. The peak area ratio which is ratio of the peak area of analytes to the peak area of internal standard was plotted against the concentration of analytes. Table 5.11 shows the calibration parameters. The calibration curves were linear over the tested concentration range. The correlation coefficients (r^2) were greater than 0.99 for all the investigated pesticides.

Table 5.11: Linearity range (µg/kg) of the Developed HS-SPME Method in 2 Representative Samples each of Fruits and Vegetables

Pesticides	Water		Apple		Tomato		Cucumber		Cabbage	
	Range (µg/kg)	r^2	Range (µg/kg)	r^2	Range (µg/kg)	r^2	Range (µg/kg)	r^2	Range	r^2
Fenobucarb	0.5 – 1000	0.9986	2.5 – 500	0.9975	2.5 – 500	0.9996	2.5 – 500	0.9985	2.5 – 500	0.9976
Ethoprophos	0.5 – 500	0.9992	2.5 – 250	0.9981	2.5 – 250	0.9986	2.5 – 250	0.9975	2.5 – 250	0.9979
Diazinone	0.5 – 500	0.9989	2.5 – 250	0.9987	2.5 – 250	0.9948	2.5 – 250	0.9981	2.5 – 250	0.9980
Chlorothalonil	2 – 1000	0.9985	10 – 500	0.9987	10 – 500	0.9975	10 – 500	0.9978	10 – 500	0.9989
Parathion-methyl	0.25 – 500	0.9988	1 – 250	0.9986	1 – 250	0.9994	1 – 250	0.9988	1 – 250	0.9964
Fenitrothion	0.5 – 500	0.9987	2.5 – 200	0.9989	2.5 – 200	0.9995	2.5 – 200	0.9983	2.5 – 200	0.9952
Chlorpyrifos	1 – 1000	0.9982	5 – 500	0.9980	5 – 500	0.9979	5 – 500	0.9981	5 – 500	0.9985
Thiobencarb	1 – 500	0.9991	5 – 250	0.9982	5 – 250	0.9950	5 – 250	0.9984	5 – 250	0.9977
Quinalphos	0.5 – 250	0.9998	2.5 – 125	0.9985	2.5 – 125	0.9991	2.5 – 125	0.9981	2.5 – 125	0.9968
Endosulfan I	5 – 250	0.9996	5 – 250	0.9980	5 – 250	0.9967	5 – 250	0.9990	5 – 250	0.9976
Endosulfan II	2 – 500	0.9991	10 – 250	0.9988	10 – 250	0.9992	10 – 250	0.9978	10 – 250	0.9987
Bifenthrin	0.25 –1000	0.9985	1 – 500	0.9985	1 – 500	0.9989	1 – 500	0.9983	1 – 500	0.9982
Fenpropathrin	0.25 – 100	0.9999	1 – 50	0.9976	1 – 50	0.9938	1 – 50	0.9984	1 – 50	0.9978
Permethrin	0.5 – 250	0.9995	5 – 100	0.9969	5 – 100	0.9976	5 – 100	0.9989	5 – 100	0.9973

5.4.2 Accuracy and Precision

This is the closeness of agreement between a series of independent measurement obtained when an analytical method is applied in replicate to multiple sampling of homologous samples. It is usually specified in terms of the relative standard deviation (RSD) (Thompson, M. et al., 2002). According to the ICH guideline, precision is divided into: repeatability also called intra-day precision (describes precision under the same operating condition over a short period of time, carried out by the same analyst); intermediate precision, also called inter-day precision (is the precision describe by with-in laboratory variation), carried out on different days; while reproducibility also known as inter-laboratory precision (describes precision obtained among laboratory in a collaborative studies), and it can be combined to the estimate of intermediate precision. In this study, repeatability and intermediate precision of the developed method were investigated.

The accuracy of a method is the degree of closeness between the measured value and the values that are accepted either as a conventional true value or an accepted reference value of analyte present in the sample. In this study accuracy of the method is determined based on the analysis of sample spiked with a known amount of pesticide and comparing the measured value with the spiked value. The accuracy is reported as the percent recovery by the analysis of a known added amount of pesticides in the sample matrix (Chan, 2008, 2011; ICH-Topic Q2(R1), 2006).

According to the ICH guideline, accuracy and precision should be assayed using a minimum of nine determinations covering the specified range, i.e. three concentration levels replicated three times for each concentration, or a minimum of six determinations of 100 % of the test concentration for precision measurement (ICH-Topic Q2(R1), 2006).

The accuracy, inter-day and the intra-day precision were determined by spiking the samples at three concentration levels and three replicates analysis were run for each concentration on the same day. The intra-day precision (n = 3) was estimated by performing three extractions in a single day, and inter-day precision (n = 9) was estimated based on three extractions per day for three days, while the accuracy was reported in terms of the average recoveries of the spiked sample at different concentration levels.

A one-way single factor ANOVA was used to estimate the variance, which gives the total sum of square, between group mean square (BMS) and within group mean square (WMS). The BMS estimates the variance that is associated with inter-day (between day variance) and a variance associated with intra-day variability (within-day). These two variances were employed in the determination of repeatability and intermediate precision (Winer, 1991). Subsequently, the repeatability (intra-day precision) and intermediate precision (inter-day) were calculated using the following equations:

$$\%\ RSD\ (\mathrm{I}ntra - day) = \frac{\sqrt{WMS}}{Average\ Relative\ Recovery}\ x\ 100 \qquad (5.1)$$

$$\%\ RSD\ (Inter - day) = \frac{\sqrt{\left(\frac{BMS - WMS}{N}\right) + WMS}}{Average\ Relative\ Recovery}\ x\ 100 \qquad (5.2)$$

where N is the number of replicates per day and the average relative recovery is the average estimated from daily average recoveries.

5.4.3 Recovery

The efficiency and accuracy of any extraction technique is determined based on the average recovery. The recovery is determined as the average relative recovery, which involves the analysis of known amounts of analytes spiked into the sample matrix, and comparing the chromatographic peak area obtained with the chromatographic peak area obtained when analyzing a standard solution of the same concentration under the same experimental conditions. Absolute recovery involves the comparison of the chromatographic peak area of the SPME injection with the chromatographic peak area of a direct injection of standard solution of analytes.

Table 5.12 and Table 5.13, showed the precisions and accuracies (relative recoveries) of the developed method in fruit and vegetable samples respectively. The intra-day precisions vary from 1.5 to 14.0 % and 0.5 to 13.9 % in fruit and vegetable samples respectively. The intermediate precisions vary from 2.4 to 14.9 % and 1.1 to 14.2 in fruit and vegetable samples respectively. The relative recoveries of the spiked fruit and vegetable samples range from 73.3 to 111 %, and 74 to 118.5 % respectively which were acceptable according to the SANCO guideline (SANCO, 2011), which stated that the method performance criteria of mean recoveries should be in the range of 70 – 120 % with RSD less than or equal to 20 %. For the apple sample, recoveries were between 73.3 and 106%, with RSD of 3.2 – 13.6 %, and 75.4 and 109.3 % in tomato (RSD = 2.4 – 14.7), while recoveries range from 76.4 – 108.9 % (RSD = 2.4 – 14.7%) and 76 to 111 % (RSD = 1.5 – 9 %) in pear and

grape samples respectively. The recoveries obtained in vegetable samples and their respective RSDs are: cucumber, 76.4 – 117 % (RSD = 2.8 – 13.9 %); cabbage, 76 – 118.5 % (RSD = 3.4 -12.7 %); lettuce, 74 – 113.6 % (RSD = 1.4 – 12.5 %); and broccoli, 75.6 – 115.7 % (RSD = 1.1 – 11.1 %) The results obtained for the precision and accuracy study are therefore in accordance with the acceptable practice and the results are satisfactory for determination of the target pesticides in the complex sample matrices with no significant matrix interference.

The recoveries obtained in vegetable samples were slightly higher than those obtained in fruit samples, this could be attributed to the presence of suspended solid particles and high molecular mass substances such as pectin and sugar present in the fruit samples (Lambropoulou & Albanis, 2003; Sang et al., 2013; Simplício & Vilas Boas, 1999), although matrix interference were completely eliminated by appropriate dilution of the samples. It was also observed that, better recoveries and precisions were achieved at higher spiked levels. All the parameters validated in this study were based on the method validation requirements of the European Union (SANCO, 2011).

Table 5.12: Accuracy, Intra- and Inter-day Precisions of the Pesticides in Fruit Samples

Pesticides	Added (µg/kg)	Apple			Tomato			Pear			Grape		
		Intra (%)	Inter (%)	Accuracy (%)	Intra (%)	Inter (%)	Accuracy (%)	Intra (%)	Inter (%)	Accuracy (%)	Intra (%)	Inter (%)	Accuracy (%)
Fenobucarb	50	11.5	12.9	80.9	2.8	6.0	105.0	8.9	13.04	99.1	4.9	6.9	90.4
	100	8.2	9.0	96.2	2.2	2.4	75.6	5.0	5.35	104.0	6.9	10.9	80.7
	150	2.74	4.0	103.5	2.2	3.1	95.5	5.8	6.59	103.5	5.0	8.4	86.8
Ethoprophos	50	13.6	14.9	79.1	13.2	14.5	80.0	7.1	7.32	106.7	9.2	10.6	76.0
	100	8.3	8.9	95.0	3.0	3.3	75.4	3.6	4.12	103.9	5.1	5.7	79.9
	150	4.3	4.5	102.4	3.2	5.0	91.6	4.7	5.10	106.4	3.1	4.7	81.9
Diazinon	50	5.5	9.2	77.8	10.4	13.7	82.3	7.7	8.09	97.8	9.0	10.2	86.7
	100	5.1	6.0	88.7	3.7	5.6	75.5	8.4	9.26	994	4.9	8.4	87.3
	150	7.4	6.3	101.9	3.2	6.0	102.8	5.8	6.38	104.2	2.7	4.5	96.7
Chlorothalonil	50	11.5	12.4	76.2	8.8	10.2	112.0	5.6	6.12	89.6	8.6	10.1	80.7
	100	5.1	9.8	81.0	7.1	7.6	109.3	10.6	11.69	82.0	5.4	6.1	78.9
	150	6.1	7.0	104.2	3.8	4.7	115.0	6.0	6.25	100.5	6.3	7.2	89.4
P. Methyl	20	6.8	10.7	73.3	10.5	11.0	80.3	7.1	8.06	76.6	5.5	6.4	83.2
	50	3.2	10.6	80.4	6.1	6.7	96.9	14.0	13.89	76.4	5.2	7.4	87.5
	100	4.3	6.4	98.0	4.4	4.7	105.0	4.1	6.62	88.6	2.2	2.9	87.3
Fenitrothion	50	5.6	6.6	102.4	12.1	13.6	85.5	5.4	5.88	109.1	6.0	10.4	95.8
	100	4.4	7.0	106.4	4.0	6.4	99.8	3.9	6.20	108.7	4.7	9.7	103.2
	150	4.3	4.3	107.6	5.5	7.4	107.6	4.1	5.17	107.5	3.7	6.3	103.5
Chlorpyrifos	50	7.8	14.3	91.6	7.4	13.6	92.2	7.9	8.87	99.8	7.6	8.4	100.7
	100	4.4	8.9	96.6	4.6	6.4	109.8	4.0	4.85	104.1	5.4	6.1	105.8
	150	6.6	6.6	98.2	3.4	7.4	107.5	3.6	3.85	102.2	2.5	4.2	111.0

Table 5.12: Accuracy, Intra- and Inter-day Precisions of the Pesticides in Fruit Samples (cont'd)

Pesticides	Added (µg/kg)	Apple			Tomato			Pear			Grape		
		Intra (%)	Inter (%)	Accuracy (%)	Intra (%)	Inter (%)	Accuracy (%)	Intra (%)	Inter (%)	Accuracy (%)	Intra (%)	Inter (%)	Accuracy (%)
Thiobencarb	50	8.2	8.2	102.4	9.50	13.0	89.1	9.7	10.8	88.0	6.2	10.7	94.8
	100	5.6	6.5	104.3	6.62	7.0	91.6	4.7	6.3	95.4	2.9	6.1	92.1
	150	4.9	5.6	106.0	3.49	6.0	101.6	5.4	5.5	95.0	3.2	3.8	98.2
Quinalphos	20	8.9	13.2	105.8	6.31	12.1	86.5	5.1	10.6	105.8	8.0	10.9	103.0
	50	7.5	11.2	89.0	9.16	11.0	88.5	2.6	4.5	105.9	1.9	5.2	108.5
	100	3.9	4.4	100.6	3.36	8.2	103.3	2.8	3.0	102.9	1.7	4.3	110.3
Endosulfan I	50	5.2	15.5	99.1	11.83	12.0	87.5	7.0	8.3	99.1	3.9	6.1	101.6
	100	6.0	6.8	102.4	7.03	8.0	98.2	5.6	6.7	102.4	2.2	7.5	102.4
	150	4.6	5.1	109.3	1.93	6.7	98.3	3.4	3.9	104.1	1.9	3.1	104.2
Endosulfan II	50	7.8	8.2	90.9	5.30	5.8	87.0	5.4	5.5	97.6	3.3	5.6	101.8
	100	5.8	6.5	99.8	2.68	3.9	96.2	4.4	4.8	103.1	2.7	4.0	105.6
	150	3.3	4.2	102.4	2.74	3.0	95.4	4.3	4.8	102.4	2.4	5.4	102.22
Bifenthrin	50	6.4	7.4	101.1	6.84	9.3	90.9	4.7	5.0	101.8	3.3	5.8	108.0
	100	6.5	6.5	106.2	4.3	6.0	96.6	3.0	3.2	104.6	3.1	5.3	107.5
	150	4.1	4.1	104.9	1.56	5.3	90.9	2.5	3.1	103.9	1.5	4.1	110.4
Fenpropathrin	5	10.8	10.6	94.3	7.21	9.1	94.8	3.4	9.2	79.9	2.9	10.5	93.3
	10	10.0	10.8	102.8	4.95	9.1	97.3	8.1	9.0	93.9	8.8	11.3	95.1
	20	10.7	11.4	98.2	7.09	8.3	97.8	9.8	10.7	96.2	7.9	9.0	98.5
Permethrin	20	8.7	8.8	101.6	11.74	13.1	102.3	11.2	12.9	95.7	8.8	8.9	102.4
	50	5.8	6.4	98.8	6.18	7.2	104.9	7.0	7.9	103.4	6.6	7.3	107.9
	100	3.4	5.01	103.5	4.71	14.7	98.4	3.3	8.1	104.6	3.1	7.6	109.9
Ranges	5 – 150	3.2 – 13.6	4.0 – 14.3	73.3 – 106.0	1.6 – 13.2	2.4 – 14.7	75.4 – 109.3	2.5 – 14.0	3.0 – 14.9	76.4 – 108.9	1.5 – 9.0	3.1 – 11.3	76.0 – 111.0

Table 5.13: Accuracy, Intra- and Inter-day Precisions of the Pesticides in Vegetable Samples

Pesticides	Added (µg/kg)	Cucumber			Cabbage			Lettuce			Broccoli		
		Intra (%)	Inter (%)	Accuracy (%)	Intra (%)	Inter (%)	Accuracy (%)	Intra (%)	Inter (%)	Accuracy (%)	Intra (%)	Inter (%)	Accuracy (%)
Fenobucarb	50	7.4	10.4	76.40	9.1	12.6	76.8	9.9	12.5	85.5	3.3	7.2	79.9
	100	5.1	6.1	80.70	4.9	6.1	77.4	7.9	10.9	80.7	1.2	1.7	76.8
	150	3.1	4.4	89.19	4.9	6.8	92.6	3.1	4.2	94.8	1.4	1.8	83.4
Ethoprophos	50	8.9	9.6	83.11	12.2	14.3	89.2	7.9	8.0	105.9	2.6	4.9	75.8
	100	3.0	3.5	86.89	7.1	8.0	81.8	3.7	4.1	85.2	1.5	1.8	75.6
	150	5.3	5.3	88.59	5.1	5.7	89.4	3.4	3.7	93.1	1.9	2.3	93.9
Diazinon	50	8.4	12.4	88.94	9.9	10.8	85.8	4.6	5.0	92.5	2.3	5.9	88.0
	100	5.0	6.4	91.72	9.0	9.3	87.6	4.2	4.6	86.4	2.1	2.4	88.3
	150	2.8	3.2	103.85	8.3	8.7	106.1	2.6	4.3	88.3	1.6	1.9	94.0
Chlorothalonil	50	11.4	13.2	81.86	12.9	14.8	113.8	7.1	8.1	82.1	5.5	5.9	90.3
	100	2.7	4.5	105.11	9.0	10.2	113.6	3.2	4.4	88.1	2.4	2.7	86.4
	150	4.2	4.5	117	7.8	8.7	90.5	2.3	4.0	87.5	1.2	1.5	97.2
P. Methyl	25	10.7	12.0	77.66	12.5	14.1	76.0	5.2	5.6	79.3	4.6	6.1	78.4
	50	8.6	9.4	78.43	10.5	12.0	78.4	4.7	5.0	74.2	3.6	4.5	88.5
	100	4.4	4.8	78.56	8.8	10.0	103.3	3.9	4.2	80.1	1.7	2.6	86.0
Fenitrothion	50	10.8	11.4	90.71	12.7	14.4	117.9	7.1	8.1	75.7	1.6	2.8	76.4
	100	4.6	5.3	109.55	9.3	10.8	106.6	2.8	3.4	86.2	1.4	3.1	86.88
	150	3.7	4.2	93.06	5.6	6.4	105.1	3.0	3.1	89.5	1.3	2.5	93.4
Chlorpyrifos	50	8.0	10.8	108.24	12.3	13.9	118.5	5.6	6.7	74.0	1.8	2.9	85.0
	100	3.2	5.6	105.72	7.9	8.5	110.2	4.5	5.0	78.6	0.5	1.8	94.9
	150	3.0	5.4	116.41	9.7	11.2	117.0	2.8	3.7	86.6	0.5	1.2	98.9

Table 5.13: Accuracy, Intra- and Inter-day Precisions of the Pesticides in Vegetable Samples (cont'd)

Pesticides	Added (µg/kg)	Cucumber			Cabbage			Lettuce			Broccoli		
		Intra (%)	Inter (%)	Accuracy (%)	Intra (%)	Inter (%)	Accuracy (%)	Intra (%)	Inter (%)	Accuracy (%)	Intra (%)	Inter (%)	Accuracy (%)
Thiobencarb	50	9.8	11.9	98.9	11.8	12.1	108.9	8.3	9.3	77.7	2.2	3.1	94.7
	100	8.5	10.5	109.6	9.1	9.8	113.3	3.9	4.9	80.0	1.7	2.7	95.8
	150	5.9	6.6	113.1	5.9	6.6	113.5	1.5	2.2	85.3	1.2	1.7	97.4
Quinalphos	25	11.7	12.7	102.7	11.0	12.4	88.3	4.0	6.1	82.1	4.1	7.6	86.5
	50	10.4	11.3	115.1	11.1	13.0	108.6	5.1	5.2	82.7	3.4	4.5	94.7
	100	8.3	8.9	112.7	4.5	7.5	111.5	3.8	4.4	78.0	1.4	4.6	92.8
Endosulfan I	50	8.3	9.3	80.8	10.0	10.2	96.8	5.0	5.4	74.4	3.4	4.5	93.4
	100	6.4	7.2	89.5	7.2	9.2	94.6	2.3	2.6	76.4	1.7	2.0	95.6
	150	6.0	7.1	87.9	4.4	4.9	90.0	1.7	2.1	91.3	1.5	2.6	98.6
Endosulfan II	50	10.3	10.8	76.7	11.0	11.9	96.7	5.5	5.7	75.6	2.3	3.4	90.4
	100	7.5	8.3	81.8	9.7	10.8	97.1	2.5	3.1	85.1	2.4	3.1	95.3
	150	6.2	6.4	85.6	5.3	6.2	107.5	1.3	1.4	91.0	1.5	3.8	96.1
Bifenthrin	50	12.4	13.1	84.7	10.3	13.3	91.3	2.1	2.4	75.3	3.9	5.1	115.7
	100	6.9	7.8	83.4	6.4	7.0	88.8	2.2	2.9	85.1	1.9	5.4	101.3
	150	6.4	6.7	87.2	6.8	7.2	98.5	1.4	1.6	91.0	2.5	4.4	94.2
Fenpropathrin	5	13.9	14.2	77.4	12.5	14.4	106.9	3.5	4.0	113.6	8.2	9.4	109.8
	10	11.7	12.5	83.3	11.2	14.1	90.7	3.8	9.6	95.5	2.6	11.1	99.8
	20	7.0	8.1	87.2	8.6	9.6	113.2	3.9	7.3	83.9	2.2	2.4	95.8
Permethrin	25	11.6	12.8	111.3	11.0	12.4	112.8	7.1	8.0	80.6	5.6	8.0	95.4
	50	7.3	10.9	108.5	10.5	12.0	88.3	5.0	5.2	76.0	3.8	4.7	79.8
	100	5.1	7.7	110.6	3.4	4.6	112.2	3.2	5.8	83.5	3.6	4.1	99.7
Ranges	5 – 150	2.8 – 13.9	3.2 – 14.2	76.4 – 117.0	3.4 – 12.7	6.1 – 14.4	76.0 – 118.5	1.3 – 9.9	1.4 – 12.5	74.0 – 113.6	0.5 – 8.2	1.1 – 11.1	75.6 – 115.7

5.4.4 Selectivity and Specificity

Specificity is defined according to the ICH document as the ability to assess the presence of an analyte unequivocally, in the presence of other interfering components, such as impurities, matrix components and/or degradation products which are expected to be present. Over the years, selectivity and sensitivity has been used interchangeably, but the International Union of Pure and Applied Chemistry (IUPAC), recommends the use of selectivity, while specificity is reserved for completely selective analytical procedures (Chan, 2008, 2011; ICH-Topic Q2(R1), 2006).

