NOVEL SPECTROPHOTOMETRIC AND HPLC METHODS FOR THE DETERMINATION OF DINITROANILINE AND ARYLOXYPHENOXY GROUPS OF HERBICIDES IN ENVIRONMENTAL SAMPLES

5/3/13.



PhD Thesis

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PAKISTAN
MARCH-2011

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FARHAT-UN-NISA SHEHZAD

Dissertation submitted to the University of Peshawar in partial fulfillment of the requirement for the Degree of

DOCTOR OF PHILOSPHY IN CHEMISTRY

INSTITUTE OF CHEMICAL SCIENCES
UNIVERSITY OF PESHAWAR
PAKISTAN

MARCH-2011

INSTITUTE OF CHEMICAL SCIENCES

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Certified that **Ms.Farhat-un-Nisa Shehzad** D/O Rafat-Ullah Khan has carried out her research and experimental work on the topic entitled **"Novel spectrophotometric and HPLC methods for the determination of dinitroaniline and aryloxyphenoxy groups of herbicides in environmental samples"** under our guidance and supervision. Her research work is original and her dissertation is worthy of presentation to the University of Peshawar for the award of degree of **Doctor of Philosophy in Chemistry**.

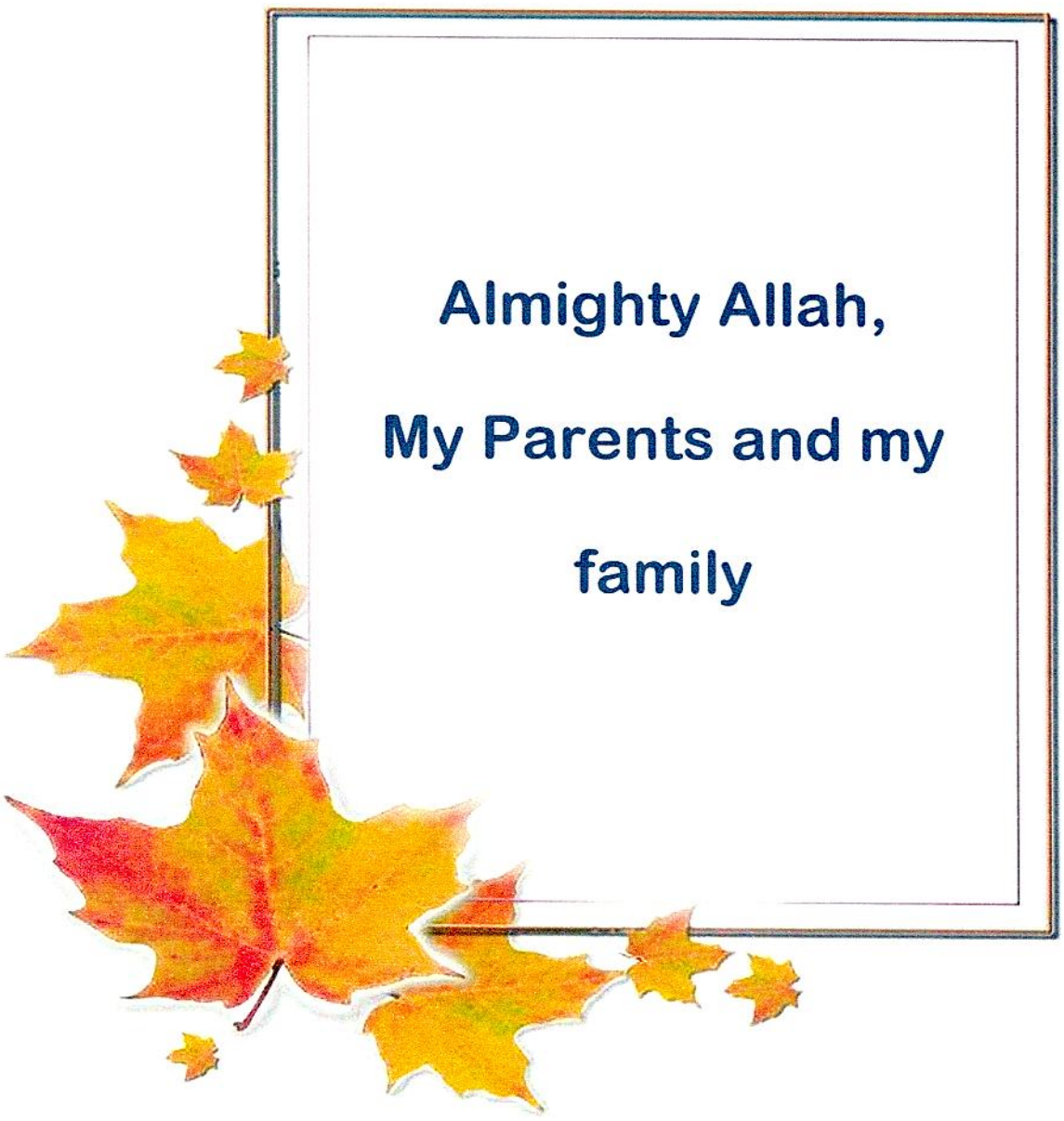
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IN THE NAME OF ALMIGHTY ALLAH THE MOST MERCIFUL AND BENIFICENT WHO BLESSED THE MANKIND WITH THE WISDOM AND ALL KIND OF POTENTIALTIES

Dedicated to



ACKNOWLEDGMENT

All praise for almighty Allah who enabled and encouraged me to complete this work. He has the knowledge of known and unknown, the kind and merciful and the Holy Prophet Hazrat Muhammad Peace Be upon Him who is forever a lantern of guidance and knowledge for humanity as a whole.

First, I would like to express my sincere gratitude and deep appreciation to my supervisor Prof. Dr. Jasmin Shah for her encouragement, guidance and direction throughout my PhD research work and during the completion of this thesis. Her help smoothed my work very much and her immense patience was essential to the completion of this Project.

I feel great pleasure and honor in expressing my sincere gratitude to my co-supervisor Prof .Dr. M. Rasul Jan for his valuable guidance and constant help. I will always remain grateful to him for the liberty he graciously granted me to encroach upon his precious time.

I owe my most sincere gratitude to Razia Khan, Samina Ashfaq, Amina Asghar, Yasir Shehzad, Ayesha Ali and all of my colleagues and faculty members of College of Home Economics and ICS for their good wishes and friendly help.

I would like to take this opportunity to extend my thanks to each one of my batch fellows specially Mian Muhammad, Bahisht Ara, Maria, Attaul Haq, and Inayatullah They always extended their cooperation for research work in Analytical section.

Thanks to laboratory assistant Mr. Asmat Ali and technician Imran Khan at CRL for cooperation throughout experimental work of this project.

The financial support of the University of Peshawar is gratefully acknowledged.

I wish to express my warm and sincere thanks to my children Shafaq, Sarim, Sundus, and Ibrahim and my niece Urooba for their love, prayers, cooperation and moral support during my PhD studies.

Last of all no acknowledgment could ever express my obligation to my loving father, brothers , sisters , mother in law, and my husband as my success is really the result of their devoted prayers. I can never compensate their unlimited love and kindness.

Farhat-un-Nisa Shehzad

SUMMARY

The importance of the role of analysis of herbicides and other toxic substances in environmental and food samples, toxicology, environmental research and health related discipline is self-evident. Analytical methods are required by various regularity agencies, for validation, which requires the demonstration of the specificity, sensitivity, calibration linearity, extraction recovery, precision and accuracy of the assay method.

It is important that the analytical methods should be specific with no interference from endogenous and exogenous substances. Some agricultural products are still being assayed by the time-honored procedures of gravimetric and titrimetric methods.

The aim of present study was to develop new Spectrophotometric and HPLC methods for the determination of dinitroaniline and aryloxyphenoxy group of herbicides in various environmental and agricultural samples.

The first chapter is concerned with the brief introduction of the groups of herbicides and specific herbicides selected for this work. It also includes information about chemical, environmental and agricultural aspects of the selected herbicides.

The second chapter covers a review of the literature reported for these herbicides. This chapter includes spectrophotometric, chromatographic, titrimetric and other analytical techniques used for the determination of dinitroaniline and aryloxyphenoxy group of herbicides. These methods have been discussed with emphasis on scope, type of sample, reagents used, brief procedure, linearity range and detection limit.

The third and last chapter contain experimental work with the results and discussion of the various methods investigated and its application for the analysis of active ingredients in formulation and residue level determination in real samples, both by spectrophotometric and HPLC methods. Part I of this chapter explains spectrophotometric method for the determination of trifluralin in commercial formulation and food samples. The method is based on the hydrolysis of trifluralin

with sodium hydroxide to form 2,6-dinitro-4-trifluromethyl aniline. The resultant aniline group is diazotized with nitrate in acidic media and the diazotized product on coupling with β-naphthol forms red colored product having λ max 550 nm. The reaction conditions were optimized for hydrolysis as well as for the diazotization reaction. Beer's law was obeyed over the range of 0.2-17 ug $mL^{-1}$with molar absorptivity of $1.5x10^4$ L $mol^{-1}cm^{-1}$. The standard deviation and relative standard deviation was found to be ± 0.0052 and 3.6% respectively. A two level factorial design of 2^3 was applied for optimization of all parameters. Recovery for the determination of trifluralin was found to be in the range 95-97%.

Part II of this chapter deals with spectrophotometric method development for pendimethalin. Pendimethalin herbicide was hydrolyzed in basic media by heating and the hydrolyzed product was used as coupling reagent for the formation of azo dye. The azo dye formed was measured at 460 nm. The limit of detection (LOD) and quantification (LOD) was found to be 0.213 μg $mL^{-1}$and 0.71 μg mL^{-1} respectively with molar absorptive of 5.6 X 10^3 L $mol^{-1}cm^{-1}$. The method was applied for residue determination in onion sample and was found to be 1.3 ± 0.31 μgg^{-1}. Similarly the method was also applied for determination of active ingredient in commercial formulation and was found in close agreement with the label value. Recoveries from real samples varied from 87-98%.

Part III of this chapter deals with method development for aryloxyphenoxy herbicides and it determination in commercial formulation and real samples. The method is based on the acid hydrolysis of fenoxaprop-p-ethyl to form 6-chloro-2, 3-dihydrobenzoxazol-2-one (CDHB) and ethyl 2-(4-hydroxyphenoxy) propionate (EHPP). The 6-chloro-2, 3- dihydrobenzoxazol-2-one was used as a coupling reagent in diazotization reaction. The absorbance of the azo dye was measured at 430 nm. Reaction conditions were optimized for hydrolysis as well as for the diazotization reaction. The Beer's law was obeyed over the concentration range of 0.2-25 ug mL^{-1} with molar absorptive of 1.4 x 10^4 L mol^{-1} cm^{-1}. The limit of detection (LOD) and quantification (LOQ) were found to be 0.029 and 0.098 ug mL^{-1} respectively. Recoveries were found in the range of 95-97%.

Part IV of chapter III deals with the investigation of a new spectrophotometric method for

determination of herbicide fenoxaprop-p-ethyl based on complexation with iron(II) after acid hydrolysis forms a yellow colored complex. The absorbance of complex was measured at 430 nm. The 4-(6-chlorobenzo[d]oxazol-2-yloxyphenolate forms a stable charge transfer complex with Fe (II) which shows molar absortivity $4.2x10^4$ L mol^{-1} cm^{-1}. Analytical parameters have were optimized and successfully applied to the determination of fenoxaprop-p-ethyl in various samples. The method shows a linear range from 2-24 μg mL^{-1}. The % recovery for determination of fenoxaprop-p-ethyl in commercial formulations was found to be 84 ± 0.067 –98 ± 0.067%. The limit of detection and quantification was found to be 0.20 μg mL^{-1} and 0.67μg mL^{-1}respectively. The results have also been statistically validated by applying analysis of variance (ANOVA) using Design Expert software (Stat-Ease).

Part V of chapter III deal with the investigation of a new HPLC method for determination of residual pendimethalin in soil and vegetable samples. The method is based on extraction of pendimethalin from samples using microwave- assisted solvent extraction (MASE) with acetone, ethanol and water as extraction solvent. Extracted pendimethalin samples were analyzed by high performance liquid chromatography with ultraviolet detector at 240 nm. The MASE parameters, temperature, heating time and solvent types were optimized for the extraction of pendimethalin from soil and vegetable samples. The maximum temperature that can be used during the heating for MASE is 60^0C, where the recovery percentages reached 97%. Linearity for pendimethalin was found in the range of 2-20 μg ml^{-1} with LOD and LOQ of 0.059 μg mL^{-1} and 0.17 μgmL^{-1} respectively.

Part VI of this chapter describes high-performance liquid chromatographic (HPLC) method for the residue determination of fenoxaprop-p-ethyl in food samples. The chromatographic analysis was carried out by HPLC, on a C_{18} packed column with 20 μl injection volume and ultraviolet detector at 280 nm. A sample was spiked with 4 to 30 μgg-1 of herbicide and was isolated from samples by using ultrasonication extraction at ambient temperature. Percent recoveries were improved by optimizing solvent types, solvent volume, and time. Calibration range determined by HPLC was 0.2-40μgmL^{-1}.The interaction of different variables for maximum % recovery response was checked by applying factorial design and was found to be in range of 91.22±0.01- 99.32±0.01. Application of this procedure to the analysis of herbicide in ester and acid form showed the effectiveness of the methodology proposed.

In part VII of this chapter gradient liquid chromatographic method for the analysis of pinoxaden and its separation from fenoxaprop-p-ethyl is described. The chromatographic conditions include the use of a C_{18} (250mmx4.6mm,5µm) column maintained at 25 ^{0}C. Mobile phase containing acetonitrile, methanol and phosphate buffer of pH3 at flow rate 0.7 mL min^{-1} and UV detector at 280 nm. Good separation of the two herbicides was achieved by changing the mobile phase composition and flow rate. System suitability test to check the quality of the separation is specified. The method shows good repeatability, linearity and robustness.

Table of Contents

S#	TITILE	Page #
CHAPTER -I		
1	**Introduction**	1
1.1	Classification based on Chemical Family	2
1.2	Classification by mode of action	4
1.3	Classification based on activity	5
1.4	Dinitroaniline herbicides	6
1.5	Aryloxyphenoxypropionate herbicides	11
1.6	Effectiveness of herbicide	15
1.7	Behavior in the environment	15
1.8	Environmental toxicity	16
	References	22
CHAPTER-II		
2	**Literature review**	27
CHAPTER-III		
	Experimental	
	Part-1	
	Spectrophotometric methods for determination of dinitroaniline and aryloxyphenoxy group of herbicides	
3.1	Investigation of spectrophotometric method for determination of dinitroaniline group of herbicides	54
3.1.2	Preliminary investigation of the hydrolysis of trifluralin and	54

	coupling of the hydrolyzed product for spectrophotometric determination of trifluralin	
3.1.3	optimization studies for spectrophotometric determination of trifluralin using base hydrolysis	56
3.1.3.4	Verifications of Beers law and determination of the limit of detection and quantification of trifluralin	72
3.1.4	Percent recovery of trifluralin from real samples and formulation by using the investigated method	75
3.1.5	Application of the investigated methods method for the analysis of trifluralin in real samples and commercial formulation	76
	Results and discussion	77
3.2.1	Investigation of spectrophotometric method for determination of pendimethalin	79
3.2.2	Preliminary investigation of the hydrolysis and coupling for spectrophotometric determination of pendimethalin	79
3.2.3	Optimization studies for the spectrophotometric determination of pendimethalin using acid hydrolysis	81
3.2.4	Verifications of Beer's law and determination of the limit of detection and quantification of pendimethalin by batch analysis	97
3.2.5	Application of the investigated method for the analysis of percent recovery of pendimethalin in real samples	100
3.2.6	Application of the investigated methods for the analysis of pendimethalin in real sample and commercial formulation	102
	Results and Discussion	103
3.3.1	Investigation of spectrophotometric method for the determination of aryloxyphenoxy propionates group of herbicides	105
3.3.2	Preliminary investigation of the possibility of acid hydrolysis and the use of hydrolyzed product as coupling reagent for spectrophotometric determination of fenoxaprop-p-ethyl	106

3.3.3	Optimization studies for spectrophotometric determination of fenoxaprop-p-ethyl	107
3.3.4	Verification of Beer's law and determination of the limit of detection and limit of quantification for the spectrophotometric determination of fenoxaprop-p-ethyl	119
3.3.5	Percent Recovery of fenoxaprop-p-ethyl using charge transfer complex	121
3.3.6	Application of the Investigated Method for the Analysis of fenoxaprop-p-ethyl in commercial formulation and real samples	126
	Result and discussion	131
3.4	Spectrophotometric method for the determination of fenoxaprop-p-ethyl using charge transfer complex	133
3.4.1	Method development strategy for the Spectrophotometric determination of fenoxaprop-p-ethyl using charge transfer -complex	133
3.4.2	Optimization of various parameters for the spectrophotometric determination of fenoxaprop-p-ethyl us using charge transfer complex	135
3.4.3	Determination of formation constant for the charge transfer complex of fenoxaprop-p-ethyl using Job's method	136
3.4.4	Extraction from real samples	151
3.4.5	Application of the investigated methods for the analysis of fenoxaprop-p-ethyl in commercial formulation and in real samples	151
	Results and discussion	154
	Experimental	
	Part-2	
	Determination of dinitroaniline and aryloxyphenoxy group of herbicides using high performance liquid chromatography	
3.5	Determination of dinitroaniline group of herbicides using high performance liquid chromatography	157

3.5.1	Investigation of suitable parameters for the HPLC determination of pendimethalin	157
3.5.2	Construction of calibration plot for the determination of pendimethalin using reverse HPLC	159
3.5.3	Optimization of extraction procedure for determination of pendimethalin using reverse phase HPLC	161
3.5.4	Determination of pendimethalin in real samples using optimized extraction procedure	168
3.5.5	Application of the investigated methods for the analysis of pendimethalin in real sample and commercial formulation	171
	Result and discussion	177
3.6	Determination of aryloxyphenoxy group of herbicides using high performance liquid chromatography	180
3.6.1	Investigation of suitable parameters for the HPLC determination of fenoxaprop-p-ethyl	180
3.6.2	Construction of calibration plot for the determination of fenoxaprop-p-ethyl using reverse phase HPLC	182
3.6.3	Optimization of extraction procedure for HPLC determination of fenoxaprop-p-ethyl	184
3.6.4	Comparison of extraction procedures for determination of fenoxaprop-p-ethyl in real samples	190
3.6.5	Application of the investigated methods for the analysis of fenoxaprop-p-ethyl in real sample and commercial formulation	194
	Result and discussion	195
3.7	separation and determination of the pinoxaden and fenoxaprop-p-ethyl herbicides using high performance liquid chromatography	203
3.7.1	Investigation of suitable parameters for separation and determination of the pinoxaden and fenoxaprop-p-ethyl herbicides using high performance liquid chromatography	204
3.7.2	Construction of calibration plot for the HPLC determination the pinoxaden and fenoxaprop-p-ethyl herbicides using high	205

performance liquid chromatography

3.7.3 Optimization of conditions for separation and determination of pinoxaden and fenoxaprop-p-ethyl herbicides using high performance liquid chromatography 207

3.7.4 Optimization of extraction procedure for the separation and determination of the pinoxaden and fenoxaprop-p-ethyl herbicides using high performance liquid chromatography 208

3.7.5 Separation and determination of the pinoxaden and fenoxaprop-p-ethyl herbicides using high performance liquid chromatography in real samples using optimized extraction procedure 212

Result and discussion 213

Conclusion 220

CHAPTER – 1

INTRODUCTION

1.0 Introduction[1-13]

According to U.S Environmental protection agency, pesticide is defined as "any substance or mixture of substances projected for preventing, destroying, repelling, or lessening the damage of any pest". A pesticide may be a chemical substance, biological agent, antiseptic or a device used to kill pests.

Agriculture has played a key role in the development of human civilization. It is an old practice that has rarely been questioned because of the increasing need of feeding the world's population. Modern agronomic techniques including the massive use of fertilizers and pesticides have sharply increased these production yields.

Pesticides, including herbicides, insecticides, ovicides, rodenticides, nematicides, and fungicides, are used extensively to improve crop yields and as a result, they accumulate in the environment and are viewed as toxic from environmental point of view. According to an estimate more than 2.5 million tons of pesticides are applied every year to agricultural crops worldwide.

Herbicide is a term that refers to the agents especially chemicals used to kill the unwanted plants. Herbicides destroy, or diminish any type of unwanted plants. They inhibit or interrupt normal plant growth and development especially of weeds. They are used widely in agriculture, industry, and urban areas for weed management.

The development and use of herbicides have played an important role in the increase of agricultural productivity on world level. Herbicides can provide cost-effective weed control with a minimum of labor. They have also been used to control aquatic weeds that block water intake or invade natural ecosystem as well as in forestry and even in swimming pools to inhibit growth of algae.

Herbicides have increasingly become a key component of weed management programs in developing countries. Almost 61% of total pesticides are used as herbicides in North America, Europe and Asia. Herbicides production is a main driver of the agrochemical industry. Compared to other methods, like tillage, herbicides have been promoted method of weed control that lessens the impact of soil erosion.

Weed is a plant that grows where it is not wanted. Weed competes with crops for water and nutrients and as a result reduces, farming yields and productivity. Weed is a plant that is out of

place and not intentionally sown. These are plants that are competitive, persistent, and interfere negatively with human activity giving the harmful economic implication of poor weed management.

An understanding of how herbicide kill weeds, is useful in selecting and applying proper herbicide for a given weed control problem and for management of weed resistance to herbicide.

Herbicides can be grouped by activity, chemical family, mode of action, and type of vegetation controlled. Although a large number of herbicides are available in the marketplaces, they can be in divided in to groups with similar chemical and phototoxic properties.

1.1 Classification based on chemical family

Herbicides with a common chemistry have been conveniently organized in to "Families". Herbicides that are chemically similar are said to belong to the same" herbicide family". The compounds in a given family typically exhibit similar characteristics and function, due to their chemical and structural similarities. Main groups including in the chemical family are given in table 1.1.

Table 1.1 : Main groups included in the chemical family based classification

Herbicide family	Herbicides with common names
1. Phenoxyacetic acids	2,4-D, MCPA, Banvel, Tordon.
2. Aryloxyphenoxy propanoic acid	Puma super,
3. Aminotriazol	Amitrol ,Cranberry
4. Substituted urea herbicides	Accent ,Beacon ,Glean, permit
5. Triazine herbicides	Simazine, Atrazine, Bladex. Evik.
6. Dinitroanilines	Trifluralin, Pendimethalin
7. Paraquat and diquat	Paraquat and diquat
8. Monsanto herbicides	Ramrod, Glyphosate, Roundup.
9. Sulfonylurea herbicide	sulfonylurea
10. Imidazolinone herbicide	Potassium cyanate,difenzoquat

1.2 Classification by mode of action

Herbicide's mode of action is the "biochemical or physical mechanism by which it kills weeds". Most herbicides destroy plants by upsetting or altering one or more of their metabolic processes. Some disrupt the cellular membranes of plants, allowing cellular contents to escape out, but do not directly disrupt other metabolic processes. Some genus or whole groups of plants are not inclined to certain herbicides because they use different biochemical pathways or have somewhat different enzymes.

Herbicide is often selected for use, based on its mode of action. Mode of action refers to the progression of events from absorption in to plant to the plant death. The mode of action is

generally dictated by its chemical structure, and therefore, herbicides in the same family, tend to have the same mode of action.

If one herbicide is useless; another herbicide with different mode of action may provide better results.

Based on the mode of action, herbicides may be classified as given below

1. Auxin mimics
2. Mitosis inhibitor
3. Photosynthesis inhibitor
4. Amino acid synthesis inhibitors
5. Lipid biosynthesis inhibitor.

1. Auxin mimics

Growth in many plant tissues is regulated by a plant hormone called Auxin.When predisposed plants are treated with these herbicides, they exhibit symptoms that could be called Auxin overdose, and ultimately die as a result of increased rates of disorganized and uncontrolled growth.

2. Mitosis inhibitor

When these herbicides are applied to plants up to two months before leaf drop, the compound is absorbed and the following spring the plant fails to leaf out because bud development is prevented and plant often dies as the season progress.

3. Photosynthesis inhibitor

These are inhibitor which inhibits the transfer of electrons in photo system. It blocks the electron transport in the chloroplast thylakoid membranes binding to the protein at the binding niche. The electrons from photo system are transferred through a series of reactions to other reactive toxic compounds. These compounds disrupt cell membrane which leads to cellular annihilation.

The second type of photosynthesis inhibitors accept electron from photo system 1 and after several cycles, generate hydroxyl radicals. These radicals are extremely reactive and destroy unsaturated lipids which lead to plant death.

4. Amino acid synthesis inhibitors

These herbicides kill plants by preventing the synthesis of certain amino acids produced by plants but not animals. Their mode of action is to inhibit the biosynthesis of certain amino acids such as phenylalanine, tyrosine, and tryptophan. These amino acids are required for protein synthesis, which in turn, is necessary for plant growth and maintenance. Other biochemical process such as carbohydrate translocation, can also be affected by these herbicides

5. Lipid biosynthesis inhibitor

Herbicides that inhibit the synthesis of enzyme required for lipid synthesis. It inhibits acetyl carboxylase, the enzyme responsible for catalyzing an early step in fatty acid synthesis. As a result the shoot and rhizome growth ceases, and shoot meristems and tuber buds begins to die back.

1.3 Classification based on activity

There are two groups of herbicides on the basis of their activity

1. Selective herbicides
2. Non –selective herbicides

1. Selective herbicides

A selective herbicide must avoid or be non toxic on the crop. The selective sprays, like sodium arsenide, copper chloride copper nitrite sulphuric acid are selective by differential wetting that is they wet and kill the weed only. These may be contact or translocated type of herbicides.

(a) Contact

Contact herbicides are those that kill plant tissues. They are thoroughly distributed on the foliage of the weed so that they may kill the meristemetic tissue in the buds of shoot tips and leaf axils of the foliage. Dinitrophenol is a well known contact selective herbicide. Another form of selective contact herbicide is oil used to kill weeds in carrot crops and in forest trees nurseries.

(b) Translocated

Selective herbicides of the translocated type must penetrate the leaf cell and move in the vascular tissue of the plant in order to kill the meristemic region of the roots. Selectivity must be therefore being of biochemical type. The first material of this general type to be used as weed killer was 2,

4-D.To fit in the category of selective translocated herbicides the compound must also be able to penetrate the cuticle, migrate across the mesophyll, enter the phloem, and move in to the roots.Chlorophenoxy compounds like dalapon, 2, 3, 6-TBA and others are used as translocated selective herbicides.

2. Non selective herbicides

Non selective herbicides are broadly classified in to two types.

(a) Contact

Nonselective herbicides are those that kill all vegetation with which it come in contact. Most are general contact herbicides that are fixed in place as they kill. All plants are protected by cuticle which is difficult to wet with aqueous spray, oil prove to be the most effective general contact spray. Oils are fortified by aromatic contents and are emulsified with water to give grater coverage more driving force to the spray.

(b) Translocated

The acid arsenical solution is the principal translocated spray of the nonselective type. Made up of an aqueous solution of 1% $As_2 O_3$ and 5 % H_2SO_4 this solution comparatively cheap, and if the condition of weed plants and the method of application right as many as 95% of the plants infestation of field bindweed may be killed depths of from 3 to 5 feet. In addition to the above mixture, ammonium sulfamate and calcium hypochlorite are also translocated by this mechanism.

The most popular substances that are used for killing the useless plants are of dinitroaniline and aryloxyphenoxy propionic groups of herbicides. They are available in the market at attractive prices. Dinitroaniline and aryloxyphenoxy propionic groups of herbicides are widely used for control of broad-leaved weeds and other vegetation in Pakistan and all over the world. A brief introduction of herbicides selected for our present investigation from these two groups is given below.

1.4 Dinitroaniline herbicides

Dinitroaniline herbicides are used to control many germinating annual grass and broadleaf weeds. The members of this family include trifluralin, benfluralin, ethafluralin, oryzalin and pendimethalin. Dinitroaniline were known as dye prior to their introduction as selective

herbicides. Most of successful dinitroaniline herbicides were yellow and easily stained many things. 2, 6-dinitroaniline herbicides had a significantly more herbicidal activity than 2, 4-or 2, 3-dinitroanilines. Dinitroaniline herbicides act as a plant growth regulator and are mitosis inhibitor. The specific mechanism of action has not been identified, but there is indication that fosamine ammonium inhibits mitosis in predisposed plants .When these herbicides are applied to the plants up to two months before leaf drop, the composite is absorbed. The following spring the plant fails to leaf out because bud maturity is prevented. Plant often dies as the season progress. It is generally renowned that herbicidal activity could vary responses due to difference in their site of action. Among this class only these herbicides like pendimethalin and trifluralin were selected and their brief description is given bellow.

Trifluralin[14-18]

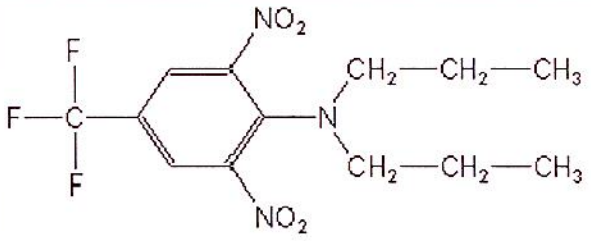
The most successful dinitroaniline were first released for use on food crops in October 1964Trifluralin that is (2, 6-dinitro-N, N-dipropyle-4-tri fluramethylbenzene) is a dinitroaniline compound which has been used extensively as an agricultural herbicide. It is a pre-emergence, soil applied and soil incorporated herbicide and is used for the control of annual grasses and certain broad leaf weeds.

Trifluralin has been used for the control of a verity of weeds in various crops especially for the control of broad leaf weeds. It is strongly absorbed by soil and can move in the soil penetrate plant shoots as gasses or liquids. It is effective for the control of annual grasses and broad leaved weeds in beans, brassica, cotton, groundnuts, foragelegumes, orchards, ornamentals, transplanted peppers, soybeans, tomatoes and vineyards. Trifluralin along with 2-4-D is used as post planting herbicide in transplanted rice.

Many soil applied herbicides are absorbed through unmerged plant shoots, and plant may be injured or killed before emergence. It's binding to soil and absorption in fruits and vegetables results in significant losses in herbicidal activity and delayed pollution problem. It is also registered for use by the general public for the control of weeds in and around gardens, lawns and residences. It is selective herbicide which by activity mode is included in contact group. They must be carefully dispersed on the foliage of the weed so that they may kill the meristemetic tissue in the buds of shoot tips and leaf axils of the foliage. Trifluralin is well known contact selective herbicide. Half-lives of trifluralin in soil vary from 45 days to 8 months.

In atmosphere trifluralin exists in gas phase. The estimated half life and life time of trifluralin due to reaction with OH radical are less than or equal to 1 hour. Data related to trifluralin is given in table 1.2.

Table 1.2 General data of trifluralin

IUPAC name	2,6-dinitro-N,Ndipropyle-4-trifluramethylbenzene
CAS name	2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl) benzenamine
CAS registry number	1582-09-8
Common name	Trifluralin
Molecular formula	$C_{13}H_{16}F_3N_3O_4$
Relative molecular mass	335.29
Structural formula	
Chemical group	Dinitroaniline
Physical characteristics	Yellow crystals,
Melting point	46-47 ^{0}C
Decomposition point	140 ^{0}C
Vapour pressure	1.99x10^{-4} mmHg

Pendimethalin[19-23]

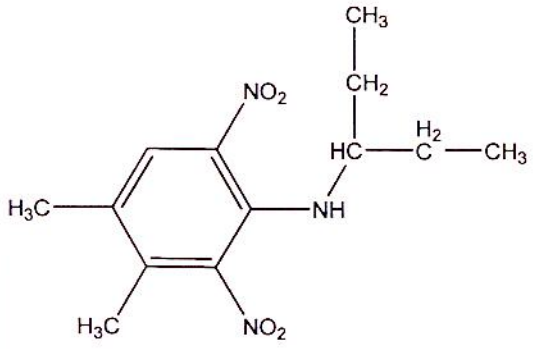
Herbicide pendimethalin, N-(1-ethylpropyl)-2,6-dinitro-3-4 xylidine is found as the preparation Stom 330, or mixed with other herbicide Stomp 330 E. Some trade names include AC 92553, Accotab,Go-Go-San, Herbadox,Penoxalin Prowl, and Way-Up.

Pendimethalin is an orange yellow crystalline solid with a faint nutty or fruit like odor. Product containing pendimethalin ranged from slightly to highly inflammable. It is stable under normal temperature and pressure but it may pose a slight fire hazard if exposed to heat and flame, it poses a fire and explosion hazard in the presence of strong oxidizers.

Pendimethalinis used for the eradication of annual grass and some broad leaf weeds in corn, Soya, potato, wheat onion, tomato cabbage and paper. It can be applied pre-emergence after seeding in cereals, maiz and rice or with shallow soil incorporation before seeding beans cotton, groundnuts, and soyabeans. In vegetable crops it can be applied pre–emergence or pre-transplanting. It is also used to control sucker in tobacco.

Field dissipation studies have revealed that pendimethalin is persistent and its half life is 98 days. Pendimethalin degrades more rapidly in anaerobic soil than in aerobic soil conditions. Acting as a microtubule inhibitor, the dinitroaniline herbicide pendimethalin has been used in agricultural applications as a selective pre-emergence herbicide in various plant cultures. It is classified as non-leaching compound. General data of pendimethalin is given in table1.3.

Table 1.3 General data of Pendimethalin

IUPAC name	N-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine
CAS name	Pendimethalin
CAS registry number	40487-42-1
Common name	Stomp
Molecular formula	$C_{13}H_{19}N_2O_4$
Relative molecular mass	281.311
Structural formula	
Chemical group	Dinitroaniline herbicide
Physical characteristics	An orange yellow crystalline solid with a faint nutty or fruit like odor
Melting point	58^0C
Decomposition point	330^0C
Solubility	Slightly soluble in water, soluble in acetone ethanol, acetonitrile.

1.5 Aryloxyphenoxypropionate herbicides[24-27]

Aryloxyphenoxy propionate (AOPP) group of herbicides have been used widely as selective post emergence graminicides. They first inhibit acetyl Co-Acarboxylase (ACCase) on the fatty acid biosynthesis with a hostile inhibition to its degraded products. The available brands with same active ingredient in different composition with trade names Puma super 75 EW, Germicide 69EW, Brake 10 EC are used as herbicides which are widely used to control the growth of broad-leaf weed in crops. Apart from their reactiveness, cheapness and effectiveness, the active ingredient of all products of AOPP herbicides are remarkable in selectivity among graminaceous plants. AOPP herbicides are rapidly absorbed by plant foliage, translocated throughout the plant and accumulated in the meristems. Herbicides of the aryloxyphenoxy class are selective herbicides which are used for the weed control of broad leaved plants. Due to their high stability and polar molecule structure these herbicides are distributed in several natural matrices like water, soil and cultivated plants .These herbicides are usually non biodegradable and persistent in the environment.

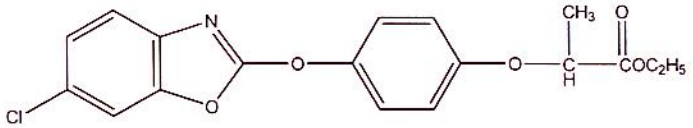
Fluazifop-p-butyl and sethoxydim are both grass specific herbicides that inhibit the synthesis of enzyme required for lipid synthesis. Both inhibit acetyl CoA carboxyl's the enzyme responsible for catalyzing an early step in fatty acid synthesis. The shoot and rhizome growth ceases, and shoot meristems and rhizome buds begins to die back.

Fenoxaprop-p-ethyl [27-30]

Fenoxaprop-p-ethyl {ethyl-2-[4-[(6-chloro-2-benzoxazolyl) oxy] phenoxy] propionates} is a member of aryloxy phenoxy-propionate class of herbicides and is used only for the control of perennial and annual grass weeds in many crops. Fenoxaprop-p-ethyl is used for post emergence control of grasses weeds in potatoes, beans barely and cotton. Fenoxaprop-p-ethyl is grass specific herbicides that inhibit the synthesis of enzyme required for lipid synthesis. Its mechanism of action is to inhibit fatty acid biosynthesis. The corresponding acid, fenoxaprop is the first degradation product in the metabolic sequence in soil. The more frequently used formulations are amines or alkaline salts, alkyl esters or free carboxylic acids. The esters, emulsified in oil, are commonly used because of their higher herbicide activity, penetration power and low vapor pressure. Due to their persistence, polar nature and water solubility, the phenoxy acids are dispersed in the environment, and their residues and transformation products

are present in several matrices like water, soil and cereals. Fenoxaprop-p-ethyl is a selective herbicide with contact and systemic action, absorb principally by leaves. It translocates both acropetally and basipetally to the roots or by leaves. It is used for post emergence control of grasses weeds in potatoes, beans barely and cotton. Data regarding fenoxaprop-p-ethyl is given in table 1.4.

Table1. 4 General data of Fenoxaprop-p-ethyl

IUPAC name	{ethyl-2-[4-[(6-chloro-2-benzoxazolyl)oxy] phenoxy] propionates
CAS name	Fenoxaprop-p-ethyl
CAS registry number	66441-23-4
Common name	Fenoxaprop-p-ethyl
Molecular formula	$C_{18}H_{16}CNlO_5$
Relative molecular mass	361.78
Structural formula	
Chemical group	Aryloxyphenoxy propionate
Physical characteristics	Coarse light beige
Melting point	242.2 ^{0}C
Decomposition point	477.5^{0}C
Solubility	Slightly soluble in water soluble in acetone, ethanol, acetonitrile.
Vapor pressure	2.79 E-09 mm Hg at 25^{0}C

Pinoxaden[31-35]

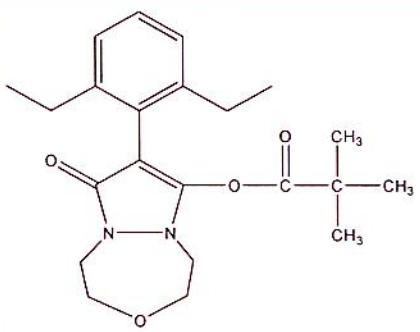
Pinoxaden (2,2-dimethyl-propionic acid 8-(2,6-diethyl-4-methyl-phenyl)-9-oxo-1,2,4,5-tetrahydro-9 H-pyrazolo [1,2-d] [1,4,5] oxadiazepin-7-yl ester) abbreviated as PXD is a member of the chemical class of the 3-hydroxi-4-phenyl-5-oxo-pyrazolines often simply called phenylpyrazolines and is marketed worldwide under the trade name AXIAL. Pinoxaden is sprayed at use rates of 30-60 g/ha against a variety of annual grass weeds such as black grass, silky bent grass, wild oats, little seed canary grass, foxtails and rye grass as well as grass found in wheat and barley crops.

Pinoxaden suppresses the function of acetyl co-A carboxylase enzyme, thereby prevents the fatty acids synthesis and accordingly the formation of bio membranes in weeds.

Pinoxaden sprayed is absorbed through leaves as well as roots by the plant, move bidirectionally through the plant body, and effectively ensure grass weeds inhibition irrespective of the level of dose and the time of application.

Pinoxaden (30 g/ha) is also effective against Isoproturon resistant weeds in barley and result in 21.6 % higher yield than Isoproturon itself. To ensure the permissiveness of primal crops, wheat and barley, a patented safener Cloquintocet-mexyl is added to the formulation. In addition, an efficient adjuvant is incorporated into the formulation to assure the optimum spread of the spray on the surface of grass leaves and to have speedy uptake of solution by the leaf tissues. General data related to pinoxaden is given in table 1.5.

Table 1.5 General data of Pinoxaden

IUPAC name	8-(2,6-diethyl-p-tolyl)1,2,4,5-tetrahydro-7-oxo-7h-pyrozolo[1,2-d][1,4,5]oxadiazopen-9-yl2,2-dimethylpropionate
CAS name	Pinoxaden
CAS registry number	243973-20-8
Common name	Axial 100 EC Selective Herbicide
Molecular formula	$C_{23}H_{32}N_2O_4$
Relative molecular mass	400.51
Structural formula	
Chemical group	Aryloxyphenoxy propionate.
Physical characteristics	White to beige colored fine powder.
Melting point	121.1
Decomposition point	No decomposition
Solubility	Slightly soluble in water soluble in acetone, dichloromethane, hexane, methanol.
Vapor pressureat25 ^{0}C	4.60X10^{-03} mm Hg

1.6 Effectiveness of herbicide[36-41]

Herbicide Effectiveness depends on the following factors.

i. Adequate plant contact

ii. Absorption by plants

iii. Translocation within the plant to the site of action

iv. Toxic level at the site of action

Factors affecting herbicide activity

Factors influencing herbicide activity include application rate, application technique, plant maturity, environmental conditions. Soil characteristics can affect soil active herbicides. Moisture and temperature are environmental factors that influence activity of soil applied herbicides.

Temperature affects the activity of soil- applied herbicides primarily because of its influence on the rate of seed germination, emergence, and growth. Seedling plants tend to be more susceptible to soil -applied herbicides under cool conditions than under warm temperatures because plant emergence is delayed and metabolism is slowed. On the other hand, extremely high temperature sometimes increases crop injury simply by placing the plant under multiple stresses.

Soil characteristics affecting herbicide activity are texture, organic matter, and pH. Herbicide adsorption is greater in fine-textured soils high in organic matter then in coarse-textured soils low in organic matter.

Soil pH influences the availability and persistence of certain herbicides in the soil. Soil pH can alter the ionic nature of the herbicide molecule, which influence adsorption, solubility and rate of herbicide breakdown.

1.7 Behavior in the environment[41]

Perhaps the most important factor determining the upshot of herbicide in the surroundings is its solubility in water. Water-soluble herbicides like herbicides of dinitroaniline group generally have low absorption capacities and are more mobile in the environment and therefore more available for microbial metabolism and other deprivation processes. Aryloxyphenoxy acid group having esters in formula group, in general, are relatively insoluble in water, and absorb quickly

to soil, penetrate plant tissues readily and are more volatile than salt and acid formulation.

a. **Effect of Soil**

Herbicide determination in soil is often described by its half life which is expressed as the DT50.The half life is the time it takes for half of the herbicide applied to the soil to dessipate. The half life gives only a rough estimate of the persistence of herbicide. Degradation rate often change with time however half life values do provide a means of comparing the relative persistence of herbicide. The allocation of an herbicide in the soil is determined primarily by the amount, type and surface area of clays and organic matter in the soil, the amount and quality of soil pH, soil moisture, and soil temperature.

b. **Level of water contamination**

Water bodies can be contaminated by directs overspray, or when herbicides drift, volatilize, leach through soil to ground water, or are carried in surface or subsurface runoff. Amount of leaching and runoff are largely depending on total rainfall the first few days after application. Total losses to runoff generally don't exceed five to ten percent of the total applied, even following heavy rains. High soil adsorption capacity, low rates of application and low rainfall reduce total runoff and pollution of local waterways. The behavior of herbicide in water is dictated by its solubility in water. Salts and acids tend to remain dissolved in water until degraded through photolysis or hydrolysis. Esters will often adsorb to suspended matter in water, and precipitate to the sediments. Once in the sediments, esters can remain adsorbed to soil particles or be degraded through microbial metabolism. Highly acidic or alkaline waters can chemically alter herbicides and change its performance in water.

1.8 Environmental toxicity

a. Birds and Mammals

Toxicity of a herbicide is described by its LD_{50}, which is the dose received either orally or dermally that kills half the population of study animals. The LD_{50} values are reported by studying the oral and dermal effects on rats, rabbits and birds'. The LD_{50} is typically reported in grams of herbicide per kilogram of animal body weight. LD_{50}s are determined under varying circumstances so comparison between different herbicides may provide only a rough sense of their relative toxicities. Dermal LD_{50} values may be more meaningful to herbicide applicators

because birds and animals are more likely to be exposed to herbicides through their skin rather than by oral digestion.

The LD_{50} does not provide any information about persistent, long-term toxic effects that may result from disclosure to smaller doses. Subleathal doses can leads to skin or eye irritation, headache, cancer and in more severe cases, birth defects, genetic disorder, paralysis, cancer, and even death. Degradation products derived from the formulation of the herbicide and the adjuvant added to the formulation may be more toxic than the herbicide compound itself, making it difficult to point out increased risks of cancer or other effects in a directly relate to a herbicide.

The most remarkable effects of herbicides on non-target plants and animals often results from the routine change they cause by killing the targeted weeds for example loss of persistent riparian plants can cause changes in water temperature and clarity that can potentially impact the entire aquatic population, and the physical structure of system through bank erosion. Removing a shrubby understory can make an environment incompatible for definite bird species and elucidation small mammals to predation.

b. Aquatic species

Toxicity of herbicide to aquatic organisms is quantified with LC_{50}, which is the concentration of herbicide in water to kill half of the study animals. The LC_{50} is naturally measured in micrograms of pesticide per liter of water. In general, ester formulations are more hazardous for aquatic species than salt and acid formulations because ester formulations are lipophilic and consequently, can pass through the skin and gills of aquatic species relatively easily. Ester formulations are not water soluble, and are less likely to be diluted in aquatic systems.

c. Human toxicology

Society gets earnings from the development of new technologies. Many of these technologies have allowed us to increase the yield from agricultural activity. Insecticides and herbicides have been used to inhibit the loss of crop yield to insects or non-food plant species. However the use of these chemical treatments is not without argument as can be seen in concerns about human risk associated with consuming food that included residue of herbicide or insecticide. The components associated with human risk with consuming food frame work includes

a) Hazard identification
b) Exposure assessment
c) Dose-response modeling
d) Risk characterization

Herbicides have the potential to contact not only humans, but non targeted flora and fauna as well. One means by which human would have ecological contact would be through the food supply with the consumption of food containing herbicide residue.

Another route would be through water that has been polluted with run-off containing herbicides. Human industrial exposure can occur in the management, manufacturing, storing or marketing of these chemicals.

Human responses to herbicides tend to be neurological and developmental neurological responses can range from short term memory loss to more extreme reactions such as seizures may occur.

A developmental response refers to the potential of certain chemicals to impact the growth, development, and functional capacity of exposed individuals. The effects of herbicides on wild life, aquatic biota and on humans are recognized in helping to inspire public awareness of the risk associated.

d. Toxic effects

A person's reaction to pesticide poisoning depends on

I. The toxicity of the herbicide
II. Duration of exposure
III. Route of absorption
IV. Efficiency with which poison is execrated by the person's body.

Different individuals can have different reactions to the same dose of herbicide. Weak peoples are in general, more sensitive to a given dose than are stronger people.

When proper safety precautions are taken, human exposure to herbicides used in natural areas should be minimal. Properly fitted personal protection equipment and well planned emergency response procedures will minimize exposure from normal use as well as emergency spill situation.

Toxicity due to selected herbicides

In recent years dinitroaniline compounds are subjected to increased toxicological and environment concerns, e.g. to cause various physiological changes and endocrine effects in the animal studies including liver parameters, kidney damage and a no of mutagenic effects. Human population is constantly exposed to chemicals present in the environment.

The level of trifluralin LD_{50} for rats1000 mg kg^{-1}; for mice 500 mg kg^{-1}; for rabbits and chicken $\geq$ 2000 mg kg^{-1}.Pendimethalin acute oral $LD_{50}$for albino rats are 1050-1250 mg/kg for mice 1340-1620mg/kg .The acute per cutaneous LD_{50} for rabbits$\geq$5000mg/kg. LC_{50} (96h) for catfish is 0.42 mg a.i $^{l-1}$and are non toxic to honeybees.

Among these compounds, chlorophenoxy acid herbicides are of significant importance because of their wide distribution and extensive use as plant growth regulators. They are easily transferred to surface and ground water due to their polar nature and relatively good solubility. High potential toxicity of fenoxaprop-ethyl which was shown in development toxicity studies in entering the risk assessment process. Toxicity studies have been conducted using fenoxaprop ethyl involving four species i.e. rat, rabbits, mouse and monkey. The studies for dietary and dermal and inhalation studies in all of these species were recognized for liver toxicity including amplified liver weight and a fall in body weight gain without a change in food consumption was reported. Oral Acute oral LD_{50} for rabbit's >2000mg/kg inhalation LC_{50} (4h) for rats while acute oral LD_{50} for bob white quill and mallard ducks> 2250 mg kg^{-1}.

Although the reported toxicity and eco toxicity of pinoxaden is minimum and below the trigger values, it is harmful if inhaled, irritates eyes and respiratory system. It is harmful to aquatic flora and fauna, and may cause long-term undesirable effects in the aquatic environment. The reported toxicity in rats is; acute oral LD_{50} (mg/kg bw)>5000, acute dermal LD_{50} (mg/kg bw)>2000, inhalation LC_{50} (mg/L)=5.2 and in birds acute LD_{50} (mg/kg bw)>2250.

Concern about the potential adverse effects of herbicides on the environment and human health have growing steadily because herbicide residues have been detected widely in the air ,water ,soil and agricultural produce.

Based on toxicology studies indicating fetal anomie-lies, maternal morality and liver toxicity field measurements studies are required. This need to develop easy analytical methods for

quantities exposure assessments.

Herbicides are one of the crucial factors worldwide increases in agricultural production. Herbicides contribute effectively and profitably to weed control and benefit society as a whole. Herbicides can provide cost-effective weed control with a minimum of labor. Use of herbicides is a main driver of the agrochemical industry. An increase in production by using herbicides for weed control is shown in Figure1.6 however use of herbicides has created considerable concern for human health and environment risk.

Despite the benefit, the use of these kinds of chemicals must be controlled because an important fraction of these herbicides are released in to the environment presenting a potential hazard risk. Considering the global consumption of herbicides per year it is not surprising that many of these compounds have been detected in natural waters and therefore, have raised considerable concern both from human health and from environmental point of view.

