SINGLE-DROP MICROEXTRACTION FOR THE DETERMINATION OF ORGANOPHOSPHORUS PESTICIDES IN WHEAT

A DISSERTATION

Submitted in partial fulfillment of the requirements for the award of the degree of

MASTER OF TECHNOLOGY

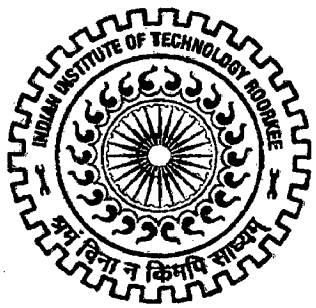
in

CHEMICAL ENGINEERING

(With Specialization in Computer Aided Process Plant Design)

By

RAHUL



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CANDIDATE'S DECLARATION

I hereby declare that the work which is being presented in this dissertation entitled **"Single-drop Microextraction for the Determination of Organophosphorus Pesticides in Wheat"** in partial fulfillment of the requirement for the award of the degree of Master of Technology in the Department of Chemical Engineering with specialization in **"Computer Aided Process Plant Design"**, submitted in **Chemical Engineering Department, Indian Institute of Technology, Roorkee, (India)**, is an authentic record of my own work carried out during the period from July 2005 to June 2006 under the guidance of **Dr. C. B. Majumder,** Assistant Professor, Department of Chemical Engineering and **Dr. Partha Roy,** Assistant Professor, Department of Biotechnology, Indian Institute of Technology, Roorkee, (India).

The matter embodied in this dissertation has not been submitted by me for the award of any other degree of this institute or any other institute.

Dated: June, 30, 2006
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CERTIFICATE

This is to certify that the above statement made by the candidate is correct to the best of my knowledge.

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ABSTRACT

An analytical procedure has been developed for determining a group of six organophosphorus pesticide commonly used in crop production. A single-drop microextraction (SDME) method was analysed for the determination of organophosphorus pesticides (OPPs) dichlorvos, phorate, fenitrothion, malathion parathion, quinalphos in wheat sample. In this work single-drop microextraction (SDME) was analysed by gas chromatography mass spectrometer (GC-MS) with electron ionization detection (GC-EID) combining positive ion mode. The significant parameters affecting the single-drop microextraction (SDME) performance such as selection of solvent type, drop volume, stirring rate, extraction time, and effect of salt concentration has been examined. An attempt was made to optimize the parameters. The most common technique was applied for extraction of organophosphorus pesticides (OPPs) are liquid phase extraction. Overall, extraction was achieved by suspending a 1.5 µl toluene drop to the tip of a microsyringe immersed in a five ml doner aqueous solution containing 2.5% NaCl (w/v) and stirred at 800 rpm. The limits of detection (LODs) in wheat sample for the six studied compounds were between 0.24 and 0.62 µg/ml with the standard deviations ranging from 1.47 to 17.02%. Linear response data was obtained in the concentration range of 0.5-50 µg/ml with correlation coefficients from 0.9526 to 0.9875. Sample of wheat grain collected from local market of Roorkee.

Overall single-drop microextraction (SDME) method proved to be a fast and simple determination of organophosphorus pesticides (OPPs) in wheat sample.

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ABBREVIATION

MRL	Maximum Residue Limit
GAP	Good Agricultural Practice
IPM	Integrated Pest Management
MTD	Maximum Tolerated Dose
NOAEL	No-Observable Adverse Effect Level
RFD	Reference Dose
TMRC	Theoretical Maximum Residue Contribution
EPA	Environmental Protection Agency
ARC	Anticipated Residue Contribution
FDA	Food and Drug Administration
LCA	Life Cycle Assessment
OPP	Organophosphorus pesticide
AChE	Acetyl-cholinesterase
Ach	Acetlycholine
LLE	Liquid–liquid extraction
SPE	Solid-phase extraction
SPME	Solid-phase microextraction
LPME	Liquid-phase microextraction
SDME	Single drop microextraction
LOD	Limits of detection

CHAPTER 1

INTRODUCTION

Pesticides are now-a-days widely used in almost all agricultural practices. Pesticides are chemicals used to protect crops from insects (insecticides), other animal pests (eg. rodenticides, miticides), weeds (herbicides) or diseases, in both dry land and irrigated agriculture. Pesticides are usually synthetic, toxic chemicals, with a wide range of differing properties designed either to kill pests or to inhibit their growth.

The main applications can be classified in production and post-harvest treatment of agricultural commodities for transport purposes. In this sense, production agriculture comprises the main category of use of pesticides subject to control requirements and, therefore, legal action levels have been fixed to assess food safety. [1]

Controls on pesticide residues in crops are generally based on Maximum Residue Limits (MRL's) which are set using field trial data for a particular pesticide to arrive at the highest residue levels expected under use according to Good Agricultural Practice (GAP). Although MRL's are a credible and useful means of enforcing acceptable pesticide use, they are inadequate as a guide to human health risks from residues. Total diet studies have consistently shown that using MRL's as a basis for calculating human dietary consumption of pesticides over-estimate actual intakes by one to three orders of magnitude.

An important factor leading to reduction of any residues left on crops at harvest are processing treatments such as washing, peeling, canning or cooking that the majority of foods receive prior to consumption. These can often substantially reduce the residue levels on or in food that has been treated with pesticides. However in some special cases more toxic by-products or metabolites can be formed during processing. Unit processes on food can also result in residues being redistributed or concentrated in various separated fractions of food or feed.

In principle, the magnitude of many of these effects can be predicted for particular pesticides from physico-chemical parameters such as solubility, hydrolytic rate constants,

volatility, and the actual physical location of residues. In practice lack of detailed data, particularly on the interactions with food components, means a more empirical approach has been followed. More research is required on some of these fundamental physico-chemical processes with pesticides in the context of food processing. However the general effects of processing may be rationalized by using these considerations.

1.1 PROFILE OF CONCERN

The food distribution network composed of farmers, grain handlers, shippers, food processors, and retailers is providing consumers year-round access to an abundant selection of vegetables, fruits, and grains never before available to the public. Encouraged by the medical and health care communities to eat a more wholesome and nutritious diet, the public has demonstrated a willingness to increase the consumption of fruits and vegetables as one method of improving personal health [13]. The selective use of pesticides to control pests (insects, diseases, weeds) of food crops has played a major role in increasing the availability of produce and grains to the consumer. Pesticides have allowed growers and handlers of food products to expand production into new geographical areas, increase production volume, extend shelf life, and improve the appearance of many of our commonly grown foods. The consequences of using pesticides for food production and the realization that some foods do contain pesticide residues are of paramount importance to health conscious consumer.

The public's concern that consuming foods containing pesticide residues may adversely impact their health is critical. Nearly four out of ten individuals presently believe that the potential risks from pesticide residues out weigh the potential health benefits of eating fresh fruits and vegetables.[10] However, nutritionists and dieticians generally agree that the issue of pesticide residues on food is not a top food issue. Rather, it is stressed that food safety issues should focus around establishing fruits, vegetables, and grains as a larger proportion of our daily diet and educating the public on the negative consequences (e.g., food poisoning) of improper preparation and/or storage of foods. It is hoped that this publication will educate the consumer to better evaluate the impact of minimal pesticide residues on human health and to weigh the benefits and risks associated with the consumption of foods containing trace pesticide residues.

1.2 PESTICIDE USE ON THE FARM

Many of today's food producers are taking an Integrated Pest Management (IPM) approach to preventing, reducing or eliminating pest problems. Growers and processors must make complicated decisions prior to planting, during the growing season, and during post harvest handling. Scientific IPM strategies give the grower economic incentives for sustaining long-term crop protection with minimal disruption to the environment. The agricultural community typically will use pesticides judiciously as part of the IPM strategy whenever proven alternatives are not available for pest control. Growers are hiring professional crop consultants with increasing frequency for advice on maintaining or increasing production through the utilization of IPM programs structured toward their specific agronomic situations. Agricultural products leaving the farm are subjected to IPM at the food processing facility. IPM practices such as plant sanitation, monitoring for insect and rodent pests, and controlled temperature strategies in the food plant environment are routinely incorporated into the food processing chain; the result is often a reduced need for pesticide application. Informed decision-making via the IPM approach ultimately effects a profitable agricultural production system and benefits the consumer by providing foods with minimal or no pesticide residue.

1.3 PROCESS FOR ALLOWING PESTICIDE USE ON FOOD

The registration of a pesticide for use in our gardens or a farmer's field requires assessment of the potential negative effects of that pesticide on human health. To anticipate how a pesticide might impact human health, laboratory animals such as mice and rats are exposed to varying dosages in their foods from very minimal to extremely high levels. Scientists and health experts then evaluate the observable effect(s) of consuming known quantities of that specific pesticide on reproduction, respiration, and the immune system. Information gained from such tests is evaluated by health professionals and medical experts to determine potential human effects.

1.3.1 Evaluation Process

1. Scientists from the Environmental Protection Agency begin the evaluation process by determining the highest pesticide dose that can be fed to laboratory animals to cause adverse health effects but not death. This dose is called the Maximum Tolerated Dose (MTD).

2. The second step in the evaluation process is the selection of the highest pesticide dose that does not cause observable harm or side effects in experimental animals. This dose level is referred to as the No-Observable Adverse Effect Level (NOAEL). The NOAEL value can be developed from acute (single incident) or chronic (multiple exposure) studies. The NOAEL is the first safety level.

3. The NOAEL usually is divided by a safety factor of 100 (safety factors range from 10 to 10,000) to take into account individual differences among people and the extrapolation of human health information from animal data. This second safety level is called the Reference Dose (RFD).

4. The RFD generally is expressed in terms of milligrams of a pesticide consumed per kilogram of body weight (mg/kg) per day. It is the amount of a pesticide residue that, if ingested daily over a 70-year lifetime, a human could consume without expecting any health related problems. It is the RFD that is used as the toxicological indicator when pesticide residues are tested on foods designated for human consumption.

5. Next, EPA scientists determine how much of a particular pesticide residue the average consumer might ingest over a life expectancy of 70 years. One measure used to calculate lifetime exposures is the Theoretical Maximum Residue Contribution (TMRC). The TMRC assumes that the foods we consume will contain maximum amounts of pesticide residues. These theoretical residue calculations assume that the maximum allowable amount of a pesticide will be applied to 100 percent of the labeled crops, that the number of pesticide

applications will be in accordance with the maximum allowed by the product label, and that the food commodities will be consumed daily for a lifetime. The TMRC is calculated by multiplying the tolerance on each crop by the average daily consumption of that crop. The individual TMRCs are then added to derive a single, Theoretical Maximum Residue Contribution which serves as one of the indicators for theoretical exposure.

6. The ultimate objective is the comparison between the total theoretical amount of that specific pesticide residue which we consume daily over a lifetime (TMRC value) and the highest safety level (RFD value). The pesticide is believed harmless to public health when the TMRC value is below the RFD safety value. If the TMRC is above the RFD, the Environmental Protection Agency reviews actual residue data or requires the development of such data to ascertain more realistic exposure estimates. This second exposure estimate incorporates "real world" residues into the calculations and is termed the Anticipated Residue Contribution (ARC). The ARC allows for a realistic refinement of the TMRC. Actual pesticide use, anticipated residues as determined in controlled field studies, the effects of processing, peeling, washing, and cooking on residues, and regulatory monitoring data represent the kinds of information used to evaluate the ARC alongside the RFD.

7. Finally, EPA examines each new request for the use of the pesticide on a food crop. The residue contribution from that use is added to the TMRC or ARC; and as long as it is below the RFD, a tolerance will be assigned for that use on that specific crop. Tolerances generally will not be approved when the ARC is above the health based RFD criteria.

1.3.2 Pesticides Residue Monitoring

The Food and Drug Administration (FDA), United States Department of Agriculture (USDA), and many states have in place a pesticide residue monitoring program aimed at detecting residues which exceed legal tolerances or for which there are

no tolerances established; in either case, food products containing illegal residues are subject to seizure and destruction. Each year, FDA samples approximately one percent of the food supply, or about 20,000 fresh food specimens grown domestically or imported. The majority of samples are derived from produce grown in other countries. These fresh vegetables and fruits are subjected to chemical analyses that can detect upwards of 268 pesticides or their metabolites. The adjacent graphs summarize results from pesticide residue studies of imported and domestic crops grown between 1987 and 1991. These FDA results are very comparable to the results obtained from USDA and state pesticide residue monitoring programs. Such residue monitoring programs provide only an overview of potential exposures to pesticide residues. They do not take into account information on the effects of washing, peeling, and processing on pesticide residues, but provide only crude estimates relative to the dietary intake of pesticide residues in food. However, the monitoring programs do deliver a very important message to consumers: that pesticide label use directions are being followed strictly by the agricultural community. Pesticide residues on the majority of foods tested were within legal tolerances; and some foods were found to contain no detectable residue. This important point clearly supports the public's view that our farmers do have the prerequisite knowledge to properly and correctly manage pesticides. This single fact gives credence to federal and state regulatory decision makers who rely on the agricultural community to follow label directions and precautions to minimize potential adverse impact on human health.

1.4 ASSESMENT OF PESTICIDS

The use of pesticides in agriculture is subject to steady observation due to the risk for human toxicity and environmental ecotoxicity. The assessment of this agriculturally important input needs adequate methodology. Developments are particularly expected in the evaluation of residue in agricultural commodities because of their toxicological risk. These requirements are also needed for the development of a tool for environmental analysis, the Life Cycle Assessment (LCA) methodology. This work is a contribution to the development of a method to assess the use of pesticides, in particular the presence of residues in agricultural commodities, according to the frame of the Life Cycle

Assessment methodology. In this introduction, the problem is exposed by a short review of:

- The conditions for the use of pesticides,
- The LCA methodology in agriculture,
- The existing methods to assess pesticide fate,
- The methodology to assess the toxicity of pesticides.

1.4.1 Life Cycle Assessment in Agriculture

The assessment of toxicity on human health is one of the components included in methods for environmental assessment. Recent developments in Life Cycle Assessment (LCA) methodology have enabled assessment of agricultural systems from an environmental point of view. LCA enables relating the environmental impacts to the main function of a studied activity. LCA consists of four phases, as described by the International Organisation for Standardisation (ISO14040) and following:

- The goal and scope of an LCA serves to define the purpose and the extent of the study. It includes a description of the system (a system, a process, a product) in terms of a functional unit.
- The inventory analysis performs a quantified inventory of the consumption of resources and of the emissions released to the natural environment. The whole life cycle from cradle to grave is taken into account: the extraction of non-renewable raw energy, the transports, the production phase, the use phase and the final disposal.
- The impact assessment is based on the inventory of emissions and resource consumptions. These impacts are classified in resource depletion, land use, greenhouse effect, photo-oxidant formation, acidification, eutrophication, aquatic ecotoxicity, terrestrial ecotoxicity and human toxicity. Within each impact category, emissions listed by the inventory analysis are multiplied by impact characterisation factors. Characterisation factors express the effect of each emission relatively to a specific environmental problem.

- Interpretation of quantitative data and qualitative information occurs at every stage of the LCA. Normalisation techniques, such as weighting indicators for the different impact categories, or multi-criteria decision making tools are applied during the interpretation phase as complementary tools.

As introduction to this present study on pesticides an LCA was completed to identify the key parameters of agricultural systems from an environmental point of view and for the role of pesticides. An environmental assessment of wheat for bread making was performed to optimize agricultural intensity of arable production systems, quality of agricultural products and environmental damages. To assess and compare different intensities of production, adequate functional units were developed to measure main functions of agricultural activity: production and upkeep of farmland. These methodological developments were applied to fertilization as a factor determining the intensity of production and the quality of the products. The following elements of this study provide a better understanding of the methodology of LCA in agriculture and introduce the development of a methodology to assess the fate of pesticides. Environmental assessment in agriculture has the particularity that the activity has a multifunctional role and evolves in a complex system close to the environment. Environmental assessment in agriculture has the particularity that the activity has a multifunctional role and evolves in a complex system close to the environment. Consequently the risk is high that the assessment is biased by reduction of system boundaries, the scenario definition, the choice of the functional unit and the considered impact indicators. The interactions between production inputs and yield are important, with influence on quantity and quality. A method has been specifically developed to take quality into account for Life Cycle Assessment of agriculture crops. Different cultural techniques are commonly used in European agriculture leading to variations in cultivation intensities, in yield quantities and qualities, and in environmental impacts. High yielding production systems maximising yield with large fertiliser supplies and crop protection interventions are economically advantageous in many European agricultural areas. On the other hand these high intensive systems are usually recognised for exposing the environment to damaging nitrogen, phosphorous and pesticides emissions. As

cultivation practices generally refer to a complex cropping system, these different factors interact and a combined assessment is therefore necessary.

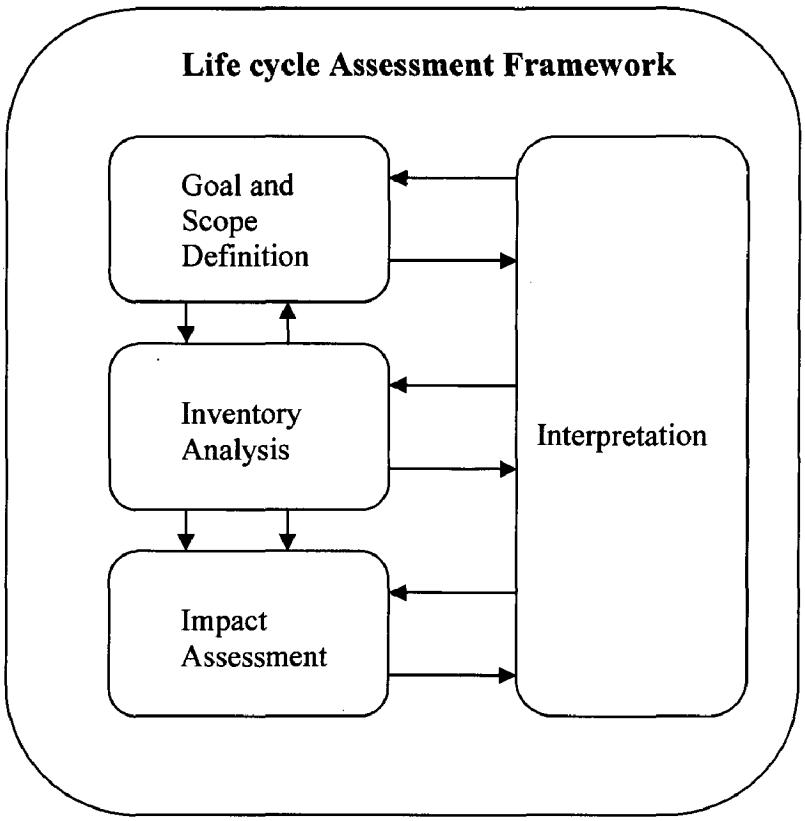


Fig. 1.1: Life Cycle Assessment

Environmental problems in arable systems are often reduced to nitrogen and pesticides problems, forgetting the specific high efficiency of these agricultural inputs to the whole production system. However the optimisation of these inputs shows that environmental optimisation of production system cannot be reduced to an optimisation of one input on its own, such as the use pesticide. A larger scale of the system is needed. Other determining agricultural parameters have to be taken into account, such as the interaction between inputs, quality requirements and the multiple function of the agricultural system. The choice of the production intensity also remains linked to the site specific potential, at field level, resulting in a combination of intensive and extensive situations. Best combination between agricultural inputs and land utilisation should therefore be explored together to design best production strategies on an environmental

point of view. High intensity level is potentially favorable per ton of product (with constant quality), when demonstrating sufficient yield increase. On the other hand environmental impact per ton of product increases with intensification if agricultural inputs are not satisfactorily combined or more generally if the intensity level exceeds the production potential. In addition, impact per hectare increases with intensification for all environmental categories except land utilization, showing that less intensive crops have to be considered for predominantly a land upkeep function. Thus pesticide or fertilizer use cannot be assessed alone, but as a whole with the rest of the system. Further studies about different utilization strategies of lower production area due to higher productivity should measure the real impact of different intensity levels.

1.5 ORGANOPHOSPHORUS PESTICIDES

Organophosphorus pesticides (OPPs) are widely used in agricultural practices because of its high effectively and relatively low price. Compared with organochlorine pesticides, OPPs demonstrate relatively low environmental persistence, but a high toxicity. Therefore, government in all countries has strictly regulated the OPPs residue in food in order to determine whether the concentrations of the pesticides used exceed their maximum residue limits (MRLs) [19]. OPPs contain, amide, sulfones and/or P=O bonds.

These groups of pesticide inhibit and inactivate the enzyme acetyl-cholinesterase (AChE). Acetlycholine (Ach) is a substance produced in nerve cell of animals that act as a chemical switch by transmitting a nerve impulse from a nerve cell to a specific receptor such as another nerve cell or a muscle cell. Organophosphorous pesticides inhibit the ability of the AChE, Ach builds up at the junction of the nerve cell and the receptor site and the nerve impulse continues, resulting the continued over-stimulation of muscles. The final result can be death by respiratory or heart failure. [35]

Most organophorous chemicals do not persist for long in the environment, ranging in soil from a few hours to as long as month after application depending on the chemical and soil conditions. They are not very mobile in soils that high organic content, and are

more stable under acidic conditions than under alkaline conditions. Degradation of Organophosphorous pesticides in surface water varies greatly with temperature.

