EGE UNIVERSITY GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

MASTER THESIS

THE PREPARATION AND CHARACTERIZATION OF SOLID PHASE MICRO EXTRACTION FIBER FOR PESTICIDE ANALYSIS

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ÖZET

KATI FAZA MİKRO ÖZÜTLEME FİBERİ YAPIMI KARAKTERİZASYONU VE PESTİSİT ANALİZİ İÇİN KULLANILMASI

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Çevre ve tarım açısından büyük önem arzeden pestisitlerin, formülasyonlarda, karmaşık yapılı gıda ve çevre örneklerinde analizi genellikle yüksek verimli, seçimli ve duyarlı yöntemlerin geliştirilmesini gerektirir. Tepe boşluğu katı faza mikro özütleme (HS-SPME) tekniği klasik örnek hazırlama tekniklerine göre birçok üstünlüğe sahiptir. Bu çalışmada pahalı ticari fiberlere alternatif oluşturmak ve özel seçimlilik kazandırmak üzere laboratuar yapımı fiber geliştirilmesi amaçlanmıştır. Fiber oluşturmada elektrokimyasal polimerizasyon tekniği ile pirol monomeri çelik tel üzerine kaplanmış ve HS-SPME koşullarına kullanımı araştırılmıştır. Adsorpsiyon sıcaklığı ve süresi, desorpsiyon sıcaklığı, karıştırma hızı ve tuz miktarı gibi denel parametreler optimize edilmiş ve sırasıyla 70^{o}C ve 45 dk. , 200^{o}C, 600 rpm ve 10 g/L olarak saptanmıştır. Bu tez kapsamında seçilen Chlorpyrifos (CP), Penconazole (PNZ), Procymidone (PRC), Brompropylate (BRP) ve Lambda-Cyhalothrin (LMD) pestisit türleri için kalibrasyon grafikleri oluşturulmuştur. Çalışmada doğruluğun artırılabilmesi için iç standart olarak aldrin(ALD) kullanılmıştır. Bu yöntemin uygulaması Türk şarap örneklerine yapıldığında, makul gerikazanım değerleri hesaplanmıştır.

Anahtar sözcükler: gaz kromatografisi, tepe boşluğu, katı faza mikro özütleme, polipirol, elektropolimerleşme, pestisit kalıntısı, şarap

ABSTRACT

THE PREPARATION AND CHARACTERIZATION OF SOLID PHASE MICRO EXTRACTION (SPME) FIBER FOR PESTICIDE ANALYSIS

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Pesticides are an important and diverse environmental and agricultural species. Their determination in formulations, in feed and food, and in complex environmental matrices often requires separation methods capable of high efficiency, unique selectivity, and high sensitivity. HS-SPME technique has great advantages over the classical sampling techniques. In this study it was aimed to prepare SPME fibers in laboratory conditions to reduce cost and improve the selectivity as well as their duration. Electrochemical polymerization is the method of choice for fiber coating. Pyrrole was electrochemically polymerized on a stainless steel wire and used as a HS-SPME. The parameters namely adsorption and time, desorption temperature, stirring speed and amount of salt were optimized as 70^{o}C and 45 min, 200^{o}C, 600 rpm and 10 g/L respectively. Calibration graphs were constructed for Chlorpyrifos (CP), Penconazole (PNZ), Procymidone (PRC), Brompropylate (BRP) and Lambda-Cyhalothrin (LMD) selected for the thesis. Aldrin (ALD) was used as the internal standard. The application of the method was made by using Turkish wine samples and acceptable recovery values were calculated for these selected pesticides.

Keywords: gas chromatography, head space analysis, solid phase micro extraction, polypyrrole, electropolimerization, pesticide residue, wine

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CONTENTS

CONTENTS (Continue)

LIST OF FIGURES

LIST OF FIGURES (Continue)

LIST OF TABLES

SYMBOLS and ABBREVIATIONS

Abbreviations	Explanations
AChE	Acetyl cholinesterase
BRP	Brompropylate
C_{fA}^{∞}	Analyte concentration on the fiber at equilibrium (mol cm^{-2})
C_{fmax}	Maximum concentration of active sites on the surface (mol cm^{-2})
C_i	Solute concentration in the sample before SPME sampling
C_{sA}^{∞}	Analyte concentration in the solution at equilibrium (mol cm^{-3}).
CCD	Catalytic combustion detector
CP	Chlorpyrifos
CV	Cyclic voltammetry
CW	Carbowax
DAD	Diode-array detection
DDT	Dichlorodiphenyl trichloroethane
DELCD	dry electrolytic conductivity detector
DID	discharge ionization detector
DVB	divinyl benzene
ECD	Electron-capture detection
EICD	Hall electrolytic conductivity detector
FID	flame ionization detector
FPD	Flame photometric detection
FTIR	Fourier Transformation Infrared spectrophotometer.
GC	Gas chromatography
HID	helium ionization detector
HS-SPME	Head space solid phase micro extraction
IRD	Infrared Detector
K	Adsorption equilibrium constant (cm^2 mol^{-1})
$Ki_{,SPME}$	Distribution constant between the absorptive SPME layer and the sample
LC	Liquid chromatography
LLE	Liquid-liquid extraction
LMD	Lambda-cyhalothrin
LOD	Limit of detection
$Mi_{,SPME}$	The amount of solute in the SPME layer at equilibrium
MRL	Maximum residue limit
MS/MS	Tandem mass spectrometry
MSD	mass selective detector
n_{fA}^{∞}	Amount of analyte adsorbed on the fiber at equilibrium
n_{fmax}	Maximum amount of the analyte that can be adsorbed on the active sites on the fiber
NHL	Non-Hodgkin's lymphoma
NPD	Nitrogen–phosphorus detection
PA	Polyacrylate
PDD	pulsed discharge ionization detector
PDMS	poly-dimethylsiloxane

SYMBOLS and ABBREVIATIONS (Continue)

Abbreviations	Explanations
PID	photo-ionization detector
PNZ	Penconazole
PPy	Polypyrrole
PPy-DS	Dodesile sulphate doped polypyrrole
PRC	Procymidone
SDS	sodium dodecylsulfate
SEM	Scanning electron microscope
TEA/TCD	thermal energy(conductivity) analyzer/detector
TGA	Thermal Gravimetric Analysis
TID	thermionic ionization detector
SPE	Solid phase extraction
SPME	Solid phase micro extraction
t_r	retention time of the compound
V_{SPME}	volume of the SPME layer

1. INTRODUCTION

1.1. Pesticides

Pesticide is any substance or mixture of substances intended for: preventing, destroying, repelling, or mitigating any pest. Though often misunderstood to refer only to insecticides, the term pesticide also applies to herbicides, fungicides, and various other substances used to control pests (U.S. EPA, 2011).

A pesticide is any substance intended to control, destroy, repel, or attract a pest (diseases and viruses). Any living organism that causes damage or economic loss or transmits or produces disease may be the target pest. Pests can be animals (like insects or mice), unwanted plants (weeds), or microorganisms (like plant diseases and viruses). People have been using chemicals to fight pests since ancient times. Some pesticides currently in use were in fact developed during the World War II for use in warfare. The organophosphate insecticides were developed as nerve gases, and the phenoxy herbicides, including 2,4-D, were created to eradicate the Japanese rice crop, and later used as a component of agent Orange to defoliate large areas in jungle warfare. After the World War II, these chemicals began to be used as pesticides in agricultural production, for environmental spraying of neighborhoods for mosquito eradication, and for individual home and garden use. They helped to increase crop yields dramatically and made available plentiful grains and a bountiful variety of inexpensive fruits and vegetables. During the 1960s and 1970s, epidemiologists in the USA noted a rise in the incidence of non-Hodgkin's lymphoma (NHL). When plotted on a map of the USA these cases were clearly clustered in agricultural areas. This increase in NHL incidence paralleled the rise in Pesticide use, prompting some epidemiologists to theorize that there was a causal link. Since then are vast majority of studies being done to determine if there is a relationship between pesticide use and human health problems (Bassil et al, 2007).

1.1.1. Classification of pesticides

Pesticides can be classified by target organism, chemical structure, and physical state. Pesticides can also be classified as inorganic, synthetic, or biological (biopesticides), although the distinction can sometimes blur. Details of chemical pesticides are given below.

Organochlorine pesticides are insecticides composed primarily of carbon, hydrogen, and chlorine. Specific uses take a wide range of forms, from pellet application in field crops to sprays for seed coating and grain storage. Organochlorine pesticides break down slowly and can remain in the environment long after application and in organisms long after exposure.

The most notorious organochlorine is the insecticide Dichlorodiphenyl trichloroethane (DDT). DDT was widely used in agricultural production around the world for many years. DDT was also the primary weapon in the global war against malaria during this period, and continues to be used for malaria control in a handful of countries. DDT was banned in many countries in the 1970s in response to public concern and mounting scientific evidence linking DDT with damage to wildlife. Other commonly known organochlorines that have been banned in the U.S. include aldrin, dieldrin, toxaphene, chlordane and heptachlor. Others that remain in use include lindane, endosulfan, dicofol, methoxychlor and pentachlorophenol (Bradman et al., 2007).

Many organochlorine pesticides are extremely persistent in the environment and in human body. Organochlorines contribute many acute and chronic illnesses. Symptoms of acute poisoning can include tremors, headache, dermal irritation, respiratory problems, dizziness, nausea, and seizures. Studies have found a correlation between organochlorine exposure and various types of cancer, neurological damage, Parkinson's disease, birth defects, respiratory illness, and abnormal immune system function.

Some of the organochlorines are known or suspected hormone disruptors, and recent studies show that extremely low levels of exposure in the womb can cause irreversible damage to the reproductive and immune systems of the developing fetus.

Organophosphate Pesticides affect the nervous system by disrupting the enzyme that regulates acetylcholine, a neurotransmitter. Most organophosphates are insecticides. Some are very poisonous and were used in World War II as nerve agents. However, these pesticides usually are not persistent in the environment.

Carbamate Pesticides are mainly used in agriculture, as insecticide, fungicide, herbicide, nematocide, or sprout inhibitors. In addition, they are used as biocides for industrial or other applications and in household products.

Carbamates are effective insecticides by virtue of their ability to inhibit acetyl cholinesterase (AChE) in the nervous system. They can also inhibit other esterases. The carbamylation of the enzyme is unstable, and the regeneration of AChE is relatively rapid compared with that from a phosphorylated enzyme. Thus, carbamate pesticides are less dangerous with regard to human exposure than organophosphorus pesticides (Fishel, 2011).

Pyrethroid Pesticides were developed as a synthetic version of the naturally occurring pesticide pyrethrin, which is found in chrysanthemums. They have been modified to increase their stability in the environment. Some synthetic pyrethroids are toxic to the nervous system.

Biopesticides are certain types of pesticides derived from such natural materials as animals, plants, bacteria, and certain minerals. For example, canola oil and baking soda have pesticidal applications and are considered biopesticides. Microbial pesticides consist of a microorganism as the active ingredient.

Biochemical pesticides are naturally occurring substances that control pests by non-toxic mechanisms. Conventional pesticides, by contrast, are generally synthetic materials that directly kill or inactivate the pest. Biochemical pesticides include substances, such as insect sex pheromones that interfere with mating as well as various scented plant extracts that attract insect pests to traps. For classification as a biochemical pesticide, EPA has established a special committee to make such decisions (U.S. EPA, 2011).

Pesticides are also referred to according to the type of pest they control. There are many classes of pesticides, each designed to destroy or repel certain species. As shown in Table 1.1, pesticides that are related because they address the same type of pests include:

1.1.2. Potential health effects of pesticides

For all pesticides to be effective against the pests they are intended to control, they must be biologically active, or toxic. Pesticides are also potentially hazardous to humans, animals, other organisms, and the environment. The World Health Organization and the UN Environment Program estimate that each year, 3 million workers in agriculture in the developing countries experience severe poisoning from pesticides (Miller, 2004).

Table 1.1 Classification of Pesticides According to Pest Types

Type	**Targets**
Algicides	algae in lakes, canals, swimming pools, water tanks, and other sites
Antimicrobials	microorganisms such as bacteria, molds, fungi
Attractants	Attract pests (e.g, to lure an insect or rodent to a trap).
Biopesticides	natural materials such as animals, plants, bacteria, and certain minerals that target a variety of pests
Biocides	Kill microorganisms.
Disinfectants	Kill or inactivate disease-producing microorganisms on inanimate objects.
Fungicides	fungi that cause plant disease/ wood rot, etc.
Fumigants	insects/fungi
Herbicides	undesirable plants/weeds
Insecticides	flying and crawling insects
Miticides	Kill mites that feed on plants and animals.
Microbial pesticides	Microorganisms that kill, inhibit, or out compete pests
Molluscicides	snails and slugs
Nematicides	invertebrates (worms)
Ovicides	Kill eggs of insects and mites
Rodenticides	mice, rats and other rodents
Repellents	Repel pests, including insects (such as mosquitoes) and birds.

Hazard depends on the toxicity of the pesticide and the amount of exposure to the pesticide and is often illustrated with the following equation:

Hazard =Toxicity x Exposure

Toxicity is the capacity of a chemical to cause harm to health. The amount needed to cause harm depends on the chemical. Like other chemicals, some pesticides are more toxic than others. A small quantity of a highly toxic pesticide can cause great harm, but almost any substance can cause harm in large enough doses (Reigart and Roberts, 1999).

Pesticide-related health problems result from exposures, which occur chiefly via one or more of the following routes; oral ingestion, inhalation and dermal (through the skin). Accidental oral contamination can occur when farmers eat, drink or smoke while spraying or do so shortly after spraying without first washing their hands. Inhalation of pesticides is promoted by spraying without protective masks, whilst absorption through the skin is made more likely when skin and clothes are wet during spraying (Kishi et al., 1995).

Although ingesting pesticides is generally the most dangerous form of exposure, inhalation and absorption through the skin are probably the major causes of occupational poisoning cases among farmers in developing countries as they are often unaware of these particular risks. Pesticides affect different people differently. Children may be more sensitive to some pesticides than adults. Effects of pesticide poisoning of a human was summarized in Table 1.2.

Pesticides can have both acute and chronic health impacts, depending on the extent of exposure. Normally, no symptoms develop in relation to each exposure. Instead, victims gradually become ill over a period of months or years. This occurs when the toxic substance either accumulates in body tissues or causes minor irreversible damage at each exposure. After a long time, enough poison has been accumulated in the body to cause clinical symptoms.

Chronic effects of long-term pesticide exposure include: impaired memory and concentration, disorientation, severe depressions, irritability, confusion, headache, speech difficulties, delayed reaction times, nightmares, sleepwalking, drowsiness and/or insomnia. An influenza-like condition with headache, nausea, weakness, loss of appetite, and malaise has also been reported.

Whether affecting adults or children, consequences of chronic pesticide exposure may only appear later in life, or even in the next generation, and include learning difficulties, behavioral and reproductive defects, and increased susceptibility to cancer (Lyons, 1999). Other long-term effects are teratogenic (inducing embryo malformation) and mutagenic (inducing genetic or chromosomal mutations).

The acute toxicity of fungicides to humans is generally considered to be low, but they can be irritating to the skin and eyes. Inhalation of spray mist or dust from these pesticides may cause throat irritation, sneezing, and coughing. Chronic exposures to lower concentrations of fungicides can cause adverse health effects.

In general, herbicides have a low acute toxicity to humans because the physiology of plants is so different than that of humans. However, there are exceptions; many can be dermal irritants since they are often strong acids, amines, esters, and phenols. Inhalation of spray mist may cause coughing and a burning sensation in the nasal passages and chest.

Prolonged inhalation sometimes causes dizziness. Ingestion will usually cause vomiting, a burning sensation in the stomach, diarrhea, and muscle twitching.

Table 1.2. Effects of Pesticide Poisoning (Seyrani, 2007)

ACUTE	CHRONIC
CENTRAL NERVOUS AND AUTONOMIC	
Exhaustion, weakness, paralysis, acute headache, nausea, vomiting, tremor, neuropathy, blurred vision, sweating	Incoordination, fits, unsteadiness, tingling, acute depression, symptoms that mimic recognized neurological diseases
EYES EARS NOSE AND THROAT	
Burning, irritating, and watering mucous membranes of eyes, ears, nose and throat	Conjunctivitis, rhinitis, sore throat and eye damage
HEART AND CARDIOVASCULAR	
Slow pulse, cardiac arrhythmias, heart block	Chest pains, circulatory failure, heart damage
LUNGS	
Shortness of breath, excess, secretions respiratory depression	Asthma, burning and irritation, lung damage
URINARY AND REPRODUCTIVE	
Dysuria, frequency of urination, uncontrolled incontinence, spontaneous abortion	Kidney damage, sterility tostus malformation
MUSKULO-SKELETAL	
Muscle cramp, tremor, paralysis, muscular twitching	Muscular tenderness, low muscle strength, muscle cramps
SKIN	
Burning, itching,	Persistent dermatitis, hands-eczema
GASTRO-INTESTINAL	
Excessive thirst, nausea, vomiting, abdominal pains and cramps, diarrhea, loss of sphincter control	Odd taste in mouth, weight loss, bleeding internally
LIVER	
Necrosis some hepatic malfunction	Distribution of enzyme systems, low tolerance to chemicals and alcohols, chemical hepatitis
ENDOCRINE	
	Suppression of adrenal cortex, hyperglycemia

Organophosphate and carbamate insecticides inhibit the activity of cholinesterase, resulting in a buildup of acetylcholine in the body. An increase in acetylcholine results in the uncontrolled flow of nerve transmissions between nerve cells. The nervous system becomes "poisoned"; the accumulation of acetylcholine causes the continual transmission of impulses across the synapses (Figure 1.1). The effects of organophosphate or carbamate poisoning can result in both systemic and topical symptoms. Direct exposure of the eye, for example, can cause topical symptoms such as constriction of the pupils, blurry vision, an eyebrow headache, and severe irritation and reddening of the eyes. Symptoms and signs of systemic poisonings are almost entirely due to the accumulation of acetylcholine at the nerve endings.