For several decades herbicides have been widely used for agricultural purposes in both in the developed and developing countries in order to control pest damage to crop yields. However the trend in the use of herbicides is increasing day by day to control different kinds of weed as part of the modern agriculture practices. This intensive use has resulted in to a serious contamination of the environment.

There are approximately 80,000 industrial and commercial chemicals in use in the world today and many more are added each year. Essentially all of these individually exhibit toxicity to humans. Toxicological information for many, but not all, of these chemicals is available in the literature.

Negative effects on human health as well as important environmental damage have been caused, especially after the second half of the 20thcentury in which great amounts of herbicides were released (and are still released) into the environment. Herbicides application over soils constitutes a threat to soil quality and their ability to provide environmental and agricultural services. In general, when herbicides enter the soil environment, they are quickly and strongly bound to soil components and their adsorption and desorption to these components govern their fate, as an example, retention by the soil can prevent their immediate access to surface or ground waters and their effects on non-target organisms. However, their persistence as harmful metabolites constitutes an ever-present hazard to the environment and also to human health. In

some cases they show residual activity in the soil and therefore, they can control and affect the germination of the crop of interest. The knowledge of the degree of herbicide pollution of a specific soil is of particular importance, since valuable data like degradation mechanisms and rate, leaching potential, accumulation, etc. can be obtained and therefore suitable actions can be taken.

The environmental fate of herbicides depends a lot on their mobility in soils and their tendency to partition into other environmental compartments, such as air and water. Transfer processes move pesticides in the environment. Concretely, leaching (the movement of water and dissolved chemicals through the soil) of herbicides through the soil profile from agricultural practices is receiving increasing attention.

With the aim of minimizing the consumers intake Environmental Protection Agency (EPA) has set guideline to limit the amount of pesticides in environmental compartments and food, through maximum residue limits (MRLs).

The fulfillment of this legislation has lead to a need of rapid, accurate, multiresidue analytical techniques for the determination of these compounds in agricultural and environmental commodities.

Most of the available methods for dinitroanilineand aryloxyphenoxy groups need expensive instrumentation and specialized technicians for its smooth running. The aim of the present investigation is to develop novel spectrophotometric and HPLC methods for identification and quantification of dinitroaniline and aryloxyphenoxy groups of herbicides coupled with more efficient and new extraction procedures. The main objective of the new methods was routine application, minimal cost, enhanced sensitivity, reproducibility and reliability with its subsequent application to environmental and agricultural samples.

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CHAPTER - 2

LITERATURE REVIEW

LITERATURE REVIEW

Ingelse et al(1) investigated method for the separation of some herbicidal enantiomers by capillary electrophoresis with 1-allylterguride as chiral selector. Baseline separation was revealed for the enantiomers of halossifop, fluazifop, and fenoxaprop. Whereas the incomplete resolution obtained for ocular isomers of flamprop. Separation times were short by applying HPLC and terguride chiral stationary phases as compared to other parallel analyses. Further they observed that the degree of dissociation of the acidic analyte as well as the amount of methanol present in the background electrolytes have a major influence on resolution.

Fayyad et al (2) reported high performance liquid chromatography for the separation and determination of 2, 4-dichlorophenol, 3, 4-dichlorophenol, 2,5-dichlorophenol, o-chlorophenol, and 2,4,5-trichlorophenol in water samples . The samples were derivatizatized with 4-aminoanti-pyrine before analysis. The substances determined are the phenolic products of the phenoxy-alkanoic acid herbicides: 2,4- dichlorophenoxyacetic acid, 2,4-dichlorophen-oxybutyric acid , and 2,4,5-trichlorophenoxy- acetic acid. For separation of phenolic product a C_{18} column was used, with gradient elution commencing at 50:50 and changing to 70:30 methanol: water. Detection was carried out at 470 nm where the 4-aminoantipyrilquinonimine derivatives show maximum absorbance. The calibration curve was linear over a concentration range of $5\text{-}10^4$ ng mL^{-1} phenols in the original water sample. The method was optimized by studying different conditions of temperature, pH and UV irradiation the degree of degradation of the phenoxy acids to phenols.

Sanchis-Mallols et al (3) determined phenoxy acid herbicides in drinking water using solid phase extraction followed by high performance liquid chromatography. Separation was performed on reverse phase column first with Lichrosphere RP select and then with, octadecyle-saline. Methanol and phosphate buffer were used as mobile phase with spectrophotometric detection at 230 nm.The samples were passed through C_{18} column for solid phase extraction. The extract was injected to HPLC for separation and quantification. % Recoveries ranged between 93-118.

Fuyu et al (4) reported the determination of dinitroaniline herbicides in human blood urine and environmental water by using headspace solid-phase micro extraction and gas chromatographic technique. The samples were collected by solid-phase micro extraction and dinitroaniline herbicides were separated from complex matrices of human urine and blood. The optimization of

conditions that were pH, salt additives, extraction temperature, and fiber contact time for water, were optimized for solid-phase micro extraction of urine and blood samples. The herbicides were extractable under these conditions, and could be determined by gas chromatography using electron capture detector. The recoveries of the herbicides, measured at the concentrations of 0.50 and 1.0 $ng.ml^{-1}$ urine or water and 1-60 ng in 0.5 mL blood, ranged from 37 to 66% for different herbicides from water or urine, and from 3.5 to 7.5 % from blood. The climax areas of the extracted herbicides were proportional to their concentrations in the range 0.1–10 ng mL^{-1} in water or urine, or 1–60 ng 0.5 mL^{-1} in blood. The lowest measurable concentration of the herbicides is in 0.1 $ngmL^{-1}$water or urine, or 0.5 ng mL^{-1} blood.

Lagana et al (5) studied isolation and trace enrichment of aryloxyphenoxy propionic acid herbicides from drinking, spring, and ground water. Volume of aqueous samples 1–2 L was passed through a disposable solid-phase extraction cartridge, packed with 500 mg of Carbograph-1 at a flow-rate of 100 $mLmin^{-1}$. After washing aryloxyphenoxy propionic acid herbicides were separated by means of a binary gradient of water and a mixture of methanol–acetonitrile. Detection and confirmation were performed by diode array detection. Recovery of the six acid herbicides ranged between 90% and 98%. The limits of detection of aryloxyphenoxy propionic acid herbicides were 8–21 ngL^{-1} for drinking water and 16–36 $ngL^{-1}$for spring water. For recovery and selectivity efficiency of Carbograph-1 cartridges was compared with that of LiChrolut EN cartridges and polystyrene divinylbenzene disks. Further the significant parameters such as pH and flow-rate for solid-phase extraction with divinylbenzene resins were optimized.

Lagana et al (6) detectedaryloxyphenoxy propionic herbicides in soil samples at ng g^{-1} levels by using soil column extraction and liquid chromatography and electrospray ionization mass spectrometry. In this method sample were simply treated for isolation and traces fortification by soil column extraction and cleans up with a Carbograph-1 cartridge. The special effects of various extracting solutions and soil type, test of extractability for aged field-treated soil and pesticide concentration on the performance of the SCE are investigated. Overall, the recoveries for each herbicide across all fortified samples 1–50 ng g^{-1}were 90±3.5% for fluazifop, 91±3.3% for haloxyfop and 90±3.1% for diclofop. To attain good liquid chromatographic separation, and to avoid ion signal moderation eluent was acidified and subsequent post column addition of a

neutralization buffer. The detection limits obtained were 0.1-0.3 ng g^{-1} by using the time-scheduled selected ion monitoring with negative ion mode of operation.

D'Ascenozo et al (7) reported the determination of arylphenoxyproionic herbicides in water by liquid chromatography with electrospray mass spectrometry. Arylphenoxy propionic acids are a new class of herbicides used for the selective removal of most grass species from any no grass crop. Eleven Surface or ground water samples and twenty one drinking-water Samples were passed through a 0.5g graphitized carbon black extraction cartridge. Reversed-phase column of 4.6-mm I.D LC C_{18} was used for extraction of arylphenoxyproionic herbicides. The analytes were passed through column with flow-rate 1 mL min^{-1} of mobile phase for separation. A flow of 20 µL min^{-1} of the column effluent was sidetracked to the pneumatically assisted electrospray source. The ion-signal optimization, the effect of the concentration of the acid in the mobile phase on the response of the pneumatically assisted electrospray source and mass spectrometer detector was investigated. Further by evaluating the specificity and sensitivity of the method, the effects of varying the orifice plate voltage on the production of the diagnostic fragment and the response of the mass spectrometer detector were also investigated. For the analyte considered, the response of the mass detector was linearly related to the amount of the analyte injected between 1 and 200 ng. In all cases, recoveries of the analytes were better than 91%. The limit of detection of the method investigated for the pesticides in drinking water sample were 4–10 ngL^{-1}.

Analysis of several herbicides used to control weeds in forest as pendimethalin, hexazinone, thiazopyr and simazine, using gas chromatography with nitrogen phosphorus detector and GC with mass spectrograph in soil were reported by Perez et al (8) The soil samples were passed through small columns for the extraction of soil samples for the determination of these compounds in soil. The solvents Acetone: water in ratio 90:10 was used for the extraction of all four compounds, and ethyl acetate when the determination of hexazinone is not required. Percent recoveries through the method were always 69-80%, and the detection limit was 0.01-5 μgmL^{-1} for gas chromatography with nitrogen phosphorus detector and. gas chromatography with mass spectrograph detector .Soil samples from tentative fields, taken at different times after treatment from the cultivate layer, were analyzed using this method.

Analysis of herbicide of the dinitroanilines group pendimethalin traces in four different natural soils by using supercritical fluid extraction with carbon dioxide was reported by

Spack et al (9) The Supercritical Fluid Extraction SFE method was compared with the classical Soxhlet and shaking methods in terms of ease to run, extraction efficiency, selectivity and reproducibility. The influence of the physico-chemical properties of the soil matrix on herbicide extraction was then evaluated with the SFE method. It was observed that supercritical fluid extraction system was much easier to run than the other two methods. It is also less time consuming and requires fewer operations as it was optimized for on-line sample clean up. supercritical fluid extraction is the most selective of the three useful methods as fewer co-extracts are obtained in the final samples. Supercritical fluid extraction with CO_2 is particularly powerful because pendimethalin is highly hydrophobic. However, this makes pendimethalin a poor choice for a selectivity study of SFE as it is very rapidly extracted at any CO_2 density. Pendimethalin extraction with supercritical CO_2 was found to be complete with average recoveries of 96–99%. The performance of SFE was shown to be unaffected by soil parameters except soil water content.

A review on the analysis of herbicides residues in cereals, fruits, and vegetables by chromatographic methods was reported by Tedeoa et al (10) The principal chemicals groups of herbicides like phenoxy acids, benzonitriles, urea, trizines, dinitrianiline, chloroacetamides, carbamates, uracils, glyphosate and bypyridylum compounds have been considered. The review briefly provides some basic information on food samples extraction, cleanup, derivitization and determination of herbicide residues.

A precise multiresidue method for the determination of acidic basic and neutral herbicides in drinking water samples was reported by Curini et al (11). The method is based on reversed phase liquid chromatograph mass spectrometry, including an ion spray interface. Fifty samples of surface water twenty of ground water forty of drinking water samples were analyzed by this procedure .Samples were passed through a 0.5g of graphitized carbon black, extraction cartridge. Acid, basic and neutral herbicides were passed through apparatus differential eluted from graphitized carbon black cartridge and were analyzed. A conventional 4.6-mm-ID C_{18} column, operating with a mobile phase flow-rate of 1 ml min^{-1}, was used to chromatograph the analytes. A flow of 100 µL min^{-1} of the column effluent was diverted to the ion spray interface source. The study demonstrates the sensitivity of the technique, with the detection limit under 10 ngL^{-1} in drinking water samples.

Gita et al (12) studied the effect of long-term field application of pendimethalin; enhanced degradation in soil. The outcome of long-term application of pendimethalin in a maize-wheat rotation on herbicides persistence was investigated. Pendimethalin was applied 1.5 $kgha^{-1}$ separately as one or two annual applications five consecutive years in the same plots. Residues of pendimethalin were determined by gas chromatography. It was observed that harvest-time residues of the herbicides decreased gradually over the years and at the end of five years 3% of applied pendimethalin was recovered from soil as against 18% in the first year. Residues were found were found distributed in the soil profile up to 90 cm depth at the end of the experiment with peak distribution of 0.03 μgg^{-1} in the surface layer of soil treated with 10 herbicide applications. The minimum distribution was, however, in the deepest soil (73-90 cm) profile. Some of the metabolites of the pendimethalin derivatives, partially reduced derivative and stylized product were also traced in surface and sub-surface soils under laboratory conditions which revealed faster degradation compared to control soils. Only the surface soils (0-15cm) showed this enhanced degradation of herbicide, which could be due to adaptability of the aerobic microorganism to degrade pendimethalin degradation was confirmed in nutrient consumption.

Ding et al (13) presented a method to analyze chlorophenoxy acid herbicides in water samples. The samples determined were 2, 4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxypropionic. The extraction of samples was performed on a graphitized carbon black cartridge, and on-line derivatization in the gas chromatographic injection port. Injection volume 10–15 mL of sample was directly injected to introduction device with tetra alkyl ammonium salts. The analytes were then identified and quantitated by ion-trap gas chromatography with mass spectrometry. The large-volume injection-port derivatization technique provides sensitivity, fast and reproducible results for Chlorophenoxy acid herbicides residues, for quantitation at 0.1 to 0.2-mgL^{-1} in 500-mL water samples. An improved characteristic mass chromatogram of molecular ions of butylated chlorophenoxy acid herbicides with a significant chlorine isotope pattern by electron impact ionization MS help us to determine herbicides residues at outline levels in aqueous samples. Recovery of the herbicide residues in spiked water samples ranged from 75 to 100% with RSDs of 1 to 15%.

Edgebretson et al (14) analyzed residues of pendimethalin in fruits, nuts, vegetables, grass, and mint using gas chromatography. Pendimethalin, in the formulation of a commercial herbicide labeled Prowl was applied to various crops. Analysis of pendimethalin and its metabolite amino-

2-methyle-3, 5 dinitrobenzyl alcohols and 4(1-ethylpropyl) was accomplished by utilizing liquid partitioning gel permeation chromatography for nuts and mint. Solid-phase extraction cleanup,and gas chromatography with a nitrogen-phosphorus detector was applied for determination. Average recoveries for pendimethalin ranged from 71% to 126% over two levels fortification while for its metabolite average recoveries ranged from 69% to 123% over two levels of fortification. The quantitation limit for mint oil was 100 μgL^{-1}. Residues greater than the limit of quantitation was found for pendimethalin in apple pomace, fresh and dry fig, wet and dry grass screenings, mint oil, almond hulls, green onion, and in tomato pomace .

Electrochemical method for the determination of dinitroaniline herbicides was reported by Sreedhar et al(15). Dinitroaniline herbicides were determined using electrochemical method by reduction mechanism at mercury electrode. The method described is based on different voltrametric techniques at different pH values in various buffer solutions. A differential pulse polarographic method was also presented for the determination of the herbicides in grains, soils as well as in water samples. The detection limits reported are 6×10^{-8} M - 1.5×10^{-8} M for dinitroaniline herbicides.

Rosales-Conrado et al (16) presented a simple method for the simultaneous determination of chlorophenoxy acid herbicides and their esters in soil by HPLC, after ultrasonic extraction, on a C_{18} packed capillary column with temperature gradient, large injection volumes and UV detection at 232 nm. Compounds determined by this method were 2,4-dichlorophenoxyacetic acid 4-(2,4-dichlorophenoxy) butanoic acid, 2,4-dichlorophenoxyacetic-1-methyl ester and 2,4-dichlorophenoxyacetic-1-butyl ester (2,4-D-1-butyl ester), Samples were spiked with herbicides in range between 2.5 and 6.0 $\mu g\ g^{-1}$ of each herbicide. Percent recoveries obtained were between 72 and 97% and detection limits were between 0.3 and 0.5 $\mu g\ g^{-1}$.

Szekacs et al (17) applied a non-labeled immunosensor method via optical wave-guide light mode spectroscopic for the trifluralin detection. The measurement is based on the diffraction of linearly polarized He- Ne laser beam at 632.8 nm laser on a diffraction grating. In a thin waveguide layer (SiO_2- TiO_2). Within the powerless antibody based immunosensor the signal measured was proportional to the trifluralin contents in the samples, but the method allowed detection of trifluralin only above 100 ng mL^{-1}. During measurements the standard solutions and samples were mixed in 1:1 ratio with antiserum containing constant amount of antibodies. The amount of free antibodies bound to the surface is inversely proportional to the trifluralin contents

in the solution. The detection limits were in range of $2x10^{-7}$ to $3x10^{-5}$ $ngmL^{-1}$. Results of trifluralin determination were compared to those obtained in parallel with enzyme linked immunosorbent assay tests and with gas chromatographic mass spectrometric analysis. Immunosorbent assay technique is highly sensitive and increase the limit of detection six times.

Brondi et al (18) developed high performance liquid chromatographic method for the determination of pesticides residues extensively in use in sugar-cane cultures in river water samples. Two extraction techniques were applied the liquid-liquid extraction and solid phase extraction followed by high performance liquid chromatography with ultraviolet detection. Water and Acetonitrile were used as mobile phase on C_{18}columnas sorbent. % Recovery obtained for residues determination was 95-98.

Albero et al (19) determined herbicides by rapid multi residue method in carrot, grape, and in vegetable juices. The analytical procedure was based on sonication assisted extraction with ethyl acetate followed by matrix solid-phase distribution of juice samples on florisil placed in glass columns. %Recoveries obtained were 84 to 110. Relative standard deviations for all herbicides studied equal or lower than 10%. The analysis of samples was proficient using gas chromatography with mass spectrometry on selected ion monitoring. Standards of herbicide were used to spike blank samples to counter act experiential and matrix effects in the chromatographic determination. The detection limits ranged from 0.1-1.6 $\mu g\ L^{-1}$. The developed method was applied to the analysis of herbicide residues in juice samples available in market.

Bermudez-Saladana et al (20) reported a method for the determination of herbicides in fish samples using biopartitioning micellar chromatography. In this technique modeling bioconcentration of herbicide is as a vitro approach to estimate the bio concentration factor. Different sets of 85 pesticides from six chemical families were used. For pesticides exhibiting bioconcentration in fish, a quantitative retention-activity relationship model was made to get log k quantitative structure-activity relationship. To avoid experimental problems the log of parameters were related with bioconcentration by using these two factors a log value was used to calculate concentration of pesticides in fish by means of biopartitioning micellar chromatography.

Golab et al (21) identified 28 degradation products of trifluralin in soil which was treated with trifluralin. Identification of the metabolites products of trifluralin and the related compounds was

made by using mass spectrograph. Compounds having nitro groups yield mass spectra containing peaks due to intra-ionic oxidation by the nitro groups. The mass spectra of the *N, N*-dialkylamines, with one exception, show major peaks due to amine α-cleavage and due to amine α-cleavage followed by carbon-nitrogen bond cleavage with rearrangement of a hydrogen atom. They presented the mass spectra of these products and a number of related compounds, which were suspected products but were not found in trifluralin-treated soil. In some cases, propionyl ions formed by this process are major ions in the spectra. Azo and azoxybenzenes resulting from condensation of two trifluralin moieties mainly through the nitrogen-nitrogen bond.

Goncalves et al (22) investigated a suitable method based on ultrasonic extraction for pesticide residue analysis in soil samples. Ultrasonic extraction was helpful in reducing the amount of sample to be collected and time consumed in the analytical process. A broad range of analytes should be preferably considered in the same analytical procedure. The analysis was done by gas chromatography–mass spectrometry. Different classes of pesticides both recent and old persistent molecules, parent compounds and degradation products were selected for analysis. The compounds studied were namely organochlorine, organophosphorous and parathyroid insecticides, triazine, acetanilide herbicides and other varied pesticides. Sensitivity for the pesticide residues detected were in the range 0.05–7.0μg kg^{-1} with good precision 7.5–20.5%, with average R.S.D13.7%. Extraction efficiency were 69–118%, average 88% for the great majority of analytes. This methodology is successful in a monitoring program of soil samples.

Song et al(23) studied biodegradation of fenoxaprop-p-ethyl by bacteria isolated from sludge. A mixed bacterial population was isolated using enrichment in basal medium containing increasing amounts of fenoxaprop-p-ethyl as sole carbon source from slug. Eight kinds of isolates could utilize fenoxaprop-p-ethyl, in pure culture, there was 46, 66.0 and 70 % loss of fenoxaprop-p-ethyl initial concentration was 100, 80, 50μgmL^{-1} as the sole carbon fenoxaprop-p-ethyl with biodegradation. Degradation kinetics obeyed the first order kinetics, the same as the degradation in soil. At least five degradation products of fenoxaprop-p-ethyl were collected by H_{uv} separated by high-pressure liquid chromatography.

Hjorth et al (24) applied an easy and quick method to determine herbicide effects in poaceae weed species by using microscopy. The substances investigated were the thiocarbamate, sulfonylurea, iodosulfuron, prosulfocarb,aryloxyphenoxypropionate and fenoxaprop-p-ethyl and

organophosphate glyphosate. The method has prospective to be used as a tool to indicate future death of grass specie due to herbicide exposure by observing phytochemical effects. Auto flurescence of leaves was studied under a microscope using ultraviolet and blue light. The fluorescence of leaves treated to enhance flavonides was also examined. To confirm the results, micro scospectro-flurometery was performed. Effects indicating future death of the grasses were observed in visually healthy leaves following handling with fenoxaprop-p-ethyl. After exposure to glyphosate and iodosulfuron, changes in the content of flavonides and other compounds with a conjugation system, structure inflexibility and decrease in the contents of chlorophyll were detected in the leaves. By observing phytochemical effects and results obtained from micro scospectro-flurometery it was indicated that flavonoid quercetin, naringenin and nitrogen were reduced by use of prosulfocarb.

Carabias et al (25) investigated a method for the determination of herbicides and thermally unstable compounds including many metabolites. In this method poly acrylate fiber was used for solid-phase micro extraction. Separation, identification and quantification were accomplished with gas chromatography followed by mass spectrometry. The herbicides of different chemical groups atrazine, alachlor, chlorotoluron, diclofop, diflufenicam, ethofumesate, isoproturon, linuron, terbutryn and trifluralin were selected for study. The chromatographic presentation of three phenylureas as a function of the medium and injection mode employed was studied. The compounds generated as a function of the solvent used in direct injection were ethyl acetate, methanol and methanol–water for the phenylureas group of herbicides. The method can be applied for the determination of stable and thermally unstable herbicides as long as a preconcentration step involving solid phase micro extraction. The limits of detection varied between 0.03 mg L^{-1} for ethofumesate and 0.13 mg L^{-1} for chlorotoluron. Herbicides in surface and ground water samples were determined by these methods. Herbicides of atrazine group were analyzed after a preconcentration step with styrene divinylbenzene sorbents, the contents of chlorotoluron found were equal to those obtained using high performance liquid chromatographic analysis.

Bruzzoniti et al (26) reported a method for determination of herbicides in drinking water using solid phase extraction followed by gas chromatography and mass spectrometry. Method was optimized for the solid phase extraction gas chromatography-mass spectrometry. Herbicides belonging to following different families trifluralin, pendimethalin (dinitroaniline), atrazine,

propazine, simizine, ametryne, cyanazine, ethylterbutylazine, ethaylatrizine (atrazine), molinate (carbamate) and alachlore, metachlor (chloroacetamide) were determined. Different solid substrates C_{18}, cyano, styrene-divinylbenzene, phenyl, graphitic carbon has been compared. The % recovery yield of the herbicides was improved by applying effect of variables as elution solvent, its volume and the sample flow rate. The optimized experimental conditions were C_{18} phase conditioned with 3 mL acetone, loaded with 1L water sample at 5mL min^{-1} and eluted with 3mL acetone. Good recoveries 79-99 % had been obtained for all analytes, except for deethylatrizine increases up to 94±17%. The optimized method was successfully checked for the identification and quantization of the selected herbicides in raw and drinking water samples, with quantization limits as low as 0.01μg L^{-1}.

Osamu et al (27) reported a highly sensitive and selective analytical method to determine levels of acetyl-CoA and malonyl-CoA in germinating rice seeds and plant tissues. The method includes a convenient extraction method for plant samples and a new liquid chromatography with mass spectrometery technique utilizing an ion pair reagent. The herbicide acyl-CoAs, present in germinating rice seeds, were then determined by the method developed. It was found that the concentrations of both acetyl-CoA and malonyl-CoA increased with time during the germination of rice seeds and also increased at the elevated cultivation temperatures among tested. These results reflect the development of enzymes that produce these acyl-CoAs in germinating rice seeds.

Fuenteset al (28) developed method to determine trifluralin, metolachlor, chlorpyiphos and triadimenton. Microwave-assisted extraction and partitioning method (MAEP) using water acetonitrile and n-hexane for desorption and simultaneous partitioning, respectively, together were used followed by gas chromatography. Pesticides trifluralin, metolachlor, chlorpyiphos and triadimenton have a broad range of physicochemical properties in agricultural soils. Three points that were instrumental and sample-associated factors affecting extraction of the target compounds considered critical in this study. Spiking procedure at trace level was carried out to reproduce the solute soil sorption taking place in the environment as closely as possible and results were analyzed taking in to account adsorption behavior of the compounds on different kinds of soils. The complete analytical procedure planned consisted of the MAEP of pesticides from 1.0 gram of soil with 1 mL of 1:1 water/acetonitrile mixture, and 5mL of hexane for trapping. The microwave heating program applied was 2 min at 250 Wand 10 min at 900 W and

130 ^{0}C maximum temperature. After extraction, the hexane layer was evaporated to dryness, the residue was redissolved and directly analyzed using gas chromatography electron capture detection (GC-ECD). Most of the compounds studied were recovered in good yields with relative standard deviations of below 9% and detection limits ranged from 0.004-0.036 μgg^{-1}.

Diez et al (29) developed and optimized dispersive solid-phase extraction and acetonitrile/partitioning extraction for the extraction and determination of herbicides in barley samples. The method developed with a minimum number of steps and a low solvent and glassware consumption provides elevated eminence results. This method, suitable for the analysis of multiple classes of pesticide residues in foods, has been given an acronymic name, QuEChERS, that reflects its major advantages (quick, easy, cheap, effective, rugged, safe). QuEChERS method, which was originally created for vegetable samples with a high amount of water was modified to optimize the extraction of a wide range of herbicides in barley. It was then compared with known conventional multi-residue extraction procedures such as the Luke method. In which simplified and shortened by eliminating the Florisil clean-up and the ethyl acetate extraction, which involves a subsequent clean-up by gel permeation chromatography. Finally, a simple acetone extraction was carried out to check the differences with the other three methods. Extracts were analyzed by liquid chromatography–tandem mass spectrometry and gas chromatography–time-of-flight mass spectrometry.

Therveni et al (30) presented a sensitive method for the determination of the herbicides nitralin and oryzalin in agricultural formulation, vegetables and grape juice samples by adsorptive stripping voltammetry. Electrode used was a hanging mercury drop electrode (HMDE) at optimized pH 6.0. A symmetric study of the various operational parameters that affect the stripping response was carried out by differential pulse voltammetry. With an accumulation potential of −0.5 V and an 80 s accumulation time, the limit of detection was 2.47×10^{-8} molL and 1.5×10^{-8} $molL^{-1}$. For both compounds the relative standard deviation ($n = 10$), correlation coefficient values were found to be 1.14%, 0.998, 1.48%, 0.999 at concentration levels of 8.3×10^{-8} molL to1.5×10^{-6} molL and 2×10^{-8} $molL^{-1}$ to 1.0×10^{-5} $molL^{-1}$ respectively. The degree of interference of some other pesticides on the differential pulse adsorptive stripping signal for nitralin and oryzalin was evaluated.

Lin et al (31) studied the hydrolysis of fenoxaprop-p-ethyl (FE), a widely used herbicide in aqueous buffer solution at pH ranging from 4.0-10.0. The degradation kinetics strongly dependent on pH value and followed first order kinetics. FE was relatively stable in natural media, where it degrades rapidly with decreasing or increasing pH values. In acidic medium (pH4, 5) the benzoxazolyl-oxy-phenyl ether linkage of FE was cleaved to form ethyl 2-(4-hydroxyphenoxy) propanoate (EHPP) and 6-chloro-2,3-dihydrobenzoxazol-2-one (CDHB). While in basic conditions (pH) 8, 9, 10) fenoxaprop- p (FA) was formed via breakdown of the ester bond of the herbicide. Both the two pathways were concurrent in natural condition (pH 6, 7). Toxicity studies on Daphnia magna showed that FE was not toxic to Daphnia magna with 48 h EC (50) of 14.3 $\mu mol\ L^{-1}$. The evolution of overall FE toxicity to Daphnia magna in the aquatic system needs to consider the degradation of FE.

Huanga et al(32) investigated and validated a sensitive method for simultaneous determination of 102 pesticide residues in teas. The multiresidue analysis of the pesticides in teas involved extraction with acetone–ethyl and acetate–hexane, clean-up using gel permeation chromatography (GPC) and solid-phase extraction (SPE), and subsequent identification and quantification of the selected pesticides by gas chromatography–mass spectrometry (GC–MS) conditions. For most of the target analytes, the optimized pretreatment processes led to no significant interference on analysis from sample matrix and the determination of 102 compounds was achieved in about 120 min. Herbicides residues could be determined in ppb range, from 0.01 $\mu g/mL$ for hexachlorobenzene to 2.5 μgmL^{-1} for propargite, with average recoveries from 59.7 to 120.9% and relative standard deviations (RSDs) in the range 3.0–20.8% for all analytes across three fortification tea levels.

Tran et al(33) described the application of different extraction techniques for the preconcentration of ten commonly found acidic and nonacidic herbicides. Liquid-liquid extraction (LLE) with dichloromethane, solid phase extraction (SPE) using Oasis HBL cartridge were compared for extraction efficiency of these herbicides in different matrices especially water samples from contaminated agricultural drainage water containing high concentration of particulate matter. Herbicides were separated and quantified by high performance liquid chromatography (HPLC) with ultraviolet detector. SPE using SDB-XC Empore ^{TM} disks was applied to determine target herbicides in the Murrumbigee irrigation Area (NSW, Australia). The

daily aqueous concentrations of herbicides from 24-h composite samples detected at two sites increased after run off from a storm event and were in the range of 0.1-17.8μg L^{-1}.

Zhang et al (34) developed a reliable, sensitive, and reproducible method for quantitative determination of nine new generation pesticides currently used in cotton agriculture. Injector temperature significantly affected analyte response as indicated by electron capture detector (ECD) chromatograms. A majority of the analytes had an enhanced response at injector temperatures between 240 and 260 ^{0}C, especially analytes such as acephate thatoverall had a poor response on the ECD. The method detection limits (MDLs) were 0.13, 0.05, 0.29, 0.35, 0.08, 0.10, 0.32, 0.05, and 0.59 $ngmL^{-1}$ foracephate, trifuralin, malathion, thiamethozam, pendimethalin, DEF6, acetamiprid, brifenthrin, and cyhalothrin respectively. This study provides a precision(0.17–13.1%), accuracy (recoveries = 88–107%) and good reproducible method for the analytes of interest. At relatively high concentrations, onlycyhalothrin was unstable at room temperature (20–25 ^{o}C) and 4 ^{o}C over 10 days. At relatively low concentrations, acephate and acetamiprid were also unstable regardless of temperature. After 10 days storage at room temperature, 30–40% degradation of cyhalothrin was observed. It is recommended that acephate, acetamiprid, and cyhalothrin be stored at -20 $^{\circ}$C or analyzed immediately after extraction.

Morozova et al (35) investigated procedures based on the ultrasonic and microwave extraction of pesticides, 2, 4-dichlorophenoxyacetic (2,4-D) and 2,4,5-trichlophenoxyacetic (2,4,5-T) acids from soils for the subsequent determination by fluorescence polarization immunoassay (FPIA). The matrix effect of soils on the FPIA results was studied, and the optimum extractants and extraction conditions were selected. The recovery of pesticides from soil was 80–132% for 2,4-D and 101–138% for 2,4,5-T. Microwave extraction is more efficient than ultrasonic extraction for the determination of 2,4-D and 2,4,5-T from soil. Results of the determination of 2, 4-D in soil by FPIA are in good agreement with the results of the determination by high-performance liquid chromatography. The procedures can be used for the rapid determination of chlorophenoxy acids in soils.

Helm et al (36) determined ethofumesate, metalaxylmethoprene, and metobromuron and piperonyl butoxide in tobacco. The pesticides were extracted with water and methanol from five different types of tobacco. The extracts were purified by partition on an extraction cartridge containing diatomaceous earth. The purified extracts were analyzed by reversed-phase high-

performance liquid chromatography connected to an atmospheric pressure ionisation–electrospraytriple quadrupole mass spectrometer operating in the positive ion mode. Two different transitions and their relative intensities were monitored for unambiguous identification. All pesticides presented overall recovery rates between 35% and 110%. The veracity is near 100% and the inter day precision is below 15%. The limits of quantifications are equal or below the guidance residue levels proposed by the Agrochemical Advisory Committee of CORESTA, an association of organizations having scientific research relative to tobacco.

Zhang et al (37) investigated a rapid multi-residue method for the determination of 16 herbicides in onion. The analytical procedure was based on preventing formation of sulfur-containing compounds in onion by microwave inactivation of the enzyme allinase. The onion samples, which had been pretreated, were extracted with acetonitrile and cleaned by solid phase extraction. The herbicide residues in onion were detected by gas chromatography/mass spectrometry with selected ion monitoring. The recoveries of 16 herbicides ranged from 69.2% to 105.0% with the relative standard deviations (RSD) below 10.7%. The limit of quantification (LOQ) ranged from 0.003 to 0.015 mg kg^{-1}. The method was applied to the analysis of herbicide residues in onion samples.

Lin et al (38) described photo degradation of fenoxaprop-p-ethyl and toxic and photo resistant effects of the degradation products. The photo degradation pathway of the commonly used herbicide fenoxaprop-p-ethyl was elucidated, and the effects of the photo degradation on its toxicity evolution were investigated. Under solar irradiation of lambda >200nm and lambda >290 nm through rearrangement, loss of ethanol after rearrangement, desertification, dechlorination, photohydrolysis, and breakdown of the ether linkage. The effects of these anthropogenic pollutants on ecosystem were studied.

Wen et al (39) presented a method on electrochemical analysis of trifluralin using a nonstructuring electrode with multi-walled carbon nanotubes. The electro analytical behaviors of the endocrine-disrupting chemical trifluralin have been studied at a nonstructuring electrode. The electrode was fabricated by coating a uniform multi-wall carbon nanotubes dihexadecyl hydrogen phosphate film on glassy carbon electrode (GCE). The reduction peak currents of trifluralin increased and the reduction peak potential shifted positively at the nonstructuring electrode, compared with that at a bare GCE. A linear response of trifluralin was obtained in the

range from 5.0×10^{-9} to 6.0×10^{-6} mol L^{-1} ($r = 0.998$) and with a limit of detect (LOD) of 2.0×10^{-9} mol L^{-1}.

Rissato et al (40) developed analytical, nutritional and clinical methods for the determination of pesticides in honey samples using gas chromatography–mass spectrometry followed by to environmental samples. The method is simple and fast multiresidue method to determine 48 pesticides within the major groups of pesticides (organohalogen, organophosphorous, pyrethroids and organonitrogen) in representative samples of honey. The recovery results found ranged from 76% to 95% and the limits of detection were lower than 0.01 $mgkg^{-1}$ for gas chromatography with electron impact mass spectrometric detection in the selected ion monitoring mode (GC–MSSIM). The results indicated that most pesticides found in the samples belonged to the organohalogen and organophosphorous groups and lower levels of residues of some organonitrogen and pyrethroids were also detected. Malathion residues were detected in all.

Nguyen et al (41) reported a rapid, specific and sensitive multi-residue method based on the QuEChERS sample preparation method followed by gas chromatography with mass spectrometric detection in the selected ion monitoring mode. Using one quantification ion and two identification ions for the routine analysis of 203 pesticides in rice paddies. Analyses of fortified rice paddy samples were performed at different levels i.e. 0.05, 0.20 and 0.50$mgkg^{-1}$. Mean recoveries from five replicates ranged from 75% to 115%, with coefficients of variation lower than 17%. The limit of quantification was in the range of 0.002–0.05mg kg^{-1} for the pesticides. A total of 1040 rice paddy samples were analyzed for method application.

Lesueur et al (42) analyzed pesticides in food samples which are becoming a major issue due to their intensive use in agriculture. Thus an appropriate control of their residues in food samples has to be operated. In this study they analyzed 105 pesticides using gas chromatography followed by mass spectrometry and 46 pesticides using liquid chromatography with mass spectrometry after extraction with the QuECheRS method in four matrices (grape, lemon, onion and tomatoes).For GC the LOD and LOQ ranged from 0.4 to 48.2 gkg^{-1} and from 1.2 to 161gkg^{-1}, respectively. For HPLC-amenable substances, they varied from 1.0 to 115lg kg^{-1} and from 3.3 to 382 gkg^{-1}, respectively. With GC/MS, 61–82% of the substances showed a recovery in the range of 70–110% and 6–30% presented a recovery higher than 110% at the 500 gkg^{-1} fortification level. With HPLC/MS, 87–93% of the substances presented recoveries in the range

of 70–110% at the 500 gkg^{-1} fortification level compared to 78–85% at the 50 $lgkg^{-1}$ fortification level. Lemon (high acidic) and onion with high sulfur content showed poor recoveries respectively.

Herbicides alachlor, mecoprop-p, pendimethaline, propanil and trifluralin were determined by Perez et al(43). They determined the absolute values of molar absorptivity coefficients ε of five herbicides in methanol to determine the concentrations in food samples. The experimental set-up consisting of two parallel absorption cells coupled to a CCD detector. The absorption spectra were measured using a deuterium lamp at room temperature (298±2K) over the wavelength range 240–340 nm. Pendimethalin and trifluralin where determined in wavelength range 240–550 nm. Absorbance was also measured at 253.7 nm using a mercury lamp in different concentrations range depending on the studied compound. Except for mecoprop-p where absorbance were too low at this wavelength, the absolute values of molar absorptivity coefficients in mol $L^{-1}cm^{-1}$ at 253.7 nm were determined according to the Beer–Lambert's law were found to be value ε 381±28 for alachlor, 11, 240±1030 for pendimethalin, 22,220±1850 for propanil and 5720±410 for trifluralin respectively.

Santilio et al (44) developed a quick and simple method for the analysis of phenoxy acid herbicides 2,4-dichlorophenoxyacetic acid, 2-(4-chloro-*o*-tolyloxy) propionic acid, and 2-(4-aryloxyphenoxy) propionic acid, 4-chloro-2-methylphenoxyacetic acid in carrots and apples by liquid chromatography coupled to triple quadruple mass spectrometry. The compounds were analyzed by QuEChERS methodology without cleanup. The recoveries were performed at two spiked levels 0.05 and 0.5 $mgkg^{-1}$ for both matrices with six replicates for each level. The mean recoveries ranged from 70-92% for both apples and carrots. The precision of the method expressed as relative standard deviation of 0.99 was obtained over the range of concentration from 0.05 μgmL^{-1} to 0.5 μgmL^{-1}, corresponding to the pesticide concentrations of 0.05 $mgkg^{-1}$ and 0.5 $mgkg^{-1}$, respectively. The quantification limits ranged from 0.01 $ngmL^{-1}$ to 1.3 $ngmL^{-1}$in solvent, whereas, the quantification limits calculated in matrix ranged from 0.05 ngg^{-1} to 21.0 ngg^{-1} for apples and from 0.06 ngg $^{-1}$to 10.2 ngg^{-1} for carrots. The standard deviation was found to be in the range 4-16%. Linearity range of concentration was from 0.05 μgmL^{-1} to 0.5 μgmL^{-1}. Corresponding to the pesticide concentrations of 0.05 $mgkg^{-1}$ and 0.5 $mgkg^{-1}$ respectively. The quantification limits ranged from 0.01 $ngmL^{-1}$ to 1.3 $ngmL^{-1}$ in solvent, whereas, the

quantification limits calculated in matrix ranged from 0.05 ngg^{-1} to 21.0 ngg^{-1} for apples and from 0.06 ngg^{-1} to 10.2 ngg^{-1} for carrots.

Meloa et al (45) described electroanalytical procedure for the determination of diquat herbicide in natural water and potato samples. Dental amalgam electrode was used to evaluate the electrochemical behavior and to develop a voltamogram. The work was based on the square wave voltammetry responses of diquat, which presented two distinct and reversible reduction peaks, at −0.56V and −1.00V. The experimental and voltammetric parameters were optimized, and the analytical curves were constructed and compared to similar curves performed by high performance liquid chromatography coupled to ultraviolet–visible Spectrophotometric detector. The responses were directly proportional to herbicide concentration in a large interval of concentration. Calculated detection limits were very similar, around $10ngL^{-1}$ for voltammetric and chromatographic experiments. These values were lower than the maximum residue limit recognized for natural water by the Brazilian Environmental Agency. The recovery percentages in pure electrolyte, natural water and potato samples showed values from 75% to 135%. The methodrepresents that the voltammetric methodology proposed is suitable for determining any contamination by diquat in different samples.

Pfligier et al (46) studied the atmospheric behavior of pesticides by adsorbing them on ozone concentration. Heterogeneous ozonolysis of three herbicides terbuthylazine, alachlor, and trifluralin were performed in a flow reactor by adsorbing herbicides on silica particles. In order to simulate atmospheric conditions the pesticides were adsorbed on particles using gas-solid adsorption equilibrium. The kinetics were calculated by comparing the initial and the remaining amounts of pesticides adsorbed on silica particles after exposure to ozone concentrations ranging from 5 to 41 μgmL^{-1} during 90 min to 6 hrs. This work offers the first results of heterogeneous ozonolysis of alachlor and trifluralin adsorbed on mineral particles. Although alachlor and terbuthylazine were expected to react with ozone, no degradation was observed which leads to a lifetime higher than 8 months towards ozonolysis for 40 $ngmL^{-1}$ of O_3. A significant degradation of trifluralin adsorbed on silica particles by heterogeneous ozonolysis was observed. The experimental data could be fit by both the Langmuir–Rideal and the Langmuir–Hinshelwood models resulting in atmospheric lifetimes of 40 and 32 days respectively for $40ngmL^{-1}$ of Ozone.

Ramos et al (47) developed a new and simple method based on the use of multi-walled carbon nanotubes as solid-phase extraction stationary phases. The determination of seven pesticides of organophosphorus group as ethoprophos, diazinon, chlorpyriphos-methyl, fenitrothion, malathion, chlorpyriphos and phosmet and one thiadiazine (buprofezin)in different kinds of soil samples was carried out. Soil samples were collected from forestall, ornamental and agricultural part and determination was performed by using gas chromatography with nitrogen phosphorus detection. Ultrasound extraction was performed by using first with 10 mL 1:1 methanol and acetonitrile (v/v) and the evaporated extract of soils was redissolved in 20 mL water at pH 6.0 extract was passed through multi-walled carbon nanotubes 100mg of 10–15 nm o.d, 2–6 nm i.d and 0.1–10 μm length. Elution was carried out with 20 mL dichloromethane. The method was validated in terms of linearity, precision, recovery, accuracy and selectivity. Matrix-matched calibration was carried out for each type of soil since statistical differences between the calibration curves constructed in pure solvent and in the reconstituted soil extract were found for most of the pesticides under study. Recovery values of spiked samples ranged between 56 and 96% for the three types of soils. Limit of detection ranges between 2.97 and 9.49 ngg^{-1}. Using a one-sample test statistical test was made by using Student's *t*-test with fortified samples at two concentration levels in each type of soil, no noteworthy differences were observed between the real and the experimental values accuracy percentages ranged between 87 and 117%.

Navaro et al (48) reported photo degradation of eight pesticides in leaching water at pilot plant scale using the tandem ZnO and $Na_2S_2O_8$ as photosensitizing oxidant and compound parabolic collectors under natural sunlight conditions. The pesticides, belonging to different chemical groups like kresoxim-methyl, hexaconazole, tebuconazole, azoxyxtrobin, apyrimethanil (fungicides), primicarb (insecticide), and propyzamide (herbicide) were used on pepper culture. As expected, the influence of the semiconductor used at concentration of 150 mg L^{-1} andsignificant degradation of pesticides was observed in all cases. Photo catalytic experiments show that the addition of photosensitizes strongly improves the removal of pesticides in comparison with photolytic tests; significantly increasing the reaction rates. The use of $Na_2S_2O_8$ implies a significant reduction in treatment time showing a quicker reaction time than ZnO alone. On the contrary, the addition of H_2O_2 into illuminated ZnO suspensions does not improve the rate of photooxidation. The vanishing of the pesticides followed first-order kinetics according to Langmuir–Hinshelwood model and complete degradation occurs from 60 to 120 min.

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CHAPTER - 3

EXPERIMENTAL RESULT AND DISCUSSION

PART I

INVESTIGATION OF SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF DINITROANILINE GROUP OF HERBICIDES

3.1 INVESTIGATION OF SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF DINITROANILINE GROUP OF HERBICIDES

3.1.1 Method development strategy for spectrophotometric determination of trifluralin

The chemical structure of trifluralin is given below in figure 3.1.1

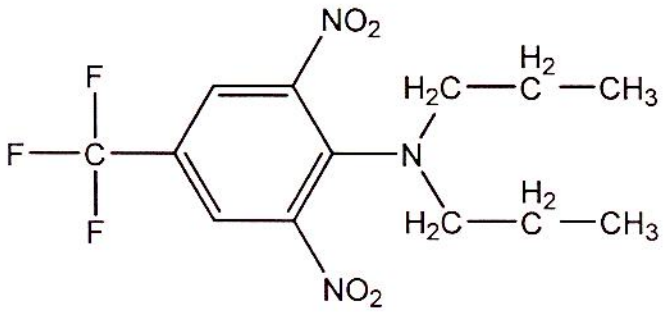


Figure 3.1.1 Chemical structure of trifluralin

The chemical name of trifluralin is 2,6-dinitro-N,Ndipropyle-4-trifluramethyl benzene. Looking in to the structure, it is clear that (2, 6-dinitro-N, N dipropyle-4-trifluramethylbenzene) could be hydrolyzed in the presence of alkali in to 2, 6-dinitro-4- trifluromethyl aniline and isopropanol. The hydrolyzed product 2, 6-dinitro-4-trifluromethyl aniline could be diazotized with nitrite in acidic media. The diazotized product could be coupled with suitable coupling reagent like β-naphthol to give azo dye. The resulting azo dye could be determined by spectrophotometric method and from the dye concentration; the amount of trifluralin could be calculated. The proposed reaction mechanism is given in Figure 3.1.2.

3.1.2. Preliminary investigation of the possibility of hydrolysis of trifluralin and coupling of the hydrolyzed product for spectrophotometric determination of trifluralin

Preliminary studies were conducted to investigate the possible hydrolysis of trifluralin and the subsequent coupling of the hydrolyzed product for the formation of azodye. Initially high concentration of trifluralin (1000 μg mL^{-1}), relatively large volume of hydrochloric acid and

sodium nitrite were used to ensure hydrolysis. With the resultant diazotized product coupling reagents like aniline, p-nitroaniline, sulfanilamide and sulfainlic acid were tried to form the expected azo dye at room temperature. The formation of colored azo dye indicated the possibility of reaction and the subsequent spectrophotometric determination of trifluralin by this method. Further studies were focused on optimization of various parameters and are discussed below.

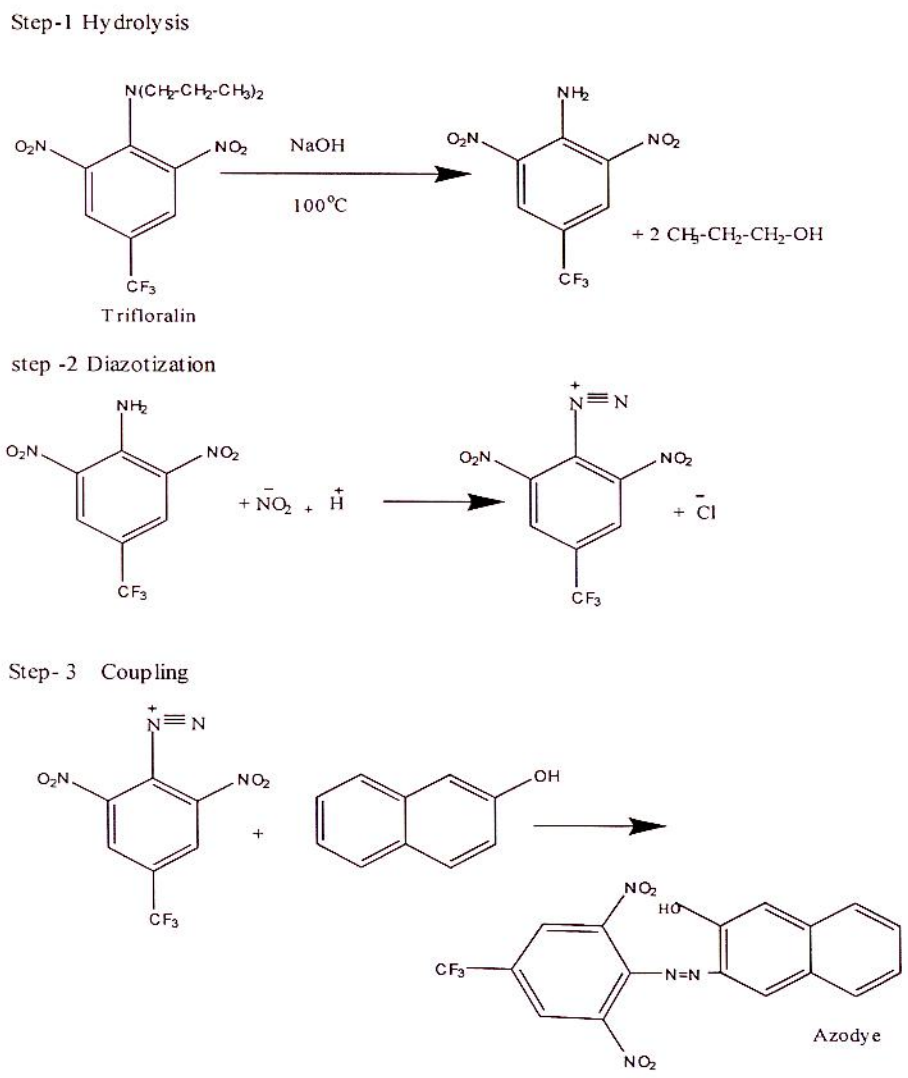


Figure 3.1.2: Proposed reaction mechanism for spectrophotometric determination of trifluralin herbicide

3.1.3: Optimization studies for Spectrophotometric Determination of Trifluralin using Base Hydrolysis

After the preliminary reaction, it was clear that trifluralin could be hydrolyzed and the hydrolyzed product could be determined by spectrophotometric method after coupling with suitable coupling reagent and formation of the azo dye. For quantitative determination it is important that each step of the proposed reaction should go toward completion. For this it was important to optimize various parameters involved in these reactions and determination.