1.6 EXTRACTION PROCESS

Methods for the determination of pesticide residues in environment and food typically require several sample preparations such as extraction, clean-up, and concentration before instrumental analysis. Liquid–liquid extraction (LLE) and solid-phase extraction (SPE) are the most useful sample preparation methods for the clean-up procedure [3, 4]. The main drawback of LLE is that it is laborious and needs a large volume of toxic organic solvents that often leads to emulsion formation, and results in analyte loss during concentration. SPE is less time-consuming than LLE but requires column conditioning and elution with organic solvents. Recent studies were focused on the development of environmentally friendly, economical, and miniaturized sample preparation methods. As a result, solid-phase microextraction (SPME) has been developed [5]. The solvent-free technique is based on the partitioning of analytes between sample matrices and the stationary phase in a fiber. Nowadays, SPME has been widely used for drugs, food, and environmental pollutants [6-8]; however, it also has some drawbacks, such as high cost, sample carry-over, and a decline in performance with time [9].

Recently, a simple, quick, inexpensive, and virtually solvent-free sample preparation method has been developed for extraction of analytes from wheat. This technique is known as liquid-phase microextraction (LPME) or single drop microextraction (SDME) [10-15]. The technique was based on the principle that distribution of analytes between a micro drop of extraction solvent at the tip of a micro syringe needle and the aqueous phase. The micro drop is exposed to an aqueous sample where analyte is extracted into the drop. After extraction, the micro drop is retracted back into the micro syringe and injected into the instruments such as gas chromatograph and high-performance liquid chromatograph for further analysis. The technique has been successfully used for the determination of dialkylphthalates, nitroaromatics, polycyclic

aromatic hydrocarbons, alcohols, cocaine, chemical warfare agents, drugs, chlorobenzenes, and also for the extraction of pesticides in water samples.

More recently, efforts have been placed on miniaturizing the LLE extraction procedure by greatly reducing the solvent to aqueous phase ratio, leading to the development of solvent microextraction methodologies. Single-drop microextraction (SDME) evolved from this novel approach and it involves extraction of organic contaminants from an aqueous donor solution into a micro drop (typically 1 μl) of an organic acceptor solvent suspended to the tip of a microsyringe [30-32]. After extracting for a prescribed period of time, the micro drop is retracted back into the micro syringe and transferred to the gas chromatograph for further analysis.

1.7 OBJECTIVE OF THE PRESENT RESEARCH WORK

The objective of the research work is to perform the single drop microextraction (SDME) for the determination of organophosphorus pesticide in wheat grain sample. The following parameters are analysed and optimized:

- Type of the organic solvent
- Extraction time
- Effect of salt concentration
- Effect of sample agitation on extraction
- Effect of solvent drop volume

CHAPTER 2

LITERATURE REVIEW

Ana Luisa Simplicio and Luis Vilas Boas (1999) developed and validated a method for the determination of organophosphorus pesticides (diazinon, fenitrothion, fenthion, quinalphos, triazophos, phosalon and pyrazophos) in fruit (pears) and fruit juice samples. The samples were diluted with water, extracted by solid-phase microextraction (SPME) and analysed by gas chromatography (GC) using a flame photometric detector in phosphorous mode. Limits of detection of the method for fruit and fruit juice matrices were below 2 mg/kg for all pesticides. Relative standard deviations for triplicate analyses of samples fortified at 25 mg/kg of each pesticide were not higher than 8.7%. Recovery tests were performed for concentrations between 25 and 250 mg/ kg. Mean recoveries for each pesticide were all above 75.9% and below 102.6% for juice, and between 70 and 99% for fruit except for pyrazophos in the fruit sample (with mean recovery of 53%). Therefore, the proposed method is applicable in the analysis of pesticides in fruit matrices and the use of the method in routine analysis of pesticide residues is discussed.

E. Psillakis and N. Kalogerakis (2002) used the continuous quest for novel sample preparation procedures that has led to the development of new methods, whose main advantages are their speed and negligible volume of solvents used. The most recent trends include solvent microextraction, a miniaturisation of the traditional liquid-liquid extraction method, where the solvent to aqueous ratio is greatly reduced. Single-drop microextraction is a methodology that evolved from this approach. It is a simple, inexpensive, fast, effective and virtually solvent-free sample pretreatment technique. A detailed and updated discussion of the developments, modes and applications of single-drop microextraction had been provided, followed by a brief description of the theoretical background of the method.

Zi-wei Yao *et.al.* (2001) described a method based on solid-phase microextraction and gas chromatography flame photometric detector for the determination of organophosphorous pesticides (OPPs) in aqueous samples. Five kinds of commercially available fibers 7, 30 and 100 μm PDMS, 85 μm PA and 65 μm PDMS-DVB were compared and 100 μm PDMS and 85 μm PA were the most sensitive fiber coatings for the analytes. The extraction time, extraction temperature, pH and content of NaCl were found to have significant influence on extraction efficiency. The optimized conditions were 100 μm PDMS fiber, 30 min extraction time at 40 °C, with 3% NaCl content and no pH adjustment. The linear range was 0.5–100 μg/l for most of the analytes. The limits of detection (LODs) ranged from 0.049 μg/l (for parathion) to 0.301 μg/l (for carbophenothion) and RSD% of repeatability at the 10 μg l−1 level were all below 8%. Environmental water samples were analyzed, but none of the analytes was detected. The recovery of spiked water samples was from 75.3 to 102.6%.

Mitsushi Sakamotoand and Taizou Tsutsumi (2004) demonstrated the applicability of headspace solid-phase microextraction (HS-SPME) to pesticide determination in water samples by evaluating the effects of temperature on the extraction of the pesticides. The evaluations were performed using an automated system with a heating module. The 174 pesticides that are detectable with gas chromatograph were selected objectively and impartially based on their physical properties: vapor pressure and partition coefficient between octanol and water of the 174 pesticides, 158 (90% of tested) were extracted with a polyacrylate-coated fiber between 30 and 100 °C and were determined with gas chromatograph–mass spectrometry. The extraction-temperature profiles of the 158 extracted pesticides were obtained to evaluate the effects of temperature on the extraction of pesticides. The pesticides were classified into four groups according to the shape of their extraction–temperature profiles. The line of demarcation between extractable pesticides and non-extractable pesticides could be drawn in the physical property diagram (a double logarithmic plot of their vapor pressure and partition coefficient between octanol and water). The plot also revealed relationships between classified extraction features and their physical properties. The new method for multi residue screening in which the analytes were categorized into sub-groups based on extraction temperature was

developed. In order to evaluate the quantitivity of the developed method, the 45 pesticides were chosen among the pesticides that are typically monitored in waters. Linear response data for 40 of the 45 was obtained in the concentration range below 5 μg/l with correlation coefficients ranging between 0.979 and 0.999. The other five pesticides had poor responses. Relative standard deviations at the concentration of the lowest standard solution for each calibration curve of the pesticides ranged from 3.6 to 18%. The value of 0.01 μg/l in the limits of detection for 17 pesticides was achieved only under the approximate conditions for screening, not under the individually optimized conditions for each pesticide. Recoveries of tested pesticides in actual matrices were essentially in agreement with those obtained by solid-phase extraction.

H. Berrada *et. al.* (2004) analyzed residues of metobromuron, monolinuron and linuron herbicides and their aniline homologous in carrots, onions and potatoes by solid-phase microextraction (SPME) performed with a polyacrylate fiber. A juice was obtained from food samples that were further diluted, and an aliquotwas extracted after sodium chloride (14%) addition and pH control. At pH 4 only the phenylureas were extracted. A new extraction at pH 11 allowed the extraction of phenylureas plus homologous aniline metabolites. Determination was carried out by gas chromatography with nitrogen–phosporus detection (NPD) the identity of the determined compounds was studied by gas chromatography–mass spectrometry. Limits of quantification (LOQs) obtained with NPD and MS (selected-ion monitoring) were in the μg/kg order allowing determination of maximum residue levels (MRLs) established in the Spanish regulations. MRLs ranged from 0.02 to 0.1 mg/kg depending on the kind of food and herbicide. Under the proposed conditions matrix effects were low enough to permit calibration with samples proceeding from ecological (non-pesticide treated) crops. Twelve commercial samples of each carrots, onions and potatoes were analyzed and only three samples of potatoes contained residues of linuron at levels below MRLs.

C. Blasco *et. al.* (2004) assayed Two approaches based on sorptive extraction, solid-phase microextraction (SPME) and stir bar sorptive extraction (SBSE), in combination with liquid chromatography (LC)–atmospheric pressure chemical ionization mass

spectrometry (MS) for analyzing chlorpyriphos methyl, diazinon, fonofos, phenthoate, phosalone, and pirimiphos ethyl in honey. In both, SPME and SBSE, enrichment was performed using a poly (dimethylsiloxane) coating. Significant parameters affecting sorption process such as sample volume, sorption and desorption times, ionic strength, elution solvent, and dilution (water/honey) proportion were optimized and discussed. Performance of both methods has been compared through the determination of linearity, extraction efficiencies, and limits of quantification. Relative standard deviations for the studied compounds were from 3 to 10% by SPME and from 5 to 9%by SBSE. Both methods were linear in a range of at least two orders of magnitude, and the limits of quantification reached ranging from 0.04 to 0.4 mg/kg by SBSE, and from 0.8 to 2mg kg^{-1} by SPME. The two procedures were applied for analyzing 15 commercial honeys of different botanical origin. SPME and SBSE in combination with LC–MS enabled a rapid and simple determination of organophosphorus pesticides in honey. SBSE showed higher concentration capability (large quantities of sample can be handled) and greater accuracy (between 5 and 20 times) and sensitivity (between 10 and 50 times) than SPME; thus, under equal conditions, SBSE is the recommended technique for pesticide analysis in honey.

J. Oliva *et. al.* (2000) described a rapid multiresidue gas chromatographic method for determining 12 insecticides in grapes, must and wine. A simple on-line microextraction method for isolating frequently applied insecticides on vineyard is used. The matrix, once extracted with an acetone–dichloromethane (1:1, v/v) mixture, was filtered and concentrated. Nitrogen–phosphorus detection (NPD) and electron-capture detection (ECD) were used to identify and quantify the insecticides, the findings being confirmed using mass spectrometric detection (MSD). No clean-up was necessary for either NPD or ECD. The regression coefficients relating to linearity were at least 0.99. Recoveries from spiked grape, must and wine samples ranged from 80 to 108% and relative standard deviations were no higher than 16% in the most unfavourable case. Individual detection limits 21 were in the range 0.02–0.1 ng. Limits of quantification varied from 0.01 to 0.05 mg kg, which are below the maximum residue limits set by the legislation of the main wine-producing countries of the European Union. Only in the case of 21 methidathion

and quinalphos were the limits of quantification equivalent to the maximum residue limits (0.05 mg/ kg) established by Spanish and French legislation, respectively.

Carlo G. Zambonin *et. al.* (2004) developed a SPME-GCMS method for the determination of a mixture of organophosphorus pesticides (phorate, diazinon, methylparathion, fenitrothion, malathion, fenthion, ethyl-parathion and methidathion) in wine and different fruit juices. The procedure is solvent-free, simple (direct SPME without further sample pre-treatment) and highly sensitive. Estimated LOD and LOQ ranged from 2 to 33 ng/ml and from 7 to 109 ng/ml, respectively, in wine, and from 2 to 90 ng/ml and from 7 to 297 ng/ml, respectively, in fruit juices. LOQ achieved by the present method are almost always below the maximum residue levels recommended by the European legislation.

Kevin N.T. Norman and Sean H.W. Panton (2001) developed an automated method using supercritical CO_2 and clean-up by solid-phase extraction (SPE) using graphitized carbon black, for the quantitative determination of organophosphorus pesticide (OPP) residues in wheat and maize. Recoveries were as good as, or better than, those obtained using liquid extraction (LE) and gel permeation chromatography (GPC) for 10 OPP's spiked at levels equivalent to 0.05 and 0.50 mg/g. Lower limits of detection were possible using supercritical fluid extraction (SFE). Incurred residues were found in wheat and maize samples, and good agreement was obtained using SFE1SPE and LE1GPC. The SFE1SPE method required less analyst time and organic solvent, and hazardous waste was reduced.

Dal Ho Kim *et. al.* (1998) described application of supercritical fluid extraction (SFE) for selective isolation of organophosphorus pesticides from a real-world matrix (wheat flour). The method uses extraction with supercritical carbon dioxide at 206.8 bar and 608°C, followed by quantitation by gas chromatography with nitrogen–phosphorous detection without clean-up of the extracts. Comparison of SFE with a method currently employed for sample preparation (i.e., organic solvent extraction followed by liquid–liquid extraction and gel permeation chromatography clean-up) shows that the SFE

technique simplifies the sample preparation step and speeds up the determination of organophosphorus pesticides in flour. Extraction times were 60 min for a 7 g sample size. This technique was able to determine organophosphorus pesticides (ethoprophos, diazinon, chlorpyrifos methyl, fenitrothion, parathion, phenthoate, EPN) in samples at the 10 ng/g level.

F. Ahmadi *et. al.* (2006) developed single drop microextraction (SDME) with modified 1.00 µl microsyringe, followed by gas chromatography with flame photometric detector (GC-FPD) for determination of 13 organophosphorus pesticides (OPPs) in water samples. By using a 1.00µl microsyringe the repeatability of drop volume and injection were improved, because of using maximum volume of microsyringe and no dead volume. On the other hand, the modification of needle tip caused increasing cross section of needle tip and increasing adhesion force between needle tip and drop, thereby increasing drop stability and achieving a higher stirrer speed (up to 1700 rpm). The method used 0.9 µl of carbon tetrachloride as extractant solvent, 40 min extraction time, stirring at 1300 rpm and no salt addition. The enrichment factor of this method ranged from 540 to 830. The linear ranges were 0.01–100 µg/l (four orders of magnitude) and limits of detection were 0.001–0.005µg/l for most of analyte. The relative standard deviation (RSD%) for 2µg/l of OPPs in water by using internal standard was in the range 1.1–8.6% (n = 5). The recoveries of OPPs from farm water at spiking level of 1.0 µg/l were 91–104%.

Min Liu *et. al.* (2005) established a liquid chromatography–mass spectrometry (LC–MS) method for the purpose of simultaneous determination of carbamate and organophosphorus (OPPs) pesticides in fruits and vegetables. Samples were extracted with acetonitrile; and then prepared by dispersive solid-phase extraction (dispersive-SPE) with primary secondary amine (PSA) as the sorbent. Four common representative samples (tomato, apple, carrot, and cabbage) were selected from the supermarket to investigate the effect of different matrices on pesticides recoveries and assay precision after spiking samples with 0.05 mg/kg. Matrix composition did not interfere significantly with the determination of the pesticides. The obtained recoveries were, with a few

exceptions, in the range of 70–110% with RSDs less than 8%. It was applied to pesticide residue monitoring in vegetables and fruits from local markets.

Heleni Tsoukali *et. al.* (2005) studied and optimised headspace-solid phase microextraction (HS-SPME) for the determination of four common organophosphorus pesticides (OPPs) in biological samples. Various parameters controlling SPME were studied: choice of SPME fiber, type and content of salt added, preheating and extraction time, desorption time, extraction temperature. Capillary gas chromatographic analysis with nitrogen phosphorus detection (GC–NPD) facilitates sensitive and selective detection of the OPPs: malathion, parathion, methyl parathion and diazinon. Fenitrothion was used as the internal standard. The method was applied to the determination of the pesticides in human biological specimens: whole blood, blood plasma, urine, cerebrospinal fluid, liver and kidney. Limits of detection ranged from 2 to 55 ng/ml depending on pesticide and type of specimen. The developed methodology overcomes limitations and obstacles of conventional methods such as the use of organic solvents, the formation of emulsions and the tedious-cumbersome procedures. The proposed protocol is seen as an attractive alternative to be used in routine toxicological analysis.

Sandra R. Rissato *et. al.* (2004) developed an analytical procedure using supercritical fluid extraction (SFE) and capillary gas chromatography with electron-capture detection to determine simultaneously residues of different pesticides (organochlorine, organophosphorus, organonitrogen and pyrethroid) in honey samples. Fortification experiments were conducted to test conventional extraction (liquid–liquid) and optimize the extraction procedure in SFE by varying the CO_2-modifier, temperature, extraction time and pressure. Best efficiency was achieved at 400 bar using acetonitrile as modifier at 90 °C. For the clean-up step, Florisil cartridges were used for both methods LLE and SFE. Recoveries for majority of pesticides from fortified samples of honey at fortification level of 0.01-0.10 mg/kg ranged 75-94% from both methods. Limits of detection found were less than 0.01 mg/kg for ECD and confirmation of pesticide identity was performed by gas chromatography–mass spectrometry in selected-ion monitoring mode. The

multiresidue methods in real honey samples were applied and the results of developed methods were compared.

Katan Patel *et. al.* (2004) reported a rapid method for the screening of organophosphorus (OP) pesticides in fruit and vegetables. Sample extracts were analysed using resistive heating-gas chromatography (RH-GC) with flame photometric detection (FPD). A Carbo Fruit insert in the GC liner allowed injection of crude extracts onto the GC system. Separation of up to 20 pesticides was achieved in 4.3 min with excellent retention time stability. Signal-to-noise ratios of 5:1 or better were obtained for the majority of the pesticides at the lowest calibrated level (LCL), 0.01μg/ml, with excellent linearity over the range 0.01-0.5 μg/ml (0.004–0.2 mg/kg equivalent). Average recoveries between 70 and 116% were obtained for pesticides spiked at 0.01 and 0.1 mg/kg with associated R.S.D. values ≤20% in the majority of cases. Estimates of relative reproducibility standard deviation (R.S.D.R), made by combining observed R.S.D. values with estimates of uncertainty associated with mean recovery allowed the determination of values which confirmed that the method is capable of producing results which are fit for purpose. The validated method was then used to screen peaches, grapes and sweet peppers for a total of 37 pesticides. Incurred residue results obtained using RH-GC–FPD were in good agreement with the results from analysis of the same samples using MS confirmation.

Manuela Schellin *et. al.* (2004) analysed eight organophosphorus pesticides (parathion-methyl, fenitrothion, malathion, fenthion, bromophos, bromophos-ethyl, fenamiphos and ethion) in aqueous samples by means of membrane-assisted solvent extraction. First a 20 ml extraction vial was filled with 15 ml of aqueous sample. Then the membrane bag consisting of nonporous polypropylene was put into the vial and filled with 800 μl of organic solvent. The analytes were separated from the aqueous layer by transporting them through the membrane material into the small amount of solvent. The techniquewas fully automated and successfully combinable with large volume extraction andGC–MS.To achieve an optimum performance several extraction conditions were investigated. Cyclohexanewas chosen as acceptor phase. Then the impact of salt, methanol, pH value,

as well asworking parameters like stirring rate of the agitator and extraction time, were studied. Moreover, the influence of matrix effectswas examined by adding different concentrations of humic acid sodium salt. Detection limits in the ng/l level were achieved using large volume injection with the injecting volume of 100 µl. The recovery values ranged from 47 to 100% and the relative standard deviation for three standard measurementswas between 4 and 12% (except for bromophos-ethyl: 22%). The linear dynamic rangewas between 0.001 and 70 µg/l. The applicability of the method to real samples was tested by spiking the eight organophosphorus pesticides to red wine, white wine and apple juice samples.

Christer Jansson *et. al.* (2004) presented a new multi-residue method for determination of pesticide residues in a wide variety of fruit and vegetables, using the National Food Administration (NFA) ethyl acetate extraction and determination by means of LC-MS/MS. The method includes pesticides normally detected by LC-UV or LC-fluorescence such as benzimidazoles, carbamates, *N*-methylcarbamates and organophosphorus compounds with an oxidisable sulphide group as well. After extraction with ethyl acetate, the extract is concentrated and an aliquot of the extract is evaporated to dryness and redissolved in methanol before injection on LC–MS/MS. The method has been validated for 57 different pesticides and metabolites. Representative species from different commodity groups were chosen as matrices in order to study the influence from different matrices on recoveries. The fortification levels studied were 0.01–0.5 mg/kg. Matrix effects were tested for all matrices by means of standard addition to blank extracts. The matrix effect, expressed as signal in solvent compared to signal in matrix, was in general found to be small. The obtained recoveries are, with a few exceptions, in the range 70-100%. The proposed method is quick and straightforward and no additional clean-up steps are needed. The method can be used for the analysis of all 57 pesticides in one single determination step at 0.01 mg/kg.

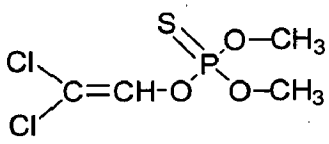
CHAPTER 3

EXPERIMENTAL SETUP AND PROCEDURE

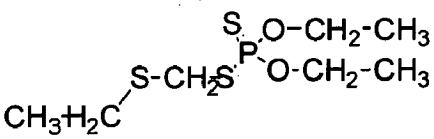
3.1 STANDARD SOLUTION AND CHEMICALS

For experimental work, six standard organophosphorus pesticides (OPPs) are used as given below:

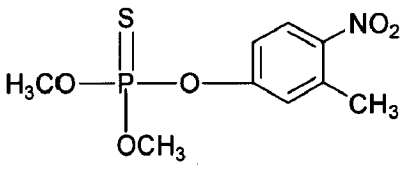
1. Dichlorvos



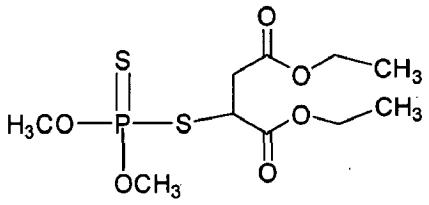
2. Phorate



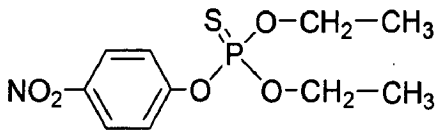
3. Fenitrothion



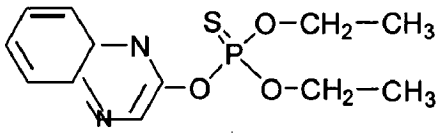
4. Malathion



5. Parathion



6. Quinalphos



All standards were 90-92% pure. Individual pesticide standard stock solutions of 1000 μg/ml in acetone were prepared and a mixed standard solution containing 10μg/ml of each of the pesticides was prepared in acetone from the individual standard stock solutions. The stock standard solution was stored in refrigerator at 4 °C. Physical properties of organophosphorus pesticides and organic solvent are shown in table 3.1 and 3.2.