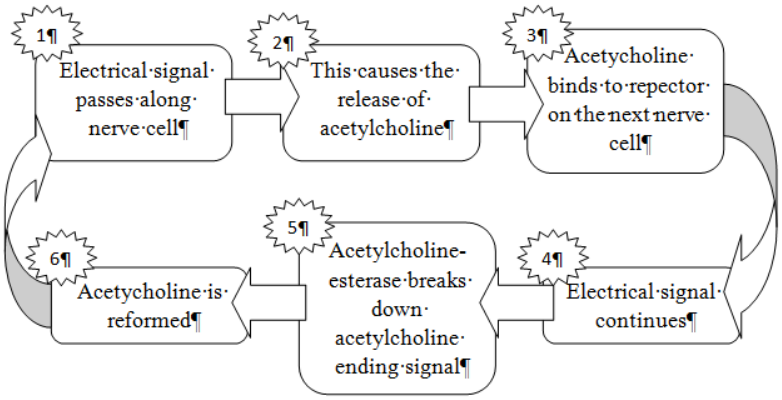


Figure 1.1 The role of acetylcholine in transmission

Organochlorine and pyrethroids also attack the nervous system, but these chemicals are not cholinesterase-inhibitors. Their main effect is on individual nerve cells, interfering with the transmission of messages along their length. Organochlorine insecticides are often extremely resistant to degradation, making them persistent in the environment. They can accumulate in animals' fat tissue, concentrating further at each level up the food chain. Harmful effects are therefore most likely to be seen at the top of the food chain, in birds of prey, or humans, for example. This is the major reason that their use has been increasingly prohibited, especially in industrialized countries.

Products are categorized on the basis of their relative acute toxicity (their LD_{50} or LC_{50} values) as given in Table 1.3. Pesticides that are classified as highly toxic (Toxicity Category I) on the basis of either oral, dermal, or inhalation toxicity must have the signal words DANGER and POISON printed in red with a skull and crossbones symbol prominently displayed on the front panel of the package label.

Table 1.3 Toxicity categories for active ingredients (Reigart and Roberts, 1999)

Routes of Exposure	**Toxicity Category**			
	I	**II**	**III**	**IV**
Oral LD_{50}	<50mg/kg	50-500mg/kg	500-5000mg/kg	>5000mg/kg
Inhalation LC_{50}	<0.2mg/L	0.2-2mg/L	2-20mg/L	>20mg/L
Dermal LD_{50}	<200mg/kg	200-2000mg/kg	2000-20000mg/kg	>20000mg/kg
Eye Effects	Corrosive corneal opacity not reversible within 7days	Corneal opacity reversible within 7 days; irritation persisting for 7 days	No corneal opacity; irritation reversible within 7 days	No irritation
Skin Effects	Corrosive	Severe irritation at 72 hours	Modarate irritation at 72 hours	Mild or slight irritation at 72 hours
Signal Word	DANGER POISON 	WARNING	CAUTION	CAUTION

Studies of farmers, mostly from industrialized countries, tend to show excesses of non-Hodgkin's lymphoma, melanoma, leukemia, multiple myeloma and soft tissue sarcoma (McDuffie et al, 1994; Davis et al., 1993).

Carcinogenesis involves irreversible alteration of a stem cell, its uncontrolled proliferation and, finally, invasion of other tissues. In this sequence there are various mechanisms by which pesticides may contribute to cancer development. The most obvious mechanism is genotoxicity, direct alteration of DNA turning harmless cells into cancer cells. Even levels of exposure to organophosphates too low to significantly decrease cholinesterase levels increased chromosomal aberrations found in blood samples from farmers. Pesticides thought to cause cancer in this way include the fumigants ethylene oxide and ethylene dibromide.

Other pesticides (e.g. DDT) have tested negative for genotoxicity but are carcinogenic in tests on animals. These may be tumor promoters. Several pesticides, particularly the organochlorine, are able to cause fixation and proliferation of abnormal cells. One possible mechanism, suggested for DDT, is inhibition of intercellular communication

Hormonal effects of some pesticides, may also promote tumours in certain tissues by stimulating hormonally-sensitive cells to carcinogenesis. Another way in which pesticides may promote cancer is through immunotoxic effects, disturbing the body's normal cancer surveillance mechanisms (Shanahan, 2003).

1.1.3. Physical and chemical properties of selected pesticides

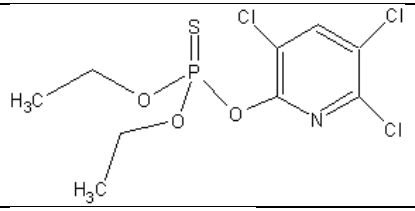
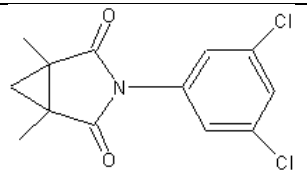
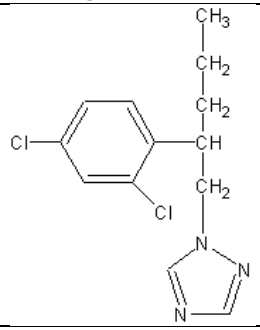
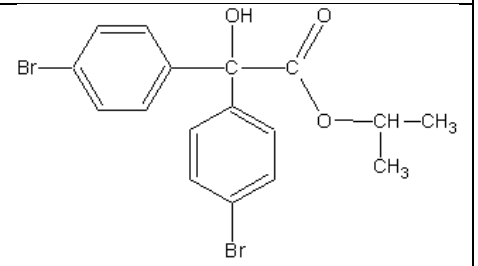
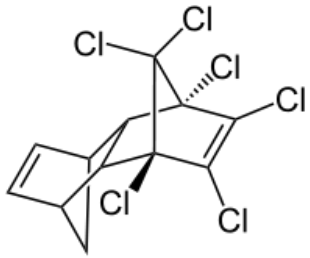
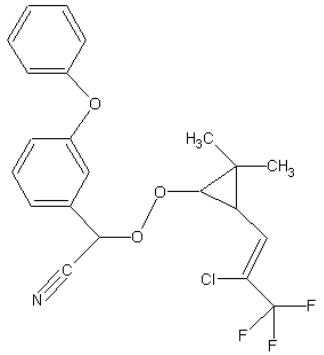
The grapevine is subject to attack numerous plant and animal parasites. A wide range of insecticides, fungicides, acaricides and herbicides are used in vineyards (Oliva et al., 2000). All pests and disease agents disrupt wine physiology and thereby can influence the fruit yield quality to some degree. Moths and mites are the most common insects that attack grapevines. Main insect attack is Lobesia botrana. However, agents that attack berries directly have the greatest impact on fruit quality. These include three of the major fungal grapevine pathogens namely; Botrytis cinerea, Plasmopara viticola and Uncinula necator (Teixeira et al., 2004). For this reason a variety of fungicides and insecticides are used to control pests that affect the vine, but the possibility exists that residues of these products can pass from grape to must and later to wine with the resulting risk to consumer's health.

Chlorpyrifos (CP), Penconazole (PNZ), Procymidone (PRO), Iprodione (IP), Bromopropylate (BRP) and Lambda-Chyalothrin (LMD) are endocrine disruptor pesticides (McKinlay et al., 2008; Zhao et al., 2008; Viswanath et al, 2010) were the most used types in grape pest control in Turkey. The efficiency of the sample preparation methods strongly dependent on the chemical nature of pesticides and therefore, physical and chemical properties of selected pesticides are given in Table 1.4.

1.1.4. Analysis methods for pesticides

As a consequence of the widespread use of pesticides, the presence of their residues in both food and the environment has become an important issue in analytical science. Nowadays, gas chromatography (GC) is the most widespread method for the separation and determination of most pesticides. However, liquid chromatography (LC) is also used for measuring levels of some pesticide residues such as carbamates and triazines, in foods of animal origin.

Table 1.4 Chemical and toxicological properties of the selected pesticides

Name	**Use**	MRL	Chemical structure	**Name**	**Use**	MRL	Chemical structure
Chlorpyrifos (CP)	Insecticide	0.50		Procymidone (PRC)	Fungucide	5.0	
Penconazole (PNZ)	Fungucide	0.20		Bromopropylate (BRP)	Acaricide	2.0	
Aldrin (ALD)	Insecticide	0.05		Lambda-Chyhalothrin (LMD)	Insecticide	0.20	

Even the generally low concentrations expected for pesticide residues in wines justify the use of sensitive analytical methods. For pesticides in wine no uniform limits have been established yet, except for procymidone for which the European Union has established maximum residue limit (MRL) of 0.5 mg/kg (EEC Directives, 2001).

Most pesticide residue detection methods for food samples comprise two main steps prior to the extraction of target analytes from the bulk of the matrix, and partitioning of the residues in an immiscible solvent and clean-up of analytes from matrix co-extractives, especially fat which interferes with assays (Bennett et al., 1997; Sannino et al., 1996; Barbini et al., 2007; Suchan et al., 2004).

Following the extraction/purification procedures, pesticide compounds are separated either on gas chromatography (GC) or liquid chromatography (LC), and then identified and quantified using different kinds of detection methods depending on the type of the molecules. Electron-capture detection (ECD), flame photometric detection (FPD), nitrogen–phosphorus detection (NPD), fluorescence detection, and diode-array detection (DAD) were mostly used for pesticide identification and quantification until recently. In addition, GC and LC methods coupled with mass spectrometry (MS) and tandem mass spectrometry (MS/MS) in residue analysis are safely used both monitoring and research applications (LeDoux, 2011).

An analytical process typically consists of separation, quantification, and data analysis. Each step is critical for obtaining accurate and reproducible results. A sample preparation step is often necessary to isolate the analyte from a sample matrix, as well as to purify and concentrate the analytes. Despite advances in instrumentation and data processing systems, however, many sample preparation practices are based on old technologies. Problems associated with these traditional sample preparation methods, such as the use of toxic organic solvents and multistep procedures that often result in loss of analytes during the process. Therefore, this step is usually the major source of error in an analysis.

Notable among recent developments are faster, greener extraction methods and microextraction techniques (Mitra, 2003). This was particularly driven by the needs of the environmental and the pharmaceutical industries, which analyze large number of samples requiring significant efforts in sample preparation.

1.2. Solid Phase Micro Extraction (SPME)

In addition to the well known liquid-liquid extraction (LLE) technique, solid phase extraction (SPE) and solid phase micro extraction techniques (SPME) were developed for semi volatile organics from liquid samples. Table 1.5 compares the extraction techniques for liquid samples.

Table 1.5 Comparsion of extraction methods for liquid samples

LLE	SPE	SPME
Use of an extractant immiscible with the sample	Wide choice solid phase	Non-solvent technique
	High selectivity	Equilibrium (nonexhaustive) extraction
Large volume of organic solvent	Small volume of organic solvent	Wide choice of fibers
Diluted extracts	High concentration factor	
Difficulty in extracting polar and ionic compounds from water		Applicable to volatile compounds
Difficult to automate	Amenable to automation	On-line coupling to GC and LC
	On-line coupling to chromatographic systems	Portable field samplers available

SPME is a relatively new method of sample introduction, developed by Pawliszyn and co-workers in 1989 (Pawliszyn, 1989 and 1990) and made commercially available in 1993. Solid-phase microextraction consists of two processes: partitioning of analytes between the coating and the sample and desorption of concentrated analytes into an analytical instrument. In the first process, the coated fiber is exposed to the sample and the target analytes are extracted from the sample matrix into the coating. However, for the extraction step with an externally coated SPME absorptive layer, the layer is exposed to a sample in a liquid or gas phase (Figure 1.2).

In the case of SPME within a coated tube, liquid or gaseous sample is simply pumped alternatively; stopped-flow sampling is also possible. The amounts of the solutes in the SPME layer gradually reach an equilibrium level with their surroundings, which represents the maximum solute amounts that can be absorbed and withdrawn under a given set of sampling conditions. The amount of solute, i.e., in the SPME layer at equilibrium ($Mi,_{SPME}$) can be approximated by the following equation:

$$Mi,_{\text{SPME}} \approx Ki,_{\text{SPME}} V_{\text{SPME}} C_{\text{i}}$$

where $Ki,_{SPME}$ is an aggregate solute distribution constant between the SPME absorptive layer and the sample, V_{SPME} is the volume of the SPME layer, and C_i is the solute concentration in the sample before SPME sampling.

Equation assumes that the sample volume is much greater than the volume of the SPME layer. SPME coatings typically have thicknesses of approximately 10–100 μm roughly 10-fold the film thickness range normally encountered in capillary GC.

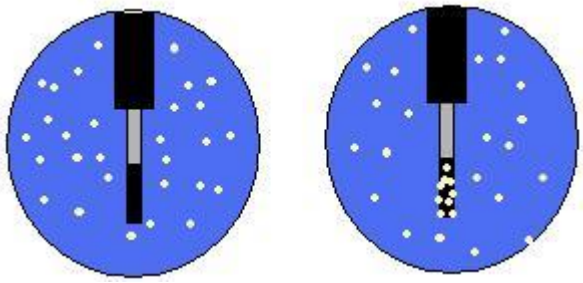


Figure 1.2 Adsorption process

The presence of a gaseous headspace over a liquid sample causes a portion of each solute to partition into the headspace in competition with the extraction process into the SPME layer. Solute mass in the SPME layer depends upon both the headspace volume and the partition ratios between the headspace or the liquid sample and the SPME layer. The fiber with concentrated analytes is then transferred to an instrument for desorption, followed by separation and quantization. Once in place at a chromatograph, the SPME layer must then be exposed to conditions that cause the absorbed solutes to desorbe with as close to 100% efficiency as possible and in a time that is short enough to be compatible with the chromatography mode in use.

First, the temperature must be high enough so that the solutes leave the SPME layer rapidly. Too-slow desorption can cause peak broadening and tailing unless additional arrangements are made for trapping solutes at the beginning of a cooled column before temperature programmed elution. Conversely, too-high inlet temperatures can induce thermal decomposition and introduce some contaminants into the column from septum bleed and from the SPME layer itself.

During sample desorption from an SPME fiber into a split–splitless inlet, the inlet split flow should be turned off so that all of the solutes can enter the column without splitting. After the SPME device has been withdrawn from an inlet splitter, the split flow can be turned on to purge the inlet of any remaining materials and prevent some degree of peak tailing.

SPME is a simple, fast, inexpensive and solventless alternative to conventional sample extraction techniques that can achieve very low levels of detection of trace organic compounds. Analytes are concentrated on the SPME fiber, and then are rapidly delivered to a capillary GC column or an HPLC column. In monitoring analytes in biological fluids, SPME is not only simpler and faster; it produces cleaner extracts than liquid-liquid or solid phase extraction. For sampling airborne compounds, such as organic pollutants or insect pheromones, the technique is very sensitive. Because analytes can be rapidly extracted from an aqueous matrix with virtually no solvent consumption, SPME saves preparation time and solvent purchase and disposal costs and can improve the limits of detection in an analysis. The technique has rapidly been established among the practical alternatives for sample preparation for gas chromatography (Woolley and Mindrup, 1996).

The primary advantages of SPME are its ability to decouple sampling from matrix effects that would distort the apparent sample composition or disturb the chromatographic separation; its simplicity and ease of use; and its reduced or non-existent solvent consumption. These characteristics combine to make SPME an attractive alternative to classic headspace or thermal-desorption sampling, solid-phase extraction and classic liquid–liquid extraction.

As with several related sample preparation and injection techniques such as headspace GC and thermal desorption, SPME lends itself well to handling difficult sample matrices and has the added benefits of low cost and simplicity. SPME doesn’t require elaborate and expensive instrument accessories for occasional use, and yet it seems to be capable of delivering very good manual results when in the hands of skilled users, which cannot necessarily be said of manual headspace or thermal-desorption sampling. Autosamplers are also available to perform repetitive unattended SPME sampling.

SPME requires careful optimization and consistent operating conditions for success, but this statement is true of the related techniques as well. Any poorly characterized sampling technique has no valid use in analytical laboratories, and the burden of developing an SPME method is no greater than for developing a method for any of the other techniques. SPME has a significant place in analysts' arrays of sample preparation techniques. (Hinshaw, 2003)

In SPME technique, 1cm length of fused silica fiber, coated with a polymer is bonded to a stainless steel plunger and installed in a holder that looks like a modified microliter syringe. In extraction procedure, the plunger moves the fused silica fiber into and out of a hollow needle. To use the unit, the analyst draws the fiber into the needle, passes the needle through the septum that seals the sample vial, and depresses the plunger, exposing the fiber to the sample or the headspace above the sample. Organic analytes adsorb to the coating on the fiber. After adsorption equilibrium is attained, usually in 2 to 30 minutes, the fiber is drawn into the needle, and the needle is withdrawn from the sample vial. Finally, the needle is introduced into the gas chromatograph injector, where the adsorbed analytes are thermally desorbed and delivered to the GC column, or into the SPME/HPLC interface. Results compare very favorably to results for other sample preparation methodology (Table 1.6).

Table 1.6 Comparision of SPME techniques with other techniques

Extraction Method	**LOD* level**	**Presicion (% RSD)**	**Expense**	**Time (min.)**	**Solvent Use**
Purge& Trap	ppb	1-30	high	30	none
Stripping	ppt	3-20	high	120	none
Headspace	ppm		low	30	none
LLE	ppt	5-50	high	60	1000mL
SPE	ppt	7-15	medium	30	100 mL
SPME	ppt	1-12	low	5	none

*Limit of detection

Langmuir model well describes the process of adsorption and desorption with a solid porous coating SPME fiber. It is assumed that a monolayer of the adsorbate can be formed at the surface. The amount of analyte adsorbed by the fiber is given by

$$C_{fA}^{\infty} = \frac{C_{fmax} K C_{sA}^{\infty}}{1 + K C_{sA}^{\infty}} \qquad (1)$$

where C_{fA}^{∞} is the analyte concentration on the fiber at equilibrium (in mol cm^{-2}), C_{fmax} is the maximum concentration of active sites on the surface (in mol cm^{-2}), K is adsorption equilibrium constant (in cm^2 mol^{-1}), and C_{sA}^{∞} is the analyte concentration in the solution at equilibrium (in mol cm^{-3}).

Equation 1 is rearranged to eq 2,

$$\frac{1}{n_{fA}^{\infty}} = \frac{1}{n_{fmax}} + \frac{1}{n_{fmax}KC_{sA}^{\infty}} \qquad (2)$$

where n_{fA}^{∞} is the amount of analyte adsorbed on the fiber at equilibrium and n_{fmax} is the maximum amount of the analyte that can be adsorbed on the active sites on the fiber, which corresponds to the maximum amount of active sites, assuming a 1:1 ratio of active sties to adsorbed analyte.

