ASSAY OF LOCALLY USED HERBICIDES USING DERIVATIZATION, COMPLEXATION AND FLOW INJECTION SPECTROPHOTOMETRIC METHODS



Ph. D Thesis

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UNIVERSITY OF PESHAWAR

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DISSERTATION

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Certified that **Mr Mian Muhammad** S/O Mian Gul Zada has carried out his research and experimental work on the topic entitled as **"Assay of Locally Used Herbicides Using Derivatization, Complexation and Flow Injection Spectrophotometric Methods"** under our guidance and supervision. His research work is original and his 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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بسم الله الرحمن الرحيم

To My Teachers & My Mentors

Prof. Dr. Muhammad Rasul Jan and Prof. Dr. Jasmin Shah,

Without whom this work would never have come into being.

To Those Closest To Me

My Parents, My Brothers, My Sisters & My Wife,

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ABBREVIATIONS

2, 4-D	2, 4-dicholrophenoxy acetic acid
2-CAA	2-cyanoacetamide
ACCase	Acetyl-CoA carboxylase
ADI	Acceptable daily intake
AFBF	American Farm Bureau Federation
AMPA	Aminomethylphosphonic acid
APCI	Atmospheric pressure chemical ionization
AR_fD	Acute reference dose
ArPP	Aryloxyphenoxypropionates
CDHB	6-chloro-2, 3-dihydrobenzoxazol-2-one
CDP-CSP	Crystalline degradation products-stationary phase
CE	Capillary electrophoresis
CE-C^4D	Capacitivity coupled contactless conductivity detection
CRs	Cross-reactivities
CTAC	Cetyl trimethyl ammonium chloride
CV	Cyclic voltammetry
DAHP	3-deoxy-D-arabino- heptulosonate-7-phosphate
DMAB	p-dimethylaminobenzaldehyde

DNOC	4,6-dinitro-o-cresol
DPs	Degradation products
E_bC_{50}	Median effective concentration for biomass (algae)
EDTA	ethylene diaminetetracetic acid
EHPP	Ethyl 2-(4-hydroxyphenoxy) propionate
EPSP	5-enolpyruvylshikimate-3-phosphate
ESI-MS/MS	Electrospray tendem mass spectrometry
EU	European Union
FI	Flow injection
FIA	Flow injection analysis
FIA-MEPIF	Flow injection analysis micellar-enhanced photochemically induced fluorescence
FID	Flame ionization detection
FMOC-Cl	9-fluorenylmethylchloroformate
FPD	Flame photometric detection
FPE	Fenoxaprop-p-ethyl
GCB	Graphitized carbon black
GC-MS	Gas chromatographic mass spectroscopic
GC-NPD	Gas chromatography using nitrogen phosphorus detector
HDME	Hanging mercury drop electrode

HRP	Horseradish peroxidase
ICP-DRC-MS	Inductively coupled plasma dynamic reaction cell mass spectrometry
IPAD	Integrated pulsed amperometric detection
IPN	Isoproturon
IPU	Isoproturon
LC	Liquid Chromatography
LC_{50}	Median lethal concentration
LD_{50}	Median lethal dose
LOD	Limit of detection
LOEL	Lowest observed effect level
LOQ	Limit of quantification
MAMPA	N-methyl amino-methylphosphonic acid
MIF	Molecularly imprinted films
MPA	Methylphosphonic acid
MRL	Maximum residue level
MTN	Metoxuron
NBD-Cl	4-chloro-7-nitrobenzofurazan
NEL	No effect levels
NMRI	Naval medical research institute

NOAEL	No observed adverse effect level
NOEL	No observed effect level
PDMA-PSS	Poly (2, 5-dimethoxyaniline)-poly (4-styrenesulfonic acid)
PLS-1	Partial least squares type 1
PUHs	Phenylurea herbicides
PXD	Pinoxaden
R_f	Retardation factor
R_fD	Reference dose
RP-HPLC	Reverse phase high performance chromatography
RPM	Revolutions per minute
RSD	Relative standard deviation
SD	Standard deviation
SDS	Sodium dodecyl sulphate
SG-IS	Sol-gel immunosorbent
SLM	Supporting liquid membrane technique
SPE-LC-ESI-MS/MS	Solid phase extraction-liquid chromatography coupled to electrospray tendem mass spectrometry
TLC	Thin layer chromatography
TRRs	Technical reports reviews
UK/EC	United Kingdom and European Commission

UNFAO	United nations food and agriculture organization
USDA	United States Department of Agriculture
US-EPA	United States Environmental Protection Agency
WSSA	Weed Science Society of America
WWTP	Waste water treatment plant

SUMMARY

Analysis of herbicides' residues in the environmental samples is important from toxicological point of view. Herbicides have the ability to be accumulated in crops and the environment and hence it is important to have suitable methods for their assessment in food, food products and other environmental samples. Analysis of herbicide residue involves a number of steps such as sample preparation, extraction, interference removal, quantification and determination of the herbicide residues and their confirmation. All these analyses are generally performed by using several analytical techniques.

The objective of the present research work was to develop analytical methods for assay of locally used herbicides which are not only simple, fast and economical but may not require expensive instrumentation.

The first chapter of this dissertation includes general introduction to herbicides focused on their history, general properties, and impact on the environment and health concerns associated with their use. A brief description of the herbicides which are the subject of this thesis like glyphosate, fenoxaprop-p-ethyl, pinoxaden and isoproturon including their physical, chemical and toxicological aspects is given in this chapter.

The second chapter covers a detailed review of the relevant literature reported for the determination of the subject herbicides. The literature covered in this chapter include methods based on chromatography, capillary electrophoresis, cyclic voltammetry scanning electron microscopy, mass spectrometry, spectrofluorimetry, spectrophotometry, nuclear magnetic resonance spectroscopy, biosensors and many others. All the reported methods have been discussed with main focus on scope, sample preparation, reagents used, procedure followed, linearity range, limit of detection (LOD), limit of quantification (LOQ) and percent recovery.

The third and last chapter comprises of experimental work with preliminary investigations of each method developed, optimization of chemical and physical variables, and application of these methods to different samples followed by results and discussion. This chapter covers work conducted based on (a) spectrophotometric method

which involves complexation reaction performed in batch, (b) flow injection spectrophotometric methods which involve complexation as well as adduct formation and (c) spectrofluorimetric method which involves a condensation reaction.

Part I of this chapter includes details about spectrophotometric method for determination of glyphosate in various environmental samples. The method is based on reaction of glyphosate with carbon disulphide to form dithiocarbamic acid which in turn forms complex with copper (II) in the presence of ammonia. Optimum parameters for the formation of the complex and its spectrophotometric determination were investigated. Beer's law was obeyed in the concentration range of 1.0 -70.0 μg mL^{-1} investigated. The composition of the complex found was glyphosate : Cu = 2:1 with a formation constant of 1.06×10^5. The LOD and LOQ of the method were found to be 1.1 μg mL^{-1} and 3.7 μg mL^{-1} respectively. The investigated method was successfully applied to the environmental samples. Recovery values in soil, wheat grains and water samples were found to be 80.0 ± 0.46 to 87.0 ± 0.28 %, 95.0 ± 0.88 to 102.0 ± 0.98 % and 85.0 ± 0.68 to 92.0 ± 0.37 %, respectively.

In Part II of chapter III a flow injection spectrophotometric method for determination of glyphosate herbicide has been described. Glyphosate was condensed with p-dimethylaminobenzaldehyde (DMAB) in alkaline media resulting in a yellow coloured product after acidification of solution with hydrochloric acid. The absorbance of the product was measured at 405 nm. The Beer's law range for the proposed method was found to be 2.0-15.0 μg mL^{-1} for batch analysis and 0.5-20.0 μg mL^{-1} for flow injection (FI) analysis with molar absorptivity values of 2.7×10^3 and 4.3×10^3 L $mol^{-1}cm^{-1}$ for batch and FI analysis respectively. The LOD and LOQ were calculated and found to be 0.2 ± 0.06 and 0.7 ± 0.1 μg mL^{-1} respectively, for batch analysis and 0.2 ± 0.07 and 0.6 ± 0.2 μg mL^{-1} respectively, for FI analysis. The proposed method was successfully applied with reasonable sample throughput (60 samples h^{-1}) for the analysis of samples. Recoveries from the real samples were found in the range of 90.0 ± 0.65 % to 93.0 ± 0.66 %.

Part III of this chapter deals with flow injection spectrophotometric method for determination of fenoxaprop-p-ethyl herbicide. The reaction involved is based on the

conversion of fenoxaprop-p-ethyl into hydroxamate salt in the presence of hydroxylamine hydrochloride in alkaline media. The hydroxamate salt produced was further reacted with Fe (III) to form red colour tris iron hydroxamate complex in acidic medium. The absorbance of the complex was measured at 500 nm using flow injection system. The method shows a linear range between 1.0 - 20.0 μg mL^{-1} with molar absorptivity of 1.81 × 10^4 L mol^{-1} cm^{-1}. The LOD and LOQ were calculated and found to be 0.29 ± 0.1 μg mL^{-1} and 0.96 ± 0.1 μg mL^{-1} respectively. Similarly, in order to check the extraction efficiency the average limit of detection and limit of quantification were calculated for residue determination and were found 0.35 μg g^{-1} and 0.45 μg g^{-1} respectively, for wheat grains and 1.2 μg g^{-1} and 1.5 μg g^{-1} respectively, for barley grains. The sample throughput was 90 samples h^{-1}. The method was successfully applied to different grain samples with recoveries in the range of 88.0 ± 1.7 % to 92.0 ± 1.0 %.

Part IV of this chapter describes a flow injection spectrophotometric method for determination of pinoxaden (PXD) herbicide. Pinoxaden was converted into hydroxamate salt, in alkaline media with hydroxylamine hydrochloride. The hydroxamate salt produced was reacted with Fe (III) and absorbance of the red colour tris iron hydroxamate complex was measured at 500 nm using a flow injection system. To minimize the interference effect of fenoxaprop-p-ethyl (FE), another related herbicide, pinoxaden was effectively separated from the former using LC with dichloromethane, n-hexane solvent system with percent recovery in the range of 86.0 ± 3.0 % - 93.0 ± 1.0 %. The linear range of the method was found to be 0.5-40.0 μg mL^{-1} with molar absorptivity of 1.53 × 10^4 L mol^{-1} cm^{-1}. The LOD and LOQ were calculated and found to be 0.1 ± 0.01μg mL^{-1} and 0.6 ± 0.05 μg mL^{-1} respectively. The method was successfully applied for the determination of PXD in soil, water and food samples with a reasonable sample through put of 60 samples h^{-1} and reasonable percent recovery in the range of 83.0 ± 6.0 % to 100.0 ± 2.0 %.

Part V of chapter III deals with spectrofluorimetric method for determination of isoproturon (IPU) herbicide. The method is based on the reaction of IPU with 2-Cyanoacetamide in the presence of ammonia (15.0 M). The product formed showed maximum fluorescence intensity at an excitation wavelength of 333 nm and an emission

wavelength of 378 nm. Various experimental parameters affecting the product yield were optimized. Under the optimized conditions maximum fluorescence intensity was a linear function of IPU concentration in the range of 0.5 – 15.0 μg mL^{-1} with correlation coefficient of 0.9991. LOD and LOQ for the method were calculated and were found to be 0.01 μg mL^{-1} and 0.032 μg mL^{-1} respectively. The effect of possible interfering agents on the determination of IPU has been studied and the proposed method has been successfully applied for determination of IPU in grains and seeds samples with recoveries in the range 85.0 ± 1.2 % - 96.0 ± 0.5 %.

INTRODUCTION

Introduction [1-41]

Human beings essentially depend directly or indirectly on agriculture, for their sustenance. World population is rapidly growing towards the expected number of 9 billion inhabitants in 2050 and at present, the world agriculture sector is facing a challenging situation. This is leading to increased pressure on the world's natural resources on one hand while on the other the arable soil surface is degraded day by day, resulting in scarcity of food commodities.

An integrated production system, that includes crop management, plant nutrition, improved crop varieties showing pest-resistance with better yield and modernization of pest control and farming techniques, is the basic demand of modern agriculture. Chemical weed management is an important component of this system.

Herbicides

In a broader sense the term 'herbicides' refers to "all those agents that destroy or inhibit the growth of plants especially of weeds or other unwanted vegetation". Thus a herbicide may be a grazing animal, a home gardener, a plant which is parasitically competing with another plant or an active chemical. Now-a-days hundreds of chemicals, with a variety of trade names and in different formulations, are marketed as herbicides.

Impacts of herbicides

Weeds, ever-present in agro-ecosystem are chemically suppressed by the use of herbicides to favour crop growth and improve farm profits. Chemical weed control is superior for several reasons as it has greater efficacy with low cost which results in a rapid, selective and long term weed control. The spraying equipments are generally cheaper than those used in mechanical weed control. In underdeveloped countries, 46 % of the inhabitants are involved in field works, mostly in weed control. Chemical weed control reduces the labor cost and this was the primary benefit of chemicals in the early era, when there was no urgent demand for more food. Aerial application for large tracts of land is extremely cost-efficient. Herbicides save energy, reduce soil erosion, reduce the cost of farming and increase crop production. Historically, in 1950-1992 global crop

yields increased from 0.45 t/A to 1.1 t/A. Based on a number of studies conducted by United States Department of Agriculture (USDA), Weed Science Society of America (WSSA) and American Farm Bureau Federation (AFBF), it has been shown that if no herbicides were used, losses in crop yield would have been 5-67 %. Unlike the agriculturally developed countries, chemical weed control is not widely practiced in Pakistan. However, reports indicate that use of herbicides result in more effective weed control in wheat and other cereals as compared to mechanical methods. Modern arable farming is strongly dependent upon herbicides for sustained and secure crop yield. However, society at large is unaware of the advantages of herbicides which result in their irresponsible consumption. Thus the use of herbicides must instantly be minimized, not only for the sake of following regulations, but for the toxicity concerns to the community which will reduce the likely adversities to the environment. Only then it can be ensured that herbicides' use will continue as an effective tool to farmers in the long run.

History of Herbicides' use

Historically before the advent of chemical use for weed control, cultural and mechanical techniques were prevailing throughout the world. The history of chemical weed control begins in the late 19th century. Until the late 19th century little progress was made in the practical use of chemicals as weed killers. The use of copper salts for the control of fungus diseases led to an accidental discovery of its selective action on broad leaved weeds in cash crops. Based on the discovery of the use of copper salts as weed killer, it was found in America, Germany and France that solutions of copper salts would kill the weeds with no harm to the cereal crops. Also iron sulphate, sulphuric acid and copper nitrate, all were used for the same herbicidal purposes. Later on solutions of sodium nitrate, ammonium sulphate and potassium salts were used as herbicides by 1900 and afterwards dry powdered kainite and calcium cyanamide were added to the list.

Following this initial period (1896-1910) of successive development in the use of chemicals as weed killers, the lack of spray machinery, lack of success due to low humidity and the discovery of cleaner seeds led to a shift of interest from chemical weed control. By the same time a new period of development had started in Europe. Working with iron sulphate, copper sulphate and sulphuric acid as weed killers, it was reported that

depending on local conditions, these chemicals killed most of the weeds, left the cereal crops safe and had a fertilizing effect on the soil which resulted in higher yield. This report was confirmed by the findings of Morettini (1915) in Italy, Rabate (1926) in France and Korsmo (1932) in Norway.

It was concluded after testing different chemicals for permanently sterilizing soil, that arsenicals show the most satisfactory results as permanent sterilizer for soil. This discovery led to the excessive use of sodium arsenite solution as a sterilizing agent and it became the standard commercial "weed killer" of that times.

Acid arsenicals spray, sodium chlorate, carbondisulphide and other chemicals were introduced into the weed control chemicals' list during the decade 1915-1925. Later on thiocyanates, dinitrophenols, different foliage-applied oils, boron compounds, ammonium sulphamates and other chemical compounds were employed as weed killers.

The first organic chemical herbicide 4,6-dinitro-o-cresol (DNOC) was introduced in 1932 followed by 2,4-Dicholrophenoxy acetic acid (2, 4-D) in the late 1940s which was introduced as weed killer by a British team during World War II and it became the 1st widely produced and used herbicide of the era. In 1951 the substituted urease and uracils came into the market as herbicides. In 1958, atrazine and in 1980s, glyphosate was introduced for non-selective control of weeds. Now-a-days the chemical weed control is continuously expanding and a large number of herbicides with a variety of characteristics are marketed throughout the world and their number and brands are increasing day by day.

General Properties of Herbicides

Behavior in the Environment

Solubility of a particular herbicide in water is probably the most important factor that determines its fate in the environment. Water-soluble herbicides with normally low adsorption capacities are more mobile in the environment and are available for nearly all degradation pathways. Esters being relatively insoluble in water rapidly get adsorbed to soils and readily penetrate plant tissues.

The half-life or half Dissipation Time of a particular herbicide, represented by DT_{50} describes its persistence in soils. Since the half-life of a specific herbicide can be significantly variable, based on soil humidity, characteristics of the soil, temperature and nature of the vegetation in the area, it gives only a coarse estimate of the persistence of the herbicide. Dissipation rates can't provide a source for comparing the relative persistence of herbicides because these frequently change with time. Various parameters like soil temperature, type, amount, and surface area of clays organic matter in the soil, soil pH and the amount and quality of soil moisture are employed to determine the dispersal of herbicides in the soil. The normal pH of most natural soils ranges between 5 and 8 but this value is strongly influenced by rainfall and the amount of leaching. Cations can be leached out in wet areas and/or coarse soils, leaving the soil acidic. Similarly in arid and semi-arid regions, soils hold cations and are alkaline.

Different processes like direct overspray, leaching through soils to groundwater, drift and volatilization or surface or subsurface runoff are responsible for contamination of water bodies. The total rainfall, following first few days after application of herbicides describes the amounts of leaching and runoff. Only about 5-10 % of the total applied, is lost due to runoff, even following heavy rains. Low rates of application, high soil adsorption capacity, and low rainfall decreases total runoff and keep the local water bodies less contaminated.

Solubility of a herbicide in water dictates its behavior in water. Acids and salts remain dissolved in water, till photolysis or hydrolysis, while esters will frequently get adsorbed to the suspended particles in water followed by precipitation to the sediments where these can be degraded through microbial activity. The average pH of surface waters ranges from 5 to 9 and highly alkaline or acidic waters can change the chemistry and thus the behavior of a herbicide in aqueous matrix.

Mode of Action

The mechanism either physical or biochemical through which a herbicide kills weeds is referred to as its mode of action. The mode of action of most herbicides involves altering or disturbing one or more of the metabolic pathways of target plants. Some cause the cellular matters of the target plant to escape by disrupting their cellular membranes but

have no direct disruption effect on other metabolic processes. The interference caused by a herbicide with the plant normal metabolism depends upon the mechanism which it disturbs. Certain species of plants are not affected by some herbicides due to difference in the biochemical pathways or enzymes they use.

Most of the currently used herbicides, predominantly affect processes limited to plants, like photosynthesis or aliphatic amino acids synthesis and animals usually suffer little or not at all. The mode of action of a herbicide determines when and how it is to be applied. Pre-emergent herbicides either kill the germinating seedling or interrupt germination while post-emergent herbicides are used directly on grown plants. Some herbicides have dual activity that is they can be used both pre- and postemergence.

Chemically and physiologically similar herbicides are grouped in the same "herbicide family". For example picloram, triclopyr and clopyralid, all have the same basic structures and chemistry, and belong to the pyridine family. The mode of action is usually related to chemical structure, and thus, herbicides of the same family show the same mode of action. For example, the members of pyridine family are all auxin mimic herbicides. However, some herbicides can have the same mode of action but belong to different families. For example, the 2, 4-D a 'phenoxy' herbicide just like the 'pyridines' is an auxin mimic.

The usual modes of action of herbicides comprise the following:

Auxin mimics imitate the hormones 'auxin' being responsible for plant growth and thus cause wild and disordered growth in target plant species. Examples are 2, 4-D, clopyralid, picloram, and triclopyr.

Mitosis inhibitors (dormancy enforcers) inhibit re-budding and new growth in spring and in summer respectively, for instance fosamine.

Photosynthesis inhibitors cause breakdown of the cells by blocking specific photosynthetic reactions, e. g. hexazinone.

Amino acid synthesis inhibitors stop amino acids synthesis which is the basic requirement for building proteins, e.g. glyphosate, imazapyr and imazapic.

Lipid biosynthesis inhibitors avoid lipids synthesis which is necessary for cell membrane's growth and maintenance, e.g. fluazifop-p-butyl and sethoxydim.

Dissipation in the Environment

The net degradation or immobilization and movement of a herbicide in the environment is termed as dissipation. Degradation involves decomposition to smaller fragments and ultimately to carbon dioxide, water and salts. This usually happens by a series of photochemical, chemical, or biological reactions. Biodegradation, which is microbial in nature, play the major role in case of most herbicides. The fragment compounds of herbicides called metabolites show its individual chemical behaviour like adsorption capacity, toxicity, degradation resistance and chemical reactivity. In some cases the metabolites show more toxic effects than the parent compound.

Photodegradation means decomposition by sunlight and depends upon the intensity of sunlight. A number of factors like season, latitude, weather, time of day, shading and pollution etc. affect the intensity of sunlight and thus the process of photodegradation of herbicides. Usually UV light is used for the studies of the photodegradation of herbicides and it is a debatable issue whether UV light actually reaches the surface of the earth or not. Therefore, the data obtained in the laboratory concerning the rates of photodegradation of herbicides may over-estimate the significance of this process in the field.

Microbial degradation involves decomposition of different herbicides by different microbes in their process of metabolism at a rate which depends upon the microbial community present under a given conditions. Warmth, high organic content and moisture enhance the process of microbial degradation. Microbial decomposition follows one of the two routes either it may be metabolized directly, or they may be co-metabolized by combining with a natural food source supporting the microbes. A lag time prior to microbial degradation always exist because the supplies of necessary enzymes or growth of populations of proper microbes take time to build-up. This time lag if long, gives way to other degradation processes to dominate over the microbial degradation.

Chemical decomposition is driven by hydrolysis, oxidation and disassociation. For herbicide degradation, the significance of these chemical reactions in the field is still under study.

Immobilization of herbicides usually takes place when they are taken up by non-susceptible plants or get adsorbed to soil particles. These processes, even reversible

isolate the herbicide and prevent them from mobility in the environment. Other degradation mechanisms are also slowed down by adsorption. The rate of adsorption is often dependent upon the characteristics of the soil and of the herbicide and on soil or water pH. Soil organic content, cation exchange capacity and clay content generally increase the adsorption rate while it decreases with increasing pH and temperature. Out of all the best determinant of herbicide adsorption rates is the soil organic content. Adsorption is also affected by the water solubility of the herbicide, the less soluble ones, with some exceptions (e.g. glyphosate) get strongly adsorbed. The adsorption/desorption process primarily determine the readiness of an herbicide for degradation and transport through the environment. The rate of microbial metabolism or chemical degradation can be significantly slowed down in some cases while in other cases it can be facilitated by adsorption.

Movement of the herbicides through the environment occurs by a number of processes taking place simultaneously and continuously. These include subsurface runoff, volatilization during or after application, leaching down into the soil or evaporation from soil and plant surfaces. Volatility of a herbicide may lead to 80-90 % losses in extreme cases.

Selectivity of Herbicides

Herbicides are selective and their selectivity depends on concentration or dosage. All herbicides are toxic to plants and their toxicity depends on the amount applied, they kill all plants on overdosing and if dosage is appropriately low no plants die. At intermediate dosages some plants are killed and some remain safe.

The selective contact herbicides kill the meristematic tissues in buds of shoot tips and leaf axils when they are very close to the plant surface. To be selective they avoid or are nontoxic to the crop. Some older selective contact sprays like copper chloride, sodium arsenite at low dosage, iron sulfate, copper nitrate and sulfuric acid were selective by differential wetting. The newer selective contact sprays, such as dinitro-sec-butylphenol, the water soluble salts of dinitrocresol and dinitro-sec-amylphenol are highly toxic to common broad-leaved weeds.

Selective translocated type herbicides enter the leaf cells and move along with foods and nutrients through the vascular tissues of the plant, killing the meristematic zones of the roots. Selectivity here is of the biochemical type and depends upon some specific characteristics of the protoplasmic constituents and enzyme system of weed and crop. Amitrol, 2, 4-D and other chlorophenoxy compounds are used as selective translocated herbicides.

The nonselective contact herbicides kill all plants with which they come in contact. The most effective general contact spray is oil because aqueous sprays cannot wet and penetrate the cuticle. Usually oils are emulsified with water and stabilized with a surfactant to give greater exposure and dynamic strength to the spray.

The principal translocated spray of the nonselective type is the acid-arsenical solution that kills as much as 95 % of the weeds to depths of from 3 to 5 feet. Similarly ammonium thiocyanate, sodium chlorate, calcium hypochlorite, arsenic acid and ammonium sulfamate are examples of the non-selective translocated type.

Controversy about herbicides use

It is a common public belief that herbicides pose a significant risk to the population mainly through ground-water contamination and residues in food and to farmers through occupational exposure and in the society, there is an excessive hostile feeling about herbicides.

Based on the statistical studies, made by herbicides manufacturers, it has been reported that more than 90 % of the people interviewed, consider herbicides hazardous for human and for the environment. The main reason for this pitiable social support is probably, the communication gap which exists between the society and the scientific world. Scientists and researchers must give complete and scientific explanations for newly developed herbicides. A rigorous testing procedure is required for an herbicide's active ingredient to be registered. These extensive tests including toxicity to animals, mode of degradation and effects on non-target organisms take several years to complete. A time span of about 7 to 10 years and a cost of 40 to 80 million dollar are needed to fulfil US-EPA regulations and to market a safe and new active ingredient. Also it is commonly seen that herbicides

are not applied according to the labelled instructions. Considering all of the above stated facts, the health and environmental threats linked with herbicide use can be minimized and the fact may become accepted that not the use but only the misuse of herbicides can be a risk to human health and environment.

Herbicides industry in global framework

A few of the developed countries utilize about 80 % of the herbicides used for agricultural purposes worldwide. About 30 % of the herbicides are consumed in Europe, 35 % in North America (USA and Canada), 15 % in Japan and Australia and 20 % in the rest of the world.

The annual sales of world's agricultural and noncrop herbicides had been reported to be $ 14-17 × 10^9 in 2001. Increased plantation throughout the world resulted in higher consumption of herbicides in the following years till 2006, after that the discovery of newer and highly active ingredients led to a decline. By the end of 2006, the herbicides' average growth rate was expected to be about 1.0 % per year in the United States. A decrease in herbicides production has been observed since 1989 in Western Europe. About 50 key herbicides are used in the world, the largest markets being France, Germany, the UK, Spain and Italy. In Japan a steady decline in herbicides production at a rate of 2.6 % per year has been observed, with no expectations of growth in the near future. In Japan about 18 major herbicides, mostly imported are used.

Risks associated with herbicides' use

Health hazards associated with the use of pesticides including herbicides is a matter of concern in the modern world. Surely, some pesticides exhibit acute toxicity and must be handled and managed like any other toxic substances. Most herbicides, fortunately, are not highly toxic and serious poisoning only occurs when the object swallow them or consume the food derived from treated crops. Based on a common public view, it is perceived that herbicides residues in food, water or air increase the risk of chronic diseases. This perception is based on the animal tests in which massive doses are administered to animals and the toxicity effects to animals are used to predict the threat to human beings. Most of the toxicologists, worldwide, unanimously agree that animal tests

are insufficient for determining actual risk to human. In light of the available evidences, the health scientists recommend not to reject the use of herbicides and all other pesticides but to use them in an appropriate way.

The active ingredients of herbicides themselves or their degradation products formed when once released into the environment may all pose a potential threat to ecosystem, immediately or in the long run. Petroleum solvents and others which are added as adjuvants to the formulations can be highly toxic. The degree of risks cannot be assessed, especially by the common man, for want of the toxicity data and the related information about the herbicides. It is a matter of great concern because large amounts of these are easily accessible.

Environmental Toxicity

Mammals and Birds: Herbicides' toxicity is reported in terms of LD_{50} being determined under dissimilar conditions. Therefore comparisons of different herbicides based on toxicity may reveal only a rough estimate of their relative toxicities. The LD_{50} does not convey any information regarding chronic, long-term toxic effects resulting from exposure to lower doses.

Sublethal doses may lead to eye or skin irritation, nausea, headache and, in some rare cases, severe effects like genetic disorders, birth defects, paralysis, cancer, and even death in mammals and in humans. Packaging Impurities in the formulations and the added adjuvants may be more lethal than the active ingredient itself, and it becomes difficult to attribute threat directly to an active ingredient solely. Herbicides kill the target weed and thus alter the habitat of non-target plants and animal and this is the most drastic effects of herbicides.

Aquatic Species: Formulations containing ester based active ingredients usually pose greater risk for aquatic species than those containing salt and acid due to their lipophilic nature and also these can pass through the skin and gills of aquatic species easily and are not liable to dilution in aquatic environment.

Soil Microbes: Soil microbes are variably affected by herbicides depending upon the concentration of herbicide and the nature of the microbes. Low residue levels can result in population's growth while higher levels may cause population drops. In most cases it is very difficult to determine the long term effects on soil microbes because the time spans of the studies are too short.

Human Toxicity

Agricultural workers specially, those who mix and apply herbicides are often exposed to herbicides mainly via skin or by inhalation. Serious eye damage and even blindness may result due to the accidental spills or splashing into the eyes.

The adverse environmental effects offset herbicides use in agriculture but with the proper regulations all over the world and improvements in the applications of biotechnology to agriculture, currently a huge figure of herbicides is approved, widely adopted and selectively used.

A brief description related to the use, structure, physical and chemical properties and environmental aspects of herbicides which are the subject of our work is given below.

1.1. Glyphosate [42-65]

Glyphosate is a foliar-applied, broad spectrum, post emergence, non-selective, contact herbicide, used to treat crops, forests, water bodies, roadside areas and public and private yards. This most widely used pesticide by volume, introduced by Monsanto Company of US origin in the early 1970s was registered by US. EPA in 1974 and re-registered in 1993.

It is stable to photodegradation, highly soluble in water, strongly adsorbed to soil particles and only broken down by soil microorganisms and has a long half-life in the environment (47 days).

Glyphosate is the only member of the 'glycines' family of herbicides and is a very polar and amphoteric compound with pKa values of 0.78, 2.29, 5.96, and 10.98.

Glyphosate being usually considered to be a nonselective herbicide is selective in transgenic crops adapted for resistance to glyphosate and kills weeds like dandelion,

nutsedge, Johnson grass and Bermuda grass. It is an acid, but typically used in a salt form, most commonly the isopropyl amine salt.

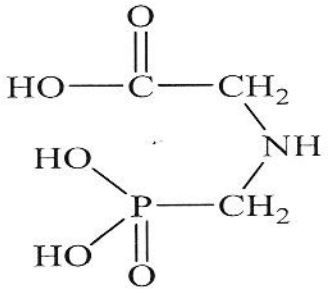
1.1.1. Physical / Chemical Properties of Glyphosate

Chemical Name (IUPAC): N-(phosphonomethyl) glycine

Molar Mass: 169.07 g mol^{-1}

Molecular Formula: $C_3H_8O_5NP$

Chemical Structure



Solubility (acid form)

Solubility in non-aqueous solvents is negligible.

Solubility in Water is 10.5 kg/L at pH 1.9 and 15.7 kg/L at pH 7.

Trade name (s): Roundup®, Rodeo, Accord and about 50 more commercial formulations are available.

Maximum Residue Levels

According to the U.S. EPA, maximum contaminant level of glyphosate in drinking water = 0.7 μg mL^{-1} and according to European Union (EU) guidelines, maximum residue level in most crops is 0.1 μg mL^{-1}. According to UNFAO, MRL of glyphosate in wheat is 5.0 mg kg^{-1}.

Mode of action: After absorption and translocation through plant tissues glyphosate disrupts shikimic acid pathway via inhibiting the enzyme, 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase. Glyphosate may also directly influence the enzyme 3-deoxy-D-arabino- heptulosonate-7-phosphate (DAHP) synthase. As a result depletion of aromatic amino acids tyrosine, tryptophan and phenylalanine needed for biosynthesis and growth occurs. The plant growth is inhibited, green colouration is lost, leaves become wrinkled and the tissues die from 4-20 days. The sodium salt of glyphosate can regulate plant growth and quickens fruit seasoning.

1.1.2. Toxicological profile

Acute Toxicity

Glyphosate exhibits low oral toxicity to rats with an acute oral LD_{50} greater than 4320 mg kg^{-1}. The acute oral LD_{50} was found >10,000 and >3530 mg kg^{-1} in mice and goats respectively. It shows low dermal toxicity to rabbits with an acute dermal LD_{50} of >2 g kg^{-1}. Glyphosate is low in toxicity for eye irritation, very low in toxicity for dermal irritation and was not observed to sensitize skin. For salt forms being considered slight eye irritants but not skin irritants, the LD_{50} in rabbits was >5000 mg kg^{-1}.

Inhalation toxicity to rats is low. Acute inhalation LC_{50} in rats is >4.43 mg L^{-1} based on a 4-hour, nose-only inhalation study. Upon exposure to the isopropylamine form of glyphosate, LC_{50} for rats was established to be >1.3 mg L^{-1} of air.

Signs of Toxicity in Humans: Indications of erosion of the gastrointestinal tract, dysphagia or trouble in swallowing and gastrointestinal hemorrhage were observed in 79 (suicidal attempts) ingestion cases. Among which seven cases died. Eye and skin irritation have seldom been reported from dermal contact to glyphosate formulations.

Chronic Toxicity

Animals: Capsules containing 0, 20,100, or 500 mg kg^{-1} day^{-1} of glyphosate were administered to beagle dogs and no effects were detected; the No Observed Effect Level (NOEL) for systemic toxicity is ≥500 mg $kg^{-1}day^{-1}$. The Lowest Observed Effect Level (LOEL) for systemic toxicity in rats was 940 and 1183 mg kg^{-1} day^{-1} for males and females, correspondingly. The NOEL for systemic toxicity is 362 mg kg^{-1} day^{-1} for males and 457 mg kg^{-1} day^{-1} for females. The chronic reference dose (R_fD) for glyphosate is 1.75 mg kg^{-1} day^{-1}.

Humans: In medical examinations and laboratory testing performed on the workers following pesticide application, no statistically substantial differences were observed.

Endocrine Disruption: Researchers have worked on rats and mice and have concluded that no indication of endocrine effects in humans or other mammals have been found from glyphosate's origin.

Carcinogenicity

Animals: A carcinogenicity study on mice showed that the systemic NOEL and LOEL were 750 and 4500 mg kg^{-1} day^{-1}, respectively. Based on these studies, EPA and an independent group of pathologists and biometricians concluded that adenoma is not caused by glyphosate. Numerous genotoxicity tests for glyphosate exhibited negative results.

Humans: The U.S. EPA classified glyphosate as Group E chemical, which is an evidence of non-carcinogenicity in humans. Researchers found that Roundup® and its constituents did not result in mutations or tumor formation while in a few cases; there was a "suggested association" between glyphosate exposure and multiple myeloma occurrence.

Teratogenic or Reproductive Effects

Animals: Researchers noticed reduced body weight gains in the dams and fetuses, higher maternal mortality, and an increased number of fetal skeletal abnormalities at the highest dose in pregnant rats dosed with glyphosate. No developmental effects were detected in rabbits. No influence on male or female sexuality and fertility was observed on dietary concentrations of up to 10,000 ppm of glyphosate in rats over two generations.

Humans: A link between preconception exposure to pesticide products containing glyphosate and elevated risks of late spontaneous abortion was proposed based on questionnaires filled out by farm operators and eligible couples collected during the Ontario Farm Family Health Study.

Fate in the Body: Based on numerous tests the researchers have concluded that, 30-36 % of glyphosate is absorbed next to ingestion while dermal absorption of glyphosate is very poor and glyphosate being non-volatile cannot be significantly absorbed from inhalation exposure.

Upon oral dosing of rats with 10 mg kg^{-1} glyphosate about 50 % of the dose was found in the gastrointestinal tract. When hens and goats were fed with glyphosate, glyphosate and its major metabolite AMPA were found in body tissues, eggs and milk.

Glyphosate has been found to undergo little metabolism and is excreted commonly unaffected in the urine and feces.

1.1.3. Environmental Fate

Soil: The average half-life of glyphosate in soil has been extensively studied and a usual field half-life of 47 days has been proposed. Glyphosate being fairly stable to chemical and photo decomposition is primarily degraded by soil microbial action, which yields AMPA and glyoxylic acid and eventually CO_2. Glyphosate adsorbs tightly to soil and is probably, immobile in soil.

Water: The average half-life of glyphosate in water ranges from a few days to 91 days and it did not get hydrolysed in buffered solution with a pH of 3, 6, or 9 at 35 °C. Being strongly adsorbed, glyphosate is unlikely to contaminate groundwater. But, aquatic uses of glyphosate and soil erosion may cause surface water contamination.

Air: Glyphosate and all its salts have little volatility with vapor pressures in the rage of 1.84×10^{-7} mm Hg to 6.75×10^{-8} mm Hg at 25 °C and are stable in air.

Plants: Glyphosate is absorbed by plant leaves and transported through the plant via phloem and is metabolized with AMPA as the only noteworthy metabolite.

1.1.4. Ecotoxicity studies

Birds: A single dose of technical grade glyphosate was found to be almost non-toxic to bobwhite quail, with an LD_{50} of >2000 mg kg^{-1}, while an 8-day dietary LC_{50} >4000 ppm for mallard ducks and bobwhite quail, showed slight toxicity. It is not likely to cause reproductive damages in birds at dose levels of about 1000 ppm. It is agreed upon that alteration of habitat is the major risk posed to birds and other wildlife by glyphosate and its formulated products.

Aquatic Life and Fish: Glyphosate was found to be a little toxic to nearly non-toxic to freshwater fish, with a 48-hour LC_{50} of greater than 24 mg L^{-1} to 140 mg L^{-1}. It is slightly toxic to freshwater invertebrates, with a 48-hour LC_{50} ranging from 55 ppm to 780 ppm. Reduction in reproductive capacity in Daphnia magna was reported based on a chronic toxicity study with glyphosate.

Terrestrial Invertebrates: Glyphosate was found to be almost non-toxic to honeybees, with acute oral and acute contact LD_{50} values >100 μg bee^{-1}. The earthworm LC_{50} in soil is >5000 ppm for Roundup®.

1.2. Fenoxaprop-p-ethyl [66-77]

Introduced in 1980s, Fenoxaprop-p-ethyl (FPE) is a foliar-applied, broad spectrum, postemergence and pre-emergence, systemic and selective herbicides. Fenoxaprop-p-ethyl is beige to brown coarse powder and has a half-life of 9 days in the environment. It is fairly stable in acidic media (pH 3), and in some cases in neutral conditions, and gets slowly hydrolyzed to the free acid in 30-1000 days. In alkaline media (pH 9), it rapidly undergoes hydrolysis in 2.2 to 60 h. The pKa values of the free acid ranges from 2.9 to 3.4.

FPE is a member of the Aryloxyphenoxypropionates (ArPP) family of herbicides including diclofop-methyl, fluazifop-P-butyl and quizalofop-P-butyl which are commonly called 'FOPs'. It is commonly known as graminicides (grass killers) and control annual and perennial grasses including wild oat, Italian ryegrass, crabgrass, goosegrass and bermudagrass in a large variety of broad-leaved crop plants like soybeans, sugar beet and cotton but are inactive towards other monocots (such as dayflower species and sedges) and to dicots. Specifically it is used to control volunteer cereals and other grass weeds in barley, wheat, rye, sunflower, legumes, fodder beet, flax, alfalfa, strawberries, beetroot, lettuce, spinach, potatoes, cucumber, peas, fennel tomatoes and peanuts. It is absorbed by plant leaves, translocated symplastically, and accumulates in meristematic areas of the root and shoot.

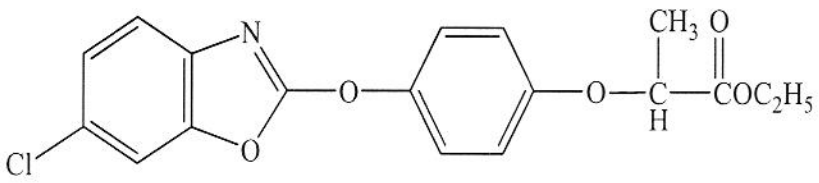
1.2.1. Physical / Chemical Properties of fenoxaprop-p-ethyl

Chemical Name (IUPAC): (+)-ethyl-2-[4-[(6-chloro-2-benzoxazolyl) oxy] phenoxy] propanoate

Molar Mass: 361.8 g mol^{-1}

Molecular Formula: $C_{18}H_{16}ClNO_5$

Chemical Structure



Solubility (at 20 °C)

Solubility in non-aqueous solvents:

Acetone and Toluene, more than 200 g L^{-1}, Ethanol 24 g L^{-1}, n-hexane 7 g L^{-1}

Solubility in Water is 0.9 mg L^{-1}

Trade name (s): Acclaim Extra, Furore Extra, Cheetah R, and Cheetah.

Residue Levels: European Union (EU), maximum residue level in most crops = 0.1 µg mL^{-1}. Data about the MRL is not yet provided by the EPA because the herbicide is currently under the registration review by US EPA and is estimated to be registered in 2013.

Mode of action: FPE functions as an acetyl-CoA carboxylase (ACCase) enzyme inhibitor. This enzyme is found in the stroma of plastids and catalyzes the first dedicated step in de novo fatty acid synthesis that is the formation of malonyl- CoA from acetyl-CoA and bicarbonate dependent on adenosine triphosphate. FPE blocks fatty acid biosynthesis and consequently the production of phospholipids used in building new membranes essential for cell growth. Within a few days after application, the younger leaves of target plants become yellow and growth ceases. This follows death of tissue (necrosis) within 1 to 3 weeks.

1.2.2. Toxicological Profile

Acute Toxicity

Oral: FPE is found to have low acute mammalian toxicity with an oral LD_{50} value of 2090 mgkg in female wister rate and 5000 mg kg^{-1} in NMRI mice. Liver toxicity of fenoxaprop-ethyl has been observed in long-term studies for mice.

Dermal and Inhalation: In rats a percutaneous LD50 value exceeding 2000 mg kg^{-1} was found and exposure to dust particles of FPE at a concentration of 0.604 mg L^{-1} caused no death in a group of ten rats. It was found slightly irritant to rabbit skin and eyes.

Chronic Toxicity

Animals: Chronic toxicity studies with FPE have been performed in mice, rats and dogs. In rats lipid lowering action was clearly observed and liver and kidney were concluded to be the target organs of toxicity. No effect levels (NEL) of 80 ppm (14 mg kg^{-1} day^{-1} and 6 mg kg^{-1} day^{-1}) for mice and rats and 400 ppm (16 mg/kg/day) for dogs were established.

Humans

Carcinogenicity: The most recent assessment in 1998 used an interim Q1 (The Carcinogenic Potential of a Compound, Quantified by the EPA's Cancer Risk Model) of 9.1×10^{-2} for FPE which was based on increases in adrenal tumors in male mice. The upper-bound (food only) carcinogenic risk estimate was 9.1×10^{-7}.

Reproductive or Teratogenic Effects: Teratogenic effects with FPE were carried out in rats and rabbits. No indication of teratogenicity at the highest dose used (100 mg kg^{-1} day^{-1}) was found. The NEL for maternal toxicity was 32 mg kg^{-1} day^{-1}, both in rats and rabbits and for fetotoxicity it was 10 mg kg^{-1} day^{-1} in rats and 100 mg kg^{-1} day^{-1} in rabbits. FPE was also thought to have reproductive toxicity and had a severe effect on motility patterns of porcine sperm.

Fate in the Body: Investigations on rates showed that FPE is rapidly absorbed and excreted only via urine. Usually 75 % of the dosed amount is recovered in the first 48 hours.