Table 3.1: Physical Properties of Organophosphorus pesticides

Pesticides	Molecular Weight	Water Solubility (g/l)	Melting Point	Vapour Pressure (mm Hg)	Boiling Point (mm Hg)	Density (at 25°C)
Dichlorvos	221	10	3.9°C	0.025 at 25°C	117°C at 10	1.415
Phorate	260.36	50	6°C	6.7 at 30°C	232°C at 760	1.61
Fenitrothion	277.23	30	4.3°C	0.0000462 at 20°C	154 at 10	1.37
Malathion	330	145	2.9°C	0.00004 at 20°C	156°C at 0.7	1.230
Parathion	291	24	6°C	0.0000378 at20°C	375°C at 760	1.267

Table 3.2: Physical properties of organic solvent

Solvent	Molar mass	Water solubility (g/l)	Melting point(^{0}C)	Boiling point(^{0}C)	Density (g/l)	Viscosity (cP)
Acetone	58.09	Immiscible	-94.0	56.30	0.790	0.320
Hexane	86.18	Immiscible	-95.0	69.00	0.654	0.294
Cyclohexane	84.16	Immiscible	6.55	80.74	0.779	1.020
Toluene	92.14	Immiscible	-93.0	110.60	0.867	0.590
Tetracholoro-methane	156.04	Immiscible	-182.5	124.80	0.717	1.065

Hexane, Toluene, Cyclohexane, Tetrachloromethane and other reagents were used AR grade with more than 99% purity. The chemicals were from M/s S.D. Fine Pvt. Ltd., India, and M/s Ranbaxy Laboratories Ltd., India. The Millipore water was used through out this work.

3.2 PREPARATION OF SAMPLES

Field samples of wheat were obtained from local grain producers. Wheat sample are sub-dividing with a grinder. A portion of the fraction (500 gm) was milled with a Laboratory disc mill on setting 99% passed through a 0.1 mm screen (125 mess size). Known amount of pesticides were added to clean extracts in acetone using a microlitre syringe. Calibration curves were prepared from sample injection.

For our experiments matrix standards and blank samples of wheat were prepared. A 25-g amount of residue-free wheat was mixed with 125 ml acetone and 25 g methanol and homogenized for 2 min. The suspension was centrifuged at 2500 rpm and filtered under vacuum. The suspension was filtered, filter cake was rehomogenised with extraction solvent, recentrifuged and the supernatant decanted as before. The volume of filtrate was reduced by evaporation to 25 ml and made-up with cyclohexane in a 50 ml volumetric flask.

3.3 EXTRACTION PROCEDURE

Single-drop microextraction is a methodology that evolved from this approach. In this process the extraction phase is a drop of a water-immiscible solvent suspended in the aqueous sample (Figure 3.1).

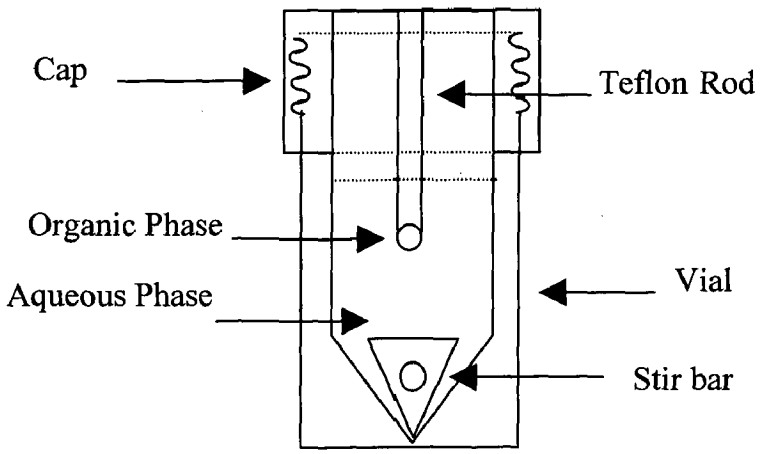


Fig. 3.1: Slide view illustration of the single-drop micro extraction system

A commercially available 10 µl micro syringe was used for microextration. Single drop microextration was performed in 10 ml vial. The vial was placed on a constant temperature magnetic stirrer with 800 rpm. This technique is performed by suspending 1.5 µl drop of organic solvent on the tip of a 10 µl micro syringe immersed in the stirred 2 ml aqueous solution of 2.5% (w/v) NaCl. For all quantification experiments, the same amount of internal standard solution was added in the aqueous samples prior extraction. Each time, the needle of the micro syringe passed through the sample vial septum and tip of the needle was immersed in the liquid phase. The analytes partition exists between the bulk aqueous phase and the organic solvent micro drop. After extraction, the micro drop was retracted into the micro syringe and transferred to the hot injector of the GC-MS for analysis.

3.4 APPARATUS AND CHROMATOGRAPHY ANALYSIS

Gas chromatography is a powerful separation technique widely applied in identification of compound analysis. Pesticide residues were determined using a Perkin Elmer Clarus 500 GC equipped with a mass spectrometry. The Perkin Elmer Clarus 500 mass spectrometry is sophisticated bench top mass spectrometry detectors that provide simple tools needed to perform routine gas chromatograph-mass spectrometer analysis as well as the sophisticated tools needed to perform the more complex analysis. Clarus 500 GC-MS runs analysis the best characterize my sample by using the electron ionization (EI+) mode. Designed as a detector Clarus 500 GC, this system provide positive identification and quantification of compound separated by the Clarus 500 GC, even those complex compound that co elute.

The chromatographic conditions were as follows: HP-5 fused silica capillary column of 30 m, 0.32 mm i.d., and 0.25 µm film thickness. Helium was used as the carrier gas. The 1µL SDME extract was injected into the GC-MS in split less Mode at 250 °C. The column oven temperature was held at 125 °C for 2 min, then programmed at 75 °C /min to 200 °C for 1 min, 10 °C/min to 220 °C and held for 3 min. Helium carrier gas was maintained at a flow rate of 1.0 ml/min.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 OPTIMIZATION OF THE SINGLE DROP MICROEXTRACTION

The parameters that influence the partition of analytes between the organic drop and the solution were optimized. In order to perform the microextraction of Organophosphorus pesticide (OPPs) from aqueous samples efficiently, several parameter that influence on the extraction efficiency should be studied and optimized. Such factors included solvent type, drop volume, stirring rate, extraction time, and effect of salt concentration. To obtain optimized extraction conditions, the ratio of peak area analyte and that of internal standard was used in GC-MS analysis of the extracts.

4.1.1 Selection of the Extraction Solvent

The selection of an appropriate extraction solvent is of major importance for the optimisation of the SDME process. The principle `like dissolves like' is applied here and for single-drop microextraction several water-immiscible solvents differing in polarity and water solubility should be tested. [18]

For the purpose of the present experiments four water-immiscible solvents (namely: hexane, cyclohexane, tetracholoromethane and toluene) differing in polarity and water solubility were tested. Solvent selectivity was evaluated after exposing for 15 min 2 µl organic solvent drop in 5 00 µg of wheat samples, stirred at 400 rpm and spiked at 5 mg/mL with all target analytes. The results revealed that cyclohexane had the lowest extraction capability when compared to the other three tested solvents (Figure 4.1.a to 4.1.f). Six analytes were being used as follows dichlorvos, phorate, parathion, fenitrothion, malathion, quinalphos.

Toluene and tetracholoromethane gave similar results for the majority of target analytes with the exception of quinalphos where toluene and tetracholoromethane respectively, showed a higher extraction capability than the other solvent. It was also

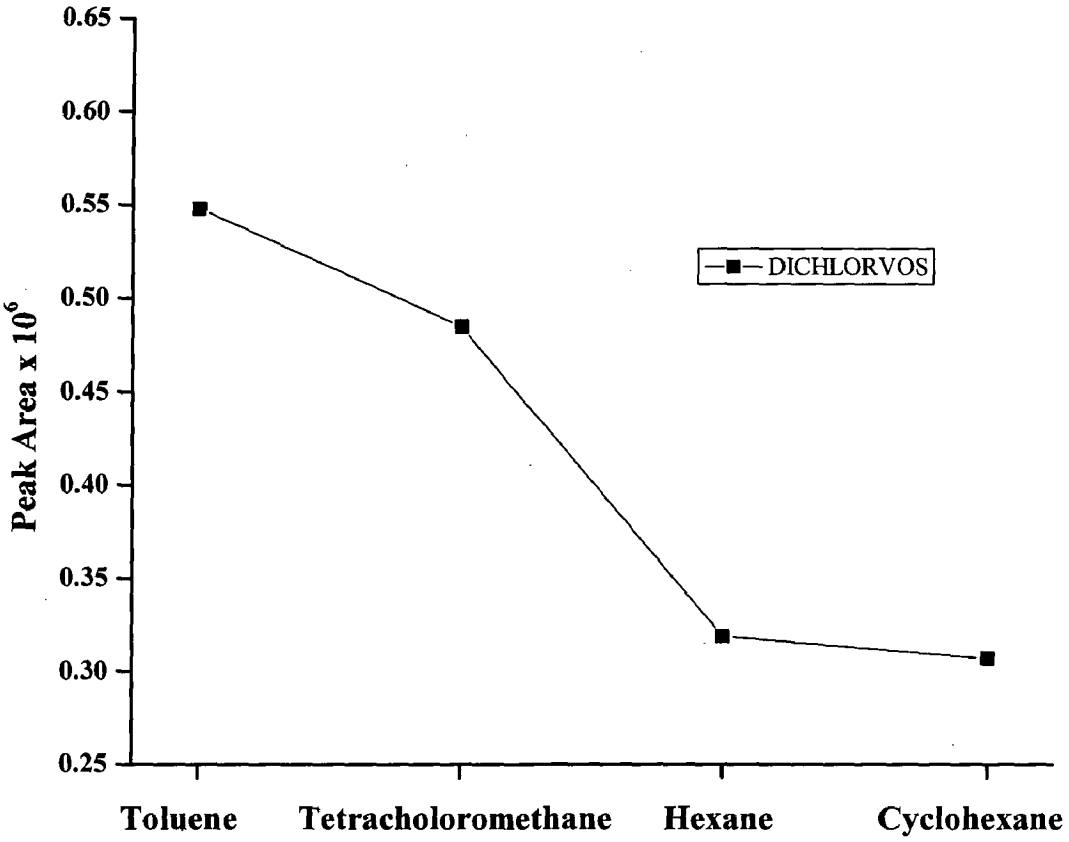


Fig. 4.1.a: Extraction efficiency of the dichlorvos pesticides with various organic solvents.

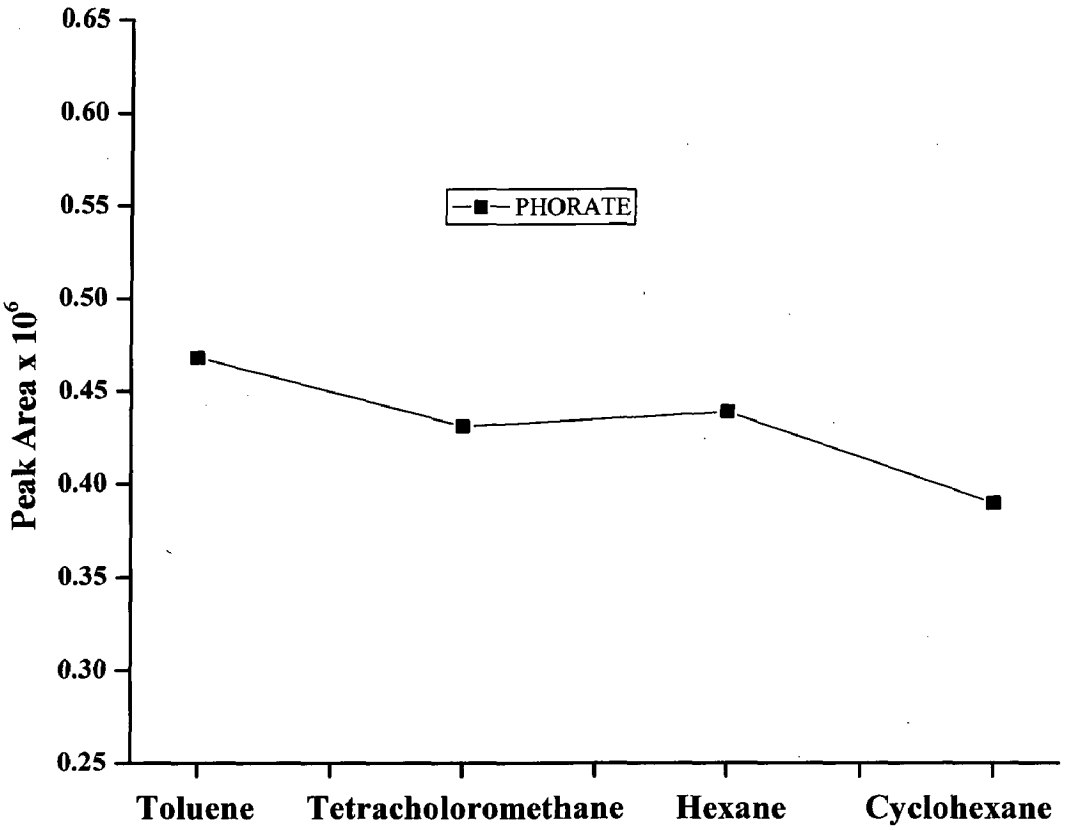


Fig. 4.1.b: Extraction efficiency of the phorate pesticides with various organic solvents.

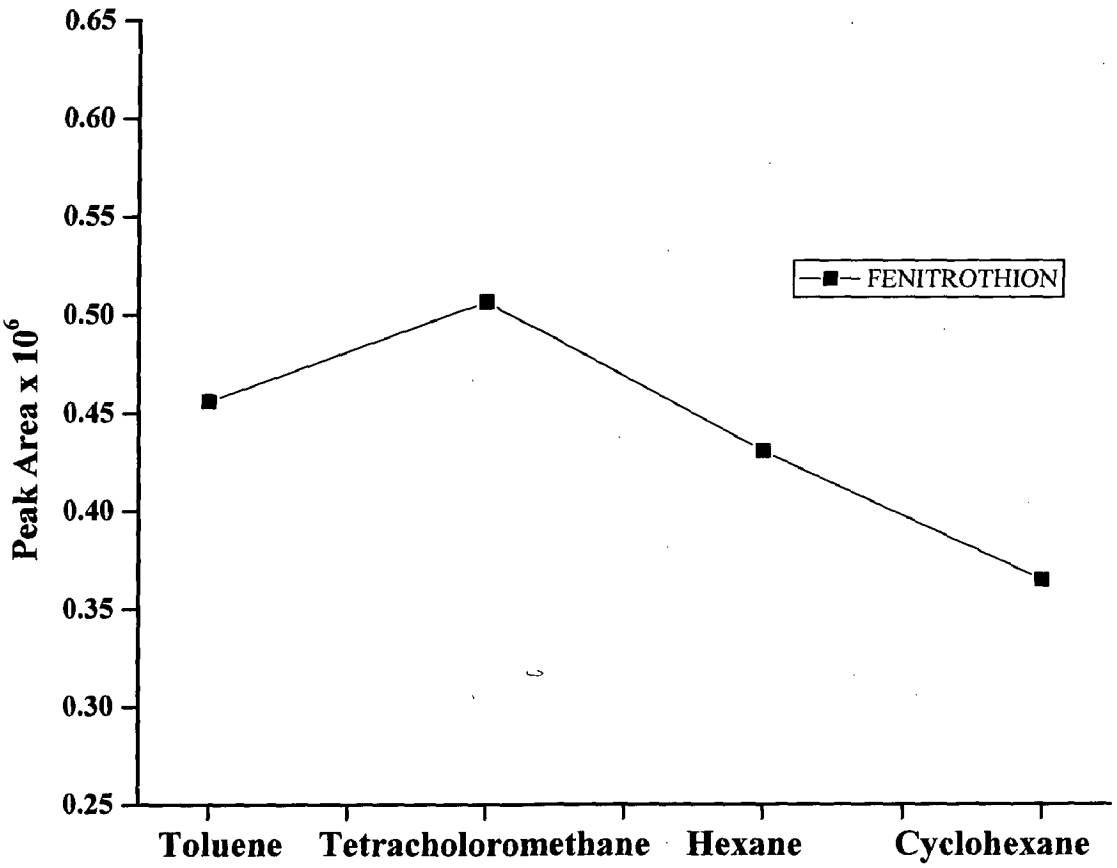


Fig. 4.1.c: Extraction efficiency of the fenitrothion pesticides with various organic solvents.

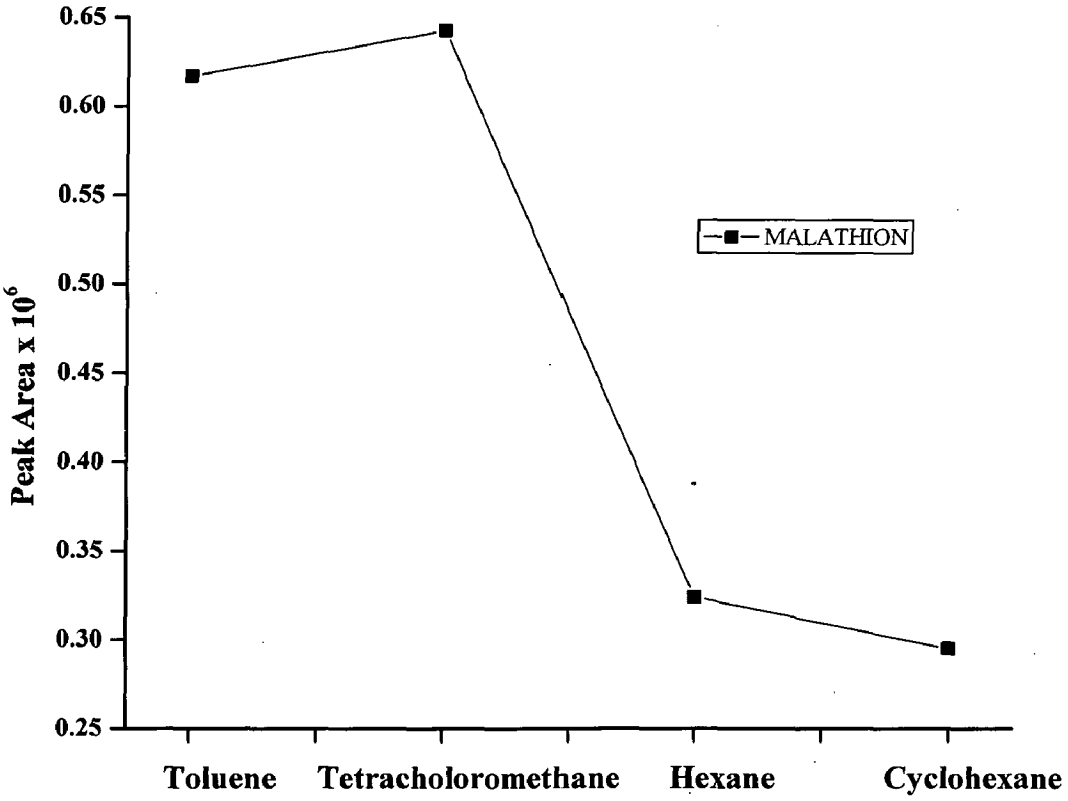


Fig. 4.1.d: Extraction efficiency of the malathion pesticides with various organic solvents.