From eq 2, a plot of $1/n_{fA}^{\infty}$ versus $1/C_{sA}^{\infty}$ yields a straight line with a slope of $1/(n_{fmax}K)$ and a y-intercept of $1/n_{fmax}$. Thus, the values of n_{fmax} and K can be calculated from the linear regression equation. This method is referred as a "reciprocal Langmuir analysis". Since C_{sA}^{∞} is equal to the initial concentration of analyte (C_0) minus n_{fA}^{∞}/V_s.

Equation 2 can be further rearranged to Eq 3, where V_s is the sample volume. If $C_0 \gg n_{fA}^{\infty}/V_s$, which is often encountered during on-site or in vivo sampling, Eq 3 can be simplified to

$$C_0 = \frac{n_{fA}^{\infty}}{K\left(n_{fmax} - n_{fA}^{\infty}\right)} + \frac{n_{fA}^{\infty}}{V_s} \quad (3)$$

$$C_0 = \frac{n_{fA}^{\infty}}{K\left(n_{fmax} - n_{fA}^{\infty}\right)} \quad (4)$$

The initial concentration of analyte in a solution can be calculated from n_{fA}^{∞}, the extracted amounts of the target analyte at equilibrium. The amount of the analyte extracted is dependent upon the distribution constant. The higher the distribution constant, the higher is the quantity of analyte extracted. Generally a thicker film is required to retain small molecules and thinner film is used for larger molecules with high distribution constants. The polarity of the fiber and the type of coating can also increase the distribution constant (Hübschmann, 2001).

Because the coatings used in SPME are selected to have strong affinities for the organic compounds they are intended to extract, K_{fs} values for these analyte are large. Consequently, SPME has a very effective concentrating effect and leads to good sensitivity. K_{fs} values usually are not sufficiently large to exhaustively extract the analyte from the matrix, however; hence the statement that SPME is an equilibrium sampling method.

There are two approaches to SPME sampling of organic molecules: Direct and headspace sampling. The types of SPME sampling methods can be seen in Figure 1.3.

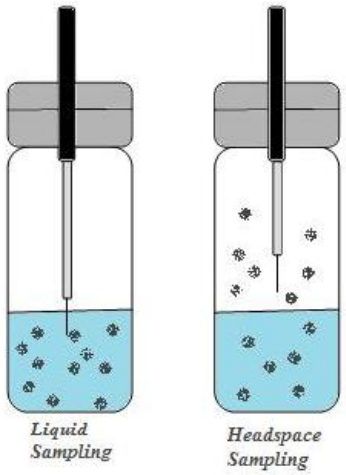


Figure 1.3 The types of SPME sampling methods

Direct (Immersion) SPME: The fiber is placed directly into the sample matrix. For high accuracy and precision from SPME, consistency in sampling time and other sampling parameters is more important than full equilibration. It also is important to keep constant the vial size and the sample volume. When sampling by immersion, the depth to which the fiber is immersed in the sample is also another parameter to be optimized. An adjustable needle guide/depth gauge on the fiber holder helps to ensure consistent depth of immersion of the fiber.

Headspace SPME: The fiber is placed in the headspace of the sample. This sampling method is suitable for the analysis of the analyte which has the normal boiling point less than 200^{o}C. The volatile organics can be analyzed easily by using this method. Equilibrium is attained more rapidly in headspace SPME than in immersion SPME, because the analytes can diffuse more rapidly to the coating on the fiber. These characteristics can be manipulated to advantage to selectively adsorb sample components, as appropriate.

Headspace SPME is ideal for minimizing interferences with an analysis, and can prolong the lifetime of the SPME fiber (Pawliszyn, 1993). As shown in Table 1.7, SPME can be used to analyze a wide range of compounds in various matrices through proper optimization or modification of SPME procedures.

Table 1.7 Applications of the SPME techniques (Pawliszyn, 1994)

Approaches	**Application**	**Analytes**	**Matrices**
Direct SPME	Routine	Most compounds	Gaseous and liquid
	In situ chemical derivatization	Polar compounds	
	In situ redox	Inorganic ions	
Headspace SPME	Routine	Volatile and semivolatile compounds	Any matrix
	Heating/ cooling	Volatile and semivolatile compounds with small partition coefficients	
	In situ chemical derivatization	Polar compounds	

Some of the SPME applications in food and environmental samples reported include flower scents (Barták et al., 2003), chemical warfare agents (Hook et al., 2003), pharmaceutical process impurities (Frost et al., 2003), organochlorine pesticides in Chinese teas (Cai et al., 2003), volatile compounds in acidic media (Araújo et al., 2003), and in cheese (Pinho et al., 2003), volatile phenols in wine (Mejas et al., 2003), environmental pollutants in water samples (Bagheri and Mohammadi, 2003), chloroanisoles in cork stoppers (Bianchi et al.,2003), volatile aliphatic amines in air (Namiesnik et al., 2003), and phenylurea herbicides in aqueous samples (Lin et al., 2003).

SPME is among the most recommended techniques, employed for the extraction and preconcentration of volatile and semi-volatile compounds at trace levels in variety of matrixes (Baciocchi et al., 2001; Yang and Peppard, 1995; Lee et al., 1996; Nagasawa et al., 1996; Lee et al 1998). To achieve more selective determination of different classes of compounds, the number of available coating materials has increased in recent time. Up to now, only seven kinds of SPME coatings are commercially available: poly-dimethylsiloxane (PDMS), PDMS-divinyl benzene (DVB), polyacrylate (PA), carboxen-PDMS, carbowax (CW-DVB), carbowax-templated resin, and stable flex DVB-carboxen-PDMS. The nonpolar PDMS fiber was the first polymer being used for SPME and, to date, this coating is the most used one, which extracts only nonpolar analytes very well (Gmeiner et al., 1998).

However, according to the principle of a like dissolves like, the polar compounds are more likely to be extracted by polar coatings such as PA and CW-DVB (Buchholz and Pawliszyn, 1994; Pawliszyn et al.1996). The CW-DVB fiber is strongly polar, but its maximum temperature is only 265^{o}C which limits its application range.

Although the use of SPME fibers is increasingly gaining in popularity, they present important drawbacks such as (i) their relatively low recommended operating temperature (generally in the range 240–280^{o}C), (ii) their instability and swelling in organic solvents (greatly restricting its use with HPLC), (iii) the breakage of the fiber, (iv) the stripping of coatings, (v) bending of the needle and their expensive cost. These common problems that SPME users contend with are recognized by researchers and constitute areas of SPME improvement. The introduction of new polymeric fibers, the development of new experimental configurations and the improvement of automatic devices will undoubtedly lead to the application of SPME to different fields of chemical analysis. Conducting polymers have increasing interest in this field.

1.3. Conductive Polymers

In recent years, intrinsic conducting polymers with conjugated double bonds have attracted much attention as advanced materials. The conducting polymers attract attention of researchers and engineers from a variety of fields in science and technology as promising electrode materials for energy storage devices (electro-chemical batteries and capacitors), electrocatalysts and biosensors based thereon, photoluminescent and electroluminescent materials, artificial muscles, gas separating membranes, anticorrosive coatings, electromagnetic shields, materials for microlithography, electrophotography, etc. There has been growing interest in conducting polymers due to their multifunctional properties and potential applications, as ion exchangers, energy-storage materials, corrosion-resistant coatings, catalysts, chemical sensors and materials for separation (Vidal et al., 1999; Campbell et al.,1999; Jerome et al.,1999; Smela, 1999 ; Alizadeh et al., 2001; Warren and Anderson, 1987; Shafiee-Dastjerdi and Alizadeh, 2004; Bagheri and Mohammadi, 2003; Djozan and Bahar, 2003; Bagheri and Saraji, 2001; Pawliszyn et al., 1999; Wu and Pawliszyn, 2001; Pawliszyn et al., 2000; Pawliszyn et al., 2000).

In addition, the possibilities to produce conducting polymers electrochemically and to control their properties by electrochemical means are of interest on their own. To date, a number of reviews and books on conducting polymers have been published. They are versatile materials in which analyte recognition can be achieved in different ways, including: (i) the incorporation of counter ions; (ii) utilizing the inherent and unusual multifunctionality of the polymer (e.g., hydrophobicity, acid–base character, π–π interaction, polar functional groups, ion exchange property, hydrogen bonding, and electroactivity) and (iii) the introduction of functional groups to the monomers.

Also, these materials have additional advantages as they can be easily synthesized in both aqueous and nonaqueous media, chemically and electrochemically. There are varieties in type of dopant and additives during synthesis. All these conditions and varieties affect the chemical, mechanical, morphological and electronic properties of the polymers. Electrochemical polymerisation provides a number of advantages over chemical methods. The first is that the reaction product is an electroactive film attached to the electrode surface and having high conductivity. Second is that the yield in charge terms is close to 100%; this provides a possibility of controlling the mass and thickness of the film. And finally, the properties of the film produced can be controlled directly in the course of preparation.

Among those conducting polymers, polypyrrole (PPy) (Figure 1.4) is especially promising for commercial applications because of its good environmental stability, facile synthesis and higher conductivity over many other conductive polymers.

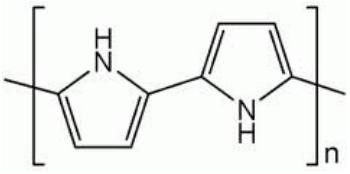


Figure 1.4 Chemical form of polypyrrole

Pyrrole can be polymerized conveniently with oxidation reactions by either an electrochemical method or a chemical method. Chemical polymerization method is capable of producing polymers on a large scale.

However, the flexibility of PPy is limited by the nature of oxidizing reagents used and the reaction conditions employed. Electrochemical synthesis is more convenient, because polymer film is directly electrodeposited on the surface of metal wires from an aqueous solution containing pyrrole and electrolyte, using one of the electrochemical techniques.

The advantages of electrochemical method are that it can be controlled easily and it is more flexible, because polymers with different functional groups can be formed conveniently by changing dopant ions or using substituted pyrrole monomers under controlled electrochemical conditions. The doping level for PPy (number of anions per monomer unit), depending on the nature of anions and conditions of synthesis, varies within 0.1 to 0.5. Switch-off of the current in the course of PPy electrosynthesis is immediately followed by the arrest of the chain growth; the mass of the polymer film is directly proportional to the charge passed (Beck and Oberst, 1987; Ko et al., 1990; Genies et al., 1983).

Electropolymerisation of pyrrole can be performed in both aqueous and non-aqueous media, such as acetonitrile (AN), propylene carbonate (PC), dichloromethane (Diaz et al., 1981; Asavapiriyanont et al., 1984; Scharifker et al., 1991; Yamaura et al., 1991). Prior to the experiment, the electrolyte solution should be purged with nitrogen or argon in order to remove oxygen, because films prepared in the presence of oxygen have inferior properties.

The oxidation of pyrrole to PPy is irreversible. The mechanism of this reaction was extensively studied (Heinze, 1991; Genies et al., 1983; Asavapiriyanont et al., 1984; Scharifker et al., 1991; Downard and Pletcher, 1986; Rodriguez et al., 1987; John and Wallace 1991; Kim et al., 1995; Beck and Oberst 1989; Waltman and Bargon 1984; Lowen and Dyke 1990; Raymond and Harrison 1990; Raymond and Harrison 1993; Reynolds et al., 1994; Tanaka et al., 1988; Hillman and Mallen 1987; Audebert and Hapiot 1995; Otero and Rodriguez 1993; Otero et al., 1991; Marcos et al., 1987; Beck et al., 1990) yet, the problem still remains not fully resolved. Among the mechanisms proposed, two have gained the greatest interest. One of those is the oxidative coupling of monomer molecules (Figure 1.5). The first stage of the reaction is the electrode oxidation of monomer molecules yielding radical cations with the radical state delocalised over the pyrrole ring (B).

The radical cations dimerise (rate-controlling stage of the reaction) and expel two protons (Genies et al, 1983; Kim et al, 1995; Beck et al., 1990). The dimers, owing to stronger conjugation, are more readily oxidised under the given reaction conditions than the monomer. The chain growth proceeds by addition of a newly formed radical cation to an oligomeric one. Some authors deny the possibility of dimerisation of radical cations because the latter experience strong Coulombic repulsion. (Asavapiriyanont et al., 1984; Scharifker et al., 1991, Rodriguez et al., 1987, Marcos et al., 1987).

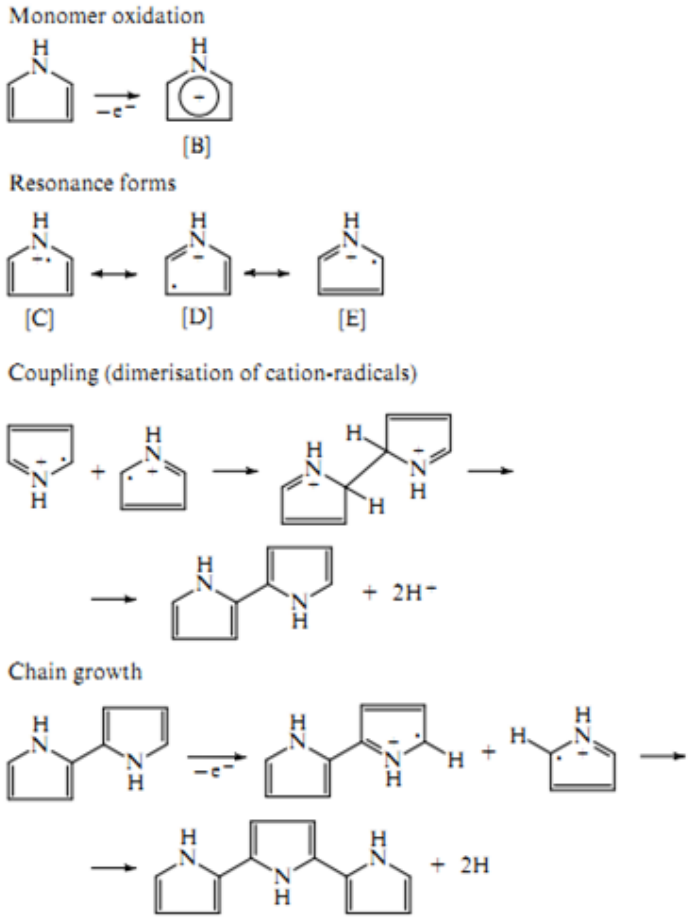


Figure 1.5 Oxidative polymerization mechanism of polypyrrole (Vernitskaya and Efimov,1997)

The alternative mechanism proposed for polymerisation of pyrrole is the free radical reaction (Asavapiriyanont et al., 1984; Rodriguez et al., 1987). This mechanism implies that formation of a radical cation on the anode is followed by the loss of a proton and attack of the radical on a neutral monomer. After reoxidation of the dimeric radical and proton loss, the dimeric molecule can experience subsequent oxidation, which results in chain growth.

The release of protons in the course of oxidation of pyrrole was observed experimentally (Raymond and Harrison 1993). Moreover, the polymer film can be coated directly on the metal wires (such as Pt) which have better mechanical strength than silica fibers. The electrochemical polymerization of pyrrole in aqueous solutions has offered the possibility for a large number of anions to be applied as dopants (Warren and Anderson, 1987). Recently, Pawliszyn and co-workers synthesized PPy on the surface of metal wires by a potentiostatic method and applied this film for SPME of some alcohols in gas phase (Pawliszyn, 1999). Thermal stability of this film was reported to be about 200°C. Obviously the lower thermal stability of the film restricts the range of applications of this film in on-fiber SPME.

It was reported that the thermal stability and mechanical property of PPy films could be greatly influenced by parameters such as type of the dopant ion, electrochemical conditions and reaction medium (Nalwa , 1997.). Therefore, the size, geometry and charge of counter ions have a significant effect on the property of PPy films.

PPy was synthesized in the presence of different dopants such as chloride (0.1 M), perchlorate (0.1 M), acetate (0.1 M), sulfate (0.1 M), and dodecylsulfate (0.007 M) ions in aqueous medium under same electrochemical condition. Polymer films doped with chloride, perchlorate, and acetate anions showed weak adhesive on the Pt wire and were unstable in high temperatures (>200^{o}C). It was reported that the fibers coated with dodecylsulfate-doped PPy were stable in high temperatures (up to 300^{o}C) and good adhesive of film to different wires were observed (Alizadeh, 2007). Some of the applications of polypyrrole fiber coating are given in Table 1.8.

Table 1.8 SPME application of polypyrrole fiber coating

Reference	Fiber Coating	Support	Target analytes	LOD	Technique
Pawliszyn et al., 2001	Polypyrrole (PPy) and poly-N-phenylpyrrole (PPPy)	Pt, Au or stainless steel wire	Methanol, acetone, hexane, tetrahydrofuran, benzene, toluene, ethylbenzene, p-xylene		GC-FID
Pawliszyn et al, 2002	Polypyrrole	Platinum wire	Sodium methylarsonate, Glutamate, perchlorate, Dopamine	ClO_4^-: 100 $ngmL^{-1}$ Dopamine: 50 ng/mL	FIA HPLC-MS
Liljegren and Nyholm, 2003	Polypyrrole	Gold microarray electrode	Chloride	10 μM	FIA
Wu and Pawliszyn, 2004	polypyrrole (PPy)	Platinum wire	methanol, acetone, hexane, tetrahydrofuran, benzene, toluene, ethyl-benzene and p-xylene, ethylamine, acetonitrile, dichloromethane, hexane, benzene, toluene, ethylbenzene and p-xylene, methanol, ethanol, 2-propanol, 1-propanol, 2-butanol, 1-butanol, 2-pentanol, 1-pentanol, 2-hexanol and 1-hexanol		FIA HPLC-MS GC-FID
Alizadeh et al., 2005	Dodecylsulfate-doped polypyrrole (PPy-DS)	Platinum wire	naphthalene, acenaphthylene , acenaphathene , flurene , phenanthrene , fluoran-thene , pyrene, chryzene	0.05-0.16 ng/mL	HS-SPME GC-FID GC-MS
Alizadeh et al.,2007	Dodecylsulfate-doped polypyrrole (PPy-DS)	Platinum wire	2-methylphenol, 3-methylphenol, 4-methylphenol, 2,6-dichlorophenol, 2,4,5-trichlorophenol, 2,3,4,6-tetrachlorophenol, 2-(1-methylpropyl)-4,6-dinitrophenol hexachlorophene	0.57-1.82 ng/mL	HS-SPME GC-FID
Mollahosseini and Noroozian, 2009	polypyrrole (PPy) doped with polyphosphate	Stainless Steel wire	lindane, heptachlor, aldrin, p,p-DDE, dieldrin, endrin, endosulfan, p,p-DDD, o,p-DDD and p,p-DDT	0.015-0.66 pg/mL	GC-ECD
Buszewski et al., 2010a	Polypyrrole	Stainless Steel wire	metoprolol, propranolol, oxprenolol, mexiletine, propaphenon (salt forms)	0.11-0.18 ng/mL	HPLC-MS
Mundkowski et al., 2010	polypyrrole (PPy)	Stainless Steel wire	Linezolid, daptomycin, and moxifloxacin		HPLC HLM
Buszewski et al., 2010b	polypyrrole (PPy) and polythiophene (PTh)	Stainless Steel wire	metoprolol, oxprenolol, mexiletine, propra-nolol, and propaphenon	12.0-63.0 ng/mL	HPLC-UV

1.4. Cyclic Voltammetry

Electroanalytical techniques are concerned with the interplay between electricity and chemistry, namely, the measurements of electrical quantities, such as current, potential, or charge and their relationship to chemical parameters. Such use of electrical measurements for analytical purposes has found a vast range of applications, including environmental monitoring, industrial quality control, or biomedical analysis (Wang, 2006).