1.2.3. Environmental Fate

Soil: The aerobic degradation of FPE studied in various types of soils showed that it broke down rapidly to the free acid which further degraded by 90 % within 13 to 38 days and no leaching in the soil was observed.

Plants: The metabolism studies of FPE in wheat and rice reveals that the parent ester degrades to its acid form in wheat which undergoes further cleavage to form 6-chloro-2,3-dihydro-benzoxazol-2-one and also 2-(4-hydroxyphenoxy)-propionic acid in rice.

Food Residue: There are no dietary risks that exceed the EPA's level of concern (LOC).

1.2.4. Ecotoxicity Studies

Birds: The toxicity of FPE to four different species, Japanese quail, Bobwhite quail, Mallard duck and partridge, was studied and it was found to be low usually about 2000 mg ai kg^{-1} bw and Japanese quail were the most susceptible of the four species studied.

Fish and Aquatic Life: The 96 hour LC_{50} values found for fenoxaprop-p-ethyl ranged from 0.0975-0.85 mg L^{-1} for fish and 0.438-3.18 mg L^{-1} for aquatic arthropods. The maximum acceptable toxicant concentration was 0.064 mg ai kg^{-1} bw for rainbow trout, based on reduction in growth and activity.

Terrestrial Invertebrates: FPE was found of low toxicity to bees and a difference of less than 10 % compared to controls was observed in a soil respiration study.

1.3. Pinoxaden[78-96]

Pinoxaden is a broad spectrum, post-emergence graminicide used in cereal crops. It is a member of the chemical class of 3-hydroxi-4-phenyl-5-oxo-pyrazolines often simply called phenylpyrazolines or DENs and is sold worldwide under the trade name AXIAL®. Pinoxaden is sprayed against a number of annual grass weeds as well grass found in wheat and barley crops.

Pinoxaden being sprayed is absorbed through foliage as well as roots by the plant and move bidirectional through the plant body and efficiently inhibits weed regardless of the level of dose and the time of application. Pinoxaden is also active against isoproturon-resistant weeds in barley. To ensure the tolerance of original crops, wheat and barley, a patented safener Cloquintocet-mexyl is added to the formulation. In addition, an effective adjuvant is combined into the formulation to guarantee the optimum spread of the spray on the grass leaves and to have fast uptake of solution by the leaf tissues.

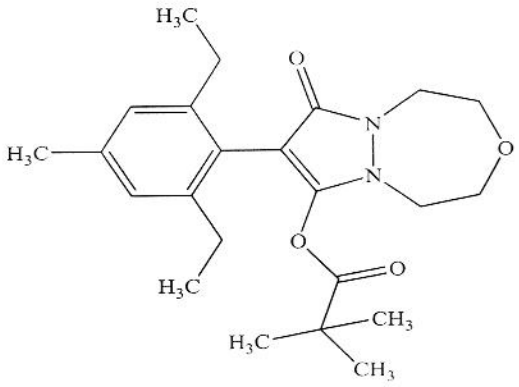
1.3.1. Physical / Chemical Properties of pinoxaden

Chemical name (IUPAC): 8-(2,6-diethyl-p-tolyl)-1,2,4,5-tetrahydro-7-oxo-7H-pyrazolo[1,2-d][1,4,5]oxadiazepin-9-yl 2,2-dimethylpropionate

Molar Mass: 400.5 g mol^{-1}

Molecular Formula: $C_{23}H_{32}N_2O_4$

Chemical Structure



Solubility (20 °C)

Solubility - In water 200 mg L^{-1}

Solubility - In organic solvents

Acetone (250000 mg L^{-1}), Dichloromethane (500000 mg L^{-1}), Hexane (1000 mg L^{-1}) and Toluene (130000 mg L^{-1})

Residue Levels: The recommended maximum residue limit MRL is 0.1, 0.01 and 0.02 mg/kg for wheat and barley, milk, and mammalian meat respectively. It is also suggested that pinoxaden MRLs be set at or about 0.04 mg kg^{-1} for eggs, poultry meat and poultry offal respectively, and 3 and 1 mg kg^{-1} for wheat and barley forage (green) and wheat and barley straw and fodder (dry) respectively.

Acceptable daily intake (ADI)

Based on a NOEL (No Observed Effect level) of 10 mg kg^{-1} bw day^{-1} in a 24-month study in rats and using a 100-fold safety factor the ADI for pinoxaden was set at 0.1 mg kg^{-1} bw day^{-1}.

Acute reference dose (AR_fD)

Using a 100-fold safety factor and the no effect level of 30 mg/kg bw/day, an AR_fD of 0.3 mg kg^{-1} bw was established for pinoxaden.

Mode of action: Pinoxaden suppresses the function of acetyl Co-A carboxylase enzyme, in that way inhibits the fatty acids synthesis and hence the development of biomembranes in weeds.

Metabolism of pinoxaden

Plants: The data found from metabolic studies in wheat indicate that parent pinoxaden (M1) is only a minor fraction of total residues. It (M1) undergoes rapid hydrolysis instantly after application, to metabolite M2 (NOA 407854). M2 undergoes further hydroxylation to form M4 (SYN 505164), and by 21 days post-treatment, the levels of M2 are non-detectable in fodder. M4 is also found to be an important residue component in wheat grain, straw and husks. Metabolism of M4 further gives a number of other metabolites.

Animals: The metabolism of pinoxaden in animals is rather comparable to that observed in plants. In milk giving goats, metabolite M2 seems to undergo conjugation with glucuronide to form metabolite M12, and these metabolites (M2 and M12) are the chief constituents of urinary TRRs. Tissues from goats were found to contain no metabolite M4.

1.3.2. Environmental Fate

Pinoxaden being moderately hydrophilic and having low volatility is likely to remain in the soil and water after application coming through spray drift or runoff. Pinoxaden is rapidly hydrolysed either abiotically or assisted by microbes, mostly in alkaline media to pivalic acid and the diketone which resist further hydrolysis. For pinoxaden the reported half-life is 17 days at pH 5 and 2 days at pH 9.

Pinoxaden also undergoes photolysis in aqueous medium. Photodegradation seems to be a minor pathway for its degradation as compared to a rapid (less than a day) abiotic and microbial hydrolysis.

The diketone moiety being herbicidally active is liable to hydroxylation through microbial activity under aerobic conditions, but is stable under anaerobic conditions. The Final metabolic products are unextractable residues and carbon dioxide.

As confirmed by laboratory studies, pinoxaden is reasonably soluble in water and has a little volatility thus; volatilization from soil or flora would not be a substantial mode of transport.

1.3.3. Toxicological profile

Acute Toxicity: Pinoxaden has a small acute toxicity in rats, following single dose with an LD_{50} of >3,129 mg kg^{-1} for oral and >2,000 mg kg^{-1} for dermal administration. It has a low inhalation toxicity of LC_{50} >5 mg L^{-1}. It shows moderate skin and eye irritation in rabbits and is not a skin sensitizer in guinea pig.

Chronic Toxicity: Based on a wide range testing on laboratory mammals and in vitro system, pinoxaden showed no significant indication of carcinogenicity, mutagenicity, teratogenicity or reproductive effects.

Short Term Toxicity: Pinoxaden orally administered at 0, 300, 600 or 1000 mg kg^{-1} bw day^{-1} for 28 days to rats showed drop in body weight gain in males and higher neutrophil and lymphocyte counts at 1000 mg kg^{-1} bw day^{-1}. A dose rate of 600 mg kg^{-1} bw day^{-1} and above resulted in increased plasma urea and creatinine levels, higher inorganic phosphate and lower chloride levels. Bilirubin levels were observed to increase in males at 1000 mg kg^{-1} bw day^{-1}. At 600 mg kg^{-1} bw day^{-1} and above urinary volume and ketones levels and kidney and liver weight were found to increase in both sexes and also Increased frequency and severity of renal tubular atrophy and dilatation and single cell necrosis was observed. Pinoxaden dosage in gelatin capsules at 0, 250, 500 or 1000 mg kg^{-1} bw day^{-1} for 28 days was given to dogs and high rate of vomiting at the dosage rate of 500 and 1000 mg kg^{-1} bw day^{-1} were observed.

1.3.4. Ecotoxicity Studies

Birds: Data obtained from standard testing measures showed that pinoxaden is nearly non-toxic to birds administered with acute oral or subacute or chronic dosage. Concentrations of 300 and 1000 mg kg^{-1} in the diet in bobwhite quail and in mallards respectively, showed no effect on reproduction.

Aquatic life: Pinoxaden was found to be slightly toxic to fish (Rainbow Trout: LC_{50} = 5.6 mg L^{-1}, 96 h) and aquatic invertebrates (Water flea: EC_{50} = 3.2 mg L^{-1}, 48 h) upon acute or chronic exposure, but highly toxic in formulation form. Technical pinoxaden was found to have slight to moderate toxicity to algae (Green algae: E_bC_{50} = 4.6 mg L^{-1}, 72 h, EC_{50} = 9.7 mg L^{-1}, 72 h) and duckweed.

Honey bees, earthworms and soil microbes: Based on standard tests, pinoxaden was found to be nearly non-toxic to earthworms, honey bees and soil microbes. Pinoxaden in formulation form was found more toxic to honeybees and toxic to earthworms and showed no toxicity to useful arthropods like parasitic wasps, green lacewings, predatory mites and rove beetles when exposed to dried foliar deposits.

1.4. Isoproturon [97-107]

Isoproturon (IPU) is a member of phenylurea herbicides (PUHs) introduced in 1960s. It is selective and systemic and is used to control annual grasses (wild oats, silky bent grass, ray grass, black grass, annual meadow grass) as well as broadleaved weeds in a variety of crops, fruits and vegetables, including wheat, winter rye, barley, citrus, asparagus, bush fruits and triticale etc. It is applied both for pre- and post-emergence control of crop and noncrop weeds.

1.4.1. Physical / Chemical Properties of Isoproturon

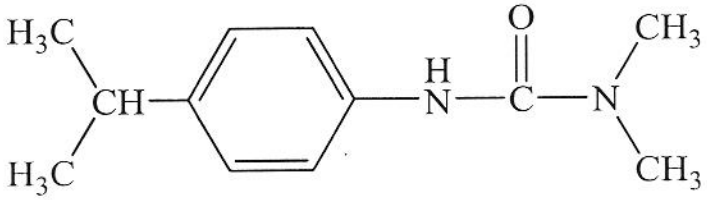
Chemical Name (IUPAC): 3-(4-isopropylphenyl)-1, 1-dimethylurea

Physical state: Colourless crystals

Molar Mass: 206.3 g mol^{-1}

Molecular Formula: $C_{12}H_{18}N_2O$

Chemical Structure



Solubility (at 20 °C)

Solubility in non-aqueous solvents: Acetone 38 g L^{-1}, Benzene 5.0 g L^{-1}, Dichloromethane 63 g L^{-1}, Methanol 75 g L^{-1}, n-hexane 0.2 g L^{-1}, Xylene 4.0 g L^{-1}

Solubility in Water: 72 mg L^{-1}

Trade name (s): Ipuron, Alon, Guideline, Panron, Passport and many others.

Residue Levels: European Union (EU), maximum admissible concentration in drinking water = 0.1 µg L^{-1}; UK/EC standards, maximum residue limit in most cereals = 0.05 mg Kg^{-1}

Mode of action: Isoproturon acts by inhibiting electron flow in photosystem II of the light reaction of photosynthesis. However, it kills plants too rapidly to be explained by inhibiting energy production via photosynthesis (starvation). The production of free radicals, resulting in blocking normal electron flow in photosystem II, is most probably responsible for the speedy plant kill.

1.4.2. Toxicological Profile

Acute Toxicity: Isoproturon is reported to have low acute oral toxicity in mammals. Oral LD_{50}s are in range of 1826-3600 mg kg^{-1} of the body weight for various species. No evidences of skin and eye irritation or sensitization after constant dermal exposure of isoproturon have been documented. Based on a 90-day dietary study in rats and beagle dogs it was observed that animals getting 400 mg kg^{-1} and higher dose exhibited a reversible rise in liver weight, induction of several hepatic enzymes and propagation of the smooth endoplasmic reticulum in hepatocytes. At a dose rate of 2000 mg kg^{-1} reversible haemolytic anaemia was observed. The NOAEL (no-observed-adverse-effect level) was 80 mg kg^{-1} and 50 mg kg^{-1} for rats and dogs respectively.

Chronic Toxicity: Rats were dosed with isoproturon at concentrations of 0, 80, 400, or 2000 mg kg^{-1} for 104 or 115 weeks. At the highest dose an increased liver weights, serum enzyme activities and cholesterol values indicating hepatic enzyme induction and marginal reduction of all red blood cells were observed.

Reproductive toxicity, embryotoxicity and teratogenicity: Isoproturon was not found to be teratogenic in rabbits or rats at doses up to 25 or 100 mg kg^{-1} of body weight per day respectively. In a two-generation study, rats were fed isoproturon at concentrations of 0, 80, 400, or 2000 mg kg^{-1} of diet per day. Highest dose resulted in significant symptoms of toxicity to the parents and pups. Maternal toxicity resulted in reduced litter sizes and numbers of implantations. A minor retardation of body weight gain was also found in the group receiving 400 mg kg^{-1} of diet. A NOAEL of 80 mg kg^{-1} of diet was identified and no effects on fertility, reproduction or sexual maturation were observed.

Mutagenicity: A number of in vitro and in vivo short-term tests for mutagenicity were conducted on isoproturon which shows that it is not mutagenic in bacterial, eukaryotic, or in vitro and in vivo mammalian test systems.

Carcinogenicity: No signal of carcinogenicity, hepatic enzyme induction or increased liver weights was seen in a two year study of isoproturon in CD-1 mice. In addition a two year dietary study in Sprague-Dawley rats, it was found to cause an increase in hepatocellular tumours at doses above 80 mg kg^{-1}. Based on these evidences isoproturon may be regarded as a tumour promoter.

Effects on humans: A three year study was conducted on a group of workers employed in various parts of the manufacturing process. Analysis of urine and blood showed no pathological defects in the peripheral blood count or any indication of haemolytic anaemia.

Fate in the Body: Isoproturon is quickly absorbed when given orally. Accumulation of isoproturon in any organ or tissue has not been reported and distribution is rapid. Experiments on rats show that it is quickly metabolized in the body via the major paths being N-demethylation and oxidation of the N-methyl groups and isopropyl moiety followed by conjugation reactions. The N-hydroxymethyl derivative of substituted urea has not been identified, though this compound may be formed at a low concentration as a short-term intermediate. All these metabolites are rapidly eliminated in the urine.

1.4.3. Environmental Fate

Soil: Isoproturon undergoes enzymatic and microbial demethylation at the urea nitrogen and hydrolysis of the phenylurea to form 4-(2- hydroxyisopropyl) aniline. Under field conditions, its half-life is about 40 and 15 days in mild and humid climates respectively.

Water: In water, it is fairly stable and undergoes hydrolysis slowly; the half-life is about 30 days.

Photodegradation: Isoproturon go through photochemical degradation to a variety of products which includes 3-(4-isopropylphenyl) urea, 3-(4-isopropylphenyl)-1-methylurea, 4, 4-diisopropylazoxybenzene and 4, 4-diisopropylazobenzene.

Environmental levels and human exposure: Due to low vapour pressure and short half-life of isoproturon in soil, the risk of human exposure from air is insignificant. Water resources may become polluted with isoproturon from manufacturing units' effluents and agricultural runoff. For the general population, diet is usually not considered as a major source of exposure to isoproturon.

1.4.4. Ecotoxicity Studies

Birds: In an assessment of acute toxicity of isoproturon to pigeons and domestic quail, based on the mortality data, the LD_{50} was found to be more than 5000 mg kg^{-1} for pigeons and 8400 mg kg^{-1} for quails.

Fish and Aquatic Life: Based on the estimation of acute toxicity of isoproturon to rainbow trout fish, irregular respiration rate, mycoid discharge and flatulence at 17.1 mg L^{-1} and loss of equilibrium and righting reflexes were observed at higher concentration. The 90 hour LC_{50}, NOEC and LOEC were calculated to be 37.22, 9.5 and 17.1 mg L^{-1} respectively.

Arthropods: For honey bees the reported acute oral toxicity, LD_{50} is 195 µg bee^{-1} and the acute contact toxicity, LD_{50} is 200 µg bee^{-1}.

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LITERATURE REVIEW

Sun et al (1) described a cheaper and faster reverse phase liquid chromatographic method for glyphosate and its byproduct aminomethylphosphonic acid (AMPA) determination in water samples. The determination involves derivatization with 4-methoxybenzensulfonyl fluoride. The limit of detection, with simple pre-concentration, for both the analytes was found to be 0.1μg L^{-1}, which testifies the sensitivity and authenticity of the method.

See et al (2) explored a fast and direct quantification method for glyphosate and its metabolites, glyfosinate and amino methylphosphonic acid in drinking water. The method involves online pre-concentration that follows capillary electrophoresis (CE) with capacitivity coupled contactless conductivity detection (CE-C^4D). Two different pre-concentration procedures, one large volume sample stacking without polarity switching and the other field-enhanced sample injection coupled with (CE-C^4D) were employed and optimized. With the former method, under optimized conditions, detection limits ranging from 0.001 to 0.1 mM with 48-53 fold higher sensitivity were obtained. Using the latter procedure, LODs as low as 0.0005-0.02 mM with 245-1002 time enhanced sensitivity was achieved. Both practices exhibited adequate reproducibility with relative standard deviation more than 10 %. The proposed methods were employed for assay of glyphosate and its metabolites in control samples of tap water.

Glyphosate and glufosinate herbicides were directly and quantitatively determined by a cheaper, fast, sensitive and simple amperometric method presented by Songa et al (3). Nanoparticles; poly (2, 5-dimethoxyaniline)-poly (4-styrenesulfonic acid) (PDMA-PSS) was produced and characterized electrochemically by cyclic voltammetry (CV) and Scanning electron microscopy. The nanoparticles were used to modify the surface of a rotating gold disk electrode. On the modified surface of the electrode the enzyme horseradish peroxidase (HRP) was electrostatically immobilized to assemble a nanobiosensor. The main principle of the method was to find out the sensor response to glyphosate and glufosinate by amperometric means. Prior to injection of the analyte into the electrolytic solution, the enzyme activity was measured by using hydrogen peroxide. The enzyme electrode was used for over 60 measurements as it was stable for long. The technique was applied to spiked corn samples with concentration ranging from 2-78 μg L^{-1}, signifying the sensitivity of the biosensor for residue detection in corn. The LODs were

found to be 0.1μg L^{-1} for both glyphosate and glufosinate, based on 20 μL sample injection volume, without any need of pre-concentration or sample clean-up.

Zelenkova and Vinokurova (4) reported an ion-exchange liquid chromatographic process aimed at the separation of glyphosate and its microbial N-acylated derivatives. Repro-Gel H column coupled to UV detection was used for elution. The elution order of the compounds was related with their structures. The limit of detection (LOD) determined in ng at 210 nm was 30 for glyphosate, 43 for sarcosine, 30 for glycine and 45 for aminomethylphosphonic acid. LOD for methylphosphonic acid was found to be 14 μg. TLC with silica gel plates and isopropanol-5 % aq NH_3 (1:1) solvent system was used for separation of glyphosate and its metabolites.

Hanke et al (5) reported a method for glufosinate, glyphosate and aminomethylphosphonic acid (AMPA) determination in water samples. The method involves derivatization with 9-fluorenylmethylchloroformate (FMOC-Cl), solid phase extraction and liquid chromatography following electrospray tendem mass spectrometry (ESI-MS/MS). Organic solvent content, retention time and amount of FMOC-Cl were optimized for enhanced sensitivity. 100 % yield for the derivatization reaction was obtained within 2 hours in ground water and surface water samples using 10 % acetonitrile contents. Solid phase extraction of 80 mL sample over 200 mg Strata-X cartridges was executed next to a twofold dilution, where low acetonitrile content allowed efficient extraction. The extract was rinsed on the SPE cartridge with dichloromethane to lessen the load of the by-products consequents to derivatization (e.g. FMOC-OH) on the LC column and mass spectrometer. In order to minimize the analyte-metal complexation, EDTA was added and the sample was acidified. The limit of quantification (LOQ), by the proposed method in ng L^{-1} was 0.8, 0.7 and 2.3 for AMPA, glyphosate and glufosinate respectively. LOD found out for both glyphosate and AMPA was 0.2 ng L^{-1} and for glufosinate it was 0.6 ng L^{-1}. The recoveries from spiked surface and ground water samples ranged from 91-107 % at 2.0 ng L^{-1} spiking level.

Hu et al (6) described an easy approach for determination of the residue of glyphosate in soil. Ammonium hydroxide (2.0 M) was employed for the extraction of residue from soil matrix. The extracted residue was derivatized with trifluoroacetic anhydride and

trifluoroethanol followed by assay on capillary gas chromatography using nitrogen phosphorus detector (GC-NPD). The results were in agreement with one obtained using gas chromatographic mass spectroscopic (GC-MS) analysis. The detection and quantification limits were observed to be 9×10^{-12} g and 0.01 mg Kg^{-1} respectively, with an average recovery in the range of 84.4-94.0 % and a relative standard deviation in the range of 8.1-13.7 %. These results conform to the method validation directives for residue analysis. Glyphosate dissipation rates and residue levels were effectively determined in the soil from an apple orchard by using the proposed method.

A novel high performance cation chromatographic technique coupled with inductively coupled plasma dynamic reaction cell mass spectrometry (ICP-DRC-MS) was presented by Popp et al (7). This approach involves simultaneous assay of glyphosate and its key metabolite, aminomethylphosphonic acid (AMPA) and is based on measurement of phosphorus atom of the analyte molecules as $^{31}P^{16}O^{+}$ fragment using O_2 as reaction gas. To achieve the European Union permissible limit of 0.1 μg $L^{-1}$for glyphosate in surface and drinking water, a two-step enrichment process was employed using Chelex 100 and AGI-X8 resins before HPIC-ICP-MS analysis. The method was applied to selected Austrian river water samples and concentration of 0.67 μg L^{-1} of glyphosate and 2.8 μg L^{-1} of AMPA were found.

Teofilo et al (8) described a voltammetric method using hanging mercury drop electrode (HDME), optimized by experimental design for glyphosate determination in water, soil and vegetable samples. The DPV variables optimized were pulse interval, pulse amplitude, voltage step, concentration of the supporting electrolyte and voltage step time. These effects were assessed by choosing 2^5 factorial design and three most important factors were investigated which were then evaluated by central composite design. The working range was found to be 0.05-100 mg L^{-1} and the LOD and LOQ achieved were 14 μg L^{-1} and 48 μg L^{-1} respectively, under the optimized conditions. After purification with an ion-exchange resin and derivatization, the optimized method was effectively used to determine glyphosate in vegetables, soil and water samples.

Ibanez et al (9) investigated an analytical technique for residue quantification of glyphosate and aminomethylphosphonic acid (AMPA) in water samples within a Round

Robinson Study. The sensitive, fine-tuned, accurate and explicitly established method being previously authenticated in various water samples involves on-line solid phase extraction-liquid chromatography coupled to electrospray tendem mass spectrometry (SPE-LC-ESI-MS/MS). Acceptable results were acquired in all cases except for glyphosate in ground water samples where in spite of using isotope-labeled glyphosate as internal standard, only 15 % recovery was achieved. The method was modified by acidifying the sample with HCl to pH 1. This followed sample neutralization and instant derivatization with 9-fluorenylmethylchloroformate (FMOC) prior to LC-MS/MS assay. This approach was applied to investigate glyphosate and its derivatives in Round Robin Study water samples with an enhanced recovery upto 98 %. The reason for the low recovery was explained to be of kinetic nature and may be due to chelate formation between glyphosate and components in the matrix which prevent glyphosates' derivatization and hence analysis. The method was also applied to re-assess previously analyzed water samples from Mediterranean area of Spain and glyphosate was found in higher concentration ranging from 2-14 times in most cases.

Glyphosate and aminomethylphosphonic acid (AMPA), with a concentration ranging from 0.1-500 μg L^{-1}, was determined in a number of aqueous media; being different in their ionic composition using an extraction and enrichment method described by Corbera et al (10). The procedure aimed at the evaluation and comparison of two anion-exchange resins; the microporous Amberlite+IRA-416 and the macroporous Amberlite+IRA-900 and commercial silica modified cartridge ISOLUTE-NH_2. The effect of various parameters like volume composition, flow rate of eluent, volume of sample, concentration and porosity of sorbent were optimized and then employed for the recovery of the analyte from the fortified natural water. The analysis involves pre-column derivatization using 9-fluorenyl methyl chloroformate (FMOC-Cl) followed by HPLC determination with fluorescence detection. Glyphosate determination at a level of 0.1 μg L^{-1} was facilitated by protonated ISOLUTE-NH_2 cartridge, with an enrichment factor up to 125. With an RSD value, less than 10 % for both glyphosate and AMPA, recovery rates of 99 % and 77 % were achieved, respectively, using the Amberlite® IRA-900 macroporous resin.

Khrolenko and Wieczorek (11) described a method for the efficient extraction of AMPA and its parent compound glyphosate from juices (apple, orange, black current and grape fruit) using supporting liquid membrane technique (SLM) followed by derivatization with p-toluenesulphonyl chloride (TsCl) and detection with HPLC-UV. The effect of different factors like concentration of analyte, flow rate and acceptor phase composition on the extraction procedure and extraction efficiency was investigated. It was found that an improved level of detection can be accomplished by proper handling of SLM parameters. By employing the optimized conditions glyphosate and AMPA were found in juices at a lower concentration range of 0.025 mg L^{-1} with recovery of 71.1, 72.1, 93.6 and 102.7 % for glyphosate and 64.1, 64.6, 81.7 and 89.27 % for AMPA from orange, grape fruit, apple and black current juices respectively. These results indicate the applicability of SLM extraction of AMPA and glyphosate pre-concentration from complex liquids.

Guo et al (12) described a trace analysis technique for quantification of glyphosate and phosphates based on ion-chromatography-inductively coupled plasma mass spectrometry (ICP-MS) detection. The analytes were separated on a polymer anion-exchange column (Dionex IonPac AS16, 4.0 mm × 250 mm) with a 20 mM citric acid solvent system at a flow rate of 0.50 ml min^{-1} followed by direct and selective detection by ICP-MS at m/z =31 . The influence of various variables on chromatographic behaviour and ICP-MS features was investigated. A linear calibration curve was found up to 400 μg L^{-1} with a limit of detection of 0.7 μg L^{-1}, for both glyphosate and phosphate, based on 500 μL sample injection volume. The interference effect of AMPA, polyphosphates, non-polar and other polar pesticides containing phosphorus, matrix anions like chlorides, nitrates, sulphate and nitrites etc. and metallic ions on the chromatographic behaviour of the analyte was studied and no interference effect on the analysis was seen. The recovery from spiked samples of certified reference material, reservoir water, Newater and drinking water gave reasonable results in the range of 97.1-107.1 % with a relative standard deviation of ≤7.4 % for triplicate analysis. The developed method based on ICP-MS is being claimed to be simple, sensitive and does not require derivatization, eluent conductivity suppression and pre-concentration.

In order to have improved sensitivity, Corbera et al (13) slightly modified the already available approach for glyphosate and AMPA determination based on capillary electrophoresis (CE). Pre-concentration step was introduced prior to CE analysis, because the detection limits found out (5.0 μg mL^{-1} and 4.0 μg mL^{-1} for glyphosate and AMPA respectively) were still not reasonable. Preconcentration of the analytes in environmental aqueous samples was achieved with Amberlite® IRA-900; an anion exchange resin. The already optimized conditions were revised except the eluent volume which was decreased as limited by CE. The method was applied for recovery from control samples of ultrapure water and recoveries in the range of 84-87 % (RSD < 6 %) and 85-98 % (RSD < 5 %) for glyphosate and AMPA respectively, were achieved with an enrichment factor up to 65. Applying this approach AMPA and glyphosate concentration of 60 ng mL^{-1} and 85 ng mL^{-1} correspondingly, was achieved. Four natural water samples, with variable conductivity, were analysed and variation in the extraction efficiency, especially the strong dependence on ionic strength of sample on AMPA was observed. In case of sample of medium and low conductivity (0-800 μS) recoveries in the range of 86-99 % were achieved for glyphosate. While studying the influence of sample salt contents on the efficiency of extraction, a linear correlation was observed for AMPA (r^2=0.996). An improved recovery was observed with the use of lower volume of sample. A comparison of the CE method based on statistical *t*-test and F-test was made using HPLC-UV method, which shows no significant differences in the results.

Safarpour and Asiaie (14) reported a capillary electrophoresis-mass spectrometry (CE-MS) approach for the rapid assay of glyphosate in a selected dried granule (DG) formulation. A bare fused-silica capillary column, run in the reversed phase mode was employed for separation purpose, using ammonium formate buffer pH 2.5. An LOD of 10 ng mL^{-1} was achieved with less than 10 minutes total analysis time. The rapid, selective, and sensitive CE-MS method involves minimum sample handling. The method was proved to be ideal for cross contamination studies in commercial formulations.

Maras et al (15) proposed a fluorimetric approach for determination of glyphosate and its primary metabolite, AMPA. The method is based on derivatization with 4-chloro-7-nitrobenzofurazan (NBD-Cl) at 90 °C under basic conditions in methanol. The binary

mixture of the two analytes was resolved by partial least square (PLS) multivariate calibration. The linearity ranges of the method were found to be 10-150 μg L^{-1} and 10-200 μg L^{-1} for glyphosate and AMPA respectively. The luminescence data of the product formed was used as a function of concentration of the analytes for calibration curve, using three dimensional excitation emission matrices. The analytical capacity of the multivariate calibration method, partial least squares type 1 (PLS-1) on two spectral data sets, synchronous and emission spectra were compared. The PLS-1 applied to the emission spectra was chosen as optimum. Glyphosate and AMPA in river water were simultaneously determined by applying the proposed method. Recoveries ranging from 83-94 % and of 104-120 % for glyphosate and AMPA correspondingly were achieved for concentration range of 100-600 μg L^{-1} of each analyte.

An HPLC method for determination of glyphosate in plant material and water was reported by Nedelkoska and Low (16). The approach is based on pre-column derivatization with 9-fluorenylmethyl chloroformate (FMOC-Cl) employing polymeric amino columns for water and plant material assay respectively. Also new findings about the optimization of the chromatographic behaviour of glyphosate and AMPA on the columns, and that of the derivatization reaction were described. Use of single polymeric amino column and 55 % (v/v) acetonitrile and 50 mM phosphate buffer at pH 10 was investigated to be the paramount HPLC conditions for separation purposes. In order to validate the method, ten spiked rain water samples at a concentration level of 1.0 μg L^{-1} were assayed. The average recovery of 94 % with RSD of 5.4 % and method LOD of 0.16 μg L^{-1} was found. Interferers-free glyphosate was efficiently separated from plant material by HPLC technique employing two polymeric amino columns joined through a switching valve. Grass samples spiked with glyphosate at a concentration level of 1.0 mg Kg^{-1} were analyzed as a validity check. Recovery of 82.4 % (RSD = 10.3 %) and a method limit of detection of 0.3 mg Kg^{-1} was achieved.

Kudzin et al (17) proposed a new method for simultaneous investigation of bioactive aminoalkanephosphonic acids including glyphosate, phosphonosarcosine, phosphono-b-alanine, phosphonoglycine, phosphonohomoalanine, glufosinate and phosphono-g-31 homoalanine. Their approach involves ^{31}P NMR analyses of these amino acids followed

by their derivatisation with trifluoroacetic acid-trifluoroacetic anhydride-trimethyl orthoacetate reagent to phosphinates. The phosphinates were subsequently assayed by MS and/ or GC-MS (electron impact ionization and / or chemical ionization).

Goodwin et al (18) reported a technique based on capillary electrophoresis coupled to mass spectrometry (CE-MS). The method involves simple microelectrospray interface for the simultaneous assay of glufosinate, glyphosate and their metabolites; aminomethyl acid and methyl phosphonicopropionic acid as the innate species. The interface is aimed to cause separation and produce the electrospray by the use of voltage applied to CE capillary and to avoid sample dilution accompanying the sheath liquid interface. In order to successfully operate the interface, the capillary should be appropriately chosen as its chemical make-up strikingly affects the peak shape. One that gives best reproducibility is a linear polyacrylamide coated capillary. It has accurate migration time and peak area ranging from 1 to 2 and RSD 7-12 %, for the four analytes. Compared to two earlier methods this technique exhibit better limit of detection, low-pg on-column and linear calibration curve over the concentration range of 1-100 mM with a correlation coefficient values above 0.97. For glyphosate in water the observed limit of detection is 1.0 mM and for water-acetone extract of wheat, it is 2.5 mM which allows the detection of non-derivatized herbicide in wheat at 10 % of the maximum residue limit.

Sparacino and Thomas (19) described a rugged double column LC approach for glyphosate analysis in ground water. The basis of the method is a formerly reported pre-column fluorescence derivatization and it involves the use of a short pre-column cartridge and a conventional reverse phase column. The precision of the method for the screening analysis was found satisfactory (RSD = 13.1 %). This method was used to study a large number of pond water samples employing the same analytical column without any reasonable cost of column efficacy. The proposed technique is claimed to be robust and efficient for glyphosate determination in ground water samples.

Kudzin et al (20) investigated an innovative methodology for the determination of glyphosate, aminomethylphosphonic acid, methylphosphonic acid (MPA) and N-methyl amino-methylphosphonic acid (MAMPA) simultaneously. The method involves an initial ^{31}P NMR analysis of mixture of the above stated analytes followed by their derivatization

with trifluoroacetic acid-trifluoroacetic anhydride-trimethyl orthoacetate reagent to volatile phosphonates. The product formed was detected by GC-flame ionization detection (FID), MS [chemical ionization (CI) MS, GC-CI-MS and GC-electron impact ionization MS]. The detection limits for all the analytes were investigated using GC-CI-MS and GC-FID. For glyphosate and its metabolites the calibration curves (GC-FID) were linear in the concentration range of 0.1-100 nmol and sufficiently reproducible for quantification purposes. Recoveries greater than 95 % were found out for the investigation of spiked water samples.

Chang and Liao (21) developed a capillary electrophoresis-indirect fluorescence technique for simultaneous determination of glufosinate, glyphosate and aminomethylphosphonic acid. The assay involves the separation of the three analytes with 1.0 mM fluorescein at pH 9.5 by CE in five minutes coupled with indirect fluorescence detector. The method was used for the quantification of glyphosate and glufosinate in commercial formulations in μM range. Precision of migration time and peaks less than 1.7 % and 7.4 % correspondingly were found. Ground water samples were also assayed by this approach.

Sato et al (22) explored a fast and practical approach for direct detection of glyphosate, glufosinate and bialaphos. The method is based on anion-exchange chromatography coupled to integrated pulsed amperometric detection (IPAD). Oxidation of the amino group in the chemical structure of these analytes exhibits catalytic current which is responsible for their electrochemical nature. In alkaline medium adsorption/desorption catalytic characteristics at gold surface, based on the Waveform in IPAD, similar to that for amino acids, were observed. The detection limit (signal to noise ratio = 3) for glyphosate, bialaphos and glufosinate was found to be respectively, 50, 65 and 25 ng mL^{-1}. The r^2 values of 0.996, 0.997, 0.995 were found over a concentration range of 0.1-50, 0.3-32 and 0.1-45 ng mL^{-1} respectively, with RSD (n=5) in the range of 1.7-3.0 %. Urine and serum samples were analyzed for glyphosate by successful application of the proposed method.

A microcolumn liquid chromatographic (μLC) and capillary electrophoresis (CE) method with on-line flame photometric detection (FPD) was reported by Hooijschuur et al (23).

The method was applied for glyphosate and its metabolites' determination of in water, fruit and vegetable samples. The limit of detection for glyphosate and its metabolites like aminomethylphosphonic acid, ethephon and fosetyl-aluminium was in the range of 7.5-500 ng mL^{-1}. LOD for RP-HPLC assay of acephate was 1.0 µg mL^{-1}. In both the methods a simple large-volume injection procedure was used.

Borjesson and Torstensson (24) described a new technique for determination of glyphosate and its degradation moiety; aminomethylphosphonic acid in soil and ground water. The method is based on ligand exchange and anion exchange followed by derivatization and final quantification and identification by GC-MS. The LOQ for both compounds in soil and water samples were found to be 0.006 µg g^{-1} and 0.1 µg g^{-1}, respectively.

Zhu et al (25) reported an ion chromatographic method with suppressed conductivity detection (DX-100) for glyphosate determination. A mixture of 4.0 mmol L^{-1} sodium hydroxide and 9 mmol L^{-1} sodium carbonate was used as eluent. A linear range, 0.042-100 µg mL^{-1} with a limit of detection 0.042 µg mL^{-1} (S/N=3), relative standard deviation 1.99 % and correlation coefficient of 0.9995 was achieved. The interference effect of common inorganic ions and organic acids was studied and found to be negligible. The method gives recoveries in the range of 96.4-103.2 %.

Shah et al (26) described a simple method, based on spectrophotometry for investigation of fenoxaprop-p-ethyl herbicide. The method involves acid hydrolysis of the analyte. Hydrolysis leads to the formation of two products; ethyl 2-(4-hydroxyphenoxy) propionate (EHPP) and 6-chloro-2, 3-dihydrobenzoxazol-2-one (CDHB). The latter was used in azo dye formation as coupling reagent. Maximum absorbance at 430 nm was observed for the azo dye. Under the optimized parameters a linear curve ranging from 0.2 to 25 µg mL^{-1} with molar absorptivity of 1.4×10^4 L $mol^{-1}cm^{-1}$ was observed. The detection and quantification limits found were 0.029 and 0.098 µg mL^{-1}, accordingly. By applying this approach to agricultural samples, recoveries of 95-97 % were achieved.

Mojtahedi et al (27) reported enantiomeric separation of three contaminants; fenoxaprop-p-ethyl, haloxyfop-methyl and indoxacarb on crystalline degradation products-stationary

phase (CDP-CSP) of high performance liquid chromatography under reverse and normal phase. A mobile phase consisting of n-hexane with alcohols including isopropanol and methanol as polar modifiers was used in normal phase mode while methanol with different ratios of triethylammunium acetate was used as eluent in reverse phase mode. The effect of the concentration of analyte, particle size of silica gel (10, 15 and 30 μm) and flow rate (0.3-0.9 ml min^{-1}) was found out. Outstanding stereoselectivity for the two enantiomers of haloxyfop-methyl and fenoxaprop-p-ethyl and chiral identification for indoxacarb under normal phase mode, was exhibited by the new chiral stationary phase, though simply indoxacarb was separated with separation factor $\alpha < 1.5$ employing reverse phase.

Ascenzo et al (28) described a sensitive method for investigating arylphenoxy propionic acid herbicide in environmental water samples, based on pneumatically assisted electrospray (ESI) liquid chromatography-mass spectrometry (LS-MS). In this approach 1.0 L of surface water and 2.0 L of drinking water sample were passed through a 0.5 g graphitized carbon black (GCB) cartridge. The analytes were then chromatographed with a traditional RP-LC C_{18} column (4.6 mm i.d.), with a flow rate of 1 mL min^{-1} of the eluent. A flow of 200 μL min^{-1} of the eluate was directed to the ESI source being operative in positive-ion mode for neutral analytes and in negative-ion mode for acidic ones. In order to optimize the ion-signal, the acid concentration in the mobile phase was scanned as a function of the signal of ESI-MS detector. A linear curve in the concentration range from 1.0 to 200.0 ng was established. Applying the method to drinking water samples, recoveries higher than 91 % and an LOD of 3-10 ng L^{-1} were achieved for all herbicides.

Balinova (29) described an approach to determine Fenoxaprop-p-ethyl and its main degradation product, fenoxaprop. The Preconcentration of the analytes were carried out by C18 solid phase extraction using two different procedures; ion-pairing by adding triethylamine and ionization suppression at low pH. Separation and detection of the analytes was carried out by HPLC-UV system at 240 nm, along with ion-pairing elution on a C-18 reverse phase column. Various parameters like sensitivity, repeatability and

linearity of the technique were assessed. The method showed sufficiently low detection limit.

Celi et al (30) reported an approach for fenoxaprop-p-ethyl and fenoxaprop residue determination in soil samples. The technique is based on two extraction procedures. In one approach 0.1 M hydrochloric acid/methanol and extraction with dichloromethane and in the other, ethyl acetate was used for extraction. Alumina or florisil cartridge was used to cleanse the extract followed by RP-HPLC with UV detection at 280 nm. The combination of alumina clean-up and ethyl acetate extraction showed best results, concerning recovery, clean-up efficiency and impartiality of soil characteristics. Recoveries higher than 70 % and a detection limit of 0.02 mg Kg^{-1} soil was found out using these conditions.

Details for an analytical method employed to determine pinoxaden herbicide's metabolites M2 and M4 in plant stuffs were described in a public release summery (31). This approach comprises of acid digestion of the homogenized sample (1.0 M HCl, reflux for 2 hours) that follows residue extraction with acetone/diethyl ether/dichloromethane (3:7:40). This follows clean up on SPE cartridge and detection with two-column HPLC-UV system at 223 nm and 243 nm for two different analytes. An analytical approach to determine pinoxaden degradation product M4 and M6 in animal commodities was also discussed. Here the acid digestion and clean up procedures followed were as above with determination by HPLC with two column switching technique and tendem MS detection (LC-MS/MS). The detection conditions; multiple reaction monitoring-m/z 333-303, positive mode for M4 and multiple reaction monitoring-m/z 345-175, negative mode for M6 were used during the analysis.

Zhao et al (32) described an electropolymerization technique for the determination of isoproturon. The method involves preparation of molecularly imprinted films (MIF) on gold electrode under weakly acidic conditions of pH 5.5. A study of the performance and the selective response to possible structurally similar interferents and imprinting effects were carried out. A probe of potassium ferricyanide was used between the electrodes and base solution. A linear correlation to isoproturon concentration from 1.0×10^{-7} to 4.0×10^{-4} mol L^{-1} with a detection limit of 2.95×10^{-8} mol L^{-1} was achieved. Farmland water

samples with recoveries from 99.0 to 102.0 % were analyzed for isoproturon by application of these sensors.

Kovalczuk et al (33) developed an HPLC-MS/MS approach coupled to LC-18 SPE cartridges with methanol-water eluent system for determination of isoproturon in poppy seeds. SPE fraction Lichrosper C18 column (25 cm × 4mm, 5 μm) was used for HPLC separation of isoproturon followed by detection and quantification with mass spectrometer coupled with ion trap analyser, run in positive atmospheric pressure chemical ionization (APCI) and MS/MS mode. The detection limit, recoveries, and repeatability obtained were 0.01 mg Kg^{-1}, 84 % and 7 %, respectively.

Zhang et al (34) proposed a sol-gel immunosorbent-based approach for isoproturon determination in surface water. A sol-gel matrix was condensed with monoclonal anti-isoproturon antibodies to form an immunosorbent. Various parameters concerning the immunosorbent like rinsing, loading and eluting procedures were optimized. Under the optimized conditions, selective extraction of isoproturon from a simulated mixture of 68 pesticides was carried out with the sol-gel immunosorbent (SG-IS). A linear range upto 2.2 μg L^{-1}, with correlation coefficient greater than 0.99, RSD lower than 5 % (n=8) and LOQ of 5 ng L^{-1} for 25 mL surface water were achieved using the proposed SG-IS coupled to LC-MS-MS technique.

A new approach for determination of isoproturon, linuron, diuron and neburon was described by Irace-Guigand et al (35). The method is based on flow injection analysis micellar-enhanced photochemically induced fluorescence (FIA-MEPIF). The analyte herbicides were irradiated for online photoconversion to a highly fluorescent product in the presence of micellar concentration of surfactants [cetyl trimethyl ammonium chloride (CTAC) or sodium dodecyl sulphate (SDS)] in buffered aqueous solution. Under optimized conditions a concentration range of 1-2 order of magnitude, detection limit of 0.33-0.92 mg L^{-1} and RSD of 1.3-11 % based on the analyte nature was found. Better recoveries in the range 90-103 % were achieved by applying the approach for the assay of spiked tap water.