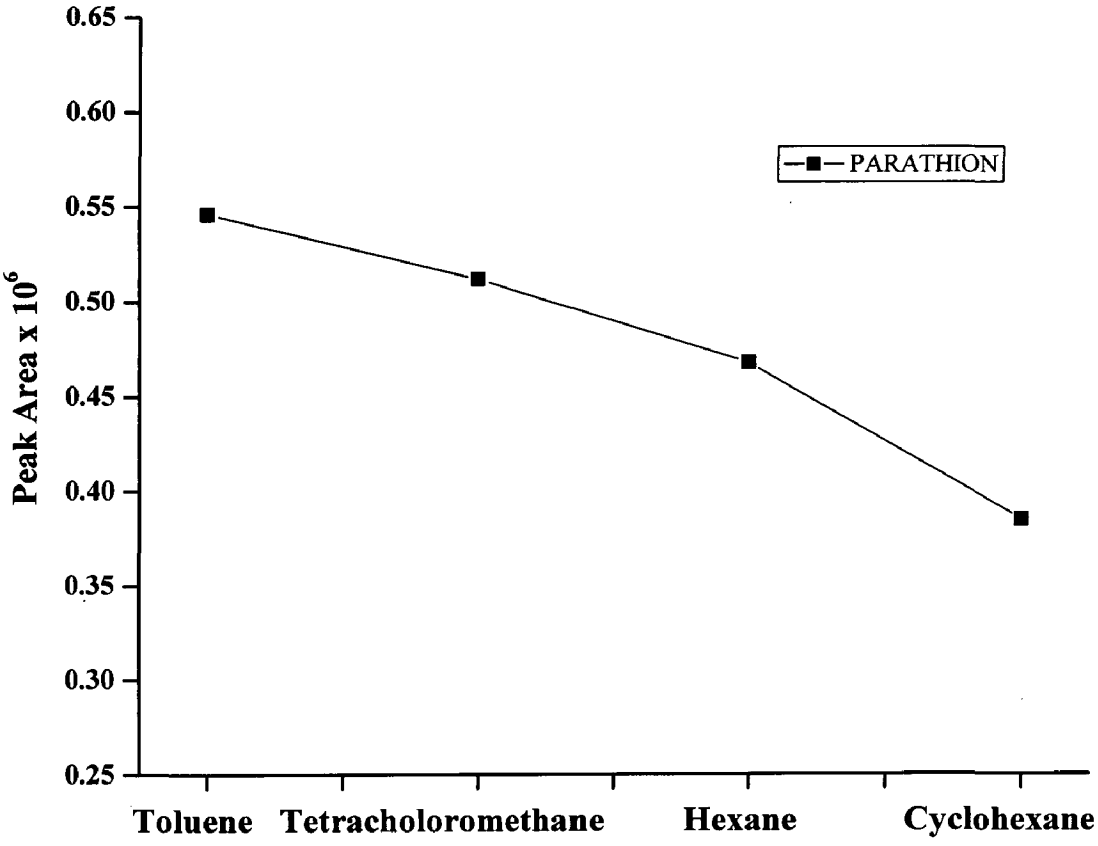


Fig. 4.1.e: Extraction efficiency of the parathion pesticides with various organic solvents.

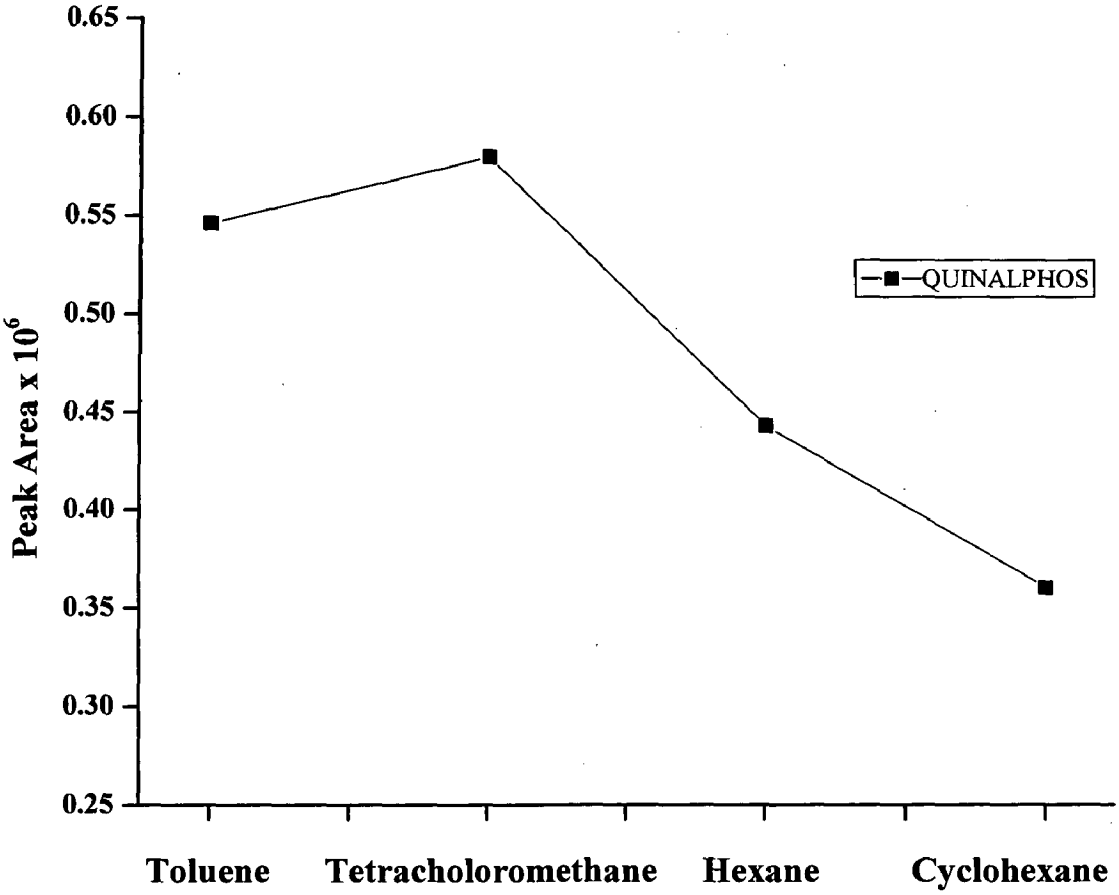


Fig. 4.1.f: Extraction efficiency of the quinalphos pesticides with various organic solvents.

found in our experiment the toluene is more stable and less toxic than other organic solvent tested. Toluene is also a very suitable solvent for pesticides GC injection.

4.1.2 Extraction Time

Single-drop microextraction is not an exhaustive extraction technique. Although maximum sensitivity is attained at equilibrium, complete equilibrium needs not to be attained for accurate and precise analysis. As prolonged extraction times may result in drop dissolution and have a high incidence of drop loss, extraction time is usually matched to the chromatography run, thus allowing maximum sample throughput.[18] However, when choosing an extraction time in the rising portion of the equilibration time profile, precise timing becomes essential for good precision.

For the purpose of the present experiments 1 µl toluene drops were exposed for sampling periods ranging from 5 to 75 min to 5 [illegible] samples spiked at 5 [illegible] and stirred at 400 rpm. After 35 min. equilibrium had not yet been reached for all the analytes, which means that it will not be practical to make use of the full capacity of the micro drop in 35 min A phenomenon of micro drop dissolution was observed (about 0.5 µl organic solvent was lost in the 35 min extraction experiments), owing to longer exposure times (Figure 4.2.a to 4.2.f). However certain extraction time was needed, for the micro drop to extract enough targets analytes. To avoid incidents of drop dissolution, due to increased exposure times, a 15 min sampling period was selected for all subsequent analyses.

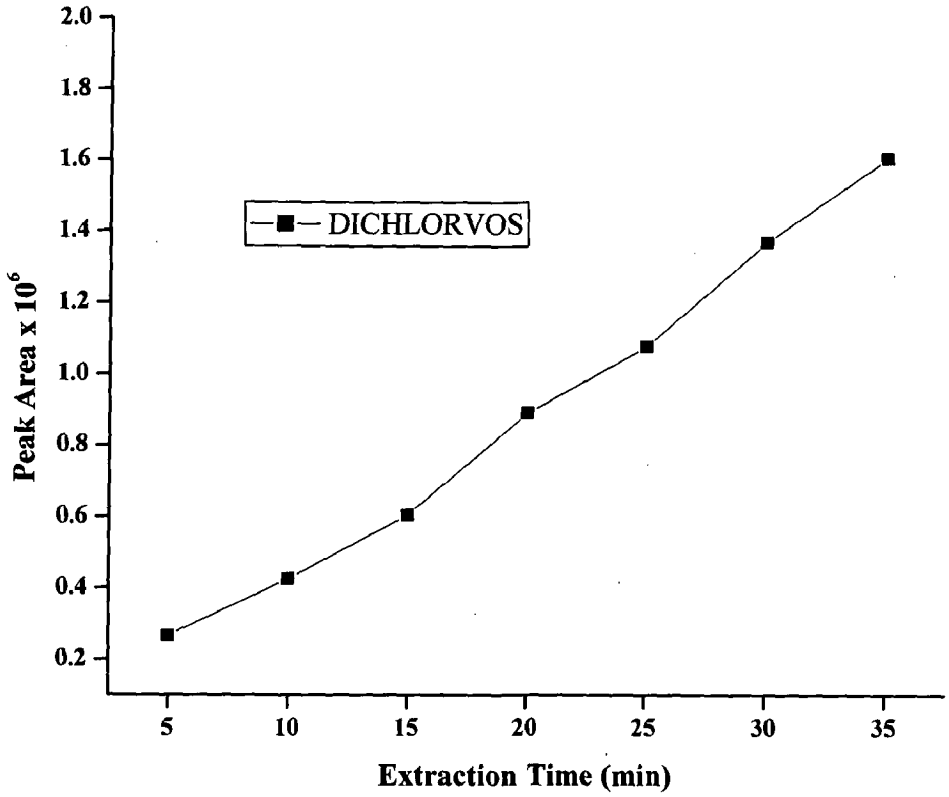


Fig.4.2.a: Plot of peak area versus extraction time for dichlorvos pesticide

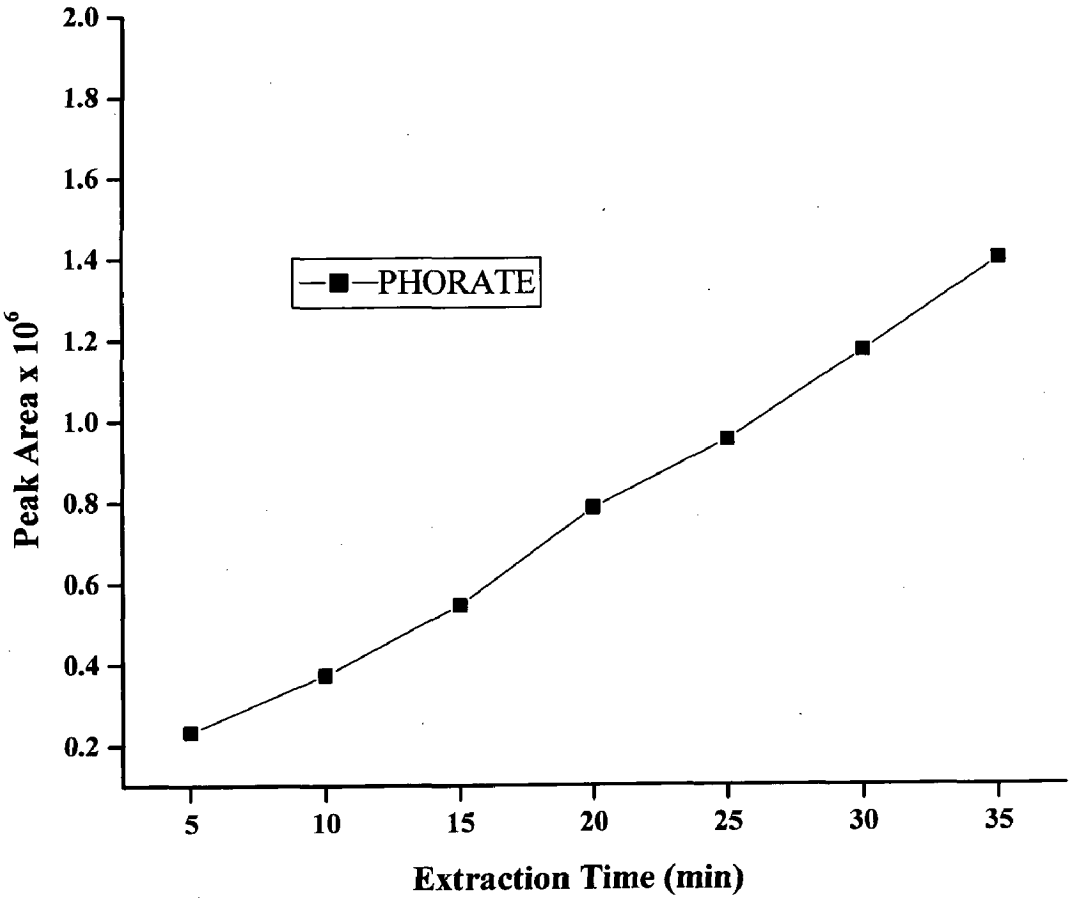


Fig. 4.2.b: Plot of peak area versus extraction time for phorate pesticides.

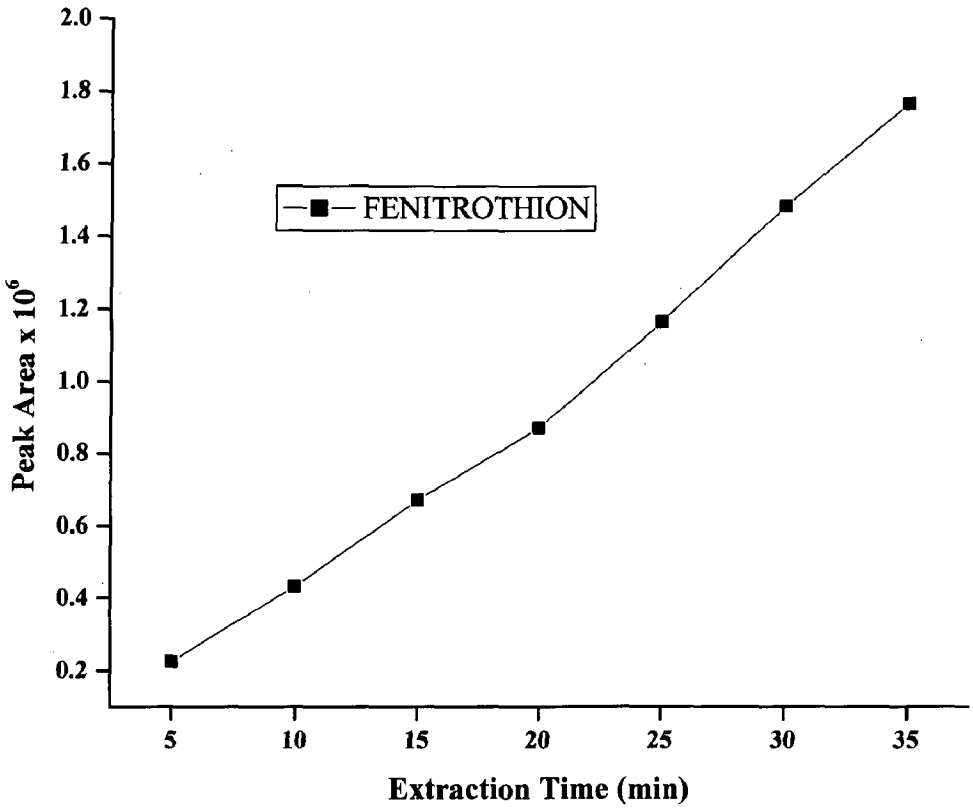


Fig.4.2.c: Plot of peak area versus extraction time for fenitrothion pesticide

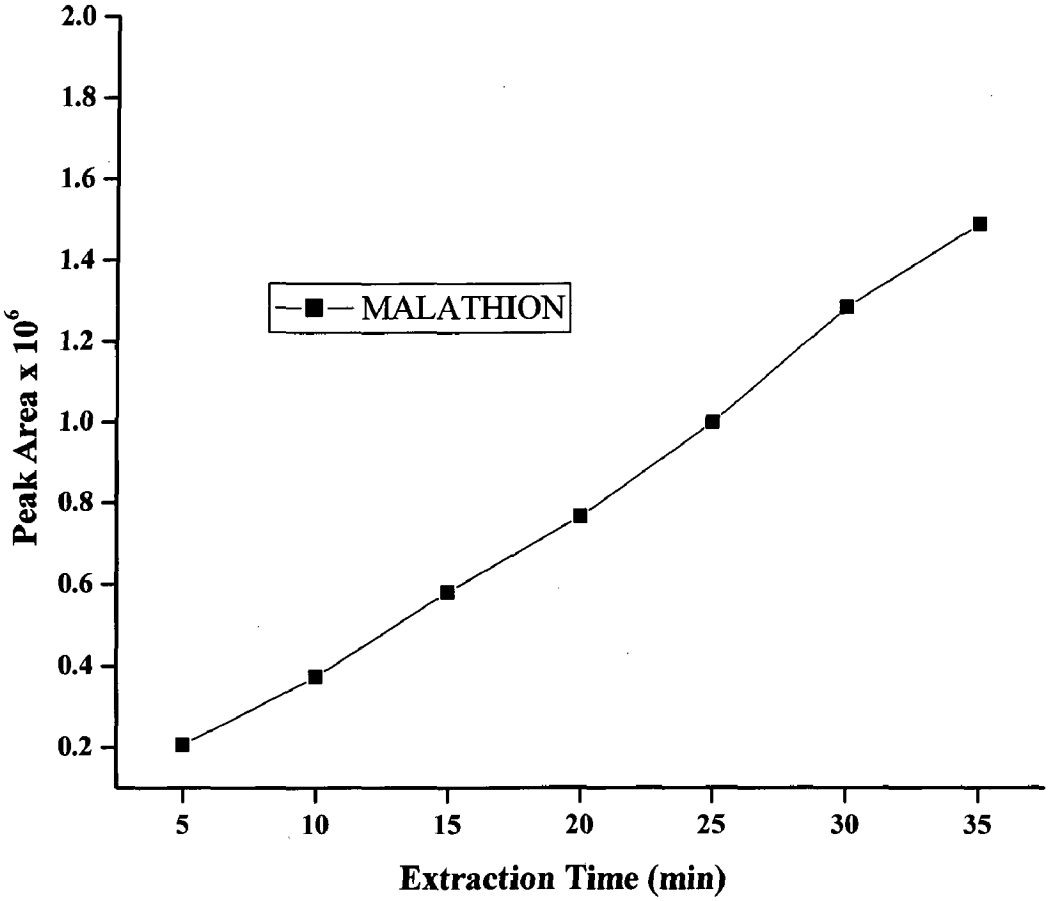


Fig.4.2.d: Plot of peak area versus extraction time for malathion pesticides.

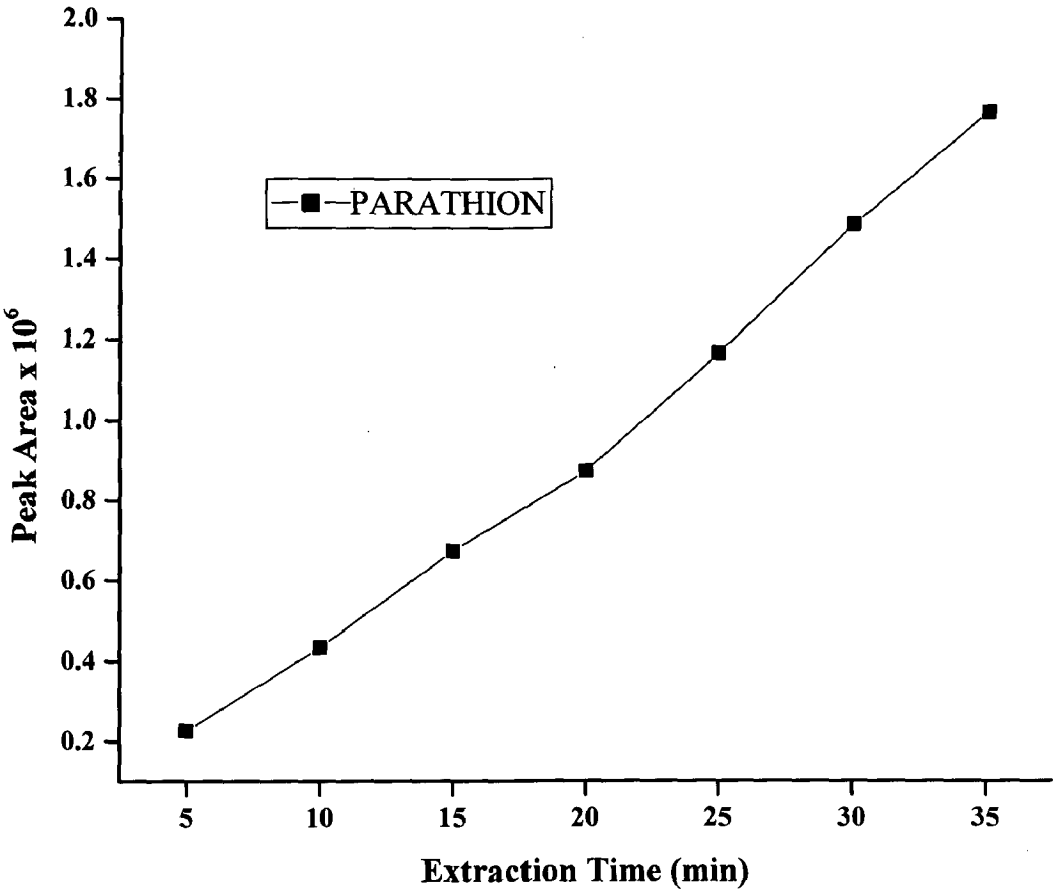


Fig.4.2.e: Plot of peak area versus extraction time for parathion pesticide

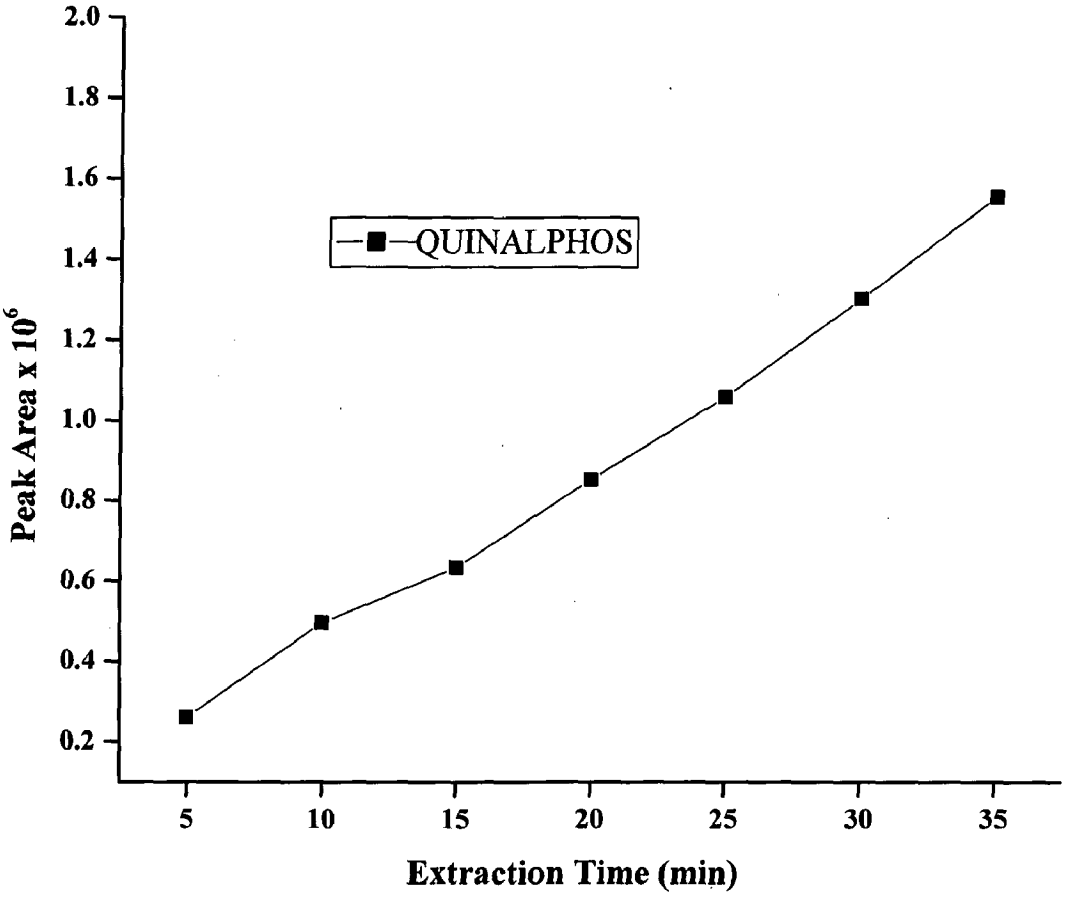


Fig.4.2.f: Plot of peak area versus extraction time for quinalphos pesticides.