Electrochemical processes take place at the electrode–solution interface. The distinction between various electroanalytical techniques reflects the type of electrical signal used for the quantitation. The two principal types of electroanalytical measurements are potentiometric and potentiostatic. Both types require at least two electrodes and a contacting sample (electrolyte) solution, which constitute the electrochemical cell. The electrode surface is thus a junction between an ionic conductor and an electronic conductor. One of the two electrodes responds to the target analyte and is thus termed the indicator (or working) electrode. The second one, termed the reference electrode, is of constant potential. Electrochemical cells can be classified as electrolytic (when they consume electricity from an external source) or galvanic.

Cyclic voltammetry (CV) is the most widely used technique for acquiring qualitative information about electrochemical reactions. The power of cyclic voltammetry results from its ability to rapidly provide considerable information on the thermodynamics of redox processes and the kinetics of heterogeneous electron transfer reactions and on coupled chemical reactions or adsorption processes. Cyclic voltammetry offers a rapid location of redox potentials of the electroactive species, and convenient evaluation of the effect of media on the redox process.

Cyclic voltammetry consists of scanning linearly the potential of a stationary working electrode in a quiescent solution, using a triangular potential waveform. Depending on the information sought, single or multiple cycles can be used. During the potential sweep, the potentiostat measures the current resulting from the applied potential. The resulting current–potential plot is termed a cyclic voltammogram. The cyclic voltammogram is a compli-cated, time-dependent function of a large number of physical and chemical parameters.

Figure 1.6 illustrates the expected response of a reversible redox couple during a single potential cycle. It is assumed that only the oxidized form O is present initially. Thus, a negative-going potential scan is chosen for the first half-cycle, starting from a value where no reduction occurs. As the applied potential approaches the characteristic E^o for the redox process, a cathodic current begins to increase, until a peak is reached. After traversing the potential region in which the reduction process takes place (at least 90/n mV beyond the peak), the direction of the potential sweep is reversed. During the reverse scan, R molecules (generated in the forward half-cycle, and accumulated near the surface) are reoxidized back to O, resulting in an anodic peak.

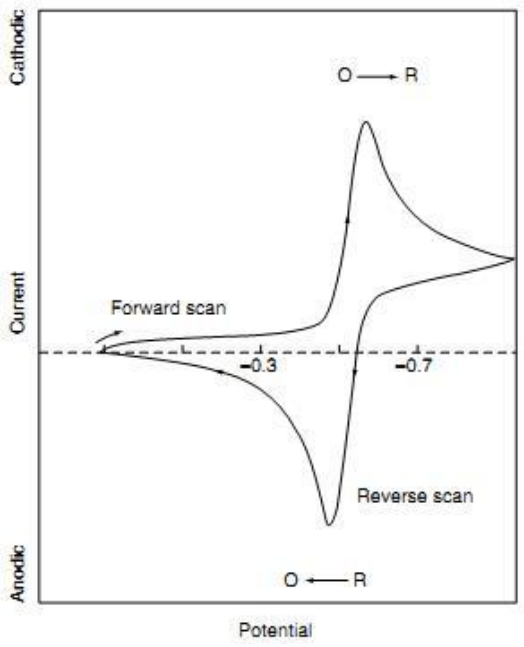


Figure 1.6 Typical cyclic voltammogram for a reversible $O + ne^- \leftrightarrow R$ redox process (Wang, 2006)

1.5. Gas Chromatography (GC)

Chromatography is a physical method of separation in which the components to be separated are distributed between two phases, one of which is stationary while the mobile phase moves in a definite direction (Poole, 2003). Chromatography is the most commonly used procedure in contemporary chemical analysis and the first configuration of chromatography equipment to be produced in a single composite unit and made commercially available was the gas chromatograph.

Gas chromatography was invented by A. J. P. Martin who, with R. L. M. Synge, suggested its possibility in a paper on liquid chromatography published in 1941. The modern gas chromatograph is a fairly complex instrument mostly computer controlled. The samples are mechanically injected, the analytical results are automatically calculated and the results printed out, together with the pertinent operating conditions in a standard format.

In gas chromatography, the mobile phase is a carrier gas, usually an inert gas such as helium or an unreactive gas such as nitrogen. The stationary phase is a microscopic layer of liquid or polymer on an inert solid support, inside a piece of glass or metal tubing called a column. The chromatogram contains a number of peaks of various sizes rising from a baseline. The number of observed peaks is an indication of sample complexity. The gaseous compounds being analyzed interact with the walls of the column, which is coated with different stationary phases. This causes each compound to elute at a different time, known as the retention time of the compound (t_r). The comparison of retention times is what gives GC its analytical usefulness.

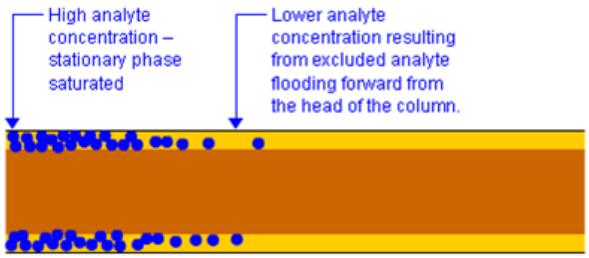


Figure 1.7 Gaseous compounds and their interaction with the walls of the column (Hinshaw and Taylor, 2009)

Most gas chromatographs consist of four units, supported by three temperature controllers and micro processor system. The first is the gas supply unit which provides all the necessary gas supplies depending on the type of detector that is chosen.

In addition the gas supply unit would be serviced by a microprocessor to monitor flow rates, adjust individual gas flows and, when and if necessary, program the mobile phase flow rate.

The second unit is the sampling unit which contains an automatic injector which is situated inside a thermostatically controlled enclosure. The injector usually has its own oven, but sometimes shares the column oven for temperature control. The injector can range in complexity from a simple sample valve, or mechanically actuated syringe to an automatic multi sampler that is microprocessor controlled.

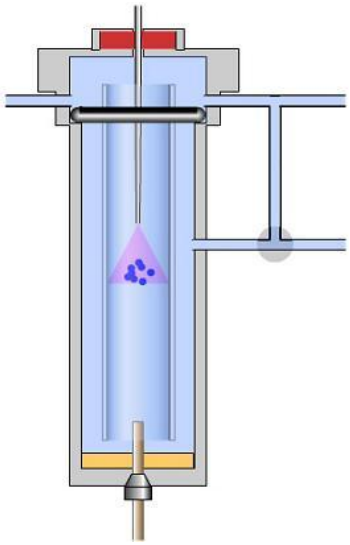


Figure 1.8 Inlet of gas chromatography (Hinshaw and Taylor, 2009)

The injection port is the interface between the environment and the separation column. It is the transfer module which brings the sample into the column and must not change the composition of the sample. Most samples are injected as liquids but there are also injection systems for gaseous samples and solids. Samples that contain volatile compounds in trace amounts are injected by head-space technique. Splitless injection means that the whole amount of injected sample will be transferred into the column. This technique is used in cases where the column will tolerate high loads of sample, e.g. for the analysis of trace components in dilute solutions. For split injections the carrier gas flow is divided so that only a partial amount will be transferred to the column. The other part will be directed out of the injector. The split ratio is usually about 1:20 to 1:100.

For this technique the sample is directly injected on the column head without using the heated injection port. After the injection the oven starts its heating ramp and the chromatographical separation begins.

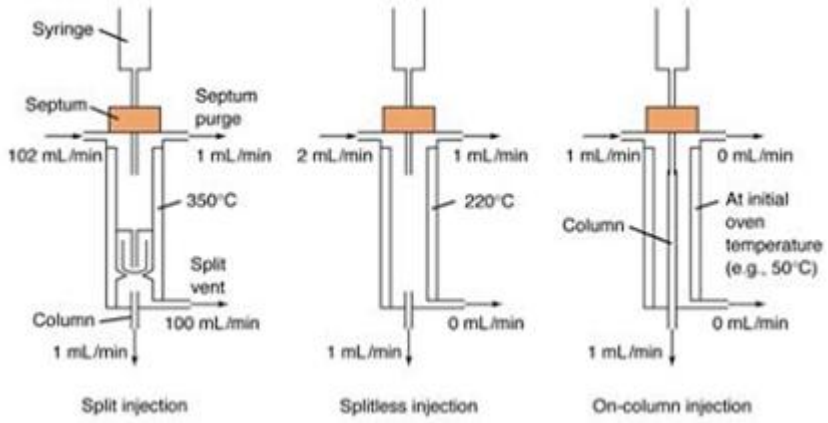


Figure 1.9 Injection systems of gas chromatography (Grant, 1996)

The sample can be introduced in a cold injector so that it will not come in contact with hot surfaces. Problems like thermal degradation, discrimination and so on can be avoided with this PTV GC-detectors measure quality differences in sample and carrier gas flow and convert this information into an electronic signal.

The third unit is the column unit which contains the column and an oven to control the column temperature. The oven also will contain a temperature sensor and a temperature programmer. The column temperature is raised continuously during development to elute the more retained peaks in a reasonable time. Modern temperature programmers contain a dedicated micro processor for the purpose.

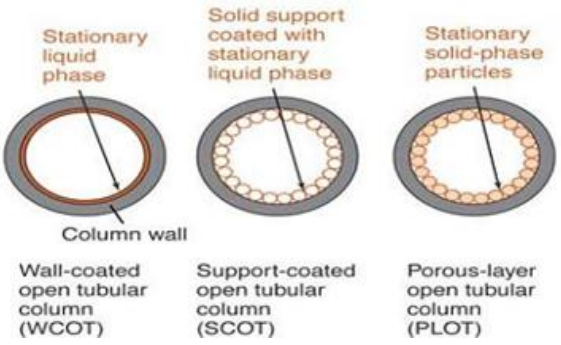


Figure 1.10 Type of open tubular columns (Grant, 1996)

The fourth unit contains the detector which is situated in its own oven. There is a wide range of detectors available each having unique operating parameters and its own performance characteristics. The detector, and the conduit connecting the column to the detector, must be maintained at a temperature at least 15^{o}C above that of the maximum temperature the oven will reach during analysis to ensure no sample condenses in the conduits or detector.

The detector oven is set at a user defined temperature and is operated isothermally, controlled by its own detector-oven temperature controller. The output from the detector is usually electronically modified and then acquired by the data processing computer which processes the data and prints out an appropriate report (Scott, 2003a).

A number of detectors are used in gas chromatography:

- catalytic combustion detector (CCD),
- discharge ionization detector (DID),
- dry electrolytic conductivity detector (DELCD),
- electron capture detector (ECD),
- flame photometric detector (FPD)
- flame ionization detector (FID)
- Hall electrolytic conductivity detector (ElCD)
- helium ionization detector (HID)
- Nitrogen Phosphorus Detector (NPD)
- Infrared Detector (IRD)
- mass selective detector (MSD)
- photo-ionization detector (PID)
- pulsed discharge ionization detector (PDD)
- thermal energy(conductivity) analyzer/detector (TEA/TCD)
- thermionic ionization detector (TID)

These detectors are classified into two main categories: destructive and non-destructive detectors. If a sample passes through the detector unchanged this will be non-destructive detection. Examples for these are the thermal conductivity detector (TCD) and the infrared detector (IRD). Examples for destructive detection are the flame ionisation detector (FID), the mass selective detector (MS) and the flame photometric detector (FPD).

In ECD, a low energy β-ray source (^{63}Ni) is used in the sensor to produce electrons and ions (Figure 1.11). The detector can function in either (direct current) DC or a pulsed mode. In the DC mode, a constant electrode potential of a few volts is employed that is just sufficient to collect all the electrons that are produced and provide a small standing current. If an electron capturing molecule enters the sensor, the electrons are captured by the molecules and the molecules become charged. The mobility of the captured electrons is much reduced compared with the free electrons and consequently, the electrode current falls dramatically.

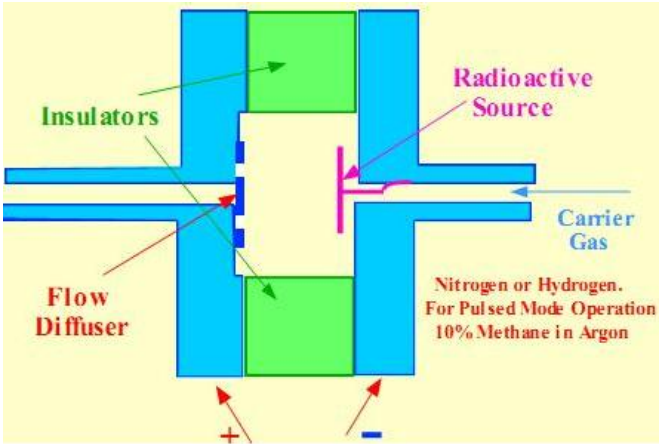


Figure 1.11 Diagram of the electron capture detector (Scott, 2003b)

In the pulsed mode of operation, a mixture of methane in argon is employed as the carrier gas. Pure argon can not be used very effectively as the carrier gas since the diffusion rate of electrons in argon is ten times less than that in a 10% methane-90% argon mixture. The period of the pulsed potential is adjusted such that relatively few of the slow negatively charged molecules reach the anode, but the faster moving electrons are all collected. During the "off period" the electrons re-establish equilibrium with the gas. The three operating variables are the pulse duration, pulse frequency and pulse amplitude. When no methane is present electron collection takes nearly 3 μs to complete. However, with 5% or 10% of methane present in the argon all the electrons are collected in less than 1 s. This reflects the increased diffusion rates of the electrons in argon-methane mixtures.

Sensor consisted of a small chamber, 1-2 mL in volume, with metal ends separated by a suitable insulator. The metal ends acted both as electrodes and as fluid conduits for the carrier gas to enter and leave the cell. The cell contained the radioactive source electrically connected to the conduit through which the carrier gas enters and to the negative side of the power supply.

A gauze "diffuser" was connected to the exit of the cell and to the positive side of the power supply. The electrode current was measured by an appropriate high impedance amplifier. This detector is extremely sensitive (10^{-13} g mL^{-1}) and is widely used in analysis of pesticides. The detector is concentration sensitive and thus the concentration of the solute for a given mass will vary with the position it is eluted in the chromatogram (Scott, 2003b).

1.6. The Aim of The Study

The main objective of this thesis is the preparation and characterization of laboratory-made SPME fiber and its usage for pesticide analysis. Since pesticides are important and diverse environmental and agricultural species, their determination in food and in complex environmental matrices is important and often requires separation methods capable of high efficiency, unique selectivity, and high sensitivity. HS-SPME technique has great advantages over the classical sampling techniques, which are time consuming and require larger samples and solvents. A number of adsorbents are available commercially as coatings for SPME fibers but special coatings that have particular volume and a selectivity towards particular analytes are being searched.

In this study it was aimed to develop SPME fibers in laboratory conditions to reduce cost and improve the selectivity as well as their duration. Electrochemical polymerization is the method of choise for fiber coating. Among various conducting polymers studied, polypyrrole (PPy) and its derivatives have been one of the most widely used classes of conducting polymers for the past decades. PPy is expected to show different extraction efficiencies towards compounds with different functional groups.

In this thesis, a number of pesticides commonly used in grapevine control namely, Chlorpyrifos (CP), Penconazole (PNZ), Procymidone (PRO), Brompropylate (BRP) and Lambda-Cyhalothrin (LMD) were selected for analysis in Turkish wine samples. Aldrin (ALD) was used as an internal standard to enhance the accuracy of the method. The performance of the SPME fibers prepared electrochemically was tested upon using these pesticides and the application of the method was made to Turkish wine samples.

2. EXPERIMENTAL

2.1 Apparatus

SPME fiber coating was carried out using a Palmsence Electrochemical Interface. A three-electrode system consisted of the stainless steel wire (316 type, i.d. 0.3 mm, working electrode), a platinum wire (auxiliary) electrode and a Ag/AgCl electrode as reference electrode was used for all CV measurements. Functional groups of PPy-DS were identified by Perkin Elmer Pyris FTIR spectrophotometer. Thermal decomposition study of PPy-DS was carried out by using Perkin Elmer Diamond TG/DTA analyzer. Philips XL30 SFEG was used for SEM measurements. The SPME holder for manual sampling was obtained from Supelco (Bellefonte, PA, USA). A heating magnetic stirrer, purchased from IKA-RCT (Germany) was used with a 10 mm stirring bar. Heraeus RT500 stove was used for fiber condition. Transsonic 460/H was used for ultrasonic treatments.

Separation and quantification of pesticides were carried out using a Agilent 6890 gas chromatograph equipped with a electron capture detector and a DB-1 (100% Dimethylpolysiloxane) fused silica capillary column (30 m x 0.32 μm I.D. and 0.25 μm film thickness, J&W Scientific, Folsam, CA). Helium and nitrogen (99.999%) were used as carrier and make-up gas, respectively.