Rodriguez-Mozaz et al (36) reported an analytical method based on optical immunosensor prototype, the so-called 'RIVer ANAlyser' (RIANA) for simultaneous assay of three different contaminants; isoproturon, estrone and atrazine. RIANA involves a fast solid phase fluoroimmunoassay taking place at an optical transducer chip, the surface of which is chemically modified with analytes derivatives placed on various distinct points. A time span of 15 min is required for each test cycle comprising regeneration step. The detection limits of the method for isoproturon, atrazine and estrone were achieved to be 0.046, 0.155 and 0.084 $\mu g\ L^{-1}$ correspondingly. A reasonable repeatability with standard deviation in the range of 1.06-6.98 % was achieved.

Winrow et al (37) described an analytical technique for determination of isoproturon in pasta and cereal grains like oats, maize and wheat. The procedure involves extraction of the analyte from samples moistened with acetone, hexane and dichloromethane mixture that follows a clean-up step with solid phase extraction by aminopropyl-bonded silica cartridge. Determination was carried out by reversed-phase liquid chromatography with photodiode array detection. The approach was validated by electrospray liquid chromatography-mass spectrometry. Recoveries from seven fortified samples for each of the four analytes with isoproturon concentration from 0.05 to 0.5 mg Kg^{-1} were found in the range of 88-104 %. Limit of detection found was 0.05 mg Kg^{-1}.

Phenylurea herbicides in water samples were determined at ultratrace level by Gerecke et al (38). Their method involves sample-enrichment by solid phase extraction on Carbopack B and alkylation with iodoethane instead of iodomethane and sodium hydride. The derivatized aqueous samples are then extracted and injected by SPME. A detection limit in the range 0.3-3.0 $\mu g\ L^{-1}$ with a standard deviation less than 10 % for samples containing about 4 ng L^{-1} was obtained in lake water, waste water treatment plant (WWTP) runoff and Nanopure water. Also the p-hydroxylated diuron's metabolite was found to be determined with the same approach. A qualitative assessment of further metabolites was also accomplished.

Mallat et al (39) described an analytical approach for determination of isoproturon in a variety of water samples. The approach is based on optical immunoassay with immobilized analyte derivatives and free, fluorescence labeled anti-isoproturon

antibodies. To evaluate the influence of compounds having similar structure on the biosensor response, cross-reactivities (CRs) of diuron, linuron and chlortoluron were carried out. At 0.1 μg L^{-1} of cross-reactant CR values of 46, 53 and 93 % for linuron and chlortoluron and diuron were observed respectively. River water samples and MilliQ were analyzed and LODs of 0.14 and 0.01 μg L^{-1} respectively, were obtained. A time period of 15 min was required for complete individual analysis along with regeneration of transducer. This River ANAlyser (RAINA) prototype with an optical immunosensor was found to be applicable for evaluating isoproturon in natural river water samples.

Corcia et al (40) reported an approach for parts per trillion level determinations of phenylurea herbicides and their degradation products (DPs). The procedure involves liquid-chromatography (33) with electrospray (ES) mass spectrometric detection which follows solid phase extraction of the analyte with Carbograph 4 cartridge. The response of the ES-MS detector for very weakly basic chloroanilines was optimized which shows a strong dependence of the ion signal intensities of the species on the composition of the LC mobile phase. A fraction of the aromatic amines, being reversibly associated with quinoidal structures of humic acids (Has) been liberated by the addition of $NaBH_4$ as reducing agent. Recoveries higher than 85 % were achieved on extraction from spiked 0.5 L river water (200 ng L^{-1} spike level), 4 L drinking water (25 ng L^{-1} spike level) and 2 L ground water (50 ng L^{-1} spike level). Relative standard deviation in the range of 5.9-13 %, 4.3-15 % and 4.6-20 % for river water, ground water and drinking water respectively, was achieved. The method's detection limit calculated for surface water, drinking water and ground water was in the range of 36-75, 3-11 and 6-21 ng L^{-1} accordingly.

Agrawal et al (41) proposed a sensitive and simple spectrophotometric technique for isoproturon's determination. The method involves base hydrolysis to primary amine that follows diazotization and coupling with p-aminoacetophenone. The resulting azo dye having λ_{max} of 525 nm is determined. The method shows linearity in the concentration range 0.02-0.2 μg L^{-1}. The molar absorptivity and Sandell's sensitivity of 8.6×10^5 L $mol^{-1}cm^{-1}$ and 0.002 μg cm^{-2} respectively, were achieved. Environmental and biological samples were efficiently analyzed for isoproturon using the proposed method.

A spectrophotometric method for assay of isoproturon and metoxuron using ethyl acetoacetate as derivatizing reagent was described by Raju et al (42). The method is based on alkaline hydrolysis of the analytes to resultant primary amines following diazotization and coupling with ethyl acetoacetate at basic pH. The coloured products follows Beer's law upto a higher concentration level of 9.0 and 15.0 µg for metoxuron and isoproturon respectively. The method was applied to technical and formulation samples with RSD in the range 0.66-0.74, 0.27-0.69 and 0.48-0.72, 0.86-1.32 for formulation and technical grade samples of isoproturon and metoxuron respectively.

Raju et al (43) reported an analytical approach for the determination of isoproturon (IPN) and metoxuron (MTN). The method is based on spectrophotometric assay of the derivatives formed when these analytes react with p-dimethylaminobenzaldehyde under acidic condition. The λ_{max} of the coloured product of IPN was shown to be at 438 nm and that of MTN at 444 nm. Molar absorptivities found were 3.09×10^4 and 3.85×10^4 L $mol^{-1}cm^{-1}$ for IPN and MTN respectively, with a relative error of less than 1.0 %.

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EXPERIMENTAL
RESULTS & DISCUSSION

3.1. SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF GLYPHOSATE HERBICIDE

The structural formula of glyphosate is shown in figure 3.1.1.

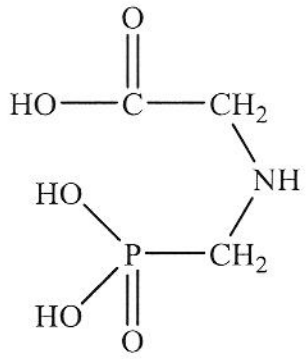
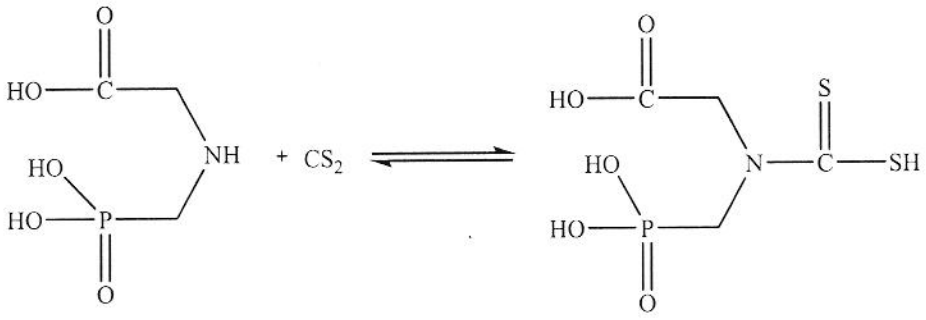


Figure 3.1.1. Structural formula of glyphosate

3.1.1. Method development strategy for the spectrophotometric determination of glyphosate

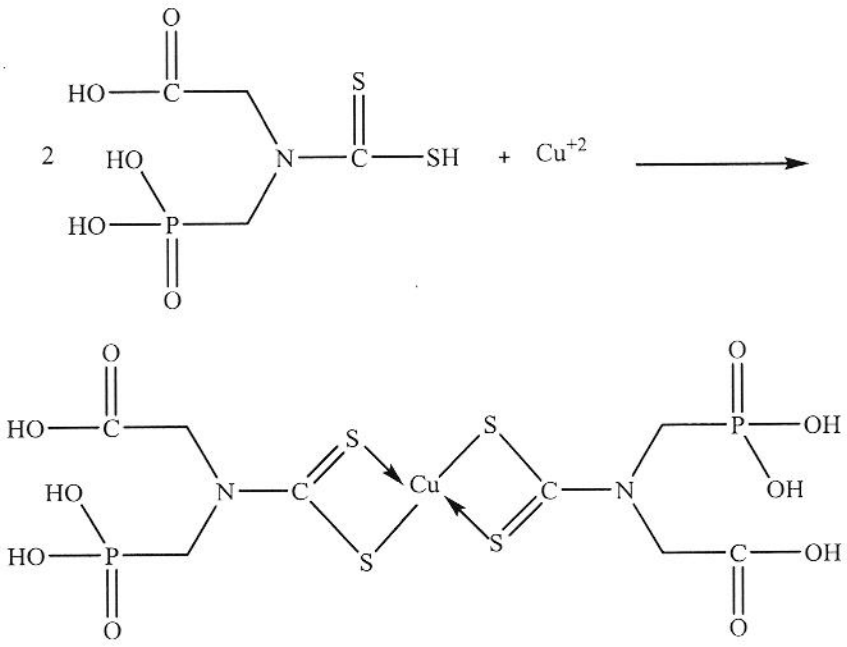
Glyphosate molecule has no chromophore or fluorophore groups as is evident from its chemical structure in figure 3.1.1. For spectrophotometric and fluorimetric determination it has to be derivatized to a suitable product. The parent molecule has three functional groups; phosphonate, amine and carboxylate. The basis of the proposed method is the conversion of the secondary amino group of glyphosate into dithiocarbamic acid group. This is accomplished by reacting it with carbon disulphide in chloroform. The dithiocarbamic acid group can then be reacted with suitable metal ion Cu (II), thus forming a coloured product suitable for spectrophotometric determination. The mechanism of the proposed two step reaction is given in figure 3.1.2.



2-(phosphonomethylamino)acetic acid

(Glyphosate)

2-(dithiocarboxy(phosphonomethyl)amino)acetic acid



Copper (II) carboxymethyl (phosphonomethyl) carbamodithioate

(Yellow)

Figure 3.1.2. Proposed reaction mechanism for the spectrophotometric determination of glyphosate

3.1.2. Preliminary exploration of the possibility of substitution and complexation reaction of glyphosate for its spectrophotometric determination

Technical grade glyphosate (1000 μg mL^{-1}) was taken (1.0 mL) in a flask containing 4.0 mL of CS_2 (2.0 %) solution in chloroform and the mixture was shaken. Then 2.0 mL

ammonical Cu (II) solution (1000 μg mL^{-1}) was added to the mixture. The mixture was vigorously shaken for three minutes leading to the appearance of yellow colour in the mixture. The mixture was transferred to a separating funnel (125 mL), shaken and allowed to stand for five minutes. The organic layer became yellow in colour due to extraction of complex which indicated the possibility of the proposed reaction and succeeding spectrophotometric determination of glyphosate by this approach. For completion of the reaction and enhanced sensitivity and repeatability, it was necessary to concentrate on the optimization of different parameters of the reaction and is described below.

3.1.3. Optimization studies for spectrophotometric determination of glyphosate

Optimization studies were carried out to achieve maximum product formation. These studies include wavelength of maximum absorbance, concentration and volume of carbon disulphide and Cu (II), pH and effect of time on stability of the complex.

3.1.3.1. Investigation of suitable wavelength for the spectrophotometric determination of glyphosate

Instruments

The absorption spectra and absorbance of solutions were measured with UV/VIS spectrophotometer (model SP-300, Optima, Japan) at 435 nm using a glass cell with an optical path length of 1.0 cm and pH was measured with pH meter, (model 7020 Kent Industrial Measurement Limited-Electronic Instruments Ltd, Chertsey Survey England) using combined glass electrode.

Chemicals and reagents

All chemicals and reagents used were of analytical reagent-grade purity or of similar purity. Commercial sample containing glyphosate was purchased from the local market. Copper nitrate (BDH), ammonia solution (BDH), carbon disulphide (Merck), chloroform (Riedel-deHaen) and distilled ethanol were used during this work. Amberlite IR-120 Na-ion exchanger resin (cationic) (ACROS ORGANICS-New Jersey, USA) was used for extraction of the herbicide from water sample. Reagent grade sodium chloride (Merck)

was used as eluent. Standard reference glyphosate was purchased from Dr.Ehrenstofer GmbH, Germany.

Solutions preparation

Standard glyphosate solution: A standard stock solution of glyphosate (1000 μg mL^{-1}) was prepared by dissolving 0.1 g of the authentic standard of glyphosate in 20 mL distilled water and diluted to 100 mL with distilled water. Working standard solutions were prepared by further dilution.

Cu (II) solution: Ammonical copper nitrate solution (100 μg mL^{-1}) was prepared by dissolving an appropriate amount of Cu $(NO_3)_2$ in 10 mL distilled water and diluted to 100 mL with 90 mL ammonia solution of 33 % purity.

Carbon disulphide (CS_2) solution: Carbon disulphide solution (1.0 %) was prepared by mixing 1.0 mL CS_2 in chloroform and diluted to 100 mL. Working standards were prepared fresh each day by further dilution.

Procedure

A known volume (2.0 mL) of glyphosate (100 μg mL^{-1}) was added to a conical flask containing 5.0 mL of CS_2 (1.0 %) and the mixture was shaken. Then 1.0 mL ammonical Cu (II) solution (100 μg mL^{-1}) was added to the mixture. The mixture was vigorously shaken for three minutes and transferred to a separating funnel (125 mL) and shaken again for the extraction of complex. After equilibration the yellow coloured chloroform layer containing the complex was collected in a 10 mL flask. The solution was diluted up to the mark with ethanol and absorbance at different wavelengths was measured against a reagent blank. The absorbance values were plotted against wavelength as shown in figure 3.1.3.

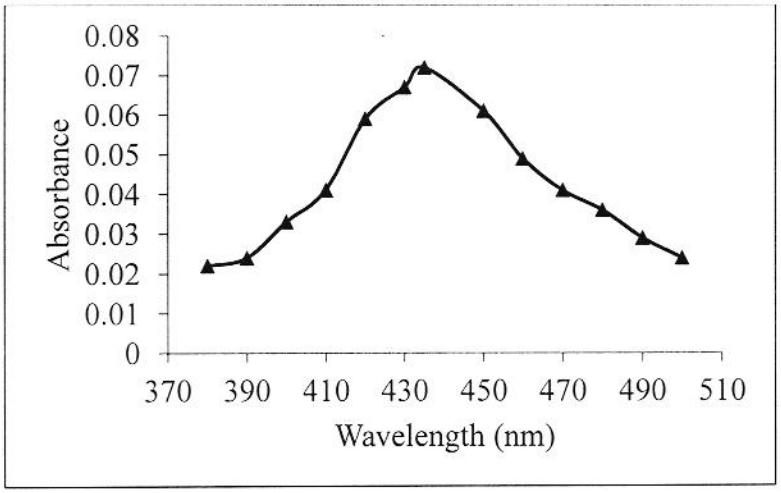


Figure 3.1.3. Wavelength optimization for spectrophotometric determination of glyphosate

3.1.3.2. Investigation of the effect of various parameters on the complex formation of glyphosate

Instrument, reagents and solutions: The same as mentioned earlier

Procedure

For finding out the effect of concentration of carbon disulphide on dithiocarbamic acid formation, 2.0 mL of glyphosate standard solution (100 μg mL^{-1}) was transferred to each of seven separate 100 mL conical flasks, followed by the addition of aliquots of carbon disulphide solution in chloroform with final concentration in the range of 0.5-3.0 %. After shaking the mixture, 1.0 mL of ammonical Cu (II) solution (100 μg mL^{-1}) was added to it. Each mixture was transferred to a separating funnel and manually shaken for 3.0 min. The lower yellow coloured chloroform layer was collected after five minutes in a 10 mL volumetric flask and diluted up to the mark with ethanol. The absorbance of the complex formed was measured against a reagent blank at 435 nm. The results are shown in figure 3.1.4. The effect of volume of carbon disulphide on the dithiocarbamic acid formation was investigated by varying the volume of carbon disulphide (1.0 %) in the range of 1-8 mL, keeping all other conditions the same. The absorbance of the complex was measured against a reagent blank and the results are shown in figure 3.1.5.

For investigating the effect of concentration of Cu (II) for complex formation, to ten separate conical flasks each containing 2.0 mL of glyphosate stock solution (100 μg mL^{-1}), 5.0 mL of carbon disulphide (1.0 %) solution was added followed by the addition of 1.0 mL of Cu (II) solution varying from 10 to 100 μg mL^{-1}. After vigorous shaking for three minutes the mixture was transferred to a separating funnel and shaken again. After five minutes the lower chloroform layer was collected in a 10 mL flask and diluted with ethanol upto the mark. The absorbance of each solution was measured against a reagent blank at 435 nm. The results are shown in figure 3.1.6. The effect of volume of Cu (II) solution (30 μg mL^{-1}) was studied by following the same procedure but only varying the volume in the range of 1-7 mL. The results are shown in figure 3.1.7.

The effect of pH on the reaction was studied by using phosphate buffer and ammonia/ammonium chloride buffer of pH ranging from 6.0-12.5. To seven separate conical flasks each containing 2.0 mL of glyphosate solution (100 μg mL^{-1}), 5.0 mL of carbon disulphide solution (1.0 %) was added followed by the addition of 4.0 mL of aqueous Cu (II) solution (30 μg mL^{-1}) and 5.0 mL of buffer solution of varying pH. The rest of the procedure followed was the same as discussed above. The results are shown in figure 3.1.8.

In order to investigate the effect of time on stability of glyphosate based Cu-dithiocarbamate complex, the optimized conditions were followed keeping glyphosate final concentration at 5.0 μg mL^{-1}. The absorbance of the complex formed was measured at different time intervals varying from no-delay to 100 minutes. The results are shown in figure 3.1.9.

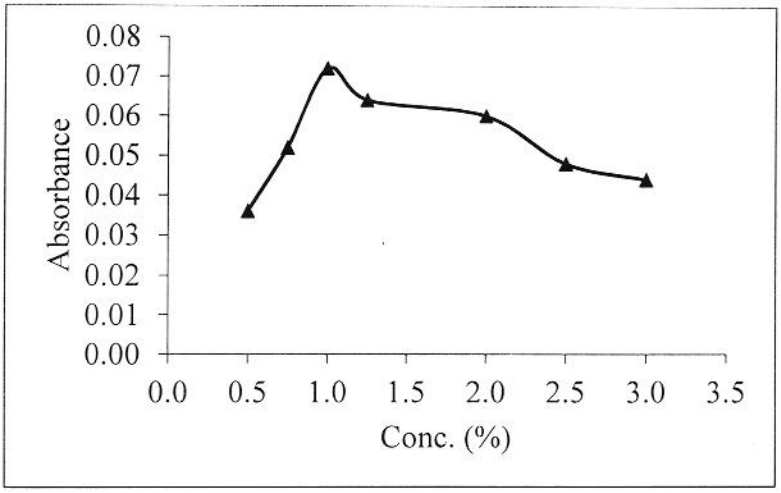


Figure 3.1.4. Effect of concentration of carbon disulphide on formation of Cu-dithiocarbamate complex

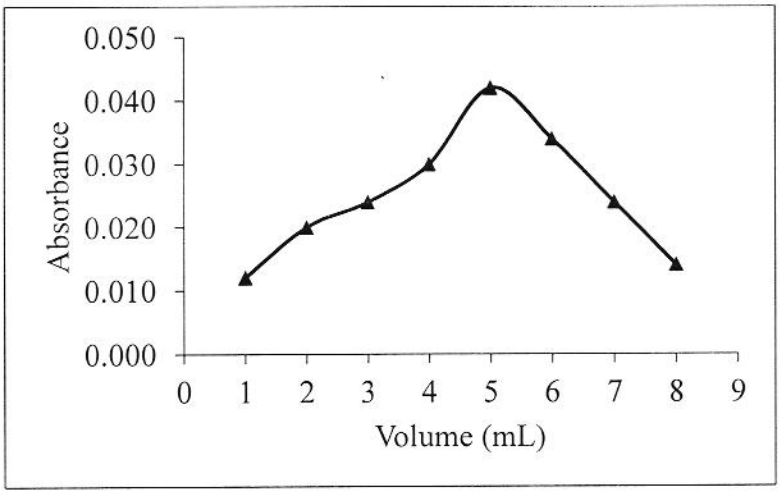


Figure 3.1.5. Effect of volume of carbon disulphide on formation of Cu-dithiocarbamate complex

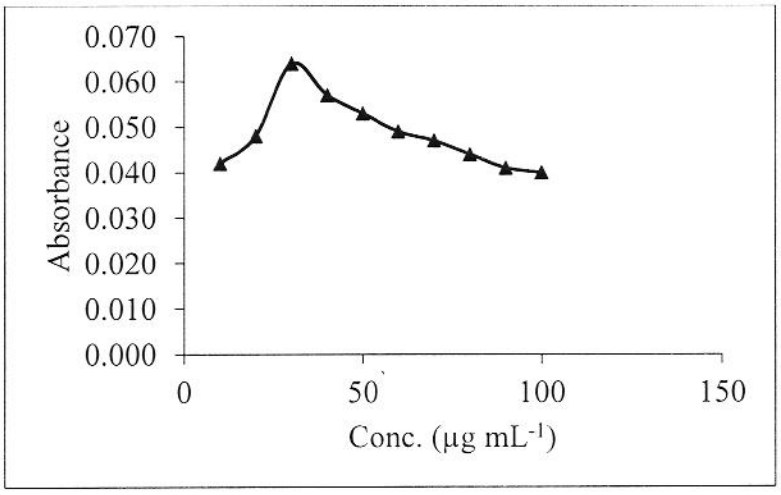


Figure 3.1.6. Effect of concentration of Cu (II) on formation of Cu-dithiocarbamate complex

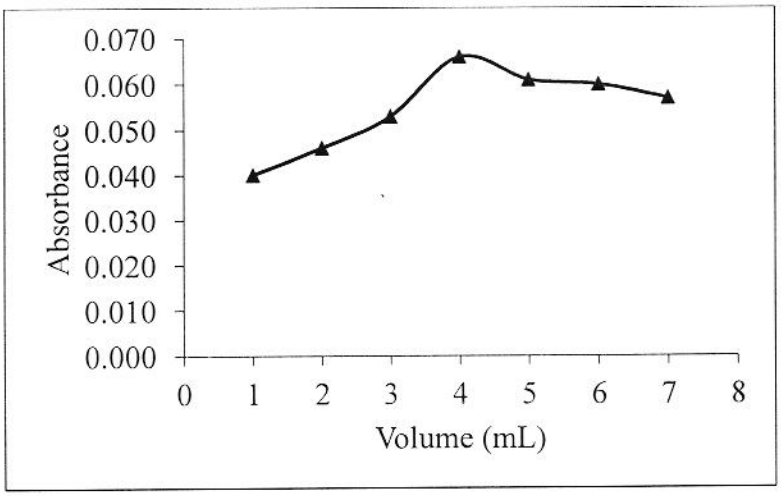


Figure 3.1.7. Effect of volume of Cu (II) on formation of Cu-dithiocarbamate complex

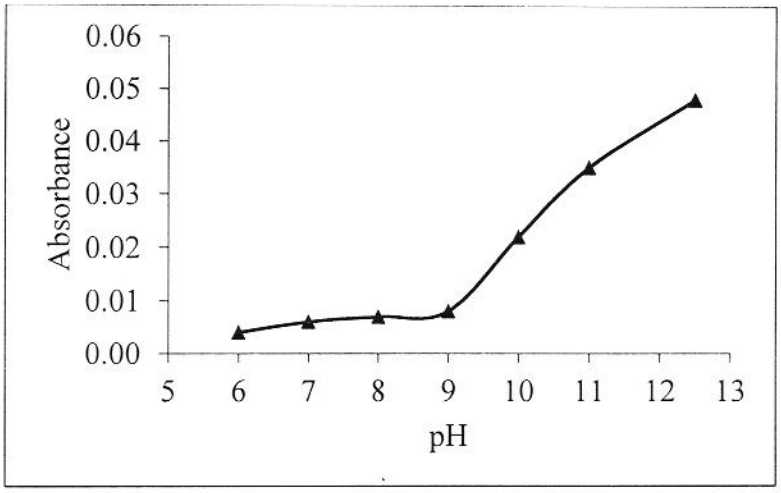


Figure 3.1.8. Effect of pH on formation of Cu-dithiocarbamate complex

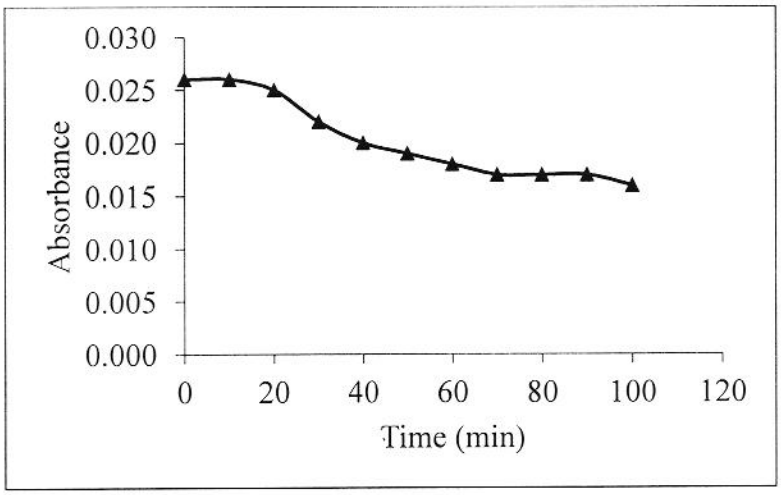


Figure 3.1.9. Investigation of the stability of the Cu-dithiocarbamate complex

3.1.4. Investigation of the empirical formula and formation constant (K_f) of the complex by Job's method of continuous variation

Instruments, reagents and solutions: The same as mentioned before

Procedure

Different volumes of glyphosate solution (0.1 M) were added to six separate conical flasks followed by the addition of 5.0 mL of carbon disulphide (1.0 %) solution to each flask. Then different volumes of Cu (II) solution (0.1 M) were added to the mixtures in such a way that the total volume and total moles of glyphosate and Cu (II) in each

mixture remained constant but the mole ratio of these two varied systematically. Each mixture was transferred to a separating funnel and after shaking for three minutes and equilibration, the lower chloroform layer in each case was collected in a 10 mL flask. Each solution was diluted to the mark with ethanol and absorbance was measured at 435 nm against a reagent blank. The absorbance was plotted against the volume ratio of Cu (II) and glyphosate. The results are given in Table 3.1.1 and shown in figure 3.1.10. The empirical formula or the combining ratio of the complex formed was determined to be ML_2 from the theoretical maximum on the curve. The formation constant K_f for the complex was evaluated from measurement of the deviations from the theoretical straight lines and the calculations are given below.

$$M + 2L \longrightarrow ML_2$$

Total metal conc. (C_M)

$C_1V_1 = C_2V_2$

$0.1 \times 1.2 = X \times 10$

$X = C_M = 1.2 \times 10^{-2}$

Total ligand conc. (C_L)

$C_1V_1 = C_2V_2$

$0.1 \times 2 = X \times 10$

$X = C_L = 2.0 \times 10^{-2}$

Conc. of the complex $[ML_2]$

$[ML_2] = (A_{actual}/A_{extrapolated}) \times C_M$

$[ML_2] = (1.22/1.35) \times 1.2 \times 10^{-2}$

$[ML_2] = 1.08 \times 10^{-2}$

Free metal conc. [M]

$[M] = C_M - [ML_2]$

$[M] = 1.2 \times 10^{-2} - 1.08 \times 10^{-2}$

$[M] = 1.2 \times 10^{-3}$

Free ligand conc. [L]

$[L] = C_L - [ML_2]$

$[L] = 2.0 \times 10^{-2} - 1.08 \times 10^{-2}$

$[L] = 9.2 \times 10^{-3}$

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

$K_f = 1.08 \times 10^{-2} / 1.2 \times 10^{-3} \times (9.2 \times 10^{-3})^2$

$K_f = 1.08 \times 10^{-2} / 1.2 \times 10^{-3} \times 8.46 \times 10^{-5}$

$K_f = 1.08 \times 10^{-2} / 1.01 \times 10^{-7}$

$K_f = 1.06 \times 10^{5}$

Table 3.1.1. Investigation of the empirical formula and formation constant (K_f) of the complex by Job's method of continuous variation

Cu (II) (mL)	Glyphosate (mL)	Absorbance
0.25	2.75	0.461
0.50	2.5	0.843
1.0	2.0	1.220
1.5	1.5	0.942
2.0	1.0	0.661
2.5	0.5	0.521
2.75	0.25	0.414

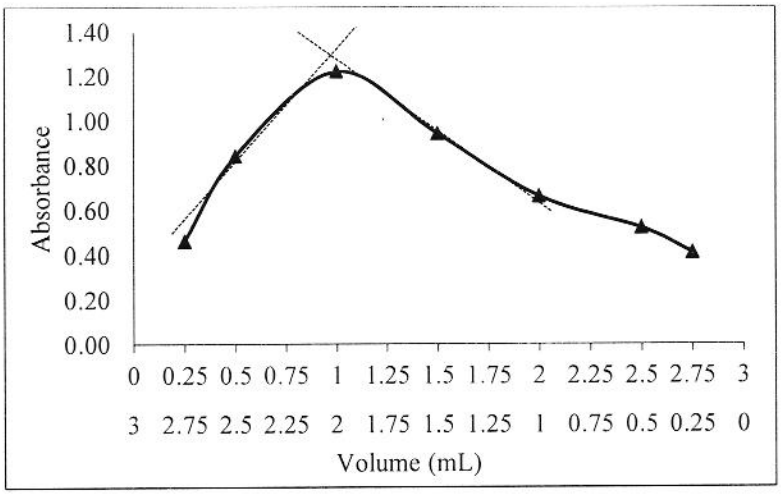


Figure 3.1.10. Investigation of the empirical formula and formation constant (K_f) of the complex by Job's method of continuous variation

3.1.5. Verification of Beer's law and determination of analytical characteristics of the method

Instruments, reagents and solutions: The same as mentioned before

Procedure

Different volumes of glyphosate (100 μg mL^{-1}) corresponding to final concentration in the range of 1-50 μg mL^{-1} were taken in seven different conical flasks. To each flask optimum volume (5.0 mL) of carbon disulphide solution (1.0 %) was added and shaken.

To the resultant dithiocarbamic acid derivative, optimum volume (4.0 mL) of 30 μg mL^{-1} Cu (II) solution was added and manually shaken for three minutes. Each mixture was transferred to a separating funnel, shaken again and after five minutes the chloroform layer was collected in a 10 mL volumetric flask and diluted with ethanol to the mark. The absorbance of the yellow coloured solution was measured at 435 nm against a reagent blank being prepared by the same procedure without the addition of glyphosate. The results are given in Table 3.1.2 and shown in figure 3.1.11. A calibration plot of concentration versus absorbance was constructed and is shown in figure 3.1.12.

The molar absorptivity (ε) was calculated based on the formula given below:

Molar Absorptivity (ε) = A/C × L

Where 'A' is the absorbance, 'C' is the Molar concentration and 'L' is the path length (cm) of the cell.

Limit of detection (LOD) and limit of quantification (LOQ) were calculated on the basis of six replicates of the lowest quantifiable concentration that can be measured with acceptable precision and accuracy (1.0 μg mL^{-1}) by applying the following formulae.

LOD = 3S/b (For concentration)

LOQ = 10S/b (For concentration)

Where 'S' is the standard deviation and 'b' is the slope of the curve

Relative Standard Deviation (RSD) was calculated by using the formula:

RSD = (S/x) ×100

Where 'x' is the mean concentration determined

At optimum conditions various analytical characteristics of the method were determined and are given in table 3.1.3.

Table 3.1.2. Verification of the Beer's law for spectrophotometric determination of glyphosate

Concentration (µg mL^{-1})	**Absorbance**
1	0.008
2	0.010
3	0.013
5	0.017
8	0.024
10	0.029
20	0.043
30	0.072
40	0.094
50	0.12

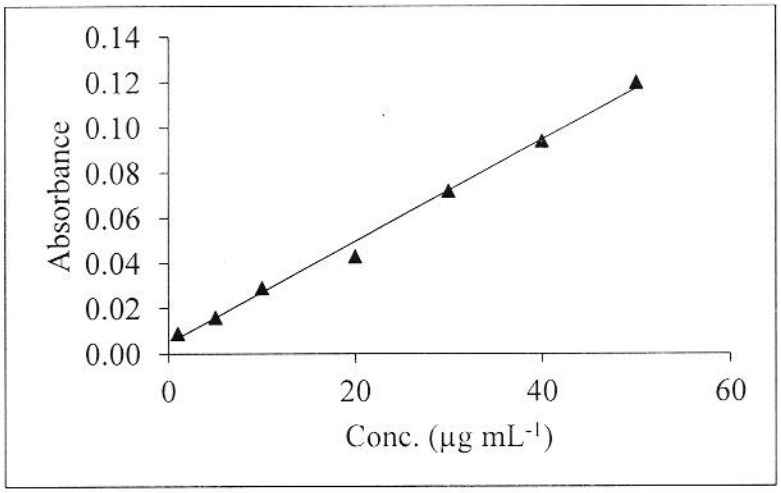


Figure 3.1.11. Verification of the Beer's law for spectrophotometric determination of glyphosate

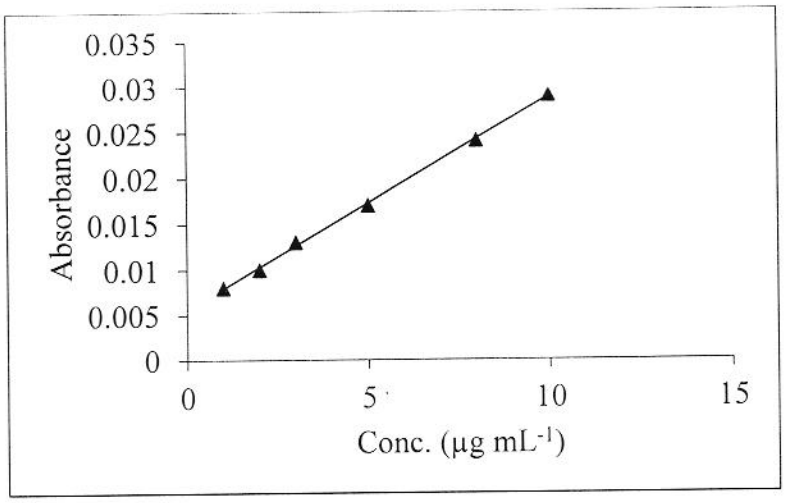


Figure 3.1.12. Calibration curve for spectrophotometric determination of glyphosate in the lower concentration region

Table 3.1.3. Analytical characteristics of the investigated method

Parameter	**Value**
λ_{max} (nm)	435
Molar absorptivity (ε) (L $mol^{-1}cm^{-1}$)	1.864×10^3
Linear range (μg mL^{-1})	1.0 - 50.0
Relative standard deviation (RSD) (%)	2.37
Limit of detection (LOD) (μg mL^{-1})	0.519
Limit of quantification (LOQ) (μg mL^{-1})	1.73
Correlation Coefficient	0.997
Slope	0.0023
Intercept	0.0056
Stability constant (K_f)	1.06×10^5

3.1.6. Investigation of the interferences effect of various compounds on determination of glyphosate using the proposed method

Instruments, reagents and solutions: The same as mentioned before.

Procedure

In order to investigate the interferences effect of various nitrogen containing compounds including urea, various pesticides and other chemical species on the spectrophotometric

determination of glyphosate, a known concentration (2.0 μg mL^{-1}) of glyphosate and different concentrations of the possible interferers in different ratios were taken in seven separate conical flasks. It was followed by the addition of optimized volumes of carbon disulphide (1.0 % solution in chloroform) and then ammonical Cu (II) solution (30 μg mL^{-1}). Each mixture was transferred to a separating funnel and shaken for three minutes. After five minutes equilibration, the lower chloroform layer was collected in a 10 mL volumetric flask and diluted up to the mark with ethanol. The absorbance of each solution was measured against a reagent blank. The results are given in Table 3.1.4 and are shown in figure 3.1.13.

Table 3.1.4. Investigation of the interferences effect of different compounds on the absorbance behavior of glyphosate based Cu (II) complex

Analyte: Interferer	1:00	1:01	1:02	1:03	1:04	1:05	1:06
	Absorbance						
Glyphosate	0.024						
Sodium Nitrate		0.024	0.023	0.024	0.025	0.022	0.024
Sodium Nitrite		0.024	0.024	0.024	0.023	0.022	0.024
Urea		0.023	0.022	0.022	0.022	0.022	0.023
Atrazine		0.025	0.026	0.028	0.03	0.033	0.033
Dimethyl amine		0.024	0.024	0.024	0.021	0.017	0.014
Acetamide		0.024	0.024	0.026	0.024	0.024	0.023
Isoproturon		0.025	0.026	0.025	0.027	0.028	0.028

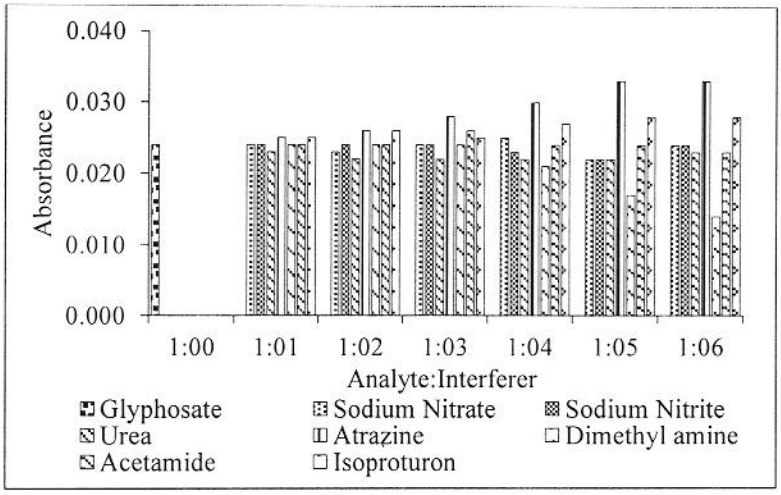


Figure 3.1.13. Investigation of the interferences effect of different compounds on the absorbance behavior of glyphosate based Cu (II) complex

3.1.7. Determination of glyphosate in spiked samples using the proposed method

Instruments and reagents: The same as mentioned before.

Recoveries of glyphosate from soil, water and wheat grain samples, spiked with known concentration of glyphosate solution, were investigated by the proposed method.

Procedure for solid-phase extraction of glyphosate from water samples

For the preconcentration and clean-up of glyphosate a cation-exchange resin was used. A slurry of the Amberlite® IR-120, Na-ion exchange resin was made in 10 mL distilled water and packed into a narrow bore glass column (4.8 mm wide, 30 cm long), plugged with glass wool at the bottom. The resin was rinsed with distilled water and then with 1.0 M HCl at a flow rate of 2.0 mL min^{-1} several times, before sample application. The pH of tap water sample spiked with glyphosate was adjusted to pH 2 in order to protonate the amine group of glyphosate to act as a cationic adsorbate. The protonated sample (25 mL) was passed through the column at a flow rate of 0.5 mL min^{-1} in order to have maximum equilibration of protonated sample. After the loading step, the column contents were eluted with 25 mL of 2.0 M NaCl solution at the same flow rate. The eluted solution was evaporated to about 10 mL at 70 °C and analysed by the proposed method following the analysis procedure described below.

Extraction from soil samples

Soil samples were collected from agricultural land in the university campus where no spray of glyphosate or any other pesticide was done. The sample collection area was manually cleaned from twigs and gravels and the surface soil was collected in glass bottles. The collected soil was dried and sieved. Control soil sample (10 g) was placed in a series of bottles and known concentration of glyphosate solutions were added to provide concentration level of 5, 10, 15 $\mu g\ g^{-1}$ in triplicate. The bottles were shaken manually for uniform mixing and kept for 24 h. The homogenized soil sample was extracted for 60 min with 25 mL of 2.0 M NH_4OH solution. The solution was evaporated to 10 mL at 70 °C. The pH of eluted sample was re-adjusted to pH 5.4 and analysed by the proposed method following the analysis procedure given below.

Extraction from wheat grains

Control samples of wheat grains (5.0 g) were washed with distilled water and known concentration of glyphosate solution was added to it in the concentration range of 5.0-15 $\mu g\ g^{-1}$. The samples were shaken and kept for 24 hr. The samples were extracted with 50 mL of 2.0 M NH_4OH solution. The solution was concentrated to 10 mL by evaporation at 70 °C and analysed by the proposed method based on the procedure given below. The extraction experiment was performed in triplicate.

Analysis Procedure

A known volume (2.0 mL) of each extract was added to a conical flask containing 5.0 mL of CS_2 (1.0 %) and the mixture was shaken. Then 1.0 mL ammonical Cu (II) solution (30 $\mu g\ mL^{-1}$) was added to the mixture. The mixture was vigorously shaken for three minutes and transferred to a separating funnel (125 mL) and shaken again for the extraction of the complex. After equilibration, the yellow coloured chloroform layer containing the complex was collected in a 10 mL flask. The solution was diluted up to the mark with ethanol and its absorbance was measured at 435 nm. The results of percent recovery of glyphosate are given in table 3.1.5.

In the second batch distilled water was used as solvent for extraction of glyphosate from wheat grains and the results of the two extraction methods were compared. The results are given in Table 3.1.6.

Table 3.1.5. Percent recovery of glyphosate from spiked samples by using the proposed method

Sample	**Amount of glyphosate**		**% Recovery ± SD**
	Added (μg g^{-1}) or (μg mL^{-1})	Found (μg g^{-1}) or (μg mL^{-1})	
Soil	05	4.0	80.0±0.46
	10	8.6	86.0±0.50
	15	13.0	87.0±0.28
Wheat grains	05	5.1	102.0±0.98
	10	9.6	96.0±0.72
	15	14.2	95.0±0.88
Water	05	4.6	92.0±0.37
	10	8.5	85.0±0.68
	15	13.0	87.0±0.44

Table 3.1.6. Determination of percent recovery of glyphosate from control samples of wheat grains using different eluents

Eluent	**Added (μg g^{-1})**	**Found (μg g^{-1})**	**% Recovery**
NH_4OH (2M)	05	5.2	104.0 ± 0.24
Distilled water	05	3.6	72.0 ± 1.40

Results and Discussion

Glyphosate molecule contains three possible reactive chemical groups i.e. amine, phosphonate and carboxylate (Fig. 3.1.1). The amino group of glyphosate was converted into dithiocarbamic acid, in the proposed method, by reacting with carbon disulphide in chloroform. The dithiocarbamic group formed was further reacted with copper metal ion in the presence of ammonia and a yellow coloured copper dithiocarbamate complex was formed. The proposed reaction mechanism for the formation of dithiocarbamic acid from glyphosate and the reaction of dithiocarbamic acid with copper (II) to form a coloured complex is shown in figure 3.1.2. The absorbance of the resultant yellow coloured complex was measured at different wavelengths in the range of 380-500 nm and maximum absorbance of complex was found at 435 nm (Fig. 3.1.3).

The effect of concentration of carbon disulphide solution on the formation of dithiocarbamic acid was studied in the range of 0.5-3.0 % (Fig. 3.1.4). It was observed that absorbance increases with increase of carbon disulphide concentration from 0.5 to 1.0 %. Beyond the optimum concentration small decrease in absorbance was observed. Similarly the effect of volume of CS_2 was also studied from 1-8 mL. Optimum volume was found to be 5.0 mL. Above 5.0 mL, a sharp decrease in absorbance was observed (Fig. 3.1.5). This decrease is due to the possible conversion of dithiocarbamate anion to neutral dithiocarbamate radical R_2NCS_2, which dimerises to stable thiuram disulphide, $R_2NC(S)S\text{-}S(S)CNR_2$. This leads to deficiency of ligands available for Cu (II) complex formation [1].