4.1.3 Effect of Salt Concentration

Addition of salt mainly NaCl to the sample may have several effects on single-drop microextraction [18] as it increases the ionic strength of the solution. Depending on the solubility of the target analytes, extraction is usually enhanced with increased salt concentration and increased polarity of the target compounds (salting - out effect). For single-drop microextraction however, the presence of salt was found to restrict extraction of chlorobenzenes and the majority of nitroaromatic explosives. It was assumed that apart from the salting-out effect, the presence of salt caused a second effect and changed the physical properties of the extraction film, thus reducing the diffusion rates of the analytes into the drop.

For the purpose of the present experiments, the effect of NaCl content ranging from 0 to 10%, w/v was investigated and the instrument's response was monitored. Each time a 1 μl toluene drop was used to extract for 15 min, 5 ml water samples spiked at 5 μg/l and stirred at 400 rpm. As shown in figure 4.3.a to 4.3.f, the results varied depending on the analyte. The addition of salt improves the extraction recovery of OPPs in many conventional extraction techniques and sodium chloride (NaCl) is commonly added to analytical samples. In this study, the effect of addition salt was tested. For dichlorvos, phorate, and quinolphos the extraction efficiency appeared to reach a maximum at 5% (w/v) of sodium chloride and subsequently decreased with increasing salt content, with the effect being more pronounced for quinolphos. A similar trend was observed for fenitrothion, and parathion but this time the extraction efficiency reached its maximum at a salt content of 10%.

Finally, in the case of parathion and quinolphos, the presence of salt caused insignificant changes on extraction efficiency. Based on the above considerations a 5% (w/v) NaCl content was used for all subsequent extractions.

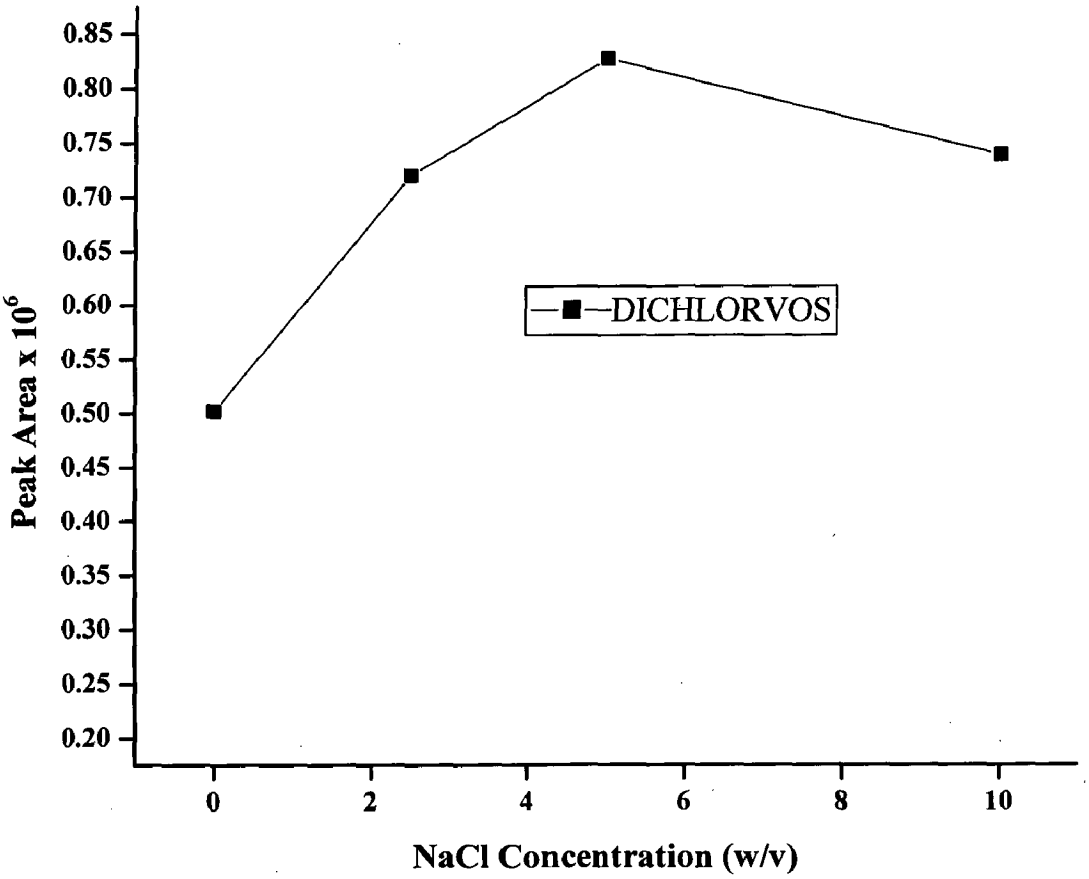


Fig.4.3.a: Plot of peak area versus NaCl concentration for dichlorvos pesticide

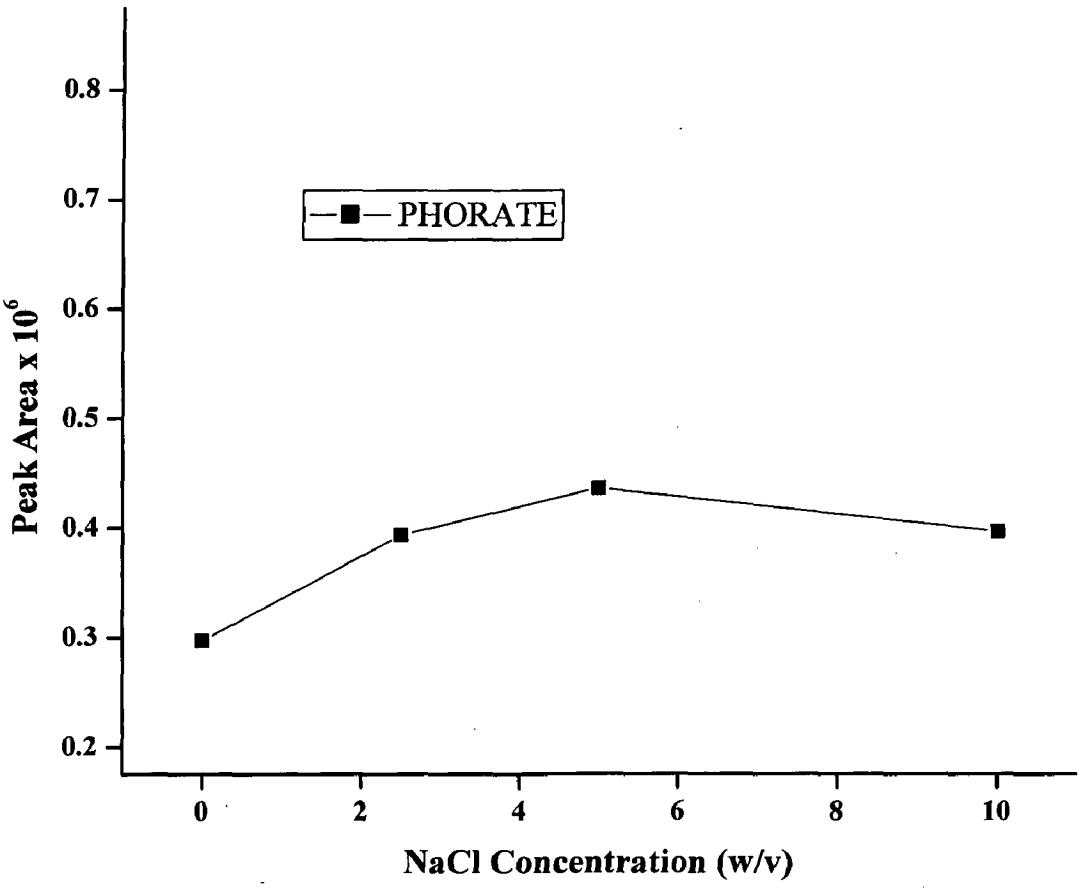


Fig. 4.3.b: Plot of peak area versus NaCl concentration for phorate pesticide

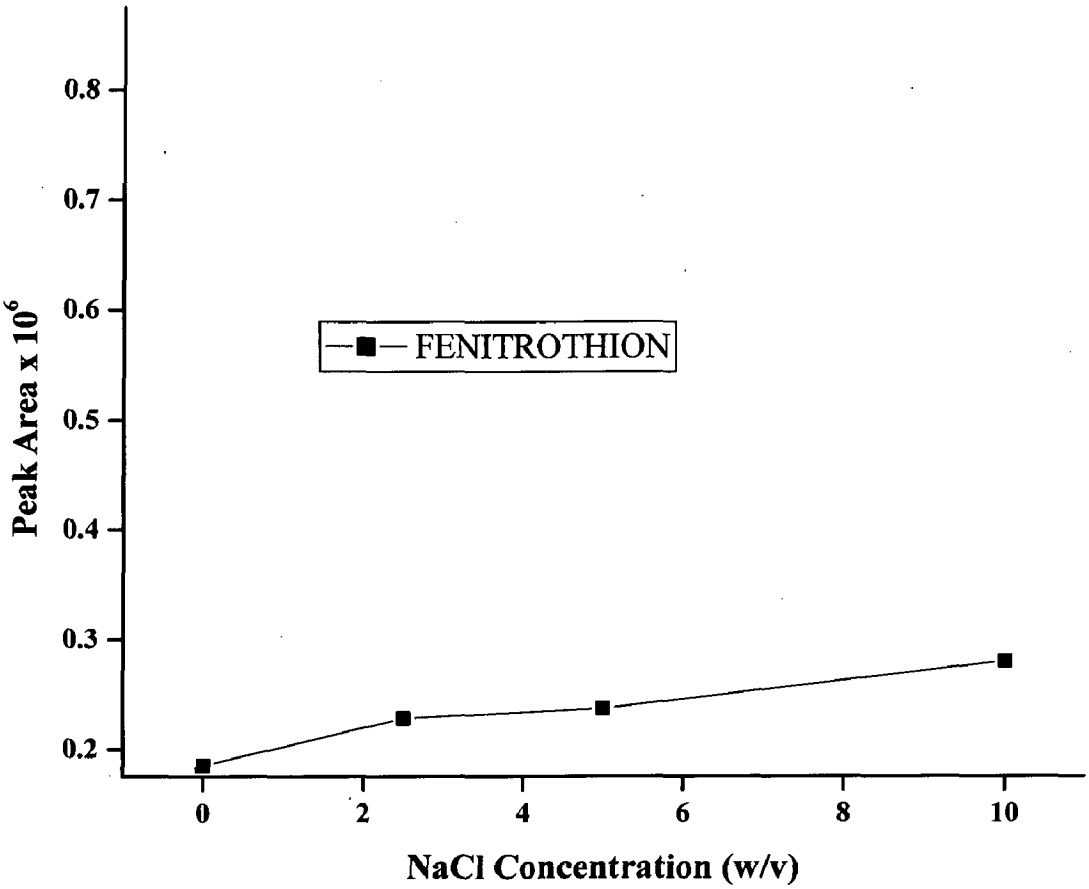


Fig. 4.3.c: Plot of peak area versus NaCl concentration for fenitrothion pesticide

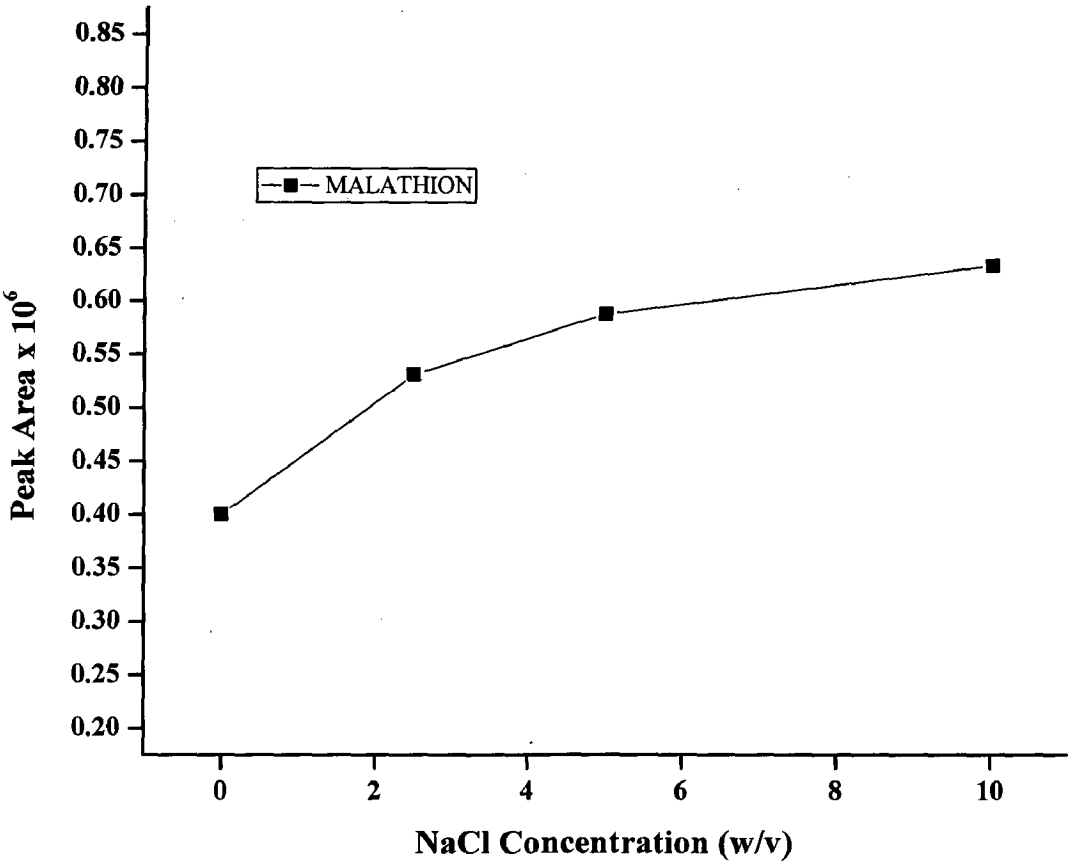


Fig.4.3.d: Plot of peak area versus NaCl concentration for malathion pesticide

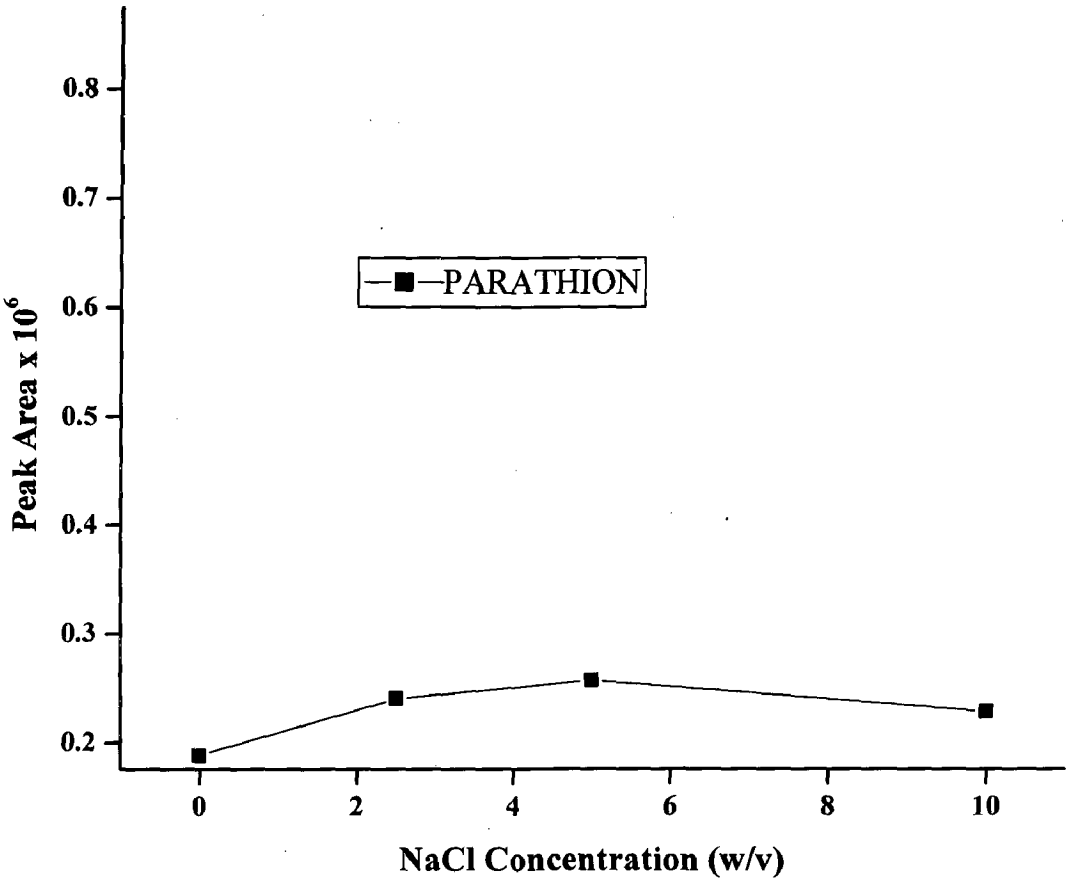


Fig. 4.3.e: Plot of peak area versus NaCl concentration for parathion pesticide

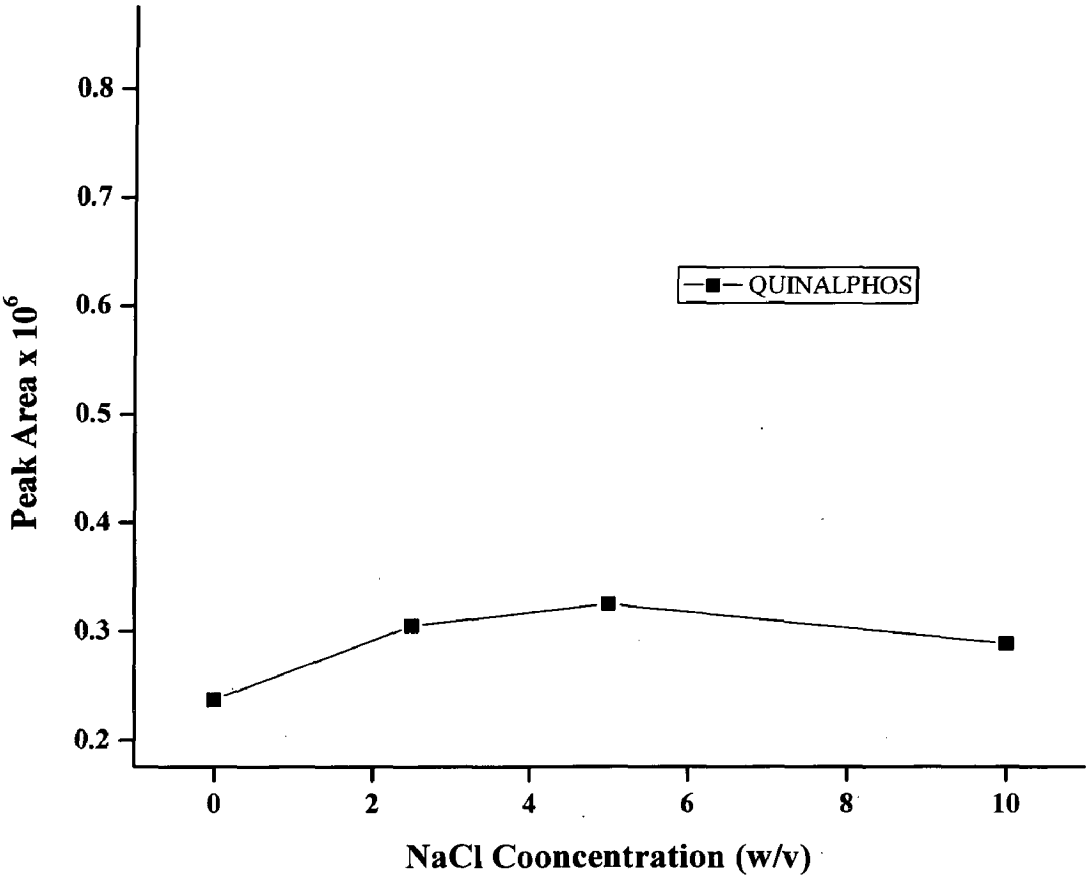


Fig.4.3.f: Plot of peak area versus NaCl concentration for quinalphos pesticide

4.1.4 Effect of Sample Agitation on Extraction

For the drop-based extraction techniques, magnetic stirring produced a dramatic increase in the analytical response of the instrument in all cases. Agitation of the sample enhances extraction and reduces the time to thermodynamic equilibrium. However, when the microdrop is directly exposed to the aqueous phase there is an upper limit as higher stirring rates result in drop dislodgement and drop dissolution especially for prolonged extraction times. The use of small stir bars, constant rotational speed and base plates thermally isolated from the sample vial are required for good precision [18].