2.2 Reagents

All reagents were of analytical reagent grade. Aquaeous solutions were prepared with ultra pure water (18.2 MΩ cm^{-1}) from a MilliPore Milli-Q Gradient water purification system. Pyrrole, obtained from Alfa-Aesar (98%), was purified by distillation under reduced pressure and stored in a refrigerator at about 4°C before use. Sodium chloride, sodium dodecylsulfate (SDS) (98%) and ethanol (99.9%). were purchased from Merck. Acetone (99.8%) was obtained from Lab-Scan Analytical Scienses.

Pesticides which are namely 2,3,4,10,10-Hexachloro-1,4,4a,5,8,8a-hexahydro-1,4:5,8-dimethanonaphthalene (Aldrin) (97%), O,O-Diethyl O-3,5,6-trichloropyridin-2-yl phosphorothioate (chloropyrifos) (98.4%), 1-[2-(2,4-dichloro phenyl)-n-pentyl]-1H-1,2,4-triazole (penconazole) (99.2%), isopropyl-4,4'-dibromobenzilate (bromopropylate) (97.5%), 3-(3,5-dichlorophenyl)-1,5-dimethyl -3-azabicyclo[3.1.0]hexane-2,4-dione (procymidone) (98%), 3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethyl-cyano(3-phenoxyphenyl) methyl cyclopropane carboxylate (lambda- cyhalothrin) (98.5%) pesticide standards were purchased

from Dr. Ehrenstarfer GmbH. A stock solution of these pesticides were prepared in ethanol and stored at 4°C for use.

2.3. Procedures

2.3.1. Fiber coating

Polypyrrole dodesile sulphate doped (PPy-DS) film was prepared electrochemically using a three electrode system. According to a former study, PPy-DS film was directly electrodeposited on the surface of stainless steel wire as working electrode from an aqueous solution contains 0.1 M pyrrole and 7.0 x 10^{-3} M SDS by using cyclic voltametry technique (Alizadeh et. al., 2005). Prior to electropolymerization, the solution was deoxygenated by purging with N_2 for five min and the stainless steel surface was cleaned in acetone using ultrasonic bath for 15 min and was subsequently washed with distilled water. The CV technique was operated using a scan rate of 20 mV s^{-1} at potential range of 0.5–1.2 V and the number of scans was set at 10 cycles

2.3.2. Pretreatment of SPME fiber

After the metal wire coated with PPy-DS film was washed with methanol, acetone, and water respectively, dried under nitrogen gas flow. Then it was heated at 100^{o}C for 20 min in oven and connected to SPME holder. Before it was used for SPME experiments the fiber conditioned at 200^{o}C in a GC injection port under helium gas for an hour until a clear blank was obtained.

2.3.3. Characterization of SPME fiber

The thickness and surface morfology of SPME fiber were characterized according to the SEM study. The surface of the PPy-coated stainless steel wire was cut into a 2 cm long piece and then analyzed on a SEM (7.0 kV accelerating potential) with different magnification. Thermal stabilty of the fiber were analyzied by TGA by using 5 mg of sample in air flow (100 mL min^{-1}) at a heating rate of 10^{o}C min^{-1}.

2.3.4. HS-SPME procedure

To examine the extraction capabilities of the PPy-DS fibers, aquoeus solutions spiked with six organochlorine pesticides including aldrin (ALD) , chloropyrifos (CP), penconazole (PNZ), bromopropylate (BRP), procymidone

(PRC), lambda- cyhalothrin (LMD) were extracted with PPy fibers using the HS-SPME mode. Because of the bad reproducibility of HS-SPME process, ALD was used as an internal standard in analysis.

10 milliliters of 12.0% ethanol solution containing the target pesticides were placed in a 20 mL glass vial with a PTFE-silicon septum. After the addition of sodium chloride and magnetic stirring bar, the vial was tightly sealed with an aluminum cap to prevent sample lossing due to evaporation. The vial was placed in two-compartment recirculation cell for 5 min before HS-SPME, to reach equilibrium. The PPy-DS fibers housed in manual SPME holder were used. When the plunger was retracted, the wire with coating was drawn into the syringe needle, thus protecting the PPy-DS coating while the syringe needle was used to pierce the septum during extraction and desorption. The PPy-DS fiber was exposed to the headspace over the stirring liquid sample for 5-45 min, depending on the experiment. After completion of sampling step, the fiber was withdrawn into the needle and removed from the sample vial. The fiber was then immediately inserted into the injection port of the GC.Fibers were conditioned prior to use by inserting it into the GC injection port for 5 min.

2.3.5. GC measurements

The injection port and detector were operated at 200^{o}C and 300^{o}C, respectively. Fiber was introduced into the chromatographic columns using splitless mode injection. SPME fiber inserted to GC injection port and GC analysis was started manually. The GC split valve was closed for 5 min, and helium was used as carrier gas with a flow rate of 40.0 mL min^{-1}. The flow rate of carrier gas was adjusted at 1.5 mL min^{-1}. The separation of pesticides on GC-ECD was performed by a temperature program as follows: 50^{o}C for 2 min increased to 150^{o}C at a rate of 10^{o}C min^{-1} and increased to 200^{o}C at a rate of 2^{o}C min^{-1} and increased to 280^{o}C at a rate of 8^{o}C min^{-1}. After GC analysis the fiber was inserted to GC injection port for 15 min to remove retained pesticide from the fiber surface.

3. RESULTS AND DISCUSSION

3.1 Polymer Film Preparation

In former studies, it was reported that the thermal stability and mechanical property of PPy films could be greatly influenced by parameters such as type of the dopant ion, electrochemical conditions and reaction medium (Nalwa, 1997.). Polymer films doped with chloride, perchlorate, and acetate anions showed weak adhesive on the Pt wire and were unstable in high temperatures (>200^{o}C). Since these fibers could be easily stripped from Pt wire surface during extraction process, the application of these coatings was not examined in this thesis.

It was also reported earliar that the fibers coated with dodecylsulfate-doped polypyrrole (PPy-DS) were stable in high temperatures (up to 300^{o}C) and good adhesive of film to different wires were observed. This stability of the PPy coating is important in practical applications of SPME to ensure a long lifetime for the SPME fiber. Initial studies have revealed that PPy-DS had good efficiency toward the pesticide compounds. For this purpose all HS-SPME analysis of target pesticides in water samples were performed by PPy-DS coated fiber.

Since the creation of a uniform and stable coating at the SPME fiber is essential for the presicion and accuracy, electropolimerization is often method of choice as the thickness of this fiber can be controlled by the number of CV cycles. PPy-DS film was prepared electrochemically using a three-electrode system according to the procedure given before (Alizadeh et al., 2005). The aqueous solution of 0.1 M pyrrole and 7.0×10^{-3} M SDS were prepared by dissolving the samples in ultrapure water. Electrodeposition of PPy-DS film on stainless steel was performed by using CV technique between 0.5–1.2 V potential ranges at 20 mV s^{-1} scan rate. Electropolymerization was completed after 10 cycles of CV measurements (Figure. 3.1).

Figure 3.1 shows the cyclic voltammograms recorded at stainless steel. Clear background signal was obtained in the absence of pyrrole (Figure 3.1.a). Upon addition of pyrrole to solution electrooxidation of pyrrole begins at 0.84 V and after this potential the current and electroplating increases linearly (Figure. 3.1.b). The rise of PPy-DS film thickness on stainless steel surface increases the effective surface area of electrode. Therefore, the current increasing after each cycle (Figure.3.1.b-f) shows the film growing on the electrode surface.

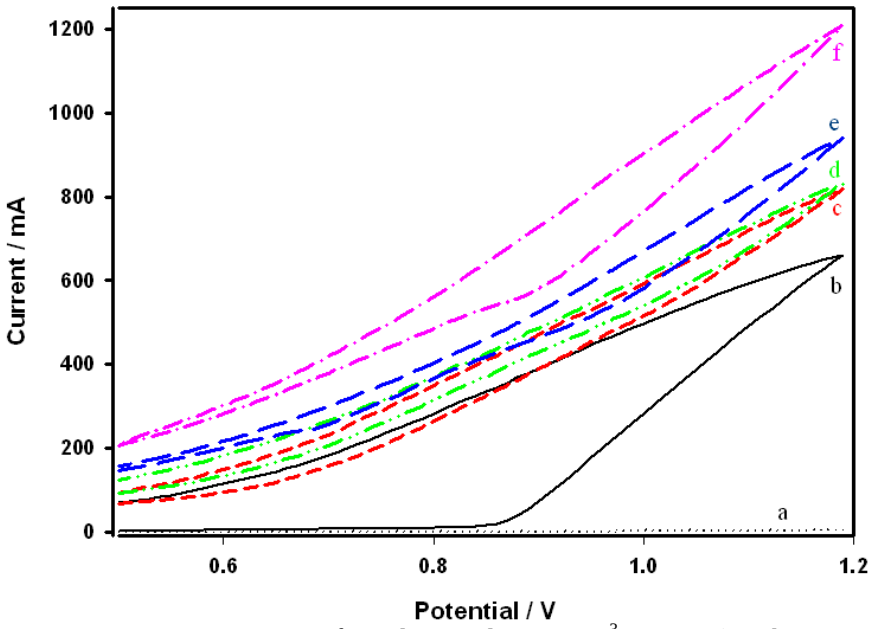


Figure 3.1 CV measurements of stainless steel in $7.0x10^{-3}$ M SDS a) without pyrrole and with 0.1 M pyrrole after b) first, ,c) second, d) fifth, e) seventh and f) tenth cycle.

3.2 Characterization of PPy-DS Film

The porosity of the structure is very important for the adsorption process, extractions occur on the active sites present on the surface. The characteristics of the surface of the polymeric film was investigated by different magnification of SEM. Figure 3.2 depicts the micrographs of PPy-DS film and shows that PPy-DS coating prepared by CV has a porous structure and the film surface is well distributed. Porous structure should significantly increase the effective surface areas of the film, therefore leading to higher extraction efficiency compared with non-porous films (Pawliszyn et al, 2001, Pawliszyn et al., 2005). The porosity of the film PPy-DS film presented higher charges in the cyclic voltammograms (Tüken, 2006).

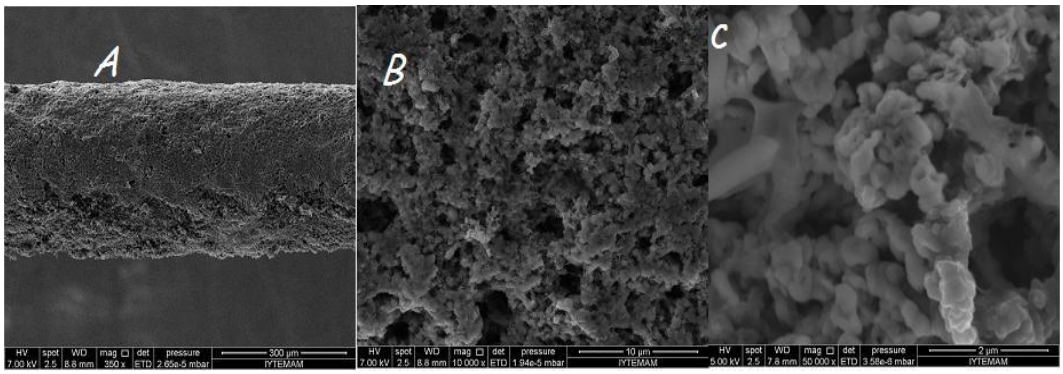


Figure 3.2 SEM images of PPy-DS coating with A) 350x B)10000x and C) 50000x magnification.

The thickness of the fiber coating can be controlled by tailoring the electrochemical conditions. The film thickness of the PPy-DS was measured from SEM images (Figure 3.3). The thickness of the PPy-DS coating from SEM measurements obtained under this condition was about 40 ± 2 μm.

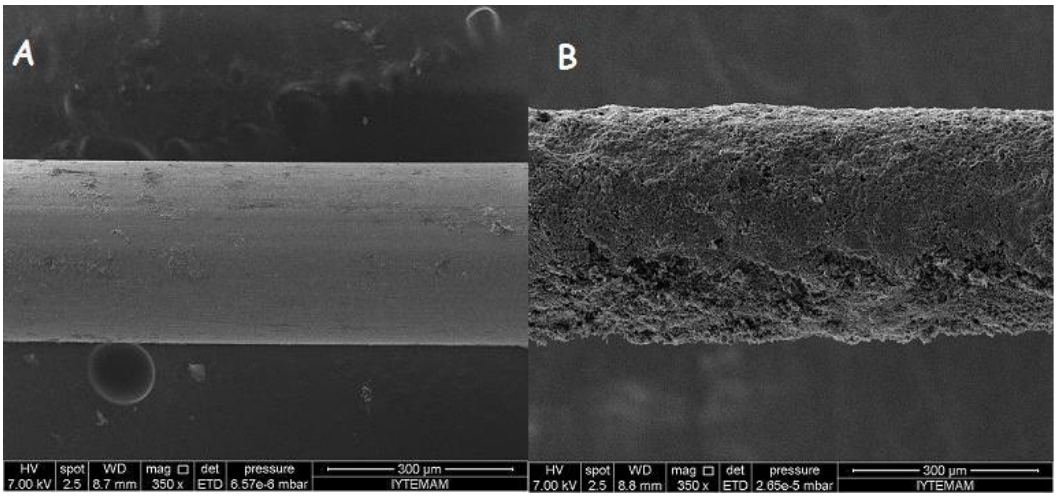


Figure 3.3 SEM images of A) stainless steel and B) PPy-DS coated stainless steel with 350x magnification.

The thermal stability of the coated film was study by TGA. Figure 3.4 presents the curves of weight loss versus temperature showing the behavior of PPy–DS coating. The first significant weight loss occurs between 30-100^{o}C. It is known that PPy-DS is hygroscopic and during the heating step, the residual water evaporates then, the main mass loss, which corresponds to the polymer degradation starts at about 200^{o}C. This result was not in agreement with the literature in which it was claimed that the fiber prepared by this procedure endures until 300^{o}C (Alizadeh et al, 2005). To prevent any possible damage of the polymer maximum desorption temperature was established as 200^{o}C.

The structural and optical properties of the samples were studied by using FTIR spectrophotometer. All spectra in the range of 450–4000 cm^{-1} with a 2 cm^{-1} spectral resolution were obtained from compressed KBr pellets in which PPy-DS powders were evenly dispersed. Two hundred scans were recorded for each FTIR spectrum. As shown in Figure 3.5, the main characteristic peaks are assigned as follows. The frequencies at 1650 and 1400 cm^{-1} were attributed to the antisymmetric and symmetric pyrrole ring vibration. The peak at 1050 cm^{-1} was ascribed to in-plane C–H stretching vibration and in-plane N–H deformation. The bands at 1000 cm^{-1} reflected the C–N stretching vibration and

the =C–H out-of-plane vibration, which implied the doping state of PPy. The peak at 650 cm^{-1} can be attributed aramotic C-H vaging.

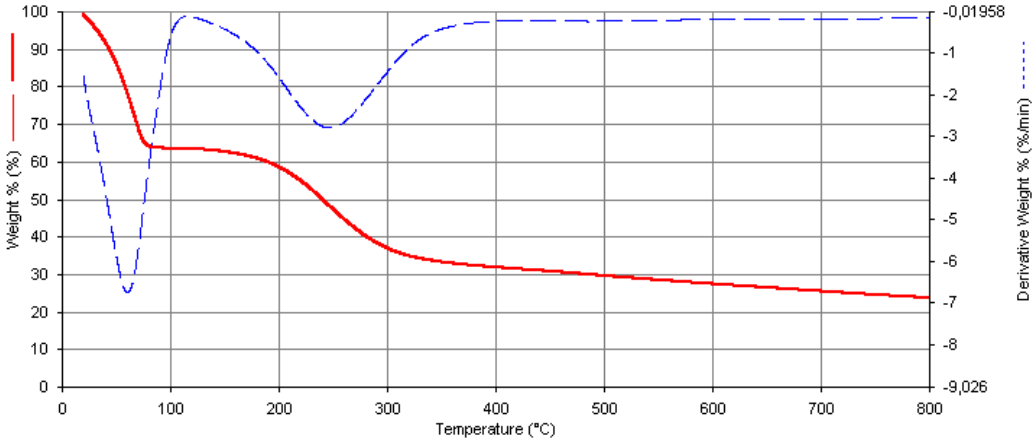


Figure 3.4 TGA curves of PPy-DS coating.

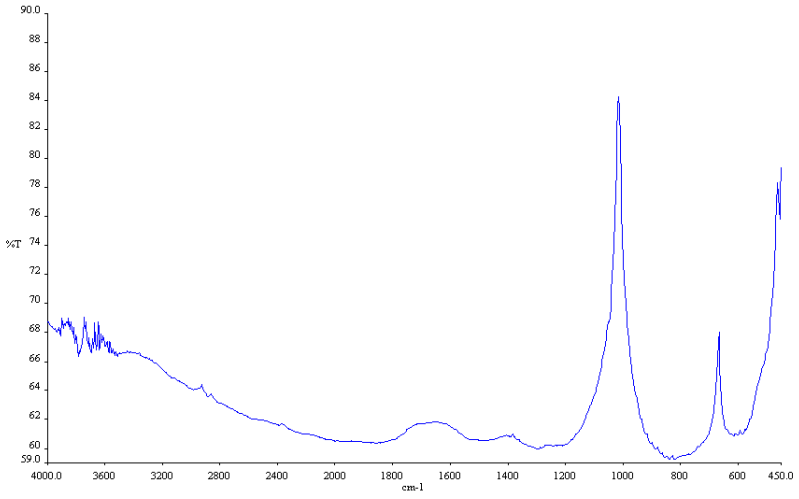


Figure 3.5 FTIR spectra of PPy-DS film.

3.3 GC Analysis of Pesticide

Typical GC–ECD chromatograms, obtained from the HS-SPME of organochlorine pesticide using PPy-DS, were similar to those obtained by direct injection of standard samples. However, some slight tailing effect, due to the low thermal desorption temperature was observed. The peaks broadening due to the desorption temperature, set at 200^{o}C, are rather acceptable, which reveals that PPy-DS desorbs the analytes in a rather convenient way. Unfortunately, using

desorption temperatures higher than 200^{o}C led to lower fiber stability and unsatisfactory and irreproducible results. The fiber could be used frequently and conveniently as long as desorption temperature not exceeded 200^{o}C.