Selectivity also describes the ability of an analytical instrument to produce a signal which represents the target analyte and not the interfering component. The selectivity of the developed method was investigated after carefully optimized extraction and desorption conditions. It has been observed that fiber coating selection is the most important aspect of SPME that govern selectivity (Li & Weber, 1999). In this study, the selectivity was determined by extracting a blank matrix containing the internal standard and an apple sample spiked with the target analyte. The resulting chromatograms are as shown in Fig 5.30, which indicates a good selectivity of SPME technique with little matrix interference. The good selectivity is achieved, because of the absence of any clean-up step (Arthur & Pawliszyn, 1990), which may cause loss of analytes and introduction of interferences.

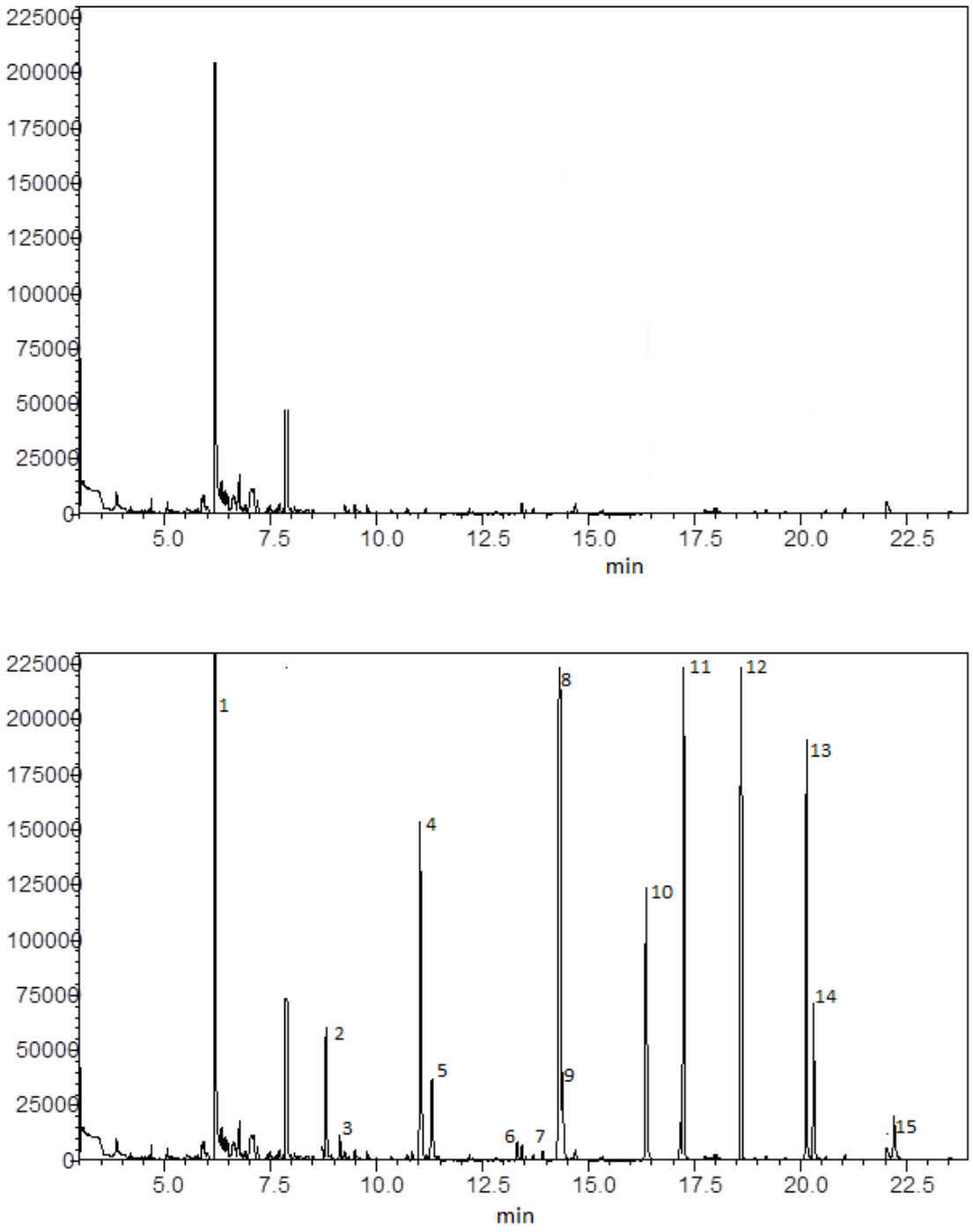


Fig 5.45: Chromatogram of the Selectivity of the Developed Method (Peak numbering as in Fig 5.29)

5.4.5 Limit of Quantification (LOQ) and Limit of Detection (LOD)

The limit of quantification (LOQ) is defined as the lowest concentration of analytes that can be quantitatively determine with an acceptable level of accuracy and precision (ICH-Topic Q2(R1), 2006), with the use of concentration response relationship (Shah et al., 2000). It is estimated as the concentration of analytes in the sample that will give a signal-to-noise ratio of 10:1, and it is affected by the sensitivity of the detector and the accuracy of the sample preparation step (Chan, 2008).

The limit of detection (LOD) is defined as the lowest concentration of analyte in a sample that can be detected but not necessarily quantified as an exact value under the optimized experimental conditions (Chan, 2008; ICH-Topic Q2(R1), 2006) and which can be reliably differentiated from background noise (Shah et al., 2000). It is estimated based on the signal-to-noise ratio of 3:1.

The following approaches can be used for the estimation of LOQ and LOD as recommended by the ICH: (i) the use of visual evaluation for non-instrumental method, which can also be extended to instrumental methods. (ii) the use of signal-to-noise ratio for instruments which exhibit background noise, which involve comparing the measured signals of the analytes with known concentrations with those of the blank samples. (iii) the use of standard deviation of response and the slope calibration curve, which can be based on standard deviation of the blank sample matrix, or the residual standard deviation of the regression line or the standard deviation of y-intercept of the regression lines (Chan, 2008; ICH-Topic Q2(R1), 2006; Miller & Miller, 2010).

The limit of quantification (LOQ) and the limit of detection (LOD) can therefore be expressed as:

$$LOQ = \frac{10\sigma}{S} \quad (5.3)$$

$$LOD = \frac{3\sigma}{S} \quad (5.4)$$

where σ is the standard deviation of the response and S is the slope of the calibration curve.

In the present study, the limits of quantification and detection were determined using the standard deviation of the y-intercept of the regression line. The LOQ and LOD values obtained (Table 5.14) are in most cases below the first calibration level. The values obtained are lower than the maximum residue levels (MRL) allowed by Codex Alimentarius and the European Union (EU, 2005). The LOD values were found ranging from 0.11 to 8.33 µg/kg, while the LOQ were between 0.38 and 27.76 µg/kg.

The analytical figures of merit obtained in this study were better or comparable with values reported in previous and recent studies for the analysis of multiclass pesticide residues in fruits and vegetable samples using solid phase microextraction technique (Abdulra'uf, Chai, & Tan, 2012; Bagheri et al., 2012; Melo et al., 2012a; Sang et al., 2013; Saraji et al., 2013), and other techniques such as liquid phase microextraction (Abdulra'uf, Sirhan, & Tan, 2012; Liu, Z. et al., 2012a; Melo et al., 2012c).

Table 5.14: Figures of Merit of the Developed Method in Fruits and Vegetable Samples

Pesticides		Apple (µg/kg)	Tomato (µg/kg)	Pear (µg/kg)	Grape (µg/kg)	Cucumber (µg/kg)	Cabbage (µg/kg)	Lettuce (µg/kg)	Broccoli (µg/kg)
Fenobucarb	LOD	2.41	2.49	2.19	2.17	1.74	2.49	2.47	2.44
	LOQ	8.03	8.33	7.31	7.22	5.81	8.33	8.22	8.13
	MRL	300	1000	300	300	300	1500	300	300
Ethoprophos	LOD	1.31	0.23	2.51	1.20	0.35	0.23	0.34	0.21
	LOQ	4.36	0.77	8.36	4.00	1.15	0.77	1.14	0.70
	MRL	20	20	20	20	20	20	20	20
Diazinon	LOD	0.88	0.21	0.51	1.05	0.32	0.21	0.23	0.21
	LOQ	2.92	0.68	1.84	3.50	1.05	0.68	0.77	0.68
	MRL	10	10	10	10	10	10	10	10
Chlorothalonil	LOD	2.16	6.94	4.76	0.43	8.33	6.80	0.51	7.34
	LOQ	7.21	23.12	15.86	1.44	27.76	22.67	1.84	24.50
	MRL	1000	2000	1000	10	1000	1000	10	5000
P. Methyl	LOD	0.24	0.62	0.27	0.22	0.50	0.53	0.59	0.55
	LOQ	0.79	2.24	0.89	0.72	1.65	1.76	1.96	1.82
	MRL	10	10	10	10	10	10	10	10
Fenitrothion	LOD	0.53	1.35	0.23	0.20	0.26	0.68	0.20	0.67
	LOQ	1.77	4.48	0.88	0.66	0.85	2.25	0.67	2.24
	MRL	10	10	10	10	10	10	10	10
Chlorpyrifos	LOD	3.30	3.71	3.17	2.79	2.96	3.30	3.52	3.32
	LOQ	11.01	12.36	10.58	9.29	9.87	11.00	11.75	11.08
	MRL	500	500	500	500	500	500	500	500

Table 5.14: Figures of Merit of the Developed in Method in Fruit and Vegetable Samples (cont'd)

Pesticides		Apple (µg/kg)	Tomato (µg/kg)	Pear (µg/kg)	Grape (µg/kg)	Cucumber (µg/kg)	Cabbage (µg/kg)	Lettuce (µg/kg)	Broccoli (µg/kg)
Thiobencarb	LOD	3.48	4.34	3.42	3.19	4.03	3.77	3.19	3.67
	LOQ	11.58	14.47	11.40	10.62	13.43	12.57	10.62	12.23
	MRL	100	100	100	100	100	100	100	100
Quinalphos	LOD	2.16	1.94	2.41	2.24	1.94	1.97	2.05	1.86
	LOQ	7.37	6.48	8.03	7.47	6.48	6.60	6.83	6.20
	MRL	50	50	50	50	50	50	50	50
Endosulfan I	LOD	2.30	3.91	2.76	3.45	3.25	3.13	2.27	2.93
	LOQ	7.67	13.14	9.20	11.50	10.83	10.43	7.57	9.77
	MRL	50	50	50	50	50	50	50	50
Endosulfan II	LOD	2.17	3.19	2.71	3.28	2.08	2.93	3.06	2.34
	LOQ	7.23	10.63	9.03	10.95	6.92	9.77	10.20	7.80
	MRL	50	50	50	50	50	50	50	50
Bifenthrin	LOD	0.11	0.99	0.17	0.75	0..89	0.74	0.64	0.67
	LOQ	0.38	3.31	0.60	2.50	2.96	2.47	2.14	2.22
	MRL	300	300	300	100	300	100	2000	200
Fenpropathrin	LOD	0.14	0.52	0.22	0.55	0.75	0.47	0.34	0.49
	LOQ	0.47	1.72	0.74	1.83	2.50	1.57	1.13	1.65
	MRL	10	10	10	10	10	10	10	10
Permethrin	LOD	1.01	1.50	2.03	1.94	2.42	1.80	1.65	1.95
	LOQ	3.36	5.00	6.78	6.44	8.05	6.00	5.50	6.50
	MRL	50	50	50	50	50	50	50	50

LOD, limit of detection
LOQ, limit of quantification
MRL maximum residue level ([a] from European Union Data (EU, 2005)

5.5 Analysis of Real Fruit and Vegetable Samples

The HS-SPME method developed in this study was subsequently applied to the analysis of fruit and vegetable samples purchased from a local wet market and also on some tomatoes obtained from Cameron Highlands, Malaysia. The real sample analysis was conducted in order to further verify the reliability and robustness of the developed method. Table 5.15 showed the amount of pesticide residues found in real samples. A total of 220 samples of fruits and vegetables were analyzed, and three samples each of tomato and cabbage were found to contain chlorothalonil, while one sample of tomato contains permethrin. One sample of apple was also found to contain chlorpyrifos, with three samples of cabbage found to contain chlorothalonil. All fruits and vegetables found to contain the target pesticides were far below the maximum residue levels allowed by the European Union and the Codex Alimentarius Commission (EU, 2005). All other pesticides investigated in the selected commodities were either not detected or were detected below the limits of quantifications and thus were not quantified.

Table 5.15: Pesticide Residues found in some Fruits and Vegetable Samples

Pesticides	Apple (µg/kg)	Tomato (µg/kg)	Pear (µg/kg)	Grape (µg/kg)	Cucumber (µg/kg)	Cabbage (µg/kg)	Lettuce (µg/kg)
Fenobucarb	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Ethoprophos	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Diazinone	n.d	n.d	n.d	n.d	2.10 (±7.4)	n.d	n.d
Chlorothalonil	n.d	80 (±10.1)	n.d	n.d	n.d	n.d	n.d
Parathion-methyl	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Fenitrothion	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Chlorpyrifos	22.4 (±5.8)	n.d	n.d	n.d	n.d	n.d	n.d
Thiobencarb	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Quinalphos	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Endosulfan I	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Endosulfan II	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Bifenthrin	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Fenpropathrin	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Permethrin	n.d	13.5(±4.9)	n.d	n.d	n.d	n.d	n.d
Fenobucarb	n.d	n.d	n.d	n.d	n.d	n.d	n.d

CHAPTER SIX

6.0 CONCLUSION

The need for frequent monitoring of pesticide residues and other contaminants in food commodities has led to the development of various sample preparation techniques. The SPME technique has proved to be efficient, fast, and accurate for qualitative and quantitative analyses of pesticide residues in fruits and vegetables, and has continued to attract the attention of various stakeholders in the agricultural and food industries.

The use of microextraction technique is emerging as a very reliable sample preparation method, while employing little or no solvent. The advantages of microextraction over the traditional methods include their simplicity of operation, rapid sampling, low cost, high recovery and enrichment factor and being environment-friendly. Solid phase microextraction remains the best environmentally friendly sample preparation and extraction technique because of its solvent-less nature. Due to its ease of automation, and less volatile analytes, that are not amenable to GC can easily be quantified with HPLC, because the extraction steps are the same irrespective of the chromatographic instruments. Food analysis is very important for quality monitoring, control, and assurance. Therefore, the SPME sample preparation described in this study has been shown to be very effective, efficient, rapid, and versatile for the analysis of pesticide residues and other contaminants from fruits and vegetables and from other food samples.

The proposed HS-SPME method demonstrates it ability for an effective screening of multiclass pesticides in fruit and vegetable samples. The use of headspace sampling technique allows for the variation of sample matrix related condition, which increases fiber life time and also ensures effective extraction. The extraction method is characterized by the absence of any clean-up step, which ensures that loss of analytes and introduction of contaminants is completely eliminated. The absence of a clean-up step results in drastic reduction in the total analysis time, improves sample throughput and reduces the consumption of large volume of toxic solvents.

The use of chemometric approach to the screening and subsequent optimization of extraction parameters has helped to reduce analysis time and also help to determine the best optimized parameters. The combination of microextraction and chemometrics, as can be observed in this study enhances better recoveries and precisions and also improves detectability of the target analytes and an improved method validation. The experimental design described in this study involved the use of a Plackett-Burman (P-B), which is a first order design, with a 2^{7-4} (resolution III) reduced factorial for the screening of the most important factors affecting the SPME efficiency and recovery of pesticide residues from fruit and vegetable samples. This helps in the estimation of the significant factors affecting extraction efficiency, although it does not yield exact quantity, but provides valuable information on each variable with relatively few and reasonable experimental runs.

The significant factors were further optimized by the use of second-order central composite design (CCD) utilizing a response surface methodology (RSM). The number of points in CCD contains a factorial run of 2^k, axial runs of 2k and C_o center point runs, which results in the total experimental runs given by: $N = 2^k + 2k + C_o$, where k and C_o are the number of variables and the number of central points respectively.

The study shows that the combination of chemometric with solid phase microextraction technique and simplex lattice mixture designs followed by GC-MS analysis results in a powerful, time-saving and cost-cutting method for the analysis of pesticide residues from complex sample matrices, especially at a very low concentration found in fruits and vegetables. The accuracies and precisions of the developed method were in accordance to International guidelines. The recoveries (74 – 118 %) for the target analytes in all the commodities analyzed were within the range as recommended by ICH. The described method can also be used for qualitative and quantitative analysis of pesticide residues in other processed food such as cocoa powder, fruit juices and spices.

The limitations encountered in this study, which include fragility of the fiber, which were handled with utmost care to avoid breakage, the quality and consistency of the fiber which differs among manufacturers and thus all the fibers employed in this study were obtained from a single manufacturer. Other limitations include low recommended operating temperature, low volume of fiber coatings and stripping of coatings in SPME when used in chlorinated solvents. The use of more selective, efficient and versatile extraction procedure and increasing interest in overcoming the aforementioned limitations and trend

towards automation will provide better integration of sampling and instrumental analysis which can be used for a wide range of analytes.

Further studies should be focused on the use of sol-gel prepared, ionic liquid, supramolecular molecules and molecularly imprinted polymer coatings as the extraction phase to increase the range of analytes that can be qualitatively and quantitatively analyzed in a wide range of environmental samples.

REFERENCES

Abdulahi, A. R. (2003). Pesticide Use in Malaysia. In M. D. Taylor, S. J. Klaine, F. P. Carvalho, D. Barcelo & J. Everaarts (Eds.), *Pesticide Residues in Coastal Tropical Ecosystems: Distribution, Fate and Effects* (pp. 19-179). London: Taylor and Francis.

Abdulra'uf, L. B., Chai, M. K., & Tan, G. H. (2012). Applications of Solid-Phase Microextraction for the Analysis of Pesticide Residues in Fruits and Vegetables: A Review. *J. AOAC Int., 95*(5), 1272-1290.

Abdulra'uf, L. B., Sirhan, A. Y., & Tan, G. H. (2012). Recent Developments and Applications of Liquid Phase Microextraction in Fruits and Vegetables Analysis. *J. Sep. Sci., 35*(24), 3540-3553.

Abhilash, P. C., & Singh, N. (2009). Pesticide Use and Application: An Indian Scenario. *J. Hazard. Mater., 165*(1-3), 1-12.

Act 1974 (2006). Laws of Malaysia: Pesticides Act 1974

Adou, K., Bontoyan, W. R., & Sweeney, P. J. (2001). Multiresidue Method for the Analysis of Pesticide Residues in Fruits and Vegetables by Accelerated Solvent Extraction and Capillary Gas Chromatography. *J. Agric. Food Chem., 49*(9), 4153-4160.

Ahmed, F. E. (2001). Analyses of Pesticides and Their Metabolites in Foods and Drinks. *TrAC - Trends Anal. Chem., 20*(11), 649-661.

Ai, J. (1997a). Headspace Solid Phase Microextraction. Dynamics and Quantitative Analysis before Reaching a Partition Equilibrium. *Anal. Chem., 69*(16), 3260-3266.

Ai, J. (1997b). Solid Phase Microextraction for Quantitative Analysis in Nonequilibrium Situations. *Anal. Chem., 69*(6), 1230-1236.

Ai, J. (1998). Solid-Phase Microextraction in Headspace Analysis. Dynamics in Non-Steady-State Mass Transfer. *Anal. Chem., 70*(22), 4822-4826.

Åkesson, M., Sparrenbom, C. J., Carlsson, C., & Kreuger, J. (2013). Statistical Screening for Descriptive Parameters for Pesticide Occurrence in a Shallow Groundwater Catchment. *J. Hydrol., 477*, 165-174. doi: 10.1016/j.jhydrol.2012.11.025

Albero, B., Sánchez-Brunete, C., & Tadeo, J. L. (2005). Multiresidue Determination of Pesticides in Juice by Solid-Phase Extraction and Gas Chromatography-Mass Spectrometry. *Talanta, 66*(4), 917-924.

ALOthman, Z. A., Dawod, M., Kim, J., & Chung, D. S. (2012). Single-Drop Microextraction as a Powerful Pretreatment Tool for Capillary Electrophoresis: A Review. *Anal. Chim. Acta, 739*, 14-24.

Altinok, I., Capkin, E., & Boran, H. (2011). Influence of Bioassay Volume, Water Column Height, and Octanol-Water Partition Coefficient on the Toxicity of Pesticides to Rainbow Trout. *Bull. Environ. Contam. Toxicol., 86*(6), 596-600. doi: 10.1007/s00128-011-0281-4

Amvrazi, E. G., Papadi-Psyllou, A. T., & Tsiropoulos, N. G. (2010). Pesticide Enrichment Factors and Matrix Effects on the Determination of Multiclass Pesticides in Tomato Samples by Single-Drop Microextraction (SDME) Coupled with Gas Chromatography and Comparison Study between SDME and Acetone-Partition Extraction Procedure. *Int. J. Environ. Anal. Chem., 90*(3-6), 245-259.