In a separate set of experiments the effect of sample agitation on extraction was investigated. For the purpose of these experiments a 1μl toluene drop was used each time to extract for 15 min water samples containing 5% (w/v) NaCl and 5ng/mL of each target analyte stirred at different agitation rates (namely, 400, 600, 800 and 1000 rpm). As expected, the results revealed that agitation dramatically enhanced extraction and according to figure , the amount of extracted analytes reached a maximum at 1000 rpm (Figure 4.4.a to 4.4.f). Nonetheless, at this stirring rate the formation of air bubbles was promoted increasing the incidents of drop loss and/or dislodgement. It was also found that at this stirring speed, the volume of the organic drop was reduced by an average value of 28% due to toluene dissolution into the aqueous phase. Based on these observations stirring of the sample at 800 rpm was selected, yielding thus acceptable results for all target analytes.

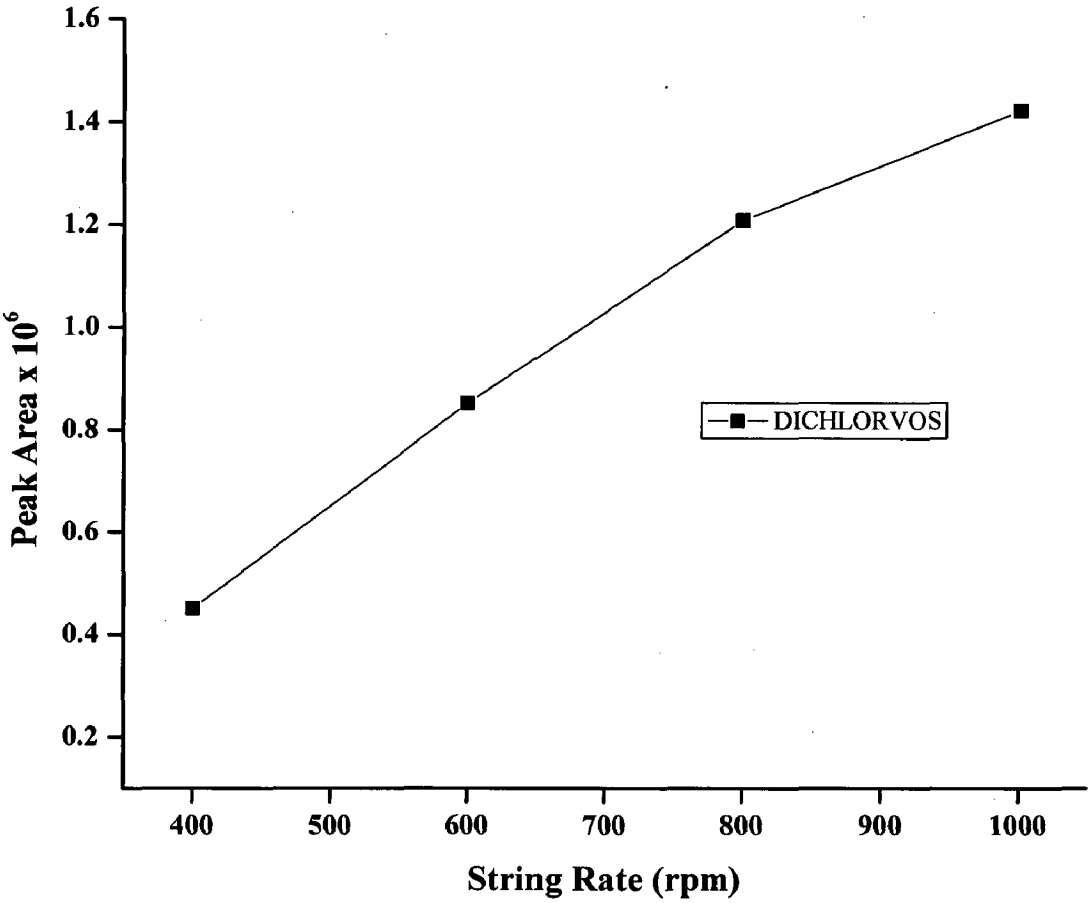


Fig. 4.4.a: Effect of string rate on the extraction efficiency for dichlorvos pesticides

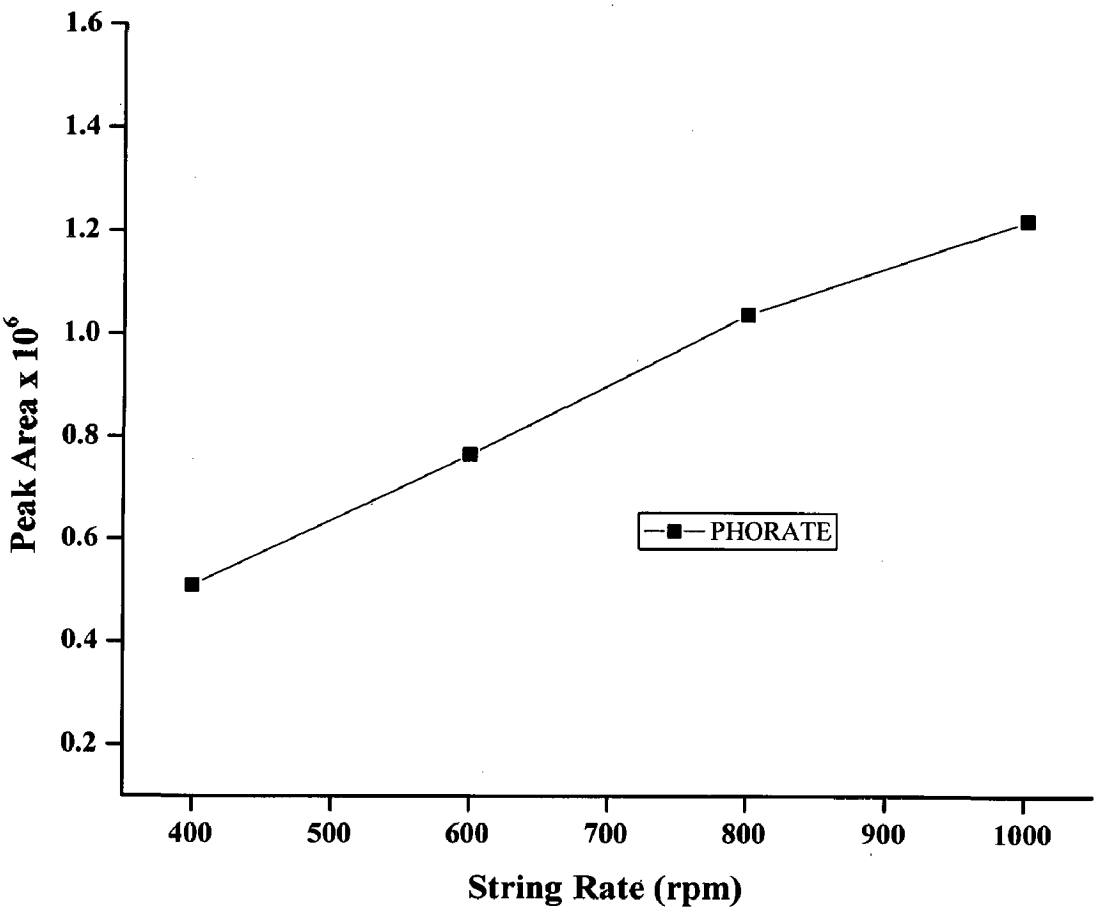


Fig. 4.4.b: Effect of string rate on the extraction efficiency for phorate pesticide

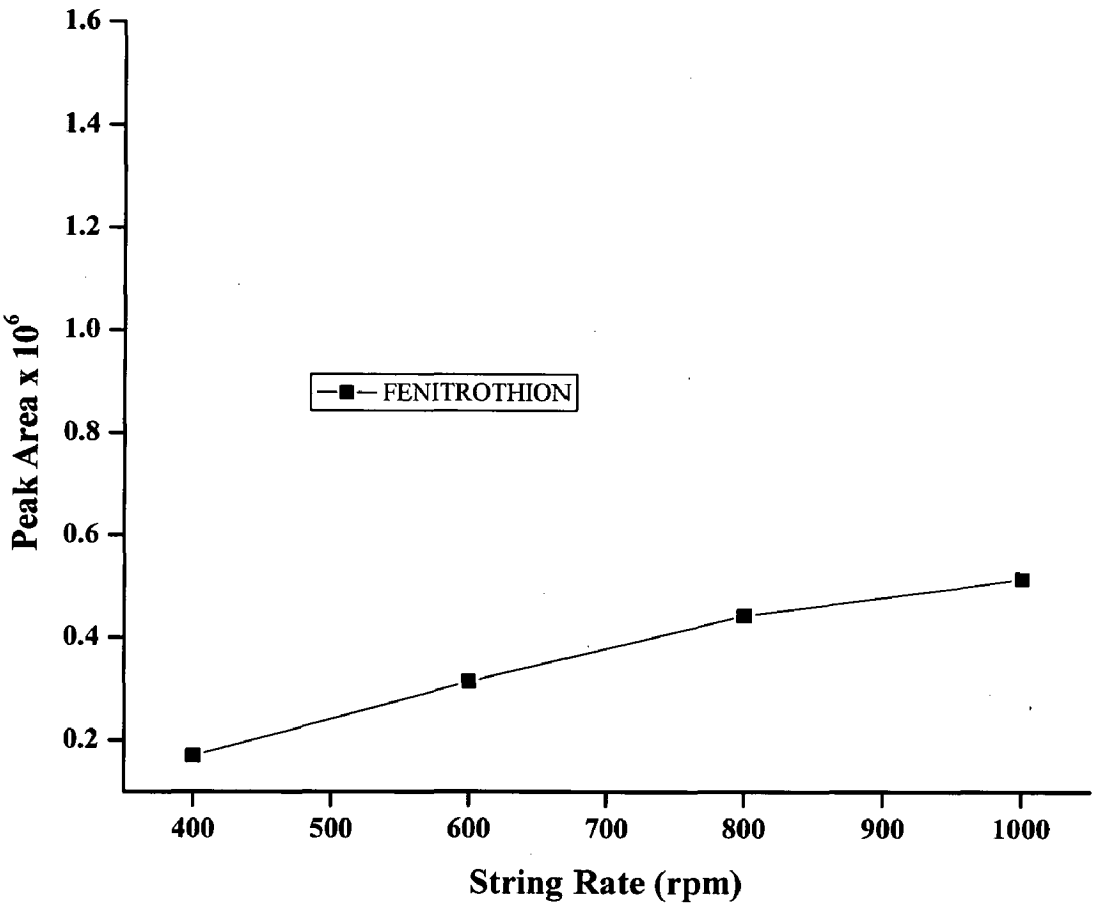
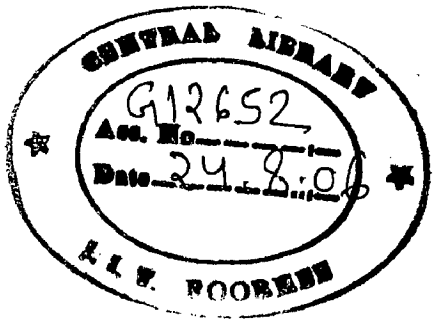


Fig. 4.4.c: Effect of string rate on the extraction efficiency for fenitrothion pesticides.



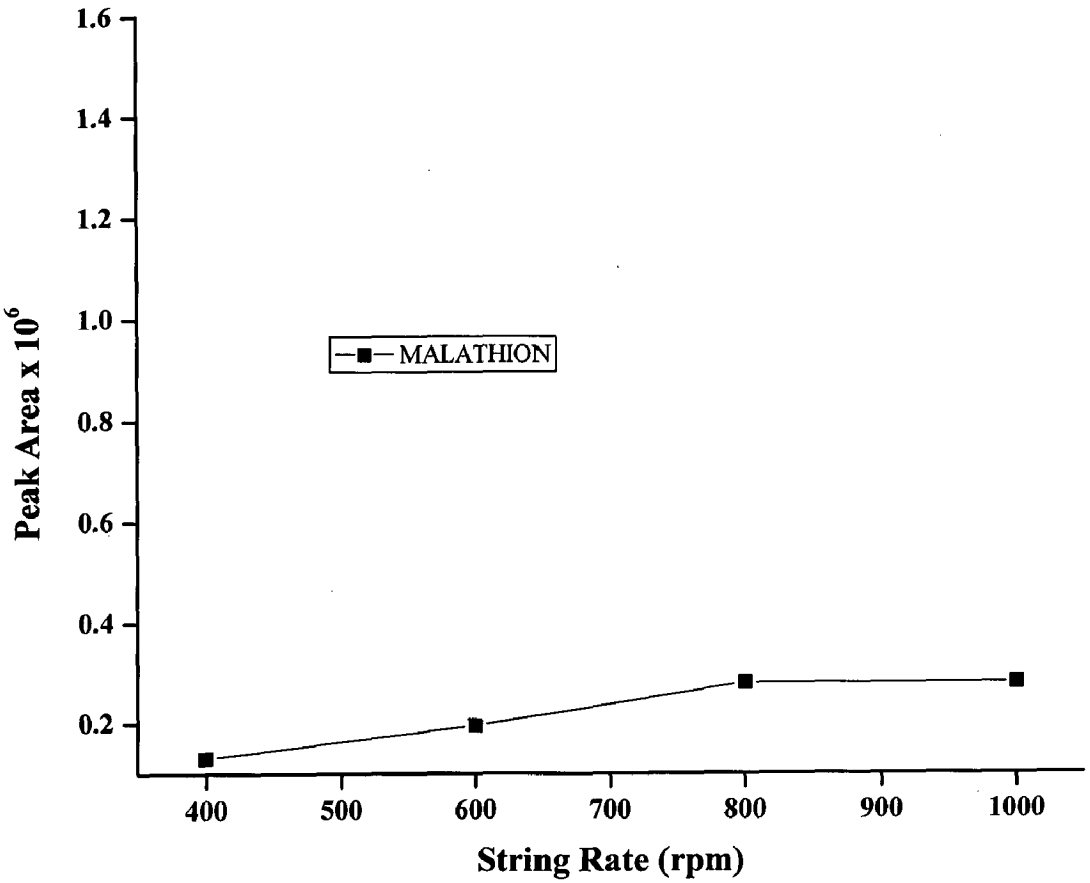


Fig. 4.4.d: Effect of string rate on the extraction efficiency for malathion pesticide

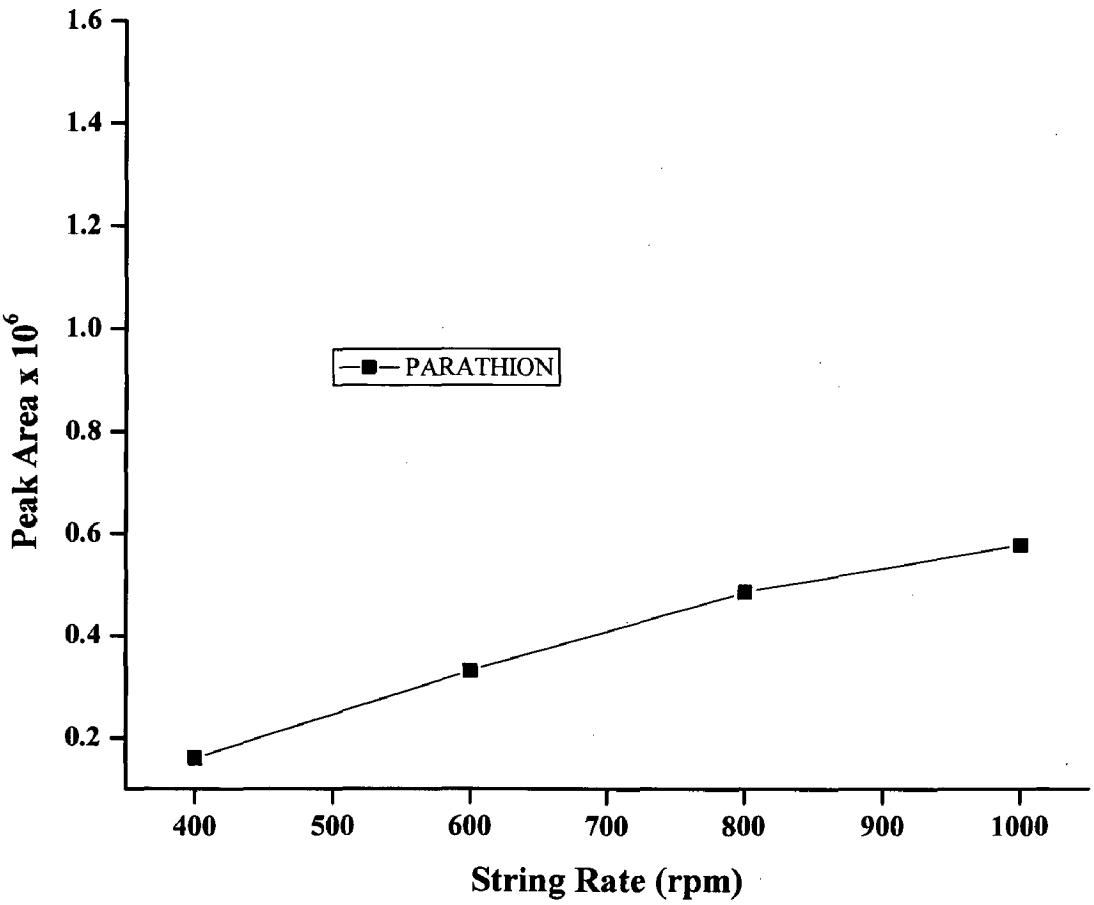


Fig. 4.4.e: Effect of string rate on the extraction efficiency for parathion pesticide

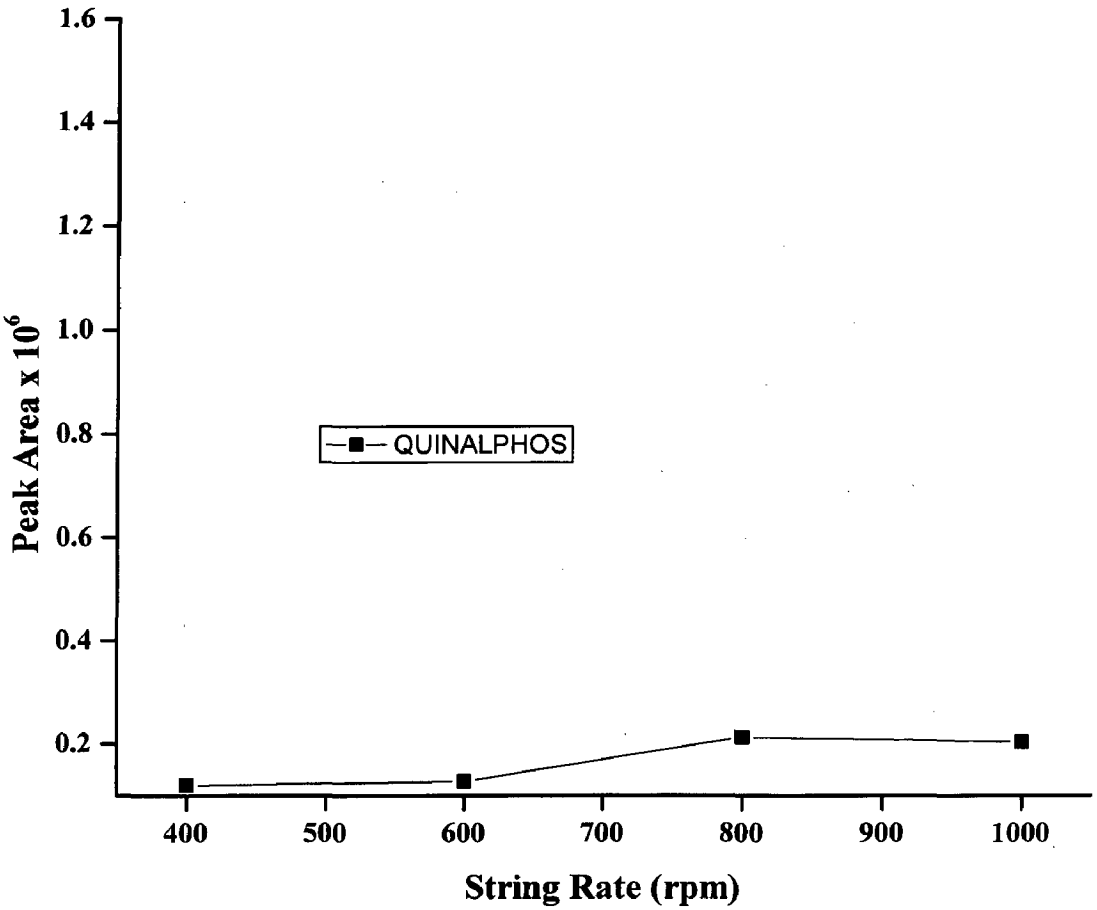


Fig. 4.4.f: Effect of string rate on the extraction efficiency for quinalphos pesticides.