The effect of concentration of copper solution was investigated in the range of 10-100 μg mL^{-1} (Fig. 3.1.6). The results show that absorbance increases with increase in Cu (II) concentration from 10-30 μg mL^{-1}, beyond which it slowly goes on decreasing because at higher metal concentration a redox reaction is possible in which Cu (II) acts as an oxidizing agent to the dithiocarbamate and gets reduced to Cu (I). This results in decrease of Cu (II) concentration and subsequent decrease in yield of the complex [1]. Volume of Cu (II) was optimized in the range of 1.0-7.0 mL (Fig. 3.1.7). Maximum absorbance was observed at 4.0 mL of copper (II) solution and further increase in volume of copper (II) solution reduces the sensitivity because of dilution effect of the free metal ions.

Complex formation of copper (II) with dithiocarbamic acid was found to be highly pH dependent. The effect of pH was investigated by using buffers of pH varying from 6.0-12.5 and it was found that the complex formation is possible only under basic conditions and absorbance of the complex increases continuously with increase in pH till pH 12.5. Thus the pH of final solution was adjusted with ammonium hydroxide at pH 12.5 (Fig. 3.1.8).

The stability of the complex is the key factor in method development which involves complexation reaction. In the proposed method the stability of the complex formed was studied by measuring its absorbance up to 100 minutes and it was observed that the complex is stable for 20 min and can be easily used for routine analysis (Fig. 3.1.9). In case of large scale batch analysis an improved stability is needed.

The stoichiometry of the complex formed between substituted glyphosate and copper (II) was investigated at the optimum pH value by applying the Job's method of continuous variation (Fig. 3.1.10). The results indicates the formation of 1:2 [copper: glyphosate] complex. The stability constant value was calculated and is given in Table 3.1.1. The high formation constant indicates the stability of the complex.

At optimum conditions a linear dependence of absorbance on concentration was observed over a wide concentration range of 1.0 to 50.0 $\mu g\ mL^{-1}$ with a molar absorptivity of 1.864 $\times\ 10^3\ L\ mol^{-1}\ cm^{-1}$ (Table 3.1.2 and Fig. 3.1.11). A calibration plot was constructed for determination of unknown concentration of glyphosate in various samples (Fig. 3.1.12). The limit of detection (3s/b), limit of quantification (10s/b), correlation coefficient (r), RSD, slope and intercept were calculated and are summarized in Table 3.1.3.

The interference effect of various compounds on the absorbance behaviour of glyphosate was also investigated (Table 3.1.4 and Fig. 3.1.13). It was found that only atrazine and isoproturon have enhancing effect on the absorbance of glyphosate. The interferences effect of these two herbicides on glyphosate determination in real environmental matrices can be considered minimum because these herbicides are insoluble in water while glyphosate is soluble, thus glyphosate can be easily extracted into the aqueous medium and then analysed by the proposed method.

The proposed method has been applied successfully for the determination of percent recovery of glyphosate from water, soil and wheat grains samples. Ammonium hydroxide solution was used as solvent for extraction of glyphosate from wheat grains and soil samples. Aliquots of the extract was analysed by the proposed method. In soil samples 80.0 ± 0.46 to 87.0 ± 0.28 % recovery was obtained while in grain samples recovery in the range of 95.0 ± 0.88 to 102.0 ± 0.98 % of glyphosate was observed. Glyphosate was also extracted from water samples using cation exchange resin and sodium chloride as an eluting solvent. Glyphosate recovery in the range of 85.0 ± 0.68 % to 92.0 ± 0.37 was obtained using 2.0 M NaCl solution (Table 3.1.5).

Comparison of the % recovery of glyphosate from control wheat grain samples is given in table 3.1.6. Higher recoveries were (104.0 %) obtained with 2.0 M ammonium hydroxide as compared to distilled water (72.01 %). The results show that glyphosate is water soluble herbicide but due to the presence of carboxyl and phosphonate groups, it forms salt with ammonium hydroxide and the extraction efficiency increases with ammonium hydroxide as compared to water as well as ammonium ion get exchanged with the protonated glyphosate molecule bound to the resin and enhance the extraction efficiency.

Reference

1. T. U. Mathai, Ph.D. Dissertation, "Studies on interaction of copper (II) with dithiocarbamates derived from primary amines", School of Chemical Sciences, Mahatma Gandhi University, Kerala India, (1999) pp. 67.

3.2. FLOW INJECTION SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF GLYPHOSATE HERBICIDE VIA CONDENSATION REACTION WITH P-DIMETHYLAMINO-BENZALDEHYDE (DMAB)

The structural formula of glyphosate is shown in figure 3.2.1.

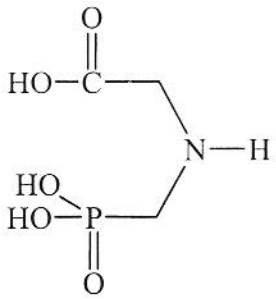


Figure 3.2.1. Structural formula of glyphosate molecule

3.2.1. Method development strategy for the flow injection spectrophotometric determination of glyphosate

Glyphosate molecule has a secondary amino and a phosphonate group. In the basic media the electron rich nitrogen atom of the glyphosate molecule is supposed to attack the electron deficient carbonyl carbon of acidified p-dimethylaminobenzaldehyde (DMAB) forming an unstable intermediate. In acidic medium condensation results in the formation of yellow coloured product which is stable for 5-10 min. The absorbance of coloured product could be used for indirect spectrophotometric determination of glyphosate herbicide. The proposed reaction mechanism is shown in figure 3.2.2.

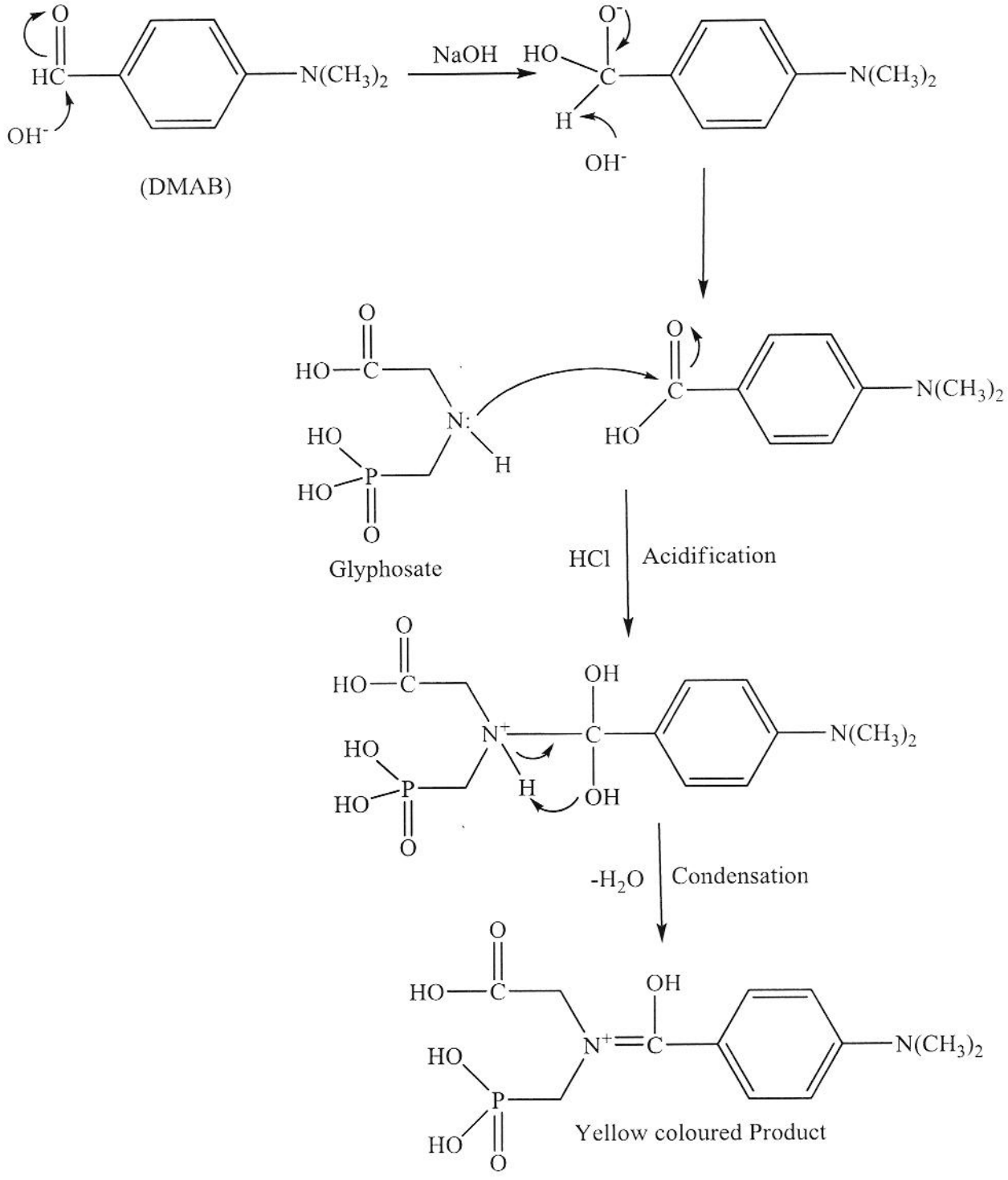


Figure 3.2.2. Proposed reaction mechanism for spectrophotometric method for determination of glyphosate

3.2.2. Preliminary investigation of the possibility of condensation reaction of glyphosate for its spectrophotometric determination

In a 100 mL conical flask 1.0 mL solution from a standard stock solution of glyphosate (1000 μg mL^{-1}) was taken and 1.0 mL of sodium hydroxide solution (1.0 M) was added to it. To this mixture 2.0 mL of 0.5 M p-dimethylaminobenzaldehyde (DMAB) solution was added. The medium was acidified by adding 4.0 mL of hydrochloric acid (3.0 M). The resulting solution turned yellow due to condensation reaction. Also a regent blank was prepared in the same manner without the addition of herbicide. The blank solution was found to be colourless. For enhanced sensitivity completion of condensation reaction was essential. Therefore it was necessary to focus on the optimization of different parameters of the reaction in order to have complete reaction and enhanced sensitivity and repeatability.

3.2.3. Optimization studies for spectrophotometric determination of glyphosate in batch method

In order to achieve maximum product formation optimization studies were conducted. These studies include wavelength of maximum absorbance, concentration and volume of sodium hydroxide, DMAB, hydrochloric acid and the effect of time on stability of the condensation adduct.

3.2.3.1. Investigation of suitable wavelength for the spectrophotometric determination of glyphosate

Instruments and equipments

In batch analysis the absorption spectra and absorbance of solutions were measured with shimadzu UV-VIS spectrophotometer (UV-1700 Pharmaspec, Japan) at 405 nm using a matched glass cells with an optical path length of 1.0 cm. In flow injection analysis absorption spectra were measured with UV/Visible Spectrophotometer (UNICO, UV-2100 United Products and Instruments Inc., Dayton, NJ, USA). Peristaltic pump (Model 124869 MP-1 pump Spectra/chrom Spectrum), Silicon tube (1.71 mm, i.d.), V-450 six-port injection valve (Upchurch Scientific Inc., USA) and PTFE Tubing (1.19 mm, i.d.,

Becton Dickson, USA) were used as main FIA components. Solutions' pH was measured with pH meter, (model 7020 Kent Industrial Measurement Limited-Electronic Instruments Ltd, Chertsey Survey England) using combined glass electrode.

Chemicals and Reagents

All reagents used were of analytical reagent-grade or similar purity. p-dimethylaminobenzaldehyde (Merck-Schuchardt; Eduard-Buchner-Str. 14-20, 85662 Hohenbrunn Germany), sodium hydroxide, hydrochloric acid, and methanol (Merck, Darmstadt Germany) were used during this work. Reference standard of glyphosate herbicide was purchased from Dr Ehrenstorfer GmbH (Augsburg, Germany). Ammonium hydroxide solution (BDH Laboratory Suppliers Poole, England) was used for extraction of herbicide from grain samples. Commercial formulation containing 41.0 % glyphosate was purchased from the local market.

Solutions preparation

Standard glyphosate solution: A standard stock solution of glyphosate (1000 μg mL^{-1}) was prepared by dissolving 0.1 g of the authentic standard in 20 mL distilled water and diluted to 100 mL with distilled water. Working standards were prepared by diluting appropriate volumes of the stock solution with distilled water.

p-dimethylaminobenzaldehyde solution: DMAB stock solution (0.2 M) was prepared by dissolving 2.98 g of the reagent in 30 mL methanol, acidified with 2.0 mL hydrochloric acid (1.0 M) and diluted upto 100 mL with methanol.

Sodium hydroxide and hydrochloric acid solutions: Sodium hydroxide (2.0 M) and hydrochloric acid (3.0 M) solutions were prepared by dissolving appropriate amounts in distilled water and diluted upto 100 mL with distilled water.

Procedure

In batch analysis 1.0 mL from a standard stock solution of glyphosate (100 μg mL^{-1}) was taken in a 100 mL conical flask followed by the addition of 1.0 mL of sodium hydroxide solution (2.0 M). To this mixture 2.0 mL of 0.2 M p-dimethylaminobenzaldehyde solution was added. The medium was acidified by adding 2.0 mL of hydrochloric acid

(3.0 M).The resulting yellow coloured solution of the condensation product was transferred to a 25 mL volumetric flask and diluted to the mark with distilled water. The absorption spectrum of the solution was measured at different wavelengths in the range of 380-440 nm. The results are shown in figure 3.2.3.

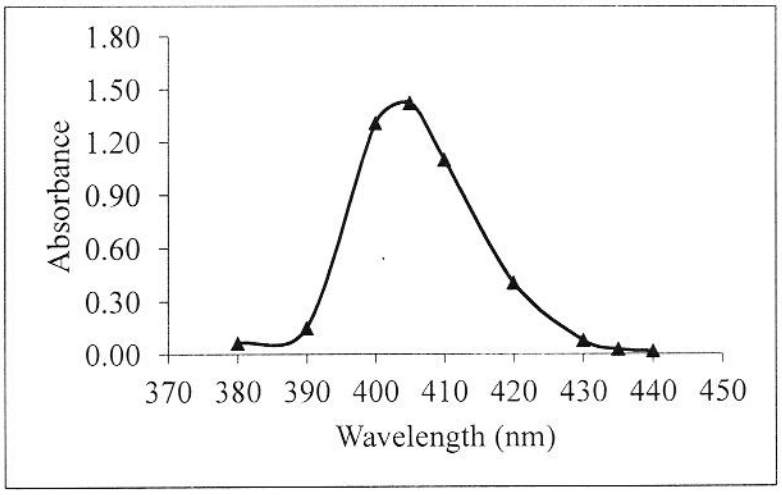


Figure 3.2.3. Wavelength optimization for spectrophotometric determination of glyphosate

3.2.3.2. Investigation of the effect of various parameters on the condensation reaction of glyphosate

Instrument, reagents and solutions: The same as mentioned before

Procedure

In order to investigate the effect of concentration of sodium hydroxide on the reaction, 1.0 mL from a standard stock solution of glyphosate (100 μg mL^{-1}) was taken in six separate 100 mL conical flasks and 1.0 mL of sodium hydroxide solution in the concentration range of 0.016-0.20 M was added to each flask followed by addition of 1.0 mL of 0.5 M p-dimethylamino-benzaldehyde. The medium was acidified by adding 1.0 mL of hydrochloric acid (3.0 M). The resulting solution of the yellow coloured condensation product was transferred to a 25 mL volumetric flask and diluted upto the mark with distilled water. The absorption spectrum of the solution was measured at 405 nm. The results are shown in figure 3.2.4. For optimizing the effect of volume of sodium

hydroxide on the reaction, only the volume of sodium hydroxide solution (0.08 M) in the range of 0.2-2.5 mL was varied. The rest of the procedure was kept the same. The absorbance versus volume plot was drawn and is shown in figure 3.2.5.

DMAB reacts with alkaline glyphosate and its concentration was optimized by adding 1.0 mL of sodium hydroxide (0.08 M) to a series of conical flasks, each containing 1.0 mL of standard glyphosate solution (100 μg mL^{-1}). DMAB solution (2.0 mL) in the concentration range of 0.004 to 0.024 M was added to each flask keeping all other parameters the same. The solution of the yellow coloured condensation product in each case was transferred to a 25 mL volumetric flask and diluted to the mark with distilled water. Absorbance of each solution was measured and the results are shown in figure 3.2.6. For optimization of volume of DMAB varying volume of DMAB solution (0.016 M) in the range of 0.5-3.0 mL was added keeping the rest of the reagents and conditions constant. The results of the absorbance as a function of volume are shown in figure 3.2.7.

Maximum condensation product formation requires acidic media. In order to study the effect of concentration of hydrochloric acid on the reaction, 1.0 mL of standard glyphosate solution (100 μg mL^{-1}) was added to six separate conical flasks. Then optimum volumes of sodium hydroxide (0.08 M) and DMAB (2.0 M) were successively added to each flask. This was followed by the addition of 1.0 mL of hydrochloric acid in the concentration range of 0.012-0.048 M. Each coloured solution was transferred to a 25 mL flask and was diluted up to the mark with distilled water. The absorbance of each solution was measured and the results are given in figure 3.2.8. Volume of HCl solution (0.024 M) was optimized by following the same procedure while varying the volume in the range of 1.0-4.0 mL. The absorbance versus volume plot is shown in figure 3.2.9.

The effect of time on the stability of the final condensation product was investigated. Under the optimized conditions the final condensation product was produced. The absorbance of the product was measured at various time intervals varying from 2.0-25.0 min. The time versus absorbance plot is shown in figure 3.2.10.

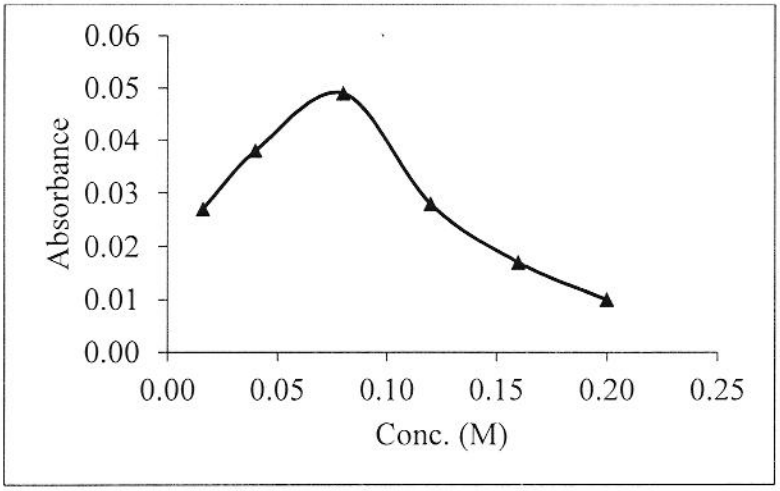


Figure 3.2.4. Effect of concentration of sodium hydroxide on the reaction for spectrophotometric determination of glyphosate

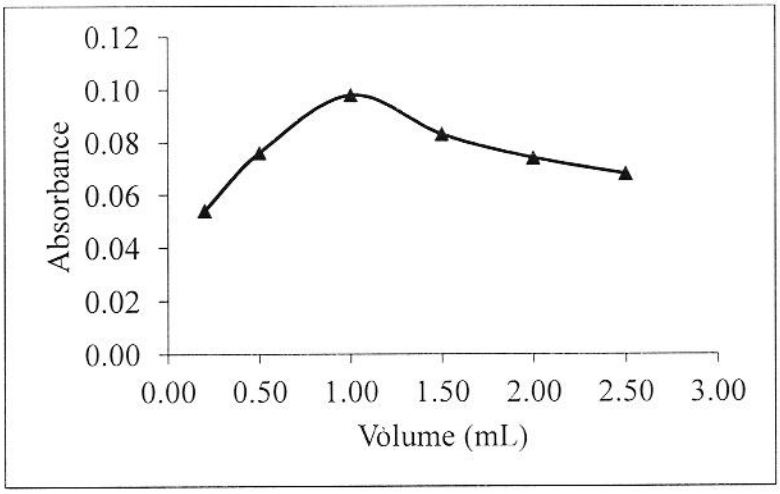


Figure 3.2.5. Effect of volume of sodium hydroxide on the reaction for spectrophotometric determination of glyphosate

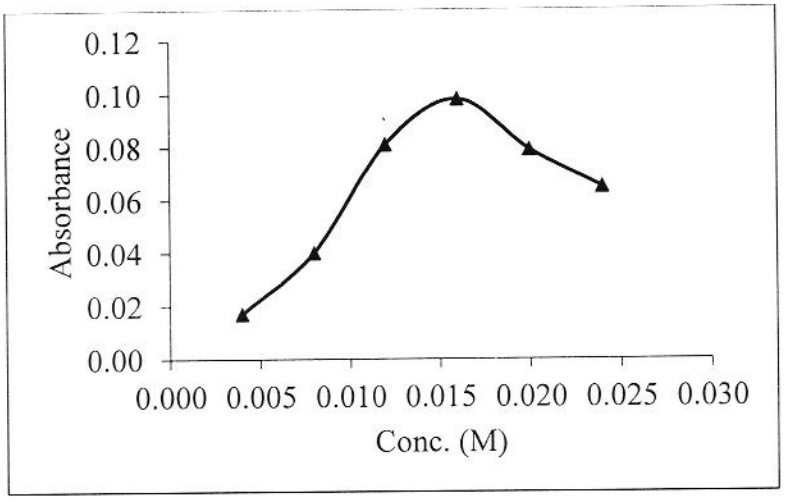


Figure 3.2.6. Effect of concentration of p-dimethylaminobenzaldehyde on the reaction for spectrophotometric determination of glyphosate

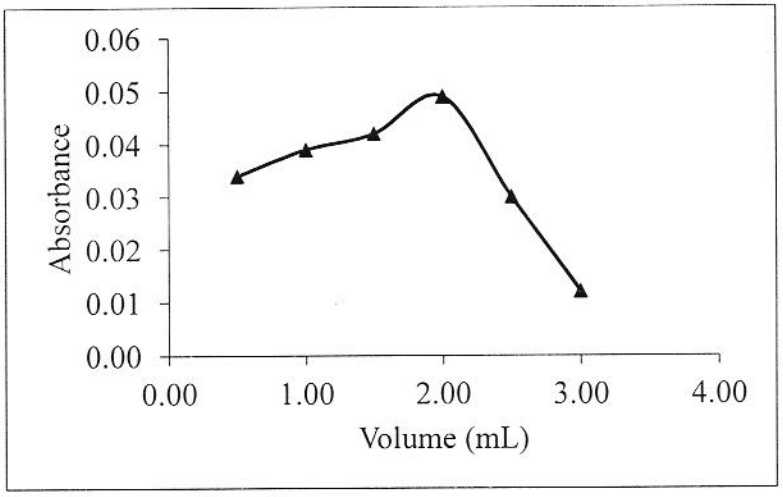


Figure 3.2.7. Effect of volume of p-dimethylaminobenzaldehyde on the reaction for spectrophotometric determination of glyphosate

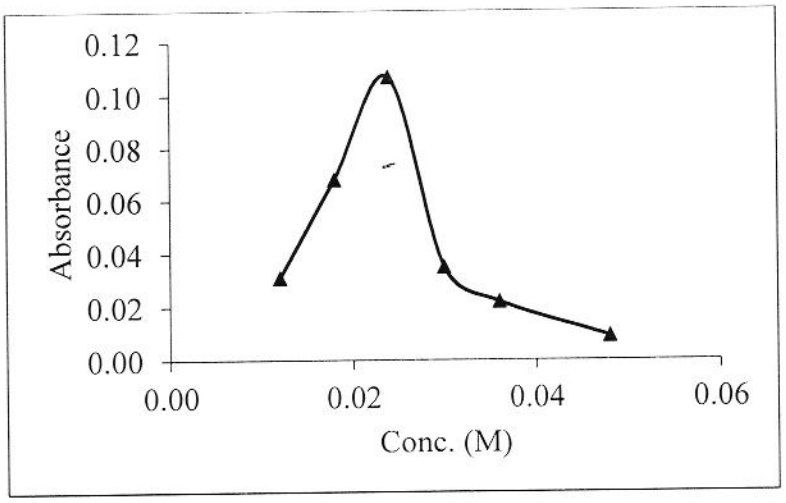


Figure 3.2.8. Effect of concentration of hydrochloric acid on the reaction for spectrophotometric determination of glyphosate

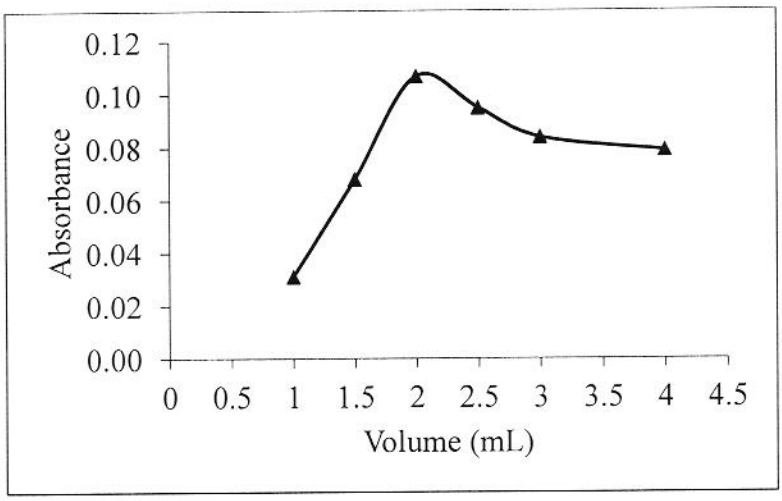


Figure 3.2.9. Effect of volume of hydrochloric acid on the reaction for spectrophotometric determination of glyphosate

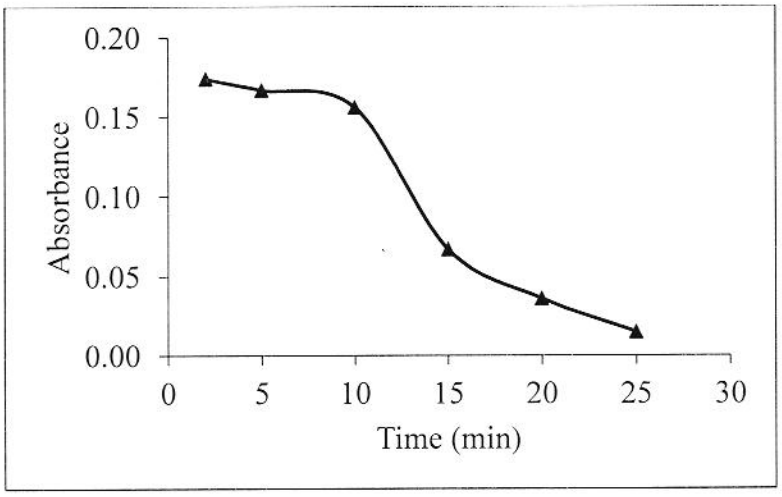


Figure 3.2.10. Effect of time on stability of the condensation product used for spectrophotometric determination of glyphosate

3.2.4. Verification of Beer's law for the spectrophotometric determination of glyphosate using batch method

Instruments, reagents and solutions: The same as mentioned earlier

Procedure

To investigate the linearity range of the proposed method varying concentrations of glyphosate (100 μg mL^{-1}) corresponding to final concentration in the range of 2-15 μg mL^{-1} were taken separate conical flasks. To each flask optimum volume (1.0 mL) of sodium hydroxide solution (0.08 M) was added followed by the addition of optimum volume (2.0 mL) of 0.016 M DMAB solution and then optimum volume (2.0 mL) of HCl (0.024 M). The resulting yellow coloured solution in each case was transferred to a 25 mL volumetric flask and diluted up to the mark with distilled water. The absorbance of each solution was measured at 405 nm against a reagent blank being prepared by the same procedure without the addition of glyphosate. The results are given in Table 3.2.1 and are shown in figure 3.2.11.

Table 3.2.1. Investigation of the effect of concentration of glyphosate on the absorbance of condensation product formed

Concentration (μg mL^{-1})	Absorbance
2.0	0.033
3.0	0.059
4.0	0.092
5.0	0.108
8.0	0.162
10	0.193
15	0.271

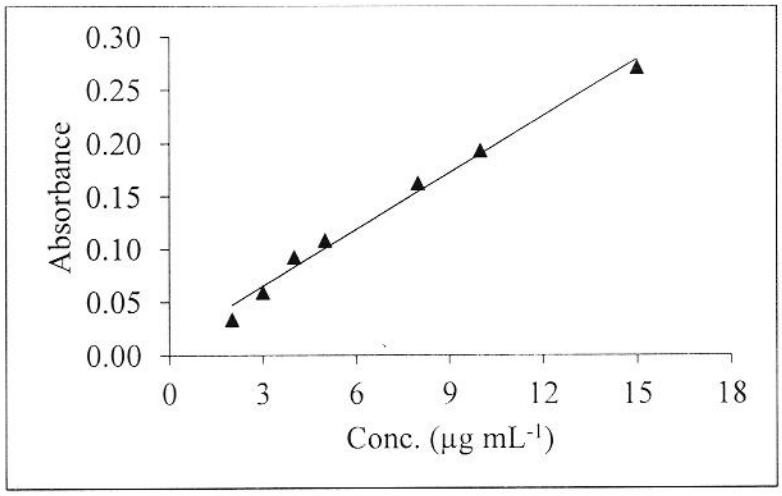


Figure 3.2.11. Investigation of the effect of concentration of glyphosate on the absorbance of condensation product formed

3.2.5. FLOW INJECTION SPECTROPHOTOMETRIC DETERMINATION OF GLYPHOSATE VIA CONDENSATION WITH DMAB

For flow injection analysis of glyphosate, a three channel FIA system was assembled as shown in figure 3.2.12. In the FI manifolds in reagent stream F-1 NaOH (0.1 M) was pumped. Sample solution of glyphosate was injected into the F-1 stream through the 6-port injection valve in a 500 μL sample injection loop. The mixture became alkaline in reaction coil R_1 and was then reacted in reaction coil R_2 with DMAB (0.016 M) flowing down in the reagent stream F-2. Finally the proposed intermediate formed was acidified

in reaction coil R_3, with HCl solution (3.0 M) flowing in reagent stream F-3, to form the condensation product. The yellow coloured product formed was continuously passed through a flow through cell and the absorbance continuously measured at λ_{max} 405 nm by UNICO UV-2100 Spectrophotometer. The chemical parameters optimized for the batch analysis of glyphosate were slightly modified according to the requirements of the setup and were applied in the FI analysis at room temperature.

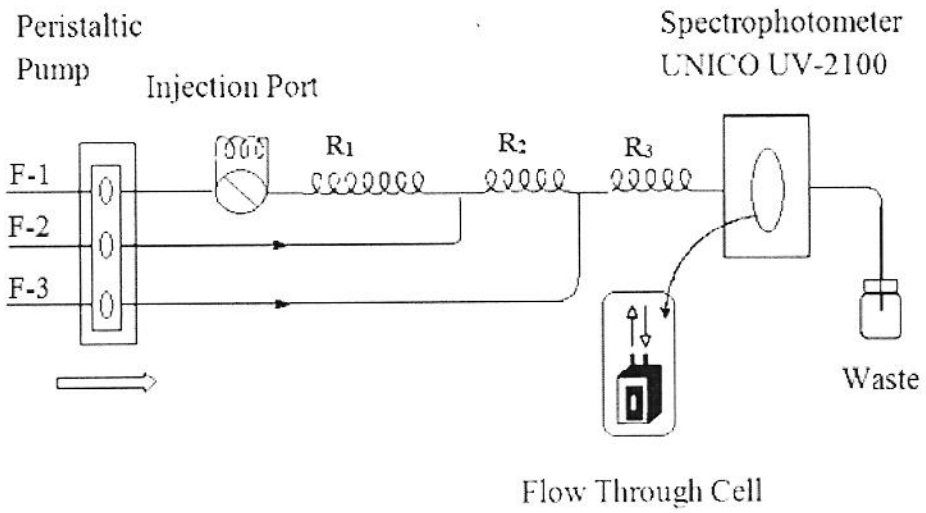


Figure 3.2.12. Scheme of flow injection system for flow injection spectrophotometric determination of glyphosate

F-1; Reagent-1(NaOH), F-2; Reagent-2 (DMAB), F-3; Reagent-3 (HCl), R_1; Reaction coil 1(addition of alkali), R_2; Reaction coil-2 (Reaction of alkaline glyphosate and DMAB), R_3; Reaction coil-3 (Acidification followed by condensation)

3.2.5.1. Investigation of the effect of physical parameters on the FI spectrophotometric determination of glyphosate

Instruments, reagents and solutions: The same as mentioned earlier

Procedure

In the flow injection system, with the reaction coils all having the same length (40 cm), sodium hydroxide (0.1 M) was pumped in the F-1 reagent stream. Into this stream 500 µL

of glyphosate solution (100 μg mL^{-1}) was loaded into the sample loop through the injection port with the help of a 5.0 cm^3 syringe and then injected to the stream. The basic mixture was allowed to react with acidified DMAB (0.016 M) being continuously pumped in reagent stream F-2 and the intermediate formed was acidified with HCl solution (3.0 M) being pumped continuously in reagent stream F-3. The absorbance of the blank was adjusted to zero by continuously pumping the reagent solutions in the assembly without injecting glyphosate and zero adjustment via computer interface. The absorbance of the product formed was continuously measured at 405 nm, while passing through a flow through cell to the waste chamber being placed outside the spectrophotometer.

The effect of flow rate on the absorbance of the final product was investigated in the range of 1.0-5.0 mL min^{-1} by adjusting the flow through the knob on the peristaltic pump. The data was displayed and recorded through a computer interface program and is shown in figure 3.2.13.

The effect of sample injection volume on the absorbance of the product was investigated by injecting different volumes of glyphosate (100 μg mL^{-1}) through the injection valve into the sample loop in the range of 200-600 μL. All the remaining physical and chemical parameters were kept the same as discussed earlier. The results are shown in figure 3.2.14.

The effects of length of the reaction coils were studied at the optimum flow rate of 1.5 mL min^{-1} and optimum sample injection volume of 500 μL keeping all the chemical variables constant. The length of each reaction coil [R_1, R_2 and R_3] was varied one by one in the range of 25-110 cm and absorption spectrum for each run was recorded. While varying the length of one reaction coil the length of the other two was kept constant. The results are shown in figure 3.2.15.

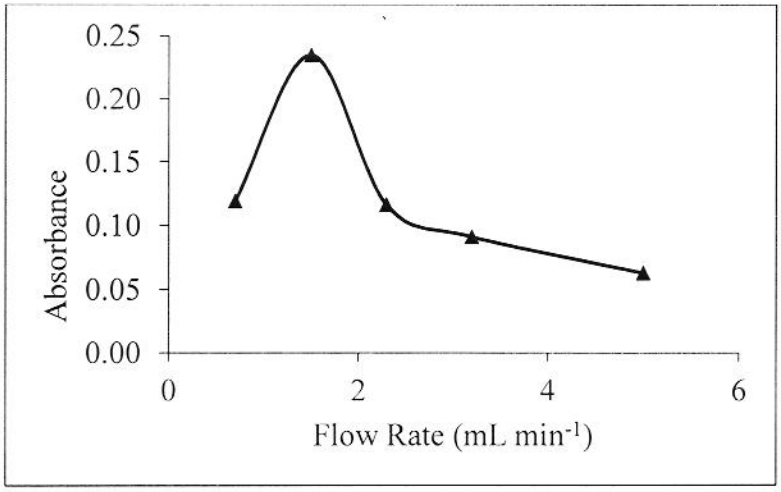


Figure 3.2.13. Effect of flow rate on the FI spectrophotometric determination of glyphosate

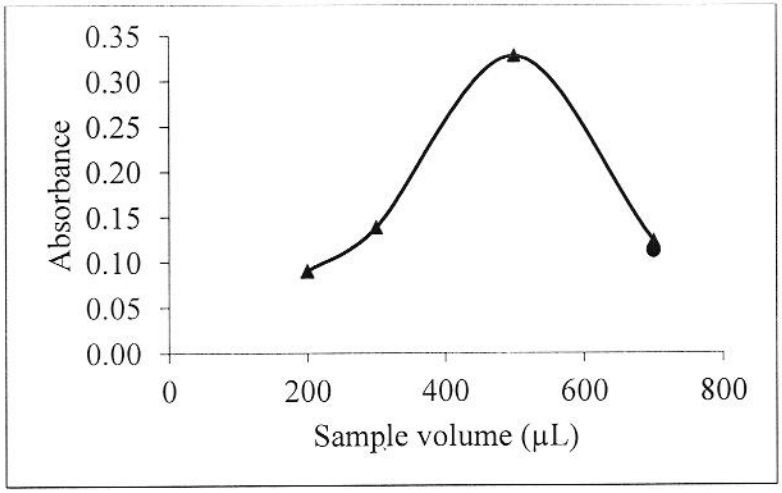


Figure 3.2.14. Effect of sample injection volume on the FI spectrophotometric determination of glyphosate

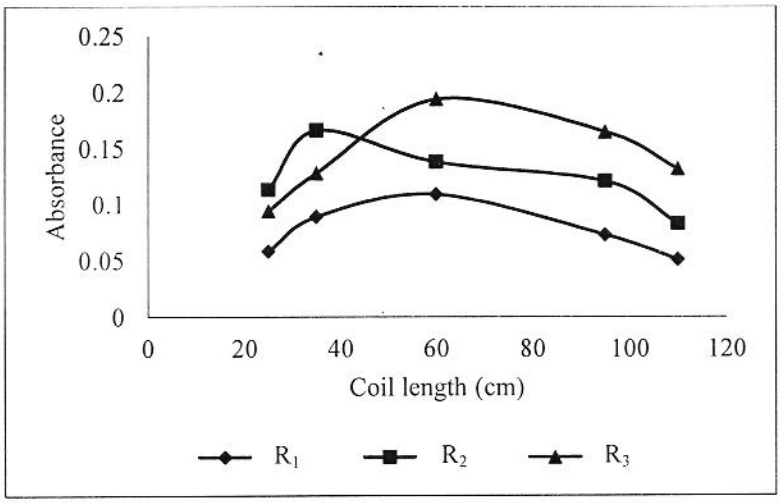


Figure 3.2.15. Investigation of the effect of reaction coil length on the FI spectrophotometric determination of glyphosate

3.2.5.2. Investigation of the effect of concentration of glyphosate on the absorbing behaviour using FI spectrophotometric determination

Instruments, reagents and solutions: The same as mentioned earlier

Procedure

Standard glyphosate solutions in the range of 0.5-20.0 μg mL^{-1} were prepared in separate 10 mL volumetric flasks by taking the required volume of standard from 10 and 100 μg mL^{-1} stock solutions and diluting with distilled water. In the flow through assembly reagents solutions of the optimized concentration were pumped through the reaction coils of optimum length i.e. R_1 = 60 cm, R_2 = 35 cm, R_3 = 60 cm, with an optimum flow rate of 1.5 mL min^{-1}. The absorbance of the blank was adjusted to zero and then optimum volume (500 μL) of each standard glyphosate solution was loaded into the sample loop through the injection port with the help of a 5.0 cm^3 syringe. In each case, sample was injected into the stream and the absorbance spectrum was recorded. Between two successive measurements the sample loop was flushed with distilled water and absorbance of the blank reagents stream adjusted to zero. With this process a sample throughput of 60 samples h^{-1} was achieved. The results are given in table 3.2.2 and a calibration plot of absorbance vs. concentration was constructed and is shown in figure 3.2.16.

Table 3.2.2. Investigation of the effect of concentration of glyphosate on the absorbance of the final product in the FI spectrophotometric determination

Conc. µg mL^{-1}	Absorbance
0.5	0.013
1.0	0.023
2.0	0.041
4.0	0.073
8.0	0.138
12	0.203
15	0.259
20	0.322

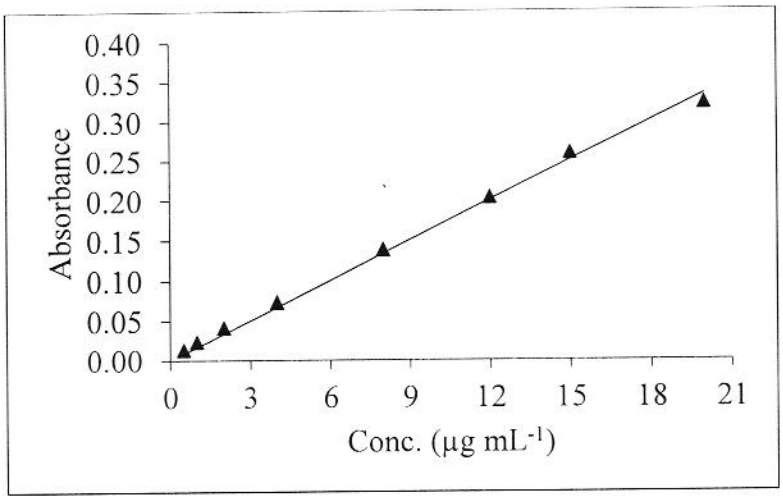


Figure 3.2.16. Investigation of the effect of concentration of glyphosate on the absorbance of the final product in the FI spectrophotometric determination

3.2.6. Investigation of various analytical features for the spectrophotometric determination of glyphosate using both batch and FI method

Different analytical parameters for methods, both batch and FI analysis, were calculated based on the formulae given below:

The molar absorptivity (ε) was calculated based on the formula given below:

Molar Absorptivity (ε) = A/C × L

Where 'A' is the absorbance, 'C' is the Molar concentration and 'L' is the path length (cm) of the cell.

LOD = 3S/b (For concentration)

LOQ = 10S/b (For concentration)

Where 'S' is the standard deviation and 'b' is the slope of the curve

Relative Standard Deviation (RSD) was calculated by using the formula:

RSD = (S/x) ×100

Where 'x' is the mean concentration determined

Limit of detection (LOD) and limit of quantification (LOQ) were evaluated for six replicates of the lowest quantifiable concentration that can be measured with acceptable precision and accuracy (2.0 μg mL^{-1} for batch and 0.5 μg mL^{-1} for FI method). At optimum conditions various analytical characteristics of the method were determined and are given in table 3.2.3.

Table 3.2.3. Analytical characteristics for the spectrophotometric determination of glyphosate by batch and FI method

Parameter	**Batch**	**FIA**
λ_{max} (nm)	405	405
Beer's law range (μg mL^{-1})	2-15	0.5-20
Molar absorptive (ε) L mol^{-1} cm^{-1}	2.7×10^3	4.3×10^3
Relative Standard Deviation (RSD) %	6.0	8.0
Limit of Detection (LOD) μg mL^{-1}	0.2 ± 0.06	0.2 ± 0.07
Limit of Quantification (LOQ) μg mL^{-1}	0.7 ± 0.1	0.6 ± 0.2
Correlation Coefficient	0.988	0.996
Slope	0.0178	0.0167
Intercept	0.0124	0.00

3.2.7. Investigation of percent recovery of glyphosate from spiked samples using the proposed batch and FI method

Instruments, reagents and solutions: The same as mentioned before

Collection of samples

For investigating the percent recovery from real samples, wheat grains was randomly collected from crop field in Malam Jaba; a hilly area of Swat (Khyber Pakhtunkhwa) after the confirmation that no herbicide had been sprayed on the field.

Sample extraction procedure

Control samples (10.0 g) of wheat grains were separately placed in bottles and known concentration of glyphosate solution having pH 4.5 was added to adjust the concentration level in the range of 44-88 $\mu g\ g^{-1}$. The samples were homogenized by mechanical shaking for uniform mixing and kept for 24 hr at ambient temperature. The homogenized samples were extracted by adding 25 mL of NH_4OH solution (2.0 M) to the bottles and then filtering on whatman® Grade 3: 6 µm. Extraction was repeated three times and the extracts were kept for analysis by the proposed method.

Analysis procedure

Percent recoveries of glyphosate from the sample were evaluated each in triplicate, by using both batch and flow injection analysis.

For batch analysis 2.0 mL of each extract was taken in a conical flask and optimum volumes of all the reagents were successively added and the absorbance of the final product was measured at 405 nm against a reagent blank.