Amvrazi, E. G., & Tsiropoulos, N. G. (2009a). Application of Single-Drop Microextraction Coupled with Gas Chromatography for the Determination of Multiclass Pesticides in Vegetables with Nitrogen Phosphorus and Electron Capture Detection. *J. Chromatogr. A, 1216*(14), 2789-2797.

Amvrazi, E. G., & Tsiropoulos, N. G. (2009b). Chemometric Study and Optimization of Extraction Parameters in Single-Drop Microextraction for the Determination of Multiclass Pesticide Residues in Grapes and Apples by Gas Chromatography Mass Spectrometry. *J. Chromatogr. A, 1216*(45), 7630-7638.

Anderson, L. I. (2006). Solid-Phase Extraction on Molecularly Imprinted Polyners: Requirements, Achievement and Future Work. In S. Piletsky & A. G. Mayes (Eds.), *Molecularly Imprinting of Polymers* (pp. 140-148). Texas, USA: Landes Bioscience.

Andraščíková, M., Hrouzková, S., & Cunha, S. C. (2013). Combination of Quechers and Dllme for Gc-Ms Determination of Pesticide Residues in Orange Samples. *Food Addit. Contam., Part A, 30*(2), 286-297. doi: 10.1080/19440049.2012.736029

Andreu, V., & Picó, Y. (2012). Determination of Currently Used Pesticides in Biota. *Anal. Bioanal. Chem., 404*(9), 2659-2681. doi: 10.1007/s00216-012-6331-x

Andruch, V., Balogh, I. S., Kocúrová, L., & Šandrejová, J. (2013a). Five Years of Dispersive Liquid-Liquid Microextraction. *Appl. Spectrosc. Rev., 48*(3), 161-259.

Andruch, V., Balogh, I. S., Kocúrová, L., & Šandrejová, J. (2013b). The Present State of Coupling of Dispersive Liquid-Liquid Microextraction with Atomic Absorption Spectrometry. *J. Anal. At. Spectrom., 28*(1), 19-32. doi: 10.1039/c2ja30175j

Andruch, V., Kocúrová, L., Balogh, I. S., & Škrlíková, J. (2012). Recent Advances in Coupling Single-Drop and Dispersive Liquid–Liquid Microextraction with UV–Vis Spectrophotometry and Related Detection Techniques. *Microchem. J., 102*(0), 1-10. doi: http://dx.doi.org/10.1016/j.microc.2011.10.006

Araoud, M., Douki, W., Rhim, A., Najjar, M. F., & Gazzah, N. (2007). Multiresidue Analysis of Pesticides in Fruits and Vegetables by Gas Chromatography-Mass Spectrometry. *J. Environ. Sci. Heal. B, 42*(2), 179-187. doi: 10.1080/03601230601123474

Arias-Estévez, M., López-Periago, E., Martínez-Carballo, E., Simal-Gándara, J., Mejuto, J. C., & García-Río, L. (2008). The Mobility and Degradation of Pesticides in Soils and the Pollution of Groundwater Resources. *Agric., Ecosyst. Environ., 123*(4), 247-260. doi: 10.1016/j.agee.2007.07.011

Arthur, C. L., Killam, L. M., Buchholz, K. D., Pawliszyn, J., & Berg, J. R. (1992a). Automation and Optimization of Solid-Phase Microextraction. *Anal. Chem., 64*(17), 1960-1966.

Arthur, C. L., Killam, L. M., Motlagh, S., Lim, M., Potter, D. W., & Pawliszyn, J. (1992b). Analysis of Substituted Benzene Compounds in Groundwater Using Solid-Phase Microextraction. *Environ. Sci. Technol., 26*(5), 979-983.

Arthur, C. L., & Pawliszyn, J. (1990). Solid Phase Microextraction with Thermal Desorption Using Fused Silica Optical Fibers. *Anal. Chem., 62*(19), 2145-2148. doi: 10.1021/ac00218a019

Asensio-Ramos, M., Ravelo-Pérez, L. M., González-Curbelo, M. T., & Hernández-Borges, J. (2011). Liquid Phase Microextraction Applications in Food Analysis. *J. Chromatogr. A, 1218*(42), 7415-7437.

Aspellin, A. L. (1997). Pesticides Industry Sales and Usage:1994 and 1995 Markets Estimates. USEPA. Retrieved 17/12/2012 from http://www.epa.gov/pesticides/pestsales/95pestsales/table_of_contents1995.htm

Aspellin, A. L., & Grube, A. H. (1999). Pesticides Industry Sales and Usage:1996 and 1997 Markets Estimates. USEPA. http://www.epa.gov/pesticides/pestsales/97pestsales/table_of_contents1997.htm

Atreya, K., Sitaula, B. K., Johnsen, F. H., & Bajracharya, R. M. (2011). Continuing Issues in the Limitations of Pesticide Use in Developing Countries. *J. Agric. Environ. Ethics, 24*, 49-62.

Aulakh, J. S., Malik, A. K., Kaur, V., & Schmitt-Kopplin, P. (2005). A Review on Solid Phase Micro Extraction—High Performance Liquid Chromatography (SPME-HPLC) Analysis of Pesticides. *Crit. Rev. Anal. Chem., 35*(1), 71-85. doi: 10.1080/10408340590947952

Bagheri, H., Es'haghi, A., Es-haghi, A., & Mesbahi, N. (2012). A High-Throughput Approach for the Determination of Pesticide Residues in Cucumber Samples Using Solid-Phase Microextraction on 96-Well Plate. *Anal. Chim. Acta, 740*(0), 36-42. doi: http://dx.doi.org/10.1016/j.aca.2012.06.001

Ballantyne, B., & Marrs, T. C. (2004). *Pesticides: An Overview of Fundamentals*. Sussex, England: John Wiley.

Baltussen, E., Cramers, C. A., & Sandra, P. J. F. (2002). Sorptive Sample Preparation - a Review. *Anal. Bioanal. Chem., 373*(1-2), 3-22.

Baltussen, E., Sandra, P., David, F., & Cramers, C. (1999). Stir Bar Sorptive Extraction (SBSE), a Novel Extraction Technique for Aqueous Samples: Theory and Principles. *J. Microcolumn Sep., 11*(10), 737-747.

Bansal, O. P. (2012). Degradation of Pesticides. In H. S. Rathore & L. M. L. Nullet (Eds.), *Pesicides: Evaluation of Environmental Pollution* (pp. 47-78). Florida, USA: CRC Press.

Barahona, F., Gjelstad, A., Pedersen-Bjergaard, S., & Rasmussen, K. E. (2010). Hollow Fiber-Liquid-Phase Microextraction of Fungicides from Orange Juices. *J. Chromatogr. A, 1217*(13), 1989-1994.

Barahona, F., Turiel, E., & Martín-Esteban, A. (2011). Supported Liquid Membrane-Protected Molecularly Imprinted Fibre for Solid-Phase Microextraction of Thiabendazole. *Anal. Chim. Acta, 694*(1-2), 83-89.

Barbash, J. E. (2006). *The Geochemistry of Pesticides*. Tacoma, USA: US Geological Survey.

Basheer, C., Alnedhary, A. A., Rao, B. S. M., & Lee, H. K. (2007). Determination of Organophosphorous Pesticides in Wastewater Samples Using Binary-Solvent Liquid-Phase Microextraction and Solid-Phase Microextraction: A Comparative Study. *Anal. Chem., 605*(2), 147-152.

Beceiro-González, E., González-Castro, M. J., Muniategui-Lorenzo, S., López-Mahía, P., & Prada-Rodríguez, D. (2012). Analytical Methodology for the Determination of Organochlorine Pesticides in Vegetation. *J. AOAC Int., 95*(5), 1291-1310.

Bedendo, G. C., & Carasek, E. (2010). Simultaneous Liquid-Liquid Microextraction and Polypropylene Microporous Membrane Solid-Phase Extraction of Organochlorine Pesticides in Water, Tomato and Strawberry Samples. *J. Chromatogr. A, 1217*(1), 7-13.

Bedendo, G. C., Jardim, I. C. S. F., & Carasek, E. (2012). Multiresidue Determination of Pesticides in Industrial and Fresh Orange Juice by Hollow Fiber Microporous Membrane Liquid-Liquid Extraction and Detection by Liquid Chromatography-Electrospray-Tandem Mass Spectrometry. *Talanta, 88*, 573-580.

Belden, J. B., Gilliom, R. J., Martin, J. D., & Lydy, M. J. (2007). Relative Toxicity and Occurrence Patterns of Pesticide Mixtures in Streams Draining Agricultural Watersheds Dominated by Corn and Soybean Production. *Integr. Environ. Assess. Manage., 3*(1), 90-100. doi: 10.1897/1551-3793(2007)3[90:RTAOPO]2.0.CO;2

Bello-López, M. A., Ramos-Payán, M., Ocaña-González, J. A., Fernández-Torres, R., & Callejón-Mochón, M. (2012). Analytical Applications of Hollow Fiber Liquid Phase Microextraction (HF-LPME): A Review. *Anal. Lett., 45*(8), 804-830.

Beltran, J., López, F. J., & Hernández, F. (2000). Solid-Phase Microextraction in Pesticide Residue Analysis. *J. Chromatogr. A, 885*(1-2), 389-404.

Beltran, J., Peruga, A., Pitarch, E., López, F. J., & Hernández, F. (2003). Application of Solid-Phase Microextraction for the Determination of Pyrethroid Residues in Vegetable Samples by Gc-Ms. *Anal. Bioanal. Chem., 376*(4), 502-511.

Berijani, S., Assadi, Y., Anbia, M., Milani Hosseini, M. R., & Aghaee, E. (2006). Dispersive Liquid-Liquid Microextraction Combined with Gas Chromatography-Flame Photometric Detection. Very Simple, Rapid and Sensitive Method for the Determination of Organophosphorus Pesticides in Water. *J. Chromatogr. A, 1123*(1), 1-9.

Bermúdez-Saldaña, J. M., Escuder-Gilabert, L., Medina-Hernández, M. J., Villanueva-Camañas, R. M., & Sagrado, S. (2005). Chromatographic Evaluation of the Toxicity in Fish of Pesticides. *J. Chromatogr. B, 814*(1), 115-125. doi: 10.1016/j.jchromb.2004.10.007

Berrada, H., Font, G., & Moltó, J. C. (2004). Application of Solid-Phase Microextraction for Determining Phenylurea Herbicides and Their Homologous Anilines from Vegetables. *J. Chromatogr. A, 1042*(1-2), 9-14.

Bicchi, C., Cordero, C., Liberto, E., Rubiolo, P., Sgorbini, B., David, F., & Sandra, P. (2005). Dual-Phase Twisters: A New Approach to Headspace Sorptive Extraction and Stir Bar Sorptive Extraction. *J. Chromatogr. A, 1094*(1-2), 9-16.

Bicchi, C., Cordero, C., Liberto, E., Sgorbini, B., David, F., Sandra, P., & Rubiolo, P. (2007). Influence of Polydimethylsiloxane Outer Coating and Packing Material on Analyte Recovery in Dual-Phase Headspace Sorptive Extraction. *J. Chromatogr. A, 1164*(1-2), 33-39.

Bidari, A., Ganjali, M. R., Norouzi, P., Hosseini, M. R. M., & Assadi, Y. (2011). Sample Preparation Method for the Analysis of Some Organophosphorus Pesticides Residues in Tomato by Ultrasound-Assisted Solvent Extraction Followed by Dispersive Liquid-Liquid Microextraction. *Food Chem., 126*(4), 1840-1844. doi: 10.1016/j.foodchem.2010.11.142

Blasco, C., Font, G., Mañes, J., & Picó, Y. (2003a). Solid-Phase Microextraction Liquid Chromatography/Tandem Mass Spectrometry to Determine Postharvest Fungicides in Fruits. *Anal. Chem., 75*(14), 3274-3283.

Blasco, C., Font, G., Mañes, J., & Picó, Y. (2003b). Solid-Phase Microextraction Liquid Chromatography/Tandem Mass Spectrometry to Determine Postharvest Fungicides in Fruits. *Analytical Chemistry, 75*(14), 3606-3615.

Blasco, C., Font, G., & Picó, Y. (2002). Comparison of Microextraction Procedures to Determine Pesticides in Oranges by Liquid Chromatography-Mass Spectrometry. *J. Chromatogr. A, 970*(1-2), 201-212.

Bojko, B., Cudjoe, E., Gómez-Ríos, G. A., Gorynski, K., Jiang, R., Reyes-Garcés, N., . . . Pawliszyn, J. (2012). SPME – Quo Vadis? *Anal. Chim. Acta, 750*(0), 132-151. doi: 10.1016/j.aca.2012.06.052

Boonchiangma, S., Ngeontae, W., & Srijaranai, S. (2012). Determination of Six Pyrethroid Insecticides in Fruit Juice Samples Using Dispersive Liquid-Liquid Microextraction Combined with High Performance Liquid Chromatography. *Talanta, 88*, 209-215.

Bordagaray, A., Garcia-Arrona, R., & Millán, E. (2011). Optimization of Solid-Phase Microextraction Procedure Coupled to Gc-Ecd for Triazole Fungicides Determination in Juice Samples. *Food Anal. Method, 4*(3), 293-299.

Borgert, C. J., Quill, T. F., McCarty, L. S., & Mason, A. M. (2004). Can Mode of Action Predict Mixture Toxicity for Risk Assessment? *Toxicol. Appl. Pharm., 201*(2), 85-96.

Bowman, B. T., & Sans, N. W. (1979). The Aqueous Solubility of Twenty-Seven Insecticides and Related Compounds. *J. Environ. Sci. Health., Part B, 14*(6), 625-634.

Brereton, R. G. (2003). *Chemometrics: Data Analyiss for the Laboratory and Chemical Plants*. West Sussex, England: Wiley.

Brereton, R. G. (2007). *Applied Chemometrics for Scientists*. West Sussex, England: Wiley.

Brondi, S. H. G., & Lanças, F. M. (2005). Optimization of a Methodology for the Determination of Organochlorine Pesticides in Surface Water by Spme-Gc/Ms. *J. Environ. Sci. Heal. B, 40*(4), 513-523. doi: 10.1081/PFC-200061508

Bruns, A. (2003). Controlling Aflatoxins and Fumonisin in Maize by Crop Management. *J. Toxicol. Toxin Rev., 22*(2), 153-173.

Buszewski, B., & Studzińska, S. (2008). A Review of Ionic Liquids in Chromatographic and Electromigration Techniques. *Chromatographia, 68*(1), 1-10. doi: 10.1365/s10337-008-0662-y

Buszewski, B., & Szultka, M. (2012). Past, Present, and Future of Solid Phase Extraction: A Review. *Crit. Rev. Anal. Chem., 42*(3), 198-213.

Cabras, P. (2003). Pesticides: Toxicology and Residues in Food. In J. P. F. D'Mello (Ed.), *Food Safety: Contaminants and Toxins* (pp. 91-124). Cambridge, MA, USA: CABI Publishing.

Cai, L., Gong, S., Chen, M., & Wu, C. (2006). Vinyl Crown Ether as a Novel Radical Crosslinked Sol-Gel SPME Fiber for Determination of Organophosphorus Pesticides in Food Samples. *Anal. Chim. Acta, 559*(1), 89-96.

Cai, L., Xing, J., Dong, L., & Wu, C. (2003). Application of Polyphenylmethylsiloxane Coated Fiber for Solid-Phase Microextraction Combined with Microwave-Assisted Extraction for the Determination of Organochlorine Pesticides in Chinese Teas. *J. Chromatogr. A, 1015*(1-2), 11-21.

Campillo, N., Viñas, P., Aguinaga, N., Férez, G., & Hernández-Córdoba, M. (2010). Stir Bar Sorptive Extraction Coupled to Liquid Chromatography for the Analysis of Strobilurin Fungicides in Fruit Samples. *J. Chromatogr. A, 1217*(27), 4529-4534.

Carlile, B. (2006). *Pesticide Selectivity, Health and the Environment*. Cambridge, UK: Cambridge University Press.

Chai, M. K., & Tan, G. H. (2009). Validation of a Headspace Solid-Phase Microextraction Procedure with Gas Chromatography-Electron Capture Detection of Pesticide Residues in Fruits and Vegetables. *Food Chem., 117*(3), 561-567.

Chai, M. K., & Tan, G. H. (2010). Headspace Solid-Phase Microextraction for the Evaluation of Pesticide Residue Contents in Cucumber and Strawberry after Washing Treatment. *Food Chem., 123*(3), 760-764. doi: 10.1016/j.foodchem.2010.05.038

Chai, M. K., Tan, G. H., & Asha, I. (2008a). Optimization of Headspace Solid-Phase Microextraction for the Determination of Pesticide Residues in Tomatoes. *Anal. Sci., 24*(2), 273-276.

Chai, M. K., Tan, G. H., & Asha, K. (2006). Method Development for the Determination of Pesticide Residues in Vegetables and Fruits by Using Solid-Phase Microextraction. *Malay. J. Chem., 8*(1), 067-071.

Chai, M. K., Tan, G. H., & Kumari, A. (2008b). Application of Solid-Phase Microextraction for the Determination of Pesticides in Vegetabble Samples by Gas Chromatography with Electron Capture Detector. *Malay. J. Anal. Sci., 12*(1), 1-9.

Chai, X., Jia, J., Sun, T., & Wang, Y. (2008). Suitability of a Novel Circulating Cooling Spme for Analysis of Organophosphorous Pesticides in Tomatoes. *Chromatographia, 67*(3-4), 309-313.

Chamberlain, E., Shi, H., Wang, T., Ma, Y., Fulmer, A., & Adams, C. (2012). Comprehensive Screening Study of Pesticide Degradation Via Oxidation and Hydrolysis. *J. Agric. Environ. Ethics, 60*(1), 354-363.

Chambers, H. W., Meek, E. C., & Chambers, J. E. (2010a). Chemistry of Organophosphorus Insecticides. In R. Krieger (Ed.), *Haye's Handbook of Pesticide Toxicology* (3rd ed., pp. 1395-1398). San Diego, USA: Elsevier.

Chambers, H. W., Meek, E. C., & Chambers, J. E. (2010b). The Metabolism of Organophosphorus Insecticides. In R. Krieger (Ed.), *Haye's Handbook of Pesticide Toxicology* (pp. 1390-1407). San Diego, USA: Elsevier.

Chan, C. C. (2008). Analytical Method Validation: Principlea and Practices. In S. C. Gad (Ed.), *Pharmaceutical Manufacturing Handbook: Regulations and Quality* (pp. 727-742). Hoboken, NJ: John Wiley.

Chan, C. C. (2011). Principles and Practices of Analytical Method Validation: Validation of Analytical Methods Is Time-Consuming but Essential. *Qual. Assur. J., 14*, 61-64.

Chen, W., Poon, K. A. F., & Lam, M. H. W. (1998). The Application of Solid Phase Microextraction in the Analysis of Organophosphorus Pesticides in a Food Plant. *Environ. Sci. Technol., 32*(23), 3816-3820.

Chen, Y., Guo, Z., Wang, X., & Qiu, C. (2008). Sample Preparation. *J. Chromatogr. A, 1184*(1–2), 191-219. doi: http://dx.doi.org/10.1016/j.chroma.2007.10.026

Chen, Y. I., Su, Y. S., & Jen, J. F. (2002). Determination of Dichlorvos by on-Line Microwave-Assisted Extraction Coupled to Headspace Solid-Phase Microextraction and Gas Chromatography-Electron-Capture Detection. *J. Chromatogr. A, 976*(1-2), 349-355.

Chong, S. L., Wang, D., Hayes, J. D., Wilhite, B. W., & Malik, A. (1997). Sol-Gel Coating Technology for the Preparation of Solid-Phase Microextraction Fibers of Enhanced Thermal Stability. *Anal. Chem., 69*(19), 3889-3898.

Codex Alimentarius Commision. (2010). *Portion of Commodities to Which MR-LS Apply and Which Is Analyzed: Cac/Gl 41-1993*. Rome.

Cooper, J., & Dobson, H. (2007). The Benefits of Pesticides to Mankind and the Environment. *Crop Prot., 26*(9), 1337-1348. doi: 10.1016/j.cropro.2007.03.022

Cortés Aguado, S., Sánchez-Morito, N., Arrebola, F. J., Frenich, A. G., & Vidal, J. L. M. (2008). Fast Screening of Pesticide Residues in Fruit Juice by Solid-Phase Microextraction and Gas Chromatography-Mass Spectrometry. *Food Chem., 107*(3), 1314-1325.

Cortés Aguado, S., Sánchez-Morito, N., Garrido Frenich, A., Martínez Vidal, J. L., & Arrebola, F. J. (2007). Screening Method for the Determination at Parts Per Trillion Levels of Pesticide Residues in Vegetables Combining Solid-Phase Microextraction and Gas Chromatography-Tandem Mass Spectrometry. *Anal. Lett., 40*(15), 2886-2914. doi: 10.1080/00032710701603934

Costa, L. G. (2008). Toxic Effects of Pesticides. In C. D. Klaassen & L. J. Casarett (Eds.), *Casarett and Doull's Toxicology: The Basic Science of Poison* (pp. 883-930). Blacklich, OH, USA: McGraw-Hill.