3.1.3.1. Investigation of suitable wavelength for the determination of trifluralin

Instruments

UV/Vis spectrophotometer (UNICO UV-2100 united product and instruments Inc, Dayton, U.S.A) with matched cells was used for all spectral measurements. A pH meter model pH-422 (Wissenschaftlich-Technische Werkstatten, W. Germany) was used for pH measurements.

Reagents and chemicals

All chemicals used were analytical reagent grade or of similar purity. Sodium nitrite, β-naphthol, hydrochloric acid, sodium hydroxide (Merck, Darmstadt, Germany) and distilled ethanol were used during this work. Commercial formulation containing trifluralin was purchased from the local market. Reference standard trifluralin was purchased from Dr. Ehrenstofer GmbH, Germany.

Solutions preparation

i) **β-naphthol solution** (1000 μg mL^{-1}): This solution was prepared by dissolving 0.1 g of β-naphthol in 2 % solution of sodium hydroxide and diluted with the same up to 100 mL.

ii) **Nitrite solution** (2000 μg mL^{-1}): Nitrite solution 2000 μg $mL^{-1}$was prepared by dissolving 0.3 g of sodium nitrite in distilled water and diluted up to 100 mL.

iii) **Sodium hydroxide solution** (2 %): 2 g of NaOH was dissolved in distilled water and diluted up to 100 mL

iv) **HCl solution (M)** 1M solution of HCl was prepared by dilution.

v) **Standard stock trifluralin solution**(1000 μg mL^{-1}): Trifluralin solution, 1000 μg mL^{-1} was prepared by dissolving 0.1 mL of trifluralin in 20 mL of ethanol and diluted up to 100 mL with distill water. From stock solution(1000 μgmL^{-1})100 and 10 μg mL^{-1} trifluralin solutions were prepared by stepwise dilution using distilled water.

Procedure

An aliquot of trifluralin solution containing 2-10 μg mL^{-1} were taken in a titration flasks. To this 10 mL of 2 % sodium hydroxide solution was added. The resulting solutions were heated on a boiling water bath for 10 min to hydrolyze trifluralin. After cooling the solutions were acidified with concentrated hydrochloric acid (37 %) to adjust the molarity up to 3 M in the final solution followed by the addition of 10 mL of 1200 μg mL^{-1} nitrite solution for diazotization. After thoroughly mixing 10 mL of 0.08 % of β-naphthol solution was added for coupling. An azo dye was formed and the solutions were diluted up to 100 mL with distilled water. The absorbance of the resulting dye was measured from 350 to 590 nm using UNICO UV-2100 spectrophotometer for finding out the optimum wavelength. The absorbance was noted with increasing wavelength and each wavelength was calibrated with blank solution. The results are given in table 3.1.1 and are shown in figure 3.1.3

Table 3.1.1: Investigation of suitable wavelength for the spectrophotometric determination of trifluralin

Wavelength(nm)	Absorbance
320	0.523
360	0.753
400	0.873
440	1.232
480	1.332
520	1.543
560	1.643
600	1.342
640	0.932

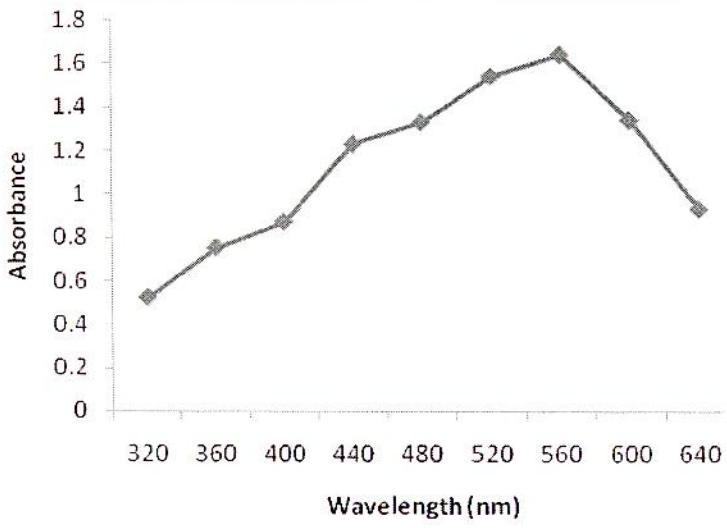


Figure 3.1.3: Investigation of suitable wavelength for the spectrophotometric determination of trifluralin

λ_{max}	Varied
Trifluralin solution	10 μg mL^{-1}
Nitrite solution (1600 μg mL^{-1})	8 mL
β-naphthol solution (1000 μg mL^{-1})	4mL

3.1.3.2: Investigation of the parameters involved in hydrolysis, diazotization and coupling of trifluralin

Instrument: The same as mentioned before

Reagents: The same as above

Solution: The same as mentioned before

Procedure:

Trifluralin standard solution 5mL from 100 μg mL^{-1}stock solution was transferred to six separate 50 mL volumetric flasks. NaOH solution in the range of 0.005-0.04%was added. Solutions were heated on boiling water bath for 10 minutes. After completion of hydrolysis time the contents of the flasks were cooled to room temperature. To each flask 6 mL of nitrite solution (1600 μg mL^{-1}) and 2mL HCl solution was added. To the resulting diazotized product 4mL of β-naphthol (1000μg mL^{-1}) prepared in different concentration of sodium hydroxide ranging from 0.005 to 0.04% was added for finding out the optimum amount of sodium hydroxide. A red colored azo dye was formed in each flask and diluted with distilled water up to the mark. Separate blank solutions were prepared using the same procedure without the addition of trifluralin. The absorbance of the azo dye was measured at 550 nm. The results are given in table 3.1.2 and are shown in figure 3.1.4.

For optimization of nitrite concentration, to each of the five flasks 5mL of different concentration of nitrite in the range of 500 to 2500 μg mL^{-1}were added and color was developed in the same way as mentioned before. The results are given in table 3.1.3 and are shown in figure 3.1.5. Similarly for finding optimum volume of nitrite solution varied volume of nitrite (1-8 mL) of 1600 μg mL^{-1} was used and absorbance for each solution was measured. The results are given in tables 3.1.4 and are shown in figure 3.1.6.

For diazotization the amount of acid was optimized. For this purpose 8mL of nitrite solution (1600 μg mL^{-1}) prepared in different concentration of hydrochloric acid (0.2-0.8 M) were used keeping the rest of the procedure same. The results are given in table 3.1.5 and are shown in figure 3.1.7.

Similarly for optimization of the amount of β-naphthol 4mL of β-naphthol from its solution of varying concentration (500-8000 μg mL^{-1}) were used, while keeping the rest of the procedure the same. The results are given in table 3.1.6 and are shown in figure 3.1.8. Similarly for finding optimum volume of β-naphthol varied volume of β-naphthol (1-8 mL) of 1000 μg mL^{-1} β-naphthol was used and absorbance were measured and the results are given in table 3.1.7 and are shown in figure 3.1.9.

Table 3.1.2: Optimization of sodium hydroxide concentration for the maximum formation of azo dye

S.No	Sodium hydroxide (%)	Absorbance
1	0.005	0.211
2	0.01	0.432
3	0.015	0.543
4	0.02	1.121
5	0.025	0.832
6	0.03	0.435

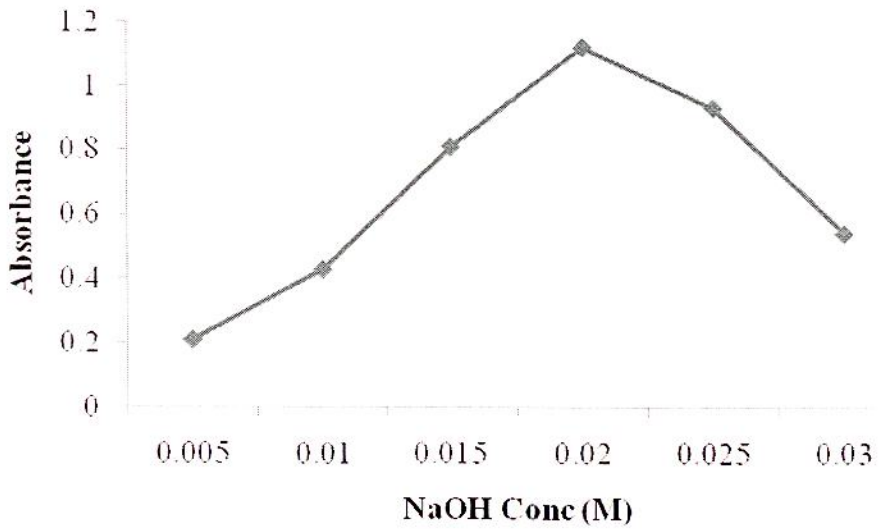


Figure 3.1.4: Optimization of sodium hydroxide concentration for the maximum formation of azo dye

Table 3.1.3: Investigation of the effect of nitrite concentration on the diazotization of trifluralin

S.NO	Nitrite conc.(µg mL^{-1})	Absorbance
1	200	0.411
2	400	0.452
3	600	0.643
4	800	0.844
5	1000	1.13
6	1200	1.823
7	1400	1.34
8	1600	1.21

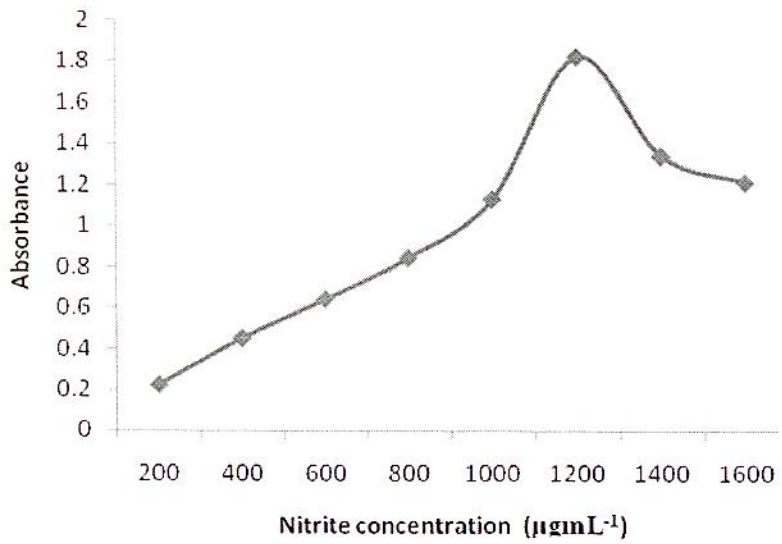


Figure 3.1.5: Investigation of the effect of nitrite concentration on the diazotization of trifluralin

Table 3.1.4: Investigation of the effect of volume of nitrite solution on the diazotization of trifluralin.

S.No	Nitrite volume (mL)	Absorbance
1	2	1.12
2	3	1.32
3	4	1.41
4	5	1.53
5	6	1.64
6	7	1.67
7	8	1.68

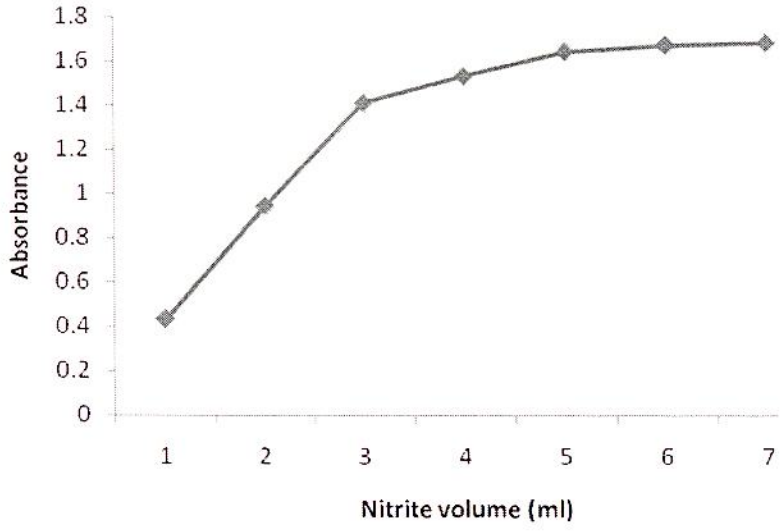


Figure 3.1.6: Investigation of the effect of volume of nitrite solution on the diazotization of trifluralin

Table 3.1.5: Investigation of the effect of acidity on the absorbance behavior of the azo dye

S.No	Concentration of HCl(M)	Absorbance
1	0.20	0.175
2	0.30	0.275
3	0.40	0.421
4	0.60	0.304
5	0.80	0.175

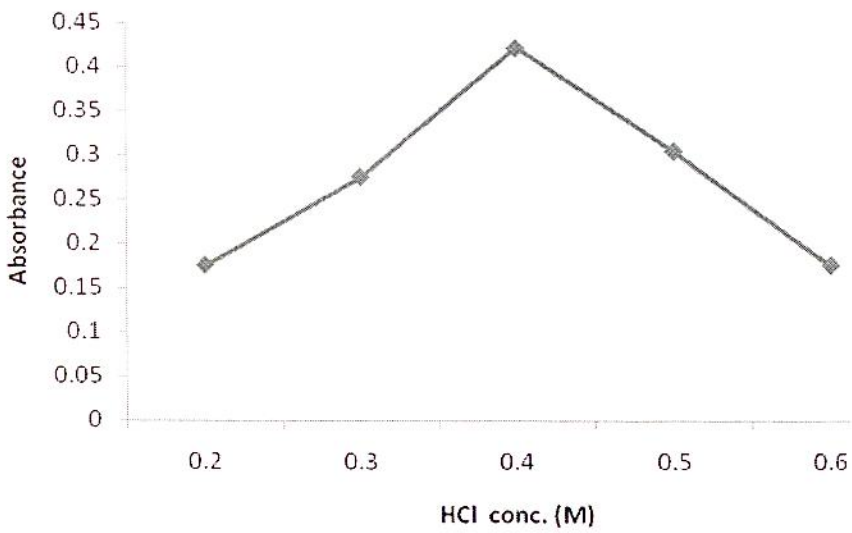


Figure 3.1.7 Investigation of the effect of HCl concentration on absorbance behavior of the azo dye

Table 3.1.6: Optimization of β-naphthol concentration for the maximum formation of azodye

S.No	β-naphthol conc.(μg mL^{-1})	Absorbance
1	500	0.213
2	1000	0.272
3	2000	1.05
4	3000	1.345
5	4000	1.45
6	5000	1.763
7	6000	1.921
8	7000	1.999
9	8000	1.999

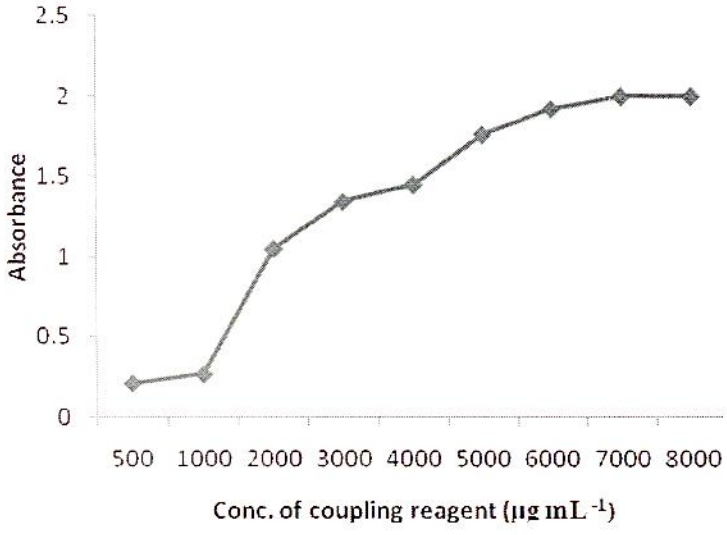


Figure 3.1.8 Optimization of β-naphthol concentration for the maximum formation of azodye

Table 3.1.7.Optimization of β-Naphthol volume for the maximum formation of azo dye

S.No	volume of β-Naphthol	Absorbance
1	2	0.563
2	3	0.872
3	4	1.752
4	5	1.432
5	6	1.032
6	7	0.94

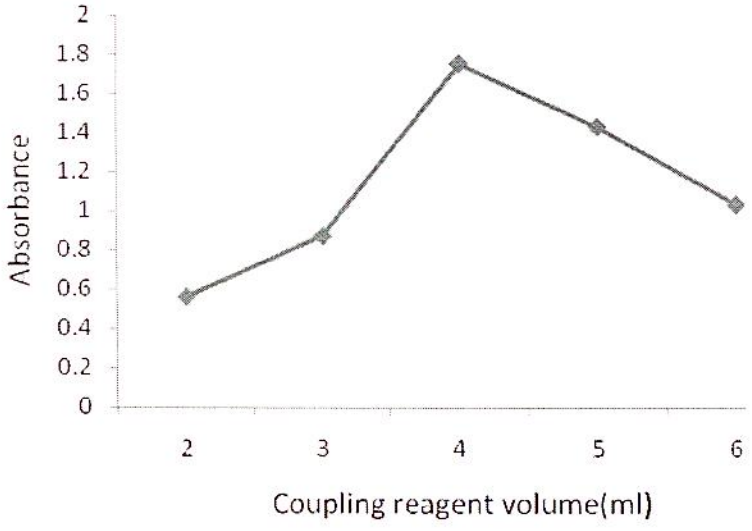


Figure 3.1.9 Optimization of β-naphthol volume for the maximum formation of azo dye

3.1.3.3 The factorial design and optimization for hydrolysis and diazotization

The relative importance of different variables and their interaction with each other for hydrolysis of trifluralin and diazotization were studied using a 2^3 factorial design. The low and high values of the factors were selected according to the studied factors. For hydrolysis of trifluralin sodium hydroxide concentration, temperature and heating time were studied. The concentration of hydrochloric acid, nitrite and β-naphthol were studied for diazotization and coupling reaction. Results for the ANOVA test are shown in table 3.1 .8 and 3.1.9. The most important effects correspond to factor C (temperature) and the BC interaction (temperature and heating time interaction) for hydrolysis of trifluralin and factor A (hydrochloric acid molarities) and the ABC interaction (HCl, nitrite and β-naphthol concentration interaction) for diazotization reaction.

The constant of the empirical model calculated from Table 3.1.8 is 1.96 which is in agreement for higher response. The magnitude of these effects, analysis of variance (ANOVA) was carried out by using Expert Design software (Stat-Ease factorial design) and results are summarized in Table 3.1.9. The results confirm that B and C are statistically the main factors and are equally significant. These effects show p-values less than 0.05 both for hydrolysis as well as for diazotization reaction which indicates that the results are statistically significant at a 95.0 % confidence level.

The results are also shown in the Pareto chart which is a vertical bar-chart for hydrolysis and diazotization reaction of trifluralin (Fig 3.1.10 and 3.1.11)

Table 3.1.8 Results of the ANOVA test for hydrolysis of trifluralin herbicide

Source	**Sum of squares**	**df**	**Mean square**	**F value**	**P value Prob > F**	
Model						
A-NaOH	0.15	3	0.49	26.11	0.0044	significant
B-Heating time	0.033	1	0.033	17.34	0.0141	
C- heating temp	0.096	1	0.096	50.85	0.0020	
AC	0.019	1	0.019	10.15	0.0333	
Residuals	7.52×10^{-3}	4	1.88×10^{-3}	_	_	
Cor. total	0.15	7	_	_	_	

Table 3.1.9 Results of the ANOVA test for Diazotization reaction

Source	Sum of squares	df	Mean square	F value	P value Prob > F	
Model	4.58	5	0.92	868.96	0.0011	significant
A-HCl	0.46	1	0.46	434.36	0.0023	
B-sodium nitrite	3.74	1	3.74	3547.6	0.0003	
C- β-naphthol	0.054	1	0.054	51.65	0.0188	
AB	0.29	1	0.29	278.28	0.0036	
BC	0.035	1	0.035	32.80	0.0292	
Residuals	2.10×10^{-3}	2	1.05×10^{-3}	–	–	
Cor total	4.58	7	–	–	–	

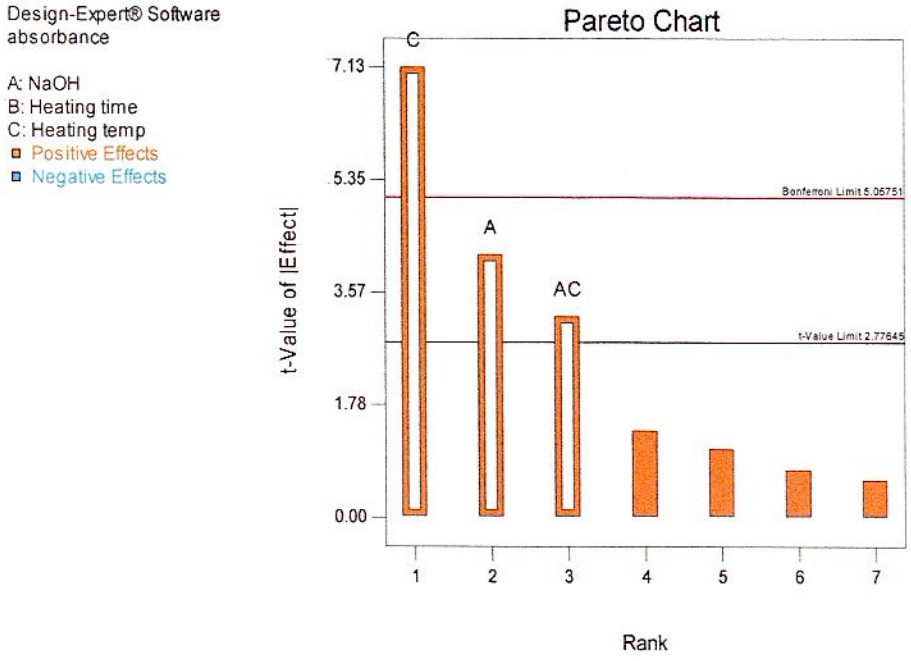


Figure 3.1.10 Standardized Pareto charts for hydrolysis reaction

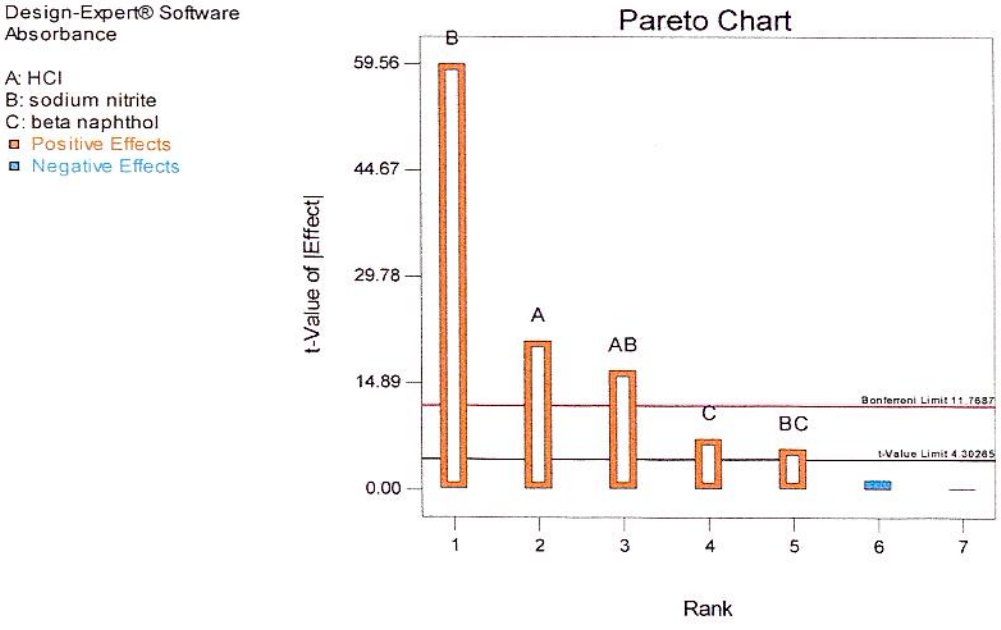


Figure 3.1.11 Standardized Pareto chart for diazotization reaction

3.1.3.4: Verifications of Beer's law and determination of the limit of detection and quantification of trifluralin

Instrument: The same as mentioned before

Reagents: The same as above

Solution: The same as mentioned before

Procedure:

From standard stock solution of trifluralin solution (100 μg mL^{-1}) different volume for 0.5 to 20 μg $mL^{-1}$was taken in 50 mL volumetric flasks. To each flask optimum volume of 1600 μg mL^{-1} nitrite solution(8 mL) was added and mixed well. To the resulting diazotized solution, optimum volume (4mL) of β–naphthol solution (1200 μg mL^{-1}) was added for the formation of azo dye. The resulting azo dye was diluted up to the mark with distilled water. Blank solution was prepared in the same way without the addition of trifluralin. The absorbance of the resulting azodye was measured at 550 nm.The results are given in table 3.1.10. A calibration plot of absorbance vs concentration was constructed and is shown in figures 3.1.12 and 3.1.13. The Molar absorptivity was calculated by using the formula

Molar absorptivity (ε) = A/ C x L

Where A= absorbance

C= Concentration (mol L^{-1})

L=path length (centimeter)

The calculated value is of 1.5×10^4 L mol^{-1} cm^{-1}

LOD and LOQ were calculated for five replicates of the lowest quantifiable concentration 0.5 μg mL^{-1}solution by using the following formula.

Limit of Detection (LOD) (for concentration) = 3 xS/ b

Limit of Quantification (LOQ) (for concentration) = 10 x S/ b

Where S = Standard Deviation

Relative standard deviation (RSD) was calculated by using the following formula.

RSD = Standard deviation /Mean x 100

Table 3.1.10 Investigation of the effect of concentration on absorbance behavior of trifluralin

Trifluralin conc. ($\mu g\ mL^{-1}$)	Absorbance	Trifluralin conc. ($\mu g\ mL^{-1}$)	Absorbance
0.2	0.008	2.0	0.532
0.3	0.041	3.0	0.654
0.4	0.082	4.0	0.723
0.5	0.123	5.0	0.843
0.6	0.183	6.0	0.993
0.7	0.234	7.0	1.231
0.8	0.321	8.0	1.283
0.9	0.372	9.0	1.352
1	0.421	10	1.781

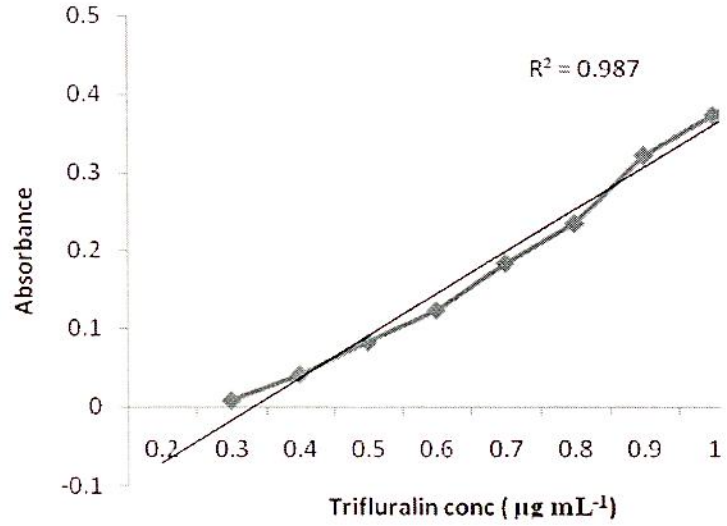


Figure 3.1.12 Investigation of the effect of concentration at low level on absorbance behavior of trifluralin

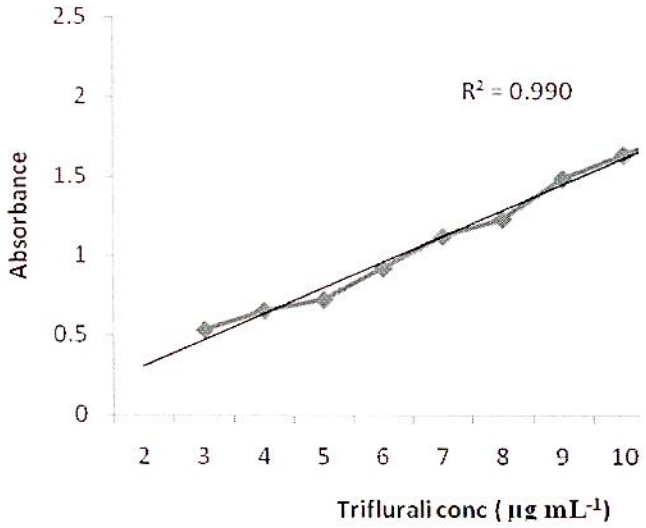


Figure 3.1.13 Investigation of the effect of concentration at high level on absorbance behavior of trifluralin

3.1.4 Percent recovery of Trifluralin from Real Samples and Commercial Formulation by using the Investigated Method

A recovery test was performed on control samples, fortified with known concentration of trifluralin solution. The solvent was evaporated at room temperature for 1 hr.

Sample extraction procedure

The vegetable samples under study were first washed properly with cold water and then with hot water in order to remove adhering dust and dirt. The vegetables samples were then peeled, chopped and were weighed in the range of 5, 10 and 15 g. To each portion different concentrations of trifluralin from 100 μgmL^{-1} stock solution and 20 mL ethanol was added and was kept for 6 hrs. After absorption the samples were shaken on shaker for 15 min and on equilibration the sample was filtered and the filtrate was evaporated on rotary evaporator up to volume of 2 mL.

The reduced volume of filtrate was preceded by the proposed method.

Results of percent recovery of trifluralin in real samples and formulation are given in table 3.1.11. Percent recovery was calculated by using formula given below.

% Recovery = µg found in fortified samples –µg found in sample / µg of standard added x 100

Table 3.1.11 Percent recovery of herbicide from real sample

S.NO	Samples	Recovery (%)
1	Onion	96.2 ± 1.24
2	Garlic	97.1 ± 2.18
3	Cabbage	95.43± 2.26
4	Potato	85.32± 3.18

3.1.5 Application of the Investigated Methods Method for the Analysis of Trifluralin in Real Samples and Commercial Formulation.

Instrument: The same as mentioned before

Samples: Vegetables samples (onion and garlic) were collected from agriculture fields, where trifluralin had been sprayed. Other vegetables cabbage and potato purchased from market. The extraction procedure was exactly the same as for sample. Six replicate of the samples were analyzed by the developed method. For analysis in commercial formulation 4 mL of trifluralin formulation was taken in four separate 50 mL volumetric flasks and dissolved in 25 mL ethanol and diluted up to mark with distilled water. From each solution 5mL were taken in four separate 50 mL volumetric flasks and color was developed in the same way as mentioned before the absorbance was measured at 550 nm.The results are shown in Table 3.1.12 and for commercial formulation in Table 3.1.13.

Table 3.1.12 Residue level of trifluralin in vegetable samples

S. No	Sample	Residue ($\mu g g^{-1}$)
1	Onion	1.35 ± 0.13
2	Garlic	1.05 ± 0.20
3	Cabbage	1.12± 0.22
4	Potato	1.32 ± 0.16

Table 3.1.13 Concentration of trifluralin in commercial formulation

Commercial formulation	Label Amount (gmL^{-1})	Batch Analysis (g mL^{-1})
Active ingredient	0.5 ± 0.46	0.42 ± 0.38

3.1.5 Result and Discussion

Trifluralin (2, 4-dinitro N-Ndiprpyle-4-trifluromethyl) was hydrolyzed in the presence of alkali in to 2, 6- dinitro-4- trifluromethyl aniline and isopropanol. The hydrolyzed product 2, 6-dinitro-4 trifluromethyl aniline was diazotized with nitrite in acidic media followed by coupling of the diazonium salt with β-naphthol. The resultant red colored azo dye was measured at 550 nm. The proposed reaction mechanism is given in Figure 3.1.2.

Hydrolysis of trifluralin to 2, 6-dinitro –4-trifluroanaline was studied at different concentrations of sodium hydroxide solution, temperature and heating time. It was observed that alkaline conditions at 100^0C were required for the hydrolysis of trifluralin (Fig 3.1.4). Maximum hydrolysis was observed with 0.025 M sodium hydroxide for 10 min heating on boiling water bath (100^0C).

Nitrite concentration for diazotization was optimized in the range of 200 μg mL^{-1}to1600 μg mL^{-1} (Fig 3.1.5.) Maximum absorbance was observed with 1200 μg mL^{-1} nitrite concentration as it gave good stability and quantitative results. The effect of acidity on the diazotization reaction was studied and it was observed that constant absorbance values were obtained at the concentration range of 3.0 to 4.0 M hydrochloric acid solution (Fig 3.1.7). Therefore, in further analyses the concentration of hydrochloric acid in solutions was adjusted at 0.3 M. The diazonium salt was coupled with β-naphthol solution to form an azo dye. The absorbance value increased with increase in concentration from 0.02 to 0.08 % of β-naphthol solution and remained constant up to 0.12% concentration (Fig 3.1.8).

All spectral measurements were carried out against reagent blank. The Beer's law was obeyed by the azo dye formed in the range of 0.2-17 μg mL^{-1} of trifluralin. The molar absorptivity was found to be $1.5x10^4$ L mol^{-1} cm^{-1}. The limit of detection (3s/b) and quantification (10s/b) were 0.15 μg mL^{-1} and 0.5 μg mL^{-1} respectively.

Precision of the method was checked by the replicate analysis of working standard solution. A recovery test was performed on the same sample after residue determination and fortified with known concentration of trifluralin solution. Six replicates of the fortified samples were analyzed using the proposed method. The results are given in Table 3.1.11. The average recoveries were found in the range of 96 -97% in all cases.

Vegetables samples (onion, garlic, cabbage and potato) were collected from agriculture fields, where trifluralin had been sprayed. The method was applied for residue determination in these samples. The values for trifluralin found in onion, garlic, cabbage and potato were found to be 1.35 ± 0.13, 1.05 ± 0.20, 1.12 ±0.02 and 1.32 ±0.16 $\mu g g^{-1}$ respectively (Table 3.1.12). The proposed method was also applied to check the purity of commercially available trifluralin herbicide. The results are given in Table 3.1.13. From the results it can be seen that they are in good agreement with the label value.

3.2 INVESTIGATION OF SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF PENDIMETHALIN

The chemical structure of pendimethalin is given below in figure 3.2.1

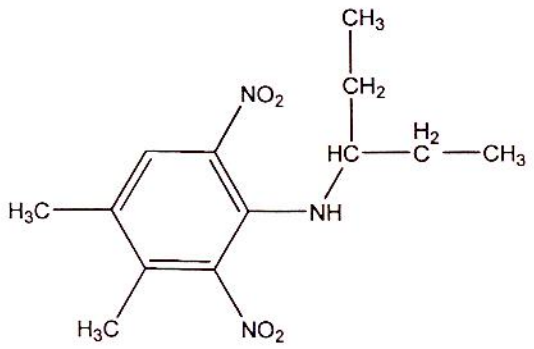


Figure 3.2.1 Chemical structure of pendimethalin

3.2.1 Method development strategy for the spectrophotometric determination of pendimethalin

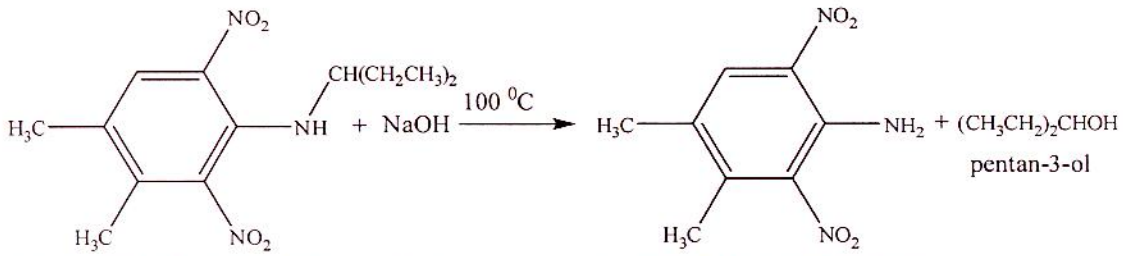
The chemical name of pendimethalin is N- (1-ethylpropyl)-2, 6- dinitro-3–4 xylidine. It is a herbicide of dinitroaniline group. It gives 3, 4-dimethyl-2,6-dinitro aniline on alkaline hydrolysis. The hydrolyzed substituted aniline when reacted with nitrite for diazotization and aniline for coupling, an orange color azo dye was formed. The proposed reaction mechanism is given in the figure 3.2.2.

3.2.2 Preliminary investigation of the hydrolysis and coupling for spectrophotometric determination of pendimethalin

Preliminary studies were conducted to investigate the possible hydrolysis of pendimethalin and the subsequent coupling of the hydrolyzed product for the formation of azodye. Initially high concentration of pendimethalin (1000 $\mu g\ mL^{-1}$), relatively large volume of sodium hydroxide,

sodium nitrite were used to ensure hydrolysis. With the resulting diazotized product coupling reagents like aniline and β-naphthol were tried to form the expected azo dye at room temperature. The formation of colored azo dye indicated the possibility of reaction and the subsequent spectrophotometric determination of pendimethalin by this method. Further studies were focused on optimization of various parameters and are discussed below.

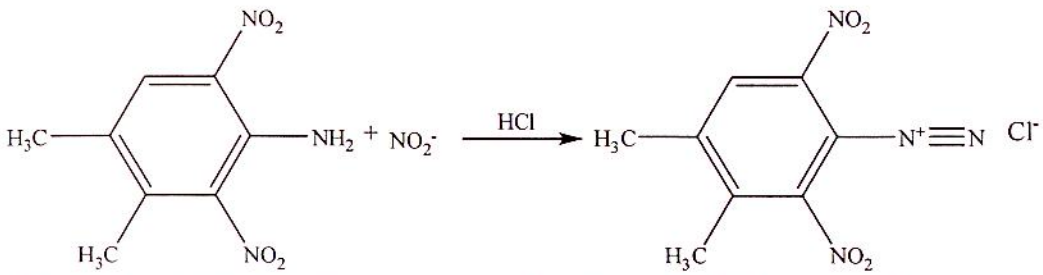
Step 1:



3,4-dimethyl-2,6-dinitro-*N*-(pentan-3-yl)aniline
Pendimethaline

3,4-dimethyl-2,6-dinitroaniline

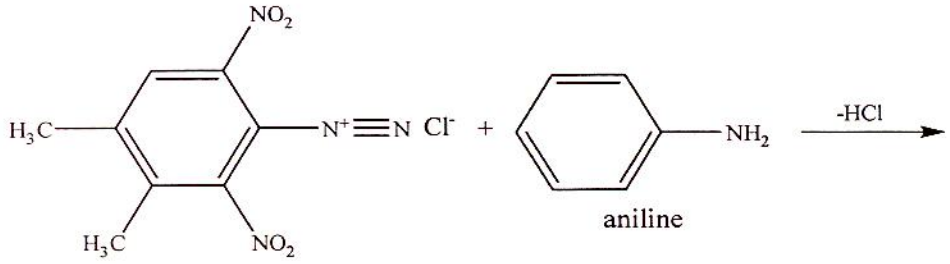
Step2:



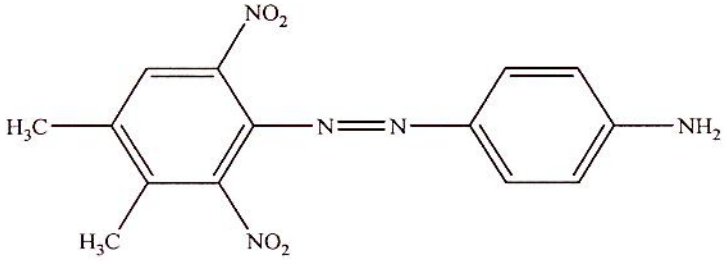
3,4-dimethyl-2,6-dinitroaniline

3,4-dimethyl-2,6-dinitrobenzenediazonium chloride

Step 3:



3,4-dimethyl-2,6-dinitrobenzenediazonium chloride



4-((3,4-dimethyl-2,6-dinitrophenyl)diazenyl)aniline

Azo Dye

Figure 3.2.2 Proposed reaction mechanism for spectrophotometric determination of pendimethalin

3.2.3 Optimization Studies for the Spectrophotometric Determination of Pendimthalin

After the preliminary reaction, it was clear that pendimethalin could be hydrolyzed and the hydrolyzed product could be determined by spectrophotometric method after coupling with suitable coupling reagent and formation of the azo dye. For quantitative determination it is important that each step of the proposed reaction should go toward completion. For this it was important to optimize various parameters involved in these reactions and determination.

3.2.3.1 Wavelength optimization for spectrophotometric determination of pendimethalin

Instruments

UV/Vis spectrophotometer (UNICO UV-2100 united product and instruments Inc, Dayton, U.S.A) with matched cells was used for all spectral measurements. A WTW pH meter model pH-422 (Wissenschaftlich-Technische Werkstatten, W. Germany) was used for pH measurements.

Reagents and chemicals

All chemicals used were of analytical reagent grade or of similar purity. Sodium nitrite, hydrochloric acid, sodium hydroxide and aniline (Merck, Darmstadt, Germany) and distilled ethanol were used during this work. Commercial formulation containing pendimethalin was purchased from the local market. Reference standard pendimethalin was purchased from Dr. Ehrenstofer GmbH, Germany.

Solutions Preparation

i) **Pendimethalin (1000 µg mL^{-1}):** 0.1g of pendimethalin was dissolved in 1000 mL volumetric flask and diluted up to mark with acetic acid. 100 µg L^{-1} solution was prepared by dilution formula from the stock solution.

ii) **NaOH (2 %):** 2 g of NaOH was dissolved in distilled water and diluted up to 100 mL with distilled water in volumetric flask.

iii) **HCl (1M)**: Concentrated HCl solution was used.

iv) **Nitrite (1000 µg mL^{-1}):** 0.15 g of sodium nitrite was dissolved in distilled water and diluted up to 100 mL with distilled water in volumetric flask.

v) **Aniline (2%):** 2mL of aniline was taken in volumetric flask (100 mL) and diluted up to mark with 50% ethanol.

Procedure

5 mL (100µg mL^{-1}) pendimethalin solution from the stock solution was taken in a conical flask. 5 mL NaOH solution (2%) was added to it, followed by heating on boiling water bath for 30 minutes. The content of the flask was cooled to room temperature and transferred to volumetric flask. 5 mL nitrite solution (1000µg mL^{-1}) was added to it, followed by the addition of 1mL HCl (conc.) and 5 mL aniline solution (2%). Reddish color azo dye appeared in the solution. 10 mL ethanol was added to obtain a clear solution. Finally the solution was diluted up to 50 mL with

distilled water. The absorbance of the resultant colored solution was measured with increasing wavelength and each wavelength was calibrated with blank solution. From 360-540 nm using UNICO UV-2100 spectrophotometer for finding out the optimum wavelength. The results are given in the table 3.2.1 and shown in fig 3.2. 3.

Table 3.2.1 Optimization of wavelength for spectrophotometric determination of pendimethalin

Wavelength (nm)	Absorbance	Wavelength (nm)	Absorbance
360	1.563	460	1.981
380	1.714	480	1.960
400	1.818	500	1.672
420	1.832	520	1.211
440	1.865	540	0.982

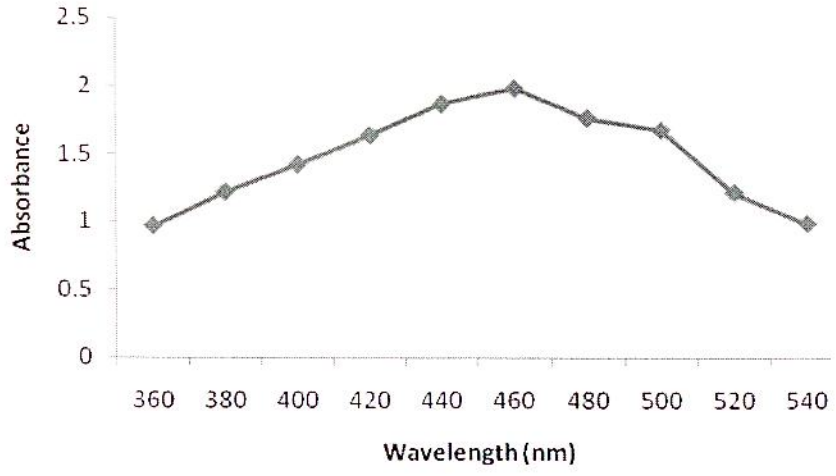


Figure 3.2.3 Optimization of suitable wavelength for spectrophotometric determination of pendimethalin

Conditions:

Wavelength	varied
Pendimethalin solution	10 $\mu g\ mL^{-1}$
Heating time	10 min
Diazotized reagent	5mL

3.2.3.2 Investigation of suitable diazotizing reagent for spectrophotometric determination of pendimethalin

Instrument: the same as mentioned before

Reagents: The same as mentioned before

Solutions:

Analytical Reagent grade purity aniline and β-naphthol were used as diazotizing reagent. Stock solution of each diazotizing reagent was prepared in four separate volumetric flasks and diazotized with nitrite solution in 1:5 ratios respectively. These stock solutions were used for further investigation.

Procedure:

From stock solution of each diazotized reagent, 5mL was taken in a 50 mL volumetric flask and 5 mL of pendimethalin solution from 100 μgmL^{-1} hydrolyzed stock solution was added to it. A red colored azo dye was formed. Separate blank solution was prepared in the same way without the addition of pendimethalin. The absorbance of the resulting azodye was measured at optimum wavelength (460nm) using UNICO-UV-2100spectrophotometer for finding out maximum absorption. The results are given in table 3.2.2 and are shown in figure 3.2.4

Table 3.2.2 Investigation of suitable diazotizing reagent for spectrophotometric determination of pendimethalin

S.NO	diazotizing reagent	Absorbance
1	β-naphthol	0.873
2	Aniline	1.421

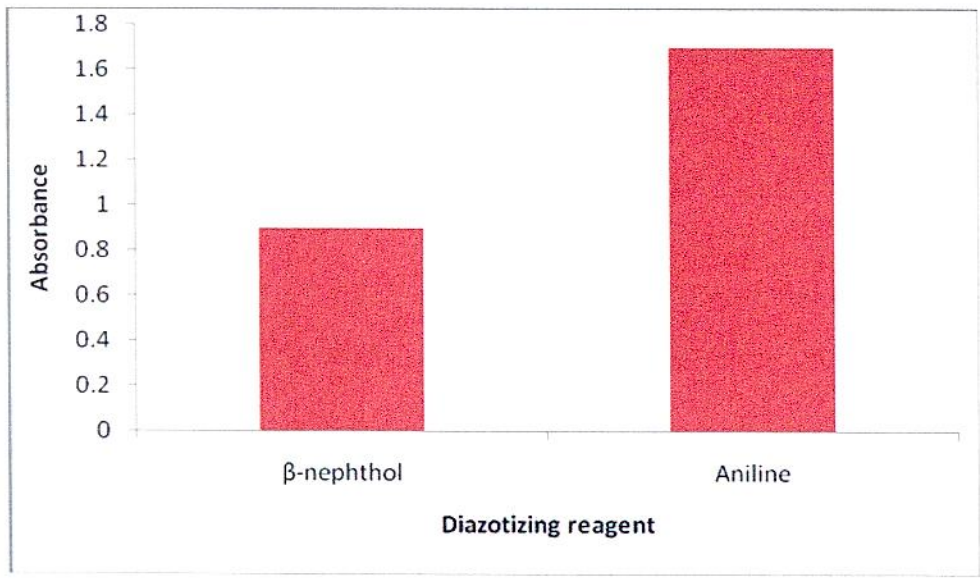


Figure 3.2.4 Investigation of suitable diazotizing reagent for spectrophotometric determination of pendimethalin

Conditions:

Wavelength	460 nm
Pendimethalin solution	10 μg mL^{-1}
Diazotized reagent	5mL

3.2.3.3 Optimization of hydrolysis time for the alkaline hydrolysis of pendimethalin

Instrument: The same as mentioned before.

Reagents: The same as mentioned before.

Solutions: The same as mentioned before.