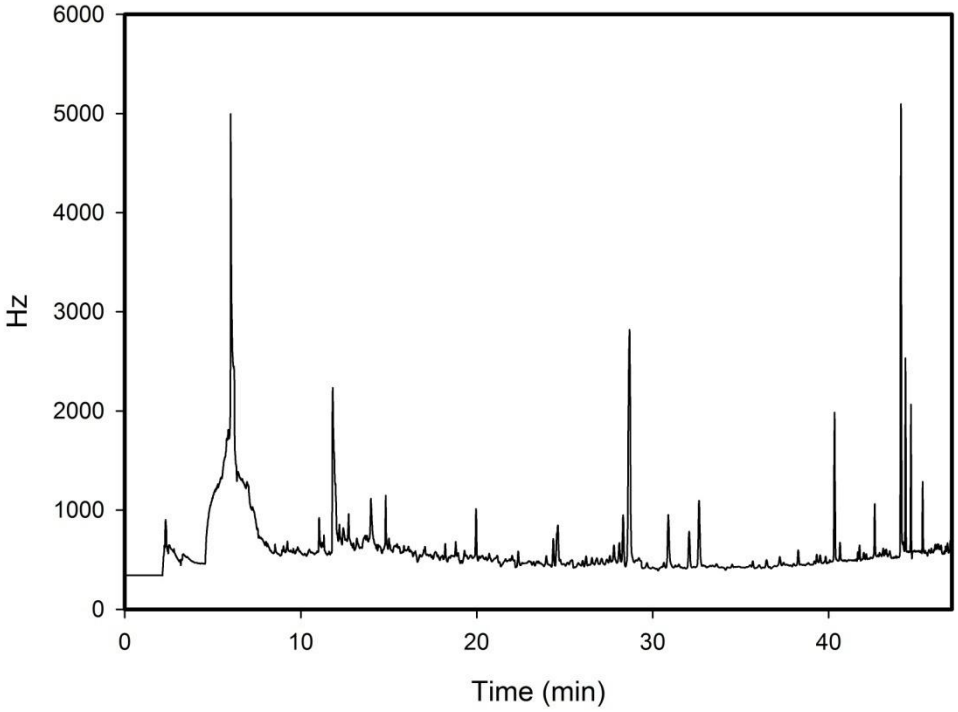


Figure 3.6 GC-ECD chromatogram of target pesticides

Table 3.1 Retention times of compounds studied by GC–ECD

Compound	**Retention time (min)**
Aldrin (ALD)	28.3
Chlorpyrifos (CP)	28.6
Penconazole (PNZ)	30.8
Procymidone (PRC)	32.0
Bromopropylate (BRP)	42.6
Lambda-cyhalothrin (LMD)	44.4; 44.7

3.4 HS-SPME of Pesticides Compounds

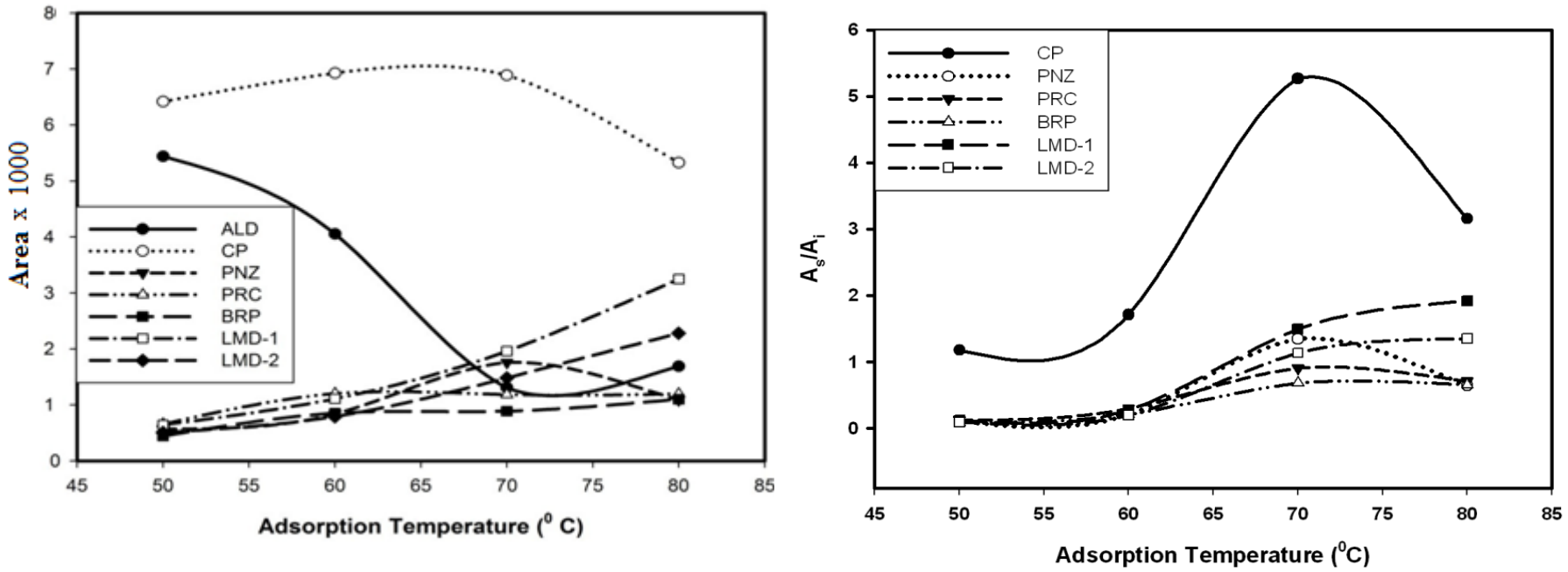
HS-SPME method was used to extract the target pesticide for the analysis. In order to extend the application of PPy-DS, a mixture of six organochlorine pesticides including: chlorpyrifos (CP), penconazole (PNZ), procymidone (PRC), brompropylate (BRP) and lambda-cyhalothrin (LMD) were tested by coupling SPME to GC-ECD. ALD was used as internal standart. The SPME of these selected analytes was performed from the headspace of water solution spiked with pesticide standard solution.

HS-SPME was performed with the synthesized PPy-DS fiber, mounted in its SPME holder. In all experiments, 10 mL of sample volume in a 20 mL vial placed on a magnetic stirrer was used. During the headspace extraction, the aqueous samples were continuously stirred with a magnetic stir bar. The extraction temperature was controlled using a hot-plate. Thermal desorption of adsorbed pesticide on fiber was carried out at GC injection port.

3.4.1. Adsorption temperature

The extraction temperature is quite important parameter in HS-SPME since it can affect the efficiency of the extraction. At elevated temperatures the analytes can effectively dissociate from the matrix and move into the headspace for rapid extraction by the fiber coatings. This part of work was carried out using a temperature range of 50-80^{o}C. Figure 3.7 represents the effect of solution temperature on the extraction ability of pesticides mixture, obtained by plotting the peak area alone and peak area ratio of pesticide (A_p) / internal standard (A_i) as a function of temperature.

As can be followed from the Figure.3.7, the peak areas of some of the pesticides have shown an increase with the temperature. However, the signal for ALD has shown a dramatic decline with the temperature and then, has become nearly the same in height in the range of 70-80^{o}C. The signals of CP and PNZ have shown a decrease after 70^{o}C. As shown in Figure 3.7 B, the signal ratios of all pesticides increased until 70^{o}C and then, decreased at 80^{o}C, except LMD. This effect can be attributed to the leakage of the pesticides at elevated temperature during the extraction process. The size of this leakage might be related to magnitude of the volatility of pesticides. The vapor pressures of the selected pesticide (CP > PNZ > PRC > BRP) were given in Table 3.2.



(A) **(B)**

Figure 3.7 Effect of adsorption temperature on the (A) peak area and (B) peak area ratio of (A_p) / (A_i). Conditions: pesticide mix concentration: 0.8 ng mL^{-1} CP, 150 ng mL^{-1} PNZ, 100 ng mL^{-1} PRC, 10.0 ng mL^{-1} BRP, 10.0 ng mL^{-1} LMD, sample volume: 10 mL, NaCl: 10 g L^{-1}, adsorption time: 15 min., stirring speed: 600 rpm, desorption temperature: 200^{o}C.

Table 3.2 Vapor pressure of selected pesticides.

Pesticide	Vapor Pressure at 25°C (mPa)
ALD	10
CP	2.5
PNZ	0.37
PRC	0.023
BRP	0.011
LMD	negligible

Relatively more volatile pesticides such as CP, PNZ have been reduced in size accordingly. Because of the low volatility of LMD, the signal loss was negligible and the extraction efficiency increased with increasing temperature, up to 80°C, due to the enhanced distribution constant of analytes between the aqueous phase and headspace. Considering the signal loss for some of the pesticides, the extraction temperature was selected as 70°C.

3.4.2. Desorption temperature

In this thesis, desorption of the pesticides adsorbed on the SPME fiber was performed in the GC injection port and the desorbed analytes were directly carried to the capillary column for analytical separation. For thermal desorption, a narrow bore (1.0 mm i.d.) unpacked injection liner was used to ensure a high linear carrier gas flow, to reduce desorption time and prevent peak broadening. Figure 3.8 shows the effect of desorption temperature on the peak areas of the pesticides.

Desorption time has a considerable effect on the amount of analytes desorbed from the fiber polymer. This parameter was selected as 5 min, by leaving the fiber in the injection port of GC while the injector temperature maintained at selected temperature. However, some of the selected pesticides retained on the fiber surface after desorption. Figure 3.9 show the chromatograms recorded by using the same fiber after desorption step. Some of the peaks disappeared but, some still remained even after second injection of the fiber. Therefore, it is necessary to increase the desorption temperature but, the stability of the fiber does not allow us to use temperature higher than 200°C. Besides, all the residual pesticides can be removed from fiber after third desorption step.

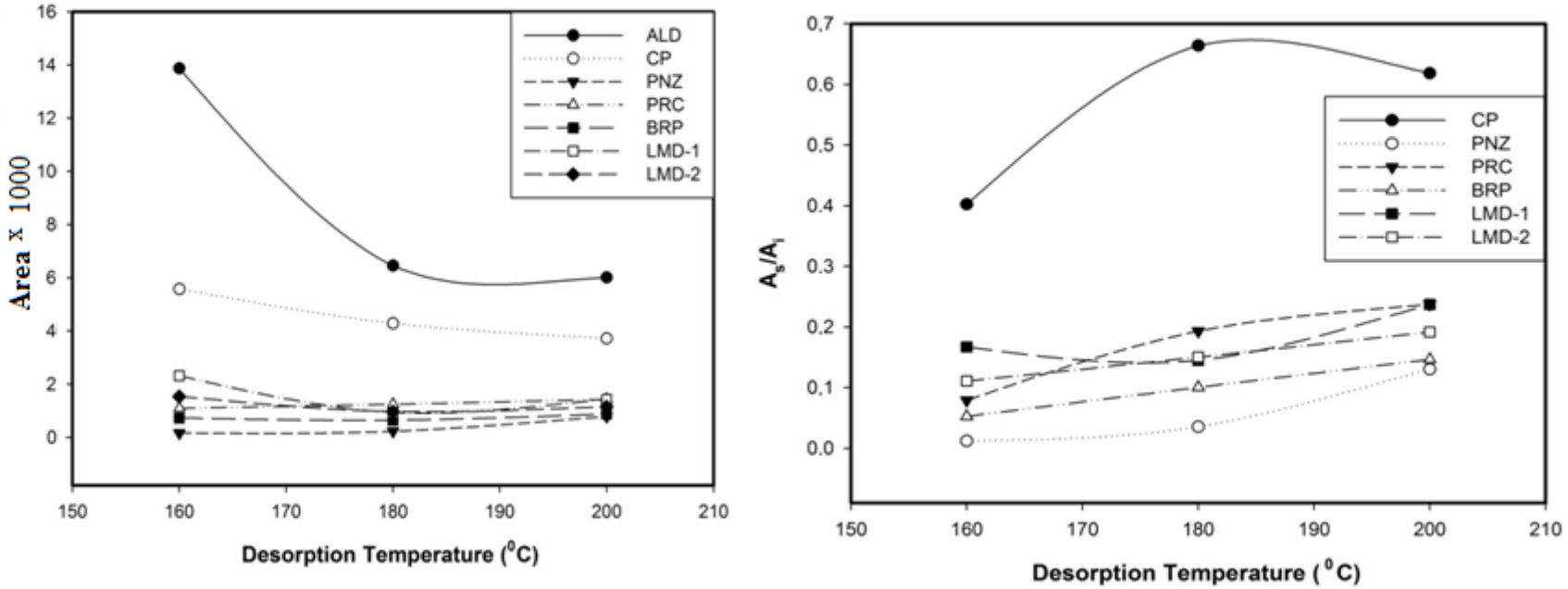


Figure 3.8 Effect of desorption temperature on the (A) peak area and (B) peak area ratio of (A_p) / (A_i). Conditions: pesticide mix concentration: 0.8 ng mL^{-1} CP, 150 ng mL^{-1} PNZ, 100 ng mL^{-1} PRC, 10.0 ng mL^{-1} BRP, 10.0 ng mL^{-1} LMD, sample volume: 10 mL, NaCl: 5 g L^{-1}, adsorption temperature: 70°C, adsorption time: 15 min., stirring speed 600 rpm.

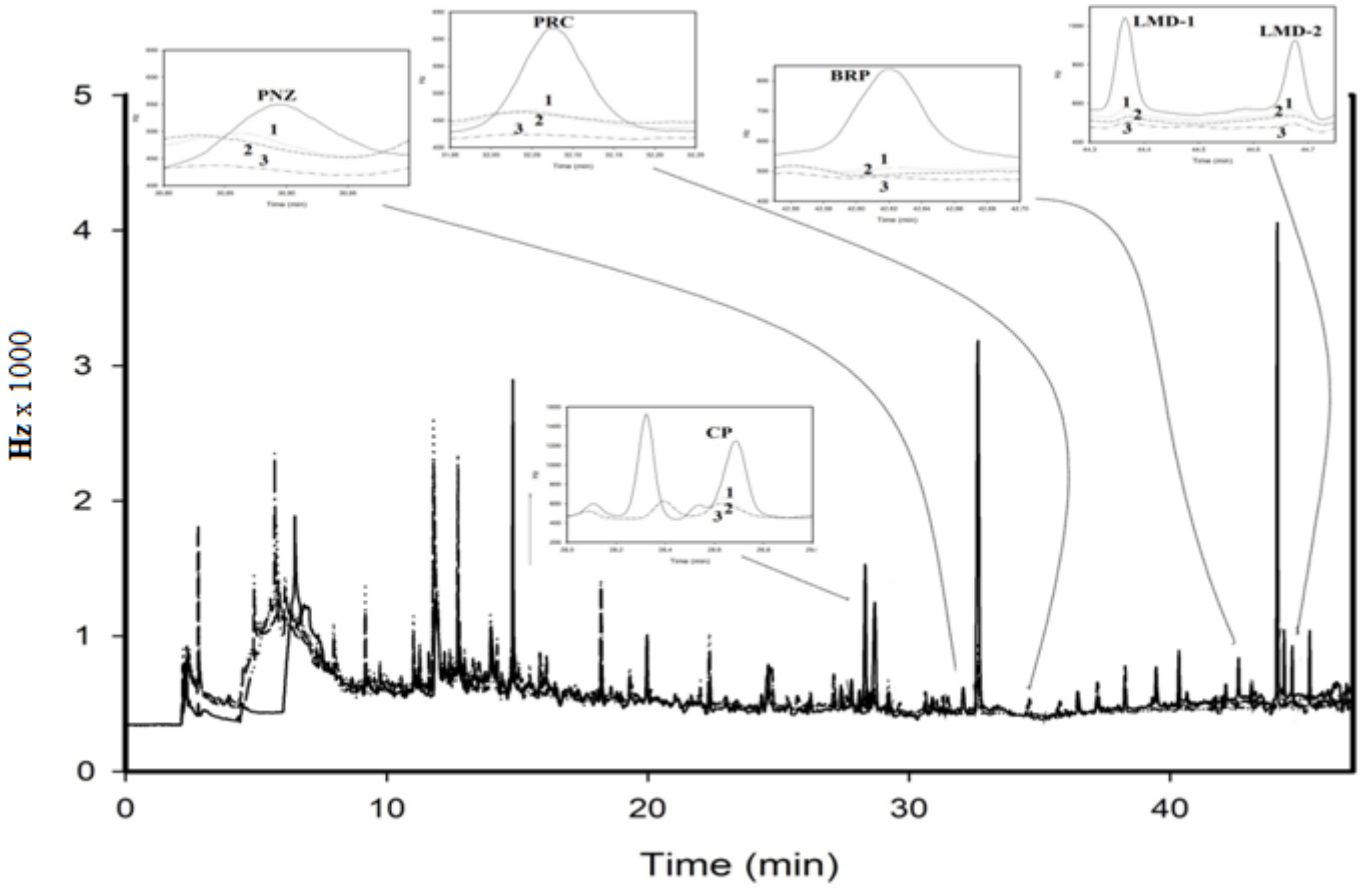


Figure 3.9 Repetitive chromatograms of the SPME fiber. Conditions: pesticide mix concentration: 00.8 ng mL^{-1} CP, 150 ng mL^{-1} PNZ, 100 ng mL^{-1} PRC, 10.0 ng mL^{-1} BRP, 10.0 ng mL^{-1} LMD,sample volume: 10 mL, NaCl: 10 g L^{-1}, ads.temperature: 70^{o}C, ads. time: 45 min., stirring speed 600 rpm, desorption temperature: 200^{o}C.

Consequently reconditioning procedure necessitates in between two consecutive analyses. To remove retained pesticide from fiber surface it conditioned at 200^{o}C for 15 min before next adsorption process.