Cunha, S. C., Fernandes, J. O., & Oliveira, M. B. P. P. (2009). Fast Analysis of Multiple Pesticide Residues in Apple Juice Using Dispersive Liquid-Liquid Microextraction and Multidimensional Gas Chromatography-Mass Spectrometry. *J. Chromatogr. A, 1216*(51), 8835-8844.

David, F., & Sandra, P. (2007). Stir Bar Sorptive Extraction for Trace Analysis. *J. Chromatogr. A, 1152*(1–2), 54-69. doi: http://dx.doi.org/10.1016/j.chroma.2007.01.032

De Fátima Alpendurada, M. (2000). Solid-Phase Microextraction: A Promising Technique for Sample Preparation in Environmental Analysis. *J. Chromatogr. A, 889*(1-2), 3-14.

De Fina, K. M., Sharp, T. L., Chuca, I., Spurgin, M. A., Acree Jr, W. E., Green, C. E., & Abraham, M. H. (2002). Solubility of the Pesticide Monuron in Organic Nonelectrolyte Solvents. Comparison of Observed Versus Predicted Values Based Upon Mobile Order Theory. *Phys. Chem. Liq., 40*(3), 255-268.

De Koning, S., Janssen, H. G., & Brinkman, U. A. T. (2009). Modern Methods of Sample Preparation for GC Analysis. *Chromatographia, 69*(SUPPL. 1), S33-S78.

Deeb, O., & Goodarzi, M. (2010). Predicting the Solubility of Pesticide Compounds in Water Using QSPR Methods. *Mol. Phys., 108*(2), 181-192. doi: 10.1080/00268971003604575

Dehghani Mohammad Abadi, M., Ashraf, N., Chamsaz, M., & Shemirani, F. (2012). An Overview of Liquid Phase Microextraction Approaches Combined with UV-Vis Spectrophotometry. *Talanta, 99*, 1-12.

del Castillo, M. L. R., Rodriguez-Valenciano, M., de la Peña Moreno, F., & Blanch, G. P. (2012). Evaluation of Pesticide Residue Contents in Fruit Juice by Solid-Phase Microextraction and Multidimensional Gas Chromatography Coupled with Mass Spectrometry. *Talanta, 89*(0), 77-83. doi: http://dx.doi.org/10.1016/j.talanta.2011.11.062

Delgado-Moreno, L., Peña, A., & Mingorance, M. D. (2009). Design of Experiments in Environmental Chemistry Studies: Example of the Extraction of Triazines from Soil after Olive Cake Amendment. *J. Hazard. Mater., 162*(2–3), 1121-1128. doi: http://dx.doi.org/10.1016/j.jhazmat.2008.05.148

Djozan, D., & Ebrahimi, B. (2008). Preparation of New Solid Phase Micro Extraction Fiber on the Basis of Atrazine-Molecular Imprinted Polymer: Application for Gc and GC/MS Screening of Triazine Herbicides in Water, Rice and Onion. *Anal. Chim. Acta, 616*(2), 152-159.

Djozan, D., Ebrahimi, B., Mahkam, M., & Farajzadeh, M. A. (2010). Evaluation of a New Method for Chemical Coating of Aluminum Wire with Molecularly Imprinted

Polymer Layer. Application for the Fabrication of Triazines Selective Solid-Phase Microextraction Fiber. *Anal. Chim. Acta, 674*(1), 40-48.

Djozan, D., Mahkam, M., & Ebrahimi, B. (2009). Preparation and Binding Study of Solid-Phase Microextraction Fiber on the Basis of Ametryn-Imprinted Polymer. Application to the Selective Extraction of Persistent Triazine Herbicides in Tap Water, Rice, Maize and Onion. *J. Chromatogr. A, 1216*(12), 2211-2219.

Donaldson, A. L., Kiely, T., & Grube, A. (2002). Pesticides Industry Sales and Usage: 1998 and 1999 Market Estimates. Usepa. Retrieved 17/12/12 from http://www.epa.gov/pesticides/pestsales/99pestsales/table_of_contents1999.htm

Dong, C., Zeng, Z., & Li, X. (2005). Determination of Organochlorine Pesticides and Their Metabolites in Radish after Headspace Solid-Phase Microextraction Using Calix[4]Arene Fiber. *Talanta, 66*(3), 721-727.

Du, J., Yan, H., She, D., Liu, B., & Yang, G. (2010). Simultaneous Determination of Cypermethrin and Permethrin in Pear Juice by Ultrasound-Assisted Dispersive Liquid-Liquid Microextraction Combined with Gas Chromatography. *Talanta, 82*(2), 698-703.

Ecobichon, D. J. (2001a). Pesticide Use in Developing Countries. *Toxicol., 160*(1-3), 27-33.

Ecobichon, D. J. (2001b). Toxic Effects of Pesticides. In L. J. Casarett (Ed.), *Casarett and Doull's Toxicology: The Basic Science of Poison* (6 ed., pp. 763-810). NY, USA: McGraw Hill.

Ehrich, M., & Jortner, B. S. (2010). Organophosphorus-Induced Delayed Neuropathy. In R. Krieger (Ed.), *Haye's Handbook of Pesticide Toxicology* (3rd ed., pp. 1479-1504). San Diego, USA: Elsevier.

Eisert, R., & Pawliszyn, J. (1997a). Automated in-Tube Solid-Phase Microextraction Coupled to High-Performance Liquid Chromatography. *Anal. Chim. Acta, 69*(16), 3140-3147.

Eisert, R., & Pawliszyn, J. (1997b). Design of Automated Solid-Phase Microextraction for Trace Analysis of Organic Compounds in Aqueous Samples. *J. Chromatogr. A, 776*(2), 293-303.

Eisert, R., & Pawliszyn, J. (1997c). New Trends in Solid-Phase Microextraction. *Crit. Rev. Anal. Chem., 27*(2), 103-135.

EPA. (2011a). 2006-2007 Pesticide Market Estimates: Usage. Retrieved 02/12/2012 from http://www.epa.gov/pesticides/pestsales/07pestsales/usage2007.htm

EPA. (2011b). Pesticide New Story: Epa Releases Report Containing Latest Estimates of Pesticide Use in the United State. Retrieved 12/12/2012 from http://epa.gov/oppfead1/cb/csb_page/updates/2011/sales-usage06-07.html

EU. (2002). Methods of Sampling Products of Plant and Animal Origin for the Determination Ofbpesticide Residues for Checking Compliance with Mrls. *Off. J. Eur. Union, Directive 2002/63/EC, L 187, 16.7.2002*, 33-43.

EU. (2005). Regulation (EC) No 396/2005. Retrieved on 24/11/2012 from http://ec.europa.eu/sanco_pesticides/public/index.cfm

EU. (2009a). Directive 2009/128/EC of the European Parliament and of the Council. *Off. J. Eur. Union, L309, 24.11.2009*, 071-086.

EU. (2009b). Method Validation and Quality Control Procedures for Pesticide Residues Analysis in Food and Feed; Sanco/10684/2009. Retrieved on 25/01/2013, from Director General Health and Consumer Protection: ec.europa.eu/food/plant/protection/.../qualcontrol_en.pdf

EU. (2009c). Regulation (EC) No 1107/2009 of the European Parliament and of the Council. *Off. J. Eur. Union, L 309, 24.11.2009*, 001-050.

EU. (2012). Commission Regulation (EU) No 899/2012 of the European Parliament and of the Council. *Off. J. Eur. Union, L 273/1, 6.10.2012*.

Falqui-Cao, C., Wang, Z., Urruty, L., Pommier, J. J., & Montury, M. (2001). Focused Microwave Assistance for Extracting Some Pesticide Residues from Strawberries into Water before Their Determination by Spme/Hplc/Dad. *Journal of Agricultural and Food Chemistry, 49*(11), 5092-5097.

FAO. (2003). International Code of Conduct on the Distribution and Use of Pesticides. Retrieved 27/12/2012 from http://www.fao.org/docrep/005/Y4544E/y4544e00.HTM

Farajzadeh, M. A., Djozan, D., & Khorram, P. (2011). Development of a New Microextraction Method Based on a Dynamic Single Drop in a Narrow-Bore Tube: Application in Extraction and Preconcentration of Some Organic Pollutants in Well Water and Grape Juice Samples. *Talanta, 85*(2), 1135-1142.

Farajzadeh, M. A., Djozan, D., & Khorram, P. (2012). Development of a New Dispersive Liquid-Liquid Microextraction Method in a Narrow-Bore Tube for Preconcentration of Triazole Pesticides from Aqueous Samples. *Anal. Chim. Acta, 713*, 70-78.

Farajzadeh, M. A., & Hatami, M. (2004). Solid-Phase Microextraction Gas Chromatography for Determination of Some Organophosphorus Pesticides. *Chromatographia, 59*(3-4), 259-262.

Fenik, J., Tankiewicz, M., & Biziuk, M. (2011). Properties and Determination of Pesticides in Fruits and Vegetables. *TrAC - Trends Anal. Chem., 30*(6), 814-826. doi: 10.1016/j.trac.2011.02.008

Fernandes, V. C., Domingues, V. F., Mateus, N., & Delerue-Matos, C. (2011). Determination of Pesticides in Fruit and Fruit Juices by Chromatographic Methods. An Overview. *J. Chromatogr. Sci., 49*(9), 715-730.

Fernandes, V. C., Subramanian, V., Mateus, N., Domingues, V. F., & Delerue-Matos, C. (2012). The Development and Optimization of a Modified Single-Drop Microextraction Method for Organochlorine Pesticides Determination by Gas Chromatography-Tandem Mass Spectrometry. *Microchim. Acta, 178*(1-2), 195-202.

Fernández, M., Padrón, C., Marconi, L., Ghini, S., Colombo, R., Sabatini, A. G., & Girotti, S. (2001). Determination of Organophosphorus Pesticides in Honeybees after Solid-Phase Microextraction. *J. Chromatogr. A, 922*(1-2), 257-265.

Ferreira, S. L. C., Dos Santos, W. N. L., Quintella, C. M., Neto, B. B., & Bosque-Sendra, J. M. (2004). Doehlert Matrix: A Chemometric Tool for Analytical Chemistry - Review. *Talanta, 63*(4), 1061-1067. doi: 10.1016/j.talanta.2004.01.015

Filho, A. M., dos Santos, F. N., & Pereira, P. A. D. P. (2010). Development, Validation and Application of a Method Based on DI-SPME and GC-MS for Determination of Pesticides of Different Chemical Groups in Surface and Groundwater Samples. *Microchem. J., 96*(1), 139-145. doi: 10.1016/j.microc.2010.02.018

Fontanals, N., Marcé, R. M., & Borrull, F. (2007). New Materials in Sorptive Extraction Techniques for Polar Compounds. *J. Chromatogr. A, 1152*(1-2), 14-31.

Fu, G.-N., He, Y.-Z., Yu, C.-Z., Gao, Y., & Gan, W.-E. (2009). Preconcentration and Determination of Carbamate Pesticide Residues in Vegetable Samples by Electrokinetic Flow Analysis with On-line Hollow Fiber Liquid–Liquid–Liquid Microextraction and Spectrophotometry. *Spectr. Lett., 42*(6-7), 305-311. doi: 10.1080/00387010903178699

Fu, L., Liu, X., Hu, J., Zhao, X., Wang, H., & Wang, X. (2009). Application of Dispersive Liquid-Liquid Microextraction for the Analysis of Triazophos and Carbaryl Pesticides in Water and Fruit Juice Samples. *Anal. Chim. Acta, 632*(2), 289-295.

Fujikawa, M., Nakao, K., Shimizu, R., & Akamatsu, M. (2009). The Usefulness of an Artificial Membrane Accumulation Index for Estimation of the Bioconcentration Factor of Organophosphorus Pesticides. *Chemosphere, 74*(6), 751-757.

Fytianos, K., Drimaropoulou, G., Raikos, N., Theodoridis, G., & Tsoukali, H. (2007). Headspace Solid-Phase Microextraction for the Gas Chromatographic Analysis of Organophosphorus Insecticides in Vegetables. *J. AOAC Int., 90*(6), 1677-1681.

Fytianos, K., Raikos, N., Theodoridis, G., Velinova, Z., & Tsoukali, H. (2006). Solid Phase Microextraction Applied to the Analysis of Organophosphorus Insecticides in Fruits. *Chemosphere, 65*(11), 2090-2095.

Gallagher, H. C., De Souza, I. E. J., & Regan, C. M. (2004). The Impact of Chemical Residues: The Case of Polychlorinated Biphenyl. In D. H. Watson (Ed.), *Pesticide, Veterinary and Other Residues in Food* (pp. 66-87). Cambridge, England: Woodhead Publishing.

Gao, J., Wang, Y., Gao, B., Wu, L., & Chen, H. (2012). Environmental Fate and Transport of Pesticides. In H. S. Rathore & L. M. L. Nullet (Eds.), *Pesticides: Evaluation of Environmental Pollution*. Florida, USA: CRC Press.

Garrido Frenich, A., Del Mar Martín Fernández, M., Díaz Moreno, L., Martínez Vidal, J. L., & López-Gutiérrez, N. (2012). Multiresidue Pesticide Analysis of Tuber and Root Commodities by QuECheRS Extraction and Ultra-Performance Liquid Chromatography Coupled to Tandem Mass Spectrometry. *J. AOAC Int., 95*(5), 1319-1330.

Ghambarian, M., Yamini, Y., & Esrafili, A. (2012). Developments in Hollow Fiber Based Liquid-Phase Microextraction: Principles and Applications. *Microchim. Acta, 177*(3-4), 271-294.

Giordano, A., Fernández-Franzón, M., Ruiz, M. J., Font, G., & Picó, Y. (2009). Pesticide Residue Determination in Surface Waters by Stir Bar Sorptive Extraction and Liquid Chromatography/Tandem Mass Spectrometry. *Anal. Bioanal. Chem., 393*(6-7), 1733-1743.

Goodle Ngram. (2013). Retrieved on 07/05/2013 www.books.google.com/ngrams/graph

Gou, Y. N., Tragas, C., Lord, H., & Pawliszyn, J. (2000). On-Line Coupling of In-Tube Solid Phase Microextraction (SPME) to HPLC for Analysis of Carbamates in Water Samples: Comparison of Two Commercially Available Autosamplers. *J. Microcolumn Sep., 12*(3), 125-134. doi: 10.1002/(sici)1520-667x(2000)12:3<125::aid-mcs1>3.0.co;2-9

Grube, A., Donaldson, A. L., & Kiely, T. (2011). Pesticides Industry Sales and Usage: 2006 and 2007 Market Estimates. USEPA. Retrieved 17/12/2012 from http://www.epa.gov/pesticides/pestsales/07pestsales/table_of_contents2007.htm

Guan, W., Wang, Y., Xu, F., & Guan, Y. (2008). Poly(Phthalazine Ether Sulfone Ketone) as Novel Stationary Phase for Stir Bar Sorptive Extraction of Organochlorine Compounds and Organophosphorus Pesticides. *J. Chromatogr. A, 1177*(1), 28-35.

Guillet, V., Fave, C., & Montury, M. (2009). Microwave/Spme Method to Quantify Pesticide Residues in Tomato Fruits. *J. Envrion. Sci. Heal. B, 44*(5), 415-422.

Guo, F. Q., Huang, L. F., Zhou, S. Y., Zhang, T. M., & Liang, Y. Z. (2006). Comparison of the Volatile Compounds of Atractylodes Medicinal Plants by Headspace Solid-Phase Microextraction-Gas Chromatography-Mass Spectrometry. *Anal. Chim. Acta, 570*(1), 73-78.

Guo, X., Zhang, X., Cai, Q., Shen, T., & Zhu, S. (2013). Developing a Novel Sensitive Visual Screening Card for Rapid Detection of Pesticide Residues in Food. *Food Control, 30*(1), 15-23.

Han, D., & Row, K. H. (2012). Trends in Liquid-Phase Microextraction, and Its Application to Environmental and Biological Samples. *Microchim. Acta, 176*(1-2), 1-22.

Han, D., Tang, B., Tian, M., & Row, K. H. (2013). Solid-Phase Extraction Combined with Dispersive Liquid-Liquid Microextraction for the Determination of Three Benzimidazole Pesticides (Carbendazim, Thiabendazole, and Thiophanate-Methyl) in Tomatoes. *Anal. Lett., 46*(4), 557-568. doi: 10.1080/00032719.2012.726682

He, L., Luo, X., Jiang, X., & Qu, L. (2010). A New 1,3-Dibutylimidazolium Hexafluorophosphate Ionic Liquid-Based Dispersive Liquid-Liquid Microextraction to Determine Organophosphorus Pesticides in Water and Fruit Samples by High-Performance Liquid Chromatography. *J. Chromatogr. A, 1217*(31), 5013-5020.

He, Y., & Lee, H. K. (1997). Liquid-Phase Microextraction in a Single Drop of Organic Solvent by Using a Conventional Microsyringe. *Anal. Chem., 69*(22), 4634-4640.

Hillocks, R. J. (2012). Farming with Fewer Pesticides: Eu Pesticide Review and Resulting Challenges for Uk Agriculture. *Crop Prot., 31*(1), 85-93.

Ho, T. S., Pedersen-Bjergaard, S., & Rasmussen, K. E. (2002). Recovery, Enrichment and Selectivity in Liquid-Phase Microextraction: Comparison with Conventional Liquid-Liquid Extraction. *J. Chromatogr. A, 963*(1-2), 3-17.

Holland, J., & Sinclair, P. (2004). Environmental Fate of Pesticides and Consequences for Residues in Food and Drinking Water. In D. Hamilton & S. Crossley (Eds.), *Pesticide Residues in Food and Drinking Water: Human Exposure and Risks* (pp. 28-62). West Sussex, England: John Wiley.

Hristovski, K. D., Westerhoff, P. K., & Posner, J. D. (2011). Octanol-Water Distribution of Engineered Nanomaterials. *J. Environ. Sci. Health., Part A, 46*(6), 636-647. doi: 10.1080/10934529.2011.562859

Hu, C., He, M., Chen, B., & Hu, B. (2013). A Sol-Gel Polydimethylsiloxane/Polythiophene Coated Stir Bar Sorptive Extraction Combined with Gas Chromatography-Flame Photometric Detection for the Determination of Organophosphorus Pesticides in Environmental Water Samples. *J. Chromatogr. A, 1275*, 25-31.

Hu, R., Hennion, B., Urruty, L., & Montury, M. (1999). Solid Phase Microextraction of Pesticide Residues from Strawberries. *Food Addit. Contam., 16*(3), 111-117.

Hu, X., Hu, Y., & Li, G. (2007). Development of Novel Molecularly Imprinted Solid-Phase Microextraction Fiber and Its Application for the Determination of Triazines in Complicated Samples Coupled with High-Performance Liquid Chromatography. *J. Chromatogr. A, 1147*(1), 1-9.

Hu, Y., Li, J., Hu, Y., & Li, G. (2010). Development of Selective and Chemically Stable Coating for Stir Bar Sorptive Extraction by Molecularly Imprinted Technique. *Talanta, 82*(2), 464-470.

Hu, Y., Wang, Y., Hu, Y., & Li, G. (2009). Liquid-Liquid-Solid Microextraction Based on Membrane-Protected Molecularly Imprinted Polymer Fiber for Trace Analysis of Triazines in Complex Aqueous Samples. *J. Chromatogr. A, 1216*(47), 8304-8311.

Hu, Y., Yang, X., Wang, C., Zhao, J., Li, W., & Wang, Z. (2008). A Sensitive Determination Method for Carbendazim and Thiabendazole in Apples by Solid-Phase Microextraction - High Performance Liquid Chromatography with Fluorescence Detection. *Food Addit. Contam. A, 25*(3), 314-319.

Huo, X., Li, Q., Lin, X., Chen, X., & Bi, K. (2011). Application of Dispersive Liquid-Liquid Microextraction for the Analysis of Six Fungicides in Fruit Samples by Gc-Ecd. *Chromatographia, 73*(3-4), 313-319.

Husbschmann, H.-J. (2009). *Handbook of Gc/Ms: Fundamentals and Applications*. Weinheim: Wiley-VCH.

Hylton, K., & Mitra, S. (2007). Barrier Film Protected, and Mixed Solvent Optimized Micro-Scale Membrane Extraction of Methyl Carbamate Pesticides. *J. Chromatogr. A, 1154*(1-2), 60-65. doi: 10.1016/j.chroma.2007.03.131

Hyötyläinen, T., & Riekkola, M. L. (2008). Sorbent- and Liquid-Phase Microextraction Techniques and Membrane-Assisted Extraction in Combination with Gas Chromatographic Analysis: A Review. *Anal. Chim. Acta, 614*(1), 27-37.

ICH-Topic Q2(R1). (2006). *Validation of Analytical Procedures: Text and Methodology*. U. S Department of Health and Human Services.

IUPAC. (2012). A Vision for Chemistry for 2050, *Chemistry International*.

Jain, A., & Verma, K. K. (2011). Recent Advances in Applications of Single-Drop Microextraction: A Review. *Anal. Chim. Acta, 706*(1), 37-65.