For FI analysis the extract was concentrated to 1.0 mL by evaporating the solvent at 60 ^{o}C in each case. Optimum volume (0.4 mL) of each extract was loaded into the sample loop through the injection port with the help of a micro syringe. The sample was then injected to the reagents stream in which optimum concentration of the reagents was flowing at an optimum flow rate. The absorbance of the product was measured at 405

nm. For each sample absorbance readings were taken in triplicate. A comparison of percent recovery investigated by both the batch and FI analysis is given in table 3.2.4.

Table 3.2.4. Investigation of percent recovery of glyphosate from spiked wheat grain sample using batch and FI method

Method of Analysis	$\mu g\ g^{-1}$ added	$\mu g\ g^{-1}$ found	% Recovery ±SD
Batch Analysis	44.0	39.06	90.2 ± 0.25
	60.0	56.0	93.4 ± 0.42
	88.0	81.3	92.4 ± 0.36
FI Analysis	44.0	41.0	93.0 ± 0.63
	60.0	56.0	93.0 ± 0.66
	88.0	79.0	90.0 ± 0.65

3.2.8. Application of the proposed batch and FI methods for glyphosate residue determination in real samples

Instruments, reagents and solutions: The same as mentioned earlier

Collection of samples

Wheat grains samples were collected from a crop field upon which the herbicide glyphosate was sprayed as a pilot project in Malam Jaba, a hilly area of Swat (Khyber Pakhtunkhwa). The herbicide was sprayed on the bare field before the cultivation of wheat crop in order to destroy the undesirable herbs.

Sample extraction procedure

Three samples of the grains (100 g each) were taken in separate bottles and spiked with a known concentration of standard glyphosate solution. The bottles were uniformly shaken manually for proper mixing and kept for two hr. The herbicide was extracted with 40 mL of NH_4OH solution (2.0 M). The concentrations in the extracts were then determined by the proposed batch and FIA method.

Procedure for analysis

For batch analysis, 20 mL of each extract was evaporated to 10 mL by heating at 60 °C, 1.0 mL of which was taken in a conical flask and optimum volumes of all the reagents mentioned before were successively added and the absorbance of the final product was measured at 405 nm against a reagent blank.

For FI analysis the extract being further concentrated to 1.0 mL was used in each case. Optimum volume (500 µL) of each extract was loaded into the sample loop through the injection port using a micro syringe. The sample was then injected to the reagents stream in which optimum concentration of the reagents was flowing at an optimum flow rate. The absorbance of the product was measured at 405 nm. For each sample absorbance readings were taken in triplicate. The residue of herbicide present in the sample was determined from the difference of the concentration of standard solution of herbicide and the concentration of herbicide in spiked sample. The results for both methods are given in table 3.2.5.

Table 3.2.5. Residue determination of glyphosate in wheat grain samples by the proposed batch and FI method.

Method of Analysis	**Average Residue ±SD**
Batch Analysis	1.07 ± 0.22
FI Analysis	1.13 ± 0.09

Results and Discussion

The polar glyphosate molecule has a secondary amino group and a phosphonate group. The electron rich nitrogen atom of the glyphosate molecule is supposed to attack the electron deficient carbonyl carbon of alkaline p-dimethylaminobenzaldehyde forming an unstable intermediate. In acidic medium condensation results in the formation of yellow colored product which is stable for 5-10 min. The absorbance of the product was scanned at different wavelengths and maximum absorbance was observed at 405 nm. The proposed reaction mechanism is shown in (Fig. 3.2.2). The absorption spectra of the condensation adduct was measured at wavelengths in the range of 380-440 nm and shows maximum absorbance at 405 nm (Fig. 3.2.3). Therefore 405 nm was selected as λ_{max} for further studies.

The physical and chemical variables were optimized for the reaction in batch and FI system by changing one variable and keeping the other variables constant. The chemical variables like sodium hydroxide, DMAB, and hydrochloric acid concentration and volume and the effect of time on the stability of the final product were investigated.

The effect of concentration of sodium hydroxide on the reaction was studied in the range of 0.016-0.20 M (Fig. 3.2.4). The absorbance was found to increase from 0.016 to 0.08 M sodium hydroxide solution and beyond this concentration a continuous decrease was observed with increase in concentration. Similarly the effect of volume of sodium hydroxide was investigated in the range of 0.2-2.5 mL (Fig. 3.2.5). It was observed that absorbance increases from 0.2 mL to 1.0 mL, beyond which it decreases slowly. This is because at higher pH of the medium the intermediate formed is unstable and gets dissociated. Also the higher concentration of the alkali creates reagent solubility problem and leads to lower yield and hence lower absorbance of the final condensation adduct.

DMAB reacts with alkaline glyphosate and its concentration was optimized in the range of 0.004 to 0.024 M (Fig. 3.2.6). Reaction starts with 0.004 M DMAB solution and maximum product formation occurs at 0.016 M concentration of DMAB beyond which the absorbance decreases. The effect of volume of DMAB was investigated in the range

of 0.5-3.0 mL. The absorbance increased with increasing volume upto 2.0 mL beyond which it decreases sharply due to dilution effect (Fig. 3.2.7).

Acidic media is required for the formation of condensation product; therefore the concentration of hydrochloric acid was optimized in the range of 0.012 - 0.048 M. Maximum absorbance was observed at 0.024 M HCl concentration. At higher concentration absorbance decreases because lower pH catalyzes breakdown of the coloured product (Fig 3.2.8). Therefore further analysis was performed with 0.024 M solution of hydrochloric acid. The effect of volume of HCl was investigated in the range of 1.0-4.0 mL and it was observed that absorbance increases from 1.0 mL to 2.0 mL of hydrochloric acid. At volume above 2.0 mL a slow decrease in absorbance was observed possibly due to dilution effect (Fig.3.2.9).

The effect of time on the absorbance of the final condensation product was investigated by measuring its absorbance at different time intervals varying from 2.0 min to 25 min after its formation (Fig. 3.2.10). The absorbance was found to remain almost constant for 10 min after the product formation and decreases steadily with further increases in time. The results suggest that the condensation product is stable upto 10 min following its formation at room temperature and the stability is gradually lost beyond 10 min. The batch method being independent of any kind of heating was found suitable to be applied for flow injection analysis of glyphosate herbicide.

The effect of concentration of glyphosate on the absorbance of the final condensation product in batch method was investigated in the range of 2.0-15.0 $\mu g\ mL^{-1}$ and a linear relationship between concentration and absorbance was obtained in this range (Table 3.2.1, Fig. 3.2.11).

For FI analysis a three channel system was assembled (Fig. 3.2.12). The effect of physical variables like flow rate, sample injection volume and reaction coil length was investigated for the FI method. Flow rate of the FI system for reaction was studied in the range of 0.7 to 5.0 mL min^{-1} (Fig 3.2.13). Increase in absorbance was observed when the flow rate increased from 0.7 to 1.5 mL min^{-1}. Above 1.5 mL min^{-1} absorbance decreases with increase in flow rates due to incomplete reaction and less efficient dispersion of the

reagents and the analyte. With this flow rate, a sampling rate of 60 samples h^{-1} was achieved.

The sample injection volume was optimized in the range of 200-700 µL and maximum signal response was observed with 500 µL which indicates efficient mixing of the analyte with the NaOH stream (Fig. 3.2.14). At 700 µL sample volume two peaks near to each other appear having lower intensity. This indicates that mixing with base occurs at the two ends of the sample stream.

The effect of reaction coils length was studied from 25 cm to 110 cm for all channels. Absorbance of the product was found to increase when coil length was increased from 25-60 cm in case of reaction coil R_1 beyond which decrease in absorbance was observed due to backward reaction of the analyte and alkali. The product formation was found maximum with 25 cm reaction coil length for reaction coil R_2 beyond which a decrease in absorbance indicates the instability of the product in alkaline media. For complete reaction the length of the reaction coil R_3 was also investigated and maximum absorbance of the product was found with 60 cm coil length which shows the optimum acidity limit. The results indicated that a condensation reaction is fast and reproducible in the FI system (Fig. 3.2.15).

In order to investigate the effect of concentration of glyphosate on the absorbance behaviour of the coloured product in FI method, the concentration was varied in the range of 0.5-20 µg mL^{-1}. A linear response of absorbance to concentration was observed over this concentration range (Table 3.2.2, Fig. 3.2.16).

Under the optimized conditions various analytical characteristics both for batch and FI methods were determined (Table 3.2.3). The molar absorptivities for batch and FI method were found to be 2.7×10^3 and 4.3×10^3 L mol^{-1} cm^{-1}, respectively. The applicability of Beer's law by batch and FIA system was confirmed. A linear relation was observed in the concentration range of 2.0-15.0 µg mL^{-1} and 0.5-20.0 µg mL^{-1} for batch and FI methods respectively. The limit of detection (3S/b) and limit of quantification (10S/b) was calculated at lower concentration that can be measured with acceptable precision and accuracy and were found to be 0.2 ± 0.06, 0.7 ± 0.1µg mL^{-1}, and 0.2 ± 0.07, 0.6 ± 0.2 µg

mL^{-1} for batch and FIA methods respectively. The sensitivity achieved with the FI method is matchable to that with the batch method. The advantages of the proposed FI method are simplicity, low reagent waste and high sample through put.

The proposed method was successfully applied for the determination of percent recovery of glyphosate from spiked wheat grains samples. Different concentrations of standard glyphosate herbicide (44-88 $\mu g\ g^{-1}$) were added to control samples. Glyphosate was extracted with NH_4OH solution following a reported method [1] and analysed by the proposed batch and FI method. The recovery from wheat grains were found to be in the range of 90.2 ± 0.25 % to 93.4 ± 0.42 % for batch analysis and 90.0 ± 0.65 % to 93.0 ± 0.66 % for FI analysis (Table 3.2.4).

The proposed method was also applied for the determination of glyphosate residue in real samples of wheat grains and the residue was found to be 1.07 ± 0.22 $\mu g\ g^{-1}$ by batch method and 1.13 ± 0.09 $\mu g\ g^{-1}$ by FI method, the results being in agreement with each other (Table 3.2.5).

Reference

1. M. R. Jan, J. Shah, M. Muhammad, B. Ara, "Glyphosate herbicide residue determination in samples of environmental importance using spectrophotometric method", Journal of Hazardous Materials, 169(4), 742-745 (2009).

3.3. FLOW INJECTION SPECTROPHOTOMETRIC DETERMINATION OF FENOXA-PROP-p-ETHYL (FPE) HERBICIDE

The structural formula of fenoxaprop-p-ethyl is given below.

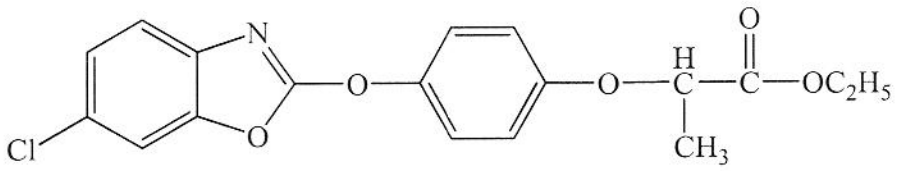
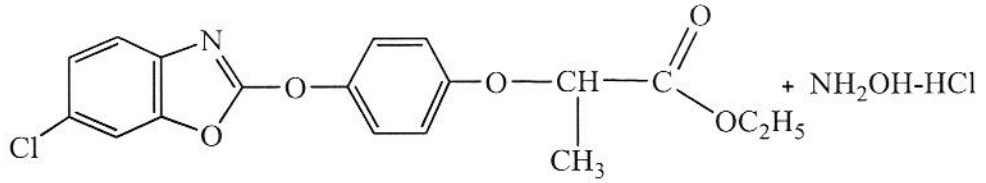


Figure 3.3.1. Structural formula of fenoxaprop-p-ethyl

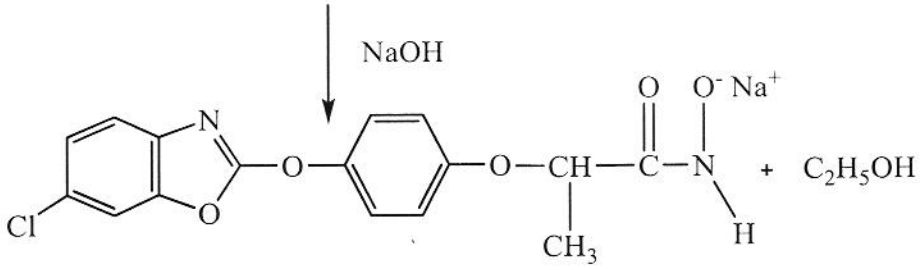
3.3.1. Method development strategy for the flow injection spectrophotometric determination of fenoxaprop-p-ethyl

The chemical structure of fenoxaprop-p-ethyl (FPE) is given in figure 3.3.1. It contains a terminal ester group. The proposed method is based on the derivatization of the molecule to a hydroxamate salt by its reaction with hydroxylamine hydrochloride in alkaline medium, accompanied by the release of ethanol moiety. The hydroxamate salt can act as a ligand of choice for complexation with Fe (III) in acidic medium. The absorbance and hence concentration of the resultant magenta coloured Fe (III) hydroxamate complex can be used as a function of the concentration of fenoxaprop-p-ethyl. Mechanism of the reaction is given in figure 3.3.2.

Step$_1$. Hydroxamate derivative formation from fenoxaprop-p-ethyl

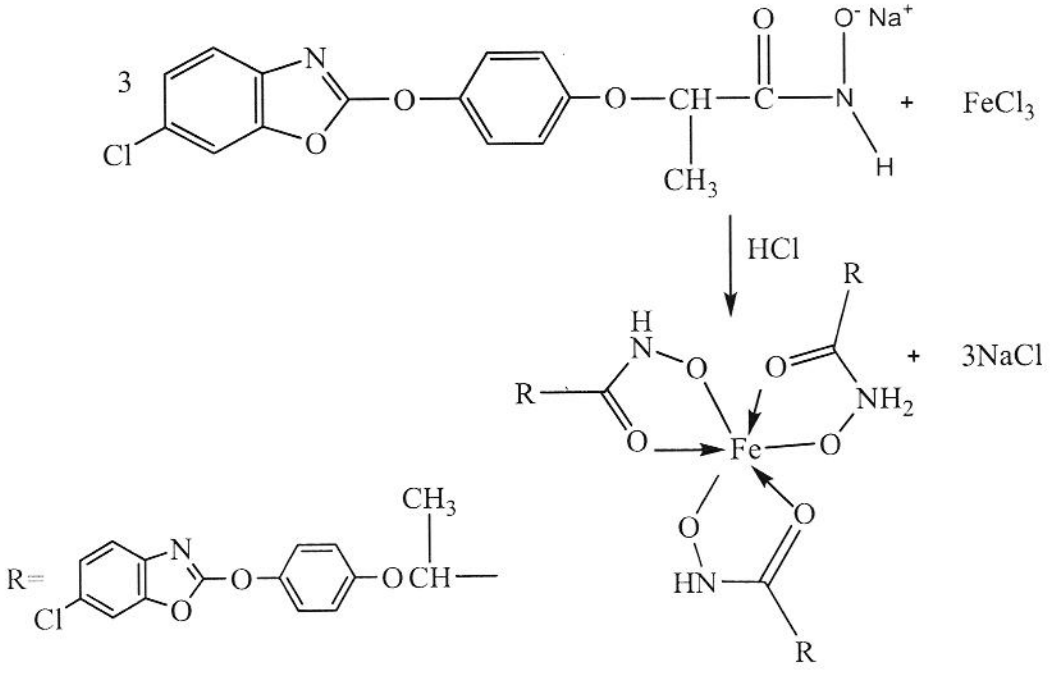


ethyl 2-(4-(6-chlorobenzo[*d*]oxazol-2-yloxy)phenoxy)propanoate
(fenoxaprop-p-ethyl)



(Hydroxamate derivative)

Step2. Complex formation of Hydroxamate ligand with Fe(III)



tris(2-(4-(6-chlorobenzo[d]oxazol-2-yloxy)phenoxy)propanamidooxy)iron

(Magenta colour complex)

Figure 3.3.2. Proposed reaction mechanism for determination of fenoxaprop-p-ethyl by spectrophotometric method

3.3.2. Preliminary investigation for the derivatization and complexation reaction of FPE with Fe (III) for its spectrophotometric determination

Standard fenoxaprop-p-ethyl (350 μg mL^{-1}) solution (2.0 mL) was added to a conical flask followed by the addition of 1.0 mL of 0.5 M hydroxylamine hydrochloride solution and 1.0 mL of 4.0 M sodium hydroxide solution. To the resultant mixture, 3.0 mL of hydrochloric acid (2.0 M) solution was added followed by the addition of a drop of iron (III) chloride hexahydrate solution (5.0 %). The formation of magenta coloured product confirmed the likelihood of the proposed reaction and the possibility of spectrophotometric determination of FPE by this method. Further studies were focused on the optimization of different physical and chemical parameters of the reaction and the detail is given below.

3.3.3. Optimization studies for spectrophotometric determination of fenoxaprop-p ethyl via batch analysis

For completion of reactions involved and maximum product formation, different physical and chemical parameters were optimized. These optimization studies include wavelength of maximum absorbance, concentration and volume of the reagents like hydroxylamine hydrochloride, sodium hydroxide and hydrochloric acid and the effect of time on the stability of the intermediate hydroxamate derivative and the final complexation product. Details of these optimizations are given below.

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

Instruments

Absorbance of the complex was measured with UV/Visible Spectrophotometer (UNICO, UV-2100 United Products and Instruments Inc., Dayton, NJ, USA) using a glass cell with an optical path length of 1.0 cm. Main components used in flow injection system were peristaltic Pump (Model 124869 MP-1 pump Spectra/chrom Spectrum), silicon tube (1.71mm, i.d.), V-450 6-port injection valve (Upchurch Scientific Inc., USA), PTFE Tubing (1.19 mm, i.d., Becton Dickson, USA).

Chemicals and reagents

All reagents used were of analytical reagent-grade purity or similar grade. Hydroxylamine hydrochloride (MP Biomedicals, LLC 1263 South Chillicothe Rd Aurora, Ohio 44202), sodium hydroxide, hydrochloric acid, ethanol, and iron (III) chloride hexahydrate (Merck, Darmstadt Germany) were used during this work. Reference standard of fenoxaprop-p-ethyl herbicide was purchased from Dr Ehrenstorfer GmbH (Augsburg, Germany). Reagent grade acetone (BDH Laboratory suppliers Poole, England) and methanol (Merck, Darmstadt Germany) were used for extraction of herbicide from grain samples. Commercial formulation containing (6.9 %) fenoxaprop-p-ethyl (FE) was purchased from the local market. All solutions used were stable at room temperature.

Solutions preparation

Standard fenoxaprop-p-ethyl (FPE) solution: A standard stock solution of fenoxaprop-p-ethyl (FPE) (350 $\mu g\ mL^{-1}$) was prepared by dissolving 0.035 g of the authentic sample in 20 mL methanol and diluted up to 100 mL with methanol. Working standard solutions were prepared by further dilution.

Hydroxylamine hydrochloride solution: Hydroxylamine hydrochloride stock solution (0.5 M) was prepared by dissolving 3.475 g of the reagent in 30 mL methanol and diluted to 100 mL with methanol. Solutions of different concentrations were prepared fresh by dilution when required.

Sodium hydroxide and hydrochloric acid solutions: Sodium hydroxide (6.0 M) and hydrochloric acid (1.0 M) solutions were prepared by dissolving appropriate amounts in distilled water and diluted up to 100 mL with distilled water.

Fe (III) chloride hexahydrate solution: Fe (III) chloride solution (5.0 %) was prepared by dissolving 5.0 g in 30 mL distilled water and diluted up to 100 mL with distilled water.

Procedure

For batch analysis, 1.0 mL was taken, from a standard stock solution of FPE (350 µg mL^{-1}), in a 100 mL conical flask followed by the addition of 1.0 mL of 0.1 M hydroxylamine hydrochloride solution. To this mixture, 0.3 mL of sodium hydroxide (6.0 M), was added and equilibrated for 5.0 min for completion of reaction. The medium was acidified by adding 2.0 mL of hydrochloric acid (1.0 M) followed by the addition of 2.0 mL ethanol. Then 0.3 mL of ferric chloride solution (5.0 %) was added. The resulting solutions of the magenta coloured complex was transferred to 10 mL volumetric flasks and diluted up to the mark with ethanol. Absorbance of the solution was measured against a reagent blank at different wavelengths. The results are shown in figure 3.3.3.

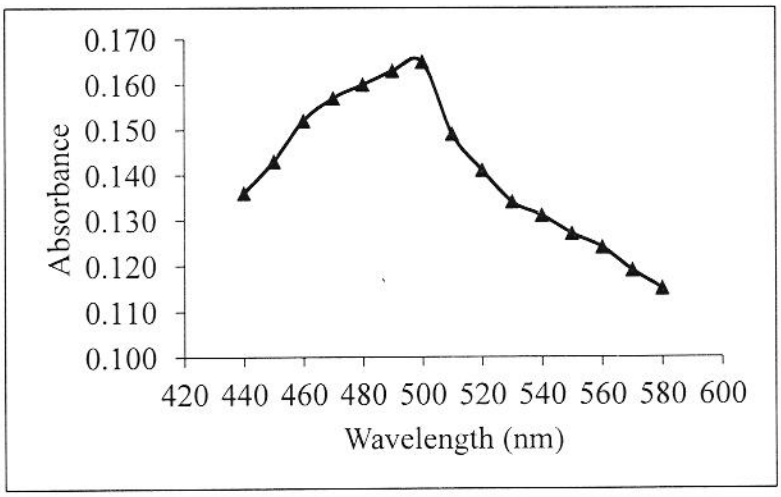


Figure 3.3.3. Wavelength optimization for the spectrophotometric determination of fenoxaprop-p-ethyl

3.3.3.2. Investigation of the effect of various parameters on the complex formation of fenoxaprop-p-ethyl in batch analysis

Instrument, Reagents and Solutions: The same as mentioned before

Procedure

Seven different 100 mL conical flasks were taken and to each flask 1.0 mL of fenoxaprop-p-ethyl stock solution (350 µg mL^{-1}) was added. Then 1.0 mL of different concentrations of hydroxylamine hydrochloride solutions in the range of 0.025 - 0.30 M

final concentration was added. To each flask 0.2 mL of sodium hydroxide (4.0 M) was added and the flasks were kept for five minutes for maximum derivatization. After five minutes to each flask, 1.0 mL of hydrochloric acid solution (2.0 M) was added followed by the addition of a small drop of Fe (III) solution (5.0 %). Each solution was then transferred to a 10 mL volumetric flask and diluted up to the mark with ethanol. Absorbance of the complex formed was measured at 500 nm against a reagent blank. The results are shown in figure 3.3.4. For volume optimization of hydroxylamine hydrochloride (0.5 M), the volume was varied in the range of 0.25-5.0 mL while keeping all other conditions the same. Absorbance was measured against a reagent blank and the results are shown in figure 3.3.5.

For optimization of the effect of concentration of sodium hydroxide solution on the derivatization reaction, 0.2 mL of sodium hydroxide solution was added from stock solutions of different concentrations to each flask so as to have final concentration of sodium hydroxide in the range of 1.0-8.0 M while keeping rest of the procedure the same. Absorbance of each solution was measured against a reagent blank at 500 nm. The results are given in table 3.3.1 and are shown in figure 3.3.6. For the effect of volume of sodium hydroxide, the whole procedure was kept the same except varying volume in the range of 0.1-0.5 mL. The results are shown in figure 3.3.7.

The effect of concentration of hydrochloric acid on the Fe-hydroxamate complex formation was investigated by taking eight separate conical flasks each containing 1.0 mL of FPE (100 μg mL^{-1}). To each flask 1.0 mL of 0.1 molar hydroxylamine hydrochloride followed by 0.25 mL of sodium hydroxide (6.0 M) solutions were added and were kept for five minutes. This was followed by the addition of 1.0 mL of hydrochloric acid from different stock solutions so as to have different final concentrations in the range of 0.6-3.5 M. After the addition of a drop of Fe (III) solution (5.0 %) the magenta coloured complex formed was transferred to a 10 mL volumetric flask and diluted up to the mark with ethanol. The absorbance was measured against a reagent blank and the results are given in table 3.3.2 and shown in figure 3.3.8. The volume of hydrochloric acid (1.0 M) was optimized by using 0.5-4.0 mL of acid keeping

rest of the procedure the same. The results are given in table 3.3.3 and are shown in figure 3.3.9.

For investigating the kinetics of the hydroxamate derivative formation, the optimized conditions were applied and the reaction progress was followed at a smaller final concentration of 6.0 μg mL^{-1}. The absorbance of the complex formed was measured at different time intervals given to the first step of the reaction, varying from 2-30 min. The results are shown in figure 3.3.10.

The stability of the Fe-hydroxamate complex was investigated by measuring absorbance of the complex formed at different time intervals in the range of 2-60 min. The results are shown in figure 3.3.11.

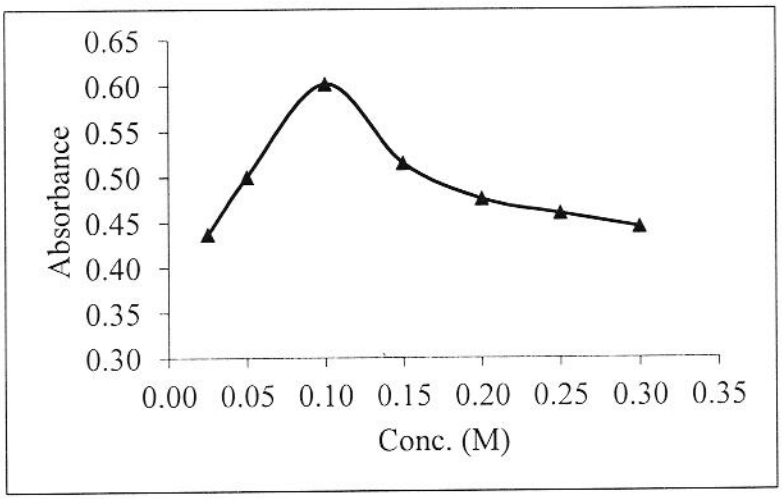


Figure 3.3.4. Effect of concentration of hydroxylamine hydrochloride on hydroxamate formation

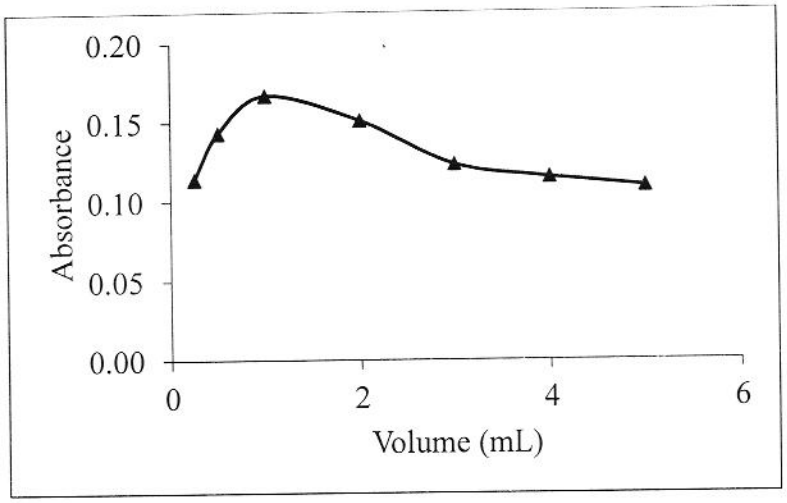


Figure 3.3.5. Effect of volume of hydroxylamine hydrochloride on hydroxamate formation

Table 3.3.1. Effect of concentration of sodium hydroxide on hydroxamate formation

Conc. (M)	Absorbance
1	No Reaction
2	No Reaction
3	No Reaction
4	0.255
5	0.300
6	0.359
7	0.353
8	0.330

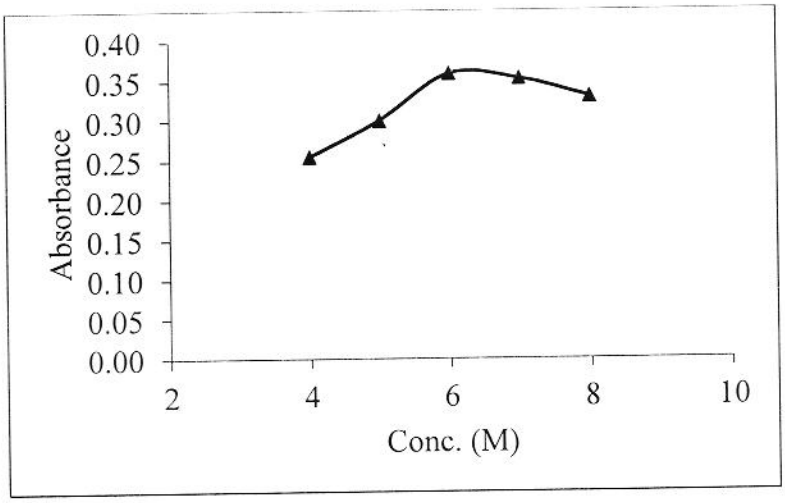


Figure 3.3.6. Effect of concentration of sodium hydroxide on hydroxamate formation

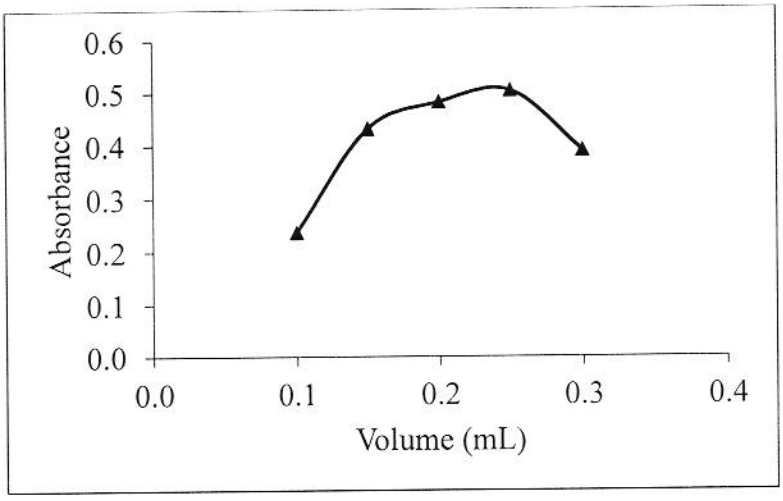


Figure 3.3.7. Effect of volume of sodium hydroxide on hydroxamate formation

Table 3.3.2. Effect of concentration of hydrochloric acid on the Fe-hydroxamate complex formation

Conc. (M)	Absorbance
0.6	Precipitate
0.8	0.471
1.0	0.688
1.5	0.63
2.0	0.434
2.5	0.332
3.0	0.288
3.5	0.148

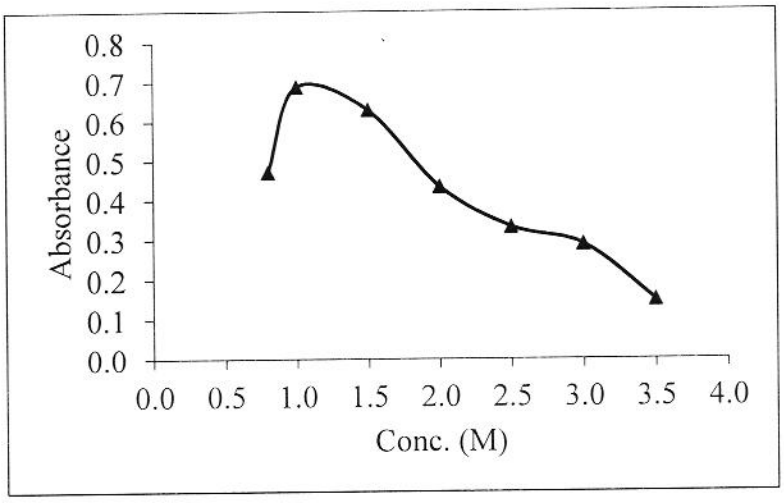


Figure 3.3.8. Effect of concentration of hydrochloric acid on the Fe-hydroxamate complex formation

Table 3.3.3. Effect of volume of hydrochloric acid on the Fe-hydroxamate complex formation

Volume (mL)	Absorbance
0.5	Precipitate
1.0	Precipitate
1.5	Precipitate
2.0	0.151
2.5	0.139
3.0	0.132
3.5	0.115
4.0	0.092

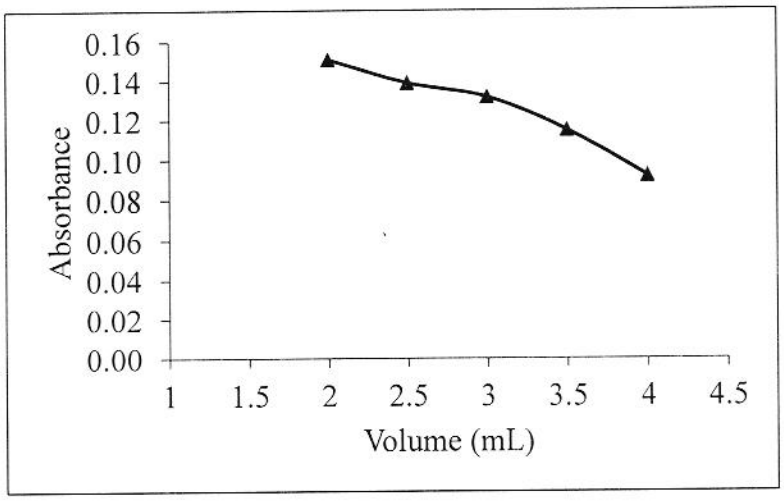


Figure 3.3.9. Effect of volume of hydrochloric acid on the Fe-hydroxamate complex formation

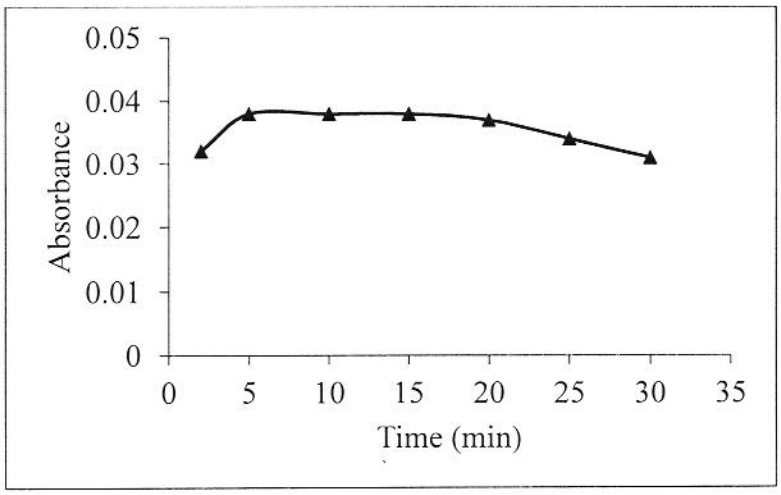


Figure 3.3.10. Effect of time on the hydroxamate derivative formation (Step-1)

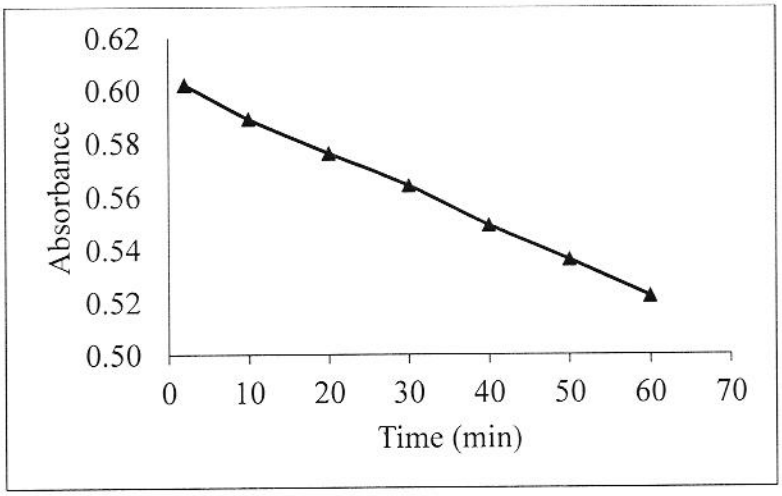


Figure 3.3.11. Effect of time on the stability of the Fe-hydroxamate complex (step-II)

3.3.4. Investigation of the empirical formula and formation constant (K_f) of the Fe-hydroxamate complex by Job's method of continuous variation

Instruments, reagents and solutions: The same as mentioned before

Procedure

Different volumes of fenoxaprop-p-ethyl (8.52×10^{-4} M) were taken in eight separate conical flasks followed by the addition of 1.0 mL of hydroxylamine hydrochloride (0.1 M) solution to each flask. Then 0.25 mL of sodium hydroxide solution (6.0 M) was added followed by the addition of 2.0 mL of HCl (1.0 M). After five minutes different volumes

of Fe (III) solution (8.52×10^{-4} M) were added to the mixtures in such a way that the total volume and total moles of FPE and Fe (III) in each mixture remained constant but the mole ratio of these two varied systematically. Each solution was transferred to a 10 mL flask and diluted up to the mark with ethanol. The absorbance in each case was measured at 500 nm against a reagent blank. The absorbance was plotted against the volume ratio of Fe (III) and FPE. The results are shown in figure 3.3.12. The empirical formula or the combining ratio of the complex formed was determined to be ML_3 from the theoretical maximum on the curve. The formation constant K_f for the complex was evaluated from measurement of the deviations from the theoretical straight lines and the calculations are given below.

From the extrapolated absorbance:

M (mL) = 0.75

L (mL) = 2.25

M/L = 0.33

M: L = 1:3

Therefore the empirical formula of the complex is ML_3

$$M + 3L \longrightarrow ML_3$$

Total Metal Conc. (C_M)

$C_1V_1 = C_2V_2$

$8.52 \times 10^{-4} \times 0.75 = X \times 10$

$X = C_M = 6.39 \times 10^{-5}$ M

Total Ligand Conc. (C_L)

$C_1V_1 = C_2V_2$

$8.52 \times 10^{-4} \times 2.25 = X \times 10$

$X = C_L = 1.92 \times 10^{-4}$ M

Conc. of the complex [ML_3]

$[ML_3] = (A_{actual}/A_{extrapolated}) \times C_M$

$[ML_3] = (0.050/0.052) \times 6.39 \times 10^{-5}$

$[ML_3] = 6.144 \times 10^{-5}$M

Free Metal [M]

$[M] = C_M - [ML_3]$

$[M] = 6.39 \times 10^{-5} - 6.134 \times 10^{-5}$

$[M] = 2.56 \times 10^{-6} M$

Free Ligand [L]

$[L] = C_L - [ML_3]$

$[L] = 1.92 \times 10^{-4} - 6.134 \times 10^{-5}$

$[L] = 1.356 \times 10^{-4}$

$K_f = [ML_3] / [M] [L]^3$

$K_f = 6.134 \times 10^{-5} / 2.56 \times 10^{-6} \times (1.356 \times 10^{-4})^3$

$K_f = 9.74 \times 10^{12}$

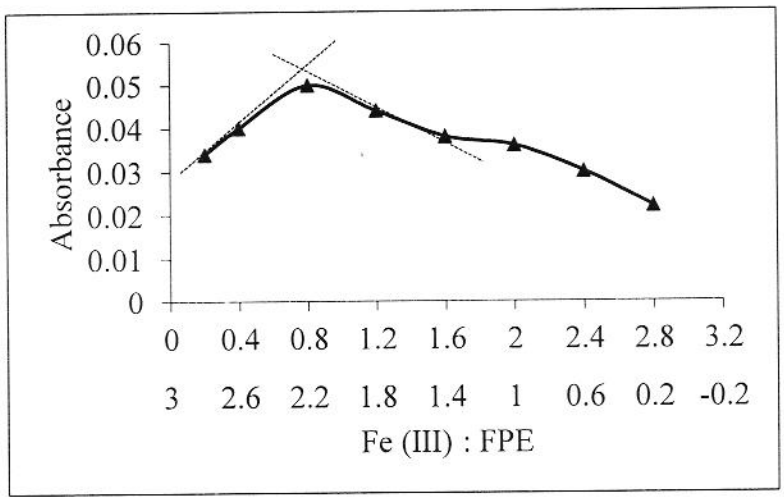


Figure 3.3.12. Investigation of the empirical formula and formation constant (K_f) of the Fe-hydroxamate complex by Job's method of continuous variation

3.3.5. Investigation of the effect of concentration of fenoxaprop-p-ethyl on the absorbance of the complex formed using batch analysis

Instruments, Reagents and Solutions: The same as mentioned before

Procedure

Different volumes of fenoxaprop-p-ethyl (100 µg mL^{-1}) corresponding to final concentration in the range of 2.0 – 50.0 µg mL^{-1} were taken in seven different conical flasks. To each flask optimum volume (1 mL) of hydroxylamine hydrochloride solution (0.1 M) was added followed by the addition of optimum volume (0.25 mL) of sodium

hydroxide (6.0 M). The flasks were kept for five minutes and then 2.0 mL of hydrochloric acid (1.0 M) and 0.1 mL of Fe (III) (5.0 %) solutions were added consecutively to each flask. Each solution was transferred to a 10 mL volumetric flask and diluted with ethanol upto the mark. Absorbance of the magenta coloured complex was measured at 500 nm against a reagent blank being prepared by the same procedure without the addition of FPE. The results are given in tables 3.3.4 and are shown in figure 3.3.13. For checking the linearity and finding out the limit of detection and limit of quantification of the method, the effect of concentration on absorbance behaviour was studied at lower concentration. A calibration plot of concentration versus absorbance was constructed and is shown in figure 3.3.14.

Table 3.3.4. Investigation of the effect of concentration of fenoxaprop-p-ethyl on the absorbance of the complex formed-Batch analysis

Concentration ($\mu g\ mL^{-1}$)	**Absorbance**
2	0.009
5	0.025
10	0.048
20	0.081
30	0.108
40	0.13
50	0.167

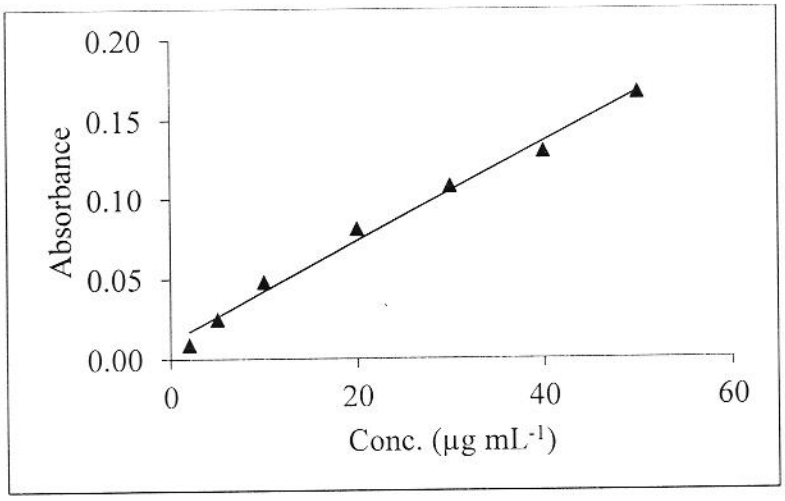


Figure 3.3.13. Investigation of the effect of concentration of fenoxaprop-p-ethyl on the absorbance of the complex formed-Batch analysis

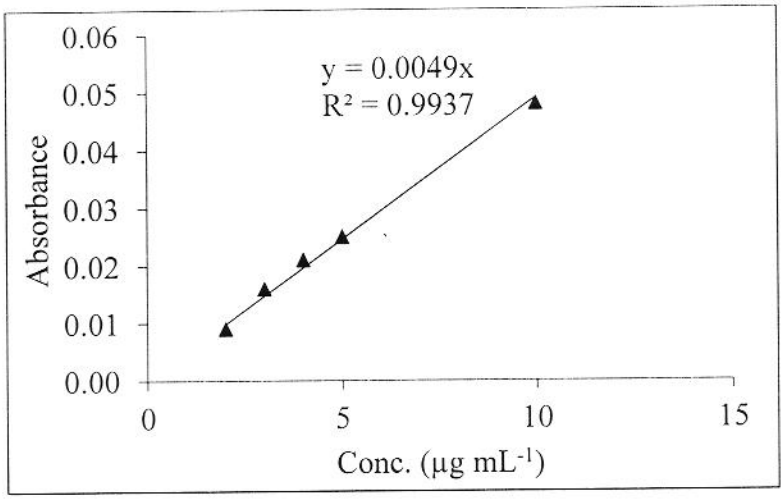


Figure 3.3.14. Calibration curve at lower concentration for spectrophotometric determination of fenoxaprop-p-ethyl-Batch analysis

3.3.6. Calculation of analytical characteristics for spectrophotometric determination of fenoxaprop-p-ethyl using batch analysis

The molar absorptivity (ε) was calculated based on the formula given below:

Molar Absorptivity (ε) = A/C × L

Where 'A' is the absorbance of the smallest concentration detected by the proposed method, 'C' is the Molar concentration and 'L' is the path length (cm) of the cell.