Procedure:

From 100 μg mL^{-1} stock solution of pendimethalin 5 mL was taken in different titration flasks. To each flask 5 mL NaOH (2 M) solution was added and heated on boiling water bath for hydrolysis in the range of 10-50 minutes. After completion of hydrolysis time, the contents of the flasks were cooled to room temperature and transferred to volumetric flasks. 5 mL nitrite solution (100μg mL^{-1}) was added, followed by the addition of 1 ml HCI solution and 5 mL of aniline solution (2 %). All the solutions were diluted up to 50 mL with distilled water. The absorbance of solution was measured at 460 nm against blanks (synthetic) using UNICO-UV-2100 spectrophotometer. The results are given in the table 3.2.3 and are shown in figure 3.2.5

Table 3.2.3 Optimization of heating time for the hydrolysis of Pendimethalin

S.NO	Heating time (min)	Absorbance
1	5	1.510
2	10	1.584
3	20	1.480
4	30	1.412
5	40	1.387
6	50	1.387

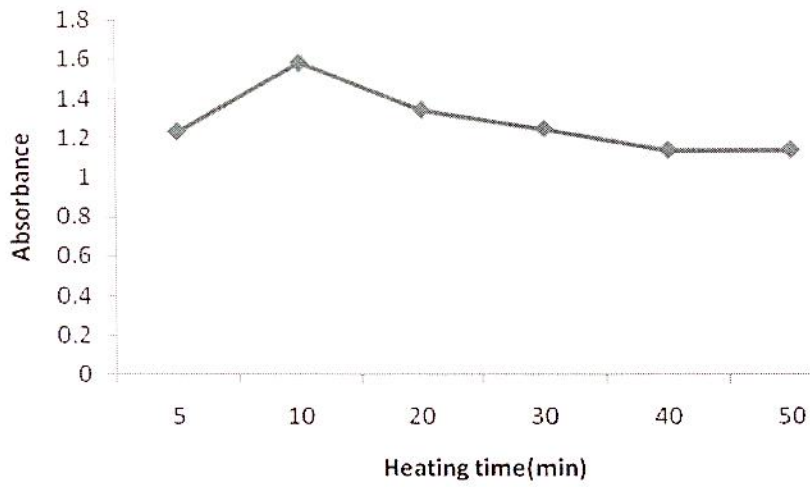


Figure 3.2.5 Heating time optimization for pendimethalin hydrolysis

3.2.3.4 Investigation of the parameters involved in hydrolysis, diazotization and coupling of pendimethalin.

For optimization ofvolume of NaOH solution required for hydrolysis of pendimethalin 5mL from 100 μg mL^{-1}stock solution of pendimethain was taken in different titration flasks. NaOH solution (2 %) in the range of 1-5 mL was added to each flask. The solutions were heated on boiling water bath for 10 minutes. After completion of hydrolysis, the contents of the flasks were cooled to room temperature. 5mL of nitrite solution was added in each flask for diazotization reaction, followed by the addition of 1mL HCl and 5 mL aniline solutions. The solutions were diluted up to 100 mL with distilled water. The absorbance of solutions were measured against blanks at 460 nm using UNICO-UV-2100 spectrophotometer the results are given in the table 3.2.4 and shown diagrammatically in figure 3.2.6.

Similarly for optimization of nitrite concentration (1000 μg mL^{-1}) volume for diazotization of hydrolyzed pendimethalin 5 mL of pendimethalin from the 100 μg mL^{-1} stock solution was taken in different titration flasks. 5 mL NaOH solutions was added to each flask followed by heating on boiling water bath for 10 minutes. After cooling, nitrite solution in the range of 2-12 ml was added for diazotization reaction. Then 1 mL HCl was added, followed by addition of 5 mL of aniline solutions. The solutions were transferred to volumetric flasks and diluted up to 50 mL with distilled water. The absorbance was measured at 460 nm using spectrophometer. The results are given in the table 3.2.5 and are shown in figure 3.2.7.

For optimization of HCl solution concentration for diazotization of hydrolyzed pendimethalin. Different concentration in the range of 0.1-0.7 M HCl solutions were used, keeping the rest of the procedure same. The results are given in the table 3.2.6 and are shown in figure 3.2.8.

Optimization of aniline concentration for coupling of diazotized pendimethalin stock solution i.e. from 0.5- 3% with 2% nitrite solution in 1:16 ratio. From each of these diazotized reagent solution 5 mL was taken in a 50 mL volumetric flask and color was developed in the same way as mentioned before. The results are given in table 3.2.7 and are shown in figure 3.2.9. Similarly for Optimization of aniline solution volume for coupling of diazotized reagent aniline solution (2 %) was added in the range of 1-12 mL for coupling with diazotized product. The absorbance was measured at 460 nm. The results are given in the table 3.2.8 and are shown graphically in fig 3.2.10.

To investigate the effect of time on the azodye, from 100 µg mL^{-1}stock solution of pendimethalin, 5 mL was taken in a titration flask. Color was developed in the same way as mentioned before. The solutions was transferred to volumetric flask and diluted up to 50 mL with distilled water. The absorbance was measured at 460 nm using spectrophotometer with different time interval. The results are given in the table 3.2.9 and are shown in fig 3.2.11.

Table 3.2.4 Optimization of sodium hydroxide solutions volume for spectrophotometer determination of pendimethalin

S.NO	NaOH Volume (mL)	Absorbance
1	1	0.232
2	2	0.265
3	4	0.453
4	6	0.892
5	8	1.563
6	10	1.563

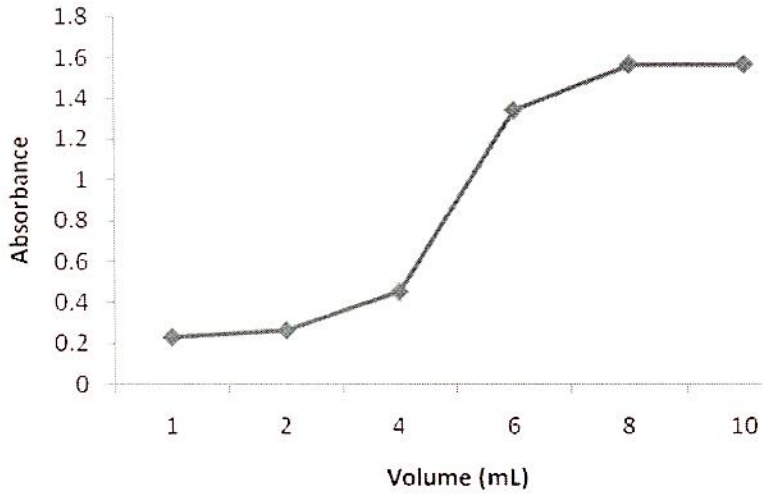


Figure 3.2.6 Optimization of NaOH solution (2M) volume for spectrophotometric determination of pendimethalin

Table 3.2.5 Optimization of nitrite concentration on the diazotization of hydrolyzed pendimethalin

S.NO	Nitrite conc. (%)	Absorbance
1	0.05	0.38
2	0.10	0.82
3	0.15	1.12
4	0.20	1.64
5	0.25	1.62
6	0.30	1.62

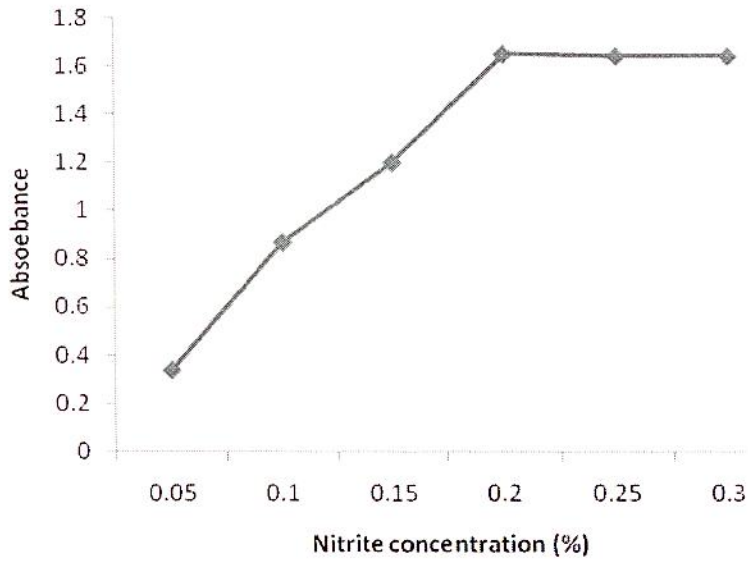


Figure 3.2.7 Optimization of nitrite concentration on the diazotization of hydrolyzed pendimethalin

Table 3.2.6 Optimization of nitrite solution volume for diazotization of hydrolyzed pendimethalin

S.NO	Nitrite volume (mL)	Absorbance
1	2	0.168
2	4	0.172
3	6	0.179
4	8	0.183
5	10	0.134
6	12	0.114

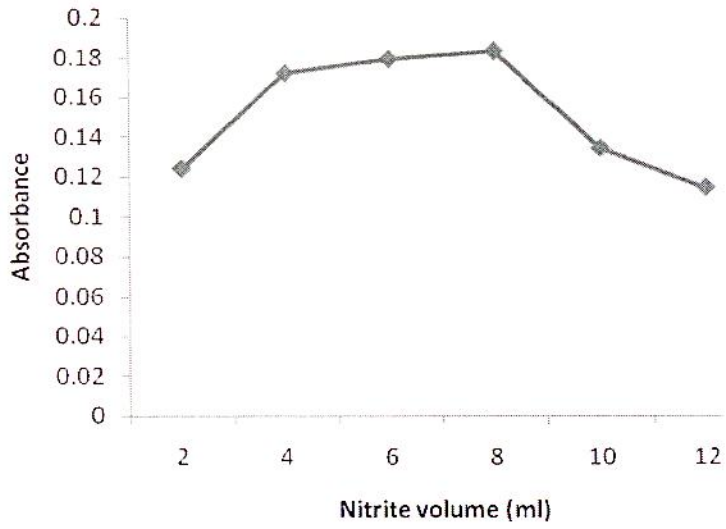


Figure 3.2.8 Optimization of nitrite solution (1000µg mL^{-1}) volume for diazotization of hydrolyzed pendimethalin

Table 3.2.7 **Optimization of HCl solution concentration for diazotization of hydrolyzed pendimethalin**

S.NO	Concentration(M)	Absorbance
1	0.1	1.322
2	0.2	1.475
3	0.3	1.523
4	0.4	1.534
5	0.5	1.534
6	0.6	1.534
7	0.7	1.534

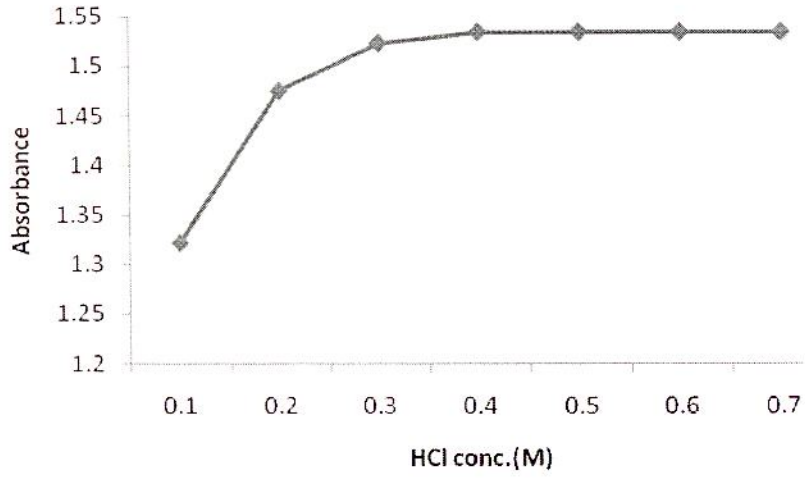


Figure 3.2.9 Optimization of HCl solution concentration for diazotization of hydrolyzed pendimethalin.

Table 3.2.8 Investigation of the effect of aniline concentration on the absorbance behavior of the azo dye

S.NO	Aniline conc. (%)	Absorbance
1	0.5	1.121
2	1.0	1.552
3	1.5	1.583
4	2.0	1.921
5	2.5	1.574
6	3	1.563

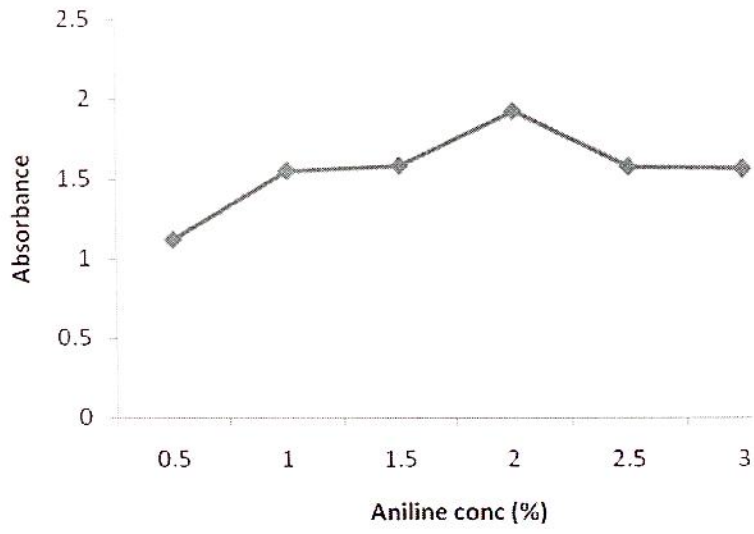


Figure 3.2.10 Optimization of aniline concentration for coupling of diazotized Pendimethalin

Table 3.2.9 Optimization of aniline solution (2%) volume for coupling of diazotized Pendimethalin

S.NO	Aniline volume(ml)	Absorbance
1	2	0.342
2	4	0.921
3	6	1.211
4	8	1.933
5	10	0.631
6	12	0.521

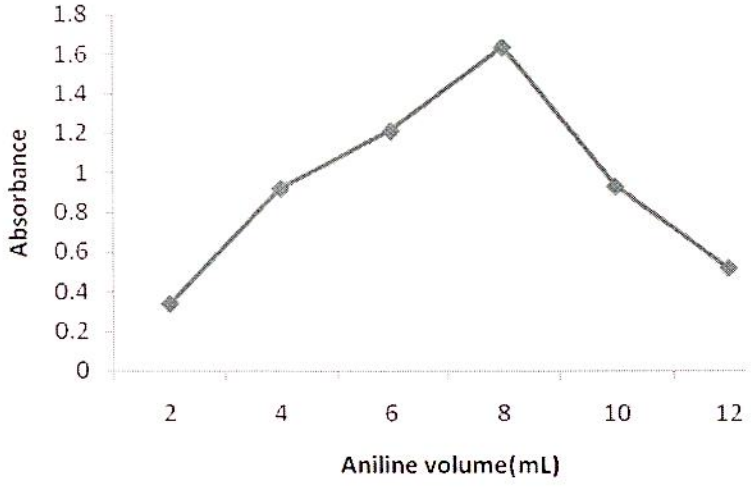


Figure 3.2.11 Optimization of aniline solution (2%) volume for coupling of diazotized Pendimethalin

Table 3.2.10 Effect of time on the formation azo dye

S.NO	Time (min)	Absorbance
1	5	1.460
2	10	1.680
3	15	1.580
4	20	1.999
5	25	1.999
6	30	1.999

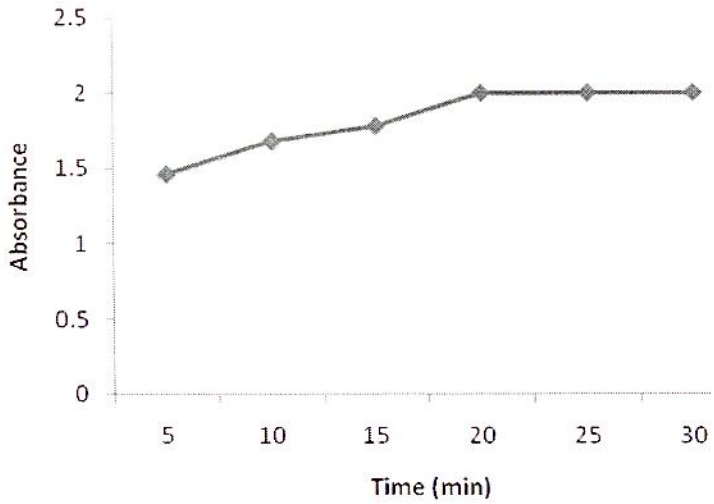


Figure 3.2.12 Investigation of the effect of time on the formation of azo dye

3.2.3.5 Verifications of Beer's law and determination of the limit of detection and quantification of pendimethalin by batch analysis

Instrument the same as motioned before

Reagents the same as mentioned before

Solutions the same as mentioned before

Procedure:

From 100µg mL^{-1} solution of hydrolyzed pendimethalin, 0.2 mL was taken in different volumetric flasks. To which added 6 mL of nitrite solution, 1 mL of HCl solutions, and 8 ml of aniline solution respectively. Finally the solutions were diluted up to 50 mL with distilled water. Blank solution was prepared in the same way without the addition of pendimethalin. The absorbance of the resulting azodye was measured at 460 nm.The results are given in table 3.2.10. A calibration plot of absorbance vs concentration was constructed and is shown in figures 3.2.12 and 3.2.13The calculated molar absorpitivity value is of 1.5×10^4 L mol^{-1} cm^{-1}.

LOD and LOQ were calculated for five replicates of 0.5 µg mL^{-1} solution by using the following formula.

Limit of Detection (LOD) (for concentration) = 3 x S =3x 0.071= 0.213

Limit of Quantification (LOQ) (for concentration) = 10 x S =10 x0.071=0.71

Where S = standard Deviation

Relative standard deviation (RSD) was calculated by using the following formula.

RSD = Standard deviation /Mean x 100= 0.071/0.71x100 =10.1

Table 3.2.11 Investigation of the effect of concentration on the absorption behavior of azodye

Pendimethalin conc. $\mu g\ mL^{-1}$	Absorbance	Pendimethalin conc. $\mu g\ mL^{-1}$	Absorbance
0.2	0.009	2.0	0.532
0.3	0.042	3.0	0.654
0.4	0.085	4.0	0.723
0.5	0.125	5.0	0.843
0.6	0.185	6.0	0.993
0.7	0.234	7.0	1.231
0.8	0.323	8.0	1.283
0.9	0.372	9.0	1.352
1	0.421	10	1.781

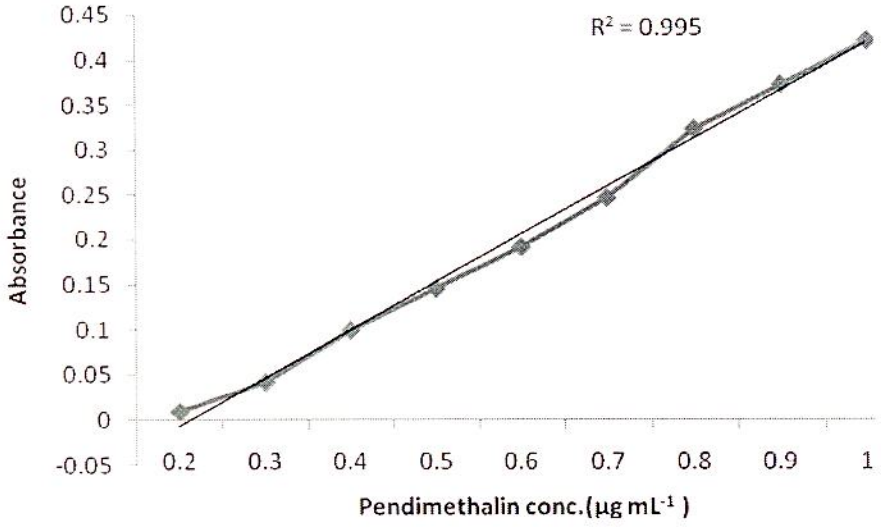


Figure 3.2.13 Effect of concentration of pendimethalin on absorbance behavior at lower level of detection.

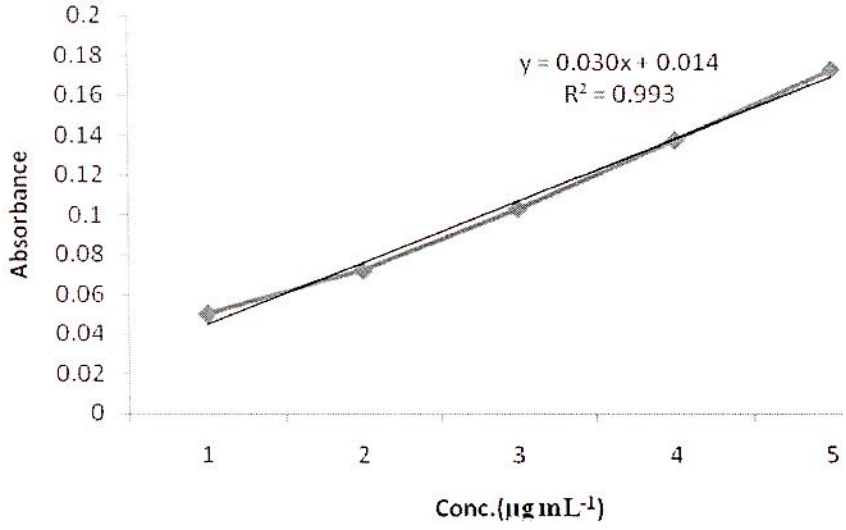


Figure 3.2.14 Effect of concentration of pendimethalin on absorbance behavior at higher level of detections

3.2.5 Application of the Investigated Method for the Analysis of Percent Recovery of Pendimethalin in Real Samples

Instrument the same as motioned before

Reagents the same as mentioned before

Solutions the same as mentioned before

Procedure:

A recovery test was performed on control samples, fortified with known concentration of pendimethalin solution. The requisite sample was homogenized with chopper and extracted with 20 mL of ethanol. The mixture was shaken for 30 minute after equilibration the, the sample was filtered and solvent was evaporated at room temperature for 1 hr. For hydrolysis 5 mL of NaOH was added and heated on boiling water bath for 10 min. After cooling, 6 mL of nitrite solution, 1 mL of HCl solution and 8 mL of aniline solution was added respectively. The solution was transferred to volumetric flask (50 mL) and diluted up to mark with distilled water. Six replicate of the fortified samples were analyzed by the same developed method. Percent recovery was calculated by using formula given below. Results of percent recovery of pendimethalin in different vegetable samples are given in table 3.2.11.

% Recovery = μg found in fortified samples –μ g found in sample /μg of standard added x 100

Table 3.2.12 Recovery(%) of pendimethalin from vegetable samples

Sample	Standard conc. added (μgmL^{-1})	Total conc. Found (μgmL^{-1})	% Recovery
Onion (10g)	4	3.5	89.3 ± 0.55
	8	7.6	79.3± 0.32
	10	8.5	89.8 ± 0.18
Garlic (10g)	10	9.3	88.6 ± 0.49
	8	7.9	95.2 ± 0.29
	4	3.7	84.3 ± 0.16
Potato (10g)	10	9.3	92.0± 0.18
	15	13.3	92.0± 0.32
	20	17.8	83.3 ± 0.16

3.2.6 Application of the Investigated Methods for the analysis of Pendimethalin in Real Samples and Commercial Formulation.

Instrument: The same as mentioned before

Reagents: The same as mentioned before

Solutions: The same as mentioned before

Standard procedure for the extraction of herbicides from real samples was used, as mentioned before for trifluralin. The results are given in the table 3.2.12.

Commercial sample (pendimethalin) was purchased from local market.1-3 mL of pendimethalin was taken in titration flask and hydrolyzed in the same way as standards. From these hydrolyzed solutions 2 mL were taken in 50 mL volumetric flasks and color was developed in the same way as mentioned before. The absorbance of the colored solution was measured at 460 nm. The results in formulation are given in table 3.2.12.

Table 3.1.12 Residue level of pendimethalin from real sample.

S.NO	Samples	Residue μgg^{-1}
1	Onion	1.37 ± 1.24
2	Garlic	1.11 ± 2.18
3	Cabbage	1.41± 1.26
4	Potato	1.32± 1.18

Table 3.2.13 Concentration of pendimethalin in commercial formulation

Commercial formulation	Label Amount (gmL^{-1})	Proposed Method (gmL^{-1})
Active ingredient	0.82 ± 0.46	0.76 ± 0.38

Result and discussion.

After preliminary investigation it was observed that the proposed reaction shown in figure 3.2.2 is feasible for Spectrophotometric determination of pendimethalin then various chemical parameters were optimized and are discussed below.

After preliminary experiments optimum wavelength for azodye formed was investigated. The results are given in table-3.2.1 and are shown in figure 3.2.3 the resulting azo dye has a maximum absorbance at 460 nm. and was used as optimum wavelength for further studies of pendimethalin. Different diazotizing reagents (table 3.2.2) were used to form an azo dye to find out the most suitable diazotizing reagent for spectrophotometric determination of pendimethalin. Aniline and β-naphthol were used for this purpose. It was observed that among all the diazotizing reagent investigated aniline was found to be the most suitable (figure 3.2.4) reagent for formation of azo dye therefore, aniline was selected as a diazotizing reagent for further studies. As hydrolysis was done on boiling water bath therefore, the time required for hydrolysis was also optimized in range 5-50 minutes (table 3.2.3). It was observed (figure 3.2.5) that maximum hydrolysis requires 10 minutes heating on boiling water bath, beyond this time it remains constant. Therefore 10 minutes heating on boiling water bath was taken as optimum heating time.

For hydrolysis of pendimethalin 8 mL of 2%NaOH solution was used (table3.2.4 and figure 3.2.6).Nitrite concentration was optimized for diazotization in the range of 0.05-0.3% (table 3.2.5) and it can be seen from figure 3.2.7 that 0.2 % was optimized concentration of nitrite for maximum diazotizing. Similarly the volume of 0.2% nitrite solution was optimized in the range of 2- 18mL (table 3.2.6). It was observed (figure 3.2.8) that 8 mL of 0.2% of nitrite solution was sufficient for maximum diazotizing and subsequently for maximum formation of azo dye.

The concentration of hydrochloric acid required for diazotization of hydrolyzed pendimethalin was optimized in range of 0.1-0.7M (table 3.2.7). An increase in absorbance was observed with increasing final acid concentration up to 0.3M of hydrochloric acid solution (figure 3.2.9) Above Similarly the volume and concentration of aniline solution was optimized (table 3.2.8 and 3.2.9). It was observed (figure 3.2.10) that 1% of aniline is the optimum concentration and above this concentration a decrease in absorption was observed. Figure 3.2.11 shows that 8 mL of 2 % aniline is the optimum volume required for the maximum formation of azo dye.

This concentration the absorbance remained constant; therefore 0.3M acidity for the final solution was used for further hydrolysis of pendimethalin herbicide.

The limit of detection (LOD) and quantification (LOD) was found to be 0.213 $\mu g\ mL^{-1}$ and 0.71 $\mu g\ mL^{-1}$ respectively with molar absorptive of 1.5 X 10^4 L. $mol.mL^{-1}$ .The validity of the proposed method was checked by standard addition method and the % recovery was found from79.0 ± 0.32 to 95.2 ±0.29%. The method was applied for residue determination in onion, garlic, cabbage and potato samples and found 1.37±1.24,1.11±2.18,1.41±1.26 and 1.32±1.18μgg^{-1} respectively.

3.3 INVESTIGATION OF SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF ARYLOXYPHENOXY PROPIONATES GROUP OF HERBICIDES

The herbicide selected from aryloxyphenoxy propionate group is fenoxaprop-p-ethyl. The chemical structure of fenoxaprop-p-ethyl is given in figure 3.3.1.

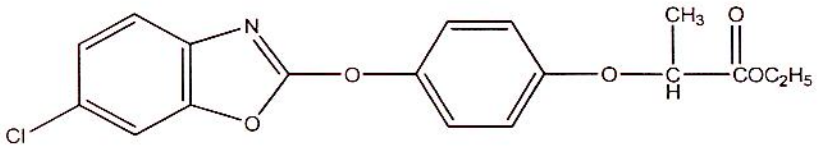


Figure 3.3.1 Chemical structure of fenoxaprop-p-ethyl.

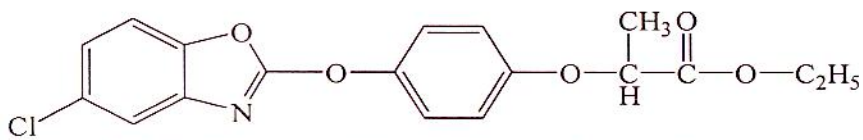
3.3.1. Method development strategy for the spectrophotometric determination of fenoxaprop-p- ethyl

Fenoxaprop-p-ethyl [-ethyl-2-[4[(6-chloro-2-benzoxazolyl)oxy]phenoxy] propionate] belongs to an aryloxyphenoxypropionate herbicide group which is used for the control of annual and perennial grass weeds in cotton, potato, wheat and soybeans as a selective herbicide. The proposed method for the spectrophotometric determination is based on the acid hydrolysis of fenoxaprop-p-ethyl to form 6-chloro-2,3-dihydrobenzoxazol-2-oneandethyl-2-(4-hydroxyphenoxy) propanate. This hydrolyzed product 6-chloro-2, 3-dihydrobenzoxazol-2-one is used as a coupling reagent with diazonium salt to give an azo dye. The azo dye is then determined and from the amount of the dye the concentration of herbicide is calculated. The proposed reaction mechanism is shown in figure 3.3.2.

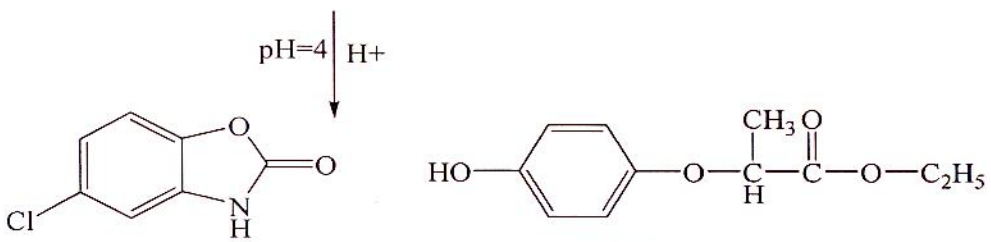
3.3.2. Preliminary investigation of the possibility of acid hydrolysis and the use of hydrolyzed product as coupling reagent for spectrophotometric determination of fenoxaprop-p-ethyl

Preliminary investigations were conducted to investigate the possible acid hydrolysis of fenoxaprop-p-ethyl and subsequent coupling of the hydrolyzed product for the formation of azo dye. High concentration of fenoxaprop-p-ethyl (100 μgmL^{-1}) and high concentration of hydrochloric acid were used to ensure complete hydrolysis. Similarly high concentration of sodium nitrite and aniline were used for the formation of diazonium salt. The diazonium salt reacted with one of the hydrolyzed product (6-chloro-2, 3-dihydrobenzoxazol-2-one) to form an azo dye. Both acid and base hydrolysis was tried by heating on boiling water bath for a time period of 40 minutes. The formation of the colored azo dye with acid hydrolysis indicated the possibility of reaction and the subsequent spectrophotometric determination of fenoxaprop-p-ethyl by this method.

Step-1 Hydrolysis



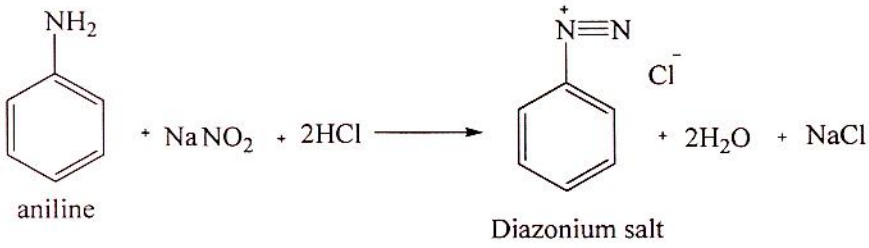
ethyl 2-(4-(5-chlorobenzo[d]oxazol-2-yloxy)phenoxy)propanoate
(fenoxaprop-p-ethyl) Herbicide



6-chloro-2,3-dihydrobenzoxazol-2-one

ethyl 2-(4-hydroxyphenoxy)propanoate

Step-2 Diazotization



Step 3 Coupling

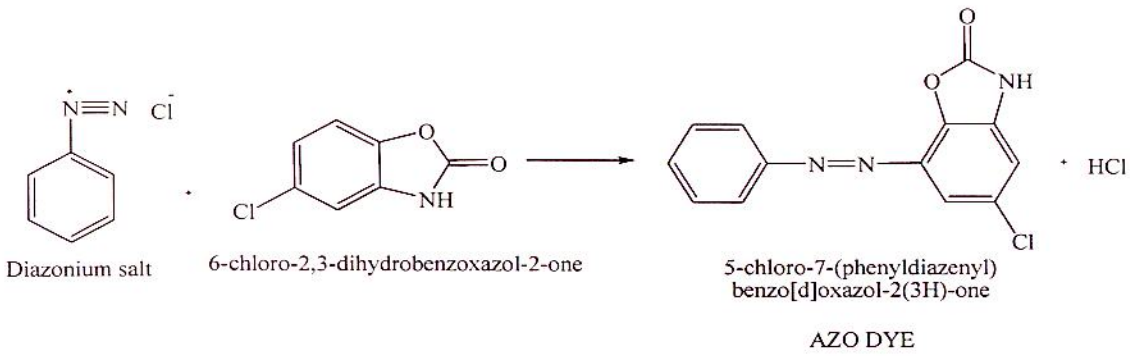


Figure 3.3.2 Proposed reaction mechanism for the spectrophotometric determination of Fenoxaprop-p-ethyl using acid hydrolysis.

3.3.3 Optimization studies for Spectrophotometric Determination of Fenoxaprop-P-Ethyl

After the preliminary reaction, it was clear that fenoxaprop-p-ethyl could be hydrolyzed and the hydrolyzed product could be determined using spectrophotometric method after coupling with suitable diazonium salt and formation of the azo dye. For quantitative determination it is important that each step of the proposed reaction should go toward completion. For this it was important to optimize various parameters involved in these reactions and determination.

3.3.3.1 Investigation of suitable wavelength for the determination of fenoxaprop-p-ethyl

Instruments

UV/V is spectrophotometer (UNICO UV-2100 united product instruments Inc, Dayton, U.S.A) with matched cells was used for all spectral measurements. A pH meter model pH-422 (Wissenschaftlich-Technische Werkstatten, W. Germany) was used for pH measurements.

Reagents and chemicals

All chemicals used were of analytical reagent grade or of similar purity. Sodium nitrite, aniline, hydrochloric acid, (Merck, Darmstadt, Germany) and distilled ethanol were used during this work. Commercial formulation containing fenoxaprop-p-ethyl was purchased from the local market. Reference standard fenoxaprop-p-ethyl was purchased from Dr. Ehrenstofer GmbH, Germany.

Solutions preparation

i) **Nitrite solution (2000 µg mL^{-1})**: 2000 µg mL^{-1} nitrite solution was prepared by dissolving 0.3 g sodium nitrite in distilled water and diluted up to 100 mL.

ii) **Aniline solution (2%):** 2 % aniline solution was prepared by adding 2 mL of aniline in 50 mL of ethanol and diluted with distilled water up to 100 mL.

iii) **Hydrochloric acid solution**: 1M HCl solution was prepared by dilution from conc.HCl.

iv) **Standard fenoxaprop-p-ethyl solution (1000 µg mL^{-1})**: 0.1 mg of fenoxaprop-p-ethyl was dissolved in methanol and diluted up to 100 mL

v) **Standard hydrolyzed Fenoxaprop-p-ethyl solution:(1000 µg mL^{-1})**

A 10 ml portion of standard fenoxaprop-p-ethyl solution (1000 µg mL^{-1}) was taken in a beaker and 3 mL concentrated solution of hydrochloric acid was added to it followed by a few mL of ethanol to get clear solution. The solution was heated on boiling water bath for 30 minutes, cooled, dissolved in 30 mLof ethanol and diluted up to 100 mL with distilled water. Working standard solutions were prepared from this hydrolyzed stock solution by dilution with ethanol and water.

Procedure

Diazonium salt of aniline was prepared by mixing aniline and sodium nitrite in equal volumes. To this 5 mL of 100 μg mL^{-1} of hydrolyzed fenoxaprop-p-ethyl was added. A red colored azo dye was formed confirming the possibility of the proposed reaction. Blank solution was prepared in the same way without the addition of fenoxaprop-p-ethyl. The absorbance of the resulting dye was measured from 350 to 590 nm using UNICO UV-2100 spectrophotometer for finding out the optimum wavelength. The absorbance was noted with increasing wavelength and each wavelength was calibrated with blank solution. The results are given in table 3.3.1: and are shown in figure 3.3.3

Table 3.3.1 Investigation of the suitable wavelength for the determination of fenoxaprop

Wave length(nm)	Absorbance	Wave length(nm)	Absorbance
360	0.632	430	1.632
370	0.722	440	1.465
380	0.943	460	1.232
390	0.982	470	0.733
400	1.354	480	0.865
410	1.121	500	0.654
420	1.522	520	0.335

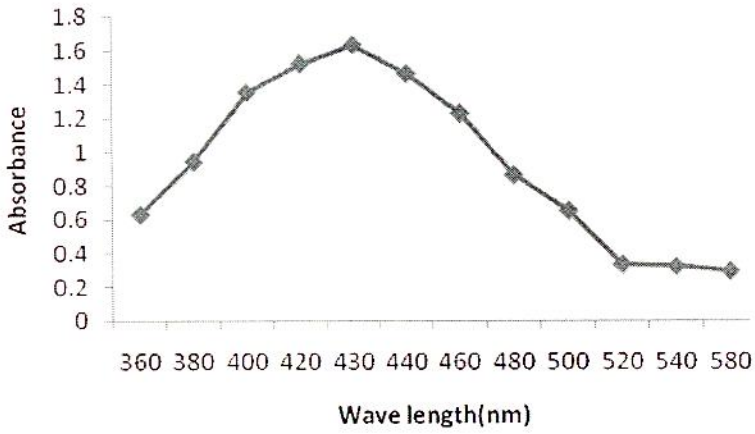


Figure 3.3.3 Investigation of the suitable wavelength for the determination of fenoxaprop-p-ethyl

Conditions:

λ_{max}	Varied
Fenoxaprop-p-ethyl solution	10 µg mL^{-1}
Diazotized reagent	5mL

3.3.2 Optimization of the parameters involved in hydrolysis, diazotization and coupling reaction for the determination fenoxaprop-p-ethyl.

Instrument: The same as mentioned before

Reagents: The same as above

Solution: The same as mentioned before

Procedure: As the first step was hydrolysis, therefore acid concentration for hydrolysis was optimized. For this10 ml of standard fenoxaprop-p-ethyl from 1000 µg mL^{-1} stock solution was taken in five separate titration flasks and varied volume of concentrated hydrochloric was added to give 0.1-0.8 M HCl solution on dilution followed by addition of 10 mL distilled ethanol. It was heated on boiling water bath for 10 minutes and on cooling 10 ml of ethanol was added to it

and diluted up to mark with distilled water in 100 mL volumetric flask. From stock solution of diazotized reagent, 8 mL was taken in 50ml volumetric flask and 5mL of Fenoxaprop-p-ethyl from 10 $\mu g\ mL^{-1}$ hydrolyzed stock solution was added to it. A red colored azo dye was formed. Blank solution was prepared in the same way without the addition of fenoxaprop-p-ethyl. The absorbance of the resulting azo dye was measured at 430 nm.The results are given in table 3.3.2 and are shown in figure 3.3.4.

For optimization of hydrolysis time, the hydrolyzed solution was heated on boiling water bath and heating time was varied from 5-60 minute. The absorbance of azo dye formed was measured at 430 nm after following the same procedure. The results are given in table 3.3.3 and are shown in figure 3.3.5.

For optimization of nitrite concentration and nitrite volume for the formation of diazonium salt stock solution of diazotized reagent was prepared by mixing various concentration of nitrite solution i.e. from 200-1600 $\mu g\ ml^{-1}$ with aniline solution in 8:1 ratio. From each of these solutions 5mL was taken in a 50 mL volumetric flask and color was developed in the same way as mentioned before. Similarly for finding optimum volume of nitrite solution varied volume of nitrite (1-10 mL) of 2000 $\mu g\ mL^{-1}$ of nitrite were used. The results are given in tables 3.3.4, 3.3.5 and are shown in figure 3.3.6, 3.3.7 respectively.

Similarly the concentration of aniline was also optimized by using varying concentration (0.5-3%) and color was developed in the same way as before .The results are given in table 3.3.6 and are shown in figure 3.3.8. Optimum volume of aniline determined by using different volumes of aniline (1-8 mL) of 2% solution were used. The absorbance of the azo dye formed were noted. The results are given in table 3.3.7 and are shown in shown in figure 3.3.9.

For optimization of the volume of diazonium salt various volumes of diazotized reagents ranging from 2-16 mL were taken in separate 50 mL volumetric flasks while keeping the rest of the procedure same . The absorbance of the resulting azo dye was measured at 430 nm. The results are given in table 3.3.8 and are shown in figure 3.3.10.

For optimization of equilibration time after color development the solution was equilibrated for different time intervals ranging from 2-60 minute. The results are shown in figure 3.3.11.

Table 3.3.2 Investigation of acid concentration for hydrolysis for Fenoxaprop-p-ethyl

S.NO	HCl conc. (M)	Absorbance
1	0.1	0.432
2	0.2	0.832
3	0.4	1.632
4	0.6	1.122
5	0.8	0.832

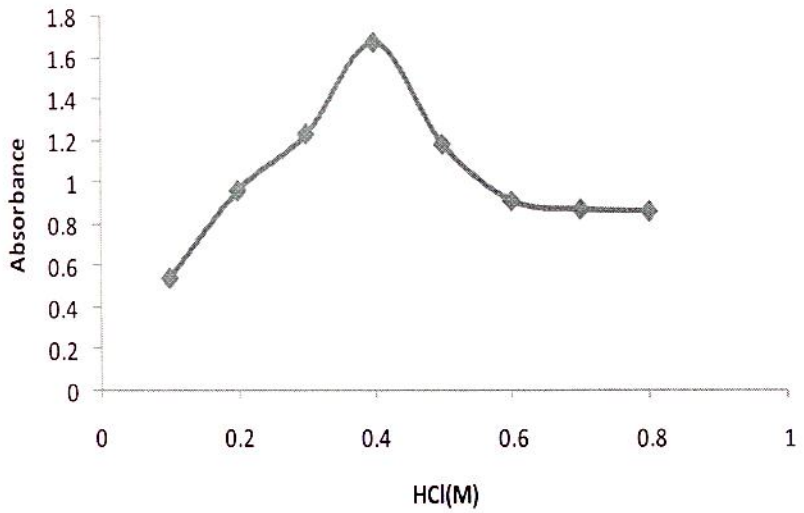


Figure 3.3.4 Investigation of acid concentration for hydrolysis of Fenoxaprop-p-ethyl

Table 3.3.3 Investigation of hydrolysis time for spectrophotometric determination of fenoxaprop-p-ethyl

S.NO	Heating time (min)	Absorbance
1	2	0.223
2	4	0.321
3	6	0.592
4	8	0.452
5	10	0.411
6	12	0.252
7	14	0.212

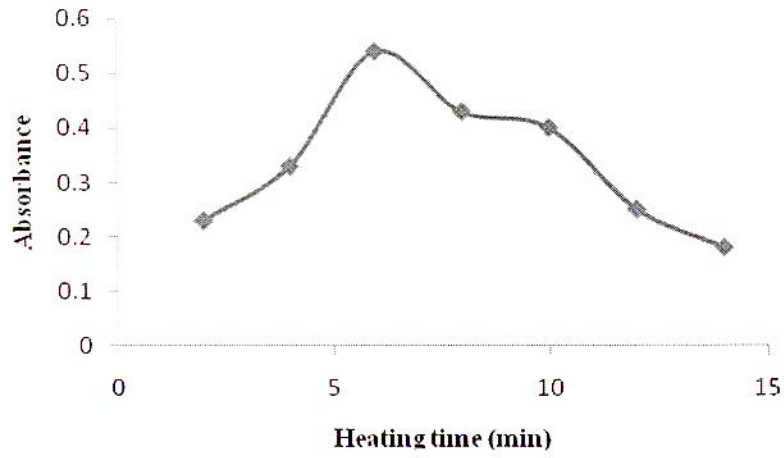


Figure 3.3.5 Investigation of hydrolysis time for spectrophotometric determination of fenoxaprop-p-ethy

Table 3.3.4 **Investigation of the effect of Nitrite concentration on spectrophotometric determination of Fenoxaprop-p-ethyl**

S.NO	Nitrite conc.(µg mL^{-1})	Absorbance
1	200	0.421
2	400	0.631
3	600	1.121
4	800	1.211
5	1000	1.012
6	1200	0.812
7	1400	0.632

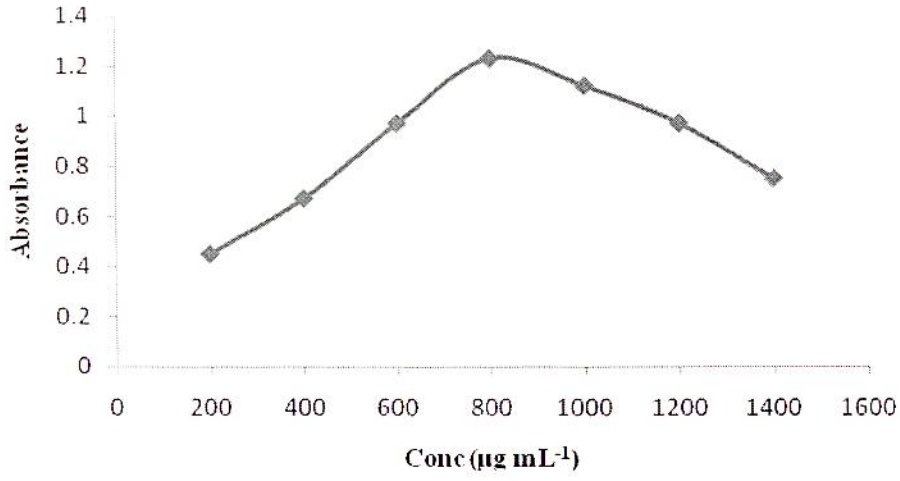


Figure 3.3.6 Investigation of the effect of nitrite concentration on spectrophotometric determination of fenoxaprop-p-ethyl

Table 3.3.5 Investigation of the effect of Nitrite volume on the absorbance behavior of the azo dye

S.NO	Nitrite volume (mL)	Absorbance
1	2	0.221
2	4	0.643
3	6	1.112
4	8	1.321
5	10	1.113
6	12	0.932
7	14	0.854

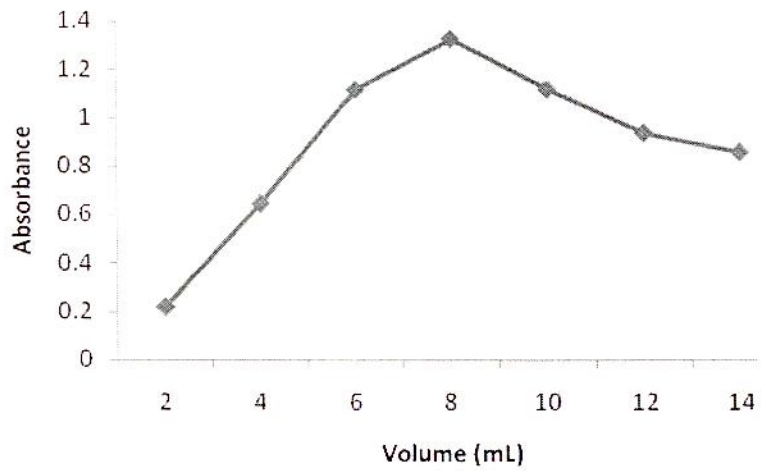


Figure 3.3.7 Investigation of the effect of Nitrite volume on the absorbance behavior of the azo dye

Table 3.3.6 **Investigation of the effect of aniline concentration on the absorbance behavior of the azo dye**

S.NO	Aniline conc. (%)	Absorbance
1	0.5	0.811
2	1	1.121
3	1.5	1.432
4	2	1.642
5	2.5	1.342
6	3	1.312

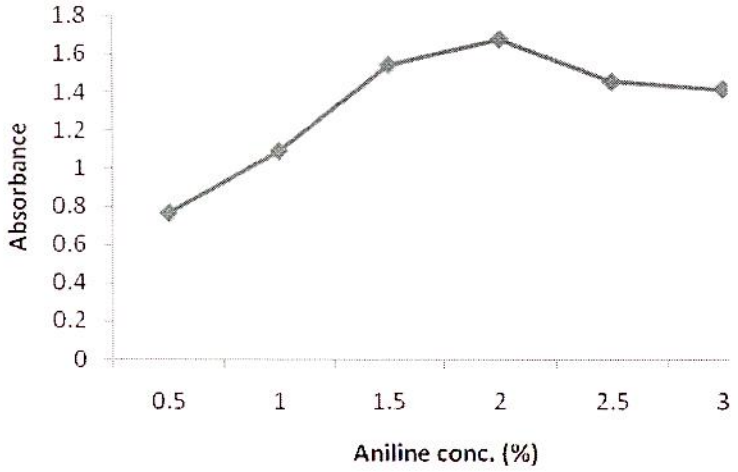


Figure 3.3.8 Investigation of the effect of aniline concentration on the absorbance behavior of the azo dye

Table 3.3.7 **Investigation of the effect of aniline volume on the absorbance behavior of the azo dye**

S.NO	Aniline volume(mL)	Absorbance
1	2	0.251
2	4	0.634
3	6	1.123
4	8	1.243
5	10	1.110
6	12	0.821
7	14	0.821
8	16	0.821

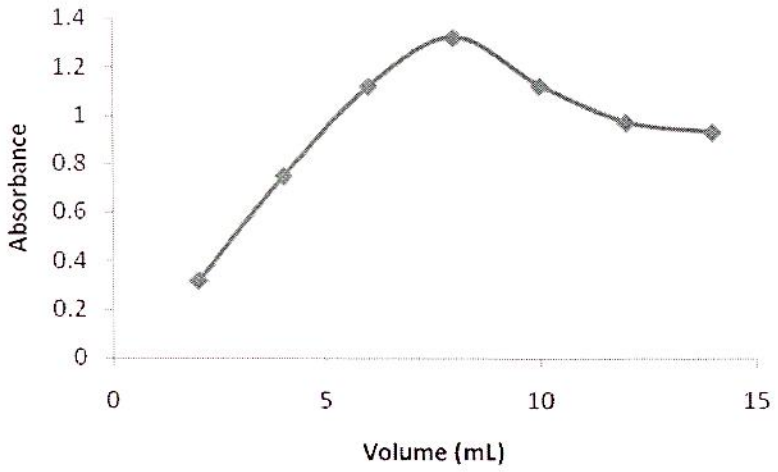


Figure 3.3.9 Investigation of the effect of aniline volume on the absorbance behavior of the azo dye

Table 3.3.8 **Investigation of the effect of volume of diazotized reagent on the absorbance behavior of the azo dye**

S.NO	Diazotized reagent volume (mL)	Absorbance
1	2	0.420
2	4	1.231
3	6	1.342
4	8	1.463
5	10	1.613
6	12	1.601
7	14	1.601
8	16	1.601

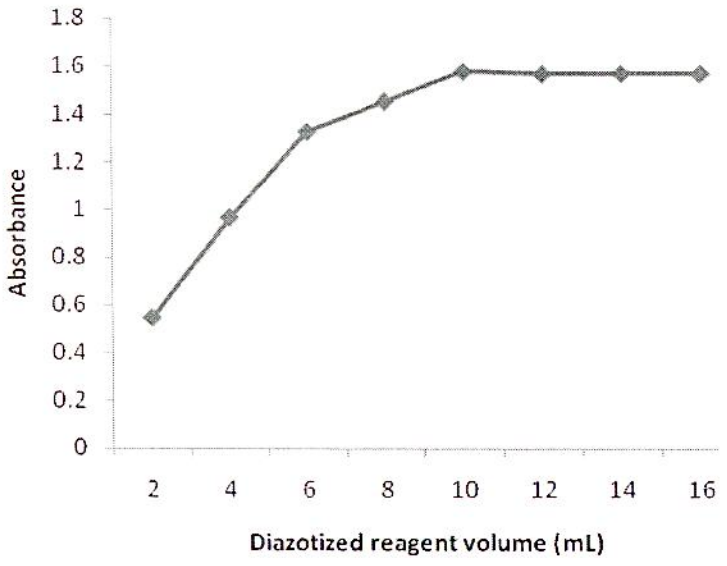


Figure 3.3.10 Investigation of the effect of volume of diazotized reagent on the absorbance behavior of the azo dye

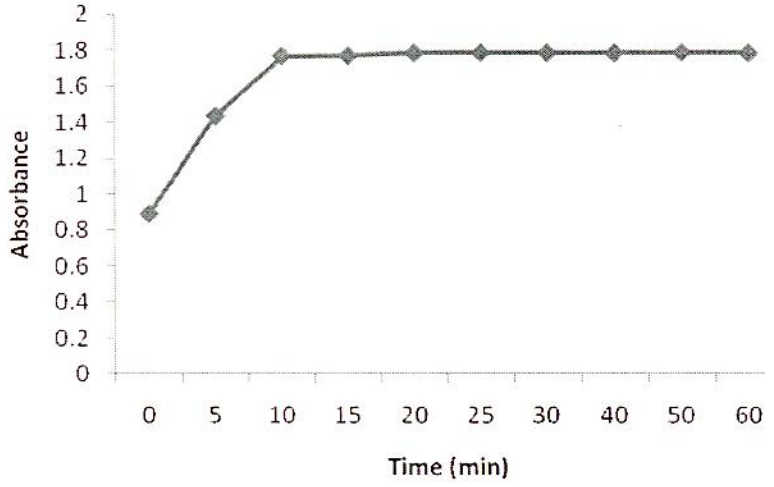


Figure 3.3.11 Investigation of the equilibration time on the absorbance behavior of the azo dye

3.3.4 Verification of Beer's law and determination of the limit of detection and limit of quantification for the spectrophotometric determination of fenoxaprop-p-ethyl.