Jeannot, M. A., & Cantwell, F. F. (1996). Solvent Microextraction into a Single Drop. *Anal. Chem., 68*(13), 2236-2240.

Jeannot, M. A., & Cantwell, F. F. (1997). Mass Transfer Characteristics of Solvent Extraction into a Single Drop at the Tip of a Syringe Needle. *Anal. Chem., 69*(2), 235-239.

Jeannot, M. A., Przyjazny, A., & Kokosa, J. M. (2010). Single Drop Microextraction-Development, Applications and Future Trends. *J. Chromatogr. A, 1217*(16), 2326-2336. doi: 10.1016/j.chroma.2009.10.089

Jenkins, J. J., & Thomson, P. A. (1999). Osu Extension Pesticide Properties Database. from Oregon State University Extension service

Jiang, R., Carasek, E., Risticevic, S., Cudjoe, E., Warren, J., & Pawliszyn, J. (2012). Evaluation of a Completely Automated Cold Fiber Device Using Compounds with Varying Volatility and Polarity. *Anal. Chim. Acta, 742*, 22-29.

Jin, B., Xie, L., Guo, Y., & Pang, G. (2012). Multi-Residue Detection of Pesticides in Juice and Fruit Wine: A Review of Extraction and Detection Methods. *Food Res. Int., 46*(1), 399-409. doi: http://dx.doi.org/10.1016/j.foodres.2011.12.003

Juan-García, A., Mañes, J., Font, G., & Picó, Y. (2004). Evaluation of Solid-Phase Extraction and Stir-Bar Sorptive Extraction for the Determination of Fungicide Residues at Low-$mgkg^{-1}$ Levels in Grapes by Liquid Chromatography-Mass Spectrometry. *J. Chromatogr. A, 1050*(2), 119-127.

Juan-García, A., Picó, Y., & Font, G. (2005). Capillary Electrophoresis for Analyzing Pesticides in Fruits and Vegetables Using Solid-Phase Extraction and Stir-Bar Sorptive Extraction. *J. Chromatogr. A, 1073*(1-2), 229-236.

Kamrin, M. A. (2000). *Pesticide Profiles: Toxicology, Environmental Impact, and Fate*. New York, USA: CRC Press.

Kaneko, H. (2010). Pyrethroid Chemistry and Metabolism. In R. Krieger (Ed.), *Haye's Handbook of Pesticide Toxicology* (3rd ed., pp. 1635-1663). San Diego: Elsevier.

Kataoka, H. (2010). Recent Developments and Applications of Microextraction Techniques in Drug Analysis. *Anal. Bioanal. Chem., 396*(1), 339-364.

Kataoka, H., Lord, H. L., & Pawliszyn, J. (2000). Applications of Solid-Phase Microextraction in Food Analysis. *J. Chromatogr. A, 880*(1-2), 35-62.

Kawaguchi, M., Ito, R., Saito, K., & Nakazawa, H. (2006). Novel Stir Bar Sorptive Extraction Methods for Environmental and Biomedical Analysis. *J. Pharm. Biomed. Anal., 40*(3), 500-508.

Kende, A., Csizmazia, Z., Rikker, T., Angyal, V., & Torkos, K. (2006). Combination of Stir Bar Sorptive Extraction-Retention Time Locked Gas Chromatography-Mass Spectrometry and Automated Mass Spectral Deconvolution for Pesticide Identification in Fruits and Vegetables. *Microchem. J., 84*(1-2), 63-69.

Kerle, E. A., Jenkins, J. J., & Vogue, P. A. (2007). *Understanding Pesticide Persistence and Mobility for Grounwater and Surface Water Protection*. Oregon, USA: Oregon State University Extention Service.

Kessler, V. G. (2005). The Synthesis and Solution Stability of Alkoxide Precusors. In S. Sakka (Ed.), *Handbook of Sol-Gel Technology: Processing, Characterization and Applications* (pp. 3-40). New York, USA: Springer.

Khodadoust, S., & Hadjmohammadi, M. (2011). Determination of N-Methylcarbamate Insecticides in Water Samples Using Dispersive Liquid-Liquid Microextraction and Hplc with the Aid of Experimental Design and Desirability Function. *Anal. Chim. Acta, 699*(1), 113-119.

Kiely, T., Donaldson, A. L., & Grube, A. (2004). Pesticides Industry Sales and Usage: 2001 and 2002 Market Estimates. USEPA. Retrieved 17/12/2012 from http://www.epa.gov/pesticides/pestsales/01pestsales/table_of_contents2001.htm

Kin, C. M., & Huat, T. G. (2009). Comparison of Hs-Sdme with Spme and Spe for the Determination of Eight Organochlorine and Organophosphorus Pesticide Residues in Food Matrices. *Journal of Chromatographic Science, 47*(8), 694-699.

Kleeberg, K. K., Dobberstein, D., Hinrichsen, N., Müller, A., P. Weber, & Steinhart, H. (2008). Sampling Procedures with Special Focus on Automatization. In F. Todra & L. M. L. Nollet (Eds.), *Advances in Food Diagnostics* (pp. 253-293). Hoboken, NJ, USA: Wiley-Blackwell.

Kloskowski, A., Chrzanowski, W., Pilarczyk, M., & Namiesnik, J. (2007). Modern Techniques of Sample Preparation for Determination of Organic Analytes by Gas Chromatography. *Crit. Rev. Anal. Chem., 37*(1), 15-38.

Knaak, J. B., Dary, C. C., Okino, M. S., Power, F. W., Zhang, X., Thompson, C. B., . . . Blancato, J. N. (2008). Parameters for Carbamate Pesticide QSAR and PBPK/PD Models for Human Risk Assessment: Reviews of Environmental Contamination and Toxicology. In D. M. Whitacre (Ed.), (Vol. 193, pp. 53-212): Springer New York.

Kocúrová, L., Balogh, I. S., Šandrejová, J., & Andruch, V. (2012). Recent Advances in Dispersive Liquid-Liquid Microextraction Using Organic Solvents Lighter Than Water. A Review. *Microchem. J., 102*, 11-17.

Kokosa, J. M. (2013). Advances in Solvent-Microextraction Techniques. *TrAC - Trends Anal. Chem., 43*, 2-13.

Kokosa, J. M., Przyjazny, A., & Jeannot, M. A. (2009). *Solvent Microextraction: Theory and Practice*. Hoboken, NJ: John Wiley.

Krutz, L. J., Senseman, S. A., & Sciumbato, A. S. (2003). Solid-Phase Microextraction for Herbicide Determination in Environmental Samples. *J. Chromatogr. A, 999*(1-2), 103-121.

Krylov, V. A., Krylov, A. V., Mosyagin, P. V., & Matkivskaya, Y. O. (2011). Liquid-Phase Microextraction Preconcentration of Impurities. *J. Anal. Chem., 66*(4), 331-350. doi: 10.1134/S1061934811040101

Kudlejova, L., Risticevic, S., & Vuckovic, D. (2012). Solid-Phase Microextraction Method Development. In J. Pawliszyn (Ed.), *Handbook of Solid Phase Microextration* (pp. 200-249). Waltham, USA: Elsevier.

Kumar, A., Gaurav, Malik, A. K., Tewary, D. K., & Singh, B. (2008). A Review on Development of Solid Phase Microextraction Fibers by Sol-Gel Methods and Their Applications. *Anal. Chim. Acta, 610*(1), 1-14.

Lambert, J. P., Mullett, W. M., Kwong, E., & Lubda, D. (2005). Stir Bar Sorptive Extraction Based on Restricted Access Material for the Direct Extraction of Caffeine and Metabolites in Biological Fluids. *J. Chromatogr. A, 1075*(1-2), 43-49.

Lambropoulou, D. A., & Albanis, T. A. (2002). Headspace Solid Phase Microextraction Applied to the Analysis of Organophosphorus Insecticides in Strawberry and Cherry Juices. *J. Agric. Food Chem., 50*(12), 3359-3365.

Lambropoulou, D. A., & Albanis, T. A. (2003). Headspace Solid-Phase Microextraction in Combination with Gas Chromatography-Mass Spectrometry for the Rapid Screening of Organophosphorus Insecticide Residues in Strawberries and Cherries. *J. Chromatogr. A, 993*(1-2), 197-203.

Lambropoulou, D. A., & Albanis, T. A. (2007a). Liquid-Phase Micro-Extraction Techniques in Pesticide Residue Analysis. *J. Biochem. Biophys. Methods, 70*(2), 195-228. doi: 10.1016/j.jbbm.2006.10.004

Lambropoulou, D. A., & Albanis, T. A. (2007b). Methods of Sample Preparation for Determination of Pesticide Residues in Food Matrices by Chromatography-Mass Spectrometry-Based Techniques: A Review. *Anal. Bioanal. Chem., 389*(6), 1663-1683.

Lambropoulou, D. A., Konstantinou, I. K., & Albanis, T. A. (2007). Recent Developments in Headspace Microextraction Techniques for the Analysis of Environmental Contaminants in Different Matrices. *J. Chromatogr. A, 1152*(1-2), 70-96.

Lavagnini, I., Urbani, A., & Magno, F. (2011). Overall Calibration Procedure Via a Statistically Based Matrix-Comprehensive Approach in the Stir Bar Sorptive Extraction-Thermal Desorption-Gas Chromatography-Mass Spectrometry Analysis of Pesticide Residues in Fruit-Based Soft Drinks. *Talanta, 83*(5), 1754-1762.

Lee, C. Y., & Smith, N. L. (2000). Apples: An Important Source of Antioxidants in the American Diet. *New York Fruits Quaterly, 8*(2), 8-10.

Lee, J., Lee, H. K., Rasmussen, K. E., & Pedersen-Bjergaard, S. (2008). Environmental and Bioanalytical Applications of Hollow Fiber Membrane Liquid-Phase Microextraction: A Review. *Anal. Chim. Acta, 624*(2), 253-268.

León, V. M., Álvarez, B., Cobollo, M. A., Muñoz, S., & Valor, I. (2003). Analysis of 35 Priority Semivolatile Compounds in Water by Stir Bar Sorptive Extraction-Thermal Desorption-Gas Chromatography-Mass Spectrometry: I. Method Optimisation. *J. Chromatogr. A, 999*(1-2), 91-101.

Lewis, N. N., & Ruud, J. J. (2004). Apples in the American Diet. *Nutr. Clin. Care, 7*(2), 82-88.

Li, S., & Weber, S. G. (1999). Selectivity in Spme. In J. Pawliszyn (Ed.), *Application of Solid Phase Microextraction* (pp. 49-56). Cambridge, UK: RSC.

Linde, C. D. (1994). *Physico-Chemical Properties and Environmental Fate of Pesticides*. California, USA: State of California EPA.

Liu, H., & Dasgupta, P. K. (1996a). Analytical Chemistry in a Drop. *TrAC - Trends Anal. Chem., 15*(9), 468-475.

Liu, H., & Dasgupta, P. K. (1996b). Analytical Chemistry in a Drop. Solvent Extraction in a Microdrop. *Anal. Chem., 68*(11), 1817-1821.

Liu, H., & Dasgupta, P. K. (1996c). A Liquid Drop: A Windowless Optical Cell and a Reactor without Walls for Flow Injection Analysis. *Anal. Chim. Acta, 326*(1-3), 13-22.

Liu, W., Hu, Y., Zhao, J., Xu, Y., & Guan, Y. (2005). Determination of Organophosphorus Pesticides in Cucumber and Potato by Stir Bar Sorptive Extraction. *J. Chromatogr. A, 1095*(1-2), 1-7.

Liu, W., Wang, H., & Guan, Y. (2004). Preparation of Stir Bars for Sorptive Extraction Using Sol-Gel Technology. *J. Chromatogr. A, 1045*(1-2), 15-22.

Liu, X. S., Tong, Z. F., Zhen, L., Huang, D. X., Gao, X., & Xu, C. Y. (2009). Simultaneous Analysis of Thiabendazole, Carbendazim and 2-Aminobenzimidazole in Concentrated Fruit Juices by Liquid Chromatography after a Single Mix-Mode Solid-Phase Extraction Cleanup. *J. Environ. Sci. Heal. B, 44*(6), 591-597.

Liu, Z., Liu, W., Rao, H., Feng, T., Li, C., Wang, C., & Wang, Z. (2012a). Determination of Some Carbamate Pesticides in Watermelon and Tomato Samples by Dispersive Liquid–Liquid Microextraction Combined with High Performance Liquid

Chromatography. *Int. J. Environ. Anal. Chem., 92*(5), 571-581. doi: 10.1080/03067311003628638

Liu, Z., Liu, W., Wu, Q., Zang, X., Zhou, X., Zeng, X., & Wang, Z. (2012b). Determination of Carbendazim and Thiabendazole in Apple Juice by Hollow Fibre-Based Liquid Phase Microextraction-High Performance Liquid Chromatography with Fluorescence Detection. *Int. J Environ. Anal. Chem., 92*(5), 582-591.

López, R., Goñi, F., Etxandia, A., & Millán, E. (2007). Determination of Organochlorine Pesticides and Polychlorinated Biphenyls in Human Serum Using Headspace Solid-Phase Microextraction and Gas Chromatography-Electron Capture Detection. *J. Chromatogr. B, 846*(1-2), 298-305.

Lord, H. (2007). Strategies for Interfacing Solid-Phase Microextraction with Liquid Chromatography. *J. Chromatogr. A, 1152*(1-2), 2-13.

Lord, H., & Bojko, B. (2012). Drug Analysis by SPME. In J. Pawliszyn (Ed.), *Handbook of Solid Phase Microextraction* (pp. 335-382). Waltham, USA: Elsevier.

Lord, H., & Pawliszyn, J. (2000). Evolution of Solid-Phase Microextraction Technology. *J. Chromatogr. A, 885*(1-2), 153-193.

Lotti, M. (2010). Clinical Toxicology of Anticholinesterase Agents in Human. In R. Krieger (Ed.), *Haye's Handbook of Pesticides Toxicology* (3 ed., pp. 1543-1589). San Diego. USA: Elsevier.

Lüthje, K., Hyötyläinen, T., Rautiainen-Rämä, M., & Riekkola, M. L. (2005). Pressurised Hot Water Extraction-Microporous Membrane Liquid-Liquid Extraction Coupled On-line with Gas Chromatography-Mass Spectrometry in the Analysis of Pesticides in Grapes. *Analyst, 130*(1), 52-58.

Lydy, M., Belden, J., Wheelock, C., Hammock, B., & Denton, D. (2004). Challenges in Regulating Pesticide Mixtures. *Ecol. Soc., 9*(6).

Magga, Z., Tzovolou, D. N., Theodoropoulou, M. A., & Tsakiroglou, C. D. (2012). Combining Experimental Techniques with Non-Linear Numerical Models to Assess the Sorption of Pesticides on Soils. *J. Contam. Hydro., 129-130*, 62-69.

Maharaj, R. (2010). Use of Insecticides for Malaria Control and Needs for Reversion of Resistance. *Indian J. Med. Res., 132*, 248-250.

Mahmoud, M. F., & Loutfy, N. (2012). Biocides. In H. S. Rathore & L. M. L. Nollet (Eds.), *Pesticides: Evaluation of Environmental Pollution* (pp. 3-28). Florida, USA: CRC Press.

Mani, V. (1999). Properties of Commercial SPME Coating. In J. Pawliszyn (Ed.), *Applications of Solid Phase Microextraction* (pp. 57-72). Cambridge, UK: Royal Society of Chemistry.

Martín-Esteban, A. (2013). Molecularly-Imprinted Polymers as a Versatile, Highly Selective Tool in Sample Preparation. *TrAC - Trends Anal. Chem.*

Massart, D. L., Vandaginste, B. G. M., Buydens, L. M. C., De Jong, S., Lewi, P. J., & Smeyers-Verbeke, J. (1997). *Handbook of Chemometrics and Qualimetrics: Part A*. Amsrterderm, The Netherlands: Elsevier.

Matsadiq, G., Hu, H. L., Ren, H. B., Zhou, Y. W., Liu, L., & Cheng, J. (2011). Quantification of Multi-Residue Levels in Peach Juices, Pulps and Peels Using Dispersive Liquid-Liquid Microextraction Based on Floating Organic Droplet Coupled with Gas Chromatography-Electron Capture Detection. *J. Chromatogr. B, 879*(22), 2113-2118.

Matsumura, F. (2010). Studies on the Action Mechanism of Benzoylurea Insecticides to Inhibit the Process of Chitin Synthesis in Insects: A Review on the Status of Research Activities in the Past, the Present and the Future Prospects. *Pestic. Biochem. Physiol., 97*(2), 133-139. doi: http://dx.doi.org/10.1016/j.pestbp.2009.10.001

Matthews, G., Zaim, M., Yadav, R. S., Soares, A., Hii, J., Ameneshewa, B., . . . van Den Berg, H. (2011). Status of Legislation and Regulatory Control of Public Health Pesticides in Countries Endemic with or at Risk of Major Vector-Borne Diseases. *Environ. Health Perspect., 119*(11), 1517-1522.

McCarty, L. S., & Borgert, C. J. (2006). Review of the Toxicity of Chemical Mixtures Containing at Least One Organochlorine. *Reg. Toxicol. Pharm., 45*(2), 104-118.

McIntyre, D., Thiede, M., Dahlgren, G., & Whitehead, M. (2006). What Are the Economic Consequences for Households of Illness and of Paying for Health Care in Low- and Middle-Income Country Contexts? *Soc. Sci. Med., 62*(4), 858-865. doi: http://dx.doi.org/10.1016/j.socscimed.2005.07.001

McNair, H. M., & Miller, J. M. (2009). *Basic Gas Chromatography* (2nd ed.). New Jersey, USA: Wiley.

McNeely Jeffrey, A., & Scherr Sara, J. (2003). *Ecoagriculture: Strategies to Feed the World and Save Wild Bioderversity*. Washington , D. C. USA: Island Press.

Melo, A., Aguiar, A., Mansilha, C., Pinho, O., & Ferreira, I. M. P. L. V. O. (2012a). Optimisation of a Solid-Phase Microextraction/Hplc/Diode Array Method for Multiple Pesticide Screening in Lettuce. *Food Chem., 130*(4), 1090-1097. doi: 10.1016/j.foodchem.2011.07.137

Melo, A., Cunha, S. C., Mansilha, C., Aguiar, A., Pinho, O., & Ferreira, I. M. P. L. V. O. (2012b). Monitoring Pesticide Residues in Greenhouse Tomato by Combining Acetonitrile-Based Extraction with Dispersive Liquid-Liquid Microextraction Followed by Gas-Chromatography-Mass Spectrometry. *Food Chem., 135*(3), 1071-1077.

Melo, A., Mansilha, C., Pinho, O., & M.P.L.V.O. Ferreira, I. (2012c). Analysis of Pesticides in Tomato Combining QuECheRS and Dispersive Liquid-Liquid Microextraction Followed by High-Performance Liquid Chromatography. *Food Anal. Method*, 1-10.

Menezes Filho, A., dos Santos, F. N., & de Paula Pereira, P. A. (2010). Development, Validation and Application of a Methodology Based on Solid-Phase Micro Extraction Followed by Gas Chromatography Coupled to Mass Spectrometry (Spme/Gc-Ms) for the Determination of Pesticide Residues in Mangoes. *Talanta, 81*(1-2), 346-354.

Miller, J. N., & Miller, J. C. (2010). *Statistics and Chemometrics for Analytical Chemisry* (6th ed.). Essex, England: Pearson.

Montes, R., Rodríguez, I., Ramil, M., Rubí, E., & Cela, R. (2009). Solid-Phase Extraction Followed by Dispersive Liquid-Liquid Microextraction for the Sensitive Determination of Selected Fungicides in Wine. *J. Chromatogr. A, 1216*(29), 5459-5466.

Moreno-González, D., Gámiz-Gracia, L., García-Campaña, A. M., & Bosque-Sendra, J. M. (2011). Use of Dispersive Liquid-Liquid Microextraction for the Determination of Carbamates in Juice Samples by Sweeping-Micellar Electrokinetic Chromatography. *Anal. Bioanal. Chem., 400*(5), 1329-1338.

Myers, R. H., Montgomery, D. C., & Anderson-Cook, C. M. (2009). *Response Surface Methodology: Process and Product Optimization Using Designed Experiments* (3rd ed.). USA: Wiley.

Natangelo, M., Tavazzi, S., & Benfenati, E. (2002). Evaluation of Solid Phase Microextraction-Gas Chromatography in the Analysis of Some Pesticides with Different Mass Spectrometric Techniques: Application to Environmental Waters and Food Samples. *Anal. Lett., 35*(2), 327-338.

Nauen, R. (2006). Insecticide Mode of Action: Return of the Ryanodine Receptor. *Pest Manag. Sci., 62*(8), 690-692.

Navalón, A., Prieto, A., Araujo, L., & Vílchez, J. L. (2002). Determination of Pyrimethanil and Kresoxim-Methyl in Green Groceries by Headspace Solid-Phase Microextraction and Gas Chromatography-Mass Spectrometry. *J. Chromatogr. A, 975*(2), 355-360.

Nerín, C., Salafranca, J., Aznar, M., & Batlle, R. (2009). Critical Review on Recent Developments in Solventless Techniques for Extraction of Analytes. *Anal. Bioanal. Chem., 393*(3), 809-833.