Limit of detection (LOD) and limit of quantification (LOQ) were evaluated by batch analysis for six replicates of the lowest quantifiable concentration that can be measured with acceptable precision and accuracy (2.0 μg mL^{-1}) by applying the following formulae.

LOD = 3S/b (For concentration)

LOQ = 10S/b (For concentration)

Where 'S' is the standard deviation and 'b' is the slope of the standard curve

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

RSD = $(S/x^{/}) \times 100$ where $x^{/}$ is the mean concentration determined.

Analytical characteristics of the method were calculated and are given in table 3.3.5.

Table 3.3.5. Analytical characteristics of the investigated batch method

Parameter	**Value**
λ_{max} nm	500
Molar absorptivity (ε) L mol^{-1} cm^{-1}	1.85×10^3
Standard deviation (S) μg mL^{-1}	0.149
Relative standard deviation (RSD) %	8.14
Limit of detection (LOD) μg mL^{-1}	0.244
Limit of quantification (LOQ) μg mL^{-1}	2.72
Correlation Coefficient	0.9937
Slope	0.0049
Intercept	0.00
Stability constant (K_f)	9.74×10^{12}

3.3.7. FLOW INJECTION SPECTROPHOTOMETRIC DETERMINATION OF FENOXAPROP-p-ETHYL

For flow injection analysis, a three channel FIA system was arranged as shown in figure 3.3.15. The chemical parameters optimized for batch analysis of fenoxaprop-p-ethyl with slight unavoidable modifications were applied in the flow system at room temperature. In the FI manifolds in reagent stream F-1 a mixture of hydroxylamine hydrochloride (0.1 M) and sodium hydroxide (4.0 M) in a ratio of 4:1 was pumped. Sample solution of fenoxaprop-p-ethyl was injected into the F-1 stream through the injection valve in an injection loop. The hydroxamate salt thus formed was then acidified with 1:1 mixture of HCl (3.0 M) and ethanol flowing down in the reagent stream F-2. Finally complexation of the acidic hydroxamate salt solution with Fe (III) (0.5 %) occurred in the stream of $FeCl_3$ solution flowing in reagent stream F-3. The magenta coloured complex formed was passed through a flow through cell and the absorbance was continuously measured at λ_{max} 500 nm.

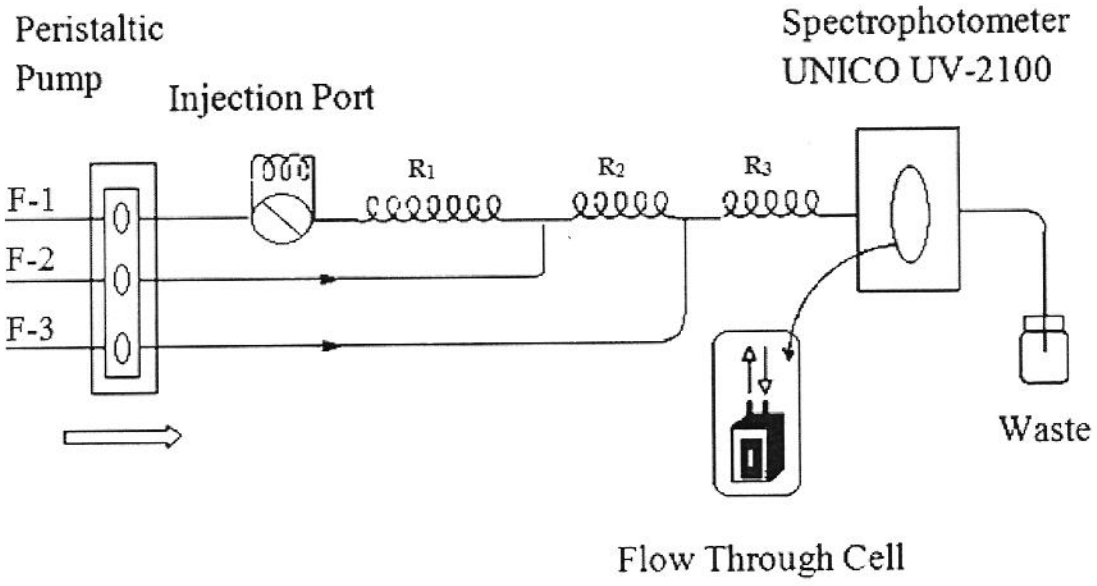


Figure 3.3.15. Scheme of manifold flow injection system for flow injection spectrophotometric determination of fenoxaprop-p-ethyl

F-1; Reagent-1($NH_2OH.HCl$ + NaOH), F-2; Reagent-2 (HCl + C_2H_5OH), F-3; Reagent-3 ($FeCl_3$), R_1; Reaction coil-1(Hydroxamate salt formation), R_2; Reaction coil-2 (Acidification), R_3; Reaction coil-3 (Complex formation)

3.3.7.1. Investigation of the effect of physical parameters on the FI spectrophotometric determination of fenoxaprop-p-ethyl (FPE)

Instruments, reagents and solutions: The same as mentioned before

Procedure

In the flow injection assembly with the same length (40 cm) of reaction coils (R_1, R_2, and R_3), 1:4 mixture of sodium hydroxide (4.0 M) and hydroxylamine hydrochloride (0.1 M) was pumped in the reagents stream F-1 and 1.0 mL of FPE solution (100 μg mL^{-1}) was introduced through the injection port with the help of a 5.0 cm^3 syringe. The derivatized product formed in reaction coil R_1 was allowed to mix with F-2 reagent stream which is a 1:1 mixture of HCl (3.0 M) and ethanol (98 %). The product was acidified in reaction coil R_2. Finally the acidified hydroxamate was reacted with Fe (III) solution (0.5 %) flowing in F-3 reagent stream and complex formation completed in R_3 reaction coil. Blank was adjusted by continuously pumping the reagent solutions in the assembly and zero adjustment via the software of the detector on the computer. Absorbance of the product formed was continuously measured at 500 nm, while passing through a flow through cell to the waste chamber being placed outside the spectrophotometer. The effect of flow rate was investigated in the range of 1.0-5.0 mL min^{-1} by adjusting the flow rate on the peristaltic pump. The data was displayed and recorded through a computer interface program and is given in table 3.3.6 and shown in figure 3.3.16.

The effect of sample injection volume on the absorbance of the product was investigated by injecting different volumes of FPE (20.0 μg mL^{-1}) through the injection valve into the sample loop in the range of 100-600 μL. All the remaining physical and chemical parameters were kept the same as discussed in the above paragraph. The results are shown in figure 3.3.17.

The effects of length of the reaction coils were studied at the optimum flow rate of 3.0 mL min^{-1} and optimum sample injection volume of 400 μL while keeping all the

chemical variables constant. The length of each reaction coil [R_1, R_2 and R_3] was varied one by one in the range of 15-95 cm and absorption spectrum for each run was recorded. While varying the length of one reaction coil the length of the other two was kept constant. The results are shown in figure 3.3.18.

Table 3.3.6. Effect of flow rate on the FI spectrophotometric determination of fenoxaprop-p-ethyl

Flow Rate (mL min^{-1})	**Absorbance**
1	0.137
2	0.201
3	1.155
4	0.117
5	0.103

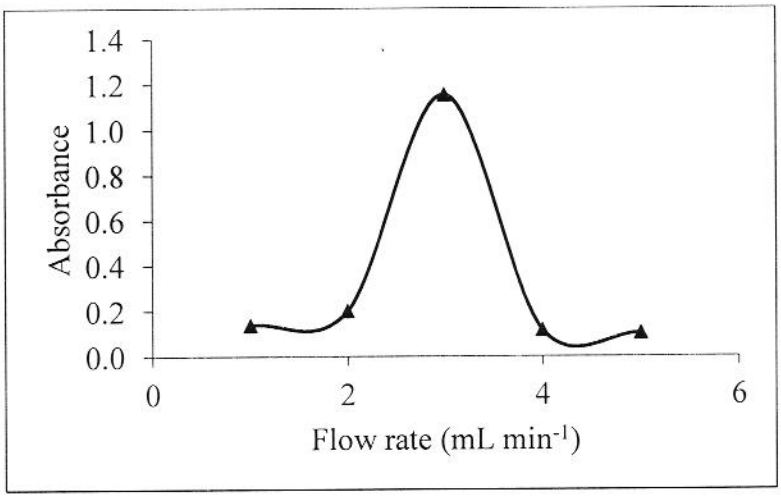


Figure 3.3.16. Effect of flow rate on the FI spectrophotometric determination of fenoxaprop-p-ethyl

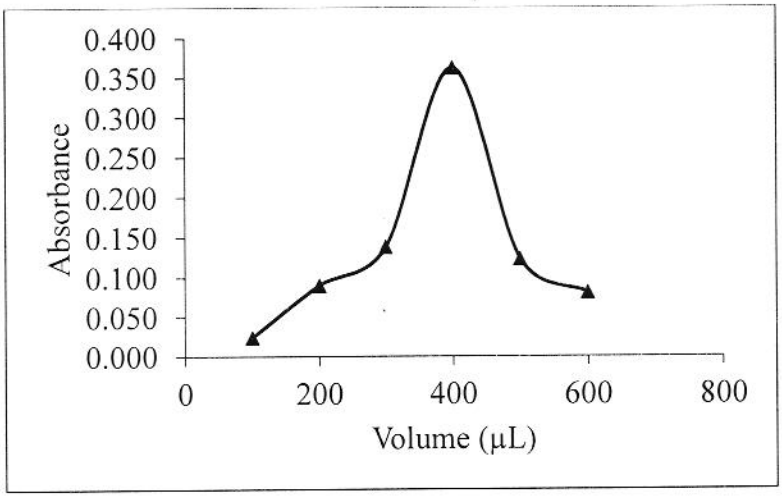


Figure 3.3.17. Effect of sample injection volume on the FI spectrophotometric determination of fenoxaprop-p-ethyl

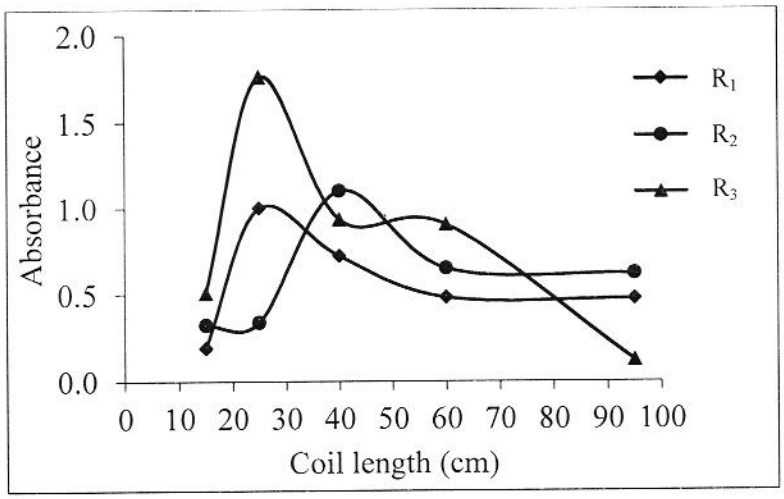


Figure 3.3.18. Effect of reaction coil length on the FI spectrophotometric determination of fenoxaprop-p-ethyl

3.3.7.2. Investigation of the effect of concentration of fenoxaprop-p-ethyl on the FI spectrophotometric determination

Instruments, Reagents and Solutions: The same as mentioned before

Procedure

Standard FPE solutions in the range of 1.0 - 20.0 µg mL^{-1} were prepared in eight separate 10 mL volumetric flasks by taking the required volume of standard from 10 and 100 µg

mL^{-1} stock solutions and diluting with ethanol. In the flow through assembly reagents solutions of the optimized concentration were pumped through the reaction coils of optimum length i.e. R_1 = 25 cm, R_2 = 40 cm, R_3 = 25 cm, with an optimum flow rate of 3.0 mL min^{-1}. The absorbance of the blank was adjusted at zero and then optimum volume (400 µL) of each standard FPE solution was loaded into the sample loop through the injection port with the help of a 5.0 cm^3 syringe. In each case the sample was injected into the stream and the absorbance spectrum recorded. Between two successive measurements the sample loop was flushed with ethanol and absorbance of the blank reagents stream adjusted to zero. With this process a sample throughput of 90 samples h^{-1} was achieved. The results are given in table 3.3.7 and shown in figure 3.3.19. A calibration plot of absorbance vs. concentration (Figure 3.3.20) was constructed at low concentration range for application of the method to real samples.

Table 3.3.7. Investigation of the effect of concentration of fenoxaprop-p-ethyl on the FI spectrophotometric determination

Conc. (µg mL^{-1})	**Absorbance**
1	0.036
2	0.053
3	0.071
5	0.108
8	0.145
10	0.177
15	0.222
20	0.279

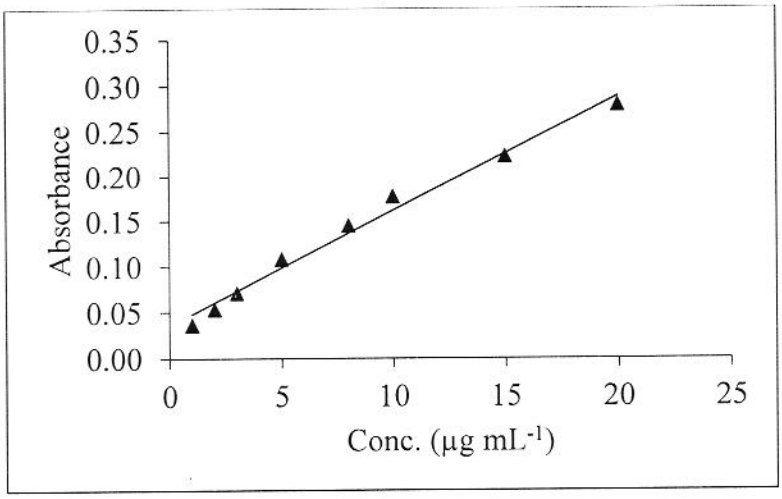


Figure 3.3.19. Investigation of the effect of concentration of fenoxaprop-p-ethyl on the FI spectrophotometric determination

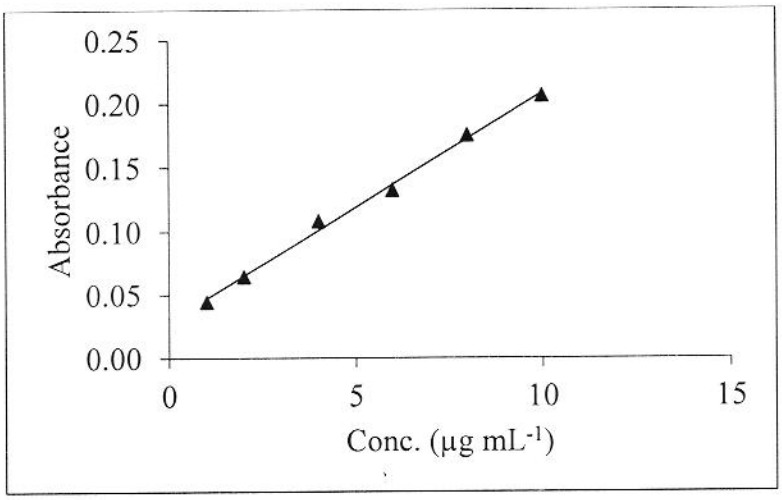


Figure 3.3.20. Calibration curve for FI spectrophotometric determination of fenoxaprop-p-ethyl

3.3.7.3. Calculation of analytical characteristics for the FI spectrophotometric determination of fenoxaprop-p-ethyl

Different analytical characteristics for FI analysis were calculated based on the formulae given earlier in section 3.3.6. Limit of detection (LOD) and limit of quantification (LOQ) were evaluated by FI analysis for six replicates of the lowest quantifiable concentration that can be measured with acceptable precision and accuracy (1.0 µg mL^{-1}). At optimum

conditions various analytical features of the method were calculated and are given in table 3.3.8.

Table 3.3.8. Analytical characteristics of the investigated FI method

Parameter	**Value**
λ_{max} nm	500
Molar absorptivity (ε) L mol^{-1} cm^{-1}	1.81×10^4
Standard deviation (S) μg mL^{-1}	0.096
Relative standard deviation (RSD) %	10.7
Limit of detection (LOD) μg mL^{-1}	0.31
Limit of quantification (LOQ) μg mL^{-1}	1.03
Correlation coefficient	0.9952
Slope	0.0179
Intercept	0.029

3.3.8. Investigation of percent recovery of fenoxaprop-p-ethyl from spiked samples by the proposed method - Batch and FI analysis

Instruments, Reagents and Solutions: The same as mentioned before

Collection of samples: Samples of wheat and barley grains were collected from crop field where no spray of the herbicide was done.

Sample extraction procedure

Control samples (10 g) of wheat and barley each were separately taken in bottles and known concentration of fenoxaprop-p-ethyl solution was added to adjust the concentration level of 5, 10, 15 μg g^{-1}. The samples were homogenized by mechanical shaking for uniform mixing and kept for 24 hours at ambient temperature. The homogenized samples were extracted for 60 min with 25 mL of each, methanol and acetone. The extraction was done in triplicates.

Analysis procedure

The percent recoveries of FPE from the samples, each in triplicate, were evaluated by the proposed method, using both batch and flow injection analysis.

For batch analysis 2.0 mL of each extract was taken in a conical flask and optimized volumes of all the reagents mentioned before were successively added and absorbance of the final product was measured at 500 nm against a reagent blank.

In case of FI analysis, the extract being further concentrated by air drying at room temperature to 1.0 mL was used in each case. Optimum volume (0.4 mL) of each extract was loaded into the sample loop through the injection port with the help of a micro syringe. The sample was then injected to the reagents stream in which optimum concentration of the reagents was flowing at an optimum flow rate. The absorbance of the product was measured at 500 nm by the method mentioned before. For each sample absorbance readings were taken in triplicate. A comparison of percent recovery investigated by both batch and FI procedure is given in table 3.3.9.

Table 3.3.9. Investigation of percent recovery of fenoxaprop-p-ethyl from spiked samples by the proposed batch and FI methods

Sample	Eluent	Added ($\mu g\ g^{-1}$)	Found ($\mu g\ g^{-1}$)		Average % Recovery ± RSD	
			Batch	FIA	Batch	FIA
Wheat grains	Methanol	5	4.4	4.5	88 ± 3.0	90 ± 1.0
		10	8.5	9.0		
		15	13.7	13.6		
	Acetone	5	4.5	4.5	89 ± 3.0	92 ± 1.0
		10	8.6	9.3		
		15	13.7	14.0		
Barely grains	Methanol	5	4.3	4.4	87 ± 0.9	88 ± 1.7
		10	8.6	9.0		
		15	13.0	13.1		
	Acetone	5	4.4	4.6	88 ± 0.5	92 ± 0.6
		10	8.7	9.2		
		15	13.3	13.8		

3.3.9. Determination of fenoxaprop-p-ethyl residue in real samples by the proposed batch and FI methods

Instruments, Reagents and Solutions: The same as mentioned before

Samples: Barley grains samples were collected from a crop field upon which the subject herbicide was sprayed as a pilot project.

Procedure

The fenoxaprop-p-ethyl residue in barely grains sample was determined by standard addition method. For this six samples of barely grains (10 g each) were taken in separate bottles and spiked with a known concentration of standard fenoxaprop-p-ethyl solution. These bottles were uniformly shaken manually for proper mixing and kept for 24 h. The herbicide was extracted with 25 mL of solvent (acetone and methanol). The extracts were evaporated at room temperature and the residue was redissolved in 25 mL methanol. The reconstituted extract was analysed by the proposed batch and FI method following the same procedure as mentioned before. The residue of herbicide present in the sample was calculated from the difference of the concentration of added standard solution and the total concentration of herbicide found in the sample. The results of the residue determination are given in table 3.3.10.

Table 3.3.10. Residue determination of fenoxaprop-p-ethyl in real samples by the proposed batch and FI methods

Method	Conc. added ($\mu g\ g^{-1}$)	Total Conc. Found ($\mu g\ g^{-1}$)	Average Residue ($\mu g\ g^{-1}$)
Batch	7.60	9.20	1.2 ± 0.25
	8.20	8.80	
	8.00	9.40	
FIA	8.25	9.40	1.15 ± 0.15
	8.30	9.60	
	8.25	9.25	

Results and Discussion

The chemical structure of fenoxaprop-p-ethyl (Fig. 3.3.1.) contains a terminal ester group which was converted into hydroxamate salt by reacting it with hydroxylamine hydrochloride in alkaline media. The hydroxamate salt formed was used as a ligand with Fe (III) in acidic media which resulted in a magenta coloured Fe (III) hydroxamate complex. The proposed reaction mechanism involved in the proposed spectrophotometric method for determination of fenoxaprop-p-ethyl is shown in figure 3.3.2. The reaction is fast at room temperature therefore; it was fit for flow injection analysis and hence was used for determination of fenoxaprop-p-ethyl herbicide. The absorption spectrum of the complex formed was scanned at a wavelength range of 440-580 nm (Fig. 3.3.3.). Wavelength of maximum absorbance was found to be 500 nm.

The concentration of hydroxylamine hydrochloride was investigated in the range of 0.025-0.3 M (Fig. 3.3.4.). The absorbance was found to increase with increase in concentration of hydroxylamine hydrochloride solution from 0.025 M to 0.1 M. A small decrease in absorbance was observed at higher concentration. Similarly the volume of hydroxylamine hydrochloride was optimized in the range of 0.25-5.0 mL (Fig. 3.3.5). The absorbance increases with increase in volume of hydroxylamine hydrochloride from 0.25 to 1.0 mL beyond which a small decrease in absorbance was observed. Higher concentration of hydroxylamine hydrochloride has two fold effects. First it causes reduction of Fe (III) to Fe (II) based on the reaction;

$$2\ NH_2OH + 2\ Fe^{3+} \longrightarrow 2\ Fe^{2+} + N_2O + 2\ H^+ + 2H_2O$$

and secondly it increases hydrogen ion concentration which in turn increases acidity. These factors lead to dissociating tendency of the complex [1].

Hydroxylamine hydrochloride reacts with fenoxaprop-p-ethyl in alkaline media and the effect of sodium hydroxide concentration was investigated in the range of 1.0 to 8.0 M solution. Reaction starts with 4.0 M sodium hydroxide solution and maximum absorbance was found at 6.0 M sodium hydroxide solution (Fig. 3.3.6.). The volume of sodium hydroxide was found to have substantial effect on the product formation. Sodium hydroxide volume was varied in the range of 0.1-0.3 mL. It was observed that absorbance

increases from 0.1 to 0.25 mL of sodium hydroxide and a higher volume result in sudden decrease in the absorbance due to greater pH change with slight increase in the volume of the base (Fig. 3.3.7.).

The hydroxamate salt formed from fenoxaprop-p-ethyl herbicide was used as a ligand and reacted with iron (III) for complex formation. Hydroxamate salt reacts with iron (III) in acidic media and hydrochloric acid was used to adjust the acidy of solution. The concentration of hydrochloric acid was varied in the range of 0.6 - 3.5 M solution. The complex formation starts with 0.8 M hydrochloric acid solution and absorbance increased with increasing acid concentration up to 1.0 M (Fig. 3.3.8.). Beyond 1.0 M concentration of acid solution the absorbance decreased, therefore further analysis was performed at 1.0 M solution of hydrochloric acid. The volume of hydrochloric acid was varied in the range of 0.5-4.0 mL. It was observed (Fig. 3.3.9.) that maximum complexation occurs at 2.0 mL of hydrochloric acid. At higher acidity of the media the complex is unstable due to competition of H^+ ion and higher acidity makes the complex unstable and the colour of the solution fade when the pH of the solution become lower than 1.2 (2).

The effect of reaction time for the hydroxamate derivative formation was investigated. It was observed (Fig. 3.3.10.) that reaction is fast and requires only two minutes for completion and beyond this time there is no substantial effect in the absorbance of the complex.

The stability of the Fe (III)-hydroxamate complex was studied from 2.0-60 minutes after formation (Fig. 3.3.11.). It was observed that the absorbance of complex linearly decreases with time. This means that the dissociation of the complex occurs at a constant rate at room temperature. Due to the instability of the complex at room temperature, flow injection analysis system was developed in which absorbance of the complex is measured immediately when it is formed.

The stoichiometric ratio of the hydroxamate salt formed from fenoxaprop-p-ethyl to iron (III) was established by the Job's method of continuous variation employing batch method (Fig. 3.3.12). The composition of the complex was found to be [Fe (III): hydroxamate anion] 1:3, means that three mole of ligand react with one mole of iron (III).

The calculated formation constant of the complex was 9.74×10^{12}, which shows high stability of the complex.

The effect of concentration of fenoxaprop-p-ethyl on the absorbance of complex was investigated (Fig. 3.3.13.). It was found that the method obeys Beer's law over a concentration range of 2-50 μg mL^{-1}. Also a calibration plot (Fig. 3.3.14.) was constructed at lower concentration for application of the method for sample analysis.

For flow injection analysis a simple three fold scheme was assembled (Fig. 3.3.15.). In FIA the effect of various physical variables like flow rate, sample injection volume and reaction coil length were investigated. Flow rate was varied in the range of 1.0-5.0 mL min^{-1}. The absorbance was observed to increase with increase in flow rate upto 3.0 mL min^{-1} above which it decreases due to incomplete reaction. The reaction in FIA is based on dispersion phenomena, therefore with high flow rates little time and space become available for reagents and analytes to react and thus decrease in dispersion of sample with reagents in a flow stream occurs (Fig. 3.3.16). Sample injection volume was studied in the range of 100-600 μL (Fig. 3.3.17). It was observed that the absorbance of the complex increases with increase in injection volume from 100 to 400 μL and then goes on decreasing and beyond 600 μL sample volume the peak splits into two and becomes less intense. This is possibly due to the reaction occurring only at the two ends of the analyte stream and the analyte in the middle of the stream remains unreacted. The effect of reaction coils length R_1, R_2 and R_3 was studied from 15 cm to 95 cm for hydroxamate salt formation, acidification and complex formation respectively, (Fig. 3.3.18). Maximum absorbance was found at 25 cm coil length in case of R_1 which indicates rapid reaction between fenoxaprop-p-ethyl and hydroxylamine hydrochloride. The second reaction coil R_2 was also optimized for changing the media from alkaline to acidic. The acidity of the reagents was found maximum with 25 cm reaction coil length. For complex formation the effect of reaction coil R_3 length was also investigated and maximum absorbance of complex was found with 40 cm. The results indicated that hydroxamic acid and complex formation reactions are fast and reproducible in the FIA system. With this setup a sampling rate of 90 samples h^{-1} was achieved in the FIA system.

In the FI analysis the effect of concentration of FPE on the absorbance behaviour of the complex was studied (Fig. 3.3.19.). The FI method was shown to have linear response in the concentration range of 1.0-20 μg mL^{-1}. A calibration plot was drawn from the same data at low concentration (Fig. 3.3.20.) for the application of the method for sample analysis.

The analytical parameters calculated under optimized conditions both for batch and FI methods are given in table 3.3.5 and 3.3.8 respectively. The applicability of Beer's law for batch and FI system was checked. A linear relation was observed in the concentration range of 2.0-50 μg mL^{-1} and 1.0-20.0 μg mL^{-1} for batch and FI methods respectively. The molar absorptivity of the resulting complex was found to be 1.85×10^3 L mol^{-1} cm^{-1} with batch method and 1.81×10^4 L mol^{-1} cm^{-1} with FIA. The Limit of detection and quantification was calculated at lower concentration that can be measured with acceptable precision and accuracy and was found to be 0.24 ± 0.15, 2.7 ± 0.22 μg mL^{-1}, and 0.3 ± 0.01, 1.03 ± 0.1 μg mL^{-1} for batch and FI methods respectively.

The validity of the proposed FI method was checked using standard addition method. The percent recovery of fenoxaprop-p-ethyl herbicide from wheat and barley samples was checked and the results are given in table 3.3.9. For extraction from wheat and barley grains different solvents like methanol, acetone, dichloromethane and 1:1 mixture of acetone and dichloromethane were used and maximum extraction was achieved with acetone and methanol. The average recoveries from wheat grains with acetone as eluent were 89.0 ± 3.0% and 92.0 ± 1.0% while with methanol it was 88.0 ± 3.0% and 90.0 ± 1.0% for batch and FI methods respectively. Similarly recoveries were found to be 88.0 ± 0.5% and 92.0 ± 0.6% for barley grains with acetone as eluent and 87.0 ± 0.0% and 88.0 ± 1.7% with methanol, using batch and FI methods respectively. The average limit of detection (LOD) and limit of quantification (LOQ) calculated is 0.35 μg g^{-1} and 1.2 μg g^{-1} respectively, for wheat grains and 0.45 μg g^{-1} and 1.5 μg g^{-1} respectively, for barley grains. The proposed method was also applied for the determination of fenoxaprop-p-ethyl residue in barley grains using acetone as extraction solvent and the residue amount found was 1.2 ± 0.25 μg g^{-1} for batch method and 1.15 ± 0.15 μg g^{-1} for FI method.

References

1. G. Aksnes, "The complex between hydroxamic acid and ferric ions and the use of the complex for quantitative determination of hydroxamic acids, acyl derivatives and ferric salts", Acta Chemica Scandinavica, 11 (4), 710-716 (1957).
2. S. Hestrin, "The reaction of acetyl choline and other carboxylic acid derivatives with hydroxylamine and its analytical applications", Journal of Biological Chemistry, 180, 249-261 (1949).

3.4. FLOW INJECTION SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF PINOXADEN HERBICIDE IN ENVIRONMENTAL SAMPLES

The chemical structure of pinoxaden is given below.

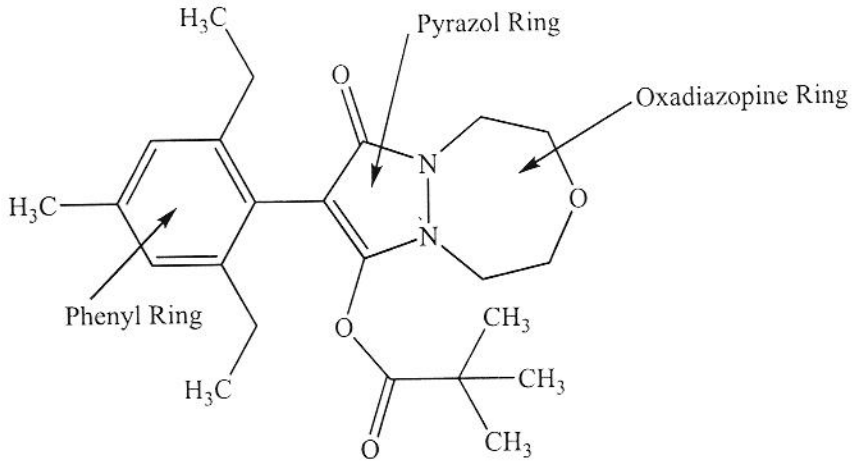
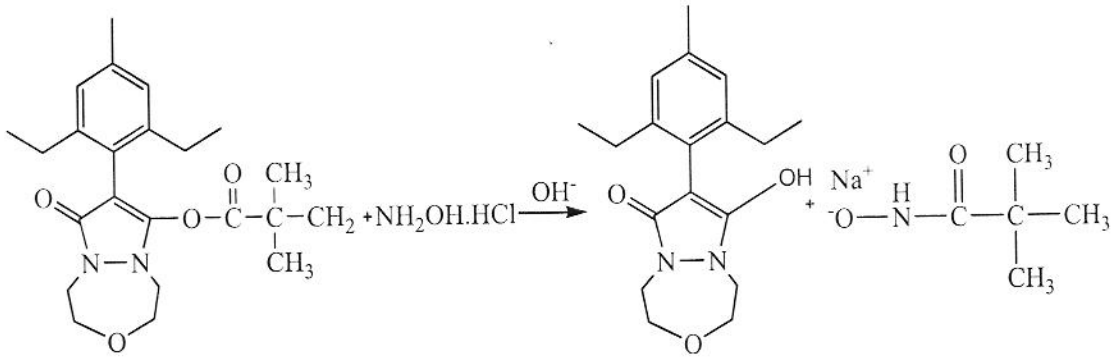


Figure 3.4.1. Structural formula of pinoxaden

3.4.1. Method development strategy for the flow injection spectrophotometric determination of pinoxaden

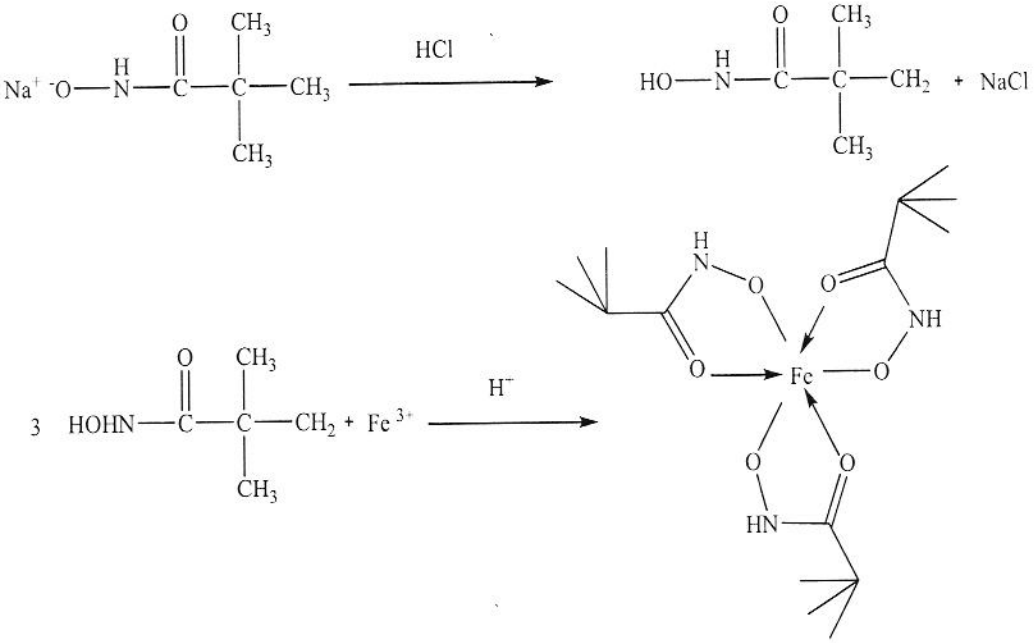
The chemical structure of pinoxaden (PXD) is given in figure 3.4.1. It contains three rings; oxadiazopine, pyrazol and phenyl and an ester side chain. The proposed method is based on the derivatization of the molecule via its ester side chain to a hydroxamate salt by reacting it with hydroxylamine hydrochloride in alkaline medium, accompanied by the release of an alcohol moiety of the rest of the molecule. The hydroxamate salt can act as a ligand of choice for complexation with Fe (III) in acidic medium. The absorbance and hence concentration of the resultant red coloured Fe (III) hydroxamate complex can be used for quantification of pinoxaden. The reaction mechanism is given in figure 3.4.2. The reaction is fast at room temperature and could be carried out in the flow injection system for determination of pinoxaden herbicide.

Step.1. Hydroxamate derivative formation from pinoxaden



(Pinoxaden)

Step 2. Complex formation of Hydroxamate ligand with Fe(III)



Magenta coloured Complex

Figure 3.4.2. Proposed reaction mechanism for the flow injection spectrophotometric determination of pinoxaden

3.4.2. Preliminary investigation of the derivatization and complexation reaction of PXD with Fe (III) for its spectrophotometric determination through batch analysis

Standard pinoxaden (100 μg mL^{-1}) solution (2.0 mL) was taken in a conical flask containing a mixture of 1.0 mL of hydroxylamine hydrochloride (0.5 M) and 0.3 mL of sodium hydroxide (4.0 M) and equilibrated for five min for completion of reaction. Then a 1:1 mixture of HCl (2.0 M) and ethanol was added to the mixture followed by the addition of two drops of Fe (III) chloride (5.0 %) premixed with 1.0 mL of Triton-X 100 (1.0 %) solution. The formation of red coloured product confirmed the possibility of the proposed reaction and succeeding spectrophotometric determination of pinoxaden by this method. Further studies were focused on the optimization of different physical and chemical parameters of the reaction and the details are given below.

3.4.3. Optimization studies for spectrophotometric determination of pinoxaden using batch analysis

In order to have maximum product formation, the effects of various physical and chemical parameters were investigated for the derivatization and complexation reactions. These optimization studies include wavelength of maximum absorbance, concentration and volume of the reagents like hydroxylamine hydrochloride, sodium hydroxide and hydrochloric acid and the effect of time on the stability of the final complexation product.

3.4.3.1. Investigation of suitable wavelength for the spectrophotometric determination of pinoxaden

Instruments and equipments

UV/Visible Spectrophotometer (UNICO, UV-2100 United Products and Instruments Inc., Dayton, NJ, USA) with a glass cell having an optical path length of 1.0 cm was used for measuring the absorbance of the coloured complex. For pumping the reagents stream in online method, a Pump (Model 124869 MP-1 pump Spectra/chrom spectrum), silicon tube (1.71mm, i.d.), V-450 6-port injection valve (Upchurch Scientific Inc., USA) and PTFE tubing (1.19 mm, i.d., Becton Dickson, USA) were used. A sintered glass crucible

(PYREX® England), metal water jet pump model C501 (Edward High Vacuum Ltd Manor Royal, Crawley, Sussex England), separating funnel (500 mL) and a glass column (PYREX) were employed in the separation process.

Chemicals and Reagents

All reagents used were of analytical grade purity. Hydroxylamine hydrochloride (MP Biomedicals, LLC 1263 South Chillicothe Rd Aurora, Ohio 44202), sodium hydroxide, Conc. hydrochloric acid, ethanol, Fe (III) chloride, Triton–X 100 and silica gel 60 (0.063-0.200) (Merck-64271 Darmstadt Germany) were used during this work. Reagent grade methanol, n-hexane (Merck-64271 Darmstadt Germany) and dichloromethane (Riedel-de Haen, Sigma-Aldrich Laborchemikalien GmbH. D-30926 Seelze Germany) were used for extraction of Pinoxaden from water, soil and cereals samples. AXIAL® commercial formulation containing (9.0 % w/v) pinoxaden as active ingredient was purchased from the local market imported from Syngenta Crop Protection Incorporated Ohawa NE 68107 USA by Syngenta Pakistan Ltd. All the solutions used were stable at room temperature.

Solutions Preparation

Standard pinoxaden (PXD) solution: In order to remove the safener cloquintocet-mexyl from the active ingredient, a milky suspension of AXIAL (1.1 mL) in distilled water (500 mL) containing 0.1 g pinoxaden was filtered through sintered glass crucible fitted with metal water jet pump. The colourless crystals of cloquintocet-mexyl remained on the filtering media. Pinoxaden from the filtrate was obtained through solvent extraction with dichloromethane (50 mL). The solvent was evaporated and standard stock solution of pinoxaden (PXD) (1000 $\mu g\ mL^{-1}$) was prepared by dissolving the residue in 100 mL methanol. Working solutions were prepared by further dilution of the stock solution.

Hydroxylamine hydrochloride solution: Hydroxylamine hydrochloride stock solution (0.1 M) was prepared by dissolving 0.69 g of the reagent in 30 mL ethanol and diluted upto 100 mL with ethanol.

Sodium hydroxide and hydrochloric acid solutions: Sodium hydroxide (6.0 M) and hydrochloric acid (1.0 M) were prepared by dissolving appropriate amounts in distilled water and diluting upto 100 mL with distilled water.

Fe (III) chloride solution: Ferric chloride solution (5.0 %) was prepared by dissolving 5.0 g in 30 mL distilled water and diluted upto 100 mL.

Triton X-100 solution: 1.0 % solution of Triton X-100 was made by dissolving 1.0 mL of the reagent in 30 mL distilled water and diluting the solution upto 100 mL with distilled water.

Procedure

For batch analysis, 1.0 mL of stock solution of PXD (100 $\mu g\ mL^{-1}$) was taken in a conical flask. To this 1.0 mL of hydroxylamine hydrochloride (0.1 M) and 0.25 mL of sodium hydroxide (6.0 M) was added and equilibrated for five min for completion of reaction, followed by the addition of 1:1 mixture HCl (1.0 M) and ethanol. Then two drops of Fe (III) chloride (5.0 %) premixed with 1.0 mL of Triton-X 100 (1.0 %) solution was added to the mixture. The resulting red coloured solution was transferred to a 10 mL volumetric flask and diluted upto 10 mL with ethanol. The absorbance of the solution was measured against a reagent blank at different wavelengths using SP 300 UV/VIS spectrophotometer. The results are shown in figure 3.4.3.

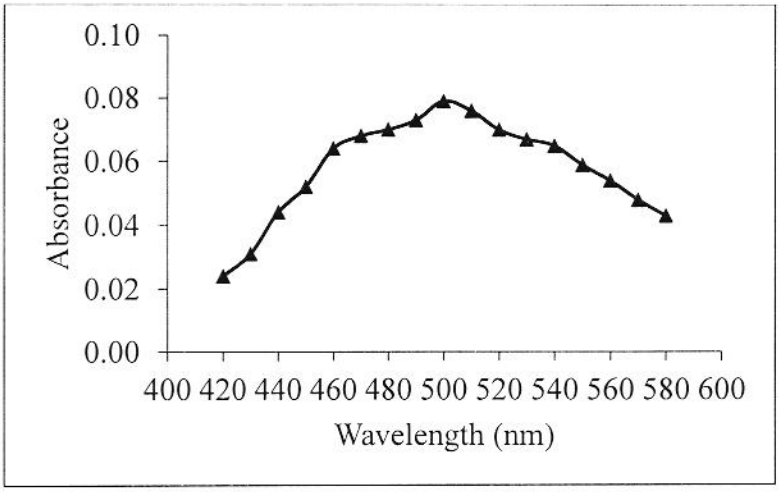


Figure 3.4.3. Wavelength optimization for the spectrophotometric determination of pinoxaden

3.4.3.2. Investigation of the effect of various parameters on the complex formation of pinoxaden in batch analysis

Instrument, Reagents and Solutions: The same as mentioned before

Procedure

To six separate 100 mL conical flasks, each containing 1.0 mL of pinoxaden (100 µg mL^{-1}), a mixture of 1.0 mL of different solution of hydroxylamine hydrochloride in concentration range of 0.025-0.80 M and 0.25 mL of sodium hydroxide (6.0 M) was added. After five minutes a 1:1 mixture HCl (1.0 M) and ethanol and then two drops of Fe (III) chloride (5.0 %) premixed with 1.0 mL of Triton-X 100 (1.0 %) solution was added to the mixtures. Each solution was then transferred to a 10 mL volumetric flask and diluted upto the mark with ethanol. The absorbance of the complex formed was measured at 500 nm against a reagent blank. The results are shown in figure 3.4.4. The effect of volume of hydroxylamine hydrochloride on the hydroxamate salt formation was studied varying the volume of hydroxylamine hydrochloride solution (0.1 M) in the range of 0.25-2.5 mL while keeping all other conditions and procedures the same. The absorbance was measured against a reagent blank and the results are shown in figure 3.4.5.