Instrument: The same as mentioned before

Reagents: The same as above

Solution: The same as mentioned before

For verification of Beer-Lambert's law at lower and higher concentration, the amount of fenoxaprop-p-ethyl was varied in the range of 8 ng mL^{-1} -100 ng mL^{-1} and 2µg mL^{-1} -12µg mL^{-1} respectively the absorbance of the resulting azo dye was measured at 430 nm. A calibration plot of absorbance vs. concentration was constructed and is shown in figures 3.3.12 and 3.3.13 LOD and LOQ were calculated by using the same formula as given before.

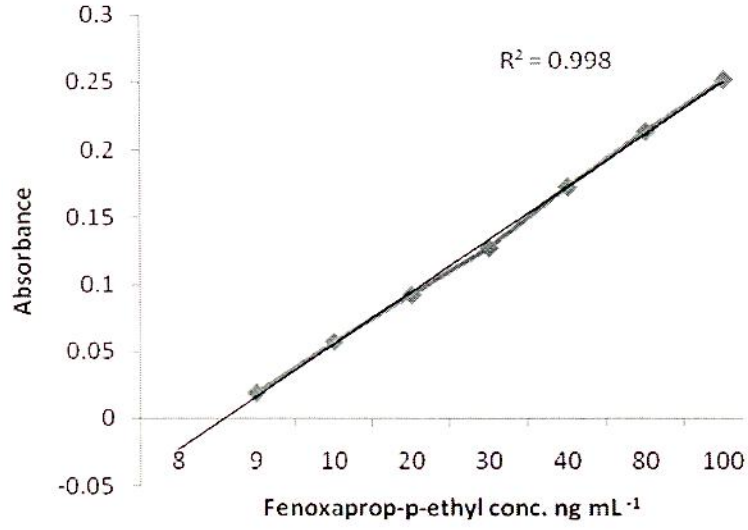


Figure 3.3.12 Investigation of the effect of concentration on absorbance behavior of fenoxaprop-p-ethyl at lower concentration.

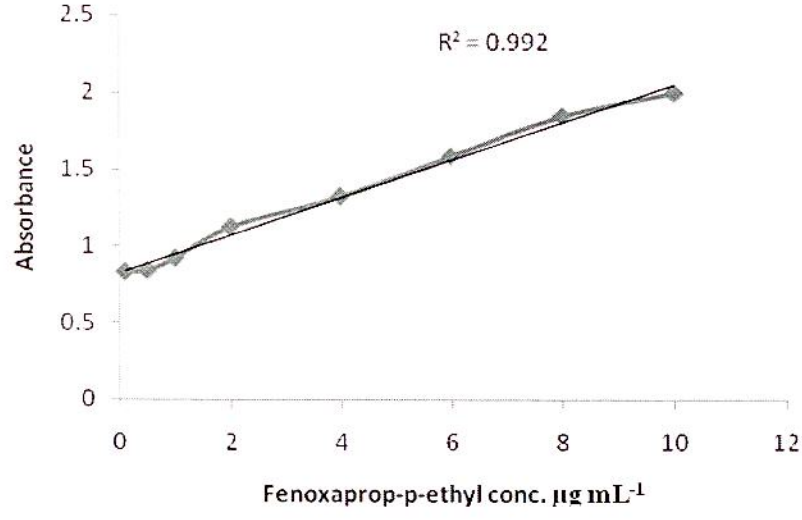


Figure 3.3.13 Investigation of the effect of concentration on absorbance behavior of fenoxaprop-p-ethyl at higher concentration

3.3.5 Percent recovery of Fenoxaprop-P-Ethyl from real samples using the Investigated Method

Instrument: The same as mentioned before

As the method investigated is meant for application to real samples, therefore it was important to optimize different extraction parameters for real samples like soil, grain and flour and check the % recovery from these samples at optimum conditions. For this purpose extraction solvent and extraction procedures were optimized for these samples. There extraction procedure i.e. mechanical, microwave assisted and sonication was compared for this purpose.

3.3.5.1 Optimization of extraction solvent.

For extraction from soil, grains and flour different solvents like ethanol, dichloromethane, n-hexane, and acetone were used. Maximum extraction was achieved with acetone and ethanol. Therefore, for residue determination acetone was used as extracting solvent. The results are shown in figure 3.5.14

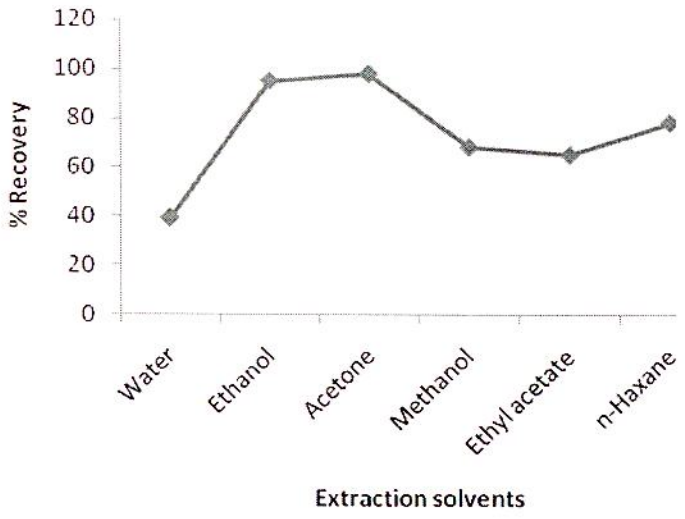


Figure 3.3.14 Investigation of suitable solvent for extraction of fenoxaprop-p-ethyl

3.3.5.2 Optimization of extraction procedures

Extraction from soil

For the analysis of fenoxaprop-p-ethyl in soil, 10 g of soil was air dried, passed through a 2 mm sieve and homogenized. Fortified samples were prepared by adding 5, 10 and 15μg $g^{-1}$of standard fenoxaprop-p-ethyl solution to soil sample. After fortification the samples were kept at room temperature for 4.0 h to allow evaporation of the solvent and different extraction procedures were used.

Mechanical extraction

For mechanical extraction 50 mL of acetone was added to each sample and after absorption the samples were shaken on shaker for 15 min and on equilibration the sample was filtered and the filtrate was evaporated on rotary evaporator up to volume of 2 mL The determination of fenoxaprop-p-ethyl in the reduced volume of filtrate was preceded by the proposed method.

Microwave extraction

Samples were fortified in the same way. After fortification the samples to get high extraction rate, microwave rapid heating were used on closed-vessel during a sample extraction procedure. The starting parameters setting in microwave system were 2.0 min at 350 W and 3.0 min at 500 W. Once the extraction program was completed the vessel were cooled down to room temperature before opening the vessel. After extraction the contents were centrifuged for 10 min and filtered. The extract was evaporated near to dryness and redissolved in acetone and diluted up to mark and analyzed by the proposed method.

Extraction by sonication

5g soil samples were directly weighed in three different flasks and were spiked at three levels (5, 10, 15 μg g^{-1}) by adding standard solution of herbicide to each samples mixing it thoroughly and were kept for 1 hr .Then 20 mL of acetone was added to each sample and sonicated for 30 min at ambient temperature. After sonication the samples were filtered on paper. The filtrate was collected in flask and was evaporated up to dryness and then reconstituted in 10 mL of acetone and diluted up to mark and analyzed by the proposed method

Extraction from grains samples

Fortified grain samples were prepared by adding 5, 10 and 15 μg $g^{-1}$of standard fenoxaprop-p-ethyl solution to barley grains. After fortification the samples were kept at room temperature for 6.0 h to allow evaporation of the solvent.

Mechanical extraction

Extraction procedure and determination was the same as for soil.

Microwave extraction

For microwave extraction barley grains samples (10g) were added to extraction vessels and fortified with 10, 20 and 30 μg g^{-1} of standard solution of herbicide to each sample followed by the addition of 50 mL of extraction solvent to each sample. The grain samples were kept in closed vessels and heated for 2-3min at optimized temperature in a microwave. This was followed by filtration and determination as mentioned before.

Extraction by sonication

Forextraction by sonication5g aliquots of the barley grains and barley flour samples were directly weighed in three different flasks and were spiked at three levels (5, 10, 15 μg g^{-1}) and were kept for 1 hr . The rest of the procedure was the same as for soil. The results are given in table 3.3.9 and fig 3.4.15

Table 3.3.9 Percent recovery comparison of herbicide from real samples

Sample	Solvent	Added ($\mu g\ g^{-1}$)	Found ($\mu g\ g^{-1}$)			Average% Recovery±RSD		
			MASE	USE	ME	MASE	USE	ME
Soil	Ethanol	5 10 15	4.4 8.5 13.7	4.5 9.3 14.6	4.0 8.0 12.6	88±3.0	93±1.0	78.3±3.0
	Acetone	5 10 15	4.5 8.6 13.7	4.7 9.6 14.0	4.0 8.3 12.0	89±3.0	96±1.0	88.0±1.7
Barely grains	Ethanol	5 10 15	4.3 8.6 13.0	4.4 9.6 14.1	3.9 8.0 12.1	87±0.0	96±1.7	75.0±1.5
	Acetone	5 10 15	4.4 8.7 13.3	4.8 9.8 14.3	3.6 7.2 12.8	88±0.5	98±0.6	73.0±0.5
Barely flour	Ethanol	5 10 15	4.5 8.6 13.7	4.8 9.6 14.4	4.5 8.6 13.7	88±1.24	96±1.0	75±1.5
	Acetone	5 10 15	4.8 9.3 14.3	4.5 9.9 13.7	3.9 8.0 12.1	93± 1.0	99±1.0	73±0.5

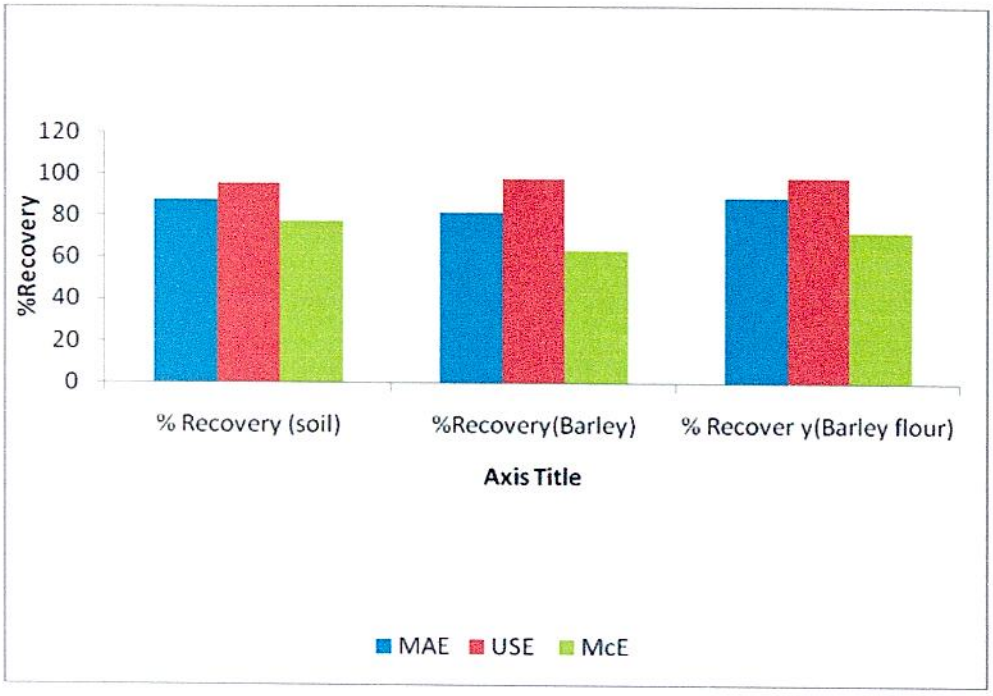


Figure.3.3.13 Investigation for best extraction method for maximum %recovery of fenoxaprop-p-ethyl from samples

MAE (Microwave assisted sample extraction)

USE (Ultrasonication sample extraction)

Mc E (Mechanical extraction)

3.3.6 Application of the Investigated Methods for the analysis of Fenoxaprop-P-Ethyl in Commercial Formulation and real samples

Instrument: The same as mentioned before

Reagents: The same as above

Solution: The same as mentioned before

Commercial samples: Commercial samples of fenoxaprop-p-ethylwere purchased from local market.

Procedure

1-4 mL of fenoxaprop-p-ethyl formulation was taken in four separate 50 mL volumetric flasks and dissolved in 25 mL acetone and diluted up to mark with distilled water. From each solution 5mLwere taken in four separate 50 mL volumetric flasks and color was developed in the same way as mentioned before. The absorbance of the colored solution was measured at 430 nm using calibration plot (Absorbance vs. concentration).The concentration of fenoxaprop-p-ethyl was calculated. The results are shown in table 3.4.10

Table 3.3.10 Concentration of fenoxaprop-p-ethyl in commercial formulation.

Commercial formulation	Label Amount ($g\ mL^{-1}$)	Proposed method ($g\ mL^{-1}$)
Active ingredient	7.50	8.2±0.31

Extraction procedure

For real samples homogenized food sample (10 g) was taken in a flask and 50 mL of acetone was added to it. Each sample was sonicated for 30 min at ambient temperature. After sonication the samples were filtered on filter paper. The filtrate was collected in flask and was evaporated up to

dryness and then reconstituted in 10 mL of acetone and diluted up to mark and analyzed by the proposed method. Each sample was analyzed in triplicates. The results aregiven intable 3.3.11.

Table 3.3.11 Residue level of fenoxaprop-p-ethyl in real samples.

S.NO	Sample	Residue ($\mu g\ g^{-1}$)
1	Barley grains	2.3±0.31
2	Wheat grains	1.2±0.22
3	Barley flour	0.15±.03

3.3.7 Factorial design for optimization conditions

The influence of different parameters and their interactions on the absorbance were studied according to the 2^3 factorial designs (state- ease). A 2^3 factorial design with a complete repetition performed in one run resulting in a total of 8 experiments was chosen for extraction conditions optimization and maximum % recovery of fenoxaprop-p-ethyl from food samples. Three-dimensional response surface (RSM) plots of absorbance versus main factors (AB and BC) and 2^3 cube model for optimized parameters for extraction and spectrophotometric studies based on experimental model are shown in figure 3.3.16 and 3.3.17The relative importance of different variables and the interaction with each other for spectrophotometric studies of fenoxaprop-p-ethyl and their impact on % recovery were studied using a 2^3 factorial design. The low and high values of the factors were selected according to the studied factors. For extraction of fenoxaprop-p-ethyl effect of different solvents, temperature and heating time were studied. Results for the ANOVA test are shown in table 3.3.13.

Table3.3.12 Analysis of variance table (ANOVA) for selected factorial model

Source	Sum of Sequeres	dF	F-VALUE	P>F VALUE	coefficient	St Error	VIF
Model	1.39	5	0.38	0.832	1.24	0.30	1.00
B	1.09	1	1.49	0.34	0.34	0.30	1.00
C	1.95	1	2.68	0.96	-0.016	0.30	
AB	0.29	1	0.40	0.59	1	-1.49	
AC	0.011	1	0.015	0.91	1	-1.33	
BC	2.62	1	3.61	0.95	1	-1.28	
Residual	1.46	2					
corr. Total	2.82	7					

Regression analysis in terms of coded factors.

A*+B*+C*+A*B*+A*C*+B*C*=1.90

+ 1.24 + 0.37 +.018+0.1 9+ 0.037 +018 = 1.90

Table 3.3.13 Factorial model for optimization study for azodye formation of fenoxaprop-p-ethyl

Source	Sum of squares	df	Mean square	F value	P value Prob > F	
Model	4.58	5	0.92	868.96	0.0011	significant
A-HCl	0.46	1	0.46	434.36	0.0023	
B-sodium nitrite	3.74	1	3.74	3547.6	0.0003	
C- Aniline	0.054	1	0.054	51.65	0.0188	
AB	0.29	1	0.29	278.28	0.0036	
BC	0.035	1	0.035	32.80	0.0292	
Residuals	2.10×10^{-3}	2	1.05×10^{-3}	–	–	
Cor total	4.58	7	–	–	–	

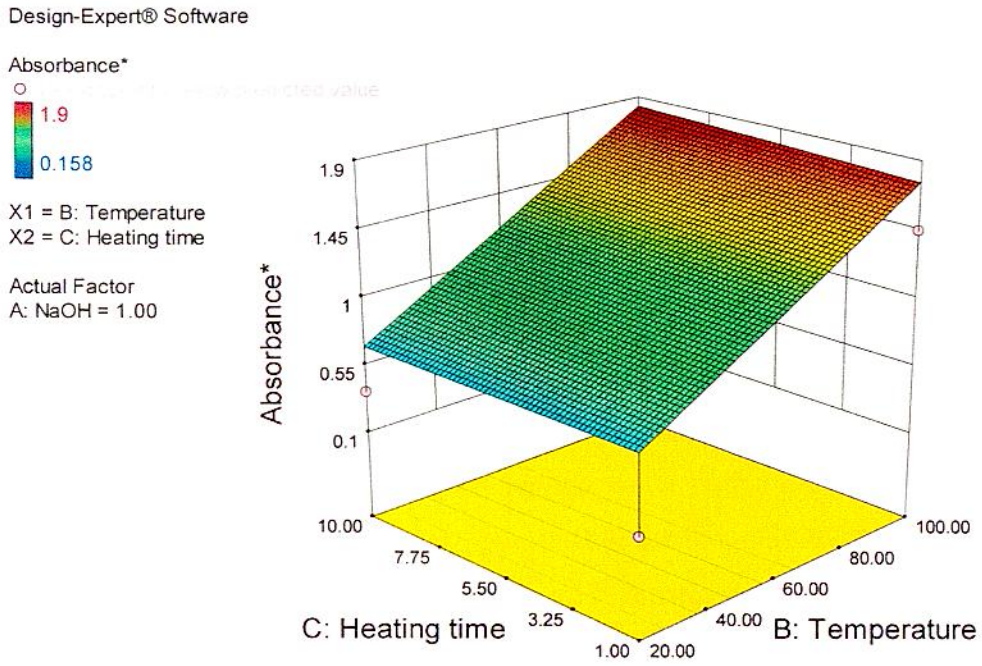


Figure 3.3.16 Response surface model (RSM) showing main factors involved for maximum absorption for spectrophotometric determination of fenoxaprop-p-ethyl.

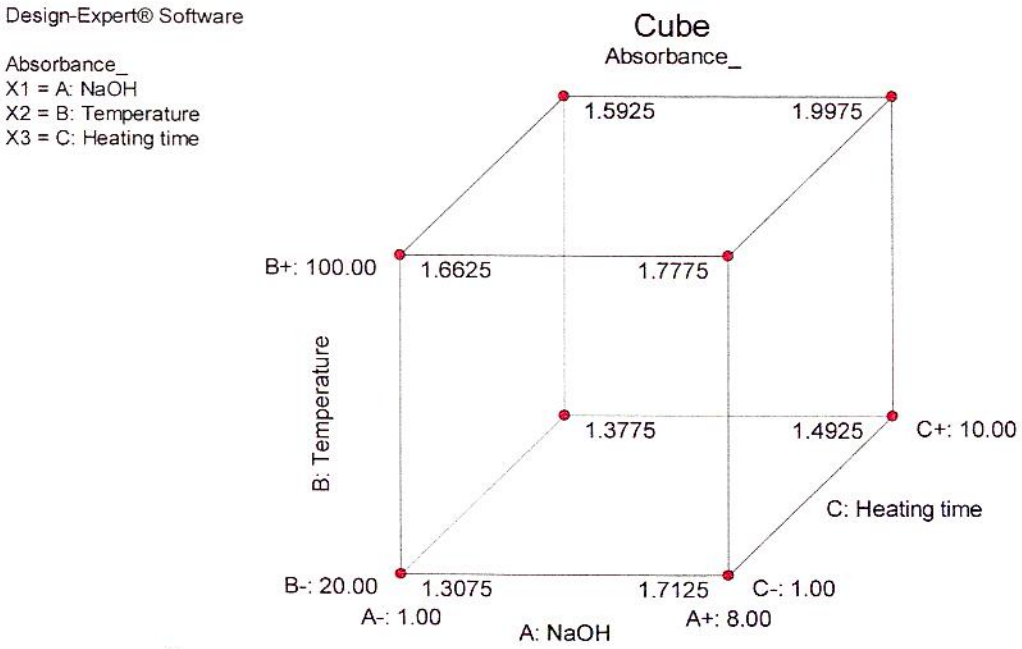


Figure 3.3.17 2^3cube model for optimized parameters for spectrophotometric determination of fenoxaprop-p-ethyl

Results and Discussion

Fenoxaprop-p-ethyl was hydrolyzed in the presence of acid to form 6-chloro-2, 3-dihydrobenzoxazol-2-one (CDHB) and ethyl 2-(4-hydroxyphenoxy) propionate (EHPP). The hydrolyzed product 6-chloro-2, 3- dihydrobenzoxazol-2-one was coupled with diazonium salt. The resultant colored azo dye absorption was measured at 430 nm. The proposed reaction mechanism is given in Figure 3.3.2.

The effect of acidity, temperature and heating time on hydrolysis of fenoxaprop-p-ethyl to 4-(5-chlorobenzo[d]oxazol-2-yloxy) phenol was studied. Maximum hydrolysis was observed with 0.4 M hydrochloric acid for 6 min heating on boiling water bath (100 °C) (Fig3.3.4 and 3.3.5). Nitrite concentration for diazotization was optimized in the range of 100 μg $mL^{-1}$to 1200 μg mL^{-1}(Fig 3.3.6). It was found that 8 mL of 800 μg mL^{-1} nitrite concentrations was sufficient as it gave good stability and quantitative results. The effect of acidity on the diazotization reaction was studied and it was observed that constant absorbance values were obtained at the concentration range of 0.1 to 0.8 M hydrochloric acid solution. Therefore, in further analyses the concentration of hydrochloric acid in solutions was adjusted at 0.4 M. Aniline solution used as a diazotizing reagent with nitrite. The diazonium salt concentration increased with increase in concentration of aniline from 0.5 to 2.0% solution and slowly decreased beyond 2.0% concentration (Fig3.3.8). Therefore 2% solution of aniline was used fordiazonium ion formation which was further coupled with hydrolyzed fenoxaprop-p-ethyl.

Precision of the method was checked by the replicate analysis of solution containing 1.0 μg mL^{-1} fenoxaprop-p-ethyl solution. The standard deviation and relative standard deviation were found to be ± 0.0098 and 3.5% respectively.

All spectral measurements were carried out against reagent blank. The Beer's law was obeyed in the concentration range of 0.2-25 μg mL^{-1} of fenoxaprop-p-ethyl. The molar absorptivity was found to be $1.5x10^4$ L mol^{-1} cm^{-1}. The limit of detection (3s/b) and quantification (10s/b) were 0.029 μg mL^{-1} and 0.098μg mL^{-1} respectively. The influence of different parameters and their interactions on the absorbance were studied according to the 2^3 factorial designs (state- ease). A 2^3 factorial design with a complete repetition performed in one run resulting in a total of 8experiments was chosen for extraction conditions optimization and maximum % recovery of fenoxaprop-p-ethyl from food samples. Three-dimensional response surface (RSM) plots of

absorbance versus main factors (AB and BC) and 2^3 cube model for optimized parameters for extraction and spectrophotometric studies based on experimental model are shown in figure 3.3.16 and 3.3.17The relative importance of different variables and the interaction with each other for spectrophotometric studies of fenoxaprop-p-ethyl and their impact on % recovery were studied using a 2^3 factorial design. The low and high values of the factors were selected according to the studied factors. For extraction of fenoxaprop-p-ethyl effect of different solvents, temperature and heating time were studied. Results for the ANOVA test are shown in table 3.3.13.

Food samples (barley and wheat) were obtained from agriculture fields, where fenoxaprop-p-ethyl had been sprayed. The method was applied for residue determination in food samples. The values for fenoxaprop-p-ethyl found in barley and wheat were 1.35 ± 0.13 μg g^{-1} and 1.05 ± 0.20 μg g^{-1}, respectively (Table 3.4.2). A recovery test was performed on the same sample after residue determination and fortified with known concentration of fenoxaprop-p-ethyl solution. Six replicates of the fortified samples were analyzed using the proposed method. The results are given in Table 3. The average recoveries were found in the range of 96 -98% in all cases. The proposed method was also applied to check the purity of commercially available fenoxaprop-p-ethyl herbicide. The results are given in Table 3.3.13and can be seen that the results are in good agreement with the label value. The azo dye formed in the proposed reaction shows maximum absorption at 430 nm.

3.4 SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF FENOXAPROP-P- ETHYL USING CHARGE TRANSFER COMPLEX.

The chemical structure of fenoxaprop-p-ethyl is given below in figure 3.4.1.

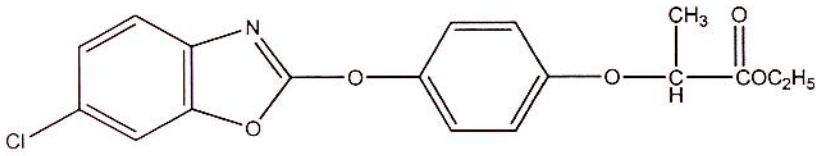
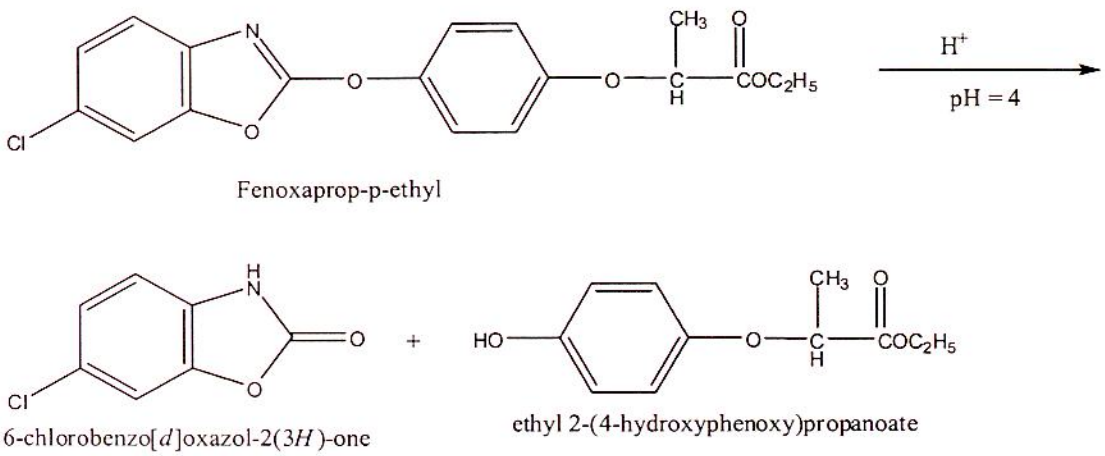


Figure 3.4.1 Chemical structure of fenoxaprop-p-ethyl.

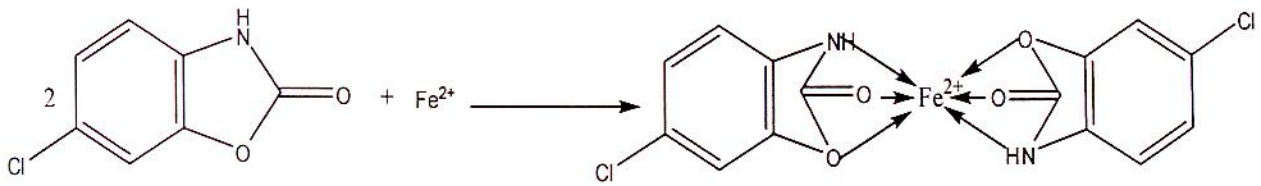
3.4.1 Method development strategy for the spectrophotometric determination of fenoxaprop-p-ethyl using charge transfer complex

The method for spectrophotometric determination is based on the acid hydrolysis of fenoxaprop-p-ethyl to form 6-chloro-2, 3-dihydrobenzoxazol-2-one and ethyl2-(4-hydroxyphenoxy) propanate. After hydrolysis, the proposed hydrolyzed product 6-chloro-2, 3-dihydrobenzoxazol-2-one is used as complexing reagent with metals. For investigation of charge transfer complex of fenoxaprop-p-ethyl with iron (II), initially ferrous sulphate solution was added drop wise to the hydrolyzed product on boiling water bath. This product forms a yellow colored charge transfer complex with iron (II), which is soluble in ethanol. The absorbance of the complex was measured at 430 nm using UNICO UV-2100 spectrophotometer. The reaction was taking place in acidic media and for pH adjustment initially H_2SO_4, HCl and acetate buffer were tried. It was observed that the charge transfer complex formed between Fe (II) and fenoxaprop-p-ethyl was not stable in strong acidic media, therefore acetate buffer was used. After confirmation of charge transfer complex in the preliminary studies, further studies were concentrated on the optimization of parameters for maximum complex formation. The proposed reaction mechanism is shown in figure 3.4.2 .

Step-1. Dissociation



Step-2. Complex formation



Yellow coloured complex

Figure 3.4.2: Proposed chemical reaction for the Spectrophotometric determination of fenoxaprop-p-ethyl by charge transfer complex formation with iron (II)

3.4.2 Optimization of various parameters for the spectrophotometric determination of fenoxaprop-p-ethyl using charge transfer complex

Instrument

UV/Vis spectrophotometer (UNICO UV-2100 united product and instruments Inc, Dayton, U.S.A) with matched cells was used for all spectral measurements. A pH meter model pH-422 (Wissenschaftlich-Technische Werkstatten, W. Germany) was used for pH measurements.

Reagents and chemicals

All chemicals used were of Analytical Reagent grade purity. Ferrous sulphate, ethanol, acetic acid, sodium acetate, acetone and ethanol (Merck), were used during this work. Reference standard fenoxaprop-p-ethyl was purchased from Dr. Ehrenstofer GmbH, Germany. Commercial formulations containing fenoxaprop-p-ethyl was purchased commercially from the local market.

Solution preparation:

i) **Standard fenoxaprop-p-ethyl solution** (1000 $ugmL^{-1)}$) was prepared by dissolving 1.0 g of fenoxaprop-p-ethyl in ethanol and diluting up to 100 mL in volumetric flask.

ii) **Ferrous sulphate solution** (1000 ug mL^{-1})**:** 1.0g of ferrous sulphate was dissolved in 30 ml of water and diluting up to 100 mL in volumetric flask.

iii) Acetate buffer (pH4) was prepared by mixing acetic acid (0.2M) solution and (0.2M) sodium acetate solution.

3.4.3 Optimization Studies for Spectrophotometric Determination of Fenoxaprop-P-Ethyl using Charge Transfer Complex

After the preliminary reaction, it was clear that fenoxaprop-p-ethyl could be hydrolyzed and the hydrolyzed product could be determined using spectrophotometric method after complexing with metal ion and formation of the charge transfer complex. For quantitative determination it is important that each step of the proposed reaction should go toward completion. For this it was important to optimize various parameters involved in these reactions and determination.

3.4.3.1: Investigation of suitable wavelength for the spectrophotometric determination of fenoxaprop-p-ethylusing charge transfer complex

Apparatus: The same as given before.

Reagents: The same as given before.

Solutions: The same as given before.

Procedure

From 100 $ugmL^{-1}$ stock solution of fenoxaprop-p-ethyl 5 mL was taken in a 100 mL volumetric flask. To this solution 5 mL of $FeSO_4$ (1000 $ugmL^{-1}$) and 10 mL of acetate buffer (pH-10) was added. The resulted yellow color charge transfer complex was diluted in 10 mL ethanol. The absorbance was measured from 350 to 460 nm using UNICO UV-2100 spectrophotometer, for finding out the optimum wavelength. Each wavelength was calibrated with blank solution. The results are given in Table- 3.4.1 and are shown in figure 3.4.3.

Table 3.4.1 **Optimization of wavelength for the extractive spectrophotometric determination of fenoxaprop-p-ethyl using charge transfer complex**

S.NO	Wave length (nm)	Absorbance
1	350	0.052
2	360	0.072
3	370	0.090
4	380	0.114
5	390	0.133
6	400	0.141
7	410	0.142
8	420	0.143
9	430	0.150
10	440	0.106
11	460	0.078

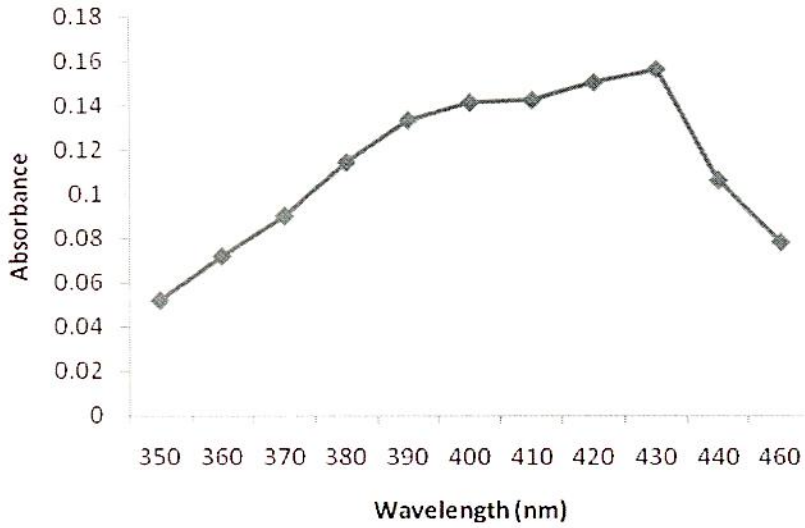


Figure 3.4.3 Optimization of wavelength forthe spectrophotometric determination of fenoxaprop-p-ethyl using charge transfer complex

Conditions:

Wavelength	varied
Volume of Fe (II) (1000 μgmL^{-1})	5mL
Volume fenoxaprop-p-ethyl(1000 μgmL^{-1})	5 mL
Volume of Acetate buffer (pH 10)	10 mL
Ethanol	10 mL

After hydrolysis the first step was maintenance of pH therefore amount of buffer was optimized. For this 10 mL fenoxaprop-p-ethyl from 100 $ugmL^{-1}$ stock solution was taken in 5 separate volumetric flasks. To each flask 10 mL of ferrous sulphate (1000 ug mL^{-1}). 5 mL solution of acetate buffer of different pH from 3.6 to 5.2 and 5 mL of ethanol was added. The resulted yellow color charge transfer complex was shacked well for 5 minutes for complete extraction. The absorbance of the complex was measured at 430 nm using UNICO UV-2100 spectrophotometer. The results are given in Table 3.4.2 and are shown in figure 3.4.4

For finding out optimum volume of buffer different volume of pH-4 acetate buffer ranging from 1 to 12 mL were added and for complex formation the above mentioned procedure was used. The results are given in table 3.4.3 and are shown in figure 3.4.5.

For the investigation of ferrous sulphate concentration for the spectrophotometric determination of Fenoxaprop-p-ethyl. Fenoxaprop-p-ethyl standard 5 mL from 10 μgmL^{-1} solution was taken into five separate volumetric flasks. To these 10 mL of Fe (II) solution of varied concentration in the range of 50 to 300 $\mu g\ mL^{-1}$ was added followed by addition of optimum volume of acetate buffer. The resulting complex was dissolved in 10 mL ethanol. The absorbance of the complex was measured at 430 nm. The results are given in Table 3.4.4 and are shown in figure 3.4.6.

For kinetic studies of the charge transfer complex of fenoxaprop-p-ethyl with Fe (II) 5 mL standard solution (3×10^{-10}M) was mixed with 10 mL of Fe (II) (3×10^{-10}M) solution. Optimum volume (1mL of pH 4 acetate buffer) was added to form complex followed by addition of ethanol. The absorbance was noted at different time interval after extraction ranging from 5 to 25 minutes. The results are given in Table 3.4.5 and are shown in figure 3.4.7.

Table3.4.2 Optimizationof acetate buffer pH for the spectrophotometric determination of fenoxaprop-p-ethyl charge transfer complex

S.NO	Acetate buffer pH	Absorbance
1	3.5	0.180
2	4.0	0.380
3	4.5	0.303
4	5	0.148
5	5.5	0.081
6	6	0.079

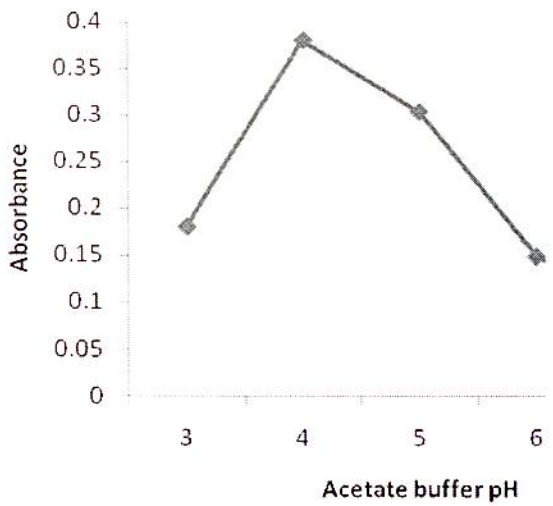


Figure 3.4.4 Optimizationof acetate buffer pH for the spectrophotometric determination of fenoxaprop-p-ethyl charge transfer complex

Table 3.4.3 Volume optimization of acetate buffer for the spectrophotometric determination of fenoxaprop-p-ethyl using charge transfer complex

S.NO	Volume of buffer	Absorbance
1	1	0.251
2	2	0.240
3	4	0.210
4	6	0.224
5	8	0.152
6	12	0.146

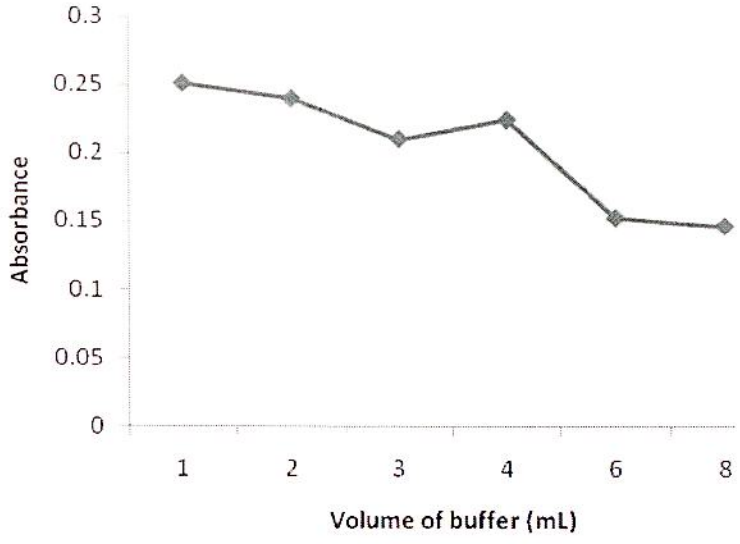


Figure 3.4.5 Volume optimization of acetate buffer for the spectrophotometric determination of Fenoxaprop-p-ethyl using charge transfer complex

Table 3.4.4 Optimization of Fe (II) Concentrations for the spectrophotometric determination of fenoxaprop-p-ethyl using charge transferring complex

S.NO	Fe(II)(μgmL^{-1})	Absorbance
1	50	0.336
2	100	0.625
3	150	0.429
4	200	0.426
5	250	0.420
6	300	0.420

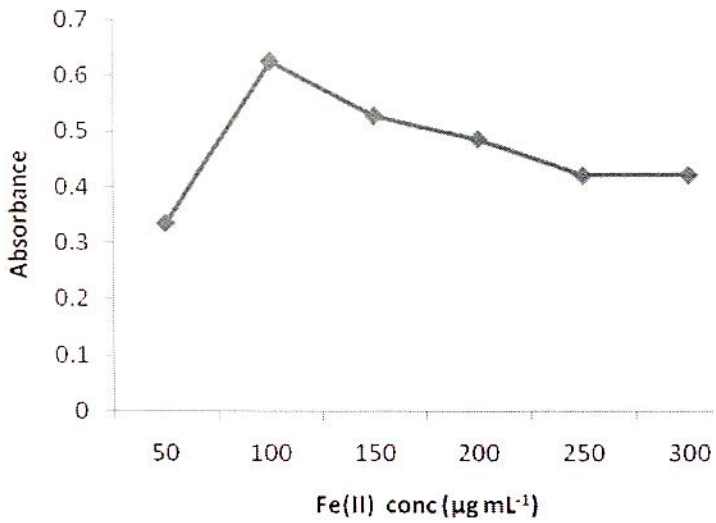


Figure 3.4.6 Optimization of Fe (II) concentration for the extractive spectrophotometric determination Fenoxaprop-p-ethyl using charge transfer complex

Table 3.4.5 kinetic studies of the charge transfer complex formed between fenoxaprop-p-ethyl and Fe (II)

Time (min)	Absorbance	Time (min)	Absorbance
0	0.036	11	0.028
1	0.034	12	0.027
2	0.033	13	0027
3	0.032	14	0.027
4	0.031	15	0.026
5	0.030	16	0.026
6	0.029	17	0.026
7	0.029	18	0.026
8	0.029	19	0.025
9	0.028	20	0.025
10	0.028		

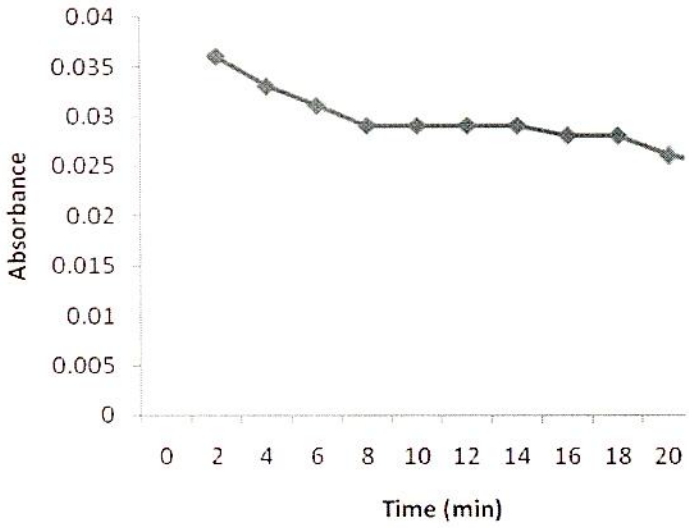


Figure 3.4.7 kinetic studies of the charge transfer complex formed between fenoxaprop-p-ethyl and Fe (II)

3.4.3.2 Determination of formation constant for the charge transfer complex of fenoxaprop-p-ethyl using Job's method.

For finding out the composition of charge transfer complex, equi molar solutions that (3×10^{-3}M) of fenoxaprop-p-ethyl and ferrous sulphate were prepared. The ratio of fenoxaprop-p-ethyl to ferrous sulphate was varied in the range from 1:9 to 9:1 followed by the addition of optimum volume of buffer. The absorbance of the complex was measured at 430 nm with respective blank using UNICO UV-2100 spectrophotometer. The results are given in table 3.4.6 and are shown in fig 3.4.8. The formula of the complex was determined using Job's method of continuous variation. After finding the formula of complex, stability constant (K_f) was calculated using the following formula.

$K_f = [ML_2]/[M]\,[L]^2$

Where K_f = Formation constant of complex formed.

$[ML_2]$ = Conc., of complex formed

$[M]$ = Conc. of metal ions

$[L]^2$ = Conc. of ligand

The Kf value calculated using the formula is 8.80×10^9

Table 3.4.6 Determination of formula of the charge transfer complex for fenoxaprop-p-ethyl using Job's method

S.NO	Volume of Fe(II) (mL)	Volume of Fenoxaprop-p-ethyl (mL)	Absorbance
1	1	9	0.11
2	2	8	0.15
3	3	7	0.30
4	4	6	0.27
5	5	5	0.21
6	6	4	0.19
7	7	3	0.15
8	8	2	0.11
9	9	1	0,09

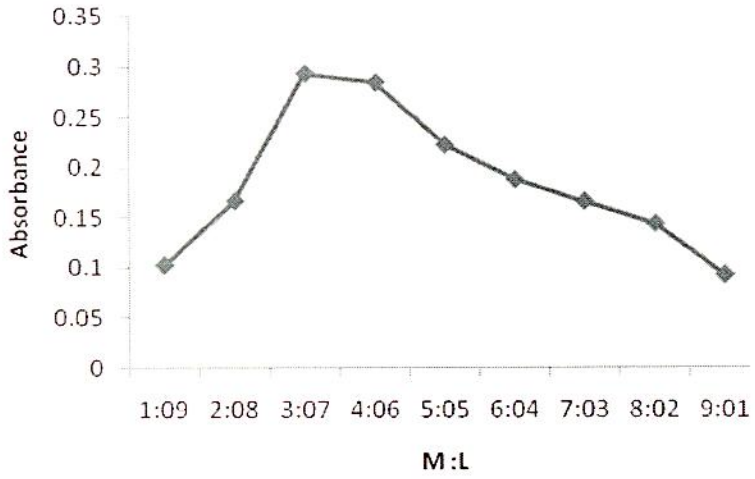


Figure 3.4.8 Determination of formula of the complex using job's method

Conditions

Fenoxaprop-p-ethyl concentration	3×10^{-5} M
Volume of Acetate buffer (pH 4)	1 mL
Concentration of FeSO4	3×10^{-5} M
Volume of ethanol	10 mL
Wavelength	430 nm

3.4.3.3 Verification of Beer's law and determination of the limit of detection and limit of quantification for the spectrophotometric determination of fenoxaprop-p-ethyl

Apparatus: The same as given before.

Reagents: The same as given before.

Solutions: The same as given before.

Procedure:

To calculate limit of detection (LOD) and limit of quantification (LOQ) six replicate solution of fenoxaprop-p-ethyl with minimum absorbance (0.2 μgmL^{-1}) was taken and color was developed using optimum conditions. The absorbance was measured at 430 nm using UNICO UV-2100 spectrophotometer. LOD, LOQ, Molar absorptivity and other statistical parameters were calculated by using the following formula.

LOD and LOQ were calculated using the following formula.

Limit of detection (for concentration) = $3 \times S$

Limit of quantification (for concentration)=$10 \times S$

Where S = Standard Deviation

Relative standard deviation (RSD) was calculated by using the following formula.

$$RSD = \frac{\text{Standard deviation}}{\text{Mean}} \times 100$$

Molar absorpitivity (ε) = A / C. L

Where A= absorbance, C stands for concentration (mol L^{-1}) and L stands for path length in centimeters.