1.5 Effect of Solvent Drop Volume

For single-drop microextraction, organic drop volumes of 1-2 μl are more commonly used as they ensure the formation of a stable /reproducible microdrop and allow fast stirring speeds [18].

The effect of solvent drop volume on SDME extraction was then investigated. For this set of experiments 500 μg wheat samples containing 5% (w/v) NaCl spiked at 5 mg/mL and stirred at 800 rpm were extracted for 15 min with organic drop volumes ranging from 1.0 to 2.0 ml. As expected increasing the volume of the organic acceptor phase resulted in an increase of the amount of extracted analytes figure 4.5.a to 4.5.f. Although at 2.0 ml extraction reached a maximum, such micro drop volumes are difficult to manipulate. Thus, 1.5 ml drop volumes were used for all subsequent extractions.

Overall, the optimised SDME experimental conditions found here were: 1.5 ml toluene micro drop, 500 μg wheat samples, 800 rpm stirring rate, 5% NaCl content and 15 min sampling time.

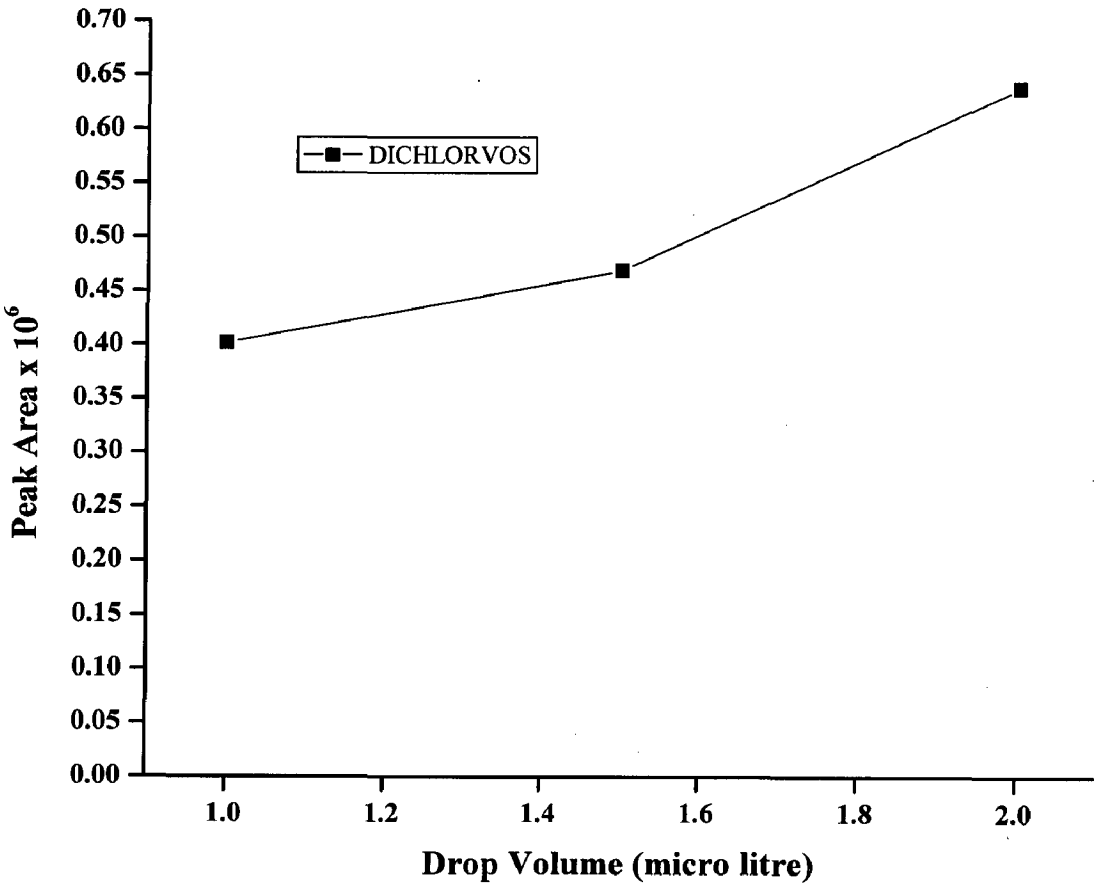


Fig.4.5.a: Effect of toluene drop volume on the extraction efficiency for dichlorvos pesticide.

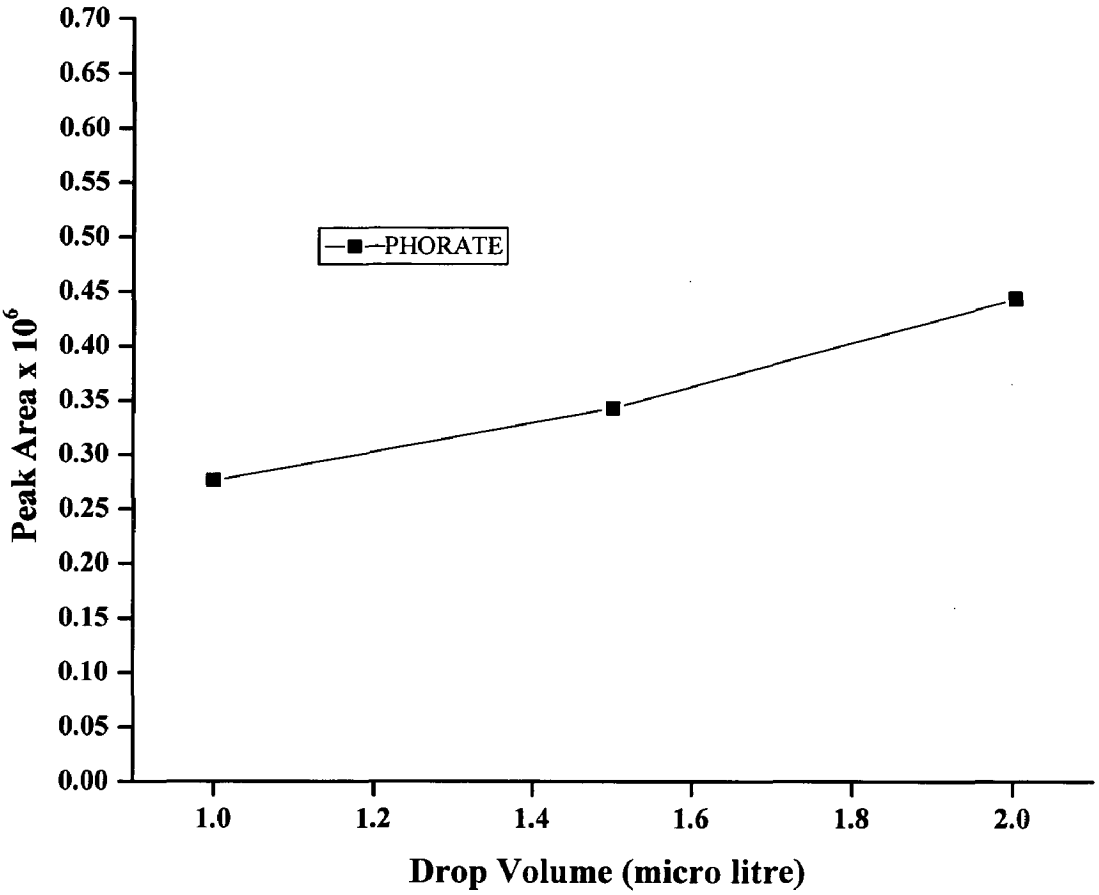


Fig.4.5.b: Effect of toluene drop volume on the extraction efficiency for phorate pesticide.

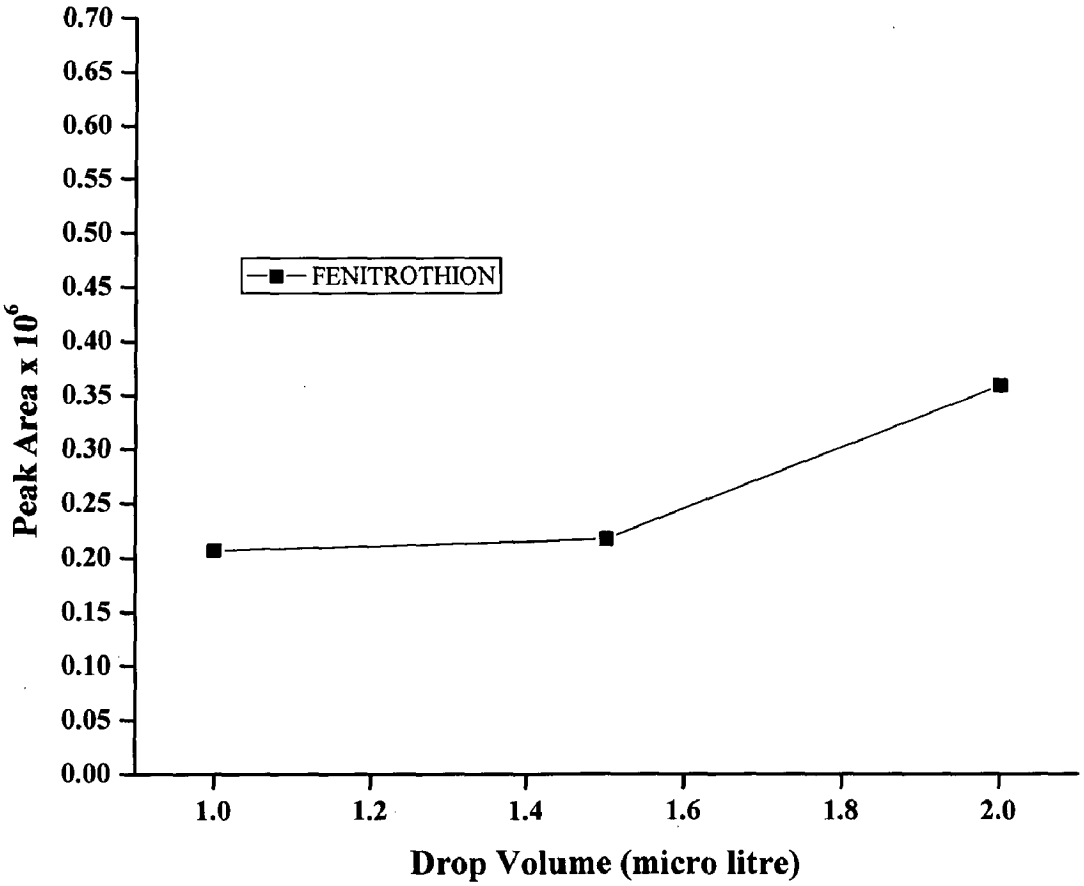


Fig.4.5.c: Effect of toluene drop volume on the extraction efficiency for fenitrothion pesticide.

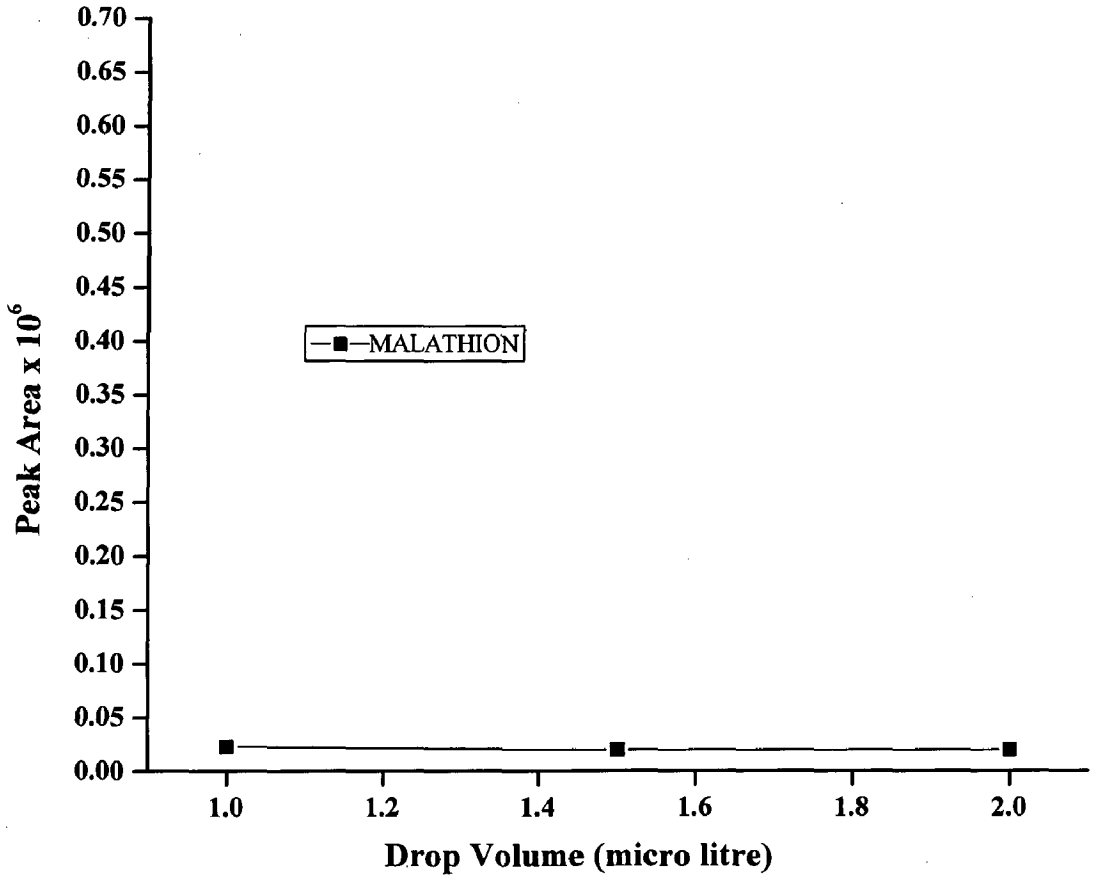


Fig.4.5.d: Effect of toluene drop volume on the extraction efficiency for malathion pesticide.

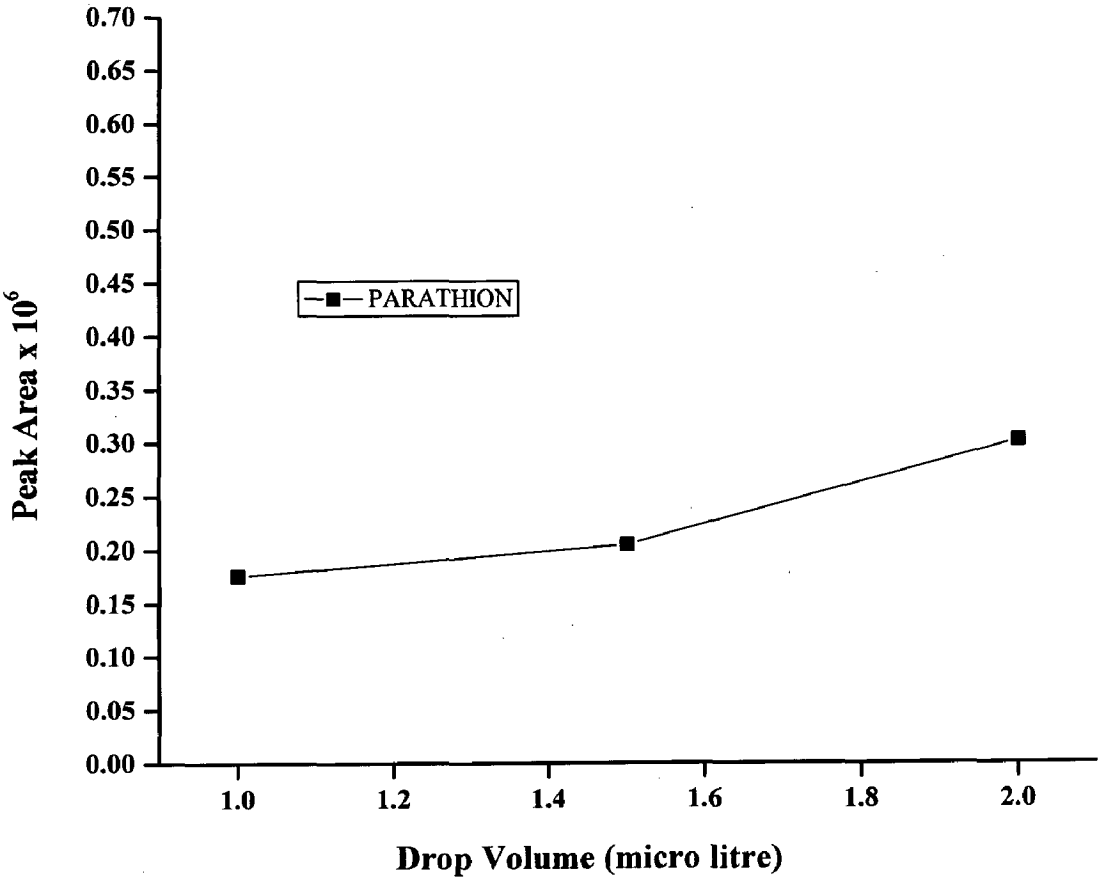


Fig.4.5.e: Effect of toluene drop volume on the extraction efficiency for parathion pesticide.

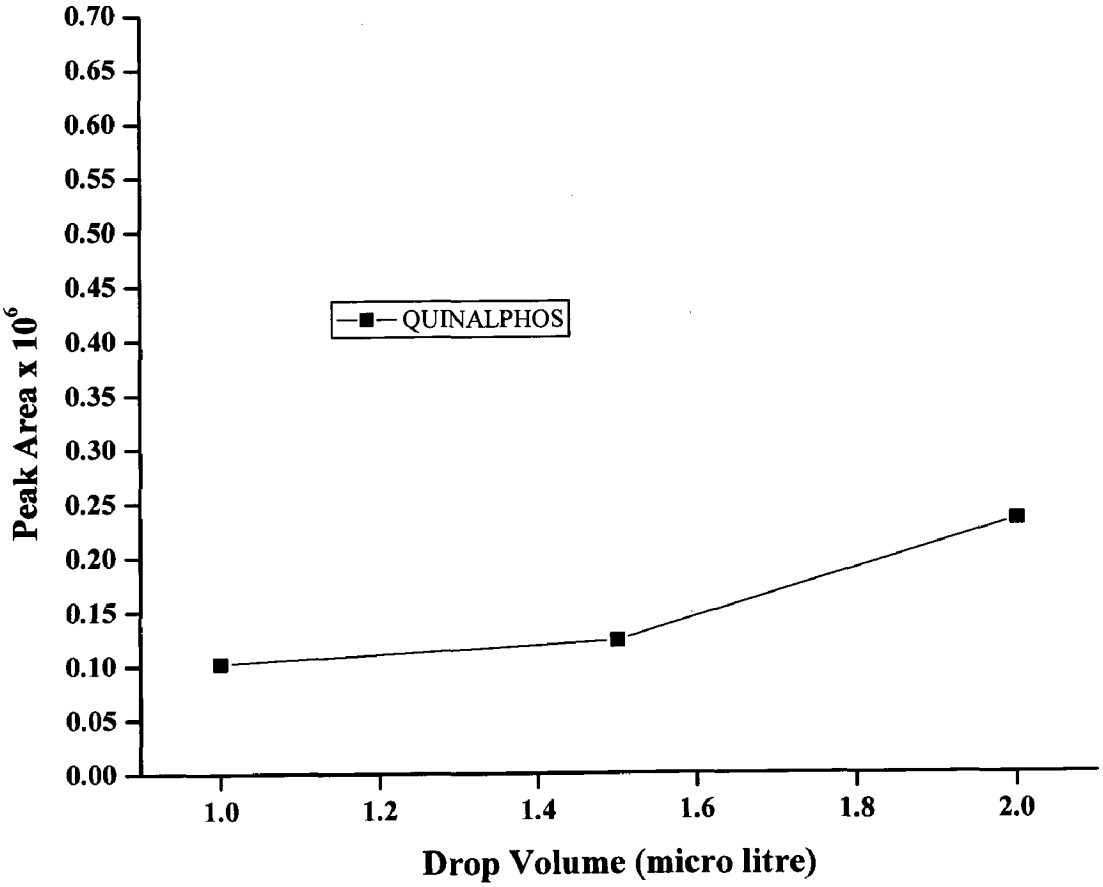


Fig.4.5.f: Effect of toluene drop volume on the extraction efficiency for quinalphos pesticide.