Ni, Y., Qiu, P., & Kokot, S. (2005). Simultaneous Voltammetric Determination of Four Carbamate Pesticides with the Use of Chemometrics. *Anal. Chim. Acta, 537*(1-2), 321-330.

Nogueira, J. M. F. (2012). Novel Sorption-Based Methodologies for Static Microextraction Analysis: A Review on Sbse and Related Techniques. *Anal. Chim. Acta*. doi: 10.1016/j.aca.2012.10.033

Novak, R. J., & Lampman, R. L. (2010). Public Health Pesticides. In R. Krieger (Ed.), *Haye's Handbook of Pesticide Toxicology* (3rd ed., pp. 231-256). San Diego, USA: Elsevier.

Ochiai, N., Sasamoto, K., Kanda, H., & Nakamura, S. (2006). Fast Screening of Pesticide Multiresidues in Aqueous Samples by Dual Stir Bar Sorptive Extraction-Thermal Desorption-Low Thermal Mass Gas Chromatography-Mass Spectrometry. *J. Chromatogr. A, 1130*(1 SPEC. ISS.), 83-90.

Ochiai, N., Sasamoto, K., Kanda, H., Yamagami, T., David, F., Tienpont, B., & Sandra, P. (2005). Optimization of a Multi-Residue Screening Method for the Determination of 85 Pesticides in Selected Food Matrices by Stir Bar Sorptive Extraction and Thermal Desorption Gc-Ms. *J. Sep. Sci., 28*(9-10), 1083-1092.

Omeroglu, P. Y., Boyacioglu, D., Ambrus, Á., Karaali, A., & Saner, S. (2012). An Overview on Steps of Pesticide Residue Analysis and Contribution of the Individual Steps to the Measurement Uncertainty. *Food Anal. Method, 5*(6), 1469-1480.

Ouyang, G. (2012). Calibration. In J. Pawliszyn (Ed.), *Handbook of Solid Phase Microextraction* (pp. 167-199). Waltham, UK: Elsevier.

Ouyang, G., & Pawliszyn, J. (2006a). Kinetic Calibration for Automated Hollow Fiber-Protected Liquid-Phase Microextraction. *Anal. Chem., 78*(16), 5783-5788.

Ouyang, G., & Pawliszyn, J. (2006b). Spme in Environmental Analysis. *Anal. Bioanal. Chem., 386*(4), 1059-1073.

Ouyang, G., & Pawliszyn, J. (2008). A Critical Review in Calibration Methods for Solid-Phase Microextraction. *Anal. Chim. Acta, 627*(2), 184-197.

Ouyang, G., Zhao, W., & Pawliszyn, J. (2005). Kinetic Calibration for Automated Headspace Liquid-Phase Microextraction. *Anal. Chem., 77*(24), 8122-8128.

Pakade, Y. B., & Tewary, D. K. (2010). Development and Applications of Single-Drop Microextraction for Pesticide Residue Analysis: A Review. *J. Sep. Sci, 33*(23-24), 3683-3691.

Paraíba, L. C. (2007). Pesticide Bioconcentration Modelling for Fruit Trees. *Chemosphere, 66*(8), 1468-1475.

Paraíba, L. C., & Kataguiri, K. (2008). Model Approach for Estimating Potato Pesticide Bioconcentration Factor. *Chemosphere, 73*(8), 1247-1252. doi: http://dx.doi.org/10.1016/j.chemosphere.2008.07.026

Pawliszyn, J. (1995). New Directions in Sample Preparation for Analysis of Organic Compounds. *TrAC - Trends Anal. Chem., 14*(3), 113-122.

Pawliszyn, J. (1997). *Solid Phase Microextraction: Theory and Practice*. New York, USA: VCH.

Pawliszyn, J. (1999). Quantitative Aspect of Solid Phase Microextraction. In J. Pawliszyn (Ed.), *Applications of Solid Phase Microextraction* (pp. 3-21). Cambridge: Royal Society of Chemistry.

Pawliszyn, J. (2012a). *Comprehensive Sampling and Sample Preparation: Analytical Techniques for Scientists*: Academic Press.

Pawliszyn, J. (2012b). Development of Spme Devices and Coatings. In J. Pawliszyn (Ed.), *Handbook of Solid Phase Microextraction* (pp. 60-97). Waltham, USA: Elsevier.

Pawliszyn, J. (2012c). Theory of Solid-Phase Microextraction. In J. Pawliszyn (Ed.), *Handbook of Solid Phase Microextraction* (pp. 13-59). Waltham, USA: Elsevier.

Pawliszyn, J., Pawliszyn, B., & Pawliszyn, M. (1997). Solid Phase Microextraction (Spme). *The Chem. Educ., 2*, 1-7.

Pawliszyn, J., Vuckovic, D., Mimaghi, F., & Risticevic, S. (2012). Automated Spme Systems. In J. Pawliszyn (Ed.), *Handbook of Solid Phase Microextraction* (pp. 135-165). Waltham, USA: Elsevier.

Pedersen-Bjergaard, S., & Rasmussen, K. E. (1999). Liquid-Liquid-Liquid Microextraction for Sample Preparation of Biological Fluids Prior to Capillary Electrophoresis. *Anal. Chem., 71*(14), 2650-2656.

Pedersen-Bjergaard, S., & Rasmussen, K. E. (2008). Liquid-Phase Microextraction with Porous Hollow Fibers, a Miniaturized and Highly Flexible Format for Liquid-Liquid Extraction. *J. Chromatogr. A, 1184*(1-2), 132-142.

Pedersen-Bjergaard, S., Rasmussen, K. E., & Grønhaug Halvorsen, T. (2000). Liquid-Liquid Extraction Procedures for Sample Enrichment in Capillary Zone Electrophoresis. *J. Chromatogr. A, 902*(1), 91-105.

Pezo, D., Salafranca, J., & Nerín, C. (2007). Development of an Automatic Multiple Dynamic Hollow Fibre Liquid-Phase Microextraction Procedure for Specific Migration Analysis of New Active Food Packagings Containing Essential Oils. *J. Chromatogr. A, 1174*(1-2), 85-94.

Picó, Y., Fernández, M., Ruiz, M. J., & Font, G. (2007). Current Trends in Solid-Phase-Based Extraction Techniques for the Determination of Pesticides in Food and Environment. *J. Biochem. Biophys. Methods, 70*(2), 117-131. doi: 10.1016/j.jbbm.2006.10.010

Popp, P., Keil, P., Montero, L., & Rückert, M. (2005). Optimized Method for the Determination of 25 Polychlorinated Biphenyls in Water Samples Using Stir Bar Sorptive Extraction Followed by Thermodesorption-Gas Chromatography/Mass Spectrometry. *J. Chromatogr. A, 1071*(1-2), 155-162.

Portugal, F. C. M., Pinto, M. L., & Nogueira, J. M. F. (2008). Optimization of Polyurethane Foams for Enhanced Stir Bar Sorptive Extraction of Triazinic Herbicides in Water Matrices. *Talanta, 77*(2), 765-773.

Portugal, F. C. M., Pinto, M. L., Pires, J., & Nogueira, J. M. F. (2010). Potentialities of Polyurethane Foams for Trace Level Analysis of Triazinic Metabolites in Water Matrices by Stir Bar Sorptive Extraction. *J. Chromatogr. A, 1217*(23), 3707-3710.

PPDB. (2009). The Pesticide Properties Database (PPDB). Available from Agriculture & Environment Research Unit (AERU), University of Hertfordshire, funded by UK National Sources and the EU-funded FOOTPRINT Project (FP6-SSP-022704). Retrieved 09/12/12 from http://sitem.herts.ac.uk/aeru/footprint/en/index.htm

Prieto, A., Basauri, O., Rodil, R., Usobiaga, A., Fernández, L. A., Etxebarria, N., & Zuloaga, O. (2010). Stir-Bar Sorptive Extraction: A View on Method Optimisation, Novel Applications, Limitations and Potential Solutions. *J. Chromatogr. A, 1217*(16), 2642-2666. doi: 10.1016/j.chroma.2009.12.051

Prieto, A., Zuloaga, O., Usobiaga, A., Etxebarria, N., & Fernández, L. A. (2008). Use of Experimental Design in the Optimisation of Stir Bar Sorptive Extraction Followed by Thermal Desorption for the Determination of Brominated Flame Retardants in Water Samples. *Anal. Bioanal. Chem., 390*(2), 739-748.

Prosen, H., & Zupančič-Kralj, L. (1999). Solid-Phase Microextraction. *TrAC - Trends Anal. Chem., 18*(4), 272-282.

Psillakis, E., & Kalogerakis, N. (2002). Developments in Single-Drop Microextraction. *TrAC - Trends Anal. Chem., 21*(1), 53-63.

Qiao, F., Zhang, X., Wang, M., & Kang, Y. (2010). Rapid Extraction of Imidacloprid in Tomatoes by Ultrasonic Dispersion Liquid–Liquid Microextraction Coupled with LC Determination. *Chromatographia, 72*(3), 331-335. doi: 10.1365/s10337-010-1661-3

Quintana, J. B., Rodil, R., Muniategui-Lorenzo, S., López-Mahía, P., & Prada-Rodríguez, D. (2007). Multiresidue Analysis of Acidic and Polar Organic Contaminants in Water Samples by Stir-Bar Sorptive Extraction-Liquid Desorption-Gas Chromatography-Mass Spectrometry. *J. Chromatogr. A, 1174*(1-2), 27-39.

Rao, P. S., & Alley, W. M. (1993). Pesticides. In W. M. Alley (Ed.), *Regional Ground Water Quality*. New York, USA: Van Nostrand Reinhold.

Ravelo-Pérez, L. M., Hernandez-Borges, J., Asensio-Ramos, M., & Rodríguez-Delgado, M. A. (2009a). Ionic Liquid Based Dispersive Liquid-Liquid Microextraction for the Extraction of Pesticides from Bananas. *J. Chromatogr. A, 1216*(43), 7336-7345.

Ravelo-Pérez, L. M., Hernández-Borges, J., Borges-Miquel, T. M., & Rodríguez-Delgado, M. A. (2008). Pesticide Analysis in Tomatoes by Solid-Phase Microextraction and Micellar Electrokinetic Chromatography. *J. Chromatogr. A, 1185*(1), 151-154.

Ravelo-Pérez, L. M., Hernandez-Borges, J., Herrera-Herrera, A. V., & Rodríguez-Delgado, M. A. (2009b). Pesticide Extraction from Table Grapes and Plums Using Ionic Liquid Based Dispersive Liquid-Liquid Microextraction. *Anal. Bioanal. Chem., 395*(7), 2387-2395.

Ray, E. D. (2004). Toxicology of Pyrethrins and Synthetic Pyrethroids. In T. C. Marrs & B. Ballantyne (Eds.), *Pesticide Toxicology and International Regulation* (pp. 128-158). West Sussex, England: John Wiley.

Renwick, A. G. (2002). Pesticide Residue Analysis and Its Relationship to Hazard Characterisation (ADI/ARFD) and Intake Estimations (NEDI/NESTI). *Pest Manage. Sci., 58*(10), 1073-1082.

Rezaee, M., Assadi, Y., Milani Hosseini, M. R., Aghaee, E., Ahmadi, F., & Berijani, S. (2006). Determination of Organic Compounds in Water Using Dispersive Liquid-Liquid Microextraction. *J. Chromatogr. A, 1116*(1-2), 1-9.

Rezaee, M., Yamini, Y., & Faraji, M. (2010). Evolution of Dispersive Liquid-Liquid Microextraction Method. *J. Chromatogr. A, 1217*(16), 2342-2357.

Rial Otero, R., Cancho Grande, B., & Simal Gándara, J. (2003). Multiresidue Method for Fourteen Fungicides in White Grapes by Liquid-Liquid and Solid-Phase Extraction Followed by Liquid Chromatography-Diode Array Detection. *J. Chromatogr. A, 992*(1-2), 121-131.

Rial Otero, R., Yagüe Ruiz, C., Cancho Grande, B., & Simal Gándara, J. (2002). Solid-Phase Microextraction-Gas Chromatographic-Mass Spectrometric Method for the Determination of the Fungicides Cyprodinil and Fludioxonil in White Wines. *J. Chromatogr. A, 942*(1-2), 41-52.

Rice, P. J., Horgan, B. P., & Rittenhouse, J. L. (2010). Pesticide Transport with Runoff from Creeping Bentgrass Turf: Relationship of Pesticide Properties to Mass Transport. *Environ. Toxicol. Chem., 29*(6), 1209-1214. doi: 10.1002/etc.180

Ridgway, K., Lalljie, S. P. D., & Smith, R. M. (2007). Sample Preparation Techniques for the Determination of Trace Residues and Contaminants in Foods. *J. Chromatogr. A, 1153*(1-2), 36-53.

Risticevic, S., Chen, Y., Kudlejova, L., Vatinno, R., Baltensperger, B., Stuff, J. R., . . . Pawliszyn, J. (2010a). Protocol for the Development of Automated High-

Throughput Spme-Gc Methods for the Analysis of Volatile and Semivolatile Constituents in Wine Samples. *Nat. Protoc.*, *5*(1), 162-176.

Risticevic, S., Lord, H., Górecki, T., Arthur, C. L., & Pawliszyn, J. (2010b). Protocol for Solid-Phase Microextraction Method Development. *Nat. Protoc.*, *5*(1), 122-139.

Risticevic, S., Niri, V. H., Vuckovic, D., & Pawliszyn, J. (2009). Recent Developments in Solid-Phase Microextraction. *Anal. Bioanal. Chem.*, *393*(3), 781-795.

Risticevic, S., Vuckovic, D., & Pawliszyn, J. (2010c). Solid Phase Microextraction. In J. Pawliszyn & H. L. Lord (Eds.), *Handbook of Sample Preparation* (pp. 81-102). New Jersey, USA: Wiley-VCH.

Rodriguez, R., Mañes, J., & Picó, Y. (2003). Off-Line Solid-Phase Microextraction and Capillary Electrophoresis Mass Spectrometry to Determine Acidic Pesticides in Fruits. *Anal. Chem.*, *75*(3), 452-459.

Romero-González, R., Pastor-Montoro, E., Martinez-Vidal, J. L., & Garrido-Frenich, A. (2006). Application of Hollow Fiber Supported Liquid Membrane Extraction to the Simultaneous Determination of Pesticide Residues in Vegetables by Liquid Chromatography/Mass Spectrometry. *Rapid Commun. Mass Sp.*, *20*(18), 2701-2708.

Rose, R. I. (2001). Pesticides and Public Health: Integrated Methods of Mosquito Management. *Emerg. Infect. Dis.*, *7*(1), 17-23.

Rozman, K. K., Doull, J., & Hayes, W. J. (2012). Dose and Time Determining, and Other Factors Influencing Toxicity. In R. Krieger (Ed.), *Hayes' Handbook of Pesticide Toxicology* (3rd ed., pp. 3-101). San Diego, USA: Elsevier.

Sagratini, G., Mañes, J., Giardiná, D., Damiani, P., & Picó, Y. (2007). Analysis of Carbamate and Phenylurea Pesticide Residues in Fruit Juices by Solid-Phase Microextraction and Liquid Chromatography-Mass Spectrometry. *J. Chromatogr. A*, *1147*(2), 135-143.

Salafranca, J., Pezo, D., & Nerín, C. (2009). Assessment of Specific Migration to Aqueous Simulants of a New Active Food Packaging Containing Essential Oils by Means of an Automatic Multiple Dynamic Hollow Fibre Liquid Phase Microextraction System. *J. Chromatogr. A*, *1216*(18), 3731-3739. doi: http://dx.doi.org/10.1016/j.chroma.2009.03.001

Sanagi, M. M., Ghani, N. F. Y. A., Miskam, M., Ibrahim, W. A. W., & Aboul-Enein, H. Y. (2010). Analysis of Organophosphorus Pesticides in Vegetable Samples by Hollow Fiber Liquid Phase Microextraction Coupled with Gas Chromatography-Electron Capture Detection. *J. Liq. Chromatogr. Relat. Technol.*, *33*(5), 693-703.

Sánchez-Rojas, F., Bosch-Ojeda, C., & Cano-Pavón, J. M. (2009). A Review of Stir Bar Sorptive Extraction. *Chromatographia, 69*(SUPPL. 1), S79-S94. doi: 10.1365/s10337-008-0687-2

Sánchez, A., Millán, S., Sampedro, M. C., Unceta, N., Rodríguez, E., Goicolea, M. A., & Barrio, R. J. (2008). Quantification of Fenitrothion and Its Main Metabolites in Poplar Leaves by Isotope Dilution Gas Chromatography-Mass Spectrometry Coupled with Solid-Phase Microextraction. *J. Chromatogr. A, 1177*(1), 170-174.

SANCO. (2009). *Method Validation and Quality Control Procedures for Pesticide Residues Analysis in Food and Feed. SANCO/10684/2009.*

SANCO. (2011). *Method Validation and Quality Control Procedures for Pesticide Residues Analysis in Food and Feed; SANCO/12495/2011*.

Sandra, P., Tienpont, B., & David, F. (2003). Multi-Residue Screening of Pesticides in Vegetables, Fruits and Baby Food by Stir Bar Sorptive Extraction-Thermal Desorption-Capillary Gas Chromatography-Mass Spectrometry. *J. Chromatogr. A, 1000*(1-2), 299-309.

Sang, Z. Y., Wang, Y. T., Tsoi, Y. K., & Leung, K. S. Y. (2013). Codex-Compliant Eleven Organophosphorus Pesticides Screening in Multiple Commodities Using Headspace-Solid Phase Microextraction-Gas Chromatography-Mass Spectrometry. *Food Chem., 136*(2), 710-717.

Sanusi, A., Guillet, V., & Montury, M. (2004). Advanced Method Using Microwaves and Solid-Phase Microextraction Coupled with Gas Chromatography-Mass Spectrometry for the Determination of Pyrethroid Residues in Strawberries. *J. Chromatogr. A, 1046*(1-2), 35-40.

Sarafraz-Yazdi, A., & Amiri, A. (2010). Liquid-Phase Microextraction. *TrAC - Trends Anal. Chem., 29*(1), 1-14. doi: 10.1016/j.trac.2009.10.003

Sarafraz-Yazdi, A., Assadi, H., & Wan Ibrahim, W. A. (2012). Determination of Triazole Fungicides Using Hollow Fiber Liquid Phase Microextraction Prior to Gas Chromatography-Mass Spectrometry Analysis. *Ind. Eng. Chem. Res., 51*(7), 3101-3107.

Saraji, M., Rezaei, B., Boroujeni, M. K., & Bidgoli, A. A. H. (2013). Polypyrrole/Sol-Gel Composite as a Solid-Phase Microextraction Fiber Coating for the Determination of Organophosphorus Pesticides in Water and Vegetable Samples. *J. Chromatogr. A, 1279*, 20-26.

Schuurmann, G., Ebert, R.-U., Nendiza, M., Dearden, J. C., Paschke, A., & Kuhne, G. (2007). Predicting Fate-Related Physicochemical Properties. In C. J. van Leeuwen & T. G. Vermeire (Eds.), *Risk Assessment of Chemicals: An Introduction* (2nd ed., pp. 375-426). Dordrecht, The Netherlands: Springer.

Seethapathy, S., & Górecki, T. (2012). Applications of Polydimethylsiloxane in Analytical Chemistry: A Review. *Anal. Chim. Acta, 750*, 48-62.

Seiber, J. N. (2002). Environmental Fate of Pesticides. In W. B. Wheeler (Ed.), *Pesticides in Agriculture and the Environment*. new York, USA: Marcel Dekker.

Séralini, G. E., Clair, E., Mesnage, R., Gress, S., Defarge, N., Malatesta, M., . . . de Vendômois, J. S. (2012). Long Term Toxicity of a Roundup Herbicide and a Roundup-Tolerant Genetically Modified Maize. *Food Chem. Toxicol., 50*(11), 4221-4231.

Serôdio, P., & Nogueira, J. M. F. (2005). Development of a Stir-Bar-Sorptive Extraction-Liquid Desorption-Large- Volume Injection Capillary Gas Chromatographic-Mass Spectrometric Method for Pyrethroid Pesticides in Water Samples. *Anal. Bioanal. Chem., 382*(4), 1141-1151.

Shah, V. P., Midha, K. K., Findlay, J. W. A., Hill, H. M., Hulse, J. D., McGilveray, I. J., . . . Yacobi, A. (2000). Bioanalytical Method Validation - a Revisit with a Decade of Progress. *Pharm. Res., 17*(12), 1551-1557.

Sharma, D., Nagpal, A., Pakade, Y. B., & Katnoria, J. K. (2010). Analytical Methods for Estimation of Organophosphorus Pesticide Residues in Fruits and Vegetables: A Review. *Talanta, 82*(4), 1077-1089.

Shen, G., & Hian, K. L. (2002). Hollow Fiber-Protected Liquid-Phase Microextraction of Triazine Herbicides. *Anal. Chem., 74*(3), 648-654.

Shirey, R. E. (2012). Spme Commercial Devices and Fiber Coatings. In J. Pawliszyn (Ed.), *Handbook of Solid Phase Microextraction* (pp. 97-133). Waltham, USA: Elsevier.