Table 3.4.7 **Verification of Beer's law for determination of fenoxaprop-p-ethyl using charge transfer complex**

S.NO	Fenoxaprop-p-ethyl (μgmL^{-1})	Absorbance
1	2	0.106
2	4	0.156
3	6	0.234
4	8	0.263
5	10	0.311
6	12	0.346
7	14	0.421
8	16	0.454
9	18	0.492
10	20	0.521

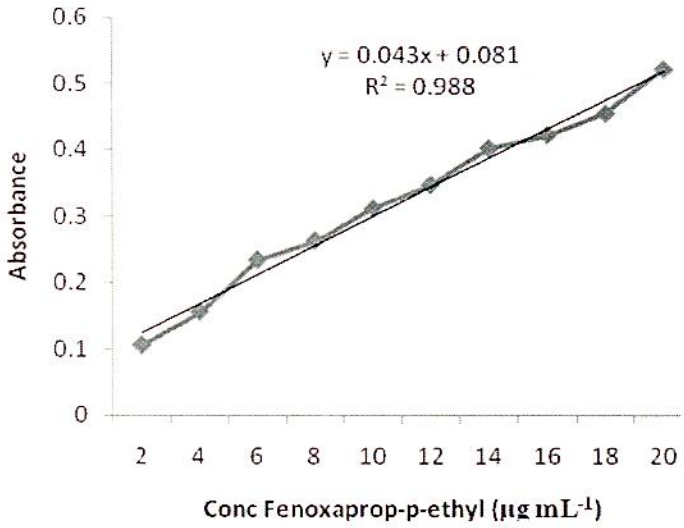


Figure 3.4.9 Verification of Beer's law for determination of fenoxaprop-p-ethyl using charge transfer complex

Table 3.4.8 **Verification of Beer's law at lower concentration for the determination of fenoxaprop-p-ethyl using charge transfer complex**

S.NO	Fenoxaprop-p-ethyl conc. (μgmL^{-1})	Absorbance
1	0.1	0.009
2	0.2	0.013
3	0.4	0.023
4	0.6	0.037
5	0.8	0.049
6	1	0.068

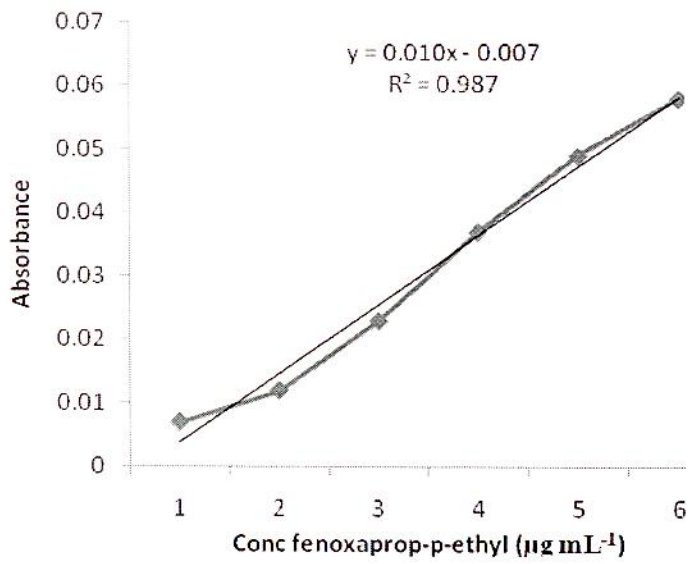


Figure 3.4.10 Verification of Beer's law at lower concentration for determination of fenoxaprop-p-ethyl using charge transfer complex

3.4.4 Extraction from real samples

Extraction from soil

For the analysis of fenoxaprop-p-ethyl in soil, 10 g of soil was air dried, passed through a 2 mm sieve and homogenized. Fortified samples were prepared by adding 5, 10 and 15 μg $g^{-1}$of standard fenoxaprop-p-ethyl solution to soil sample. After fortification the samples were kept at room temperature for 4.0 h to allow evaporation of the solvent. Then 20 mL of acetone added to each sample and sonicated for 30 min at ambient temperature. After sonication the samples were filtered on filter paper. The filtrate was collected in flask and was evaporated up to dryness and then reconstituted in 10 mL of acetone and diluted up to mark and analyzed by the proposed method

Extraction from grains samples

Fortified grain samples were prepared by adding 5, 10 and 15 μg $g^{-1}$of standard fenoxaprop-p-ethyl solution to of barley grains and wheat. After fortification the samples were kept at room temperature for 6.0 h to allow evaporation of the solvent. Then 20 mL acetone was added to each sample and the sonication extraction procedure was used as mechanical for soil samples. The results are given in table 3.4.9.

3.4.5 Application of the investigated methods for the analysis of Fenoxaprop-P-Ethyl in Commercial Formulation and in real samples

To check the validity and applicability of the investigated method it was important to apply it on available commercial formulations. For this purpose commercially available samples of fenoxaprop-p-ethylwere purchased from local market.

1-4 mL of fenoxaprop-p-ethyl formulation was taken in four separate 50 mL volumetric flasks and dissolved in 25 mL acetone and diluted up to mark with distilled water. From each solution 5ml were taken in four separate 50 mL volumetric flasks and color was developed in the same way as mentioned before. The absorbance of the colored solution was measured at 430 nm using

the calibration plot (absorbance vs. concentration) the concentration of fenoxaprop-p-ethyl was calculated. The results are shown in Table 3.4.10

Table 3.4.9 % Recoveries of fenoxaprop-p-ethyl from real samples

Real samples	Taken (g)	Added (ug mL^{-1})	Found (ug mL^{-1})	% Recovery
Soil	10	5	4 .7	94.00±0..09
		10	8.92	89.90 ±0..09
		15	13.5	90.20 ±0..01
Barelygrain	10	5	4.71	95.00 ±.017
		10	9.32	93 00±.0.11
		15	14.21	94 ±.0 .009
Wheat grain	5	10	9.62	96.00 ±.021
		15	14.73	98.00 ±0.30
		20	18.87	94.30 ±0 .92

Table 3.4.10 Concentration of fenoxaprop-p-ethyl in commercial formulation.

Commercial formulation	Label Amount (g mL^{-1})	Proposed method (g mL^{-1})
Active ingredient	7.5	7.8±0.31

Residue determination

For real samples homogenized food sample (10 g) was taken in a flask and 50 mL of acetone was added to it. Each sample was sonicated for 30 min at ambient temperature. After sonication the samples were filtered on filter paper. The filtrate was collected in flask and was evaporated up to dryness and then reconstituted in 10 mL of acetone and diluted up to mark and analyzed by the proposed method. Each sample was analyzed in triplicates. The results are given in table 3.4.11

Table 3.4.11 Residue level of fenoxaprop-p-ethyl in real samples

S.NO	Sample	Residue (µg g^{-1})
1	Barley grains	2.7±0.318
2	Wheat grains	1.5±0.35
3	Wheat flour	0.19±.054

Results and discussion

Fenoxaprop-p-ethyl on acid hydrolysis at different condition gave the product 6-chloro-2, 3-dihydrobenzoxazol-2-one (CDHB) and ethyl 2-(4-hydroxyphenoxy) propionate (EHPP). The reaction is based on complexing of fenoxaprop-p-ethyl with Fe (II). Its λ_{max} was found to be 430 nm (table 3.4.1 and fig.3.4.3).

The influence of various experimental parameters on the absorbance of the charge transfer complex of fenoxaprop-p-ethyl with Fe (II) was studied. It was observed that the solution acidity has a great effect upon the reaction of Fe (II) with fenoxaprop-p-ethyl. Acetate buffer was selected for maintaining the pH and pH optimization studies were carried out in the range of 3.5 - 5.5 pH. Maximum charge transfer complex formed at pH 4.0. The higher or lower pH value caused a decrease in the absorbance value (Table 3.4.2 & 3.4.3 and fig 3.4.4 & 3.4.5). The volume of pH 4 acetate buffer was also optimized from 1-12 mL. One mL was found to be the best for completion of the reaction. The effect of concentration of $FeSO_4$ was studied using different volumes of 0.01 M solution in the range of 0.5-3.0 mL. The highest absorbance was using 3 mL (Table 3.4.4 and fig 3.4.6). The charge transfer complex of fenoxaprop-p-ethyl with Fe (II) was formed at room temperature and was stable for 30 mints (table 3.4.5 and fig 3.4.7) Composition of charge transfer complex was investigated by applying Job's method of continues variations. The plot reached a maximum value at mole fractions of 2.66: 6.67, which indicated the formation of 1:2 complexes. The proposed reaction path way is shown in scheme 1 (fig 3.4.2). The stability constant (k_f) of the charge transfer complex was calculated from the continuous variation plot and found to be 8.80×10^9.

The effect of absorbance verses concentration for fenoxaprop-p-ethyl was investigated using charge transfer complex and a linear behavior was observed in the range 0.2-12 $\mu g\ mL^{-1}$. Statistical parameters were calculated and are given in the table 3.4.11 .The linearity of calibration graphs was proved by the high value of correlation coefficient (r) 0.987. The molar absorptivity of the resulting colored charge transfer complex was found to be 1.75×10^4 L mol^{-1} cm^{-1}.The limit of detection and quantification were calculated and found to be 0.07 $\mu g\ mL^{-1}$ and 0.23 $\mu g\ mL^{-1}$ respectively.

The validity of the proposed method was tested for the determination of fenoxaprop-p-ethyl in real samples. Standard addition technique was applied to test the reliability and recovery of the

proposed method in which known concentration of fenoxaprop-p-ethyl was added to the previously analyzed portion of commercial preparations. The results are given in table 3.4.9. The percent recoveries were found to be in the range of 89.9±0.09–.98.0±0.30 %.

For further confirmation of fenoxaprop-p-ethyl it was determined in commercial formulations (Table 3.4.10). The proposed method is sensitive; therefore it could be easily applied for routine analysis in pure form and in commercial preparations.

EXPERIMENTAL- 2

DETERMINATION OF DINITROANILINE AND ARYLOXYPHENOXY GROUP OF HERBICIDES USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

3.5 DETERMINATION OF DINITROANILINE GROUP OF HERBICIDES USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

The herbicide selected from dinitroaniline group for HPLC studies was pendimethalin. The chemical structure of pendimethalin is given below in figure 3.5.1.

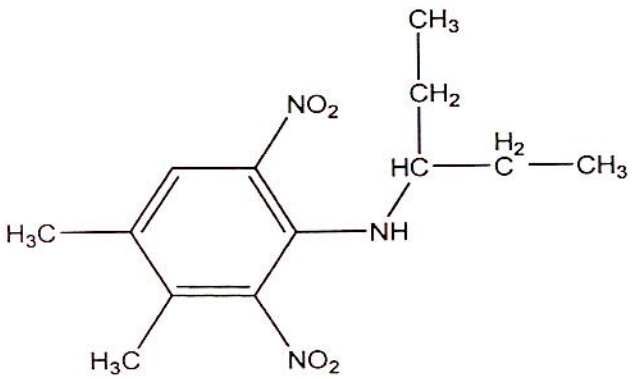


Figure 3.5.1 Chemical structure of Pendimethalin

As pendimethalin is soluble in polar solvents, therefore reverse phase assembly was set up for its determination.

3.5.1 Investigation of suitable parameters for the HPLC determination of Pendimethalin

Instrument

The HP series 1100 LC system (Perkin Elmer Series 200 CA, USA) equipped with gradient pump (Model 600), an auto sampler (Model 717 plus) with 20 µL injection loop and ultraviolet detector at 240 nm was used. Zorbax SB- C_8 (80 A^0, 4.6 X 150 mm, 5mm particle size) was used as a separation column with a Zorbax C_{18} (4x4 mm) as a guard cartridge. Microwave extractions were performed with KEN ST/SS25 KENWOOD (China) microwave with temperature control system.

Reagents and Chemicals

Pendimethalin, N-(1-ethylpropyl)-2, 6- dinitro-3–4 xylidine 99 % was purchased as standards from Dr.Ehrenstofer GmbH, Germany.

HPLC-grade acetonitrile and ethanol were obtained from Rathburn, Walkerburn, UK. LC grade water was used by purifying distilled water with a Milli-Q water purification system (Millipore Co, USA). All other chemicals used were of analytical- reagent grade purity obtained from Merck (Darmstadt, Germany).

Standard pendimethalin solution (1000 $\mu g\ mL^{-1}$)

Stock solution of pendimethalin was prepared by dissolving 10 mg of pendimethalin in 10 mL of acetonitrile and diluted with acetonitrile. Working standards were prepared from this stock solution in range of 2-40 $\mu g\ mL^{-1}$ by dilution with acetonitrile.

All physical parameters such as wavelength, flow rate, injection volume, solvent elution mode, and time for elution and chemical parameters as solvents, composition of solvents for gradient elution were optimized for the HPLC determination of pendimethalin.

The choice of wavelength is based on where the compounds of interest have the best response and interfering compounds have the lowest response. One of the advantages of the UV detector is that it can simultaneously collect different chromatograms at different wavelengths during a single run. Wavelength optimization studies were conducted in the wavelength range of 220-280 nm and 240 nm was found as optimum.

For optimization of flow rate of mobile phase flow rate was varied from range 0.5 mL min^{-1} to 2 mL min^{-1} and 1 mL min^{-1} was chosen as optimum

Similarly Injection volume was optimized in range 0.5µL-20 µL, and 20 µL was selected as the optimum injection volume.

All the optimized parameters are presented in table 3.5.1 and were used for subsequent analysis.

Table3.5.1 Optimum conditions for HPLC for determination of pendimethalin using reverse phase HPLC

Parameter	Optimized volume (gradient elution)		
Eluents used	Mobile phase 1 H_2O	Mobile phase 2 Acetonitrile	Time (min)
	80	20	5
	50	50	7
	0	100	10
Flow rate ($mLmin^{-1}$)	1		
Injection Vol.(µL)	20		
Wave length	240nm		

3.5.2 Construction of Calibration Plot for the determination of Pendimethalin using reverse HPLC

Instrument: **The same as mentioned before**

Reagents: **The same as mentioned before**

Solution: **In different concentration range.**

From standard stock solution of pendimethalin (100µg mL^{-1})working standard solutions were prepared in concentration range of 5.0 µgmL^{-1} to30 µg mL^{-1} using acetonitrile for dilution. HPLC system was equilibrated with the mobile phase prior to analysis. After equilibration

solutions were injected one by one and the peak height was noted for each concentration. Accuracy and precision of method were determined on three replicates. A calibration plot of peak hight vs. concentration was constructed and is shown in figure 3.5.2. Based on IUPAC recommendation points were considered to be in the linear range if their values did not differ by more than 5% from the slop. The linearity of the curve was verified by the correlation coefficient of 0.995.

The limits of detection (LOD) and limits of quantification (LOQ) were calculated from minimum concentration for which a proper peak was observed using the following formula.

LOD = 3s/b and LOQ = 10s/b

Where b is slope of the curve

The LOD and LOQ were found to be 0.059 μgmL^{-1} and 0.17 μgmL^{-1} respectively.

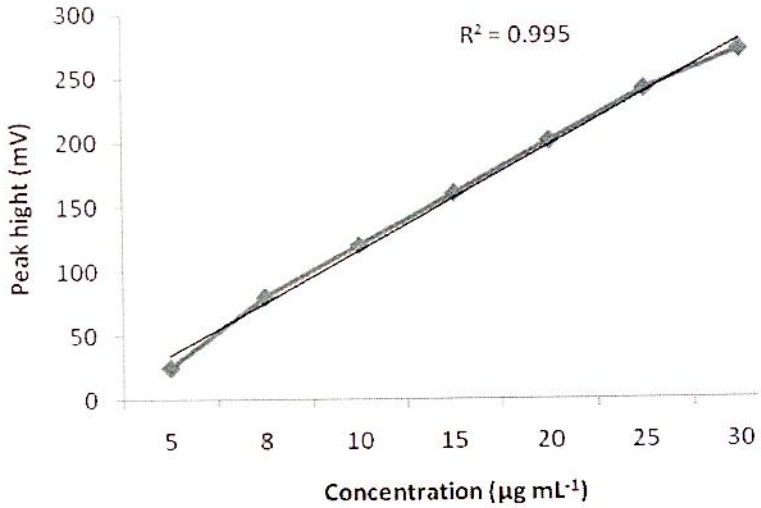


Figure 3.5.2 Calibration plot for pendimethalin determination using HPLC

3.5.3 Optimization of extraction procedure for determination of Pendimethalin using reverse phase HPLC.

After optimization of instrumental and physical parameters it was important to optimize extraction procedure for pendimethalin. For this purpose the effects of various experimental parameters of mechanical extraction and microwave extraction (MASE) were studied. After optimization of instrumental and physical parameters it was important to optimize extraction procedure for pendimethalin. For this purpose the effects of various experimental parameters for and microwave extraction (MASE) were studied. Parameter optimized were solvent type, ratio of mixtures of solvents, MASE time, and temperature.

Preliminary experiments were performed to select the optimum operation conditions for the microwave-assisted solvent extraction of pendimethalin. The parameters like temperature, time and solvent type for extraction were optimized

Pendimethalin is partially soluble in water and highly soluble in acetone ethanol and methanol. These solvents were used for extraction of pendimethalin from soil, vegetables and water samples. . The results are given in table 3.5.3 and are shown in Fig 3.5.3For investigation of the effect of temperature for MASE on the extraction efficiency of, extractions pendimethalin were carried out at 25, 40, 60, 80 and 100 °C, respectively. The results of extraction recoveries are shown in (Fig 3.5.4)Similarly for the effect of extraction time, extraction of spiked samples was carried out at 60 °C for 3, 5, 7, 9 and 11 min. using acetone as the extraction solvent(Fig 3.5.5).

Similarly the volume of optimum solvent was optimized for maximum extraction. The results are shown in fig (3.5.6) The % Recoveries in the range of 60-78% were obtained with mechanical extraction which was improved to 920-98% by microwave assisted solvent extraction in different samples.

Effect of pH on extraction efficiency of pendimethalin was investigated in the range of 3-11 and the result are shown in fig (3.5.7)

The influence of different parameters and their interactions on the % recoveries were studied according to the 2^3 factorial designs (state- ease). A 2^3 factorial design with a complete repetition

performed in one run resulting in a total of 8 experiments was chosen for extraction conditions optimization and maximum % recovery of pendimethalin from soil and vegetable samples.

The ANOVA results are given table 3.5.3 Three-dimensional response surface (RSM) plots of % recoveries versus main factors (AB and BC) for extraction and HPLC study based on experimental model (fig 3.5.7 and 3.5.8)

Table 3.5.2 Results of the ANOVA for selected factorial model for HPLC determination of Pendimethalin

Source	Sum of Squares	df	MeanSquares	F Value	p-value Prob > F	
A-Temperature	1027.00	6	171.17	342.33	0.0413	
B- Time	40.50	1	40.50	81.00	0.0172	
C-Solvent volume	684.50		684.50	81.00	0.0704	significant
AB	40.50	1	40.50	361.00	0.0704	
AC	40.50	1	40.50	81.00	0.0335	
BC 1	80.50	1	180.50		0.0704	
ABC	40.50	1	40.50			
Residual	0.50	1	0.50			
Cor Total	1027.50	7				

Response%Recovery

Conclusion

Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case BC (Solvent volume and time for heating) are significant model terms.

Std. Dev.	0.71	R-Squared	0.9995
Mean	83.75	Adj R-Squared	0.9966
C.V. %	0.84	Pred R-Squared	0.9689

The "Pred R-Squared" of 0.9689 is in reasonable agreement with the "Adj R-Squared" of 0.9966.

Final Equation in Terms of Coded and actual Factors:

%Recovery =+83.75+2.25+9.25-2.25-2.25+4.75+2.25=98

* A * C * A * B * A * C * B * C * A * B * C= 98

Table 3.5.3 Investigation of suitable solvent for pendimethalin extraction

S.No	Solvent	% Recovery
1	Water	52
2	Ethanol	60
3	n-Hexane	68
4	Acetic acid	70
5	Acetonitrile	81
6	Acetone	92

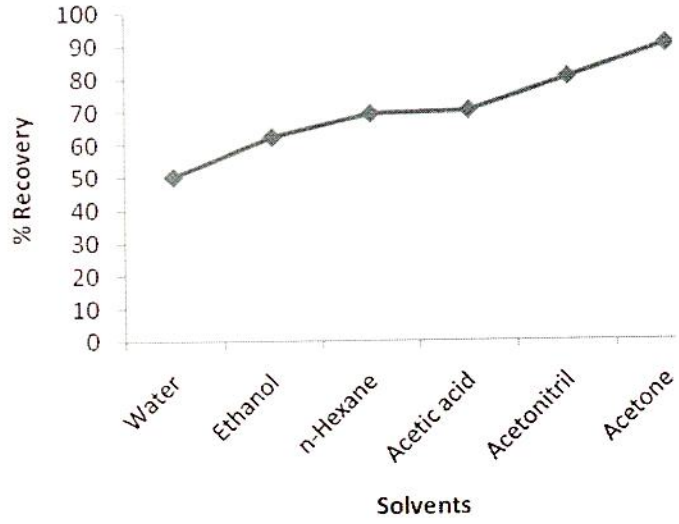


Figure 3.5.3 Investigation of suitable solvent for pendimethalin extraction

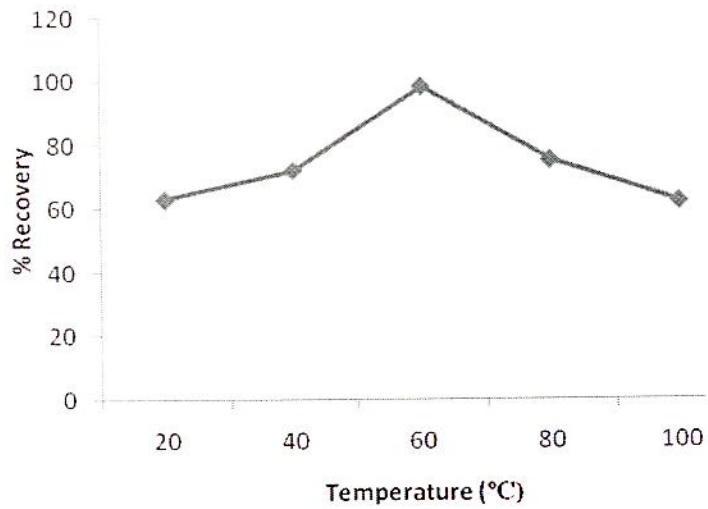


Figure 3.5.4 Optimization of temperature using MASE for maximum % recovery of pendimethalin

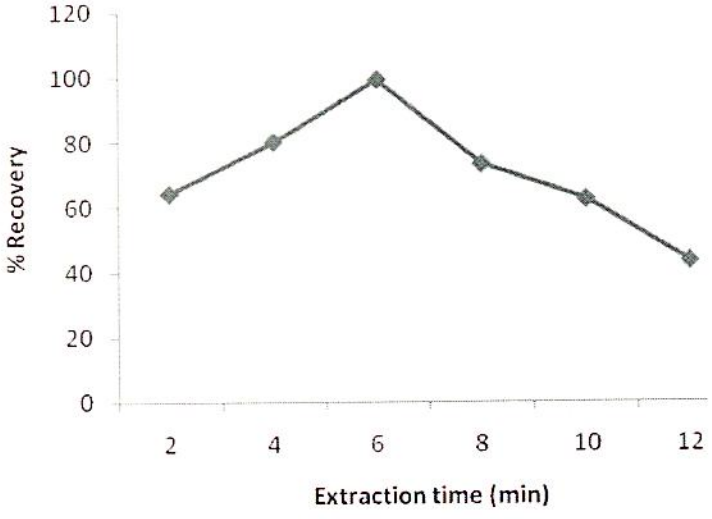


Figure 3.5.5 Optimization of extraction time for MASE

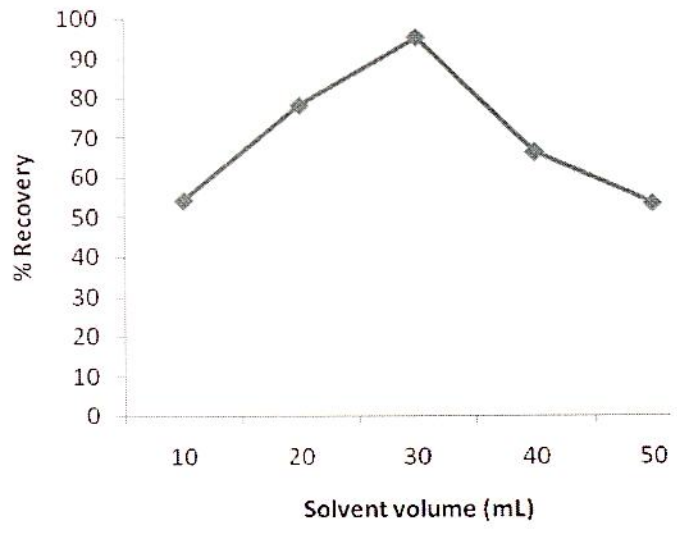


Figure 3. 5.6 Investigation of suitable volume of solvent for pendimethalin extraction

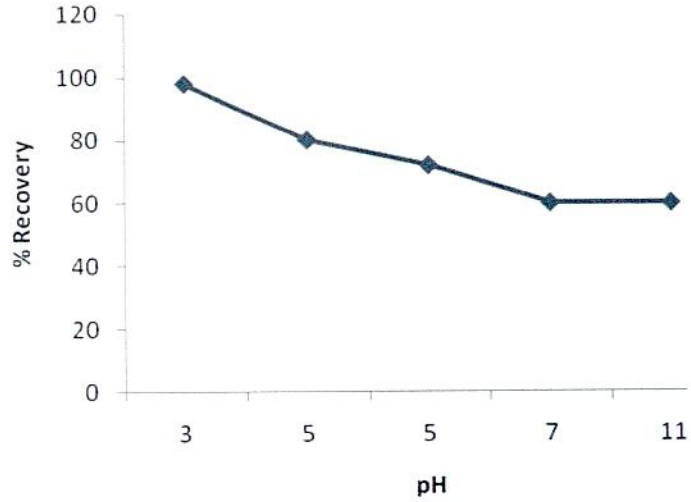


Figure 3.5.7 Effect of pH on % recovery of pendimethalin

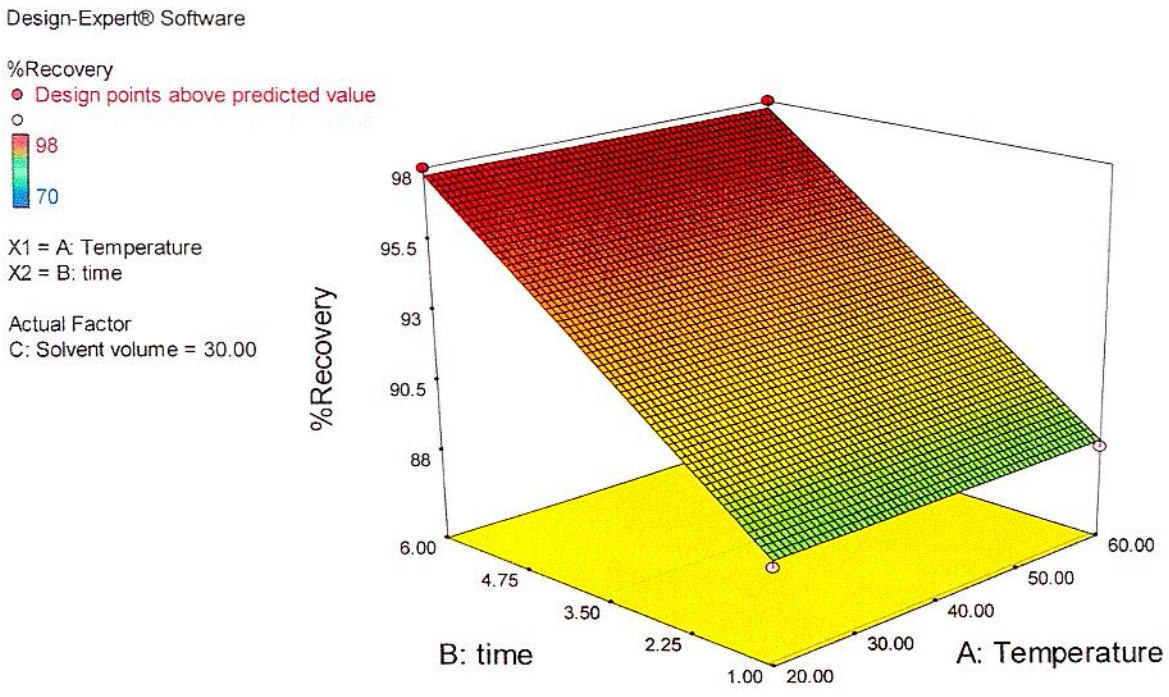


Figure 3.5.8 Response surface plot optimization of temperature and time for maximum %recovery of pendimethalin

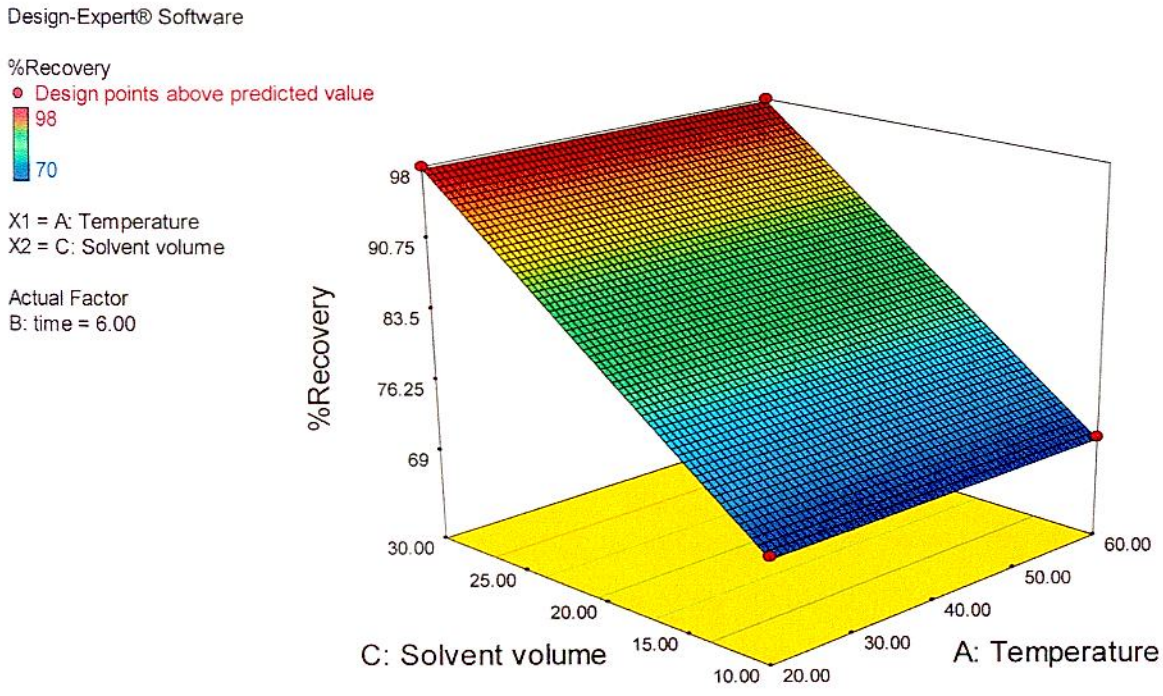


Figure 3.5.9 Response surface plot optimization of temperature and solvent volume for maximum % recovery of pendimethalin

3.5.4 Determination of Pendimethalin in real samples by using Optimized Extraction Procedure

Instrument: The same as mentioned before

As the method investigated is meant for application to real samples, therefore it was important to optimize different extraction parameters for real samples like soil, vegetable and water and check the % recovery from these samples at optimum conditions. For this purpose extraction of solvent and extraction procedures were optimized for these samples. Extraction procedure i.e. mechanical, microwave and sonication were compared for this purpose.

Extraction from samples

For the analysis of pendimethalin in soil, 10 g of soil was air dried, passed through a 2 mm sieve and homogenized. Fortified samples were prepared by adding 5, 10 and 15 μg $g^{-1}$of standard pendimethalin solution to soil sample. After fortification the samples were kept at room temperature for 4.0 h to allow evaporation of the solvent and different extraction procedures were used. For mechanical extraction 50 mL of acetone was added to each sample and after absorption the samples were shaken on shaker for 15 min and on equilibration the sample was filtered and the filtrate was evaporated on rotary evaporator near to dryness. Than it was redissolved in 10 mL acetonitrile. The determination of pendimethalin in filtrate was preceded by the proposed method.

For microwave extraction

Samples were fortified in the same way. After fortification 10 mL of acetone was added and to get high extraction rate, microwave rapid heating were used on closed-vessel during a sample extraction procedure. The starting parameters setting in microwave system were 2.0 min at 350 W and 3.0 min at 500 W. Once the extraction program was completed the vessel were cooled down to room temperature before opening the vessel. After extraction the contents were centrifuged for 10 min and filtered. On equilibration the sample was filtered and the filtrate was evaporated on rotary evaporator up to volume of 2 mL then 5mL of methanol was added to it centrifuged and passed through0.45 μm pore.The extract was evaporated near to dryness and redissolved in 10 mL of acetonitrile before HPLC analysis.

Similarly for extraction from vegetable samples, fortified vegetable samples were prepared by adding 5, 10 and 15 μg $g^{-1}$of standard pendimethalin solution to onion and garlic samples. After fortification the samples were kept at room temperature for 6.0 h to allow evaporation of the solvent. The rest of the procedure was the same as mentioned for soil. For microwave extraction vegetable samples (10g) were added to extraction vessels and 10 mL of acetone was added In case of vegetable samples were kept in closed vessels and heated for 2-3min at optimized temperature in a microwave. This was followed by filtration and determination as above. Similarly for water samples the same optimized extraction procedure was applied to 10 mL of water samples.

On equilibration the sample was filtered and the filtrate was evaporated on rotary evaporator up to volume of 2 mL. Then 5 mL of methanol was added to it centrifuged and passed through0.45 μm pore. Filtrate was dried and redissolved in 10 mL of acetonitrile before HPLC analysis. Vegetables samples onion and garlic were homogenized with chopper and fortified with known concentration of pendimethalin solution extracted by the same method as mentioned for soil. For chromatographic analysis the extracts were further filtered through 0.45 μm membrane. The extract was evaporated near to dryness and redissolved in 5 mL of acetonitrile and diluted up to 10 mL.

Analysis of soil, vegetable and water samples followed equilibration and injections of the standards and blanks preceded injections of the sample extracts. The concentration of pendimethalin in final extract was calculated using the following formula.

Concentration (μg g^{-1}) = μg mL^{-1}.Peak hight.Dilution.Total volume of the extract/peak highs for standard.Sample weight.

Where: μg mL^{-1} = concentration of the standard pendimethalin solution.

Peak hight = peak hight of the analyte in the sample

Dilution =Dilution factor, if the sample was diluted prior to analysis.

Peak hight of standard = peak hight of the standard in the sample

Sample weight = weight of the sample extracted

% Recovery = μg g^{-1}concentration of standard x 100/Sample weight

The control samples of soil and onion and water were spiked at three levels (5, 10 and 15 μg g^{-1}) of pendimethalin concentration and were analyzed in replicates. The results are given in Table 3.5.4. Chromatograms of pendimethalin for 10 μgmL^{-1} of standard solution of pendimethalin chromatogram of 10 μgmL^{-1} of standard solution of pendimethalin is shown in fig 3.5.9 .chromatograms for shown for residue in figure 3.5.10 chromatograms5, 10 and 15 μg g^{-1} spiked soil samples in fig 3.5.11 (a,b and,c) chromatogram for 5,10,15 μg mL^{-1} spiked onion samples and figure 3.5.12 (a,b and,c) and chromatograms for spiked samples in water in 3.5.13 (a,b and c)

Table 3.5.4 Percent recovery of pendimethalin from real samples

S.No	Spiked Sample	Added (μgmL^{-1})	Found (μgmL^{-1})		% Recovery	
			ME	MAE	M E	MAE
01	Soil	5	4	4.5	80±0.38	
		10	9	9.7	90±0.41	
		15	12	15.6	80±0.21	
02	Vegetables	5	4	5.2	80±0.38	
		10	9	9.75	90±0.41	
		15	12	15.10	80±0.21	
03	water	5	4.5	5.12.5	90±0.37	
		10	9.5	9.25	105±0.27	
		15	16	14.75	106±0.15	

3.5.5 Application of the investigated methods for the analysis of Pendimethalin in real sample and Commercial Formulation

Instrument: The same as mentioned before

Sample collection and sample preparation

Soil and vegetable sample were collected from a field upon which the herbicide was sprayed as a pilot project and pendimethalin was determined by standard addition method. Five samples of vegetable (10 g each) were taken in separate beakers and spiked with a known concentration of standard pendimethalin solution. These beakers were uniformly shaken on mechanical shaker for proper mixing and kept in microwave with 10 mL of acetone for 6 min for extraction. The herbicide was extracted with 25 mL of solvent (acetone). The extract of acetone was evaporated at room temperature and the residue was dissolved in 10 mL methanol. The solution were filtered through membrane, evaporated to dryness and redissolved in acetonitrile. The residue of herbicide present in the sample was determined from the difference of the concentration of added standard solution and the concentration of herbicide in spiked sample. The results in real samples and formulations are given in tables 3.5.5 and 3.5.6.

Table 3.5.5 Residue level of herbicide found in real sample

S.NO	Samples	Residue μgg^{-1}
1	Soil	1.97 ± 1.24
2	Onion	1.31 ± 2.18
3	Garlic	1.41± 1.26

Table 3.5.6 Concentration of pendimethalin in commercial formulation

Commercial formulation	Label Amount (gmL^{-1})	HPLC Analysis (gmL^{-1})
Active ingredient	0.82 ± 0.46	0.80 ± 0.38

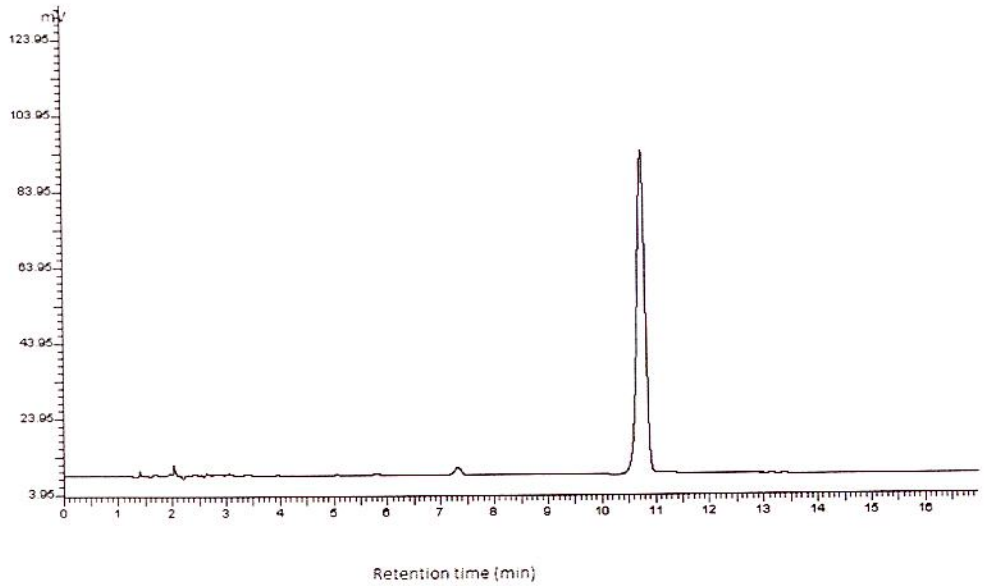


Figure 3.5.9 Chromatogram of standard pendimethalin ($10\mu g\ mL^{-1}$) using reverse phase HPLC

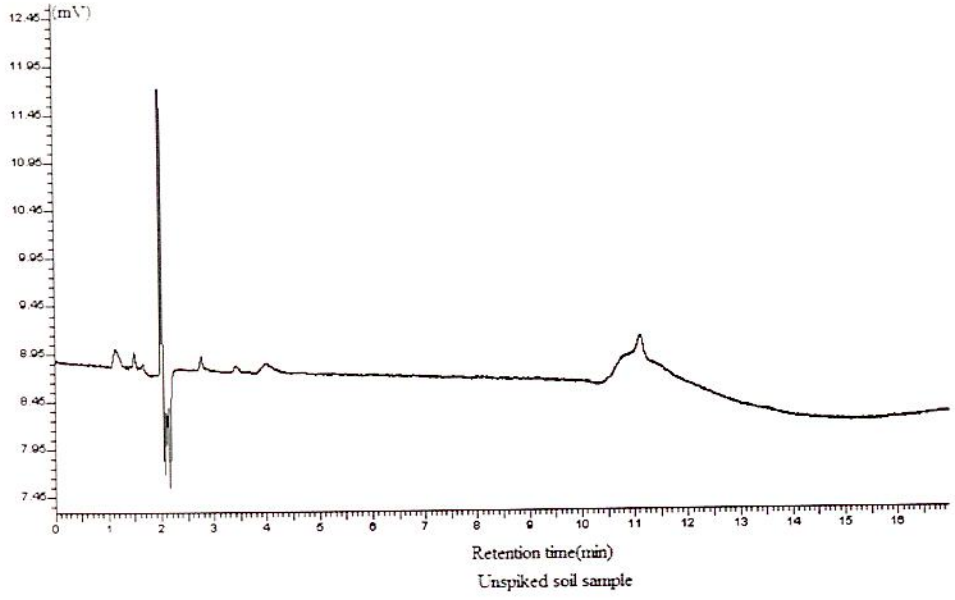


Figure 3.5.10 Chromatogram for un spiked soil samples using reverse phase HPLC

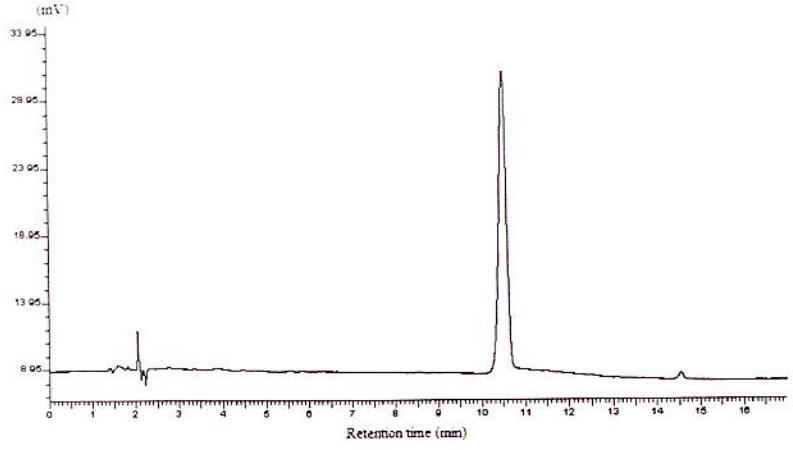


Figure 3.5.11 (a) Chromatogram for 5 μg mL^{-1} fortified soil samples using reverse phase HPLC

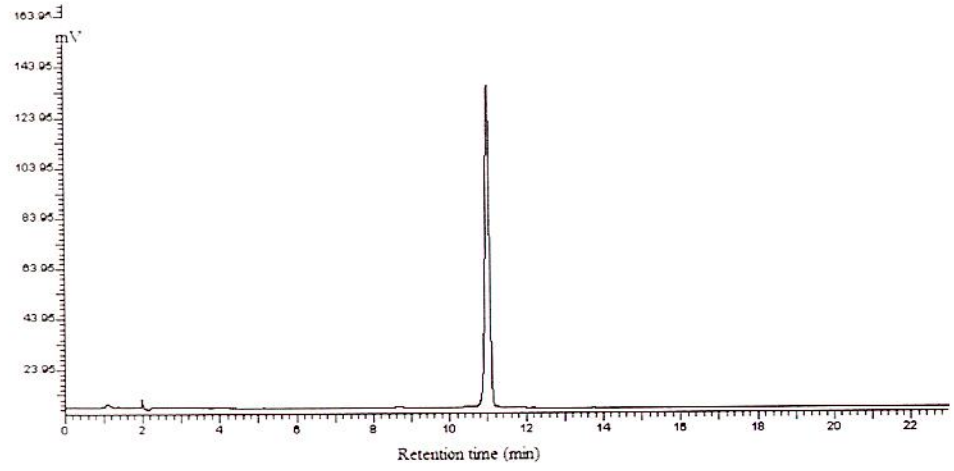


Figure 3.5.11(b) Chromatogram for 10 µg mL^{-1} fortified Soil samples using reverse phase HPLC

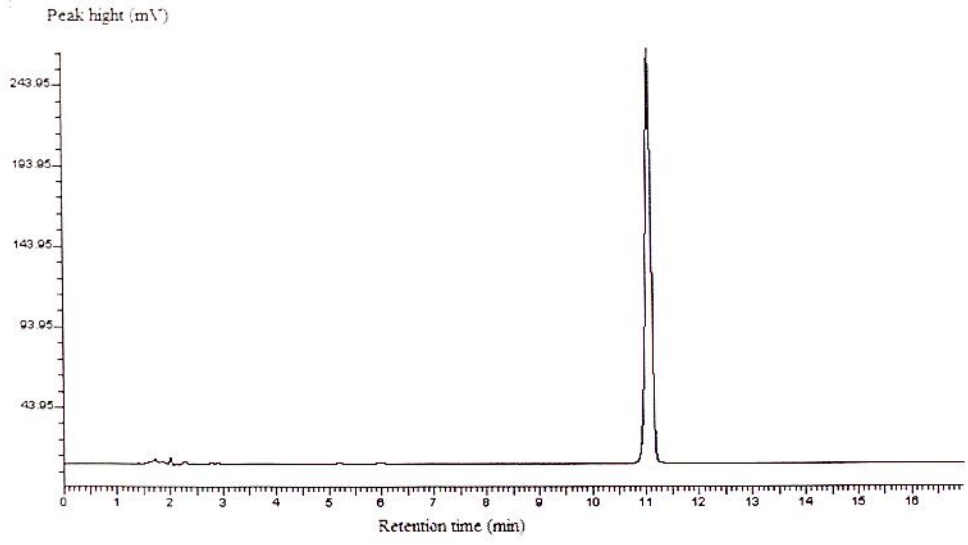


Figure 3.5.11(c) Chromatogram for 15 µgmL^{-1} fortified soil samples using reverse phase HPLC

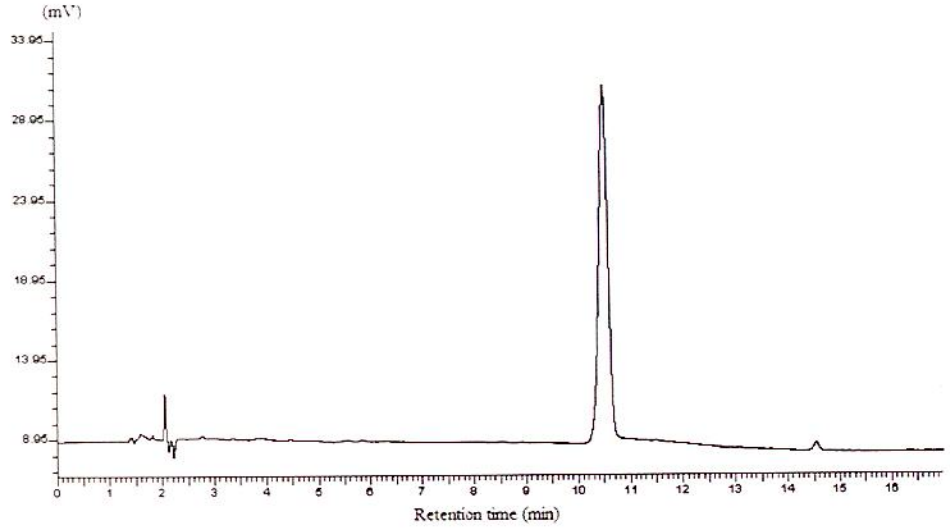


Figure3.5.12.(a) Chromatogram for 5 μgmL^{-1} fortified onion samples using reverse phase HPLC

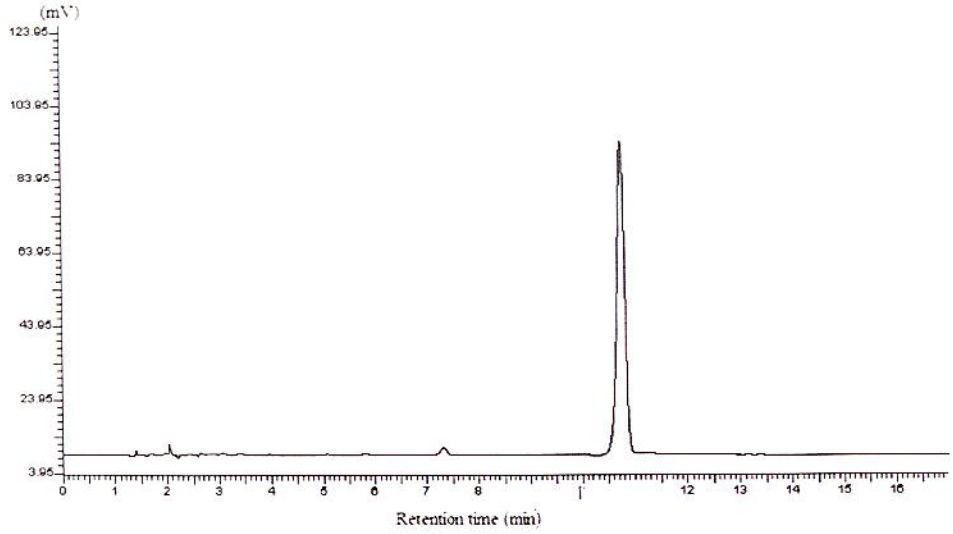


Figure 3.5.12(b) Chromatogram for 10 μgmL^{-1} fortified onion samples using reverse phase HPLC

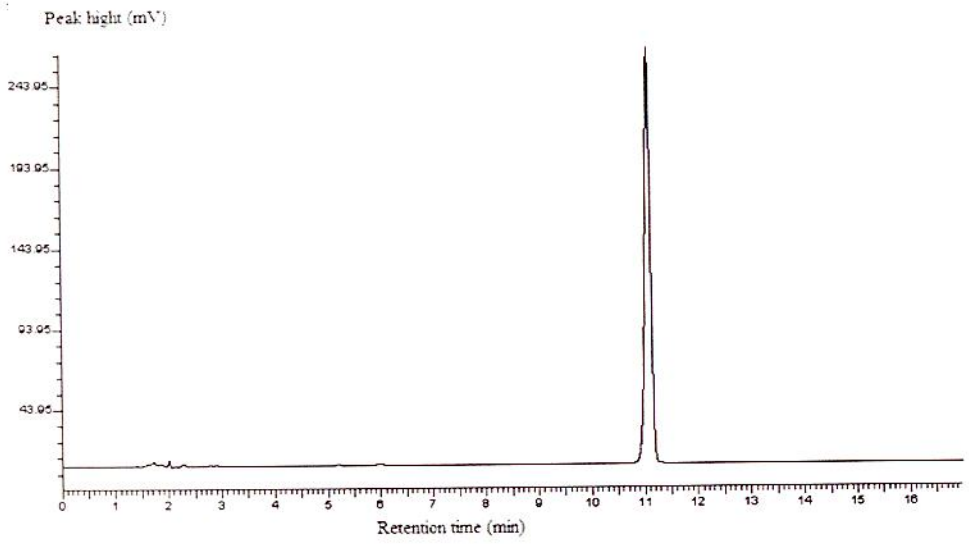


Figure 3.5.12(c) Chromatogram for 15 μg mL^{-1} fortified onion samples using reverse phase HPLC

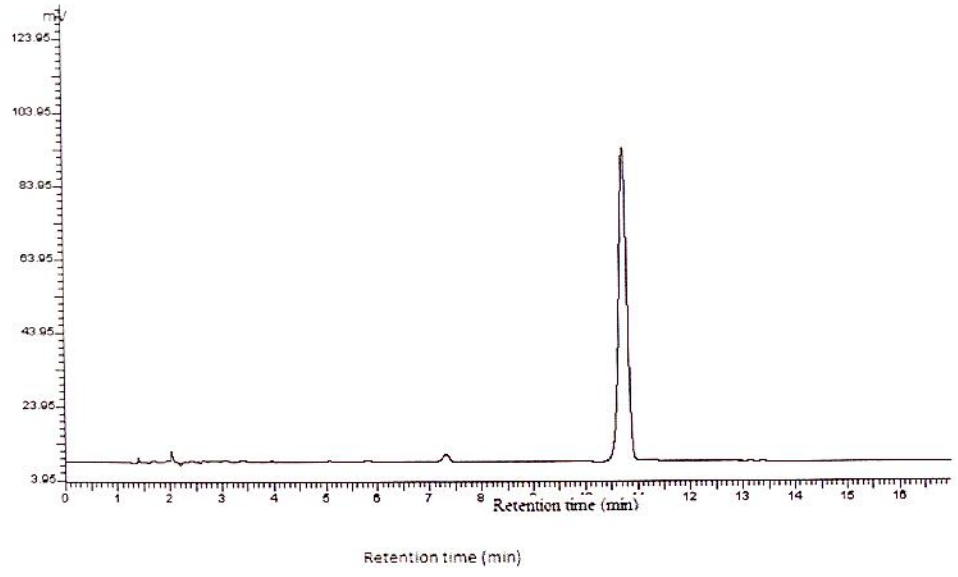


Figure 3.5.13(a) Chromatogram for 8 μgmL^{-1} fortified water samples using reverse phase HPLC

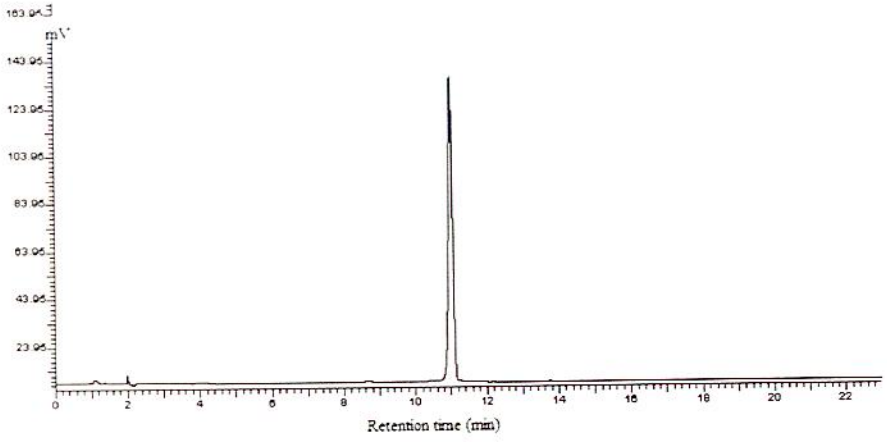


Figure 3.5.13(b) Chromatogram for 10 μgmL^{-1} fortified water samples using reverse phase HPLC

Results and discussion

HPLC method was developed for pendimethalin using reverse phase Zorbax SB-C8 column. Sample injection was 20 µL. The mobile phase consisted of acetonitrile and water. The gradient elution was carried out according to the programme; 5 min at 80% acetonitrile with 20% water and 100% acetonitrile with 0% water for 12 min. The flow rate was kept at 1 ml min^{-1}. The ultraviolet detector was adjusted at 240 nm for absorption measurement.