Effect of the concentration of sodium hydroxide was studied by adding constant-volume (0.25 mL) of sodium hydroxide solutions from varied concentrations of in the range of 4.0 - 8.0 M, while keeping the rest of the procedure the same. The absorbance of each solution was measured against a reagent blank at 500 nm. The results are shown in figure 3.4.6. The effect of volume of sodium hydroxide solution was studied by varying volume in the range of 0.1-0.5 mL with all the remaining steps and conditions the same and in the same order. The results are shown in figure 3.4.7.

In order to study the effect of concentration of hydrochloric acid on the pinoxaden based Fe-hydroxamate complex, seven separate conical flasks each containing 1.0 mL of pinoxaden (100 μg mL^{-1}) stock solution were taken. To each flask 1.0 mL of 0.1 M hydroxylamine hydrochloride and then 0.25 mL of sodium hydroxide (6.0 M) solutions were added and kept for five minutes. After equilibration, 1.0 mL of hydrochloric acid from different stock solutions was added to each flask, so as to have different final concentrations in the range of 0.5-3.0 M. Finally a drop of Fe (III) solution (5.0 %) premixed with Triton X-100 was added to each flask and red coloured complex formed was transferred to a 10 mL volumetric flask and diluted upto the mark with ethanol. Absorbance was measured against a reagent blank and the results are shown in figure 3.4.8. Similarly the effect of volume of hydrochloric acid (1.0 M) was investigated by varying the acid volume in the range of 1.4-3.5 mL keeping all the remaining conditions the same. The results are shown in figure 3.4.9.

To study the effect of time on the stability of the final product, under the optimized conditions the absorbance of the complex formed was measured at different time intervals in the range of 2.0-30.0 min. The results are shown in figure 3.4.10.

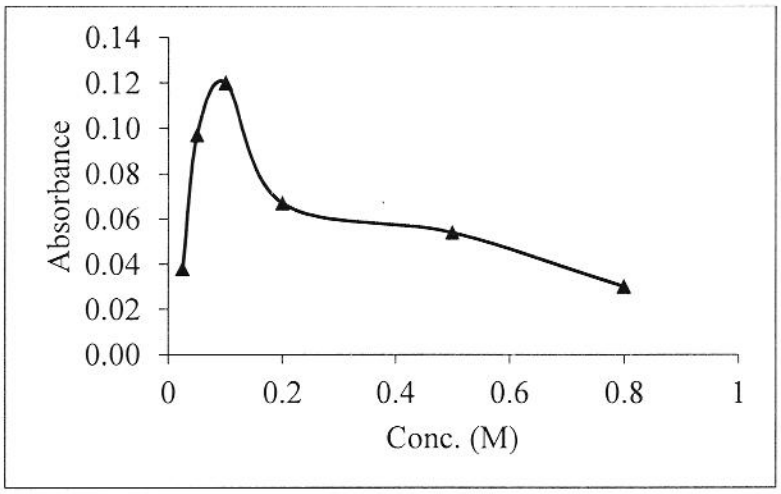


Figure 3.4.4. Effect of hydroxylamine hydrochloride concentration on hydroxamate salt formation

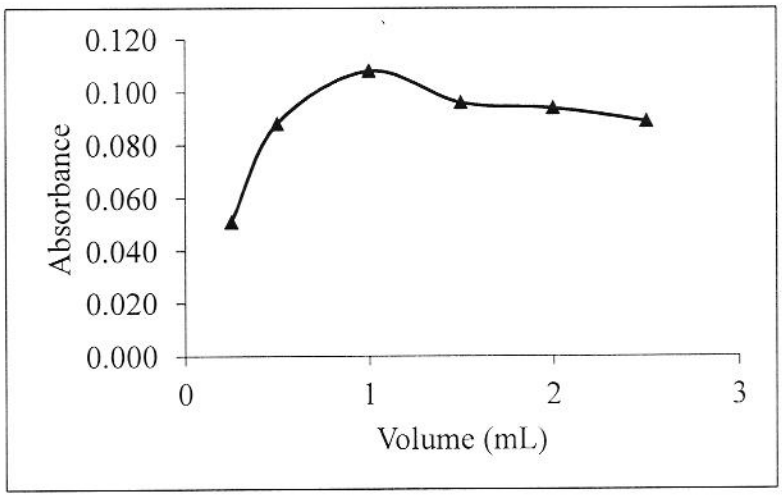


Figure 3.4.5. Effect of volume of hydroxylamine hydrochloride on hydroxamate salt formation

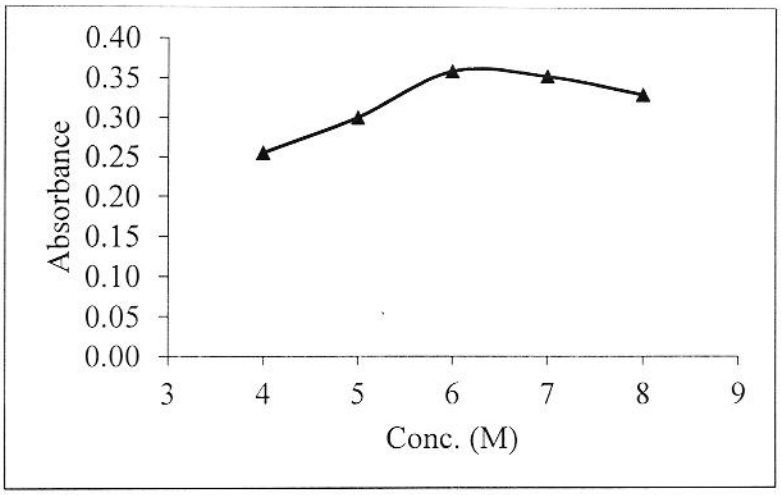


Figure 3.4.6. Effect of sodium hydroxide concentration on hydroxamate salt formation

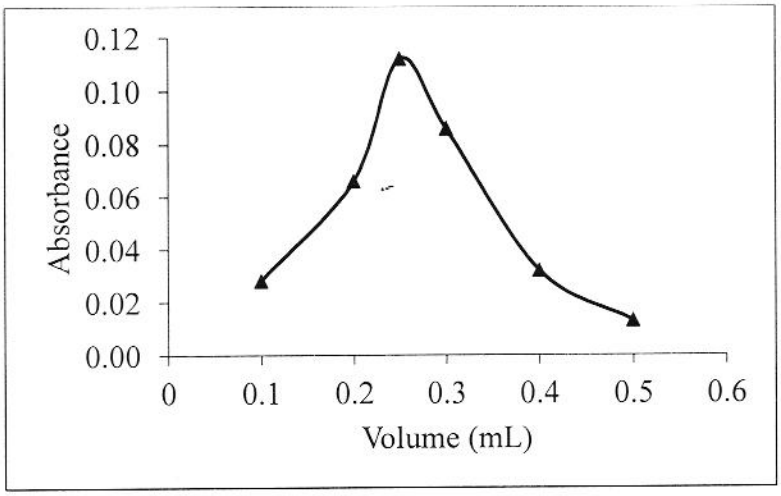


Figure 3.4.7. Effect of volume of sodium hydroxide on hydroxamate salt formation

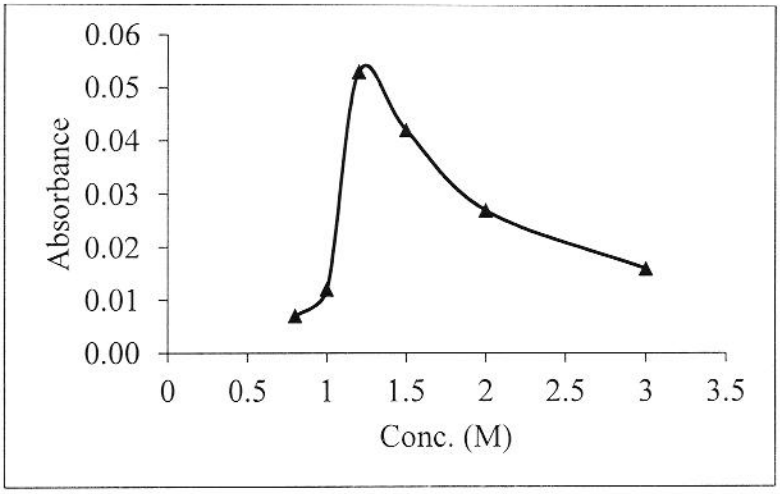


Figure 3.4.8. Effect of hydrochloric acid concentration on the Fe-hydroxamate complex formation

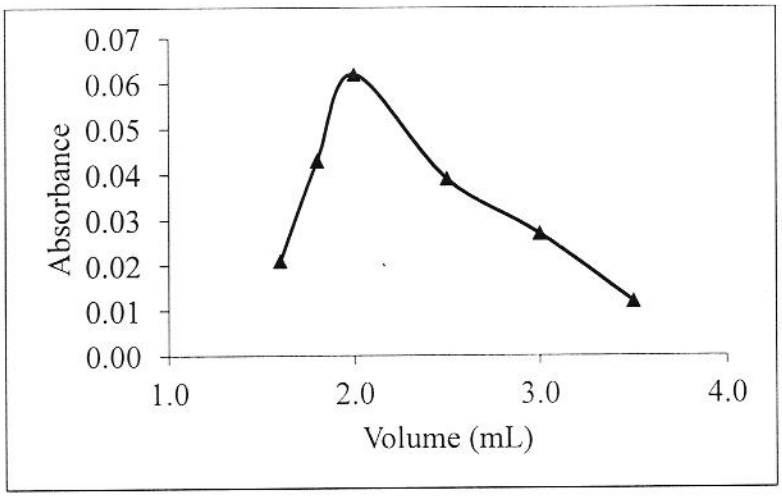


Figure 3.4.9. Effect of volume of hydrochloric acid on the Fe-hydroxamate complex formation

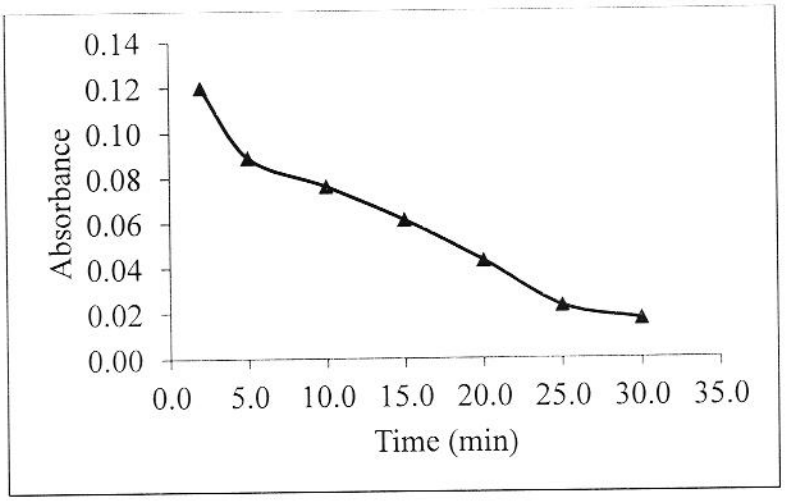


Figure 3.4.10. Effect of time on the stability of the pinoxaden based Fe-hydroxamate complex.

3.4.4. Investigation of the empirical formula and formation constant (K_f) of the pinoxaden based Fe-hydroxamate complex by Job's method of continuous variation

Instruments, Reagents and Solutions: The same as mentioned before

Procedure

To investigate the combining mole ratio of the ligand and the metal, different volumes of pinoxaden (0.01 M) were added to eight separate conical flasks followed by the addition of a mixture of 1.0 mL of hydroxylamine hydrochloride (0.1 M) solution and 0.25 mL of sodium hydroxide solution (6.0 M) to each flask. After five min equilibration time, 2.0 mL of hydrochloric acid (1.2 M) was added to each flask followed by addition of different volumes of Fe (III) solution (0.01 M) premixed with Triton X-100 (1.0 %) to each flask, in such a way that the total volume and total moles of PXD and Fe (III) in each mixture remained constant but the mole ratio of these two varied systematically. Each solution was transferred to a 10 mL flask and diluted upto the mark with ethanol. The absorbance in each case was measured at 500 nm against a reagent blank. The absorbance was plotted against the volume ratio of Fe (III) and PXD. The results are given in table 3.4.1 and shown in figure 3.4.11. The combining ratio of the complex formed was calculated from the theoretical maximum on the curve. The formation

constant K_f for the complex was evaluated from measurement of the deviations from the theoretical straight lines.

From the extrapolated value of absorbance the concentration of free metal (Fe (III)) was calculated and is given as:

M (mL) = 0.78

L (mL) = 2.22

M: L = 0.78:2.2 or 1:2.82 = or 1:3

Therefore, the formula of the complex is ML_3

$$M + 3L \longrightarrow ML_3$$

Total Metal Conc. (C_M)

$C_1V_1 = C_2V_2$

$0.01 \quad 0.75 = X \times 10$

$X = C_M = 7.8 \times 10^{-4}$ M

Total Ligand Conc. (C_L)

$C_1V_1 = C_2V_2$

$0.01 \quad 2.22 = X \times 10$

$X = C_L = 2.22 \times 10^{-3}$ M

Conc. of the complex [ML_3]

$[ML_3] = (A_{actual}/A_{extrapolated}) \times C_M$

$[ML_3] = (0.263/0.275) \times 7.8 \times 10^{-4}$

$[ML_3] = 7.4 \times 10^{-4}$ M

Free Metal Conc. [M]

$[M] = C_M - [ML_3]$

$[M] = 7.8 \times 10^{-4} - 7.4 \times 10^{-4}$

$[M] = 3.5 \times 10^{-5}$ M

Free Ligand Conc. [L]

$[L] = C_L - [ML_3]$

$[L] = 2.22 \times 10^{-3} - 7.4 \times 10^{-4}$

$[L] = 1.46 \times 10^{-3}$

$K_f = [ML_3] / [M] [L]^3$

$K_f = 7.4 \times 10^{-4} / 3.5 \times 10^{-5} \times (1.46 \times 10^{-3})^3$

$K_f = 6.8 \times 10^9$

Table 3.4.1. Investigation of the empirical formula and formation constant (K_f) of the PXD based Fe-hydroxamate complex by Job's method of continuous variation

Fe (III) (mL)	PXD (mL)	Absorbance
0.2	2.8	0.125
0.4	2.6	0.166
0.8	2.2	0.263
1.2	1.8	0.254
1.6	1.4	0.237
2	1	0.218
2.4	0.6	0.181

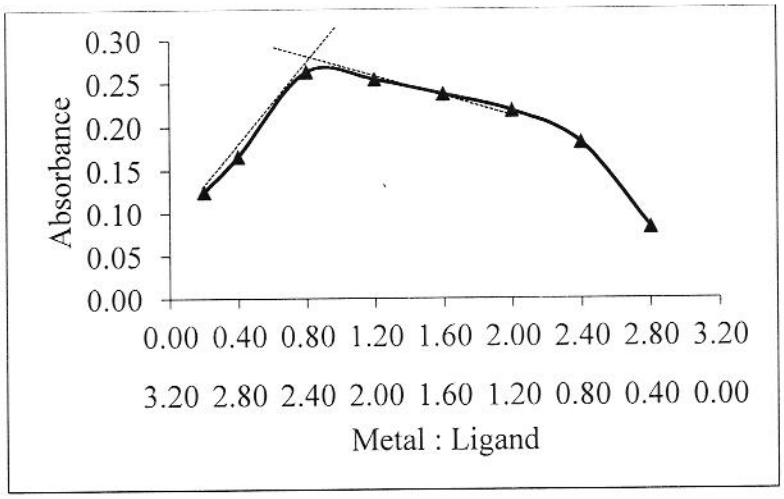


Figure 3.4.11. Investigation of the empirical formula and formation constant (K_f) of the PXD based Fe-hydroxamate complex by Job's method of continuous variation

3.4.5. Investigation of the effect of concentration of pinoxaden on the absorbance of the complex formed using batch method

Instruments, Reagents and Solutions: The same as mentioned before

Procedure

Different volumes of pinoxaden (100 μg mL^{-1}) corresponding to final concentration in the range of 0.5-40 μg mL^{-1} were taken in ten separate conical flasks. To each flask, 1.0 mL

of 4:1 mixture of hydroxylamine hydrochloride solution (0.1 M) and sodium hydroxide (6.0 M) was added and the flasks were kept for five minutes. Then 2.0 mL of hydrochloric acid (1.2 M) and 0.1 mL of Fe (III) (5.0 %) solutions premixed with Triton X-100 (1.0 %) were added consecutively to each flask. Each solution was transferred to a 10 mL volumetric flask and diluted with ethanol upto the mark. The absorbance of the red coloured complex formed was measured at 500 nm against a reagent blank, prepared by the same procedure without the addition of PXD. The results are given in table 3.4.2 and are shown in figure 3.4.12. A calibration plot of concentration versus absorbance was constructed at lower concentration level and is shown in figure 3.4.13.

Table 3.4.2. Investigation of the effect of concentration of pinoxaden on the absorbance of the complex formed using batch method

Concentration ($\mu g\ mL^{-1}$)	**Absorbance**
0.5	0.012
1.0	0.016
2.0	0.024
5.0	0.060
10.0	0.120
15.0	0.171
20.0	0.243
25.0	0.276
30.0	0.332
40.0	0.421

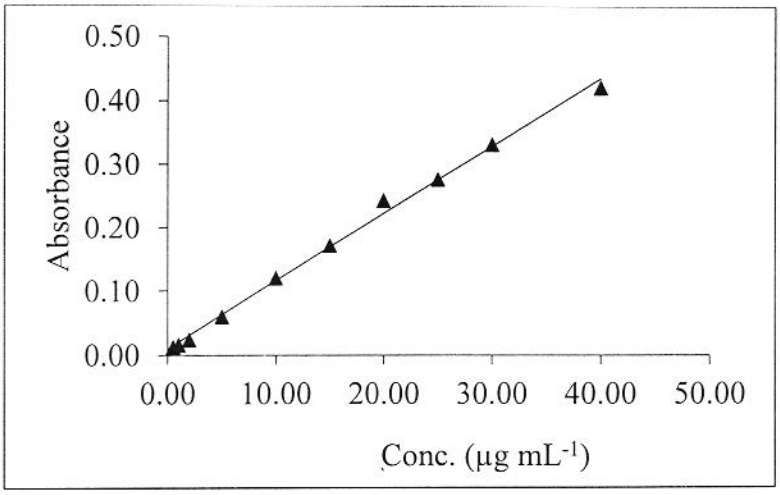


Figure 3.4.12. Investigation of the effect of concentration of pinoxaden on the absorbance of the complex formed using batch method

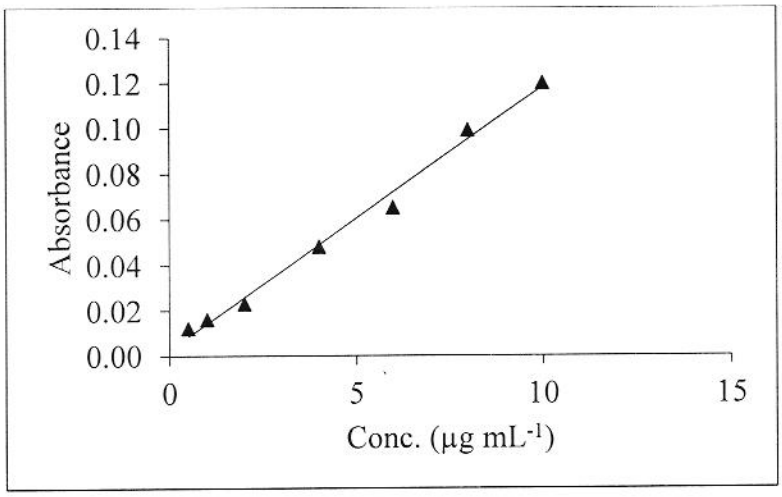


Figure 3.4.13. Calibration curve for spectrophotometric determination of pinoxaden using batch analysis

3.4.6. Investigation of analytical features for the spectrophotometric determination of pinoxaden using batch method

The molar absorptivity (ε) was calculated using the formula given below:

Molar Absorptivity (ε) = A/C × L

Where 'A' is the absorbance of the smallest concentration detected by the proposed method, 'C' is the molar concentration and 'L' is the path length (cm) of the cell.

Limit of detection (LOD) and limit of quantification (LOQ) were calculated for batch method based on six replicates of the lowest quantifiable concentration that can be measured with acceptable precision and accuracy (0.5 μg mL^{-1}) by applying the following formulae.

LOD = 3S/b (For concentration)

LOQ = 10S/b (For concentration)

Where 'S' is the standard deviation and 'b' is the slope of the standard curve

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

RSD = (S/x) ×100

Where 'x' is the mean concentration determined

All analytical characteristics of the method were determined at optimum conditions and are given in table 3.4.3.

Table 3.4.3. Analytical characteristics of the investigated batch method

Parameter	**Value**
λ_{max} (nm)	500
Beer's law range (μg mL^{-1})	0.5-40
Molar absorptivity (ε) (L mol^{-1} cm^{-1})	9.6×10^3
Relative standard deviation (RSD) (%)	13.7
Limit of detection (LOD) (μg mL^{-1})	0.2 ± 0.02
Limit of quantification (LOQ) (μg mL^{-1})	0.7 ± 0.03
Correlation coefficient	0.997
Slope	0.0116
Intercept	0.0027
Stability constant (K_f)	6.8×10^9

3.4.7. FLOW INJECTION (FI) SPECTROPHOTOMETRIC DETERMINATION OF PINOXADEN

For flow injection analysis, a three channel FIA system was arranged as shown in figure 3.4.14. The chemical parameters optimized for the batch analysis of pinoxaden with slight modification as needed were applied for the flow injection analysis at room temperature. In the FI manifolds in reagent stream F-1 a mixture of hydroxylamine hydrochloride (0.1 M) and sodium hydroxide (4.0 M) in a ratio of 4:1 was pumped. Sample solution of pinoxaden was injected into the F-1 stream through the injection valve in an injection loop. The hydroxamate salt thus formed was then acidified with 1:1 mixture of HCl (3.0 M) and ethanol flowing down in the reagent stream F-2. Finally complexation of the acidic hydroxamate salt solution with Fe (III) (0.5 %) occurred in the stream of $FeCl_3$ (0.5 %) solution flowing in the channel F-3. The absorbance of the red coloured complex passing through a flow through cell was continuously measured at λ_{max} 500 nm.

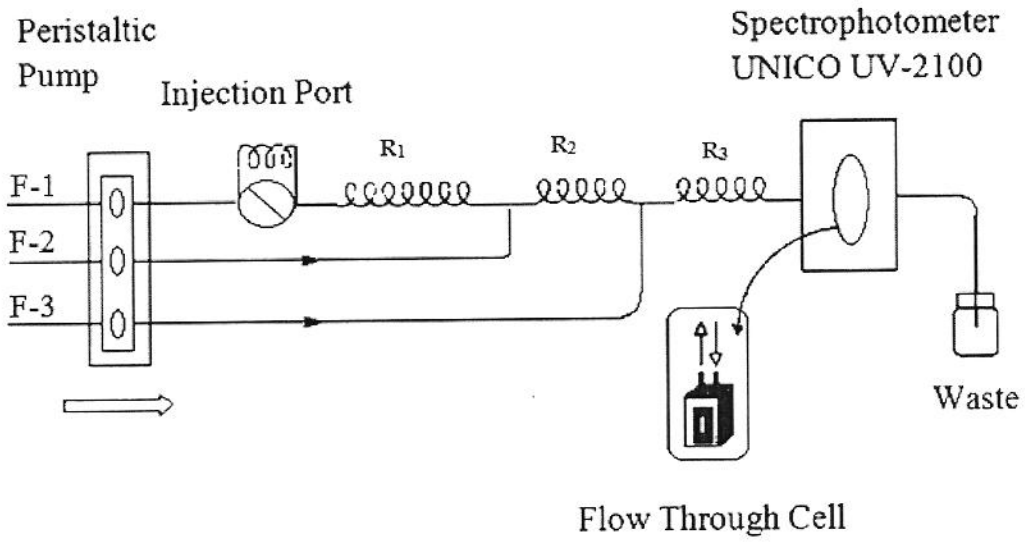


Figure 3.4.14. Scheme of manifold flow injection system for flow injection spectrophotometric determination of pinoxaden

F-1; Reagent-1($NH_2OH.HCl$ + NaOH), F-2; Reagent-2 (HCl + C_2H_5OH), F-3; Reagent-3 ($FeCl_3$), R_1; Reaction coil-1(Hydroxamate salt formation), R_2; Reaction coil-2 (Acidification), R_3; Reaction coil-3 (Complex formation)

3.4.7.1. Investigation of the effect of physical parameters on the FI spectrophotometric determination of pinoxaden

Instruments, reagents and solutions: The same as mentioned before

Procedure

In the flow injection assembly with the same length (40 cm) of reaction coils (R_1, R_2, and R_3), 1:4 mixture of sodium hydroxide (4.0 M) and hydroxylamine hydrochloride (0.1 M) was pumped in the reagents stream F-1 and 1.0 mL of PXD solution (100 µg mL^{-1}) was introduced through the injection port with the help of a 5.0 cm^3 syringe. The derivatized product formed in reaction coil R_1 was allowed to mix with F-2 reagent stream which is a 1:1 mixture of HCl (3.0 M) and ethanol (98 %). The product was acidified in reaction coil R_2. Finally the acidified hydroxamate was reacted with Fe (III) solution (0.5 %) flowing in F-3 reagent stream and complex formation completed in R_3 reaction coil. Blank was adjusted by continuously pumping the reagent solutions in the assembly and zero adjustment via the software of the detector on the computer. Absorbance of the product formed was continuously measured at 500 nm, while passing through a flow through cell to the waste chamber being placed outside the spectrophotometer. The effect of flow rate was investigated in the range of 1.0-5.0 mL min^{-1} by adjusting the flow rate on the peristaltic pump. The data was displayed and recorded through a computer interface program and is given in table 3.2.6 and shown in figure 3.2.15.

The effect of sample injection volume on the absorbance of the product was investigated by injecting different volumes of pinoxaden (100 µg mL^{-1}) in the range of 200-700 µL. All the remaining physical and chemical parameters were kept the same and the absorbance was measured. The results are shown in figure 3.4.16.

The effects of length of the reaction coils were investigated at the optimum flow rate of 3.2 mL min^{-1} and optimum sample injection volume of 500 µL keeping the chemical

variables constant. The length of each reaction coil [R_1, R_2 and R_3] was varied one by one in the range of 15-95 cm in a way that while varying the length of one reaction coil the length of the other two was kept constant and the absorption spectrum for each run was recorded. The results are shown in figure 3.4.17.

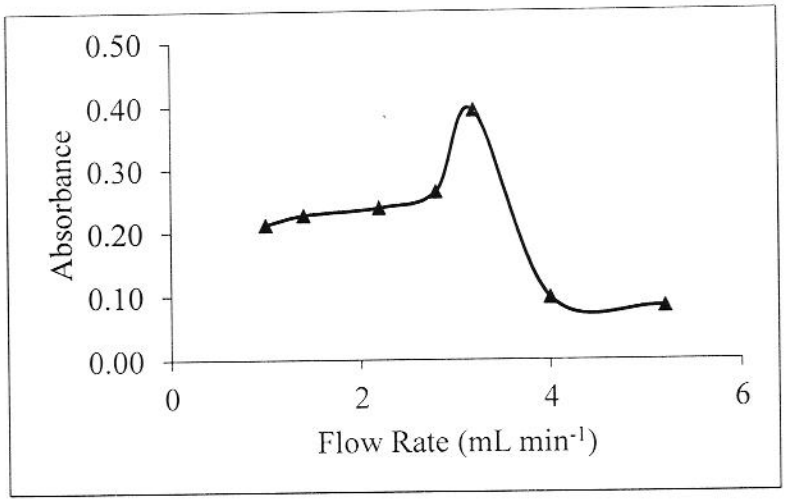


Figure 3.4.15. Effect of flow rate on the FI spectrophotometric determination of pinoxaden

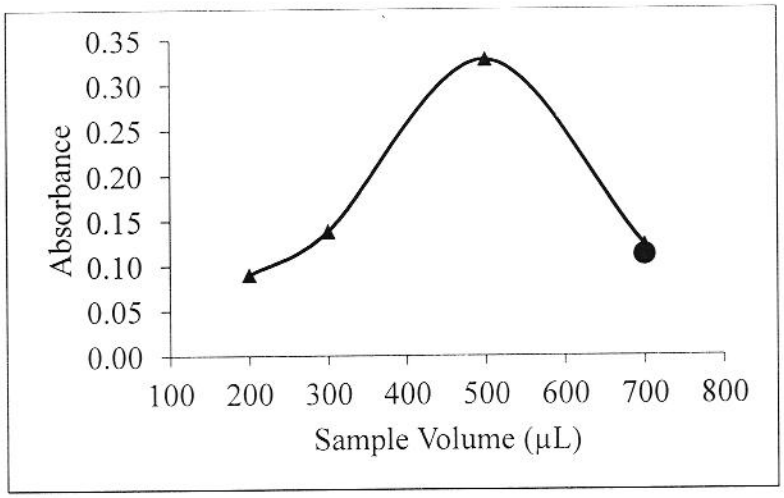


Figure 3.4.16. Effect of sample injection volume on the FI spectrophotometric determination of pinoxaden

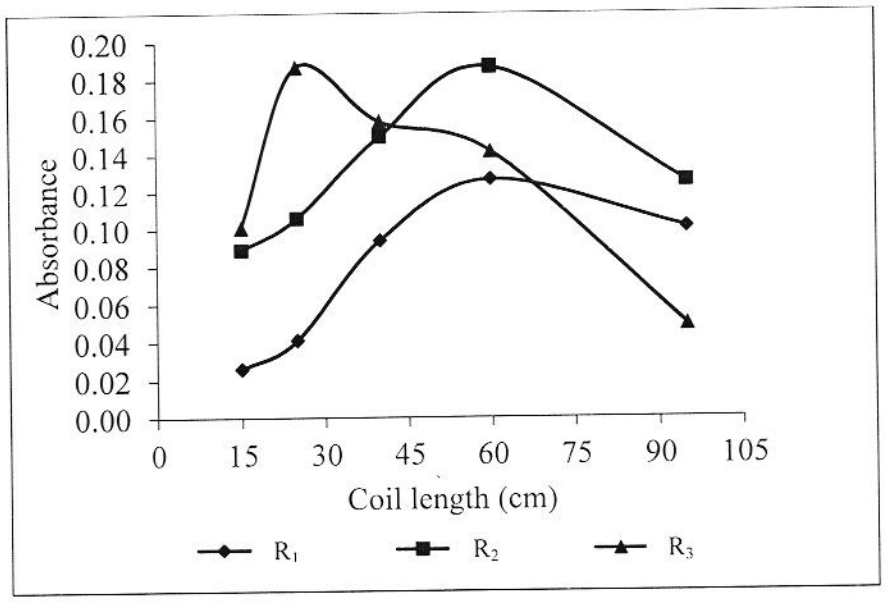


Figure 3.4.17. Effect of reaction coil length on the FI spectrophotometric determination of pinoxaden

3.4.7.2. Investigation of the effect of concentration of pinoxaden on the FI spectrophotometric determination

Instruments, Reagents and Solutions: The same as mentioned before

Procedure

Standard pinoxaden solutions in the range of 0.5 - 30.0 μg mL^{-1} were prepared in nine separate 10 mL volumetric flasks by taking the required volume of standard from 10 and 100 μg mL^{-1} stock solutions and diluting with ethanol. In the flow through assembly reagents solutions of the optimized concentration were pumped through the reaction coils of optimum length i.e. R_1 = 25 cm, R_2 = 40 cm, R_3 = 25 cm, with an optimum flow rate of 3.2 mL min^{-1}. The absorbance of the blank was adjusted at zero and then optimum volume (500 μL) of each standard pinoxaden solution was loaded into the sample loop through the injection port with the help of a 5.0 cm^3 syringe. In each case the sample was injected into the stream and the absorbance spectrum recorded. Between two successive measurements the sample loop was flushed with ethanol and absorbance of the blank

reagents stream adjusted at zero. With this process a sample throughput of 60 samples h^{-1} was achieved. The results are given in table 3.4.4 and shown in figure 3.4.18. Also a calibration plot was constructed at low concentration for application of the method to real samples. The calibration curve is shown in figure 3.4.19.

Table 3.4.4. Investigation of the effect of concentration of pinoxaden on the FI spectrophotometric determination

Conc. (µg mL^{-1})	**Absorbance**
0.5	0.016
1	0.021
2	0.033
3	0.043
4	0.054
5	0.066
10	0.121
20	0.215
30	0.326

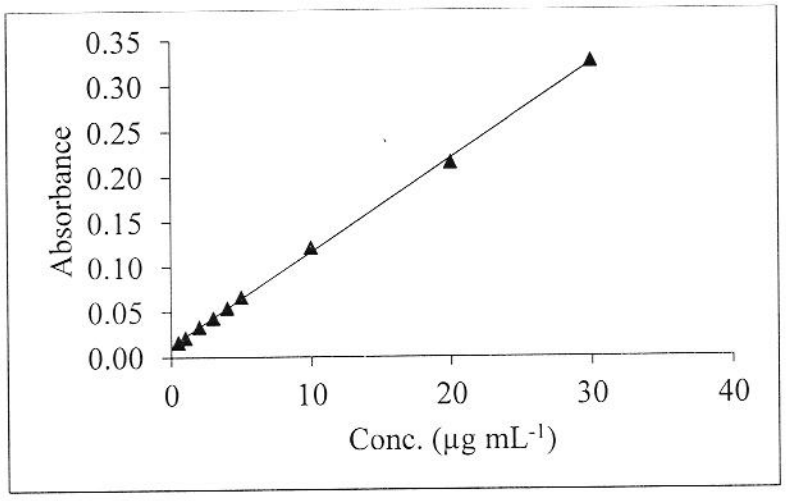


Figure 3.4.18. Investigation of the effect of concentration of pinoxaden on the FI spectrophotometric determination

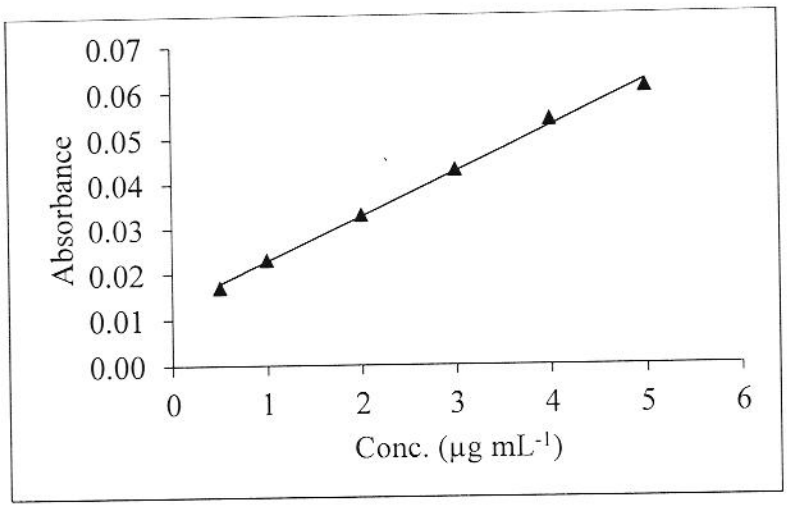


Figure 3.4.19. Calibration curve for FI spectrophotometric determination of pinoxaden

3.4.7.3. Investigation of various analytical features for the flow injection spectrophotometric determination of pinoxaden

Different analytical parameters for the FI analysis of pinoxaden were calculated based on the formulae given earlier in section 3.4.6. Limit of detection (LOD) and limit of quantification (LOQ) were evaluated for six replicates of the lowest quantifiable concentration that could be measured with acceptable precision and accuracy (0.5 µg mL^{-1}). At optimum conditions, analytical features of the method determined are given in table 3.4.5.

Table 3.4.5. Analytical characteristics of the investigated FI method

Parameter	FIA
λ_{max} (nm)	500
Molar absorptivity (ε) (L mol^{-1} cm^{-1})	1.53×10^4
Beer's law range (µg mL^{-1})	0.5-30
Relative standard deviation (RSD) (%)	8.0
Limit of detection (µg mL^{-1})	0.1± 0.01
Limit of quantification (µg mL^{-1})	0.6 ± 0.05
Correlation coefficient	0.973
Slope	0.0142
Intercept	0.0129

3.4.8. Separation of fenoxaprop-p-ethyl herbicide; a possible interferer, from pinoxaden by column chromatography

Procedure

TLC Analysis

For preliminary investigation of the solvent system for separation, TLC plates were developed for pinoxaden and fenoxaprop-p-ethyl individually and as a mixture, using a variety of solvent systems. A mixture of dichloromethane and n-hexane (1:1) showing better results was selected to be the best solvent system.

Column Chromatographic Analysis

Slurry of 1.0 g of the silica gel 60 was made in 1.0 mL of n-hexane and packed into a narrow bore glass column (4.8 mm wide, 15 cm long), plugged with glass wool at the bottom. The column was washed with 10 mL of n-hexane before sample application. Stock solution (9.0 mL) of PXD (100 μg mL^{-1}) was dried at room temperature, dissolved in n-hexane (5.0 mL) and loaded onto the column. To ensure maximum retention the column was equilibrated for 10 min. The column was then rinsed with 10 mL of n-hexane in five steps, using 2.0 mL aliquots each time, and the eluents were collected. These aliquots were evaluated by the proposed method for estimation of PXD retained. After the loading step, the retained sample was eluted with 10 mL of a 1:1 mixture of dichloromethane and n-hexane at a flow rate of 0.2 mL min^{-1} and collected the aliquots (each 2.0 mL) separately in glass vials. These aliquots were dried at room temperature, dissolved in methanol (2.0 mL) and evaluated by the proposed method. The same procedure was repeated for FE (3.0 mL of 350 μg mL^{-1}) and mixture of PXD (8.0 mL, 100 μg mL^{-1}) and FE (4.0 mL, 350 μg mL^{-1}) respectively. The results are given in table 3.4.6.

Table 3.4.6. Separation of pinoxaden and fenoxaprop-p-ethyl by column chromatography

Analyte	Added (µg)	Retention				Recovery		
		No.	µg unadsorbed	Total µg Retained	% Retention	No	µg Recovered	% Recovery
Pinoxaden (A) R_f =0.3	900	1 2 3 4 5	16 62 60 82 44 Σ=264	900 (-) 264 = 636	70.6	1 2 3 4 5 6	70 90 122 200 66 30 Σ=578	91.0 ± 2.0
Fenoxaprop-p-ethyl (B) R_f = 0.6	1050	1 2 3 4 5	42 64 60 56 40 Σ=262	1050 (-) 262 = 788	75.0	1 2 3 4 5 6	80 400 80 44 42 40 Σ=686	87.0 ± 3.0
Mixture of (A) and (B)	B=490 A=800	1 2 3 4 5 6	40 54 46 Σ=140 64 40 10 Σ=114	490-140 =350 800-114 =686	71.4 85.7	1 2 3 4 5 6	200 70 32 Σ=302 3.2 9.4 19.2 Σ=636	86.0 ± 3.0 93.0 ± 1.0

3.4.9. Investigation of percent recovery of pinoxaden from spiked samples using batch and FI methods

Instruments, Reagents and Solutions: The same as mentioned before

Samples: Soil and wheat grains samples were collected from crop field after confirmation that pinoxaden herbicide was not sprayed the current year. The university campus tap water was used as a control sample.

Sample extraction procedure

In triplicate each control sample (10 g soil and wheat grains, 100 mL tap water) were taken in separate bottles and known amounts of pinoxaden in the range of 5-15 $\mu g\ g^{-1}$ was added to these samples. The bottles were manually shaken for uniform mixing and kept for two hour. The homogenized soil and wheat grains samples were extracted with 50 mL methanol, while pinoxaden from water samples were extracted with dichloromethane (50 mL). Dichloromethane was then evaporated and the residue dissolved in methanol (10 mL). Percent recovery was evaluated by the proposed method, using both batch and flow injection methods.

Analysis procedure

For batch analysis 2.0 mL of each extract was taken in a conical flask and optimum volumes of all the reagents mentioned before were successively added and the absorbance of the final product was measured at 500 nm against a reagent blank.

FI analysis of the extract, being further concentrated to 2.0 mL, in each case was done by loading optimum volume (0.4 mL) of each extract into the sample loop through the injection port with the help of a micro syringe. The sample was then injected to the reagents stream in which optimum concentration of the reagents was flowing at an optimum flow rate. The absorbance of the product was measured at 500 nm by the method mentioned before. For each sample absorbance readings were taken in triplicate. A comparison of percent recovery investigated by both the batch and FI analysis is given in table 3.4.7.

Table 3.4.7. Investigation of percent recovery of pinoxaden from spiked samples using batch and FI methods

No	Spiked Sample	Added ($\mu g/g^{-1}$ or $\mu g/mL^{-1}$)	Found (µg)		% Recovery		Average % Recovery	
			Batch	FIA	Batch	FIA	Batch	FIA
01	Soil	5.0	5.0	4.8	100.0 ±0.1	96.0±1.6	92.0±8.0	98.0±2.0
		10	8.5	9.85	85.0±0.3	98.5±0.9		
		15	13.5	15.25	90.0±0.5	100.6±0.6		
02	Wheat Grains	5.0	4.0	5.1	80.0±0.4	102±0.8	83.0±6.0	100.0±2.0
		10	9.0	9.75	90.0±0.4	97.5±1.0		
		15	12.0	15.1	80.0±0.2	100.6±1.0		
03	Tap water	5.0	4.5	5.12	90.0±0.4	102.5±0.7	100±9.0	98.0±5.0
		10	10.5	9.25	105.0±0.3	92.5±0.4		
		15	16.0	14.7	106.0±0.1	98.3±0.1		

Results and Discussion

The chemical structure of pinoxaden (Fig. 3.4.1) contains an ester group like propanoate which was easily converted into hydroxamate salt in alkaline media by reaction with hydroxylamine hydrochloride. The hydroxamate was reacted in acidic media with Fe (III) which results in the formation of a red coloured Fe (III) hydroxamate complex. The proposed reaction mechanism is shown in Figure 3.4.2. The reaction is rapid at room temperature therefore applied for the flow injection analysis of pinoxaden herbicide.

The wavelength of maximum absorbance was investigated in the range of 420-580 nm and it was observed that the complex shows absorption maximum at 500 nm (Fig. 3.4.3).

The chemical variables like hydroxylamine hydrochloride, sodium hydroxide and hydrochloric acid concentrations were studied in a batch system for complex formation. NH_2OH-HCl concentration was investigated in the range 0.025-0.8 M (Fig. 3.4.4). The absorbance increases with increase in concentration of the reagent upto 0.1 M and decreases beyond this limit. Therefore further study was carried out with 0.1 M NH_2OH-HCl solution. Similarly the volume of hydroxylamine hydrochloride (0.1 M) was varied in the range of 0.25 - 2.5 mL keeping all the remaining conditions the same (Fig. 3.4.5). The absorbance increases from 0.25 mL to 1.0 mL beyond which it decreases slowly. Higher concentration of hydroxylamine hydrochloride has two fold effects. First it causes reduction of Fe (III) to Fe (II) based on the reaction;

$$2\ NH_2OH + 2\ Fe^{3+} \longrightarrow 2\ Fe^{2+} + N_2O + 2\ H^+ + 2H_2O$$

and secondly it increases hydrogen ion concentration which in turn increases acidity. These factors lead to dissociating tendency of the complex (1).