3.4.3. Stirring rate

Magnetic stirrer is widely used for agitation in HS-SPME. The extraction efficiency is significantly affected by the stirring speed. It is generally accepted that the reduction of the diffusion layer is essential in order to reach equilibrium faster, which is easily achieved by sample agitation (Mohammadi, 2005). Although the equilibration time progressively decreases with increasing agitation rate, faster agitation tends to be uncontrollable and the rotational speed might cause a change in the equilibration time resulting poor measurement precision. Figure 3.10 shows the effect of strirring rate on the peak area and peak area ratios for the pesticides studied. As the peak ratios give a maximum at 600 rpm, this rate was chosen for further studies.

3.4.4. Ionic strength

For many organic analytes, aqueous solubility decreases with increasing ionic strength, and thus, the partitioning from the aqueous solution to the head space is improved. To raise the ionic strength, an inorganic salt is often added to the aqueous matrix. The addition of NaCl to the sample increases the efficiency of the extraction for all analytes. Figure 3.11 shows the effect of salt amount on the final signals of the pesticides. Peak areas and peak ratios have given a maximum at 10 g L^{-1} NaCl and this amount of salt was used for further studies.

3.4.5. Adsorption time

HS-SPME is an equilibrium-based technique and there is a direct relationship between the amount extracted and the adsorption/extraction time. Optimal time was determined by varying the contact time between the fiber and a standard aqueous solution between 0 and 60 min. Figure 3.12 shows the extraction time profiles for the studied pesticides from aquatic samples. For CP, ALD and LMD there is only a small increase in response after the initial 20 min, but for BRP and the higher extraction times led to greater extraction efficiencies. Therefore, an extraction time of 45 min was selected to shorten the analysis time.

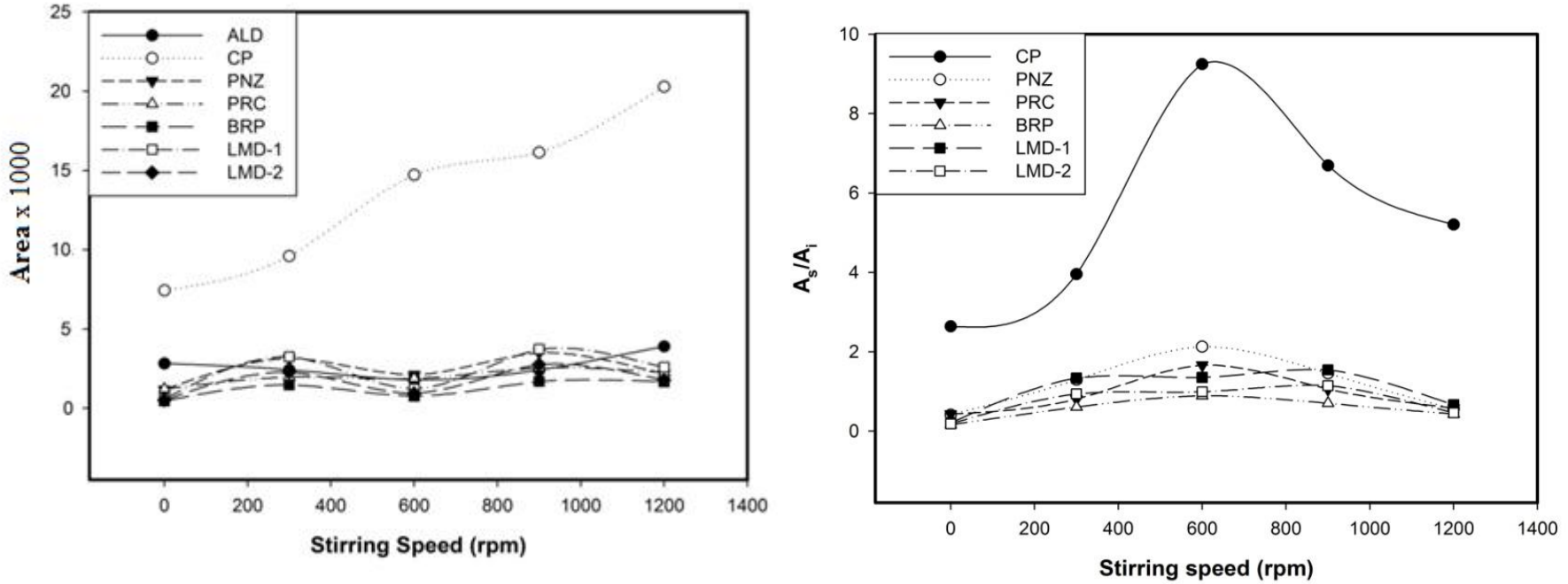
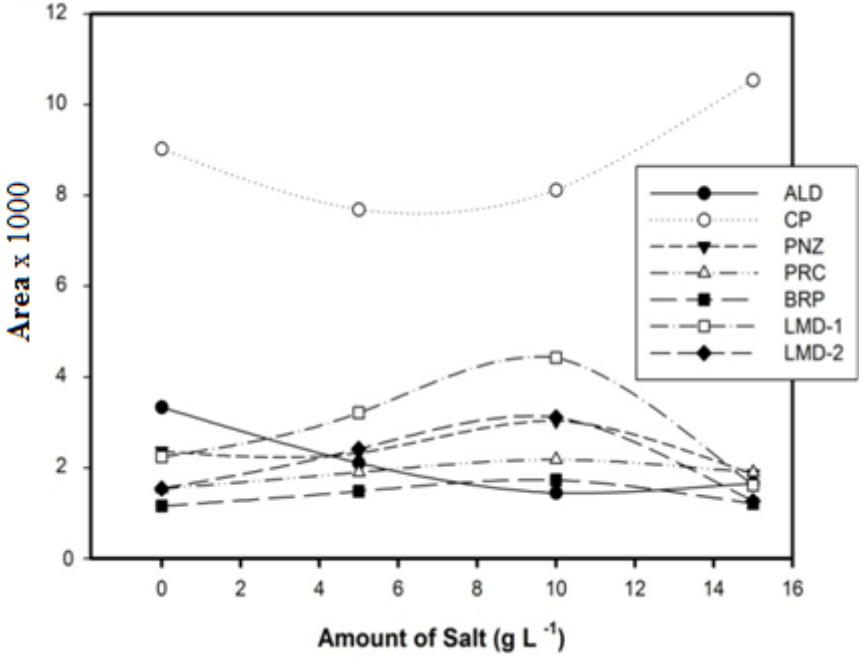
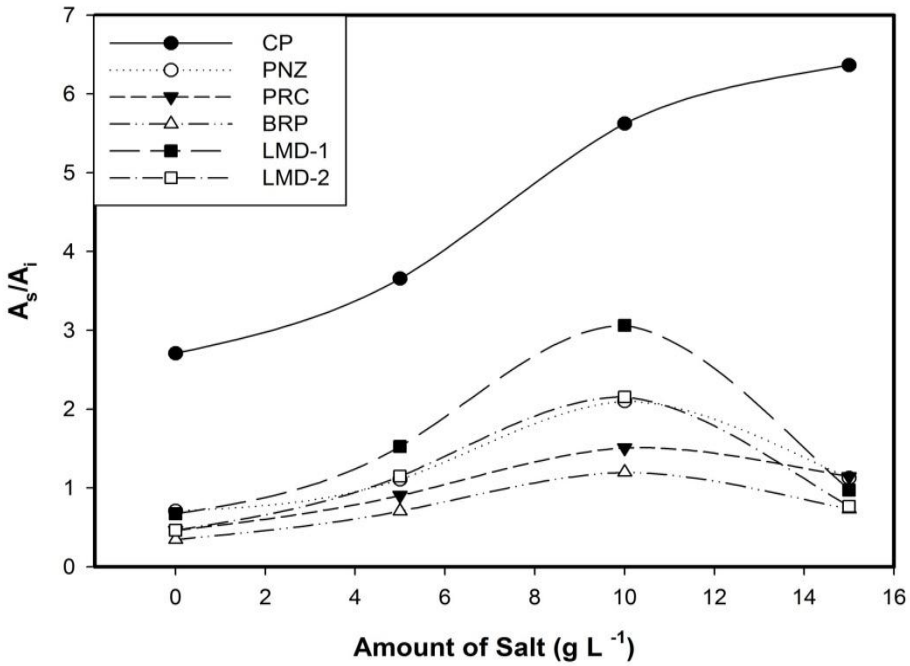


Figure 3.10 Effect of the stirring speed on the (A) peak area and (B) peak area ratio of $(A_p) / (A_i)$. Conditions: pesticide mix concentration: 0.8 ng mL^{-1} CP, 150 ng mL^{-1} PNZ, 100 ng mL^{-1} PRC, 10.0 ng mL^{-1} BRP, 10.0 ng mL^{-1} LMD, sample volume: 10 mL, NaCl: 10 g L^{-1}, adsorption temperature: 70°C, adsorption time : 15 min., desorption temperature: 200°C

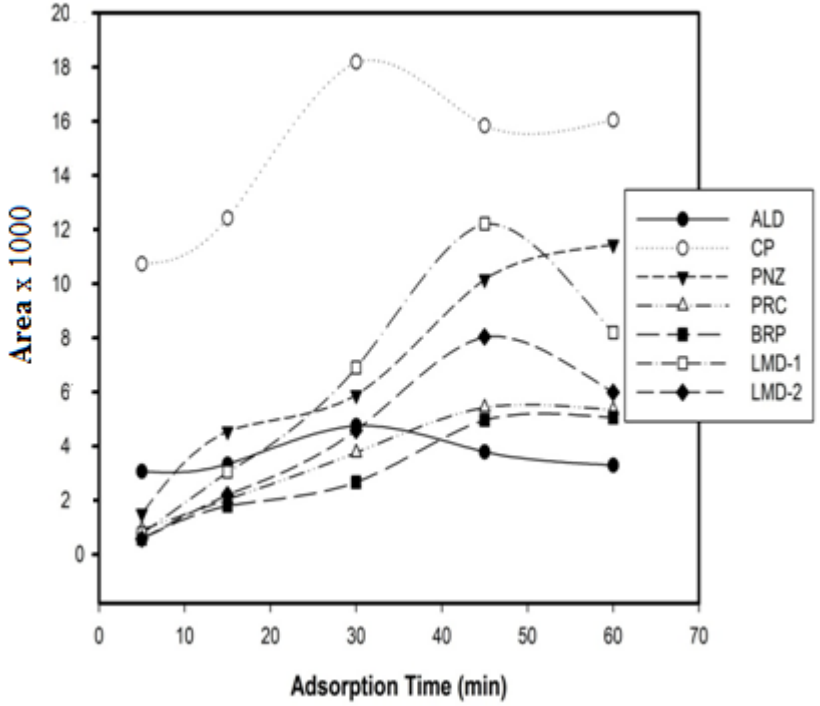


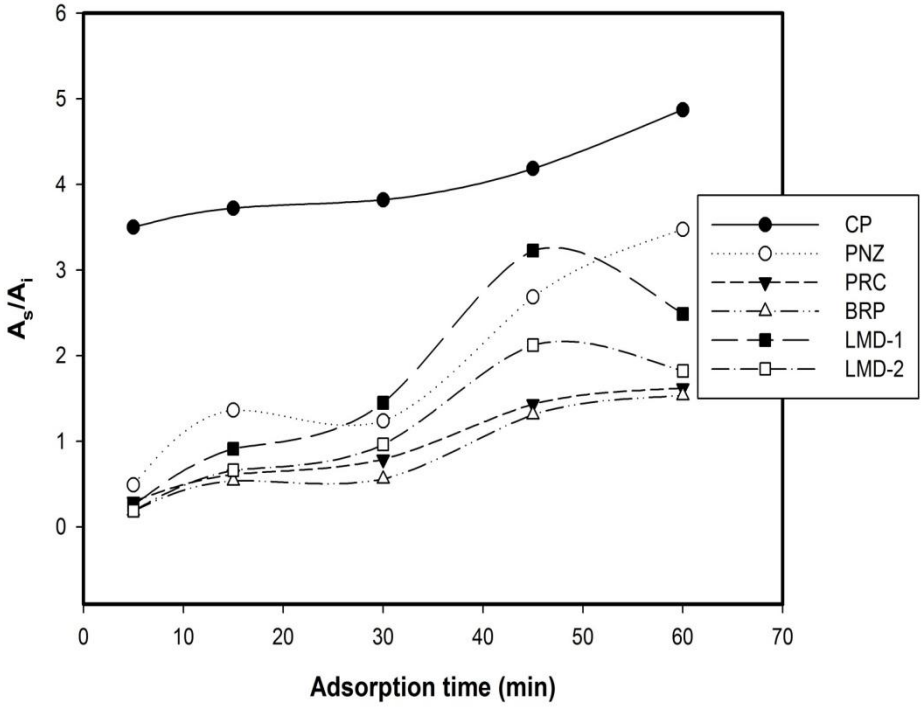
(A)



(B)

Figure 3.11 Effect of the amount of salt on the (A) peak area and (B) peak area ratio of (A_p) / (A_i). Conditions: pesticide mix concentration: 0.8 ng mL^{-1} CP, 150 ng mL^{-1} PNZ, 100 ng mL^{-1} PRC, 10.0 ng mL^{-1} BRP, 10.0 ng mL^{-1} LMD, sample volume: 10 mL, adsorption temperature: 70^{o}C, adsorption time: 15 min., stirring speed: 600 rpm, temperature: 70^{o}C, desorption temperature: 200^{o}C





(A) **(B)**

Figure 3.12 Effect of adsorption time on the (A) peak area and (B) peak area ratio of (A_p) / (A_i). Conditions: pesticide mix concentration: 00.8 ng mL^{-1} CP, 150 ng mL^{-1} PNZ, 100 ng mL^{-1} PRC, 10.0 ng mL^{-1} BRP, 10.0 ng mL^{-1} LMD, sample volume: 10 mL, NaCl: 10 g L^{-1}, adsorption temperature: 70°C, stirring speed: 600 rpm, , desorption temperature: 200°C

3.5. Analytical Characteristics of The Method

Under optimized conditions, the performance of the method was tested upon using pesticide standards and calibration graphs have been constructed. Although, the internal standard was used to improve the accuracy and precision of the analysis, the calibration graphs drawn by using the peak area ratios have resulted a poor correlation coefficient values. Therefore, the peak areas of the pesiticides were plotted against the concentration (Figure 3.13(A-B)). The calibration curve was obtained by subjecting same aliquots of sample (10 mL) added with different amount of pesticides mixture and a constant amount of IS (0.05 ng/mL).

The detection and quantification limits of all pesticides (LOD and LOQ) were estimated according to the relationships LOD = 3.3xSD / slope and LOQ = 10xSD /slope. In different range of concentration, the correlation coefficient, LOD and LOQ values of target pesticeds were listed in Table 3.3.

Precision of the method was expressed as the relative standard deviation (RSD) obtained from the determination of three consequtive analysis. The concentration and RDS of pesticides were also present at Table 3.4.

As can be seen from the results, good correlations were obtained all target pesticides. LOD of target pesticide were range in PNZ~PRC>LMD~BRP>CP. The sensitivity of CP was approximately 20 times higher than PNZ and PRC and approximately 2 times higher than BRP and LMD. Low %RSDs were obtained for CP and PRC. Hovewer, %RSD of LMD, PNZ and BRP were higher than 10%. LMD-1 has shown good correlation than LMD-2. On the other hand the peak area of LMD-1 was used for calibration curves.

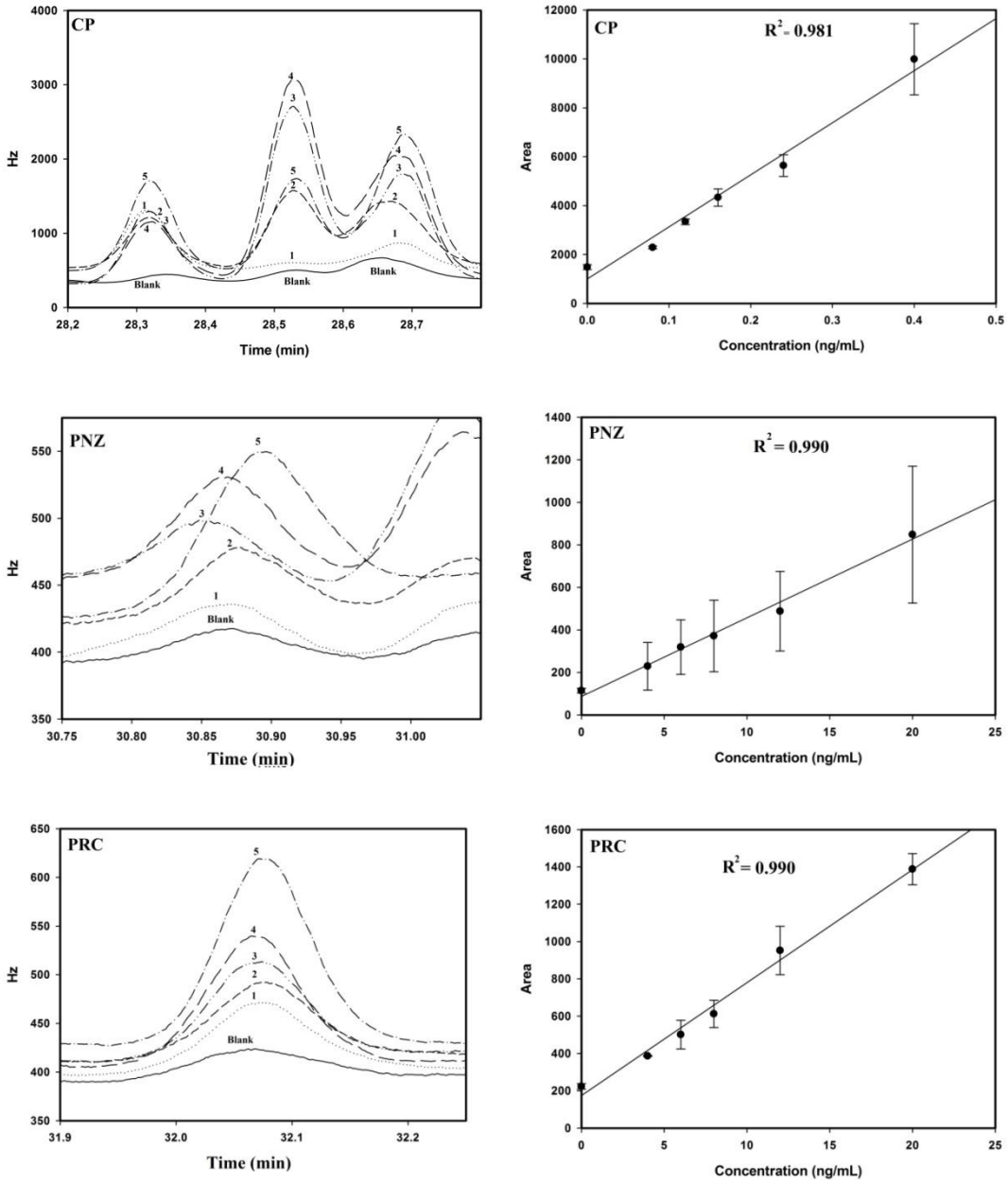


Figure 3.13 (A) The chromatograms and calibration curves of CP, PNZ and PRC pesticides.