4.2 PERFORMANCE OF THE SDME METHOD

4.2.1 Linearity of Calibration Curve

This is the most common method where interference effects are known to be absent .The calibration curves were prepared for each OPPs by plotting peak area verses concentration. Calibration curve is the ratio of the peak area for the target pesticide to plotted on the *y*-axis, and concentration of the target pesticide was plotted on the *x*-axis. This optimized single-drop microextraction (SDME) condition were the used to evaluate the linearity of the proposed method over the concentration range 0.5-50 μg/l for all target pesticide. The correlation coefficients were calculated from the six data (triplicates in six points) on the calibration curve, and ranged between 0.9526 and 0.9875 as shown in figure 4.6.a to 4.6.f.

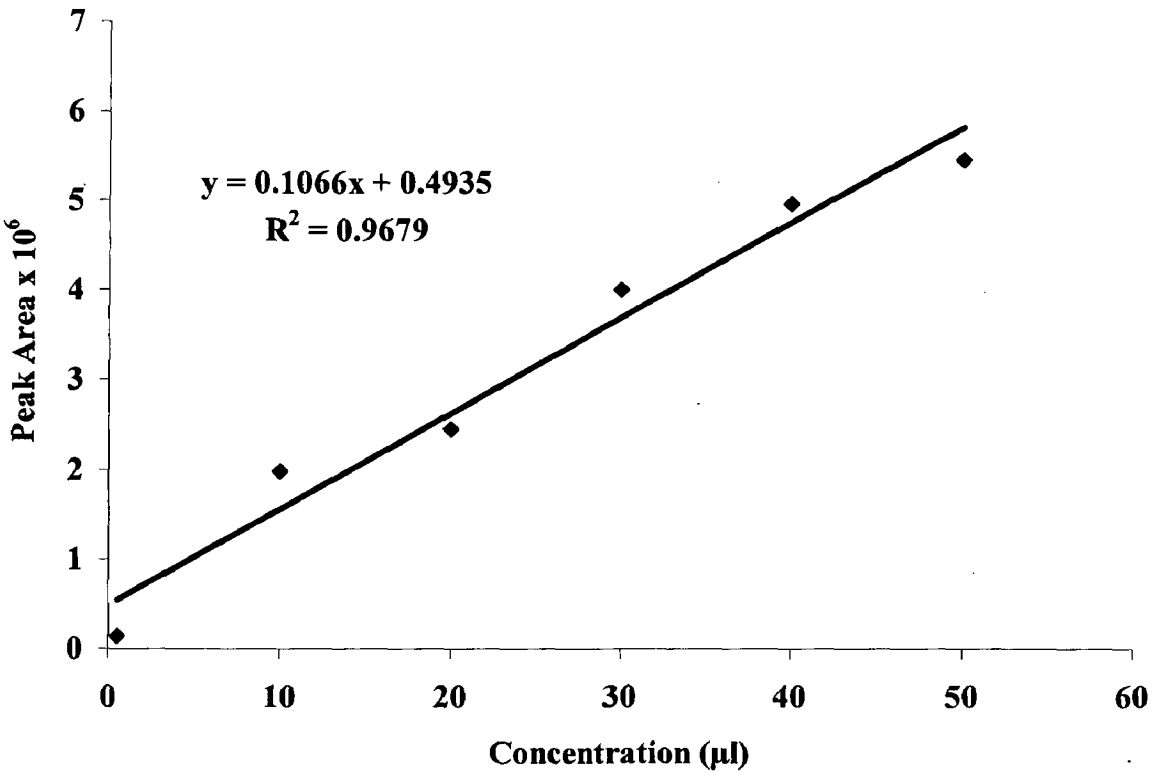


Fig.4.6.a: GC-MS external standard calibration curves for Dichlorvos

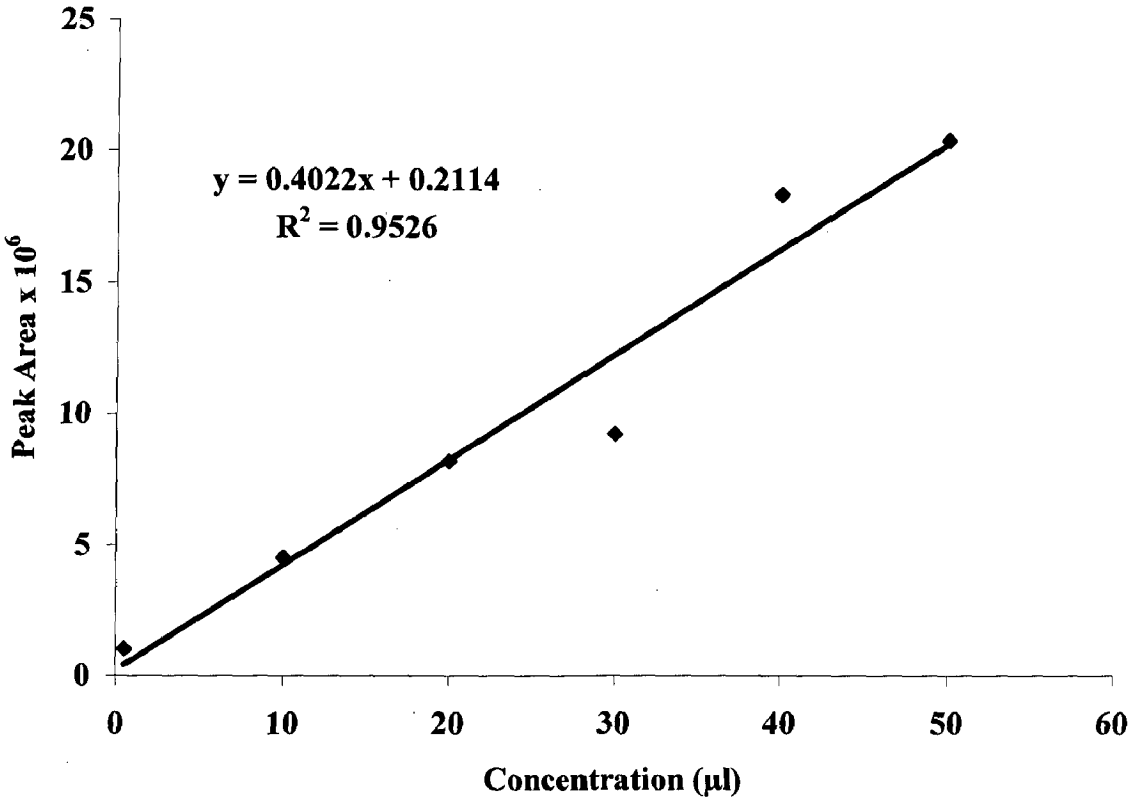


Fig. 4.6.b: GC-MS external standard calibration curves for Phorate.

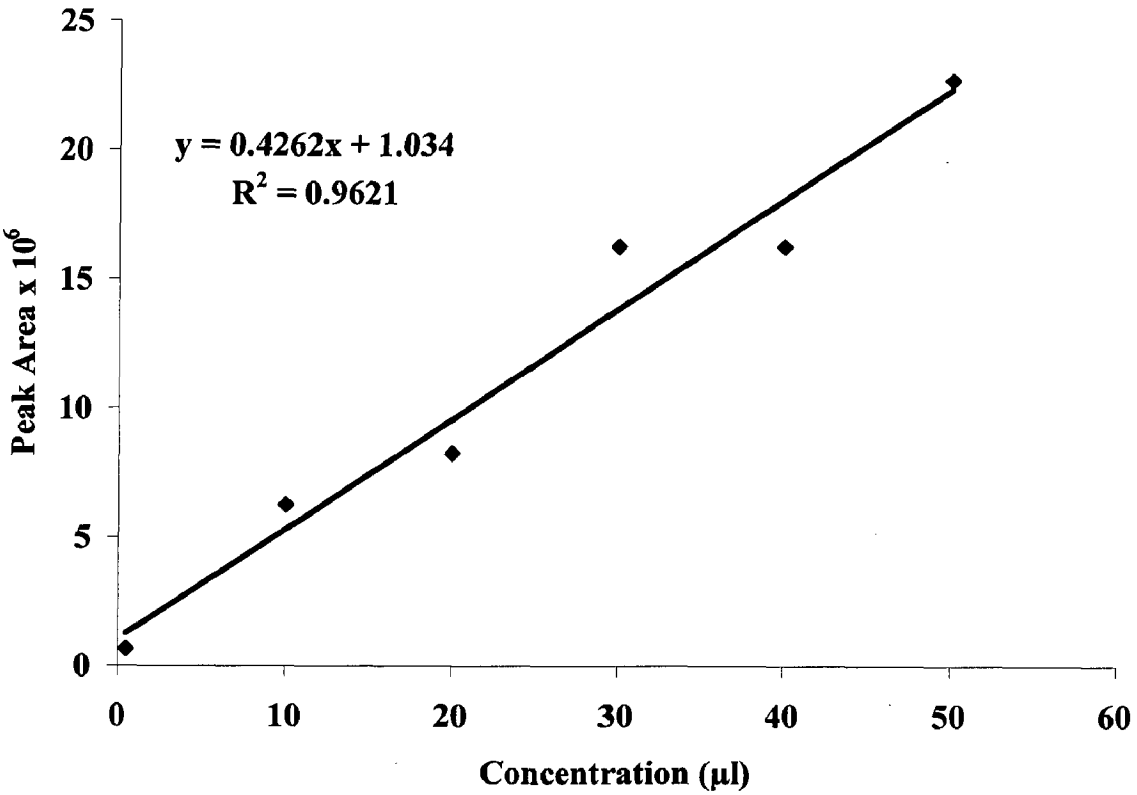


Fig. 4.6.c: GC-MS external standard calibration curves for Fenitrothion

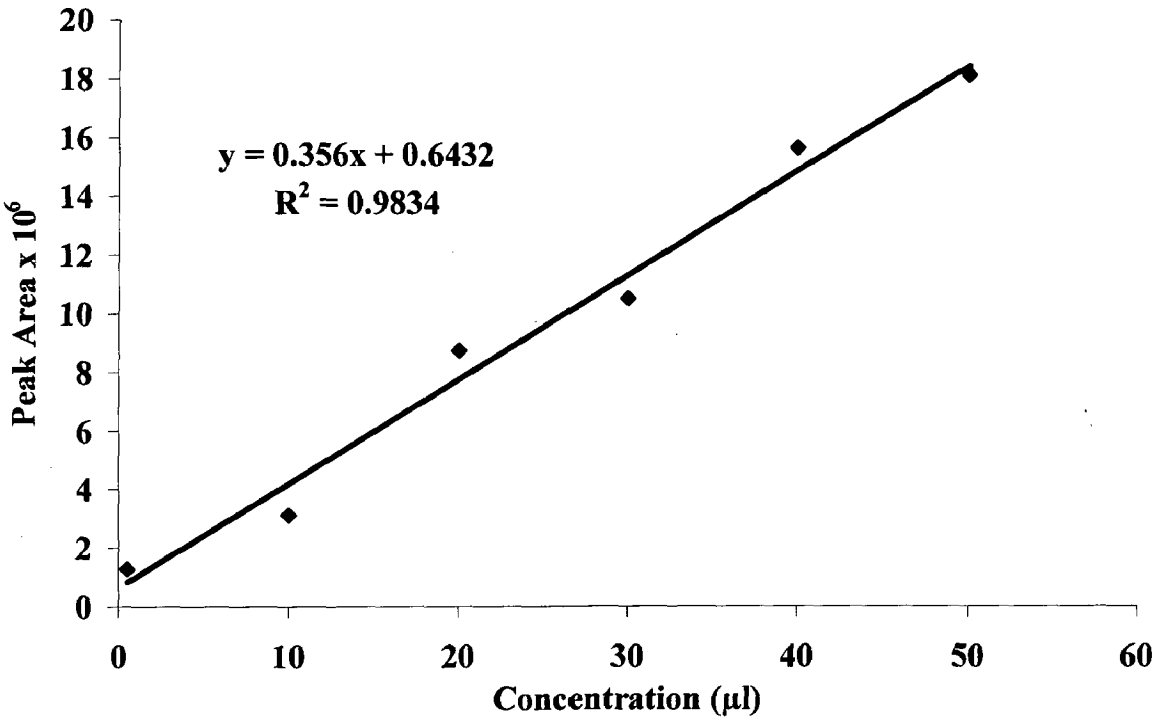


Fig. 4.6.d: GC-MS external standard calibration curves for Malathion

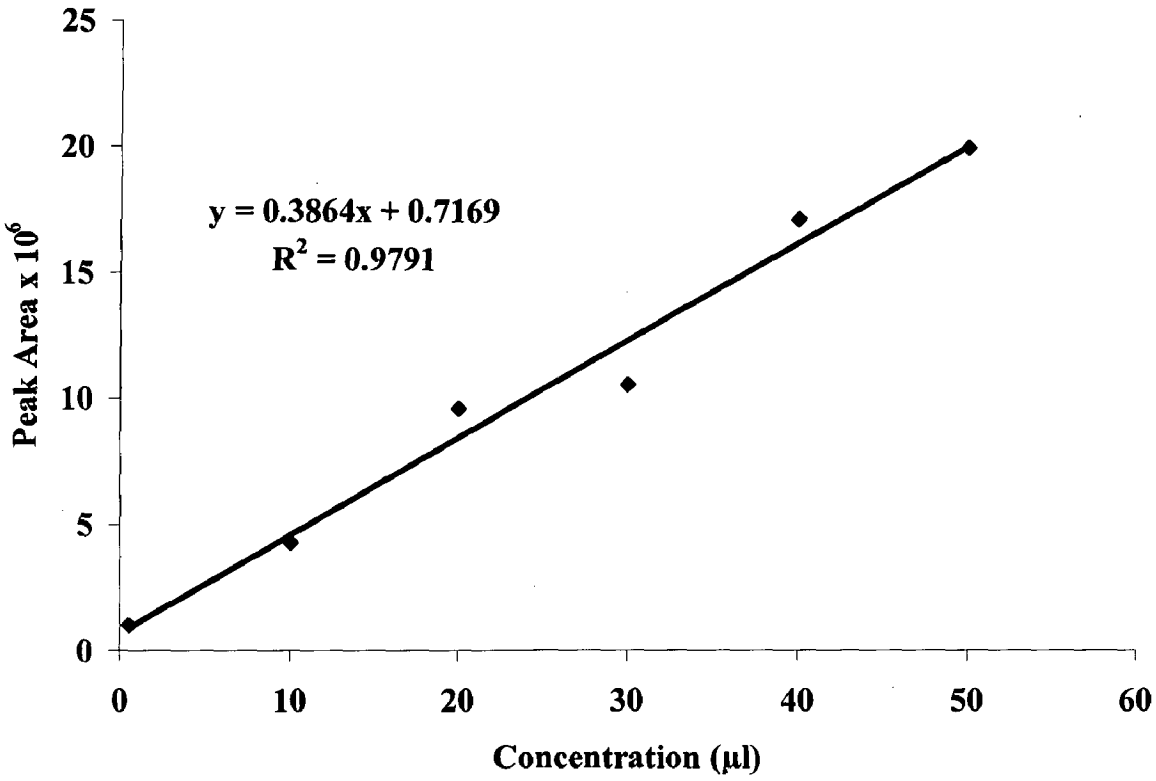


Fig. 4.6.e: GC-MS external standard calibration curves for Parathion

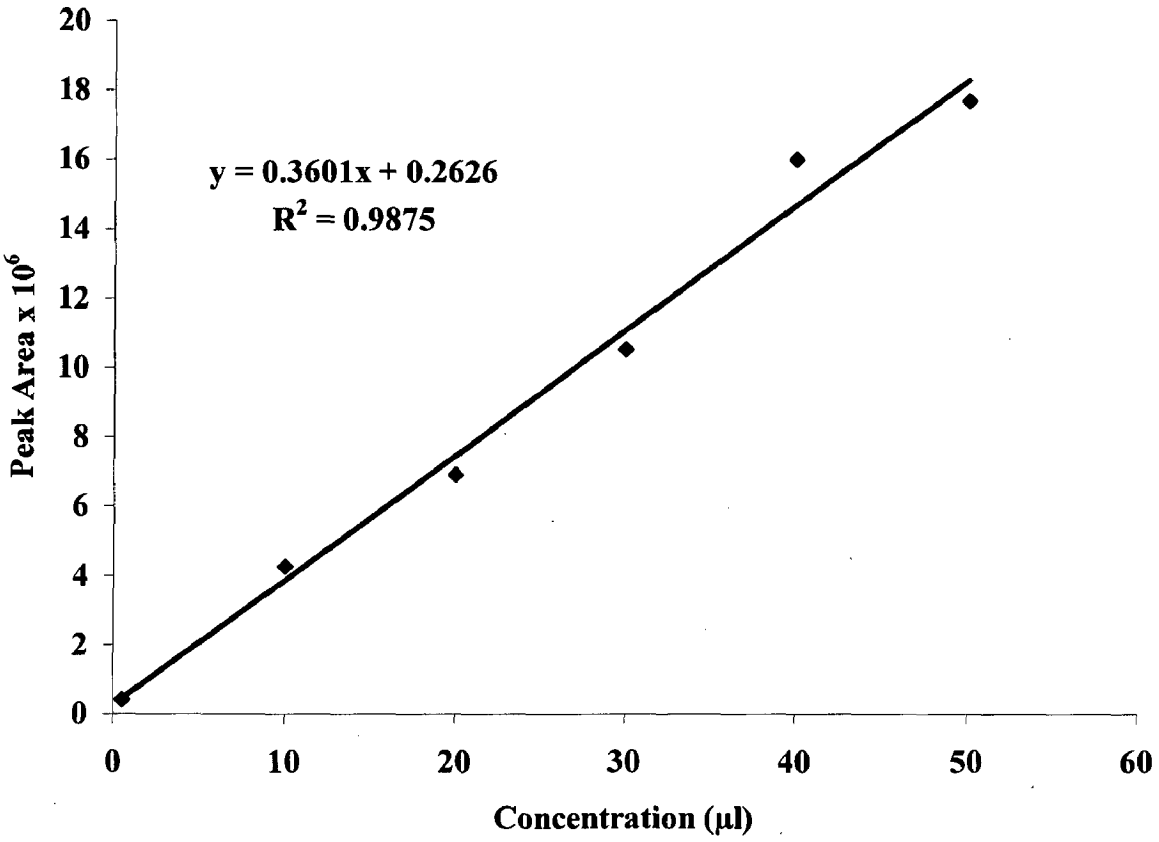


Fig. 4.6.f: GC-MS external standard calibration curves for Quinalphos

4.2.2 Limit of Detection

The calculations of the limits of detection (LOD) were based on a three N/m ratio, where N is the background noise and m is the slope of the respective calibration equation. Table 3 shows the LOD values for each analytical system calculated using 1 µl injection of the matrix matched standard containing 0.05 ng of pesticide. Values given are the highest LOD measured in a chromatography run. It was possible to determine OPPs residue at a level of 0.05 µg/ml for wheat.

Table 3: Analytical performance data for six OPPs by SDME-GC-EID

OPPs	Equation	Correlation coefficient (R^2)	Linearity (µg/ml)	S.D. (%)	Detection Limit(mg/kg)
Dichlorvos	Y=0.1066x + 0.4935	0.9679	0.5-50	17.0195	0.62
Phorate	Y=0.4022x + 0.2114	0.9526	0.5-50	15.5900	0.32
Fenitrothion	Y=0.4262x + 1.034	0.9620	0.5-50	15.3335	0.35
Malathion	Y=0.3560x + 0.6432	0.9834	0.5-50	15.5770	0.36
Parathion	Y=0.3864x + 0.7169	0.9791	0.5-50	1.4750	0.24
Quinalphos	Y=0.3601x + 0.2626	0.9875	0.5-50	15.650	0.34

CHAPTER 5

CONLUSIONS AND RECOMMENDATION

5.1 CONCLUSION

Single-drop microextraction (SDME) process to a gas chromatography mass spectrometer (GC-MS) with EID detection allows determination of organophosphorus pesticides (OPPs) from wheat sample.

Gas chromatography is the technique of choice for determining pesticides because its favorable combination of very high selectivity resolution, good accuracy and precision, wide dynamic concentration range and high sensitivity for thermostable and volatile molecule.

In this study, be further optimized operating parameter and evaluated performance characteristics of GC-MS EID for the analysis of multiple pesticide residues in food crop.

In addition to the pesticide solution prepared in solvent, which were used for initial optimization and evaluation, separation efficiency and peak characteristics (including the no. of point across peak).

Once the sample is prepared the extraction and determination can be perform automatically. SDME has demonstrated to be fast, simple, solvent free method for extracting pesticide form wheat sample.

5.2 RECOMMENDATION

Although a thorough study was made on the laboratory scale on Single-drop microextraction (SDME), still some modification are needed to implement in the system for industrial application. Following are some of the recommendation needed in the Single-drop microextraction system:

- Due to the lack of time, the performance of Single-drop microextraction for the change in extraction solvent, drop volume, string rate, salt concentration are not studied in depth. Therefore these studies should be continued for further work.
- The SDME process can also studied by using other detector like Nitrogen Phosphorus Detector, Flam Photometry Detector.
- Further research work on different food materials are required to explore the potential of Single-drop microextraction process.

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