Simplício, A. L., & Vilas Boas, L. (1999). Validation of a Solid-Phase Microextraction Method for the Determination of Organophosphorus Pesticides in Fruits and Fruit Juice. *J. Chromatogr. A, 833*(1), 35-42.

Smith, A. G. (2004). Toxicology of Organochlorine Insecticides. In T. C. Marrs & B. Ballantyne (Eds.), *Pesticide Toxicology and International Regulation* (pp. 27-87). West Sussex, England: John Wiley.

Souza-Silva, É. A., Lopez-Avila, V., & Pawliszyn, J. (2013). Fast and Robust Direct Immersion Solid Phase Microextraction Coupled with Gas Chromatography–Time-of-Flight Mass Spectrometry Method Employing a Matrix Compatible Fiber for Determination of Triazole Fungicides in Fruits. *J. Chromatogr. A*(0). doi: http://dx.doi.org/10.1016/j.chroma.2013.07.071

Souza Silva, E. A., & Pawliszyn, J. (2012). Optimization of Fiber Coating Structure Enables Direct Immersion Solid Phase Microextraction and High-Throughput Determination of Complex Samples. *Anal. Chem., 84*(16), 6933-6938.

Štajnbaher, D., & Zupančič-Kralj, L. (2003). Multiresidue Method for Determination of 90 Pesticides in Fresh Fruits and Vegetables Using Solid-Phase Extraction and Gas Chromatography-Mass Spectrometry. *J. Chromatogr. A, 1015*(1-2), 185-198.

Stalikas, C., Fiamegos, Y., Sakkas, V., & Albanis, T. (2009). Developments on Chemometric Approaches to Optimize and Evaluate Microextraction. *J. Chromatogr. A, 1216*(2), 175-189.

Stark, G. (2011). EU Pesticide Legislation - An Update. *Aspects Appl. Bio., 106*, 259-262.

Stenersen, J. (2004). *Chemical Pesticides: Mode of Action and Toxicology*. New York, USA: CRC Presss.

Stoyanov, K. S., & Walmsley, A. D. (2006). Response Surface Modelling and Experimental Designs. In P. Gemperline (Ed.), *Practical Guide to Chemometrics* (2nd ed.). Boca Raton, FL, USA: Taylor and Francis.

Surgan, M., Condon, M., & Cox, C. (2010). Pesticide Risk Indicators: Unidentified Inert Ingredients Compromise Their Integrity and Utility. *Environ. Manage., 45*(4), 834-841.

Tadeo, J. L., Pérez, R. A., Albero, B., García-Valcárcel, A. I., & Sánchez-Brunete, C. (2012). Review of Sample Preparation Techniques for the Analysis of Pesticide Residues in Soil. *J. AOAC Int., 95*(5), 1258-1271.

Tan, G. H., & Abdulra'uf, L. B. (2012). Recent Developments and Applications of Microextraction Techniques for the Analysis of Pesticide Residues in Fruits and Vegetables. In M. Stoytcheva (Ed.), *Pesticides - Strategies for Pesticides Analysis* (pp. 171-190): InTech.

Tan, G. H., & Chai, M. K. (2011). Sample Preparation in the Analysis of Pesticide Residues in Food for Chromatographic Techniques. In M. Stoytcheva (Ed.), *Pesticides - Strategies for Pesticides Analysis* (pp. 28-59). Rijeka, Croatia: InTech.

Tankiewicz, M., Fenik, J., & Biziuk, M. (2011). Solventless and Solvent-Minimized Sample Preparation Techniques for Determining Currently Used Pesticides in Water Samples: A Review. *Talanta, 86*(1), 8-22. doi: 10.1016/j.talanta.2011.08.056

Theis, A. L., Waldack, A. J., Hansen, S. M., & Jeannot, M. A. (2001). Headspace Solvent Microextraction. *Anal. Chem., 73*(23), 5651-5654.

Thompson, C. M., & Richardson, R. J. (2004). Anticholinesterase Insecticides. In T. C. Marrs & B. Ballantyne (Eds.), *Pesticide Toxicology and International Regulation* (pp. 89-128). Sussex, England: John Wiley.

Thompson, M., Ellison, S. L. R., & Wood, R. (2002). Harmonized Guides for Single-Laboratory Validation of Method of Analysis. *Pure Appl. Chem., 74*(5), 835-855.

Thorbek, P., & Hyder, K. (2006). Relationship between Physicochemical Properties and Maximum Residue Levels and Tolerances of Crop-Protection Products for Crops Set by the USA, European Union and Codex. *Food Addit. Contam., 23*(8), 764-776. doi: 10.1080/02652030600643377

Turiel, E., & Martín-Esteban, A. (2010). Molecularly Imprinted Polymers for Sample Preparation: A Review. *Anal. Chim. Acta, 668*(2), 87-99.

Turiel, E., Tadeo, J. L., & Martin-Esteban, A. (2007). Molecularly Imprinted Polymeric Fibers for Solid-Phase Microextraction. *Anal. Chem., 79*(8), 3099-3104.

Turner, C. (2006). Overview of Modern Extraction Techniques for Food and Agricultural Samples. In C. Turner (Ed.), *Modern Extraction Techniques* (Vol. 926, pp. 3-19): ACS, Washington DC, USA.

Tuzimski, T. (2012). Application of RP-HPLC-Diode Array Detector after SPE to the Determination of Pesticides in Pepper Samples. *J. AOAC Int., 95*(5), 1357-1361.

Ulrich, U., Dietrich, A., & Fohrer, N. (2013). Herbicide Transport Via Surface Runoff During Intermittent Artificial Rainfall: A Laboratory Plot Scale Study. *Catena, 101*, 38-49. doi: 10.1016/j.catena.2012.09.010

Urbanowicz, M., Zabiegała, B., & Namieśnik, J. (2011). Solventless Sample Preparation Techniques Based on Solid- and Vapour-Phase Extraction. *Anal. Bioanal. Chem., 399*(1), 277-300. doi: 10.1007/s00216-010-4296-1

Van Eck, W. H. (2004). International Standards: The International Harmonization of Pesticide Residue Standards for Food and Drinking Water. In D. Hamilton & S. Crossley (Eds.), *Pesticide Residues in Food and Drinking Water: Human Exposure and Risks*. Sussex, England: John Wiley.

Vapnek, J., Pagotto, I., & Kwoka, M. (2007). *Designing National Pesticide Legislation*. Rome: FAO.

Vázquez, P. P., Mughari, A. R., & Galera, M. M. (2008). Solid-Phase Microextraction (SPME) for the Determination of Pyrethroids in Cucumber and Watermelon Using Liquid Chromatography Combined with Post-Column Photochemically Induced Fluorimetry Derivatization and Fluorescence Detection. *Anal. Chim. Acta, 607*(1), 74-82.

Viñas, P., Aguinaga, N., Campillo, N., & Hernández-Córdoba, M. (2008). Comparison of Stir Bar Sorptive Extraction and Membrane-Assisted Solvent Extraction for the Ultra-Performance Liquid Chromatographic Determination of Oxazole Fungicide Residues in Wines and Juices. *J. Chromatogr. A, 1194*(2), 178-183.

Viñas, P., Martínez-Castillo, N., Campillo, N., & Hernández-Córdoba, M. (2010). Liquid-Liquid Microextraction Methods Based on Ultrasound-Assisted Emulsification and Single-Drop Coupled to Gas Chromatography-Mass Spectrometry for Determining

Strobilurin and Oxazole Fungicides in Juices and Fruits. *J. Chromatogr. A, 1217*(42), 6569-6577.

Walker, R. (1998). Toxicity Testing and Derivation of the Adi. *Food Addit. Contam., 15*(SUPPL.), 11-16.

Wan Ibrahim, W. A., Farhani, H., Sanagi, M. M., & Aboul-Enein, H. Y. (2010). Solid Phase Microextraction Using New Sol-Gel Hybrid Polydimethylsiloxane-2-Hydroxymethyl-18-Crown-6-Coated Fiber for Determination of Organophosphorous Pesticides. *J. Chromatogr. A, 1217*(30), 4890-4897. doi: 10.1016/j.chroma.2010.05.050

Wan Ibrahim, W. A., Wan Ismail, W. N., Abdul Keyon, A. S., & Sanagi, M. M. (2011). Preparation and Characterization of a New Sol-Gel Hybrid Based Tetraethoxysilane-Polydimethylsiloxane as a Stir Bar Extraction Sorbent Materials. *J. Sol-Gel Sci. Technol., 58*(3), 602-611.

Wang, J., Du, Z., Yu, W., & Qu, S. (2012). Detection of Seven Pesticides in Cucumbers Using Hollow Fibre-Based Liquid-Phase Microextraction and Ultra-High Pressure Liquid Chromatography Coupled to Tandem Mass Spectrometry. *J. Chromatogr. A, 1247*, 10-17.

Wang, S., Liu, C., Yang, S., & Liu, F. (2012). Ionic Liquid-Based Dispersive Liquid-Liquid Microextraction Following High-Performance Liquid Chromatography for the Determination of Fungicides in Fruit Juices. *Food Anal. Method*, 1-7.

Wang, Z., Hennion, B., Urruty, L., & Montury, M. (2000). Solid-Phase Microextraction Coupled with High Performance Liquid Chromatography: A Complementary Technique to Solid-Phase Microextraction-Gas Chromatography for the Analysis of Pesticide Residues in Strawberries. *Food Addit. Contam., 17*(11), 915-923.

Wardencki, W., Michulec, M., & Curyło, J. (2004). A Review of Theoretical and Practical Aspects of Solid-Phase Microextraction in Food Analysis. *Int. J. Food Sci. Technol., 39*(7), 703-717.

Wasik, S. P., Miller, M. M., & Tewari, Y. B. (1983). Determination of the Vapor Pressure, Aqueous Solubility, and Octanol/Water Partition Coefficient of Hydrophobic Substances by Coupled Generator Column/Liquid Chromatographic Methods. *Residue Rev., Vol. 85*, 29-42.

Watson, M. (2004). Toxicology Assessment of Agricultural Pesticides. In D. Hamilton & S. Crossley (Eds.), *Pesticide Residues in Food and Drinking Water: Human Exposure and Risks*. West Sussex, England: John Wiley.

Waxman, M. F. (1998). *Agrochemical and Chemical Safety Handbook*. Boca Raton, Florida, USA: CRC Press.

Wennrich, L., Popp, P., & Breuste, J. (2001). Determination of Organochlorine Pesticides and Chlorobenzenes in Fruit and Vegetables Using Subcritical Water Extraction Combined with Sorptive Enrichment and Cgc-Ms. *Chromatographia, 53*(SPEC. ISS), S380-S386.

Whitford, F., Fuhremann, T., Rao, K. S., & Klaung, J. E. (2007). Pesticide Toxicology: Evaluating Safety Risk. Available from Purdue Pesticide Program Retrieved on 14/01/2013, from Purdue University Cooperative Extension Service www.ppp.purdue.edu/Pubs/ppp-40.pdf

WHO. (1995). Vector Control for Malaria and Other Mosquito-Borne Diseases. Retrieved on 29/11/2012 from http://apps.who.int/iris/bitstream/10665/41726/1/WHO_TRS_857.pdf

WHO. (2006). *Preventing Disease through Healthy Environments: Towards an Estimate of the Environmental Burden of Disease*. Geneva, Switzerland: World Health Organization of the United Nations.

WHO. (2010). The Who Recommended Guideline Classification of Pesticides by Hazards and Guideline to Classification 2009. Retrieved 27/12/2012 from, from IPCS http://www.who.int/ipcs/publications/pesticides_hazard_2009.pdf

Wilson, B. W. (2010). Cholinesterases. In R. Krieger (Ed.), *Haye's Handbook of Pesticide Toxicology* (3rd ed.). San Diego, USA: Elsevier.

Winer, B. J. (1991). *Statistical Principles in Experimental Design* (3rd ed.). New York: McGraw-Hill.

Wu, J., Luan, T., Lan, C., Hung Lo, T. W., & Chan, G. Y. S. (2007). Removal of Residual Pesticides on Vegetable Using Ozonated Water. *Food Control, 18*(5), 466-472.

Wu, Q., Li, Z., Wang, C., Wu, C., Wang, W., & Wang, Z. (2011). Dispersive Solid-Phase Extraction Clean-up Combined with Dispersive Liquid-Liquid Microextraction for the Determination of Neonicotinoid Insecticides in Vegetable Samples by High-Performance Liquid Chromatography. *Food Anal. Method, 4*(4), 559-566.

Xiao, Q., Hu, B., Yu, C., Xia, L., & Jiang, Z. (2006). Optimization of a Single-Drop Microextraction Procedure for the Determination of Organophosphorus Pesticides in Water and Fruit Juice with Gas Chromatography-Flame Photometric Detection. *Talanta, 69*(4), 848-855.

Xiong, J., & Hu, B. (2008). Comparison of Hollow Fiber Liquid Phase Microextraction and Dispersive Liquid-Liquid Microextraction for the Determination of Organosulfur Pesticides in Environmental and Beverage Samples by Gas Chromatography with Flame Photometric Detection. *J. Chromatogr. A, 1193*(1-2), 7-18.

Xu, L., Basheer, C., & Lee, H. K. (2007). Developments in Single-Drop Microextraction. *J. Chromatogr. A, 1152*(1–2), 184-192.

Yang, X. M., Wang, O., Wang, M. Z., Hu, Y. X., Li, W. N., & Wang, Z. (2008). Determination of Metolcarb and Diethofencarb in Apples and Apple Juice by Solid-Phase Microextraction-High Performance Liquid Chromatography. *J. Chromatogr. Sci., 46*(8), 751-756.

Yu, J., Wu, C., & Xing, J. (2004). Development of New Solid-Phase Microextraction Fibers by Sol-Gel Technology for the Determination of Organophosphorus Pesticide Multiresidues in Food. *J. Chromatogr. A, 1036*(2), 101-111.

Yu, M.-H. (2001). *Environmental Toxicology: Impacts of Environmental Toxicants on Living Systems*. Florida, USA: CRC Press.

Yu, S. J. (2008). *The Toxicology and Biochemistry of Insecticides*. New York, USA: CRC Press.

Zacharia, J. T. (2011). Identity, Physical and Chemical Properties of Pesticides. In Stoytcheva (Ed.), *Pesticides in the Modern World - Trends in Pesticides Analysis* (pp. 1-19). Rijeka, Croatia: InTech.

Zambonin, C. G., Cilenti, A., & Palmisano, F. (2002). Solid-Phase Microextraction and Gas Chromatography-Mass Spectrometry for the Rapid Screening of Triazole Residues in Wine and Strawberries. *J. Chromatogr. A, 967*(2), 255-260.

Zambonin, C. G., Quinto, M., De Vietro, N., & Palmisano, F. (2004). Solid-Phase Microextraction - Gas Chromatography Mass Spectrometry: A Fast and Simple Screening Method for the Assessment of Organophosphorus Pesticides Residues in Wine and Fruit Juices. *Food Chem., 86*(2), 269-274.

Zang, X., Wang, J., Wang, O., Wang, M., Ma, J., Xi, G., & Wang, Z. (2008). Analysis of Captan, Folpet, and Captafol in Apples by Dispersive Liquid-Liquid Microextraction Combined with Gas Chromatography. *Anal. Bioanal. Chem., 392*(4), 749-754.

Zeng, J., Chen, J., Lin, Z., Chen, W., Chen, X., & Wang, X. (2008). Development of Polymethylphenylsiloxane-Coated Fiber for Solid-Phase Microextraction and Its Analytical Application of Qualitative and Semi-Quantitative of Organochlorine and Pyrethroid Pesticides in Vegetables. *Anal. Chim. Acta, 619*(1), 59-66.

Zgoła-Grześkowiak Agnieszka, A., & Grześkowiak, T. (2011). Dispersive Liquid-Liquid Microextraction. *TrAC - Trends Anal. Chem., 30*(9), 1382-1399.

Zhang, J., & Lee, H. K. (2006). Application of Liquid-Phase Microextraction and on-Column Derivatization Combined with Gas Chromatography-Mass Spectrometry to the Determination of Carbamate Pesticides. *J. Chromatogr. A, 1117*(1), 31-37.

Zhang, L., Chen, F., Liu, S., Chen, B., & Pan, C. (2012). Ionic Liquid-Based Vortex-Assisted Dispersive Liquid-Liquid Microextraction of Organophosphorus Pesticides in Apple and Pear. *J. Sep. Sci, 35*(18), 2514-2519.

Zhang, M., Huang, J., Wei, C., Yu, B., Yang, X., & Chen, X. (2008). Mixed Liquids for Single-Drop Microextraction of Organochlorine Pesticides in Vegetables. *Talanta, 74*(4), 599-604.

Zhang, S., Li, C., Song, S., Feng, T., Wang, C., & Wang, Z. (2010). Application of Dispersive Liquid-Liquid Microextraction Combined with Sweeping Micellar Electrokinetic Chromatography for Trace Analysis of Six Carbamate Pesticides in Apples. *Anal. Method, 2*(1), 54-62.

Zhang, X., Knaak, J. B., Tornero-Velez, R., Blancato, J. N., & Dary, C. C. (2010). Application of Physiologically Based Pharmacokinetic/Pharmacodynamic Modeling in Cumulative Risk Assesment for N-Methyl Carbamate Insecticides. In R. Krieger (Ed.), *Haye's Handbook of Pesticide Toxicology* (3rd ed., pp. 1591-1605). San Diego: Elsevier.

Zhang, Z., & Pawliszyn, J. (1993). Headspace Solid-Phase Microextraction. *Anal. Chem., 65*(14), 1843-1852.

Zhang, Z., Yang, M. J., & Pawliszyn, J. (1994). Solid-Phase Microextraction. *Anal. Chem., 66*(17), 844A-853A.

Zhao, E., Han, L., Jiang, S., Wang, Q., & Zhou, Z. (2006). Application of a Single-Drop Microextraction for the Analysis of Organophosphorus Pesticides in Juice. *J. Chromatogr. A, 1114*(2), 269-273.

Zhou, J., Liu, R., Song, G., & Zhang, M. (2009). Determination of Carbamate and Benzoylurea Insecticides in Peach Juice Drink by Floated Organic Drop Microextraction-High Performance Liquid Chromatography. *Anal. Lett., 42*(12), 1805-1819.

Zhou, Y., Han, L., Cheng, J., Guo, F., Zhi, X., Hu, H., & Chen, G. (2011). Dispersive Liquid–Liquid Microextraction Based on the Solidification of a Floating Organic Droplet for Simultaneous Analysis of Diethofencarb and Pyrimethanil in Apple Pulp and Peel. *Anal. Bioanal. Chem., 399*(5), 1901-1906. doi: 10.1007/s00216-010-4567-x

Zhu, X., Cai, J., Yang, J., Su, Q., & Gao, Y. (2006). Films Coated with Molecular Imprinted Polymers for the Selective Stir Bar Sorption Extraction of Monocrotophos. *J. Chromatogr. A, 1131*(1-2), 37-44.