Preliminary experiments were performed to select the optimum operation conditions for the microwave-assisted solvent extraction of pendimethalin. The parameters like temperature, time and solvent type for extraction were optimized.

Selection of solvent for extraction is important because it affects the percent recoveries of herbicides. Pendimethalin is partially soluble in water and highly soluble in acetone, ethanol and methanol. These solvents were used for extraction of pendimethalin from soil and vegetables. The maximum extraction was achieved with acetone as extraction solvent (Fig 3.5.2). The % recoveries were in the range of 70-78 in mechanical extraction and were improved to 92-97% by microwave assisted solvent extraction in different samples.

The temperature of microwave irradiations control the energy supplied to the sample under analysis. To study the effect of extraction temperature on the extraction efficiency of pendimethalin, extractions were carried out at 25, 40, 60, 80 and 100 °C, respectively. The results of extraction recoveries are shown in Fig 3.5.3. The recovery of pendimethalin increased as the temperature of extraction increased from 25 °C to 60 °C and the recovery decreased above 60 °C. This is probable due to degradation of pendimethalin at higher temperatures.

For optimization of time of extraction spiked samples were extracted at 60 °C after 3, 5, 7, 9 and 11 min. using acetone as the extraction solvent. When the extraction time was increased from 3 min to 11 min the recovery of pendimethalin decreased (Fig 3.5.4). This is due to degradation of pendimethalin caused by increasing irradiation time. Therefore, 3 min irradiation time was selected for MASE.

Factorial design for optimization for determination of pendimethalin using reverse phase HPLC.

The relative importance of different variables and the interaction with each other for HPLC determination of pendimethalin and their impact on % recovery were studied using a 2^3 factorial design. The low and high values of the factors were selected according to the studied factors. For extraction of pendimethalin the effect of different solvents, temperature and heating time were studied. Results for the ANOVA test are shown in table 3.5.3. The most important effects correspond to factor C (solvent volume) and the BC interaction (temperature and heating time interaction) for extraction of pendimethalin and factor A (temperature) and the ABC interaction (solvent volume, temperature and heating time) for extraction and its impact on % recovery was optimized by statistic model .

The % recovery of the empirical model calculated from Table 3.5.3 is 98 which is agreement for higher response. The magnitude of these effects, using analysis of variance (ANOVA) was carried out by using Expert Design software (Stat-Ease factorial design) and the results are summarized in table 3.5.2 The results confirm that B and C are statistically the main factors and are equally significant. These effects show p-values less than 0.05 for optimized studies which indicates that the results are statistically significant at a 95.0 % confidence level.

The results of factorial design are also shown in the figure 3.5.7 (a&b) which is three-dimensional response surface (RSM) plots of % recoveries versus main factors (AB and BC) for

extraction and HPLC study based on experimental model. It is clear that % recovery increases as temperature and time increases.

Calibration curve was obtained at concentration range 2.0-25 μgmL^{-1} using HPLC with ultraviolet detector at 240 nm(fig3.5.7). The linearity of the curve was verified by the correlation coefficient of 0.995. The limits of detection (LOD) and limits of quantification (LOQ) were calculated using the following formula.

LOD = 3s/b and LOQ = 10s/b

Where b is slope of the curve.

The LOD and LOQ were found to be 0.059 $\mu g\ mL^{-1}$ and 0.17 $\mu g\ mL^{-1}$ respectively.

The control samples of soil, water and onion were spiked at three levels (5, 10 and 15 $\mu g\ g^{-1}$) of pendimethalin concentration and were analyzed in triplicate. The results are given in table 3.5.4 and chromatograms are shown. The method validation studies for spiked samples indicated that the MASE method gives good recoveries and precision for pendimethalin at three levels (5, 10, 15 $\mu g\ g^{-1}$).

The recoveries were found to be in the range of 90-100 % for soil samples and 97-100 % for onion samples and 92-102%for water samples. The developed microwave-assisted solvent extraction procedure using acetone as extracting solvent for extraction of pendimethalin from soil and vegetable samples was found to be the best for soil and vegetables samples. The results indicated that the MASE method is precise and efficient. MASE are now preferred over the traditional laborious techniques of blending, shaking or soxhlet extraction of analyte.

The influence of different parameters and their interactions on the % recoveries from soil and vegetables samples were studied according to the 2^3 factorial designs (state- ease). A 2^3 factorial design with a complete repetition performed in one run resulting in a total of 8 experiments was chosen for extraction conditions optimization and maximum % recovery of pendimethalin from samples .

3.6 DETERMINATION OF ARYLOXYPHENOXY GROUP OF HERBICIDES USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

The herbicide selected from aryloxyphenoxy propionate group for HPLC studies was fenoxaprop-p-ethyl. The chemical structure of fenoxaprop-p-ethyl is given below in figure 3.6.1.

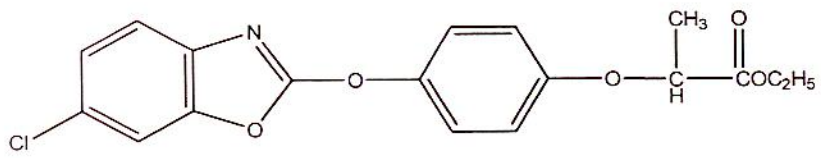


Figure 3.6.1 Chemical structure of fenoxaprop-p-ethyl.

As fenoxaprop-p-ethyl is soluble in polar solvents, therefore reverse phase assembly was setup for its determination.

3.6.1 Investigation of Suitable Parameters for the HPLC determination of fenoxaprop-p-ethyl

Instrument

The HP series 1100 LC system (Perkin Elmer Series 200 CA, USA) equipped with gradient pump (Model 600), an auto sampler (Model 717 plus) with 20 µL injection loop and ultraviolet detector at 280 nm was used. Zorbax SB- C_8 (80 A^0, 4.6 X 150 mm, 5mm particle size) was used as a separation column with a Zorbax C_{18} (4x4 mm) as a guard cartridge. Microwave extractions were performed with KEN ST/SS25 KENWOOD (China) microwave with temperature control system.

Reagents and Chemicals

Fenoxaprop-p-ethyl {ethyl-2-[4-[(6-chloro-2-benzoxazolyl) oxy] phenoxy] propionate} (99 %) was purchased as standards from Dr. Ehrenstofer GmbH, Germany.

HPLC-grade acetonitrile and methanol used were products of Rathburn, Walkburn, UK. LC grade water was obtained by purifying distilled water with a Milli-Q water purification system. All other chemicals used were product of analytical reagent grade purity manufactured by Merck (Darmstadt, Germany).

Standard solution and sample preparation

Stock solution of fenoxaprop-p-ethyl (1000 μgmL^{-1}) was prepared by dissolving 0.1 g of fenoxaprop-p-ethyl in 10 mL of acetonitrile and diluted up to 100 mL with acetonitrile. Working standards were prepared from this stock solution in range of 5-80 $\mu g\ mL^{-1}$ by dilution with acetonitrile.

All physical parameters such as wave length, flow rate, injection volume, solvent elution mode, and elution time, solvents and composition of solvents for gradient elution were optimized for the HPLC determination of fenoxaprop-p-ethyl.

The choice of wavelength is based on where the compounds of interest have the best response and interfering compounds have the lowest response. Wavelength optimization studies were conducted in the wavelength range of 220-300 nm and 280 nm was found as optimum.

For optimization of flow rate of mobile phase flow rate was varied from range 0.5 mL min^{-1} to 2.0 mL min^{-1} and 0.7 mL min^{-1} was chosen as optimum.

Similarly injection volume was optimized in a range 0.5µL-20µL, and 20µL was selected as the optimum injection volume.

All the optimized parameters are presented in table 3.6.1 and were used for subsequent analysis.

Table 3.6.1 Optimum conditions for determination of fenoxaprop-*p*-ethyl using reverse phase HPLC.

Parameter	Optimized volume (gradient elution)	
Eluents used	Mobile phase 1: Mobile phase 2 Methanol : Acetonitrile	Time (min)
	100: 0	5
	30:70	8
	0: 100	12
Flow rate ($mLmin^{-1}$)	0.7	
Injection Vol.(µL)	20	
Wavelength	280nm	

3.6.2 Construction of calibration plot for the determination of fenoxaprop-p-ethyl using reverse phase HPLC.

From standard stock solution of fenoxaprop-p-ethyl ($100\mu gmL^{-1}$) working standard solutions were prepared in concentration rang of 5.0 - 30 $\mu g\ mL^{-1}$ using acetonitrile for dilution.

HPLC System was equilibrated with the mobile phase prior to analysis. After equilibration solution were injected one by one and the peak height was noted for each concentration. Accuracy and precision of method were determined using replicates. A calibration plot of peak height vs. concentration was constructed and shown in figure 3.6.2. Based on IUPAC recommendation points were considered to be in the linear range if their values did not differ by more than 5% from the slope. The linearity of the curve was verified by the correlation coefficient of 0.998.

The limit of detection (LOD) and limit of quantification (LOQ) were calculated from minimum concentration for which a proper peak was observed using the following formula.

LOD = 3s/b and LOQ = 10s/b

Where b is slope of the curve

The LOD and LOQ were found to be 0.029 μg mL^{-1} and 0.098 μg mL^{-1} respectively

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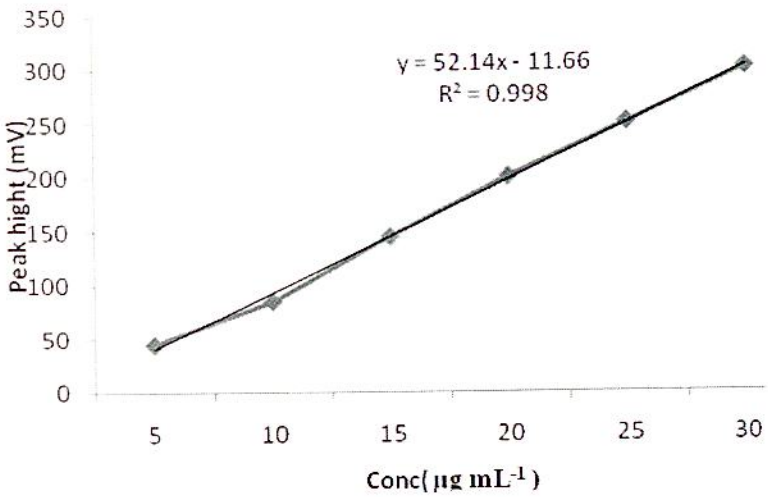


Figure 3.6.2 Calibration plot for fenoxaprop-p-ethyl determination

3.6.3 Optimization of microwave extraction procedure for HPLC determination of fenoxaprop-p-ethyl.

HPLC conditions

After optimization of instrumental and physical parameters it was important to optimize extraction procedure for fenoxaprop-p-ethyl. For this purpose the effects of various experimental parameters for and microwave extraction (MASE) were studied. Parameter optimized were solvent type, ratio of mixtures of solvents, MASE time, and temperature.

Preliminary experiments were performed to select the optimum operation conditions for the microwave-assisted solvent extraction of fenoxaprop-p-ethyl. The parameters like temperature, time and solvent type for extraction were optimized

Fenoxaprop-p-ethyl is partially soluble in water and highly soluble in acetone,n-hexane ethanol and methanol. These solvents were used for extraction of fenoxaprop-p-ethyl from soil, vegetables and water samples. . The results are given in table 3.6.2 and are shown in Fig 3.6.3.Similarly the volume of optimum solvent was optimized for maximum extraction. The results are shown in figure 3.6.4 The % Recoveries in the range of 70-78% were obtained with mechanical extraction which was improved to 92-98% by microwave assisted solvent extraction in different samples.

For investigation of the effect of temperature for MASE on the extraction efficiency of, extractions fenoxaprop-p-ethyl were carried out at 25, 40, 60, 80 and 100 °C, respectively. The results of extraction recoveries are shown in (Fig 3.6.5)

Similarly for the effect of extraction time, extraction of spiked samples was carried out at 60 °C for 3, 5, 7, 9 and 11 min. using acetone as the extraction solvent(Fig 3.6.6).

The influence of different parameters and their interactions on the % recoveries were studied according to the 2^3 factorial designs (state- ease). A 2^3 factorial design with a complete repetition performed in one run resulting in a total of 8 experiments was chosen for extraction conditions optimization and maximum % recovery of fenoxaprop-p-ethyl from soil and vegetable samples.

The ANOVA results are given table 3.6.3 Three-dimensional response surface (RSM) plots of % recoveries versus main factors (AB and BC) for extraction and HPLC study based on experimental model (fig 3.6.7 and 3.6.8)

Table 3.6.2 Investigation for suitable solvent for Fenoxaprop-*p*-ethyl extraction

S.No	Solvent	% Recovery
1	Ethanol	71
2	n-Hexane	72
3	Actone	97
4	Acetonitril	85
5	Actic acid	75
6	Methanol	67

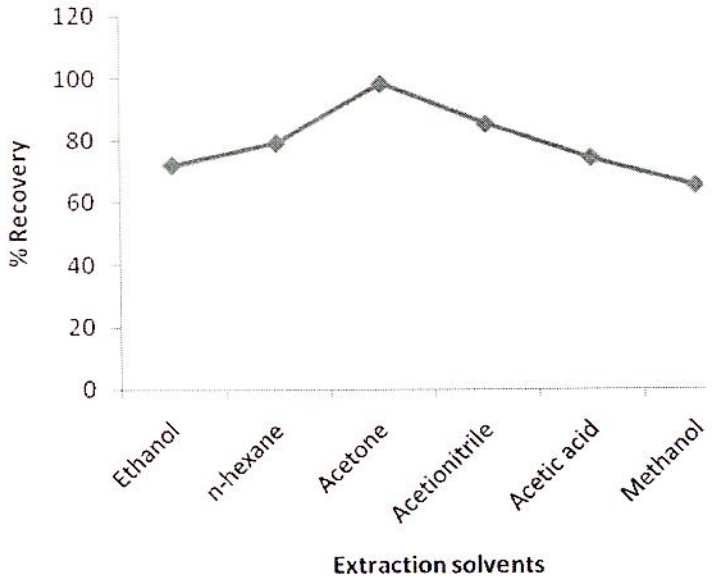


Figure 3.6.3 Investigation for suitable solvent for extraction of fenoxaprop-*p*-ethyl

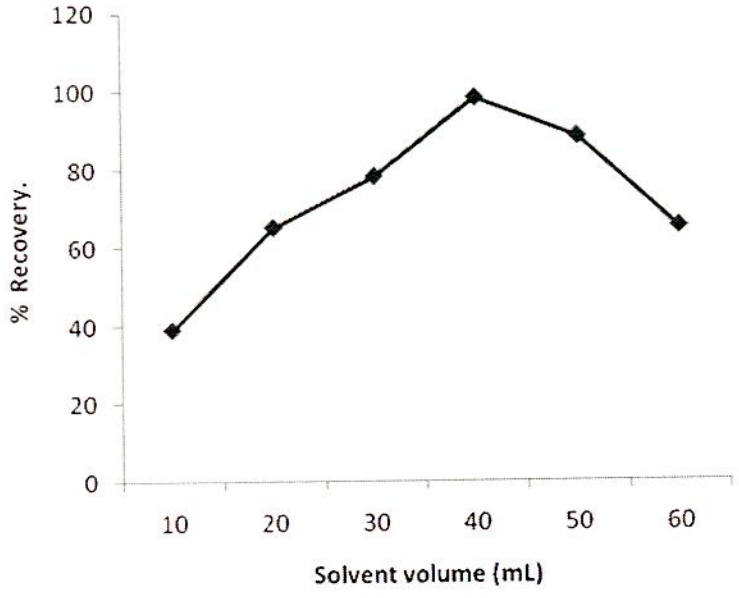


Figure 3.6.4 Investigation of suitable volume of solvent for extraction of fenoxaprop-*p*-ethyl

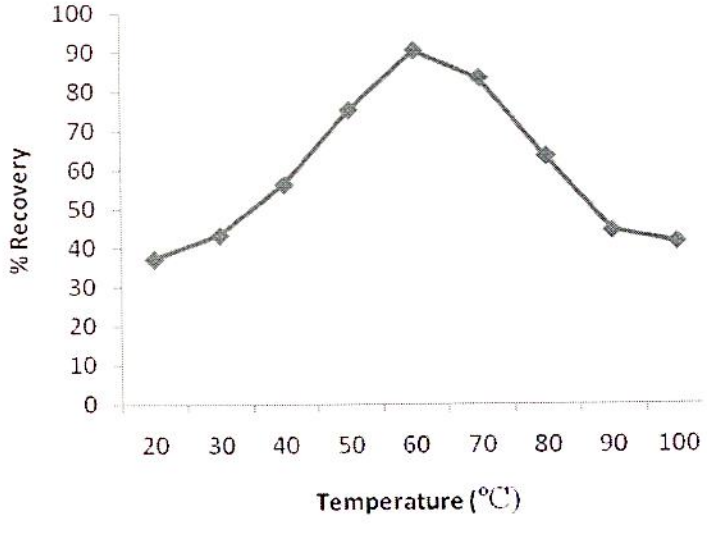


Figure 3.6.5 Effect of microwave temperature on %recovery of fenoxaprop-p-ethyl

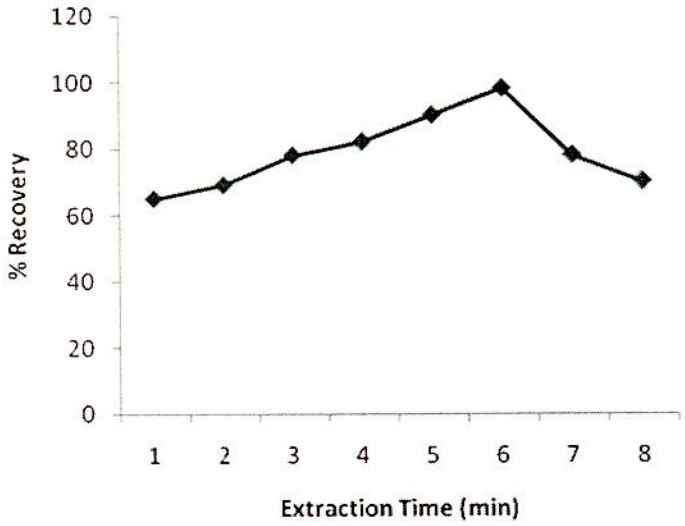


Figure 3.6.6 Effect of microwave extraction time on % recovery of fenoxaprop-*p*-ethyl

Table 3.6.3 Results of the ANOVA for selected factorial model for HPLC determination of fenoxaprop-*p*-ethyl

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	1027.00	6	171.17	342.33	0.0413	
A-Temp	40.50	1	40.50	81.00	0.0704	
B-Time	40.50	1	40.50	81.00	0.073	
C-Solvvol	684.50	1	684.50	1369.00	0.0172	significant
AB	40.50	1	40.50	81.00	0.0704	
AC	40.50	1	40.50	81.00	0.0704	
BC	180.50	1	180.50	361.00	0.0335	
ABC	40.50	1	40.50	81.00	0.0704	
Residual	0.50	1	0.50			
Cor Total	1027.50	7				

Conclusion

Values of "Prob > F" less than 0.0500 indicate model terms are significant.

In this case BC (Solvent volume and time for heating) are significant model terms.

Std. Dev.	0.023	R-Squared	0.9998
Mean	83.75	Adj R-Squared	0.9966
C.V. %	0.84	Pred R-Squared	0.9689

The "Pred R-Squared" of 0.9689 is in reasonable agreement with the "Adj R-Squared" of 0.9966.

Final Equation in Terms of Coded and actual Factors:

%Recovery =+83.75+2.25+9.25-2.25-2.25+4.75+2.25=98

* A * C * A * B * A * C * B * C * A * B * C= 98

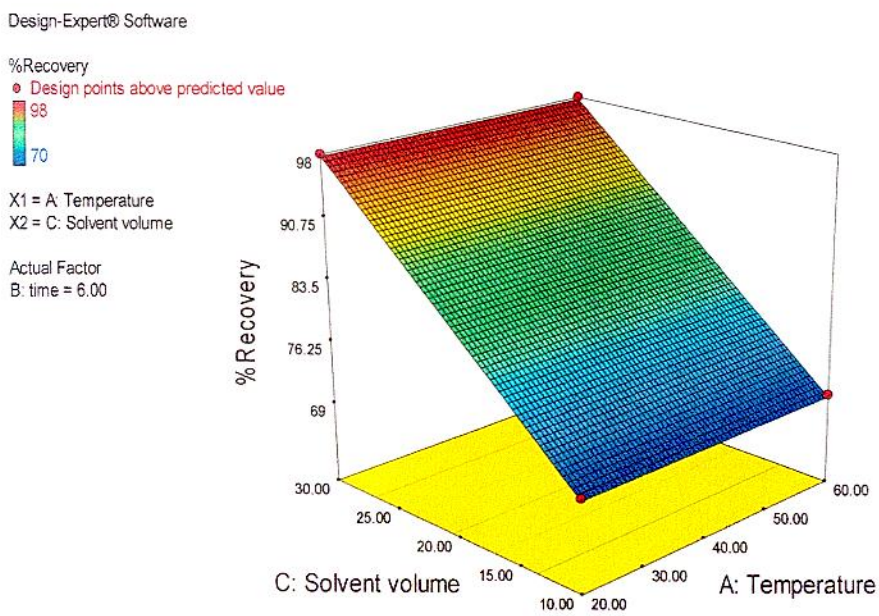


Figure 3.6.7 Response surface plot for % recovery of fenoxaprop-p-ethyl

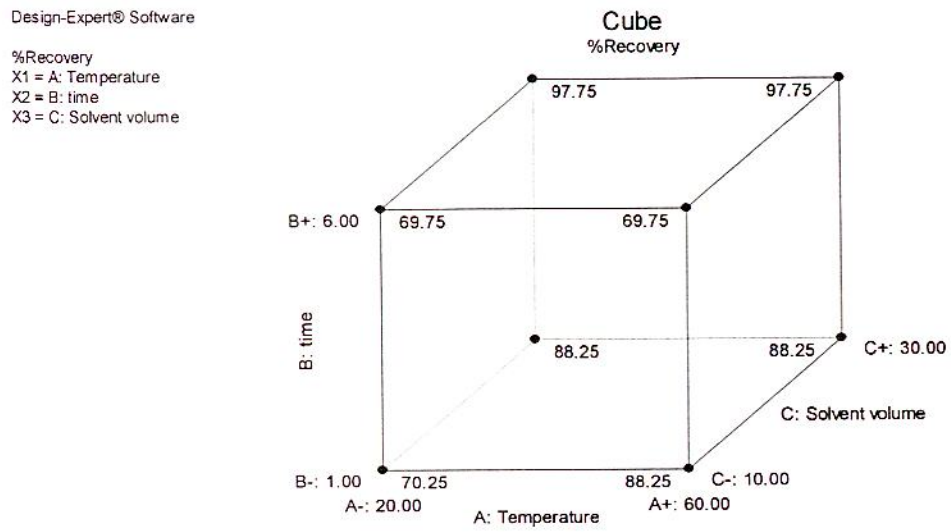


Figure 3.6.8 Graphical 2^3 factorial designs for determination of fenoxaprop-p-ethyl

3.6.4 Comparison of extraction procedures for determination of fenoxaprop-p-ethyl in real samples

Instrument: The same as mentioned before

The application of optimized HPLC procedure to real samples is dependent on the extraction efficiency from the real samples therefore it was important to optimize different extraction parameters for real samples like soil, vegetable and water and check the % recovery from these samples at optimum conditions. For this purpose extraction procedure of mechanical, microwave and sonication were compared for these samples.

Sample preparation for extraction

For the extraction of fenoxaprop-p-ethyl from control soil sample, 10 g of soil was air dried, passed through a 2 mm sieve and homogenized. Fortified samples were prepared by adding 5, 10 and 15 μg $g^{-1}$of standard fenoxaprop-p-ethyl solution to soil sample. After fortification the samples were kept at room temperature for 4.0 h to allow evaporation of the solvent and different extraction procedures were used.

For mechanical extraction 50 mL of acetone was added to each sample and after mixing the samples were shaken on shaker for 15 min equilibration the samples were filtered and the filtrate was evaporated on rotary evaporator near to dryness. Then it was redissolved in 10 mL acetonitrile and determination of fenoxaprop-p-ethyl in filtrate was preceded by the proposed method.

For microwave extraction optimum volume of acetone was added to each fortified sample in a microwave closed-vessel. The starting parameters setting in microwave system were 2.0 min at 350 W and 3.0 min at 500 W. Once the extraction program was completed the vessel were cooled down to room temperature before opening the vessel. After extraction the contents were centrifuged for 10 min and equilibrated. After equilibration the samples were filtered and the filtrate was evaporated on rotary evaporator up to volume of 2 mL then 5 mL of methanol was added to it centrifuged and passed through0.45 μm micro pore. The extract was evaporated near to dryness and redissolved in 10 mL of acetonitrile for HPLC analysis.

Similarly for extraction from grain samples fortified grain samples were prepared by adding 5, 10 and 15 μg $g^{-1}$of standard fenoxaprop-p-ethyl solution to barley samples. After fortification the samples were kept at room temperature for 4.0 h to allow evaporation of the solvent. The rest of the procedure was the same as mentioned for soil. For microwave extraction grain samples (10g) were added for extraction to closed vessels and heated for 2-3min at optimized temperature in a microwave. Followed by filtration and determined as mentioned above. Similarly for water samples the same optimized extraction procedure was applied using 10 mL of fortified water samples. On equilibration the sample was filtered and the filtrate was evaporated on rotary evaporator up to volume of 2 mL then 5 mL of methanol was added to it centrifuged and passed through0.45 μm micro pore. The filtrate was dried and redissolved in 10 mL of acetonitrile for HPLC analysis.

In case of vegetables samples were homogenized with chopper and fortified with known concentration of fenoxaprop-p-ethyl solution extracted in same method as mentioned for soil. For chromatographic analysis the extracts were further filtered through 0.45 μm membrane. The extract was evaporated near to dryness and redissolved in 5 mL of acetonitrile and diluted up to 10 mL.

Analysis of soil, grain, vegetable and water samples followed equilibration and injections of the standards and blanks preceded injections of the sample extracts. The concentration of fenoxaprop-p-ethyl in final extract was calculated using the following formula.

Concentration ($\mu g\ g^{-1}$) = $\mu g\ mLl^{-1}$.Peak height.Dilution. Total volume of the extract/ peak height for standard. Sample weight

Where: $\mu g\ L^{-1}$ = concentration of the standard fenoxaprop-p-ethyl solution.

Peak height = peak height of the analyte in the sample

Dilution =Dilution factor, if the sample was diluted prior to analysis.

Peak height of standard = peak height of the standard in the sample

Sample weight = weight of the sample extracted,

% Recovery = $\mu g\ g^{-1}$.Concentration of standard.100/Sample weight.

The results are given in Table 3.6.4. Fig 3.6.8 shows the chromatogram for standard fenoxaprop-p-ethyland Fig 3.6.9 (a,b,c) shows the chromatograms for fenoxaprop-p-ethyl of 5,10,15 $\mu g\ mL^{-1}$ spiked fenoxaprop-p-ethyl soil samples. fig 3.6.10 (a,b,c) chromatogram of for 5,10,15 $\mu g\ mL^{-1}$ spiked fenoxaprop-p-ethyl barley grain samples figure 3.6.11and for spiked water samples figure 3.6.12 (a,b,c) 10 , 15 $\mu g\ mL^{-1}$ spiked chromatograms for vegetables samples.

Table-3.6.4 Comparison of different extraction procedure for the extraction of fenoxaprop-*p*-ethyl from real samples

Sample	Added ($\mu g\ g^{-1}$)	Found ($\mu g\ g^{-1}$)			Average% Recovery±RSD		
		MASE	USE	SFE	MASE	USE	M E
Soil	5 10 15	4.5 8.6 13.7	4.7 9.6 14.0	4.0 8.3 12.0	89±3.0	96±1.0	88±1.7
Barley grains	5 10 15	4.3 8.6 13.0	4.4 9.6 14.1	3.9 8.0 12.1	87±0.0	96±1.7	75±1.5
Vegetables	5 10 15	4.4 8.7 13.3	4.8 9.8 14.3	3.6 7.2 12.8	88±0.5	98±0.6	73±0.5
Water	5 10 20	4.5 8.6 18.7	4.8 9.6 14.4	4.5 8.6 13.7	88±1.24	96±1.0	75±1.5

MASE (Microwave assisted sample extraction)

USE (Ultrasonication sample extraction)

ME (Mechanical extraction)

3.6.5 Application of the investigated methods for the analysis of Fenoxaprop-P-Ethyl in real sample and Commercial Formulation

Instrument: The same as mentioned before

Sample collection and sample preparation.

For real samples homogenized food sample (10g) was taken in a flask and 50 mL of acetone was added to it and mechanically shaken vigorously. Extraction in microwave was performed for 4 minute. After extraction the samples were filtered on filter paper first and then through micro porous membrane .The filtrate was evaporated to dryness and redissolved in acetonitrile. The residue of herbicide present in the sample was determined from the difference of the concentration of added standard solution and the concentration of herbicide in spiked sample. .The results in real samples and formulations are given in tables 3.6.5 and 3.6.6. Selected chromatograms in different fortified samples are shown in figures 3.6.9 to 3.6.12.

Table 3.6 .5 Residue level of fenoxaprop-*p*-ethyl in real samples

S. No	Sample	Residue ($\mu g\ mL^{-1}$)
1	Barley grains	1.35 ± 0.13
2	Barley flour	1.05 ± 0.20
3	Soil	1.12± 0.22
4	Water	1.32 ± 0.16

Table 3.6.6 Concentration of fenoxaprop-p-ethyl in commercial formulation

Commercial formulation	Label Amount ($g\ mL^{-1}$)	HPLC Analysis ($g\ mL^{-1}$)	Spectrophotometric method ($g\ mL^{-1}$)
Active ingredient	0.75 ± 0.46	0.74 ± 0.38	0.72 ± 0.38

Result and discussion

HPLC method was optimized for determination of fenoxaprop-p-ethyl using reverse phase Zorbax SB-C_8 column attached with a sample loop of 20 µL capacities. The mobile phase consisted of acetonitrile and methanol. Gradient elution was carried out according to the programme; 5 min at 100% acetonitrile, 12 min 30% methanol and 70% acetonitrile and 100% methanol with 0% acetonitrile for 8 min. The flow rate was kept at 0.7 mL min^{-1} using ultraviolet detector at 280 nm for absorption measurement Standard curve was constructed to encompass anticipated range of fenoxaprop-p-ethyl concentration in range of 5-30 μgmL^{-1} in different samples. The peak height was noted for each concentration. Accuracy and precision of the method was determined using replicates. Calibration curve was prepared by plotting peak heights versus concentrations (Fig 3.6.2)

The effects of various experimental parameters of microwave-assisted extraction step were studied. Parameters optimized were solvent type, ratio of mixtures of solvents, time, and temperature.

Selection of solvent for extraction is important because it affects the % recoveries of herbicides. Fenoxaprop-p-ethyl is partially soluble in water and high solubility in acetone, methanol and ethanol. These solvents were used for extraction of fenoxaprop-p-ethyl from soil, vegetables and barley. Maximum extraction was achieved using acetone. Recoveries in range of 70-78 without microwave and improved to 92-100% were achieved by microwave assisted extraction and Ultrasonication methods in different samples. (Fig 3.6.4)

Volume of solvent for MASE was optimized to select the minimum volume of solvent required for maximum % recovery.40-50 mL is the optimum volume for MASE and ultrasonication assisted extraction (fig 3.6.4)

Extraction time optimizations were carried out for 1-6 min using acetone as the extraction solvent. The extraction time at room temperature at which herbicide shows maximum recovery is 6 min and % recovery is improved by 1min microwave heating at 60^0C. As can be seen that the recovery of increased as the temperature of fenoxaprop-p-ethyl extraction increased from 25 °C to 60 °C and the recovery decreased beyond this temperature that could be due to degradation of fenoxaprop-p-ethyl at higher temperatures. At high temperature degradation starts and peak broadening is observed (fig 3.6.5) It was observed that when the extraction time was increased from 3 min to 6 min the recovery of fenoxaprop-p-ethyl was increases but at higher time decrease in % recovery observed (Fig3.6.6) due to degradation of fenoxaprop-p-ethyl for increasing irradiation time. Therefore, 3 min irradiation time was selected for MASE

Three factors were defined to evaluate for ANOVA using (factorial design) and their contribution to herbicide extraction efficiency from soil and food samples A temperature, B time of extraction and C solvent volume. The ANOVA test ($p<0.05$) showed that amount of solvent and temperature had significantly positive effect on fenoxaprop-p-ethyl extraction (Table 3.6.2). Recoveries higher then 98%were observed in preliminary studies on partitioning of herbicides between methanol and acetonitrile assisted by microwave.

A central composite design was applied based on a two level factorial design, supported the low numbers of factors to be optimized. The result of ANOVA for solvent volume, extraction time and temperature appeared to have significant effect on extraction efficiency with 2^3 factorial (Table 3.6.3) response surface were obtained and all these responses were well fitted with R^2 (0.9998).Figures 3.6.7 and 3.6.8 shows response surface and residual plot of the composite desirability function estimated from design.

Fig 3.6.8 shows HPLC chromatograms, recorded at 280 nm for standard solution of fenoxaprop-p-ethyl prepared in acetonitrile. The retention time for fenoxaprop-p-ethyl standard is 4.37 min. Similar chromatograms were obtained for soil, vegetable barley and water samples for percent

recoveries .Gradient elution with the acetonitrile and methanol system enabled an efficient separation and well shaped peaks of all fenoxaprop-p-ethyl. Samples were analyzed

Chromatograms obtained for fortified soil 3.6.9 (a, b,c) barley grains 3.6.10 (a,b,c) water 3.6.11 and cabbage 3.6.12 (a,b,c) were used for identification and quantification 3.6.13 were used for residual studies.

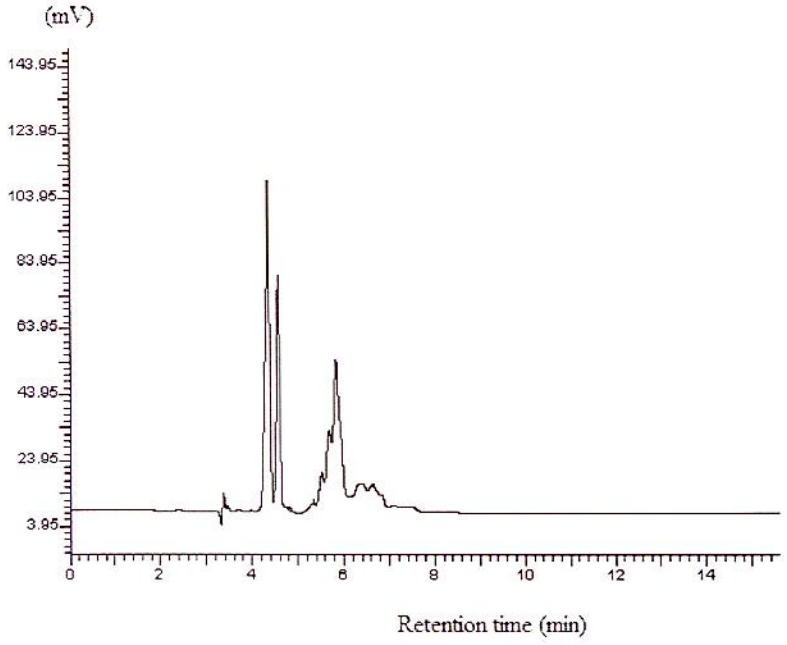


Figure 3.6.8 Chromatogram of standard fenoxaprop-*p*-ethyl herbicide using reverse-phase HPLC

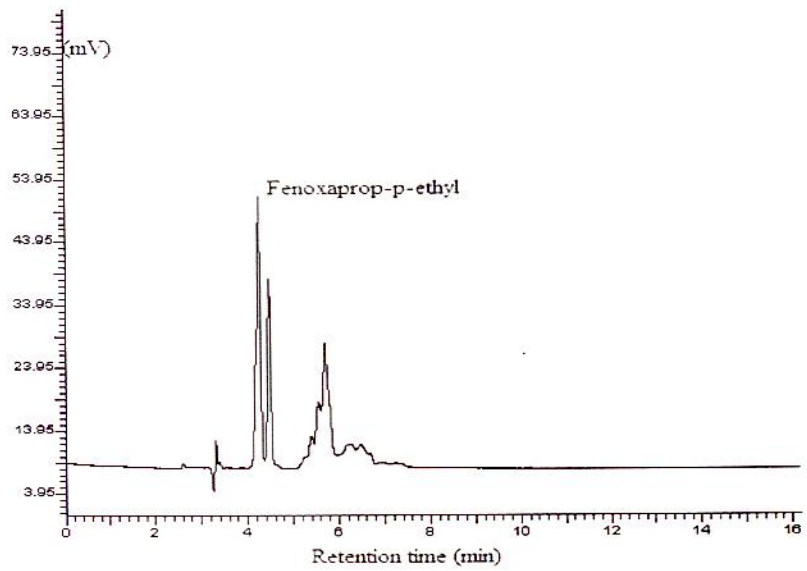


Figure 3.6.9(a) Chromatogram for 5μgg^{-1} fenoxaprop-p-ethyl fortified soil sample using reverse-phase HPLC

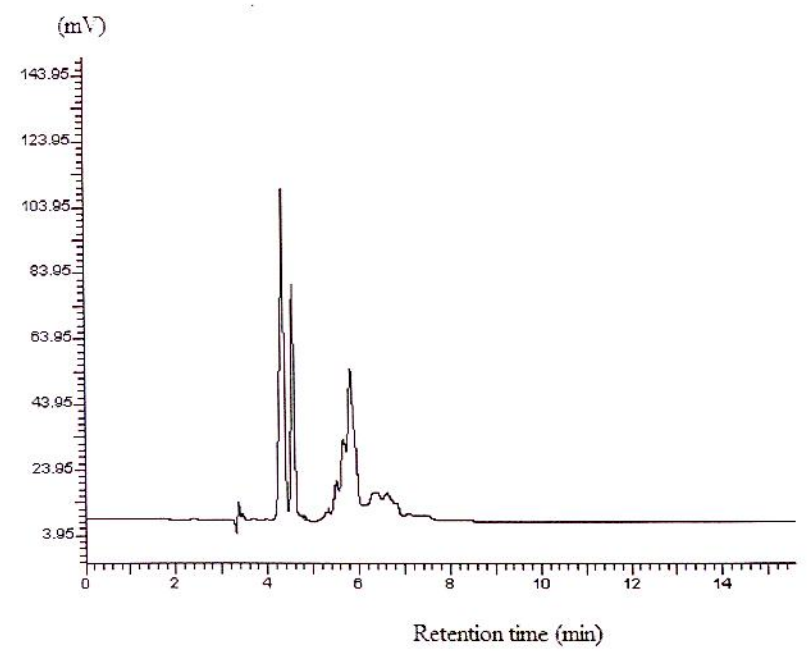


Figure 3.6.9(b) Chromatogram for 10 μgg^{-1} fenoxaprop-p-ethyl fortified soil sample

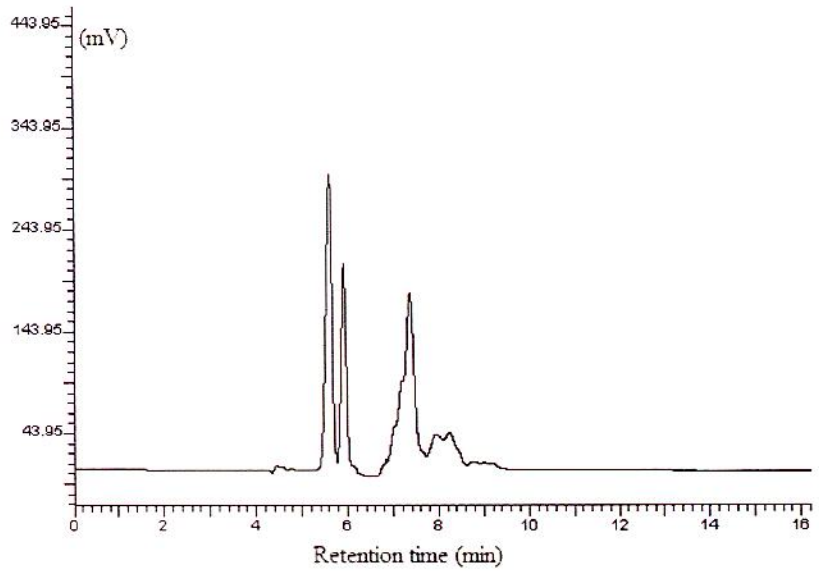


Figure 3.6.9(c) Chromatogram for 15 μgg^{-1} fenoxaprop-p-ethyl fortified soil sample using reverse-phase HPLC.