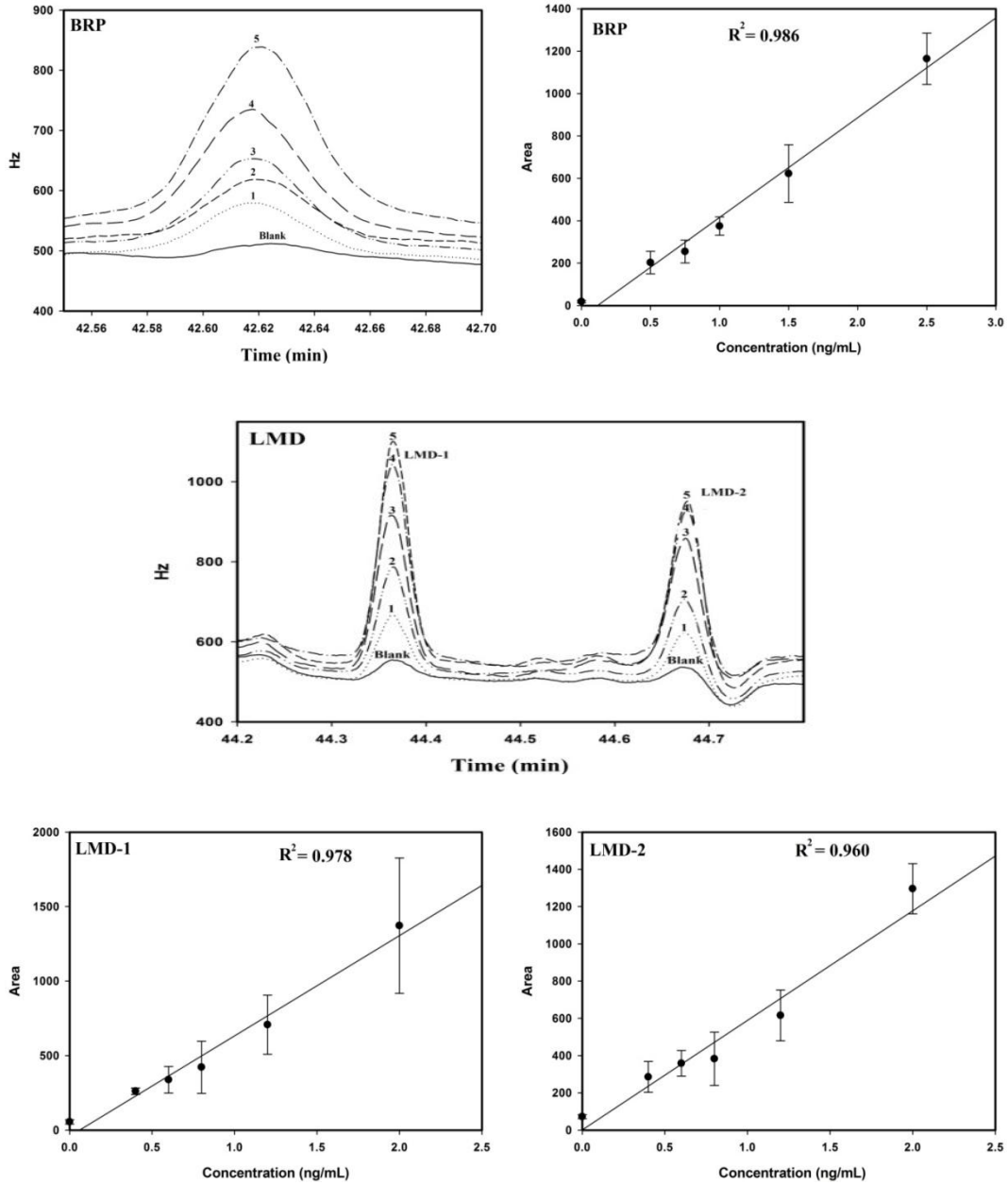


Figure 3.13 (B) The chromatograms and calibration curves of BRP and LMD pesticides

Table 3.3 The equation, correlation coefficient, LOD and LOQ of target pesticides

Pesticide	Equation	R^2	LOD (ng/mL)	LOQ (ng/mL)
CP	y= 21710.282x+892.953	0.981	0.073	0.089
PNZ	y= 36.437x+91.402	0.990	1.642	3.690
PRC	y= 60.732x+171.425	0.990	1.659	3.289
BRP	y= 463.887x-43.513	0.986	0.174	0.256
LMD-1	y= 660.936x-24.471	0.978	0.193	0.339
LMD-2	y= 598.919x+3.112	0.960	0.142	0.258

Table 3.4 % RSD of target pesticides with different concentration (C).

CP		PNZ		PRC		BRP		LMD-1		LMD-2	
C	%RSD	C	%RSD	C	%RSD	C	%RSD	C	%RSD	C	%RSD
0.08	2.40	4.00	48.86	4.00	0.64	0.50	26.24	0.40	8.26	0.40	29.01
0.12	3.38	6.00	40.11	6.00	15.39	0.75	21.23	0.60	26.29	0.60	19.21
0.16	8.17	8.00	45.26	8.00	11.94	1.00	11.51	0.80	41.37	0.80	37.38
0.24	7.87	12.0	38.33	12.00	13.57	1.50	21.78	1.20	28.06	1.20	22.14
0.40	14.59	20.0	37.95	20.00	6.02	2.50	10.41	2.00	33.09	2.00	10.42

3.6. Application of The Method to The Real Sample

The method was applied for Turkish wine sample. Commercial a red wine sample from İzmir was obtained from market place. The wine was stored at 4^{o}C before analysis. Ten milliliters of wine sample, 1.0 g of NaCl and 0.05 ng/mL of internal standard solution were placed in 20mL sample vials. The vials were tightly capped with teflon faced silicone septa. The sample vials were placed in the headspace sampling system and the same SPME fiber as that used in the calibration curve was used. The SPME and GC-ECD conditions were set as mentioned before.

Because of the matrix effect of wine samples on signal standard addition method was applied. Four standard additions with triplicate analysis were performed on all samples. Chromatograms and standard addition curves were also present at Figure 3.14(A-B). Equation and correlation coefficients were listed in Table 3.5. RSD of target pesticides for all concentration were also aviable at Table 3.6.

According to the tables, the calibration graphs have shown good linearity with reasonable correlation coefficients and rather low RSD values. In the red wine sample studied, very low PNZ and BRP contents were detected (Table 3.7). The level of these residual pesticides were found well below the limit set by Turkish Food Administration.

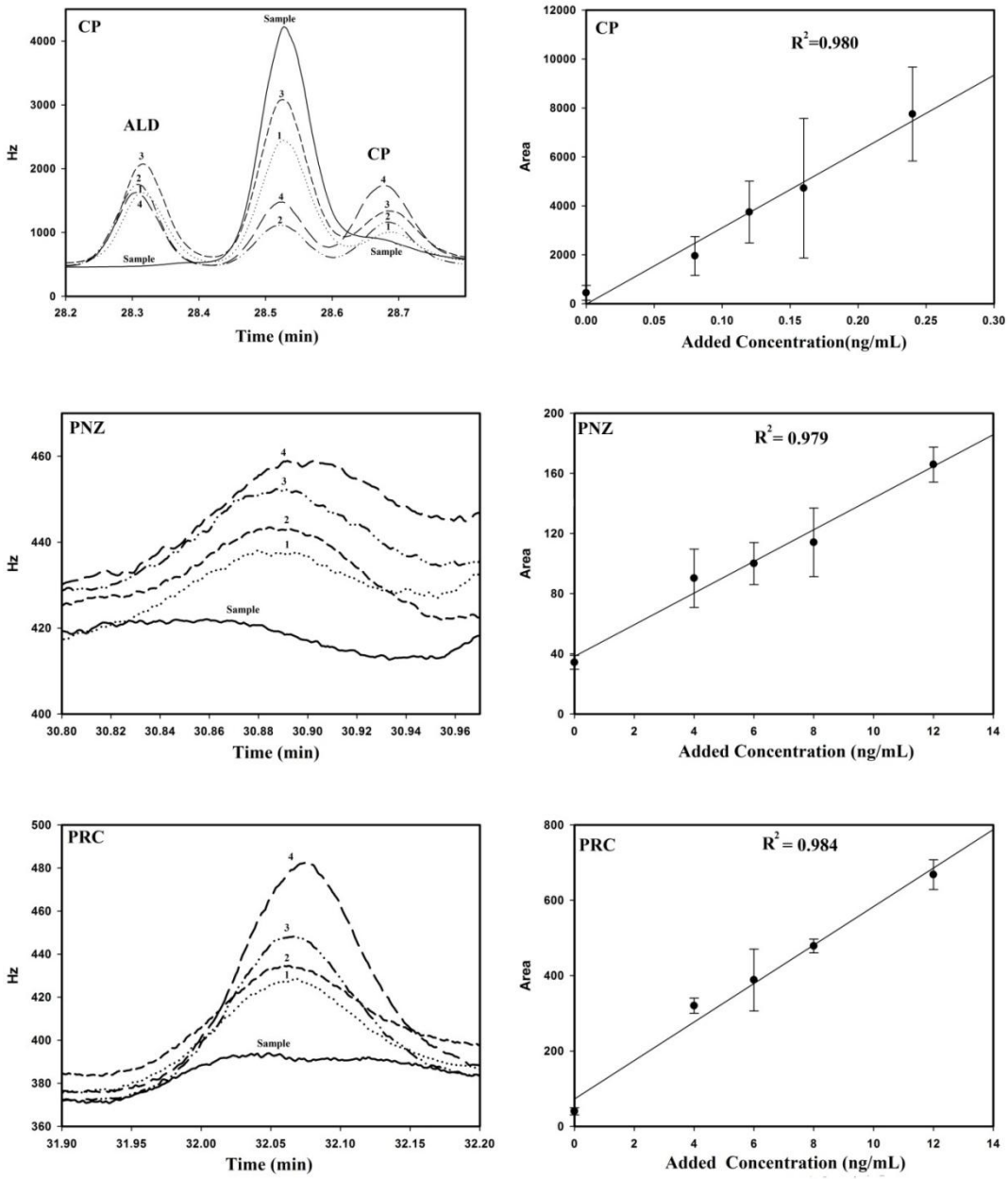


Figure 3.14 (A) The chromatograms and standard addition curves of CP, PNZ and PRC pesticides in red wine sample spiked with standard pesticide solutions.

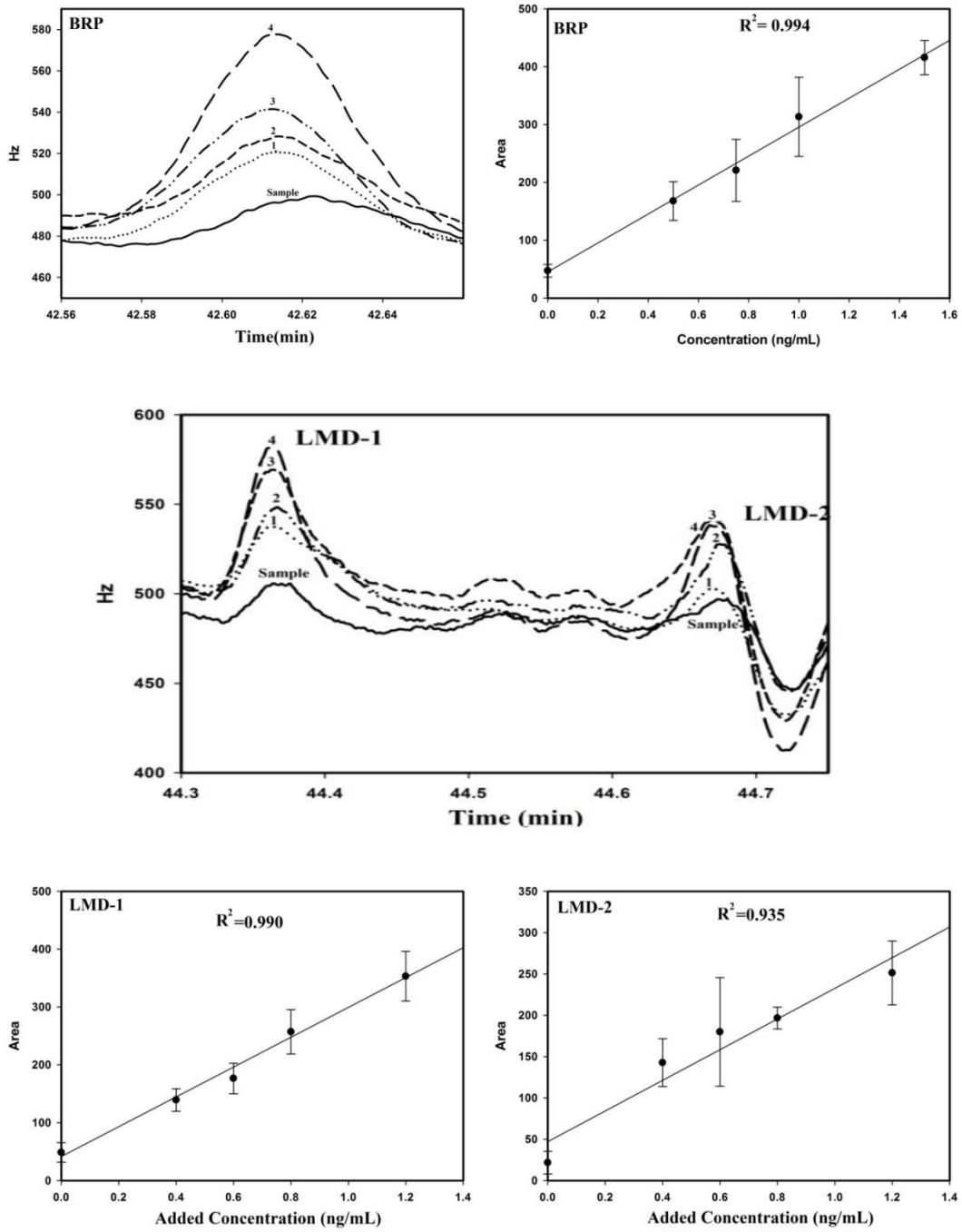


Figure 3.14 (B) The chromatograms and standard addition curves of BRP and LMD pesticides in red wine sample spiked with standard pesticide solutions.

Table 3.5 The equation, correlation coefficient of standard addition curves for red wine sample spiked with standard pesticide solutions

Pesticide	Equation	R^2
CP	y= 30856.684x+18.406	0.980
PNZ	y= 10.461x+91.402	0.979
PRC	y= 51.068x+72.638	0.984
BRP	y= 250.213x+45.388	0.994
LMD-1	y= 257.871x+40.267	0.990
LMD-2	y= 185.610x+47.073	0.935

Table 3.6 % RSD of target pesticides with different concentration (C) in wine sample

CP		PNZ		PRC		BRP		LMD-1		LMD-2	
C	%RSD	C	%RSD	C	%RSD	C	%RSD	C	%RSD	C	%RSD
0.08	40.68	4.00	21.45	4.00	6.33	0.50	19.88	0.40	14.05	0.40	20.31
0.12	33.70	6.00	13.99	6.00	21.13	0.75	24.23	0.60	14.94	0.60	36.53
0.16	60.42	8.00	20.02	8.00	3.83	1.00	21.81	0.80	14.94	0.80	6.72
0.24	24.76	12.0	6.99	12.0	5.91	1.50	7.12	1.20	12.14	1.20	15.36

Table 3.7 Concentration of target pesticides in wine sample

Pesticide	C (ng/mL)
CP	<LOD
PNZ	3.65
PRC	<LOD
BRP	0.18
LMD-1	<LOD

The influence of the wine matrix was investigated by determining the recovery percentages of the pesticides from spiked samples. Table 3.8 shows the recovery ratios of target compounds in wine samples. As can be followed from the table, the recovery ratios of CP and LMD in the wine sample were found very close to 100%. The ratios for PRC and BRP were found in the range of 80–120%. However, poor recovery values were obtained for PNZ. In conclusion, this method is reliable to quantify the amount of pesticide compounds in wine matrix. During the repetitive analysis, high mechanical stability of the fiber was observed and even after over 250 injections, the fiber can be used safely with a good precision.

Table 3.8 Recovery results of target pesticides in red wine

Pesticide	**C (ng/mL)**	**Recovery (%)**
CP	0.16	98.6
PNZ	4.00	48.8
PRC	12.00	86.2
BRP	1.00	84.5
LMD-1	0.80	95.9
LMD-2	0.80	68.5

4. CONCLUSION

In this study, pyrrole was subjected to electropolymerization onto the steel wire for developing a SPME fiber for analytical purposes. The fiber was placed over the vial that contains mixed standard of pesticide solutions and heated for an effective extraction in head space conditions. Gas chromatographic measurements have made for simultaneous determination of 6 pesticides those commonly detected in Turkish wine samples. The parameters namely adsorption temperature and time,desorption temperature, strring speed, amount of salt were optimized and calibration graphs were constructed for Chlorpyrifos (CP), Penconazole (PNZ), Procymidone (PRC), Brompropylate (BRP) and Lambda-Cyhalothrin (LMD) selected for the thesis. Aldrin was used as the internal standard. The application of the method was made by using Turkish wine samples. Recovery values except PNZ were found acceptable.

The method offers an inexpensive alternative for fiber making reducing cost considerably and improving the selectivity as well as their duration. Even after 250 usages, the fiber can be used sensitively with a reasonable precision. Further studies will cover alternative polymeric matrixes for volatile and semivolatile pesticide analysis in food samples.

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