LIST OF PUBLICATIONS

- **ISI CITED JOURNALS**

1. Abdulra'uf L. B. and Tan G. H. (2013) "Multivariate Study Of Parameters in the Determination of Pesticide Residues in Apple by Headspace Solid Phase Microextraction Coupled to Gas Chromatography-Mass Spectrometry using Experimental Factorial Design" *Food Chemistry* **141(4)** 4344 - 4348

2. Abdulra'uf, L. B, Ala, Y. S. and Tan, G. H. (2012) "Recent Developments and Applications of Liquid Phase Microextraction in Fruits and Vegetables Analysis" *Journal of Separation Science* **35(24)** 3540 - 3553

3. Abdulra'uf, L. B.; Chai, M. K. and Tan, G. H. (2012): Applications of Solid Phase Microextraction for the Analysis of Pesticide Residues in Fruits and Vegetables: A Review. *Journal of Association of Official Analytical Chemists Int.* **95(5)** 1272-1290

4. Abdulra'uf, L. B.; Adebayo, W. H and Tan, G. H. (2012): SPME Fibers for the Analysis of Pesticide Residues in Fruits and Vegetables: A Review. *Critical Review in Analytical Chemistry*, **42(2),** 152 – 161

5. Abdulra'uf L. B. and Tan G. H. (2013) "Chemometric Study and Optimization of Headspace Solid Phase Microextraction Parameters for the Determination of Multiclass Pesticide Residues in Cocoa Powder using Gas Chromatography-Mass Spectrometry. *Journal of Association of Official Analytical Chemists Int.* **(Under Review)**

6. Abdulra'uf L. B. and Tan G. H. (2013) "Importance and Environmental Fate of Pesticides" *Applied Ecology and Environmental Research* **(Under Review)**

7. Abdulra'uf L. B. and Tan G. H. (2013) "Review of SBSE Technique in the Analysis of Pesticide Residues in Fruits and Vegetables" *Chromatographia* **(Under Review)**

8. Abdulra'uf L. B. and Tan G. H. (2013) "Chemometric Approach to the Optimization of HS-SPME/GC-MS for the Determination of Multiclass Pesticide Residues in Fruits and Vegetables" *Journal of Agriculture and Food Chemistry* (**Under Review**)

- **BOOK CHAPTER**

  Tan G. H. and Abdulra'uf L. B. (2012) "Recent Developments and Applications of Microextraction Techniques for the Analysis of Pesticide Residues from Fruits and Vegetables, In M. Stoytcheva (Ed.), Pesticides - Strategies for Pesticides Analysis" *Rijeka, Croatia:* InTech. Pp. 171-190

- **CONFERENCE PROCEEDINGS**

  Abdulra'uf L. B. and Tan G. H. (2012) "Application of Factorial Design to the Study of Parameters in the Determination of Pesticide Residues in Apple by Headspace Solid Phase Microextraction Coupled to Gas Chromatography-Mass Spectrometry" *Pure and Applied Chemistry International Conference (PACCON 2012) proceedings,* pp. 748 – 751

LIST OF CONFERENCES

1. Abdulra'uf L. B. and Tan G. H.(2013) "*Application of Factorial Design to the Study of Parameters in the Determination of Pesticide Residues in Apple by Headspace Solid Phase Microextraction Coupled to Gas Chromatography-Mass Spectrometry*" A paper presented at the National Postgraduate Fundamental and Applied Sciences Seminar 2013 (UTAR NFPASS 2013) held at University Tunku Abdulrahman, Perak Campus, Malaysia, from May 13 – 14, 2013

2. Abdulra'uf L. B. and Tan G. H. (2012) "*Development of HS-SPME-GC/MS Method for the Analysis of Multiclass Pesticides in Grape Samples using Experimental Factorial Design*. An oral paper presented at the Malaysian Chemical Congress (17th MCC) Conference, held in Kuala Lumpur, Malaysia, Sept 15 – 17, 2012

3. Abdulra'uf L. B. and Tan G. H. (2012) "*Application of Factorial Design to the Study of Parameters in the Determination of Pesticide Residues in Apple by Headspace Solid Phase Microextraction Coupled to Gas Chromatography-Mass Spectrometry*" An oral paper presented at the Pure and Applied Chemistry International Conference (PACCON 2012), held in Chiang Mai, Thailand, Jan 11 – 13, 2012

4. Abdulra'uf L. B. and Tan G. H. (2011) *"Headspace Solid Phase Microextraction (HS–SPME) coupled to Gas Chromatography Mass Spectrometry (GC–MS) for the Analysis of Organophosphorus, Organochlorine and Carbamate Pesticides"* A paper presented at the 7th Mathematics and Physical Sciences Graduate Conference (7th MPSGC), held at National University of Singapore, December 12 – 14, 2011

5. Abdulra'uf L. B. and Tan G. H. (2011) *"Headspace Solid Phase Microextraction (HS–SPME) coupled to Gas Chromatography Mass Spectrometry (GC–MS) for the Analysis of Organophosphorus, Organochlorine and Carbamate Pesticides"* A paper presented at the 13th International Symposium on Advances in Extraction Technologies (EXTECH 2011), Kuala Lumpur, Malaysia. September 27 – 29, 2011

APPENDIX I

Food Chemistry 141 (2013) 4344–4348

Contents lists available at SciVerse ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Multivariate study of parameters in the determination of pesticide residues in apple by headspace solid phase microextraction coupled to gas chromatography–mass spectrometry using experimental factorial design

Lukman Bola Abdulra'uf [a,b,*], Guan Huat Tan [a,*]

[a] Department of Chemistry, Faculty of Science, University of Malaya, Lembah Pantai, 50603 Kuala Lumpur, Malaysia
[b] School of Basic and Remedial Studies, Kwara State College of Education, P.M.B. 1527, Ilorin 240244, Kwara State, Nigeria

ARTICLE INFO

Article history:
Received 11 August 2012
Received in revised form 30 April 2013
Accepted 3 July 2013
Available online 12 July 2013

Keywords:
SPME
Multivariate analysis
Factorial design
Pesticide residues

ABSTRACT

Solid-phase microextraction (SPME) is a solvent-less sample preparation method which combines sample preparation, isolation, concentration and enrichment into one step. In this study, multivariate strategy was used to determine the significance of the factors affecting the solid phase microextraction of pesticide residues (fenobucarb, diazinon, chlorothalonil and chlorpyrifos) using a randomised factorial design. The interactions and effects of temperature, time and salt addition on the efficiency of the extraction of the pesticide residues were evaluated using 2^3 factorial designs. The analytes were extracted with 100 μm PDMS fibres according to the factorial design matrix and desorbed into a gas chromatography–mass spectrometry detector. The developed method was applied for the analysis of apple samples and the limits of detection were between 0.01 and 0.2 μg kg^{-1}, which were lower than the MRLs for apples. The relative standard deviations (RSD) were between 0.1% and 13.37% with average recovery of 80–105%. The linearity ranges from 0.5–50 μg kg^{-1} with correlation coefficient greater than 0.99.

1. Introduction

Fruits and vegetables provide the body with micronutrients (minerals and vitamins) that are very vital to the body but are required in small quantities (Lewis & Ruud, 2004). This has led to their production in large quantities to meet the ever growing demand. To achieve this, pesticides were introduced to protect fruits and vegetables on the farm and during storage. Pesticides, including organophosphorous pesticides (OPP), organochlorine pesticides (OCP), carbamate pesticides (CP), phenyl urea pesticides (PUP), pyrethroid pesticides (PP) and carbamate pesticides (CP) have been used effectively in controlling pests, fungi and weeds, thereby beneficial to the steady increase in agricultural production (Chai & Tan, 2010; Vazquez, Mughari, & Galera, 2008). However, they persist in the food chain, due to their penetrating effect into the tissues of fruits and vegetables and therefore there is a need to analyse the level of pesticide residues in food.

Sample preparation is the most crucial and critical steps in the analysis of pesticide residues from complex fruit and vegetable matrices (Menezes Filho, dos Santos, & de Paula Pereira, 2010). The introduction of solid phase microextraction (SPME) technique in 1990 (Arthur & Pawliszyn, 1990), has helped to overcome the problems inherent in the solvent-based sample preparation techniques. Solid phase microextraction is a solvent free sample preparation and extraction technique developed by Pawliszyn and his co-workers (Arthur & Pawliszyn, 1990). It is an efficient, simple, versatile and effective adsorption/absorption and desorption technique with minimum matrix interference. It eliminates the use of toxic solvents and combines sampling, isolation, concentration and enrichment in one step (Ouyang & Pawliszyn, 2008; Pawliszyn, 1997). It was developed to overcome the problems associated with solvent-based, time consuming techniques, such as, liquid–liquid extraction (LLE), solid phase extraction (SPE), supercritical fluid extraction (SFE), and accelerated solvent extraction (ASE) (De Koning, Janssen, & Th. Brinkman, 2009). The traditional techniques also required a large volume of sample and solvents which impose environmental pollution and health hazards (Kataoka, Lord, & Pawliszyn, 2000). The SPME technique is based on the use of a fused silica or metal alloy that is coated on the outside with an appropriate polymerized stationary phase, attached to a stainless steel, mounted on a fibre holder housed in a modified syringe (Arthur, Killam, Buchholz, Pawliszyn, & Berg, 1992; Beltran, Peruga, Pitarch, & Lopez, 2003). The SPME process involves two basic steps which

* Corresponding authors. Address: Department of Chemistry, Faculty of Science, University of Malaya, Lembah Pantai, 50603 Kuala Lumpur, Malaysia. Tel.: +60 102343957 (L.B. Abdulra'uf).
E-mail address: abdulrauflukman@siswa.um.edu.my (L.B. Abdulra'uf).

0308-8146/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodchem.2013.07.022

Lukman Bola Abdulra'uf
Ala' Yahya Sirhan
Guan Huat Tan

Department of Chemistry, University of Malaya, Lembah Pantai, Kuala Lumpur, Malaysia

Received May 4, 2012
Revised August 27, 2012
Accepted August 27, 2012

Review Article

Recent developments and applications of liquid phase microextraction in fruits and vegetables analysis

The sample preparation step has been identified as the bottleneck of analytical methodology in chemical analysis. Therefore, there is need for the development of cost-effective, easy to operate, and environmentally friendly miniaturized sample preparation technique. The microextraction techniques combine extraction, isolation, concentration, and introduction of analytes into analytical instrument, to a single and uninterrupted step, and improve sample throughput. The use of liquid-phase microextraction techniques for the analysis of pesticide residues in fruits and vegetables are discussed with the focus on the methodologies employed by different researchers and their analytical performances. Analytes are extracted using water-immiscible solvents and are desorbed into gas chromatography, liquid chromatography, or capillary electrophoresis for identification and quantitation.

Keywords: Liquid-phase microextraction / Microextraction / Pesticide residues
DOI 10.1002/jssc.201200427

1 Introduction

The jointFood and Agricultural Organization and World Health Organization (FAO/WHO) Food Standard established the Codex Alimentarius Commission that set the maximum residue limit (MRL) for pesticides and other contaminants in fruits and vegetables. Other bodies including the European Union (EU) and United State Environmental Protection Agency (EPA) have also set directives for the MRL of pesticide residues on fruits and vegetables [1]. The pesticide residues are present at trace concentration levels (usually in mg/kg or less) and thus different MRL directives for pesticides were set at low concentration levels for various fruit and vegetable samples. The various legislations were proposed in order to check the misuse of pesticides in the environment and curb the adverse effects of the pesticides on human health, therefore the need for their accurate assessment [2]. The accurate evaluation of pesticide residues is aimed at quality control and quality assurance measures to regulate pesticide residues in foods.

The presence of the pesticide residues at trace levels embedded in complex food samples has encouraged the development of various environmental friendly sample preparation techniques that make use of microliter volume of solvents. Sample preparation step has been identified as the bottleneck of any analytical methodologies in the determination of contaminants from foods. Prior sample preparation before instrumental analysis helps to extract, isolate, and concentrate the analytes of interest from complex food matrices containing other interfering high molecular mass compounds. Therefore, the current trend of sample preparation techniques is focused on the simplifications, miniaturization, and combination of different steps, such as extraction, concentration, isolation of analytes, cleanup, and instrumental analysis in one single step. The microextraction techniques, including solid-phase microextraction (SPME) [3–5], microextraction in packed sorbent [6–9], and liquid-phase microextraction (LPME) [10–30], allow for greater sensitivity, low detection limit, and are fast and economical with high sample throughput.

The different microextraction techniques were developed in order to overcome the limitations inherent in the conventional sample preparation, which are tedious, time consuming, multistep, and use large volume of solvents and samples. They were developed to corroborate the recent advances in analytical instrumentations, such as gas chromatography (GC), liquid chromatography (LC), and capillary electrophoresis (CE), coupled to different detectors, and are highly sensitive, efficient, and compatible with various microextraction techniques. They are used for both qualitative and

Correspondence: Dr. Lukman Bola Abdulra'uf, Department of Chemistry, University of Malaya, Lembah Pantai, Kuala Lumpur, WP 50603, Malaysia
E-mail: lbarchem@yahoo.com; abdulrauflukman@siswa.um.edu.my
Fax: +60-3-79674193

Abbreviations: **DI**, direct immersion; **DLLME**, dispersive liquid–liquid microextraction; **EU**, European Union; **LLSME**, liquid–liquid–solid microextraction; **LPME**, liquid-phase microextraction; **MIP**, molecularly imprinted polymer; **MM-LLE**, microporous membrane liquid–liquid extraction; **MRL**, maximum residue limit; **MWCNT**, multiwalled carbon nanotube; **OCP**, organochlorine pesticide; **PHWE**, pressurized hot water extraction; **SDME**, solvent drop microextraction; **SLM**, supported liquid membrane; **sMEKC**, sweeping electrokinetic chromatography; **SPME**, solid-phase microextraction; **USAEME**, ultrasound-assisted emulsification microextraction.

SPECIAL GUEST EDITOR SECTION

Applications of Solid-Phase Microextraction for the Analysis of Pesticide Residues in Fruits and Vegetables: A Review

LUKMAN BOLA ABDULRA'UF
University of Malaya, Department of Chemistry, 50603, Lembah Pantai, Kuala Lumpur, Malaysia
MEE KIN CHAI
Universiti Tenaga Nasional, College of Foundation and General Studies, Department of Science, Mathematics and Computing, Jalan IKRAM-UNITEN, Malaysia
GUAN HUAT TAN
University of Malaya, Department of Chemistry, 50603, Lembah Pantai, Kuala Lumpur, Malaysia

This paper reviews the application of various modes of solid-phase microextraction (SPME) for the analysis of pesticide residues in fruits and vegetables. SPME is a simple extraction technique that eliminates the use of solvent, and it is applied for the analysis of both volatile and nonvolatile pesticides. SPME has been successfully coupled to both GC and LC. The coupling with GC has been straightforward and requires little modification of existing equipment, but interfacing with LC has proved challenging. The external standard calibration technique is widely used for quantification, while standard addition and internal or surrogate standards are mainly used to account for matrix effects. All parameters that affect the extraction of pesticide residues from fruits and vegetables, and therefore need to be optimized, are also reviewed. Details of the characteristics of analytical procedures and new trends in fiber production using sol-gel technology and molecularly imprinted polymers are discussed.

A nutritious, balanced diet plays a vital role in human health and well-being. Fresh fruits and vegetables constitute an important part of a balanced diet, as they contain a significant amount of nutrients and minerals (1). The production of fruits and vegetables in large quantities involves the use of toxic pesticides, and the increase in world population has led to greater demand for food production. This, in turn, has led to the introduction of pesticides, which are used to protect plants before and after harvest. The continuous use of pesticides has contributed to the steady increase in food production (2). Use of pesticides plays a beneficial role in providing large quantities of a low-cost supply of fruits and vegetables (3), but also comes with side effects.

Pesticides, including insecticides, herbicides, and fungicides, have been used effectively to control pests, fungi and weeds. Pesticides may penetrate the tissues of fruits and vegetables, where they remain as residues, result in the contamination of foods, and pose a potential risk to human health due to their toxicity. Therefore, there is a need to strike a balance between their expected benefits and possible risks (4). Hence, their concentration must always be minimal in fruits and vegetables and must be below the maximum residue limits. Today, the monitoring of pesticide residues in food is a priority objective in pesticide research in order to allow extensive evaluation of food quality and contamination. Therefore, the analysis of pesticide residues in fruits and vegetables is essential for monitoring and safety purposes.

The purpose of any analytical study is to obtain information about substances. Analytical process involves several steps: sampling, sample preparation, separation, quantification, and data analysis (5). Sample preparation is a crucial step and, indeed, is the bottleneck of analytical methodologies in the analysis of fruits and vegetables for pesticide residues, volatile compounds, contaminants, flavors, and food additives. The current trend aimed at reliable and accurate analysis of fruits and vegetables is to focus on the simplification, miniaturization, and improvement of sample preparation, such as extraction, concentration, isolation of analytes, and cleanup (6, 7), with microextraction methods. Fruit and vegetable samples are usually analyzed with a preliminary step of sample preparation, since contaminants are present at trace concentration levels (usually μg/g or less) in complex matrixes. The analysis usually requires various matrix pretreatment methods (8), such as liquid–liquid extraction, SPE, supercritical fluid extraction (9, 10), matrix solid-phase dispersion (11), and accelerated solvent extraction (12).

Solid-phase microextraction (SPME) is a solvent-free sample preparation technique (13) developed by Pawliszyn and his coworkers in 1990. Its application has been examined (14), optimized, automated (15), and reviewed (7, 16, 17). It is a simple and effective sorption (adsorption/absorption) and desorption technique that eliminates the need for solvents and combines sampling, isolation, concentration, and enrichment in one step (18–20). It was developed to overcome the problems associated with solvent-based, time-consuming techniques, usually multistep techniques that require the use of large volumes of samples and solvents, which cause environmental pollution and health hazards.

This article reviews various aspects of the SPME technique used in the analysis of fruits and vegetables. The review includes modes and types of SPME, including fiber types, optimization of extraction conditions, calibration methods, and the various

Guest edited as a special report on "Methods of Pesticide Residue Analysis" by Tomasz Tuzimski.

Corresponding author's e-mail: abdulrauflukman@siswa.um.edu.my

DOI: 10.5740/jaoacint.SGE_Abdulrauf

Critical Reviews in Analytical Chemistry, 42:152–161, 2012

ISSN: 1040-8347 print / 1547-6510 online
DOI: 10.1080/10408347.2011.632315

SPME Fibers for the Analysis of Pesticide Residues in Fruits and Vegetables: A Review

Lukman Bola Abdulra'uf,[1,2] Wasiu Adebayo Hammed,[1] and Guan Huat Tan[1]

[1]*Department of Chemistry, University of Malaya, Kuala Lumpur, Malaysia*
[2]*School of Basic and Remedial Studies, Kwara State College of Education, Ilorin, Nigeria*

Food samples are usually analyzed with a preliminary step of sample preparation, because pesticide residues are present in low concentrations and embedded in complex matrixes. Solid phase microextraction (SPME) is a solvent-free extraction technique that is fast, relatively inexpensive, easily automated, and versatile with high sample throughput. Analyte extraction, pre-concentration, and introduction into the analytical instrument are achieved in a single uninterrupted process. SPME uses fibers coated with a polymeric stationary phase that can be either a solid, liquid, or a combination of both. The fiber coating extracts the target analytes from a complex food matrix by absorption in the case of liquid coatings or adsorption in the case of solid coatings. The SPME fiber is then transferred into the analytical instrument for desorption and analysis of the target analytes. The use of sol-gel technology in the production SPME coated fiber has helped to increase the range of pesticide residues that can be extracted with the SPME technique.

Keywords SPME, coated fiber, sol-gel, pesticide residues

INTRODUCTION

An accurate assessment of pesticide residues and other contaminants in fruits and vegetables is very important due to increasing awareness of the effects of these chemicals on human health. The need to satisfy the drastic increase in the demand for fruits and vegetables by a growing population has led to an increase in the use of pesticides. Although the use of pesticides has helped to increase food production, it is necessary to strike a balance between their benefits and the possible risk to human health. Therefore, there is an urgent need for quality control monitoring of the use of such pesticides on fruits and vegetables for safety purposes.

The analysis of fruits and vegetables for the evaluation of pesticide residues is an important aspect of quality control measures put in place to prevent the misuse of pesticides. Although various analytical techniques have been developed for food analysis, most analytical instruments cannot handle the sample matrixes directly (Kataoka et al., 2000). However, this is not a simple task as fruits and vegetables contain a wide range of complex compounds that cause interference with instrumental analysis. Food analysis involves several steps: sampling, sample preparation, separation, detection, quantification, and data analysis, the purpose of which is to obtain information on the quality and quantity of pesticide residues present in the fruit and vegetable samples.

The sample preparation step is a most critical step in the qualitative and quantitative analysis of pesticide residues in fruits and vegetables. To achieve this, several extraction methods such as solid phase extraction, liquid-liquid extraction, supercritical fluid extraction, and pressurized liquid extraction (De Koning et al., 2009) have been developed. However, these sample preparation techniques are tedious, time consuming, and use large volumes of toxic solvents (Kataoka et al., 2000), and could also lead to loss of analytes, giving rise to analytical errors. An ideal, effective, efficient, and simple sample preparation technique should combine sample isolation, concentration and qualitative and quantitative determination of pesticide residues in a simple step, irrespective of the complexity of the sample matrixes.

Fruit and vegetable samples are usually analyzed with a preliminary sample preparation step (Ahmed, 2001), since pesticide residues are usually present at a trace concentration level, embedded in complex sample matrixes. This requires an efficient and effective extraction technique that allows for greater selectivity in the concentration of analytes, thus the need for various sample matrix pretreatment methods. The current reliable and accurate analysis of fruits and vegetables is focused on the

Address correspondence to Lukman Bola Abdulra'uf, Dept. of Chemistry, University of Malaya, Lembah Pantai 50603, Kuala Lumpur, Malaysia. E-mail: abdulrauflukman@siswa.um.edu.my

Chapter 9

Recent Developments and Applications of Microextraction Techniques for the Analysis of Pesticide Residues in Fruits and Vegetables

Guan Huat Tan and Lukman Bola Abdulra'uf

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/46843

1. Introduction

Analysis of pesticide residues and other contaminants in fruit and vegetable samples is becoming increasingly important due to the health hazards caused by their accumulation in human tissue. The body requires some important nutrients which can be provided by the consumption of fruits and vegetables. The purpose of any analytical study is to obtain information about substances and analytes present in the sample. Analytical process involves several steps: sampling, sample preparation, separation, quantification and data analysis [1]. Sample preparation is a very important step and indeed the bottleneck of analytical methodologies, in the analysis of fruits and vegetables for the presence of pesticide residues in fruit and vegetable samples [2].

The first step in any instrumental analysis is sample preparation, which involves the isolation or extraction of the desired analytes from the sample matrix, since they are present at trace concentration (usually μg.kg or less). This helps in the elimination of any interferences and also reduces the volume of extracts, thereby concentrating the analytes [2]. The type, nature and composition of sample and the nature and concentration of analytes to be isolated or extracted determines the choice of separation and detection method to be used, and this also dictates the type of sample preparation to be employed [3, 4], since the efficiency of any analysis is determined by the sample preparation step. The current trend of microextraction techniques is aimed at a reliable and accurate analysis of contaminants from complex samples. It is focused on the reduction of sample and solvent volume, with the automation/coupling of the sampling step to the analytical instruments, while maintaining the high throughput performance, low cost operation and improvement of the sample preparation, such as extraction, concentration, isolation of analytes, and clean-up [5, 6].