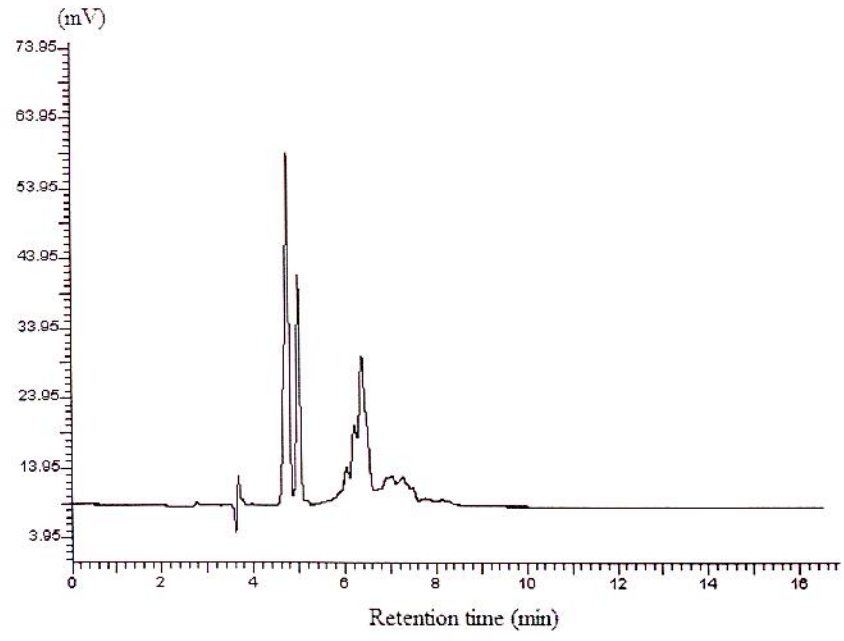


Figure 3.6.10(a) Chromatogram for $5\mu gg^{-1}$ fenoxaprop-p-ethyl fortified barley grain sample using reverse-phase HPLC

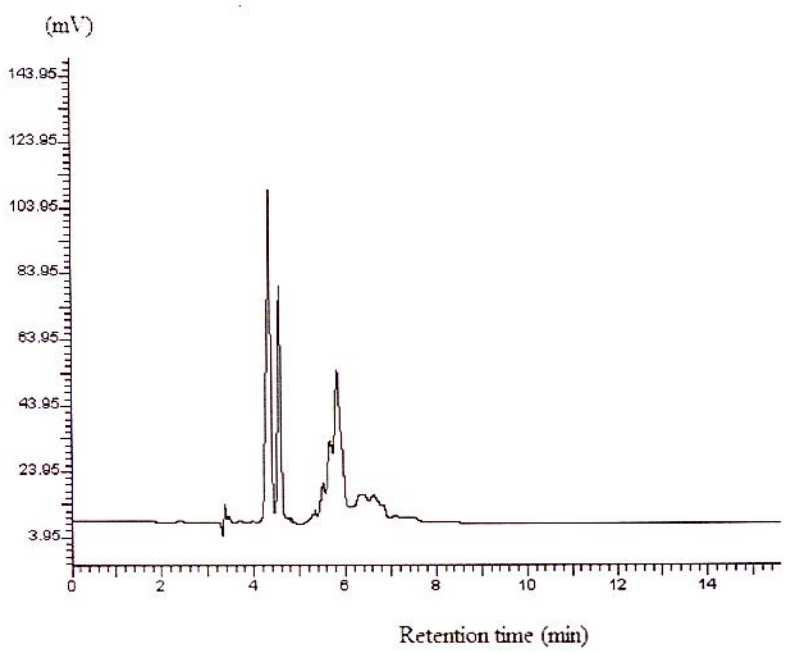


Figure 3.6.10(b) Chromatogram for 10 μgg^{-1} fenoxaprop-p-ethyl fortified barley grain sample using reverse-phase HPLC

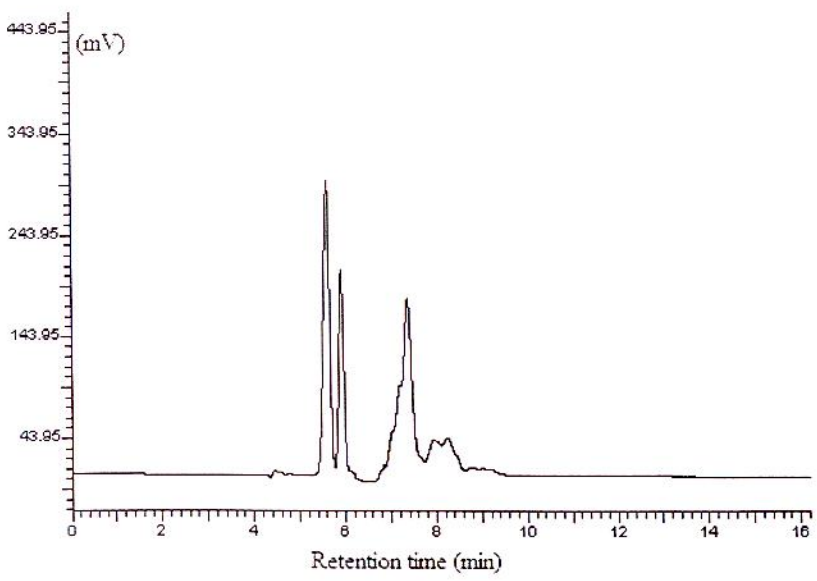


Figure 3.6.10(c) Chromatogram for1 5μgg^{-1} fenoxaprop-p-ethyl fortified barley grain sample using reverse-phase HPLC

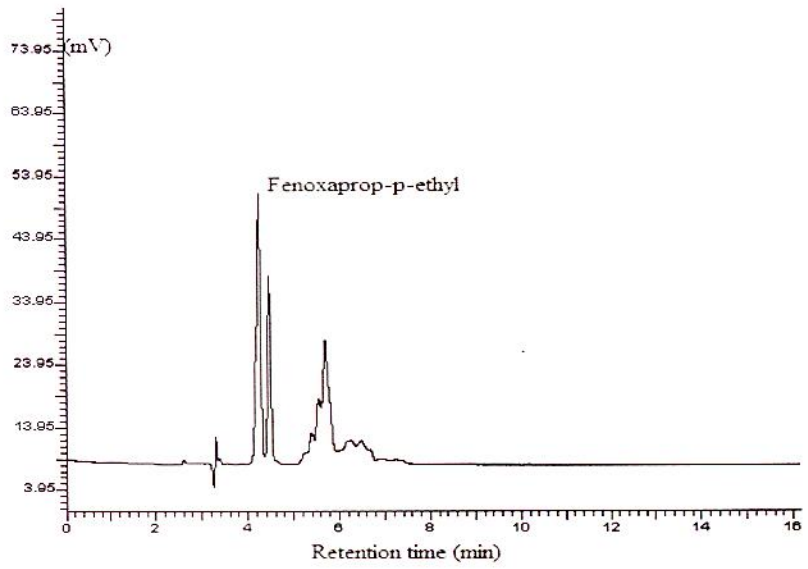


Figure 3.6.11(a) Chromatogram for 5μgg^{-1}fenoxaprop-p-ethyl fortified water sample using reverse-phase HPLC

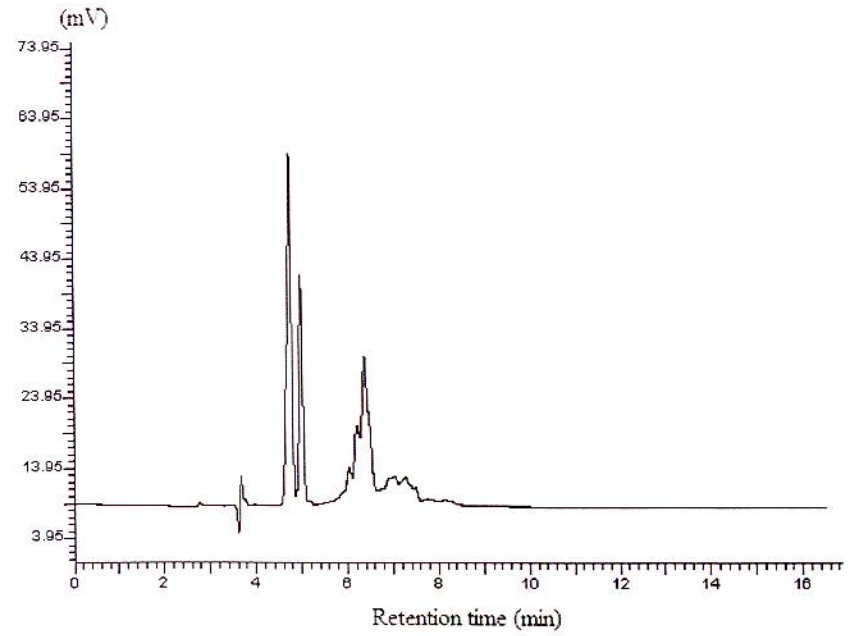


Figure 3.6.12(a) Chromatogram for 5μgg^{-1} fenoxaprop-p-ethyl fortified cabbage sample using reverse-phase HPLC

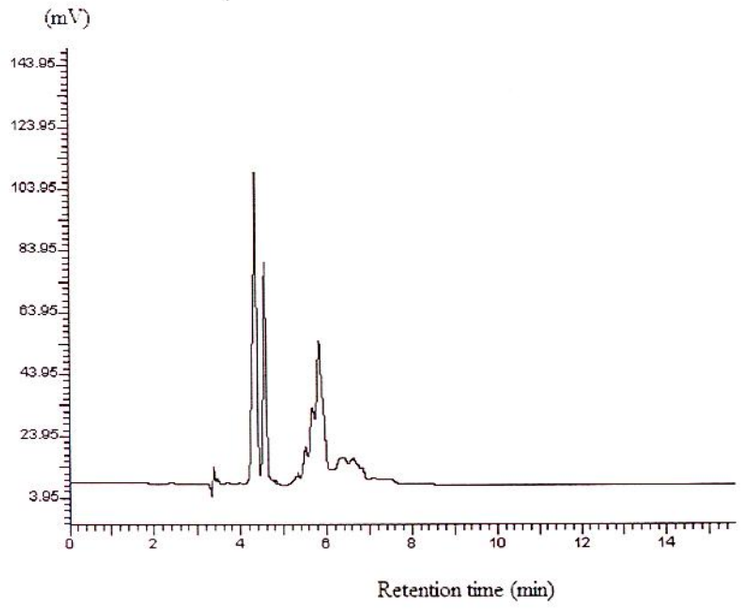


Figure 3.6.12(b) Chromatogram for 10 $\mu g g^{-1}$ fenoxaprop-p-ethyl fortified cabbage sample using reverse-phase HPLC

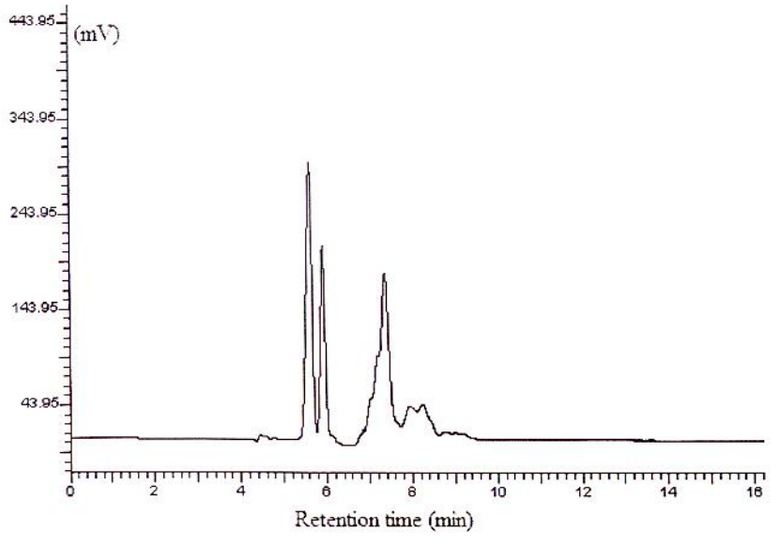


Figure 3.6.12(c) Chromatogram for(15 μg^{-1})fenoxaprop-p-ethyl fortified cabbage sample using reverse-phase HPLC

3.7 SEPARATION AND DETERMINATION OF PINOXADEN AND FENOXAPROP-P-ETHYL HERBICIDES USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

The herbicides selected from aryloxyphenoxy group for HPLC studies were pinoxaden and fenoxaprop-p-ethyl. The chemical structures of pinoxaden and fenoxaprop-p-ethyl are given below in figure 3.7.1 and 3.7.2 respectively

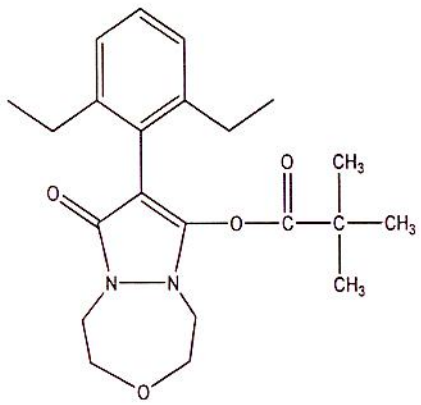


Figure 3.7.1 Chemical structure of pinoxaden

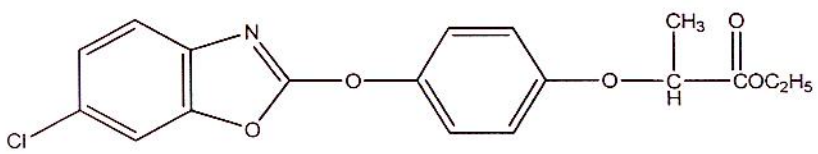


Figure 3.7.2 Chemical structure of fenoxaprop-p-ethyl

3.7.1 Investigation of suitable parameters for separation and determination of Pinoxaden and Fenoxaprop-P-Ethyl Herbicides.

Instrument

The HP series 1100 LC system (Perkin Elmer Series 200 CA, USA) equipped with gradient pump (Model 600), an auto sampler (Model 717 plus) with 20 µL injection loop and ultraviolet detector at 240 nm was used. The separation of aryloxyphenoxy herbicides was performed using Zorbax SB- C_8 (80 A^0, 4.6 X 150 mm, 5mm particle size) with a Zorbax C_{18}(4x4 mm) guard cartridge. Microwave extractions were performed with KEN ST/SS25 KENWOOD (China) microwave with temperature control system.

Reagents and materials

Standard fenoxaprop-p-ethyl [-ethyl-2-[4[(6-chloro-2-benzoxazolyl) oxy] phenoxy] propionate] 99 % and pinoxaden 98% was purchased from Dr.Ehrenstofer GmbH, Germany.

HPLC-grade acetonitrile and ethanol were obtained from Rathburn, Walkerburn, UK. LC grade water was used by purifying distilled water with a Milli-Q water purification system (Millipore Co, USA). All other chemicals used were of analytical reagent grade purity obtained from Merck (Darmstadt, Germany).

Fenoxaprop-p-ethyl and Pinoxaden standard stock solutions (400 µg mL^{-1})

For preparation of standard the stock solution of pinoxaden and fenoxaprop-p-ethyl 10 mg of standards were dissolved in 10 mL of acetonitrile and diluted with acetonitrile up to 25mL working standards were prepared from this stock solution in the range of 5-40 µg mL^{-1} by dilution with acetonitrile in 10 mL volumetric flask.

Mixture solution

The mixture solution of both herbicides was prepared in methanol. Mixed stock solution containing each analyte at 0.1, 0.25, 0.5, 1.0, 2.5, 5.0 and 10 µg mL^{-1} were also prepared in methanol and these were used for separation, optimization and construction of calibration curves.

Table3.7.1 Optimum conditions for HPLC determination of pinoxaden and its separation from fenoxaprop-p-ethyl using reverse phase HPLC.

Parameter	Optimized value	(gradient elution)
Eluents used	Mobile phase 1: Mobile phase 2 Methanol : Acetonitrile	Time (min)
	80 : 20	5
	50 : 50	10
	20 : 80	5
Flow rate ($mLmin^{-1}$)	0.7	
Injection Vol.(µL)	20	
Wave length	280nm	

3.7.2 Construction of calibration plot for the determination of pinoxaden and fenoxaprop-p-ethyl using reverse-phase HPLC

From standard stock solutions of pinoxaden and fenoxaprop-p-ethyl (400µg mL^{-1})working standard solutions were prepared in concentration range of 2.0 µgmL^{-1} to 50 µgmL^{-1} for pinoxaden and 5-50 µgmL^{-1} for fenoxaprop-p-ethyl using acetonitrile for dilution.

HPLC system was equilibrated with the mobile phase prior to analysis. After equilibration standard solution were injected one by one and the peak height was noted for each concentration. Accuracy and precision of the method were determined on three replicates. A calibration plot of peak height vs. concentration was constructed and is shown in figure 3.7.3 and 3.7.4. The

linearity of the curve was verified by the correlation coefficient (0.987) for pinoxaden and 0.998 for fenoxaprop-p-ethyl. The limits of detection (LODs) and the limits of quantification (LOQs) were calculated using peak height ratio of 3 and 10 times, respectively. The LODs of fenoxaprop-p-ethyl and pinoxaden found are 0.014 μg mL^{-1}, 0.088 μg mL^{-1} respectively while their LOQs are 0.022 μg $mL^{-1}$and 0.16 μg mL^{-1} respectively.

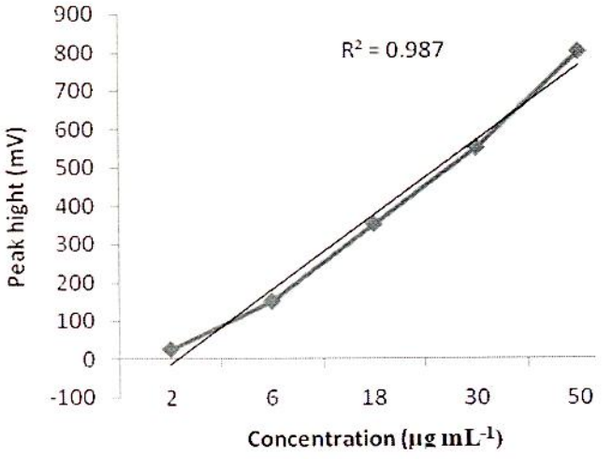


Figure 3.7.3 Calibration plot for the determination of pinoxaden herbicide

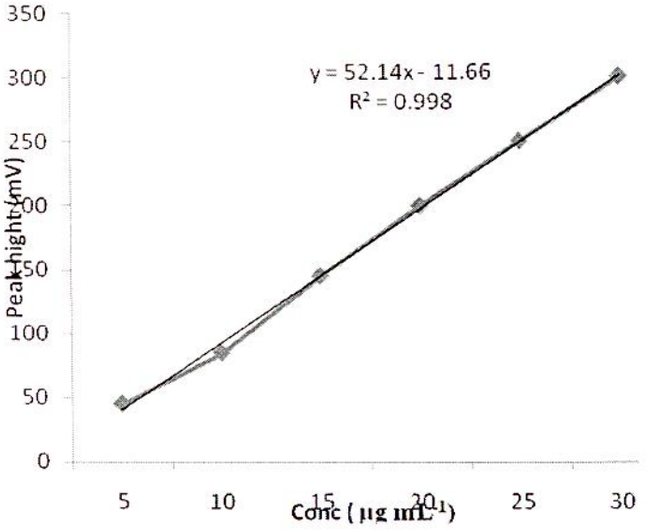


Figure 3.7.4 Calibration plot for the determination of fenoxaprop-p-ethyl herbicide

3.7.3 Optimization of conditions for the separation and determination of pinoxaden and fenoxaprop-p-ethyl using reverse-phase HPLC

In order to improve the separation of two herbicides and detect ability of the eluting substances, composition of the mobile phase and pH were investigated.

For optimization of solvent composition different mobile phase compositions, such as methanol/water, acetonitrile water, methanol acetonitrile mixtures were investigated. The chromatographic results of good separation and better resolution were achieved by using acetonitrile/methanol mixture. Since methanol and acetonitrile possess different hydrogen bonding capability, it can be inferred that hydrogen bonding might play an important role in the separation of components of mixtures of herbicides. The water contents affect the sensitivity of the detector response with low peak height where sensitive response is obtained in acetonitrile and methanol mixture. The results are shown in figure 3.7.5.

The mixture of two herbicides was subjected for separation on column, under acidic, basic, and natural conditions by changing the composition of buffer. The stress of change in flow rate was carried out at room temperature. For thermal stress the herbicides samples were kept in microwave oven for 4min. The concentration used was 1mg mL^{-1}.

After preliminary investigation of HPLC studies and reproducible chromatograms which shows variation in height on change in concentration of herbicides. The HPLC analysis was performed by using the mobile phase consisted of acetonitrile and methanol. The gradient elution was carried out according to the programme for 5,10,5 min. The flow rate was 0.7 mL min^{-1}.The amount injected was 20 µl. Wavelength 280 nm as working wave length for both herbicides.

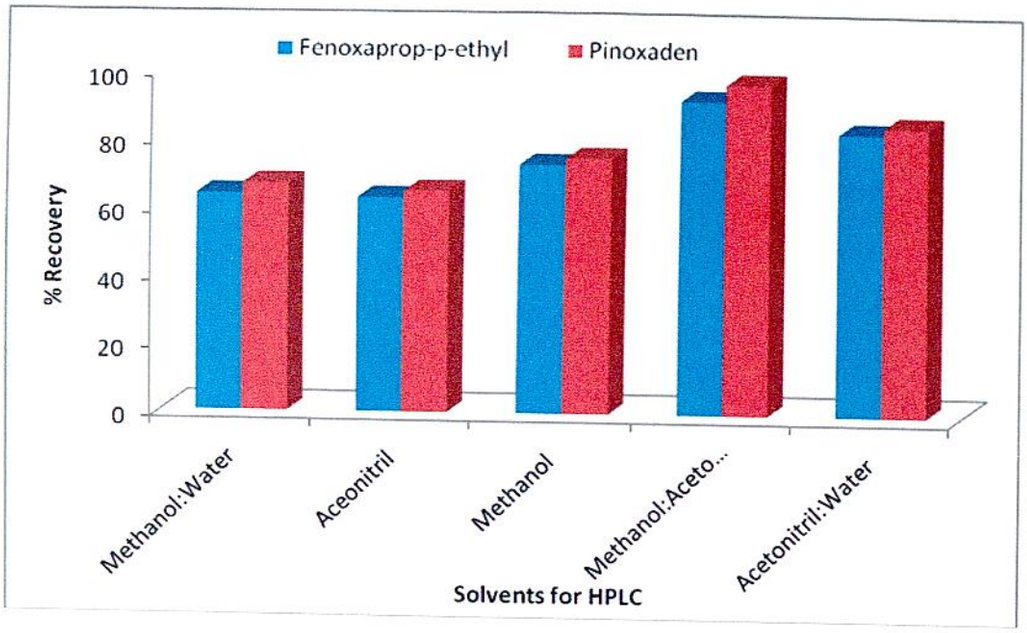


Figure 3.7.5 Investigation of suitable solvent system for separation and determination of fenoxaprop-p-ethyl and pinoxaden by reverse-phase HPLC method.

3.7.4 Optimization of extraction procedure for the separation and determination of pinoxaden and fenoxaprop-p-ethyl

The effects of various experimental parameters for mechanical extraction and microwave extraction were studied. Parameter optimized were solvent type, ratio of mixtures of solvents, MASE time, and temperature.

For optimization of extraction solvents organic solvents such as methanol, acetonitrile, n-Hexane, chloroform, dichloromethane acetone and ethanol were studied for extraction of both herbicides because pinoxaden and fenoxaprop-p-ethyl are partially soluble in water and highly soluble in acetone, ethanol and methanol. These solvents were used for extraction of these herbicides from soil, vegetables and water samples. The results are shown in figure 3.7.6

Effects of temperature for microwave assisted extraction (MAE) on the extraction efficiency of both herbicides were carried out at 25, 40, 60, 80 and 100 °C, respectively. The results of extraction recoveries are shown in (Fig 3.7.7)

For optimization of extraction time, extraction of spiked samples was carried out at 60 °C from 1-6 min. using methanol as the extraction solvent. When the extraction time was increased from 1 min to 6 min the recovery of both herbicides was decreased (Fig 3.7.8) due to degradation of herbicides for increasing irradiation time. Therefore, 3 min irradiation time was selected for MASE.

Effect of solvent volume on extractions studied using efficiency w four different volumes of extractant solvents, ie10, and 20,30,40,50 mL (Fig 3.7.9) and 20 mL was found to be the optimum extractant.

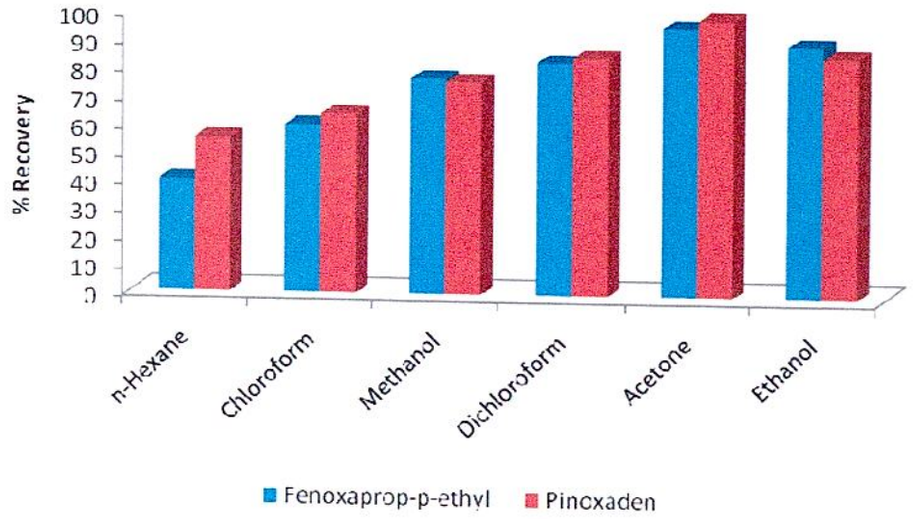


Figure 3.7.6 Optimization of solvent for extraction of fenoxaprop-p-ethyl and pinoxaden

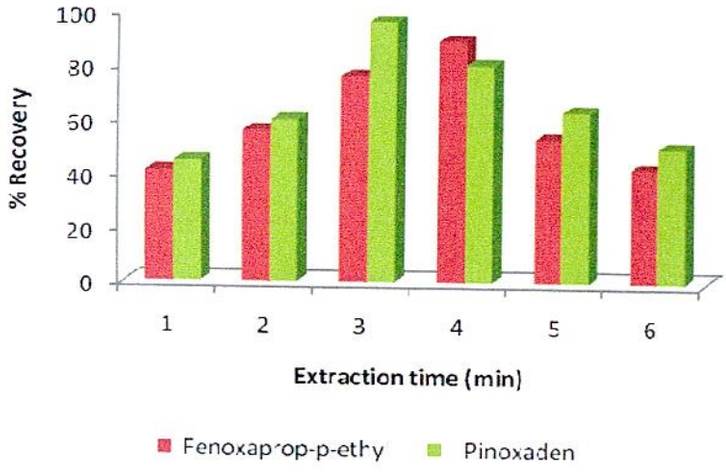


Figure 3.7.7 The effect of extraction time on the extraction recoveries of fenoxaprop-p-ethyl and pinoxaden

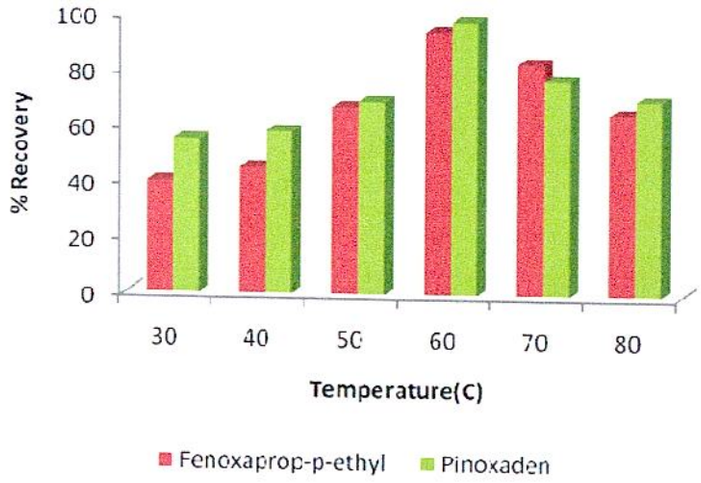


Figure 3.7.8 Investigation of suitable temperature for extraction and determination of fenoxaprop-p-ethyl and pinoxaden by reverse-phase HPLC method.

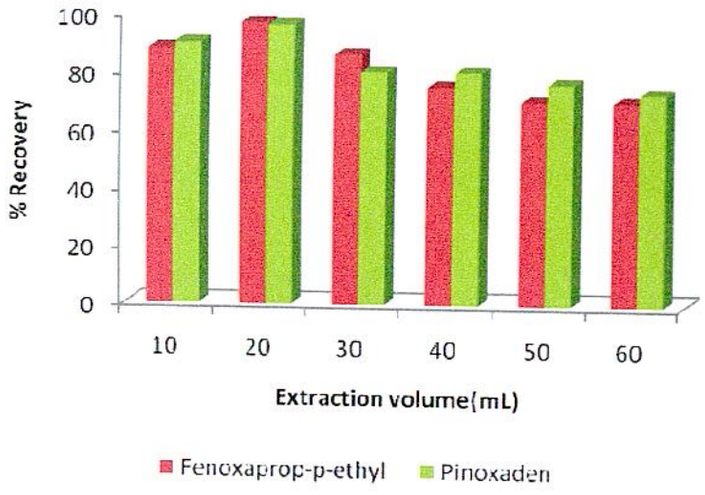


Figure 3.7.9 Investigation of suitable volume of solvent for extraction and determination of fenoxaprop-p-ethyl and pinoxaden by reverse-phase HPLC method

3.7.5 Separation and determination of aryloxyphenoxy group of herbicide in real samples by using reverse-phase HPLC

Sample preparation(Wheat and vegetables samples)

Recovery studies were carried out using three control samples (10g) of wheat grains and cabbage fortified with (5 and 10 μg g^{-1}) of pinoxaden and fenoxaprop-p-ethyl mixture in 1:1 ratio. Two parts of spiked wheat and cabbage samples (10 μg g^{-1} and 5 μg g^{-1}) were prepared by adding an appropriate volume of spiking solution of both fenoxaprop-p-ethyl and pinoxaden herbicides to the wheat sample, then whisking carefully to homogenize it. The other part was used as a blank. All of these samples were stored in the dark by wrapping sample flasks with aluminum foil. There were no detectable levels of the target analyte in the wheat and vegetables before spiking. The samples were kept for four hours for sorption and were shaken occasionally for uniform mixing. A mixture of 20 mL methanol and acetone was added to each sample and kept in microwave for 4min .On cooling the grain samples and the filtrate was evaporated up to dryness. Then the residue was redissolved in acetonitrile, filtered, centrifuged and analyzed using HPLC at optimized conditions. The results are given in table 3.7.2 and table 3.7.3.

Table 3.7.2 % Recoveries of fenoxaprop-p-ethyl and pinoxaden from real samples

Real samples	Added (ug mL^{-1})	Found (ug mL^{-1})	% Recovery
Wheat grain	5	4.82	97.00 ±0.017
	10	9.57	94.00±0.11
	15	14.61	95.00 ±0.009
Cabbage	5	9.52	95.00 ±0.021
	10	9.73	98.00 ±0.30

Table 3.7.3 Residue level of fenoxaprop-p-ethyl and pinoxaden in real samples

S.NO	Sample	Residue (μg g^{-1}) Fenoxaprop-p-ethyl	Residue (μg g^{-1}) Pinoxaden
1	Cabbage	2.6 ±0.29	1.13±0.20
2	Wheat grains	1.7±0.25	1.15±0.22

3.7.6 Results and Discussion

Separation of pinoxaden and fenoxaprop-p-ethyl was done by liquid column chromatography after preliminary analysis by TLC. Different solvents separately and n mixture were screened for the best separation of pinoxaden and fenoxaprop-p-ethyl herbicides.

Instrumental parameters of HPLC were optimized for the separation of fenoxaprop-p-ethyl and pinoxaden like mobile phase solvents, mobile phase flow rate, ratio of solvents and gradient elution. Flow rate of mobile phase was varied in the range of 0.5-1.0 mL min^{-1} using methanol/acetonitrile in the ratio 20:80, 50:50 and 80:20 for the separation of both herbicides. The complete resolution of mixture was achieved using 0.7 mL min^{-1} flow rate and mobile phase ratio 50:50 in time range of 15 min and gradient elution for 5, 10, and 5 min was suitable for clear separation.

The conditions for microwave –assisted extraction were also optimized such as solvent type, volume, extraction time and temperature. Organic solvents such as methanol, acetone, acetonitrile, chloroform,n-hexane, dichloromethane and ethanol were studied for extraction of both herbicide separately at 850 W of microwave for 3 minutes and average recoveries found are given in figure.3.7.6. Fenoxaprop-p-ethyl was found to be well extracted in acetone with average recoveries of 98% recovered in acetone. Recoveries in the range of 86-90 % were obtained with

ethanol as extraction solvent. Similarly, it was observed that pinoxaden is 86 % recovered in methanol and acetonitrile, respectively.

For investigation of the effect of temperature on the extraction efficiency, extractions were carried out at 30-80 ^{0} C. The extraction recoveries are shown in figure 3.7.7. For both herbicides the recoveries increased clearly as the extraction temperature increased from 30 °C to-80°C and the recoveries decreases with further increase in temperature from 70 °C to 80 °C.

Extraction time optimizations were carried out at 850 W for 1-6 min using acetone/methanol as extraction solvent. Under the condition of changing the extraction time, the results of the MAE recoveries of fenoxaprop-p-ethyl and pinoxaden from wheat grains are shown in figure 3.7.8.When the extraction time was increased from 1 to 3 min, the recoveries of both herbicides increased, however further increase in extraction time did not lead to any significant increase in recovery. The optimum extraction time was selected to be 3 min for further studies and the recoveries of both herbicides were higher than 90%.

Large amounts of volume of solvent may be make procedure complex and leads to unnecessary waste however if solvent volume is not enough the target extraction would not be complete. Therefore different volumes of extract were studied. The effect of the extractant and its volume on the extraction efficiency for MAE is shown in 3.7.9.The recoveries of fenoxaprop-p-ethyl and pinoxaden increased when the solvent volume was increased from 10 mL to 20 mL and then small decrease in recoveries observed with increase in further in further volume of extracting solvent.

Figure3.7.10 and 3.7.11 show the chromatograms of fenoxaprop-p-ethyl and pinoxaden standards solution 20 μgmL^{-1} and Figure 3.7.12 shows chromatogram for separation and determination of fenoxaprop-p-ethyl and pinoxaden in a mixture using optimized reverse phase HPLC method.

The applicability of the method of separation in real samples was checked by analyzing food samples purchased from local market. Three different concentrations (5,10,15 $\mu g\ g^{-1}$) were used for checking of samples

Figure 3.7.13, 3.7.14 and 3.7.15 shows the chromatograms of mixtures of (5,10 and 15 $ug\ g^{-1}$) spiked wheat sample. While Figure 3.7.16, 3.7.17 (5,10 $\mu g\ g^{-1}$) spiked vegetables samples. The

method validation studies for spiked samples indicated that the present method provides good recoveries (90–97%) of fenoxaprop-p-ethyl and pinoxaden. The results obtained for recovery, precision and accuracy show that the proposed HPLC method for separation and determination of herbicides is an efficient and easy method for identification and quantification of theses aryloxyphenoxy herbicides in agricultural samples.

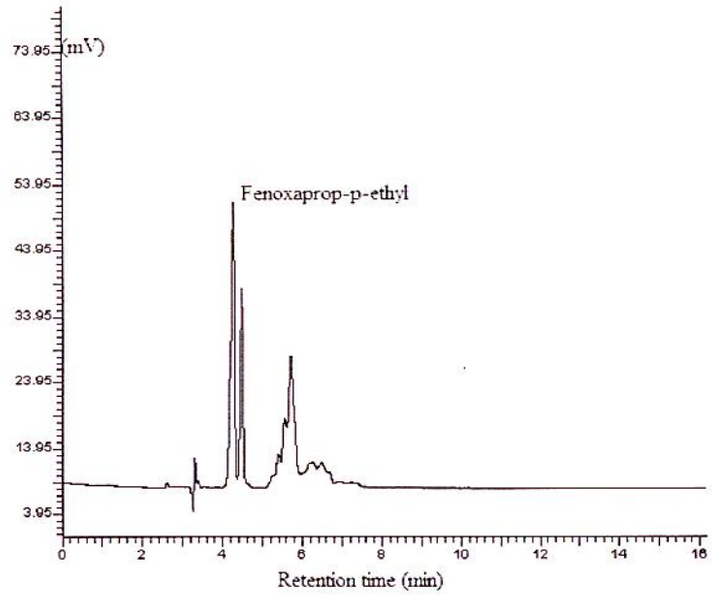


Figure 3.7.10 Chromatogram for standard fenoxaprop-p-ethyl herbicide using reverse-phase HPLC

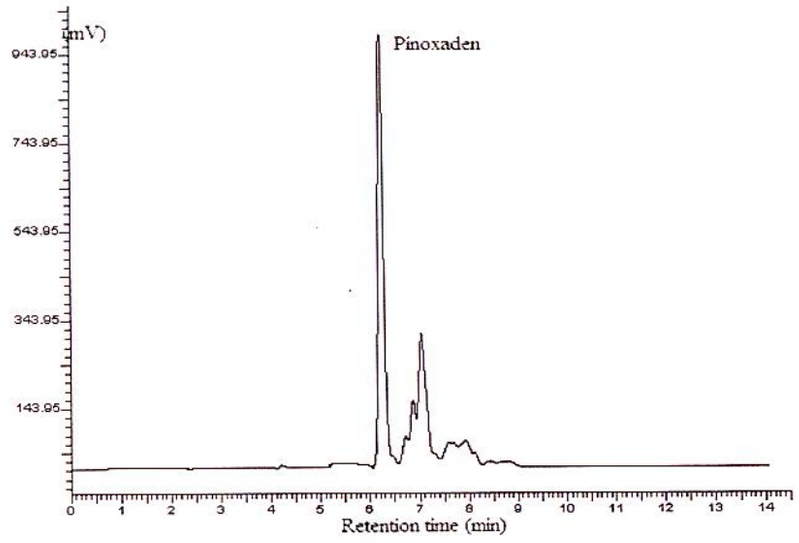


Figure 3.7.11 Chromatogram for standard Pinoxaden herbicide using reverse-phase HPLC

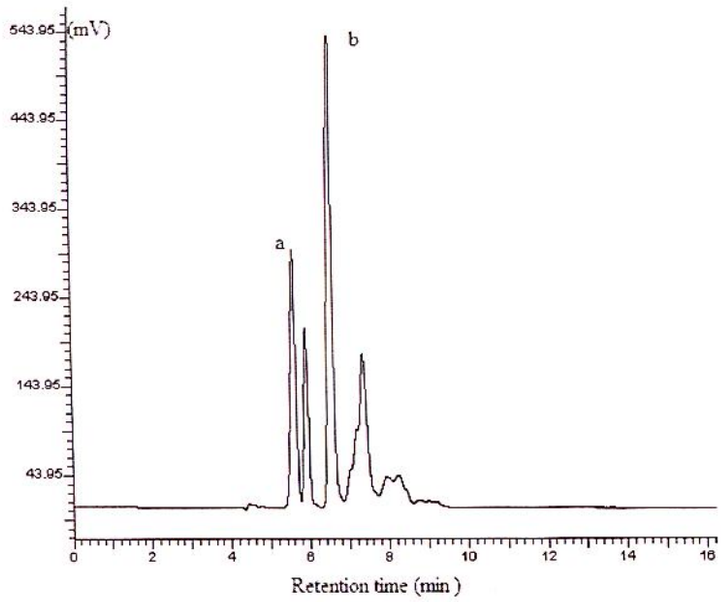


Figure 3.7.12 Typical separation Chromatogram of fenoxaprop-p-ethyl (a) and pinoxaden (b) mixture (20µg mL^{-1}) using reverse-phase HPLC

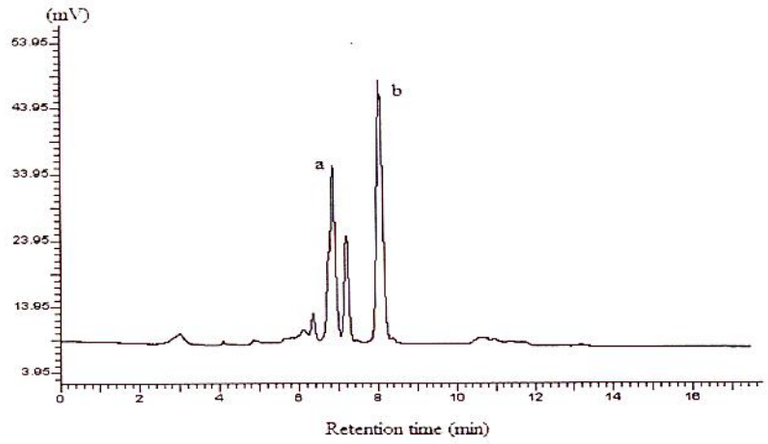


Figure 3.7.13 Chromatogram for fenoxaprop-p-ethyl (a) and pinoxaden (b) fortified 5µg g^{-1} wheat samples using reverse-phase HPLC

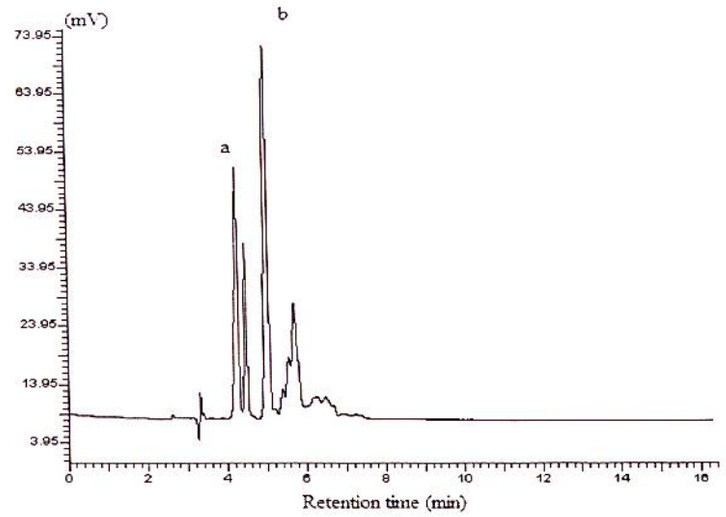


Figure 3.7.14 Chromatogram for fenoxaprop-p-ethyl (a) pinoxaden (b) fortified (10µg g^{-1}) wheat samples using reverse-phase HPLC

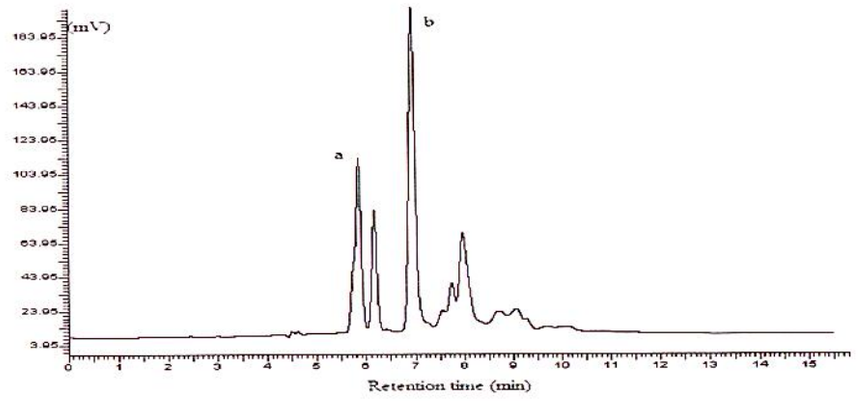


Figure 3.7.15 Chromatogram for fenoxaprop-p-ethyl (a)and pinoxaden (b)fortified 15µg g^{-1} wheat grain samples using reverse-phase HPLC

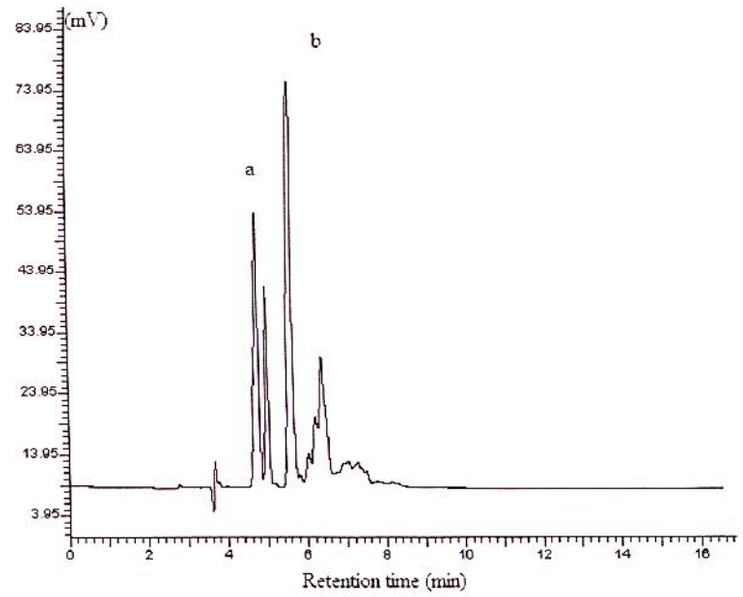


Figure 3.7.16 Chromatogram for fenoxaprop-p-ethyl (a) pinoxaden (b) fortified (10µg g^{-1}) cabbage samples using reverse-phase HPLC

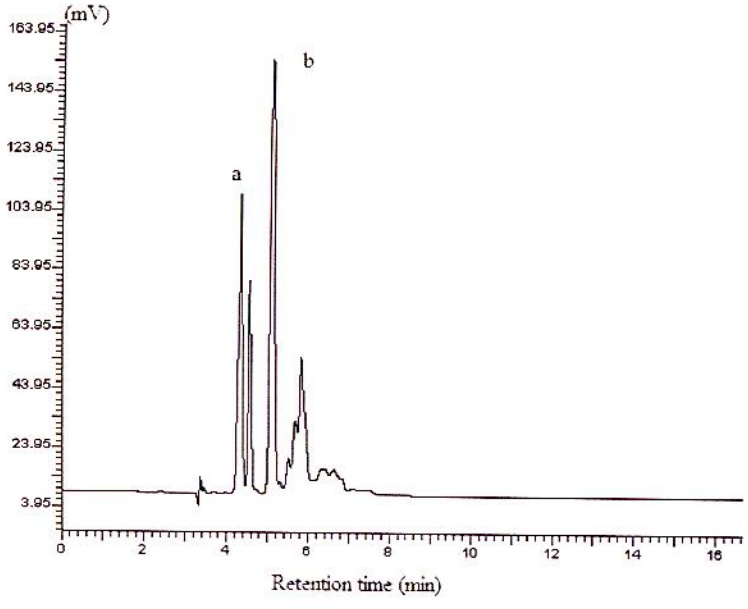


Figure 3.7.18 Chromatogram for fenoxaprop-p-ethyl (a) pinoxaden (b) fortified (15µg g^{-1}) cabbage samples using reverse-phase HPLC

Conclusion

Four different spectrophotometric methods and three HPLC methods were developed for residue determination and analysis of active ingredients in commercial formulation each for dinitroaniline and aryloxyphenoxy propionate group of herbicides.

The method for trifluralin determination is based on base hydrolysis of trifluralin in the presence of alkali to form 2, 6- dinitro-4- triflurомethyl aniline and isopropanol. The hydrolyzed product 2, 6-dinitro-4 triflurомethyl aniline was diazotized with nitrite in acidic media followed by coupling of the diazonium salt with β-naphthol. The resultant red colored azo dye was measured at 550 nm. The molar absorprtivity was found to be $1.5x10^4$ L mol^{-1} cm^{-1}. The limit of detection (3s/b) and quantification (10s/b) were 0.15 μgmL^{-1} and 0.5 μgmL^{-1} respectively.

Similarly spectrophotometric method has been investigated for the determination of Pendimethalin in formulation and for residue analysis. Pendimethalin was hydrolyzed with base by heating on boiling water bath for 10 minutes followed by diazotization and coupling reaction. The resulting azo dye was measured at 430 nm the LOD and LOQ were found to be 0.213 μg mL^{-1} and 0.71μgmL^{-1} respectively with molar absorpitivity of $5.6x10^4$ L mol^{-1} cm^{-1}. The method was applied for the determination of residue in the onion samples and was found to be 91.83±0.005% and 1.3±0.313 % 5 μgg^{-1}.

The spectrophotometric method for fenoxaprop-p-ethyl is based on acid hydrolysis fenoxaprop-p-ethyl followed by coupling of hydrolyzed product with diazotized aniline solution and the resulting azo dye was measured at 470 nm. Various analytical conditions like concentration of nitrite and aniline solutions were optimized. It was found that 8 mL of aniline and 10 mL of nitrite solution is sufficient for formation of azo dye with maximum absorption Molar absorptivity was calculated and found to be $1.6.10^5$ L .$mol.^{-1}$ cm^{-1}.The method is simple, fast and reliable and can be used for fenoxaprop-p-ethyl herbicide.

Similarly fenoxaprop-p-ethyl on acid hydrolysis gave the product 6-chloro-2, 3-dihydrobenzoxazol-2-one (CDHB) and ethyl 2-(4-hydroxyphenoxy) propionate (EHPP). In the 4^{th} method the reaction is based on complexing of 6-chloro-2, 3- dihydrobenzoxazol-2-one (CDHB) with Fe (II). Its λ_{max} was found to be 430nm. The influence of various experimental parameters on the absorbance of the charge transfer complex of fenoxaprop-p-ethyl with Fe (II)

was studied. The method is applicable to environmental samples and applied to wheat and barley grains with recoveries in the range of 88.0 ±1.7% to 92.0 ± 1.0%. Compared to gas chromatographic and liquid chromatographic methods the proposed method is simple, sensitive and reproducible.

HPLC method was developed for the determination of herbicide pendimethalin using reverse phase Zorbax SB-C_8 column. Sample injection was 20 µL. The mobile phase consisted of acetonitrile and water. The gradient elution was carried out according to the programme; 5 min at 80% acetonitrile with 20% water and 100% acetonitrile with 0% water for 12 min. The flow rate was kept at 1 mL min^{-1}. The ultraviolet detector was adjusted at 240 nm for absorption measurement.

Similarly HPLC method was developed for determination of fenoxaprop-p-ethyl using reverse phase Zorbax SB-C_8 column attached with a sample loop of 20 µL capacities. The mobile phase consisted of acetonitrile and methanol. Gradient elution was carried out according to the programme; 5 min at 100% acetonitrile. 12 min 30% methanol and 70% acetonitrile and 100% methanol with 0% acetonitrile for 8 min. The flow rate was kept at 0.7 mL min^{-1}. Using ultraviolet detector at 280 nm for absorption measurement. Standard curve was constructed to encompass anticipated range of fenoxaprop-p-ethyl concentration in range of 5-30 µgmL^{-1} in different samples. The peak height was noted for each concentration. Taking in to account the better selectivity of the HPLC method proposed for separation of herbicides of the same family first the instrumental parameters were optimized to get clear separation. Reversed-phase HPLC, with UV detection, is a good alternative for herbicide determination because derivitization step is not required. Solvents provide the gross overall control of the retention of the analyte. A series of preliminary experiments were conducted in selecting conditions for the microwave-assisted extraction step. The method validation studies for spiked samples indicated that the present method provides good recoveries and reasonable precision for aryloxyphenoxy of two levels (10 µg g^{-1} and 5 µgg^{-1}). The recoveries of aryloxyphenoxy herbicides are 90–97%. The results obtained for recoveries, precision, and accuracy show that the proposed extraction method (MASE) followed by spectrophotometric and HPLC methods are efficient, sensitive, reproducible methods for identification, determination and quantification of herbicides in environmental samples.

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2. Jasmin Shah, M.Rasul Jan,**Farhat-un-NisaShehzad** and Mian Muhammad, "Spectrophotometer method for quantification of Fenoxaprop-p-ethyl herbicide" *Journal of Chemical Society of Pakistan* 32(4), 557-540,2010

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12. **Farhat-un-NisaShehzad***, JasminShah, M.Rasul Jan,"Spectrophotometric Method for Quantification of Pendimethalin Herbicide in Commercial Formulation and food Samples, *Pakistan Journal of Chemical Society* (Accepted)

13. Jasmin Shah, M. Rasul Jan,**Farhat-un-NisaShehzad** and Mian Muhammad, "Separation and determination of pinoxaden and fenoxaprop-p-ethyl herbicides using high performance liquid chromatography." Toxicological & Environmental Chemistry(Submitted)

14. **Farhat-un-Nisashehzad***, Jasmin shah, M. Rasul Jan,"Quantification of Fenoxaprop-p-ethyl herbicide in water and food samples by microwave-assisted solvent extraction and HPLC method",*Pakistan Journal of Chemical Society* (Submitted)