Hydroxylamine hydrochloride reacts with pinoxaden in alkaline media and sodium hydroxide concentration was studied in the range of 2.0 - 8.0 M. Hydroxamate salt formation starts when the solution is 4.0 M in sodium hydroxide and increases with increase in concentration upto 6.0 M solution. A small decrease in absorbance above 6.0 M was observed (Fig. 3.4.6). Volume of sodium hydroxide solution has a substantial effect on the final product formation. The effect of volume of sodium hydroxide solution

(6.0 M) in the range of 0.1-0.5 mL was also investigated and 0.25 mL of sodium hydroxide solution was found optimum for further studies (Fig. 3.4.7). Higher concentration of alkali results in the formation of Fe (III) hydroxide instead of hydroxamate complex. This subsequently reduces the efficiency of complexation reaction.

The hydroxamate salt formed from PXD herbicide was used as a ligand and Fe (III) as metal for complex formation. Hydroxamate salt reacts with Fe (III) in acidic media and hydrochloric acid was used for adjustment of solution acidity. The concentration of hydrochloric acid was varied from 0.5 M to 3.0 M to check the effect of acidity (Fig. 3.4.8).The complex formation was observed to start at 0.8 M hydrochloric acid concentration and becomes maximum at 1.2 M. Therefore, 1.2 M hydrochloric acid was selected for complex formation in batch analysis. In case of FI analysis 1.2 M HCl is unable to acidify the hydroxamate salt solution flowing down in F1 and the alkaline solution form precipitate of Fe (III) hydroxide in the tubing system and leads to erroneous results, therefore high concentration of HCl (4.0 M) was used for the said acidification process in FIA system. Volume of the acid was varied in the range of 1.4-3.5 mL (Fig. 3.4.9) and it was found that the reaction starts at 1.6 mL of the acid, becomes maximum at 2.0 mL and then decreases because higher acidity makes the complex unstable and the colour of the solution fade when the pH of the solution become lower than 1.2 (2).

The stability of the pinoxaden based Fe (III)-hydroxamate complex was studied from 2.0-30 minutes after formation (Fig. 3.4.10). It was observed that the absorbance of the complex decreases linearly with time. This means that the dissociation of the complex occurs at a constant rate at room temperature.

The stoichiometric ratio of the complex formed from hydroxamate derived from PXD to Fe (III) was established by the Job's method of continuous variation. The composition of the complex was found to be [Fe (III): hydroxamate] 1:3 that is three mole of ligand react with one mole of the metal. The formation constant of the complex was calculated and found to be 6.8×10^9. The data is given in table 3.4.1 and shown in figure 3.4.11.

For verification of Beer's law for the method, the effect of concentration of pinoxaden on the absorbance of the complex was investigated and the results are given in table 3.4.2 and shown in figure 3.4.12. It was found that the method obeys Beer's law over a concentration range of 0.5-40 μg mL^{-1}. Also a calibration plot (Fig. 3.4.13) was constructed at lower concentration for application of the method to real samples.

For flow injection analysis a simple three channel scheme was assembled (Fig. 3.4.14) and due to the instability of the complex at room temperature the absorbance of the complex is measured directly without any delay.

The physical parameters were optimized for the reaction in FI system by changing one variable and keeping the other variables constant. Maximum absorbance of the complex was found at 500 nm. Flow rate of the FI system for the reaction was studied in the range of 1.0 to 5.2 mL min^{-1} (Fig. 3.4.15). The absorbance was found to increase with increase in flow rate from 1.0 to 3.2 mL min^{-1} and decrease with higher flow rates because at higher flow rate the reagents and analyte dispersion is inefficient. A sampling rate of 60 samples h^{-1} was achieved with 1.0 mL min^{-1} flow rate. The sample injection volume was optimized in the range of 200-700 μL and maximum signal response was observed with 500 μL which indicates efficient mixing of the analyte with the reagent stream. At 700 μL sample volume, mixing of sample with the reagent stream is incomplete. Only mixing occurs at two ends of the sample zone present in the reagent stream. Therefore, instead of a single peak two peaks near to each other appeared with lower intensity (Fig. 3.4.16).

The effect of length of reaction coils for the two step reaction was investigated (Fig. 3.4.17). The effect of reaction coil length R_1 for hydroxamate formation from PXD with hydroxylamine hydrochloride in alkaline media was studied from 15 cm to 95 cm. Maximum absorbance of reaction product was found at 60 cm coil length, which indicates medium reaction rate between PXD and hydroxylamine hydrochloride. For the flow of acid to acidify the solution, the R_2 reaction coil length was optimized and 60 cm reaction coil length was found to be the optimum for adjustment of acidity of the reaction. Finally, the length of coil R_3 for complex formation was investigated and with 25 cm coil length maximum absorbance was found. The results indicated that

hydroxamate and complex formation reactions are rapid and reproducible in the FI system at room temperature.

In the FI analysis the effect of concentration of pinoxaden on the absorbance behaviour of the complex was investigated (Table 3.4.4, Fig. 3.4.18). The FI method was shown to obey Beer's law in the concentration range of 0.5-30.0 μg mL^{-1}. A calibration plot was drawn from the same data at low concentration (Fig. 3.4.19) for the application of the method to real samples.

Under the optimized conditions the analytical parameters were investigated for the batch and FI methods and are shown in table 3.4.3 and 3.4.5 respectively. A linear relation between concentration and absorbance was observed in the concentration range of 0.5-34.0 μg mL^{-1} and 0.5-30.0 μg mL^{-1} for batch and FI methods respectively. The molar absorptivity of the resulting complex was found to be 9.6×10^3 L mol^{-1} cm^{-1} for batch method and 1.53×10^4 L mol^{-1} cm^{-1} for FI method. The Limit of detection and quantification calculated at lower concentration with acceptable precision and accuracy were found to be 0.2 ± 0.02, 0.7 ± 0.03 μg mL^{-1}, and 0.1 ± 0.01, 0.6 ± 0.05 μg mL^{-1} for batch and FI methods respectively. The advantages of the developed FI method are high sensitivity, low reagent waste and high sample through put.

Fenoxaprop-p-ethyl (FE) and pinoxaden both contains an ester group which leads to Fe (III) hydroxamate complex by following the proposed method. Though the hydroxamate ligands formed in both cases are different i.e. in case of pinoxaden the ligand is N-hydroxypivalamide derivative while in case of FE the ligand is 2-(4-(6-chlorobenzo[d]oxazol-2-yloxy) phenoxy)-N-hydroxypropanamide derivative, still if both of the herbicides are present in the same sample, FE may act as interferent and should be removed. To avoid interferences effect of fenoxaprop-p-ethyl on pinoxaden determination, separation step was used before analysis by the proposed method. Separation of PXD and FE was achieved by liquid column chromatography (LC) after preliminary analysis by TLC. The R_f values of PXD and FE (0.3, 0.6) as predicted by TLC are in close agreement with the results obtained through LC. The data in table 3.4.6 shows that maximum amount of the fenoxaprop-p-ethyl is eluted prior to the second aliquot as compared to PXD which is eluted in fourth aliquot. Pinoxaden was

successively separated from fenoxaprop-p-ethyl with percent recovery in the range of 86.0 ± 3.0% and 93.0 ± 1.0 % with the suggested LC system.

Standard addition method was used for checking the validity of the proposed method. The percentage recovery of PXD herbicide from control soil, wheat grains and water samples was determined and the results are given in table 3.4.7 The average % recovery from soil samples were 92.0 ± 8.0 % and 98.0 ± 2.0 %, from wheat grains 83.0 ± 6.0 % and 100.0 ± 2.0 % and from water sample 100.0 ± 9.0 % and 98.0 ± 5.0 % for batch and FI methods respectively. The lower efficiency of the batch method may be attributed to the dissociation of the complex during the solution transfer and dilution steps.

References

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2. S. Hestrin, "The reaction of acetyl choline and other carboxylic acid derivatives with hydroxylamine and its analytical applications", Journal of Biological Chemistry, 180, 249-261 (1949).

3.5. SPECTROFLUORIMETRIC METHOD FOR DETERMINATION OF ISOPROTURON (IPU) HERBICIDE IN SPIKED GRAIN SAMPLES

The chemical structure of isoproturon (IPU) is given below.

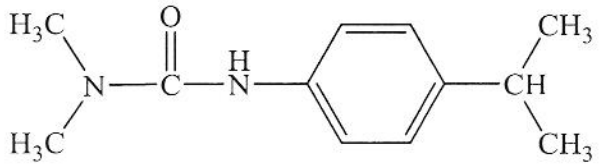


Figure 3.5.1. Chemical structure of isoproturon

3.5.1. Method development strategy for the spectrofluorimetric determination of isoproturon

Isoproturon is a phenylurea herbicide. Its structural formula as shown in figure 3.5.1 contains an electron deficient carbonyl carbon. The method development strategy for the proposed method was to add another species to IPU which form a chemical bond with the carbonyl carbon and make a rigid structure which is a basic requirement for fluorescence activity. For this purpose 2-cyanoacetamide (2-CAA) was used. Here 2.0 mL of 0.1 M 2-CAA was reacted with 2.0 mL of ammonia solution (33.0 %). Ammonia molecule abstracts a proton from the acidic methylene group on 2-CAA and form a carbene intermediate. This electron rich species was used to attack the carbonyl carbon of isoproturon molecule at high temperature when 2.0 mL of standard solution of IPU (600 μg mL^{-1}) was added to the mixture. The resulting adduct undergoes rearrangement to form the final product which is colourless and could not be used for spectrophotometric assay of IPU. However it was found to be fluorescence active and was decided to be used for spectrofluorimetric determination of IPU herbicide. The proposed reaction pathway is shown in figure 3.5.2.

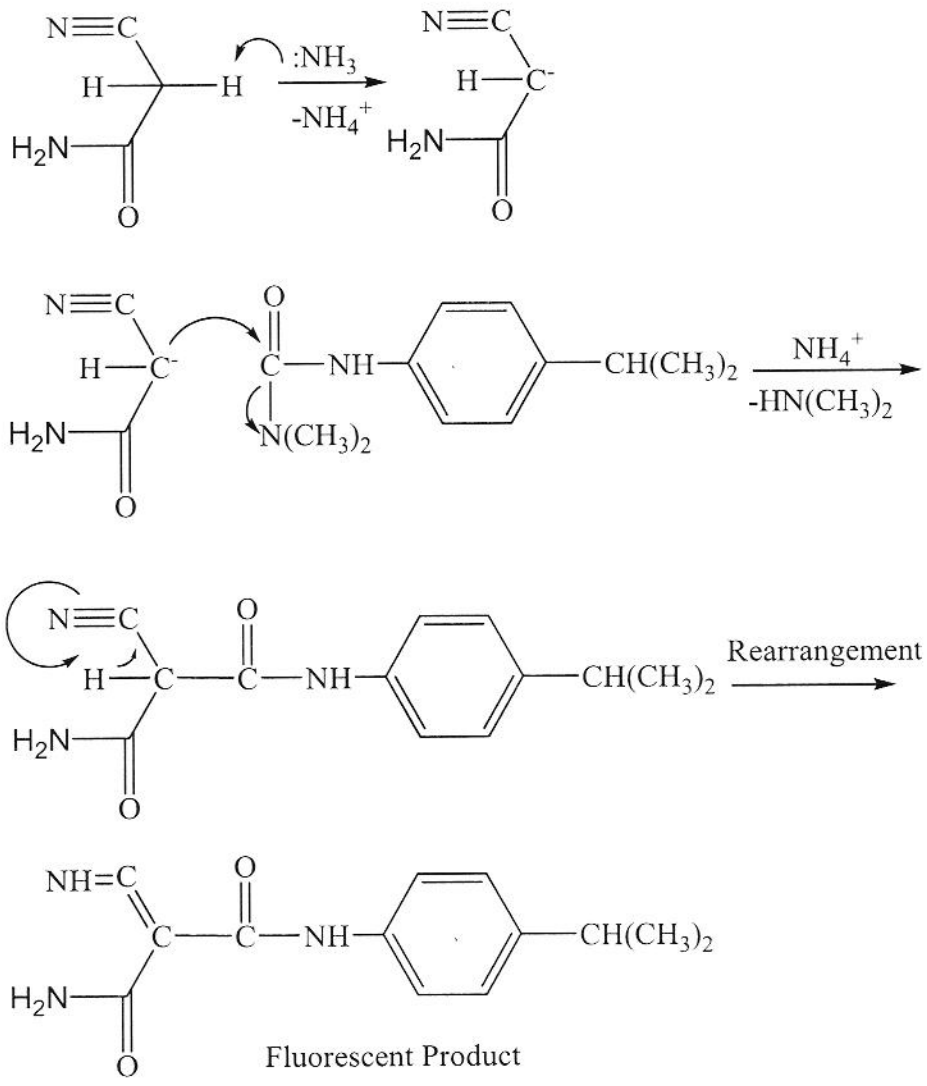


Figure 3.5.2. The proposed mechanism for the chemical reaction involved in the spectrofluorimetric determination of Isoproturon

3.5.2. Primary investigation of the possibility of fluorophore formation for spectrofluorimetric determination of isoproturon

In order to investigate the possibility of the proposed reaction, 1.0 mL of a standard isoproturon solution (600 μg mL^{-1}) was added to a flask containing a mixture of 2.0 mL of 2-CAA (0.1 M) and 2.0 mL of ammonia (33.0 %). The solution was diluted to 10.0 mL with distilled water. The fluorescence spectrum for the solution was recorded and no substantial peak was observed in the spectra. Once again in a second turn the reagents were mixed in the order, 2.0 mL of 2-CAA (0.1 M) and then 2.0 mL of ammonia (33.0

%) and the mixture was heated on a boiling water-bath for 10 minutes. The contents of the reaction flasks were transferred to 10 mL volumetric flask after cooling to room temperature and diluted up to the mark with distilled water. The resulting product was found to be fluorescence active against a reagent blank. It had good fluorescence activity and showed good separation in the excitation and emission wavelengths. The fluorigenic behaviour of the resulting product indicates the possibility to use this method for spectrofluorimetric determination of IPU herbicide. An analytical method should be reproducible and sensitive and this is possible under certain optimized conditions. Therefore, it was mandatory to focus on the optimization of various physical and chemical parameters. The details of the optimization study are described below.

3.5.3. Optimization of different physical and chemical parameters for spectrofluorimetric determination of isoproturon

Optimization studies were conducted for maximum product formation. These studies include wavelengths of maximum excitation and emission, concentration and volume of 2-CAA and ammonia solutions, heating temperature and heating time and the effect of time on stability of the fluorophore.

3.5.3.1. Investigation of suitable excitation and emission wavelength for the spectrofluorimetric determination of isoproturon

Instruments

All fluorescence intensities were measured with RF-5301 PC Spectrofluorophotometer Shimadzu Japan, equipped with 150-watt Xenon discharge lamp, excitation, emission grating monochromator and 1×1 cm quartz cell. The instrument was operated at low sensitivity with excitation and emission slit width set at 5.0 nm. A six-hole electrical thermostatic water bath (YuJia china) with temperature range of 37 °C-100 °C was used for heating. Lab Orbital Shaker (Model No. OS-340C, Digisystem Laboratory Instrument Inc. Taiwan R. O. C) was used for shaking the suspensions. A centrifuge (Model No. 98274 China) and a microwave oven (Model No. KEN ST/SS 25, DSG Retail Ltd. China under license from Kenwood Appliances Luxembourg SA) were employed during samples analysis.

Chemicals and reagents

All chemicals used in the present work were of analytical grade or similar purity and were used as such without further purification. Reagents like 2-CAA (Across organics New Jersey USA), Ammonia (BDH, 33.0 %) and methanol (analytical grade, Merck) were used in this work. The isoproturon standard (purity 99.0 %) was purchased from Dr. Ehrenstrofer (Germany) and was used as received. Florisil-30-60 U.S. mesh (BDH Chemicals LTD. Poole England) was used as adsorbent.

Solutions preparation

Standard isoproturon solution: Standard isoproturon stock solution (600 μg mL^{-1}) was prepared by dissolving 0.03 g of authentic standard in 10 mL of methanol and dilution up to mark with methanol in 50 mL volumetric flask. Working standards were prepared when needed, by diluting appropriate quantity of the stock solution to 50 mL with methanol.

2-Cyanoacetamide solution: 2-CAA solution (0.05 M) was prepared by dissolving 0.42 g of the reagent in distilled water and diluting up to 100 mL with distilled water.

Ammonia solution: Ammonia solution (15.0 M) was prepared by diluting 82.9 mL of 33.0 % (18.1 M) ammonia to 100 mL with distilled water in 100 mL volumetric flask.

Procedure

In a 100 mL conical flask, 2.0 mL of 2-CAA (0.05 M) and 2.0 mL of ammonia (15.0 M) were taken and known volume (1.0 mL) of standard solution of isoproturon (100 μg mL^{-1}) was added to it. The flask was heated on a boiling water-bath for 10 minutes. The contents of the reaction flask was transferred to 10 mL volumetric flask after cooling at room temperature and diluted up to the mark with distilled water. The fluorescence spectra of the resulting fluorescent product were measured against an appropriate blank. The results are shown in figure 3.5.3.

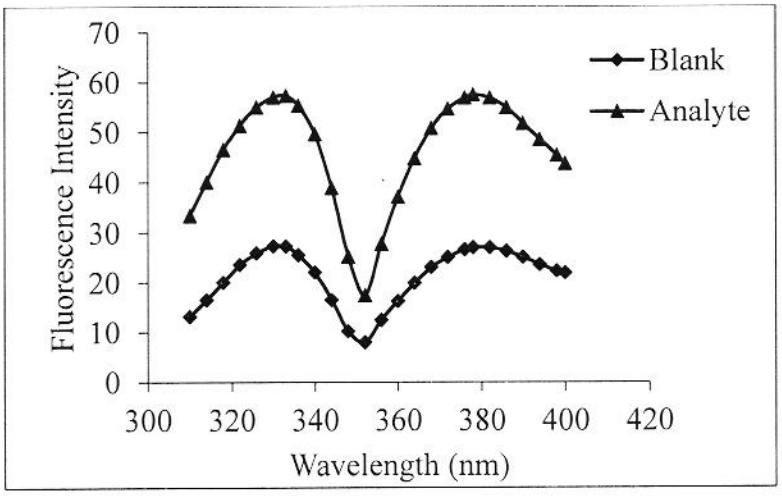


Figure 3.5.3. Fluorescence spectra of the product for spectrofluorimetric determination of isoproturon

3.5.3.2. Investigation of the effect of various physical and chemical parameters on the spectrofluorimetric determination of isoproturon

Instrument, reagents and solutions: The same as described before

Procedure

In order to investigate the effect of concentration of 2-CAA on the fluorescence intensity of the final product, eight separate conical flasks were taken. To each flask 2.0 mL of variable concentration of 2-CAA solution in the concentration range of 0.003-0.20 M was added followed by the addition of 2.0 mL of ammonia solution (33.0 %) and 1.0 mL of standard solution of isoproturon (100 μg mL^{-1}). Each flask was heated on a boiling water bath for 10 min. Then after cooling at room temperature the contents of each flask were transferred to a 10 mL volumetric flask and diluted up to the mark with distilled water. The fluorescence intensity of the product was measured against a reagent blank at the optimized excitation and emission wavelengths. The results are shown in figure 3.5.4. Similarly the effect of volume of 2-CAA on the fluorophore formation was studied by varying the volume of optimum concentration of 2-CAA solution (0.05 M) in the range of 0.5-6.0 mL keeping all the remaining conditions constant. The results are shown in figure 3.5.5.

The effect of concentration of ammonia solution on the fluorescence intensity of the product was studied by taking 2.0 mL of 2-CAA (0.05 M) in a series of six separate conical flasks. This was followed by the addition of 2.0 mL of ammonia solution in the concentration range of 3.0-18.0 M and then 1.0 mL of standard isoproturon solution (100 $\mu g\ mL^{-1}$) to each flask. Each mixture was then heated on a boiling water bath for 10 min. The mixture in each case was cooled at room temperature and transferred to 10 mL volumetric flasks. After dilution upto the mark with distilled water fluorescence intensity of each solution was measured against a reagent blank. The results are shown in figure 3.5.6. For investigating the effect of volume of ammonia on the reaction, different volume of ammonia solution (15.0 M) in the range of 0.5-5.0 mL was added to different flasks following the remaining procedure as discussed above. The results are shown in figure 3.5.7.

For investigating the effect of temperature on the proposed reaction, a series of six separate conical flasks were taken and 2.0 mL of 2-CAA (0.05 M) and 2.0 mL of ammonia (15.0 M) solutions were added to each flask. Then 1.0 mL of isoproturon standard solution (100 $\mu g\ mL^{-1}$) was added to each flask and was heated for 10 min at a temperature ranging from 25-100 ^{o}C. Each solution was transferred to 10 mL volumetric flask and after cooling to room temperature, the solution was diluted up to the mark with distilled water. Fluorescence intensity of the product in each case was measured. The results are shown in figure 3.5.8.

For investigating the effect of heating time on the reaction, the fluorescence intensity of the product was measured by following the same procedure as discussed above, without heating and with heating for different time intervals (upto 40 min). The results are shown in figure 3.5.9.

The effect of time on stability of the fluorophore was investigated by measuring the fluorescence intensity of the product at different time intervals ranging from 1.0-80 min after its formation. The results are shown in figure 3.5.10.

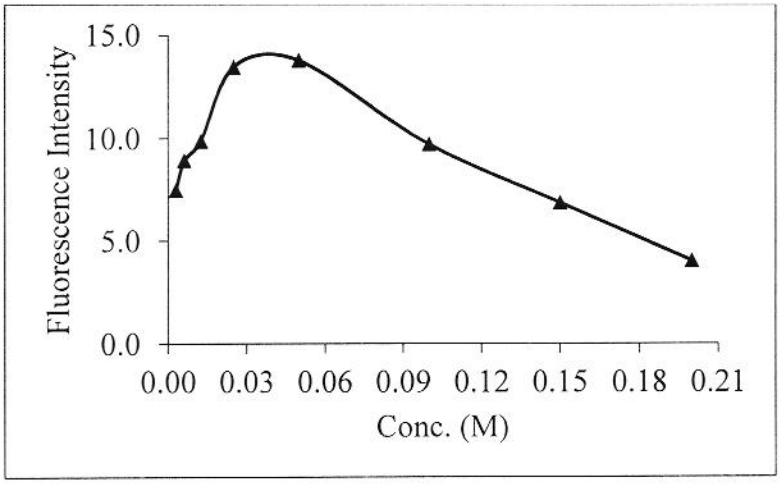


Figure 3.5.4. Effect of concentration of 2-CAA solution on the fluorescence intensity of fluorophore

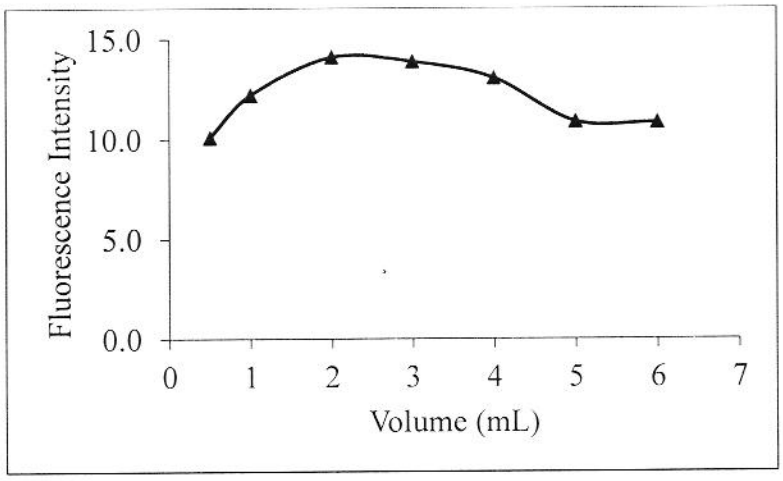


Figure 3.5.5. Effect of volume of 2-CAA solution on the fluorescence intensity of fluorophore

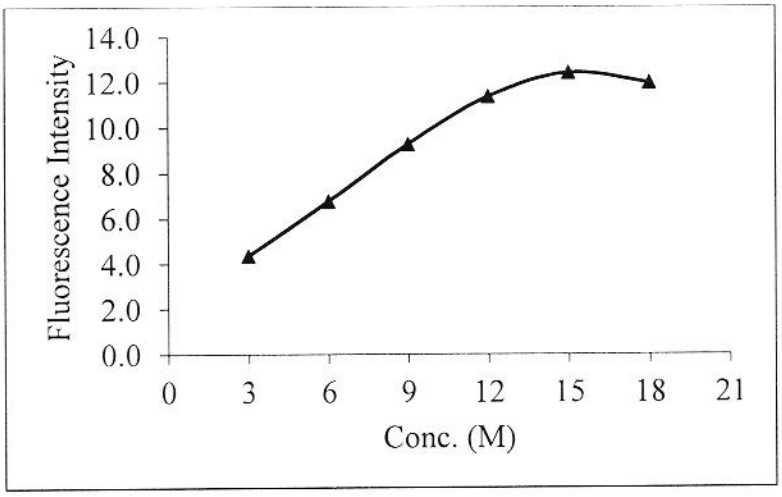


Figure 3.5.6. Effect of concentration of ammonia solution on the fluorescence intensity of fluorophore

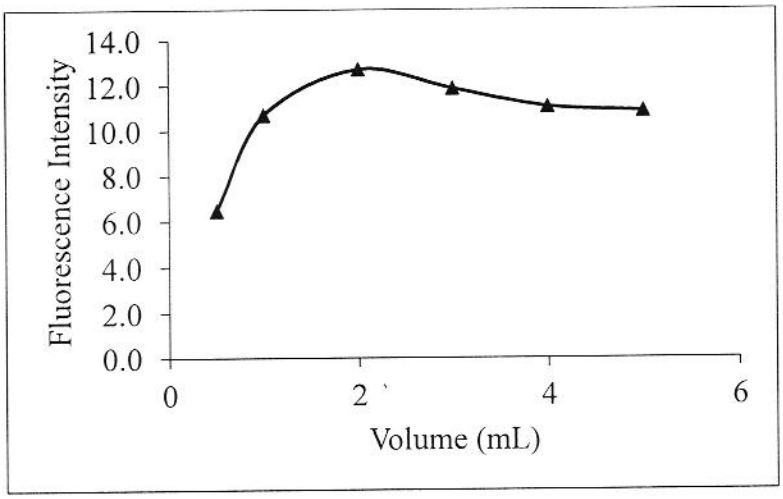


Figure 3.5.7. Effect of volume of ammonia solution on the fluorescence intensity of fluorophore

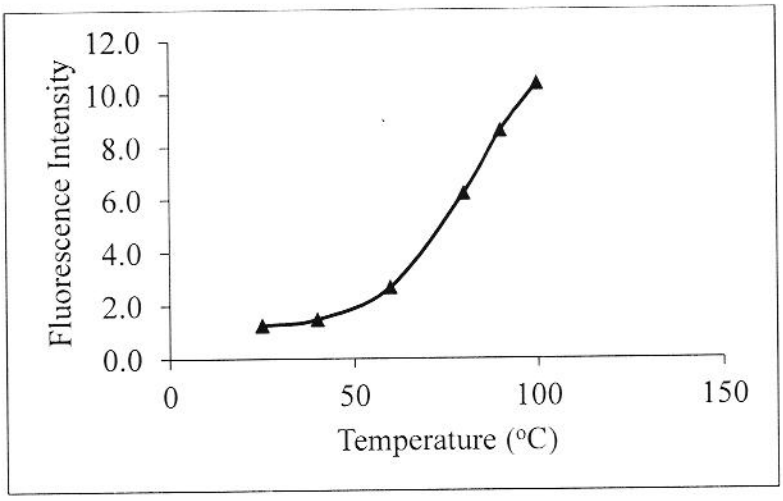


Figure 3.5.8. Effect of heating temperature on the fluorescence intensity of fluorophore

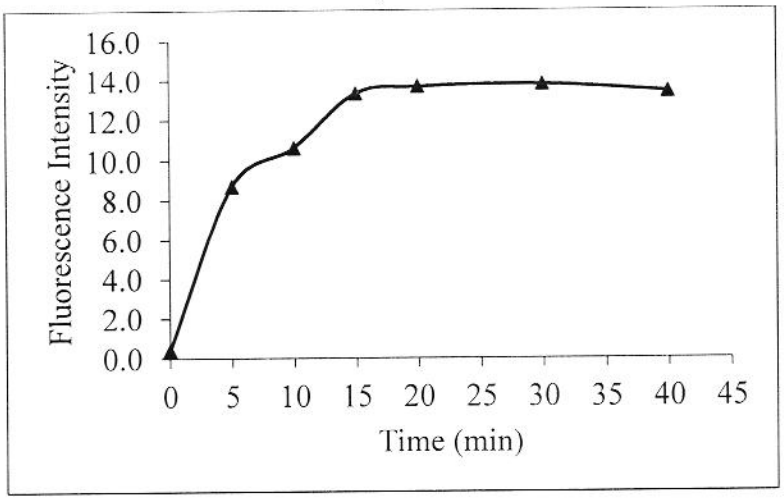


Figure 3.5.9. Effect of heating time on the fluorescence intensity of fluorophore

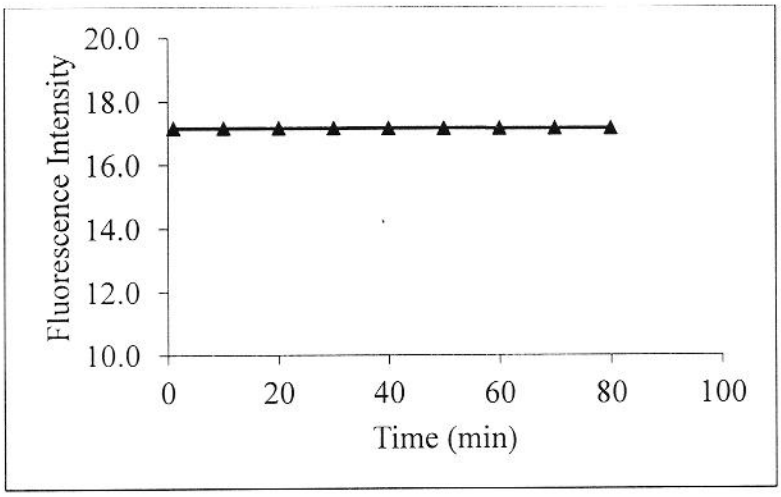


Figure 3.5.10. Effect of time on stability of the IPU based fluorophore

3.5.4. Investigation of the effect of concentration of isoproturon on the fluorescence intensity of the fluorophore formed

Instruments, reagents and solutions: The same as mentioned earlier

Procedure

In order to investigate the effect of concentration of isoproturon on the fluorescence intensity of the fluorophore formed, 2.0 mL of 2-CAA (0.05 M) and 2.0 mL of ammonia (15.0 M) were taken in a series of separate conical flasks followed by the addition of standard solution of isoproturon in concentration range of 0.5-20.0 μg mL^{-1}. These flasks were heated on a boiling water-bath for 15 minutes. The contents of the reaction flasks were transferred to 10 mL volumetric flask after cooling to room temperature and diluted up to the mark with distilled water. The fluorescence intensity of the resulting fluorescent product was measured at λ_{ex} 333 nm and λ_{em} 378 nm. All fluorescence intensity measurements were corrected for the solvent signal with the appropriate blank. Each measurement was carried out in triplicate in all cases and the results were expressed as mean values. The results are given in table 3.5.1 and shown in figure 3.5.11. A calibration plot of fluorescence intensity vs. concentration of IPU was constructed at lower concentration for application to real samples and is shown in figure 3.5.12.

Table 3.5.1. Effect of concentration of IPU on the fluorescence intensity of the fluorophore formed

Conc. (μg mL^{-1})	Fluorescence Intensity
0.5	8.506 ± 0.211
1.0	10.209 ± 0.137
3.0	15.701 ± 0.122
5.0	19.646 ± 0.135
10	29.756 ± 0.218
15	39.067 ± 0.111
20	46.418 ± 0.106

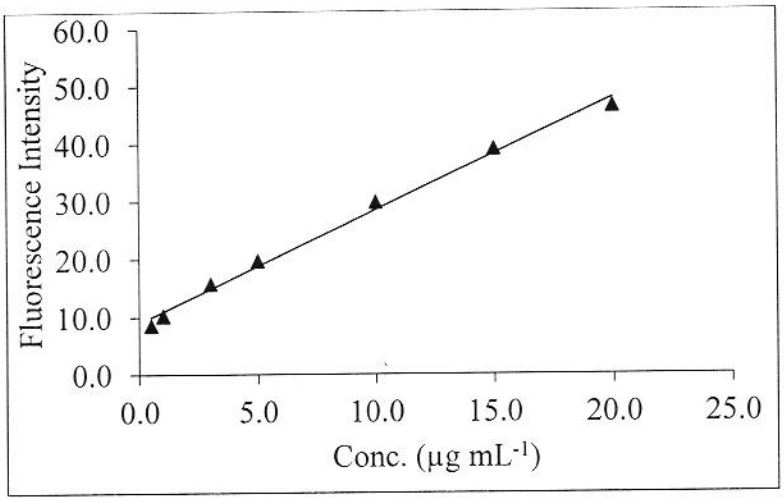


Figure 3.5.11. Effect of concentration of IPU on the fluorescence intensity of the fluorophore formation

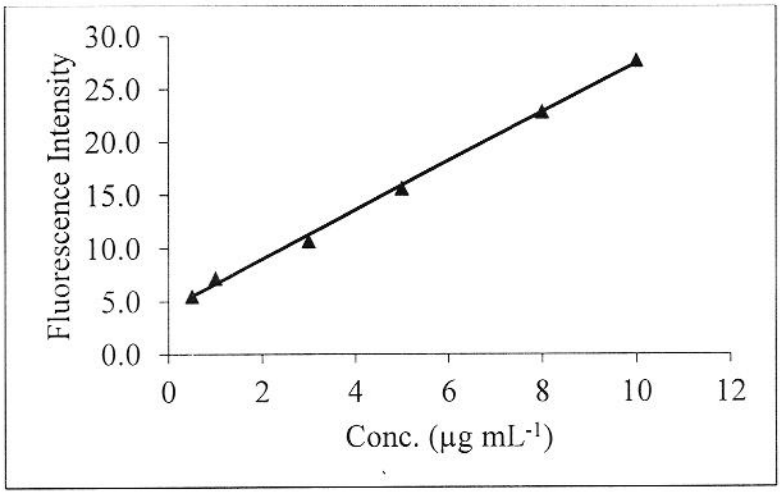


Figure 3.5.12. Calibration curve for spectrofluorimetric determination of IPU in spiked samples

3.5.5. Determination of limit of detection, limit of quantification and other analytical characteristics of the proposed spectrofluorimetric method for determination of IPU

Instruments, reagents and solutions: The same as mentioned earlier

Procedure

Six replicates with lowest quantifiable concentration of IPU (0.5 µg mL^{-1}) which can be measured with acceptable precision and accuracy were analysed by the proposed method applying the same optimized physical and chemical parameters. Limit of detection (LOD), limit of quantification (LOQ) and other analytical characteristics based on these analyses were calculated for the proposed method in the same way as mentioned before and are given in table 3.5.2.

Table 3.5.2. Analytical characteristics for spectrofluorimetric determination of Isoproturon

Analytical Characteristics	Value
λ_{ex} (nm)	333
λ_{em} (nm)	378
Linear Range (μg mL^{-1})	0.5 - 15.0
Limit of detection (3S/b) (μg mL^{-1})	0.01
Limit of quantification (10S/b) (μg mL^{-1})	0.032
Relative standard deviation (%)	0.6 %
Regression equation (y)	Y=2.3167 X + 4.3263
Slope (b)	2.3167
Intercept (c)	+4.3263
Correlation coefficient (r)	0.9991
Standard deviation (μg mL^{-1})	$3.2x10^{-3}$

3.5.6. Investigation of the interferences effect of various related compounds on the spectrofluorimetric method for determination of IPU

Instruments, reagents and solutions: The same as described before.

Procedure

The interferences effect of various related compounds like urea and different locally used herbicides on the spectrofluorimetric determination of IPU was investigated. For this purpose a known concentration of IPU and different concentrations of the possible interferers in different ratios were added to a series of conical flasks each containing optimized volume of 2-CAA solution (0.05 M) and ammonia solution (15.0 M). Each mixture was heated on a boiling water bath for 15 min. After cooling to room temperature each solution was transferred to a 10 mL volumetric flask and diluted up to the mark with distilled water. The fluorescence intensity of each solution was measured against a reagent blank. The results are given in Table 3.5.3 and are shown in figure 3.5.13.

Table 3.5.3. The interferences effect of different compounds on the fluorescence intensity of isoproturon based fluorophore

Analyte: Interferer	1:01	1:02	1:03	1:04
urea	95.2	94.3	94.4	93.4
Glyphosate	50.5	33.3	19.7	10.2
pinoxaden	93.4	75.4	95.3	92.8
Atrazine	97.3	73.1	73.3	70.7
Metribuzin	78.0	91.8	95.1	91.2
Terbutryn	82.6	82.4	79.1	82.5
Mandipropamide	87.0	93.4	88.2	73.2

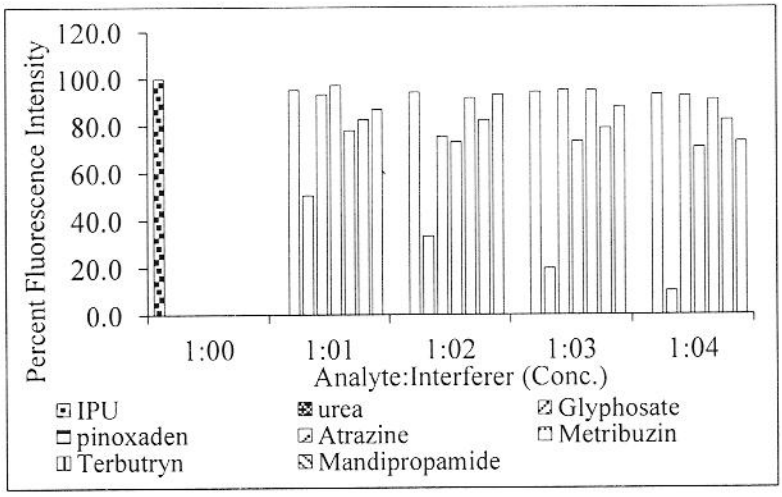


Figure 3.5.13. The interferences effect of different compounds on the fluorescence intensity of isoproturon based fluorophore

3.5.7. Investigation of percent recovery from spiked samples using the proposed method

Instruments and reagents: The same as mentioned before

Samples preparation

Various grain samples, such as barley, wheat, beans, poppy seeds and mustard seeds were collected from different fields in Swat valley of Khyber Pakhtun Khwa after it was confirmed that isoproturon was not sprayed on the field at least for two years prior to

sample collection. The samples were properly cleaned and chaff separated by winnowing. Triplicates of each sample (3.0-5.0 g) were taken in separate conical flasks, spiked with standard IPU solution to give final concentrations of 4.0, 8.0 & 10.0 μg g^{-1} in case of barley, 1.0, and 3.0 & 15.0 μg g^{-1} in case of wheat and 5.0, 10.0 & 15.0 μg g^{-1} in all other cases. Also a blank of each grains sample (3.0-5.0 g) was taken in separate conical flask and only methanol was added to it instead of the analyte. All samples were shaken for 10 minutes on the orbital shaker prior to extraction procedure to achieve maximum mixing.

Procedure for extraction of isoproturon from grain samples

Microwave extraction of IPU from wheat, barley and beans

To the bottles containing spiked grains and bean, methanol (20 mL) was added and were kept in a microwave oven for four minutes with addition of 2.0 mL additional methanol after every minute. The cooled mixtures were filtered and the filtrate being coloured due to presence of coextractants from the grains was passed through a florisil column (10 × 0.5 cm) to remove the interfering materials. The column was washed with 10 mL methanol. The washings were collected in conical flasks, evaporated and the residues were redissolved in 5.0 mL methanol. Aliquots of these samples were analysed by the proposed method.

Extraction of IPU from poppy seeds and mustard seeds

IPU from poppy seeds and mustard seeds was extracted following a reported method [1] with certain modifications. Samples (5.0 g each) were taken in 100 mL conical flasks and 20 mL of methanol: water (1:1, v/v) mixture was added to each flask. All the mixtures were shaken (150 RPM) by an orbital shaker for 30 min and then solid particles, in case of poppy seeds were separated by centrifugation at 2000 RPM for 10 min. The supernatant was transferred into the volumetric flask. The suspension was twice rinsed by 5.0 mL of methanol. The mixture of supernatants was evaporated to 5.0 mL and analysed by the proposed method. In case of mustard seeds the mixtures containing oily fractions were filtered through a sintered glass crucible fitted with metal water jet pump. The filtrates were evaporated to 5.0 mL and analysed by the proposed method.

Analysis Procedure

A known volume (5.0 mL) of each extract was added to a conical flask containing a mixture of 2.0 mL of 2-CAA solution (0.05 M) and 2.0 mL of ammonia solution (15.0 M). Each solution was heated on a boiling water bath for 15 min. After cooling at room temperature the solution was transferred to a volumetric flask in each case and diluted up to the mark with distilled water. Then fluorescence intensity of each solution was measured against a reagent blank prepared in the same manner except adding methanol to the samples instead of isoproturon. The results of percent recovery of isoproturon are given in table 3.5.4.

Table 3.5.4. Percent recovery of isoproturon from spiked samples using the proposed method

Sample	$\mu g\ g^{-1}$ added	$\mu g\ g^{-1}$ found	% Recovery	Mean ± SD
Barley†	04	3.72	93.0	95 ± 2.0
	08	7.76	97.0	
	10	9.68	97.0	
Wheat†	01	0.89	89.0	93 ± 4.0
	03	2.93	98.0	
	15	14.01	93.0	
Poppy seeds‡	05	4.78	96.0	96 ± 0.5
	10	9.57	96.0	
	15	14.57	97.0	
Mustard Seeds‡	05	4.20	84.0	85 ± 1.2
	10	8.69	87.0	
	15	12.8	85.0	
Beans†	05	4.75	95.0	94 ± 1.0
	10	9.32	93.0	
	15	14.26	95.0	

† Microwave Extraction

‡ Mechanical Extraction

Results and Discussion

The carbonyl group of phenylurea moiety in the chemical structure of IPU (Fig. 3.5.1) was reacted with 2-CAA in the presence of ammonia. Ammonia abstracts a proton from the methylene group of 2-CAA forming a carbanion intermediate which attacks the carbonyl carbon of IPU, releasing dimethyl amine and produces cyanomalonamide derivative. This derivative on rearrangement gives a fluorescent product as given in the proposed reaction mechanism (Fig. 3.5.2). The fluorescence spectrum of the resultant fluorophore shows strong fluorescence at λ_{em} 378 nm with excitation at λ_{ex} 333 nm (Fig. 3.5.3).

The effect of the concentration of 2-CAA solution (2.0 mL) on the fluorescence intensity of the final product was investigated in the range of 0.003-0.20 M. It was observed that fluorescence intensity increased rapidly with increase in reagent concentration upto 0.05 M, beyond which it steadily decreases because of certain physical interaction between excess 2-CAA molecules and the excited singlet state of the fluorophore molecule. Also an unwanted side reaction which may takes place at high reagent concentration of 2-CAA cause fluorescence quenching (Fig. 3.5.4). In order to investigate the effect of volume of 2-CAA solution, the course of the reaction was followed by varying the volume in the range of 0.5-6.0 mL keeping all the remaining conditions the same. Fluorescence intensity of the resultant fluorophore was found to slowly increase form 0.5-2.0 mL. Above 2.0 mL a slow decreases in fluorescence intensity was observed due to dilution effect (Fig. 3.5.5)

Ammonia is used as a catalyst in the first step of the derivatization reaction. The effect of the concentration of ammonia (2.0 mL) on the reaction in the concentration range of 3.0-18.0 M was investigated. Maximum fluorophore formation was observed at 15.0 M concentration of ammonia beyond which the fluorescence intensity remains nearly constant (Fig. 3.5.6). The effect of volume of ammonia (15.0 M) on the reaction was studied in the range of 0.5-5.0 mL solution (Fig. 3.5.7). It was found that maximum product formation takes place when 2.0 mL of ammonia solution is used. Beyond this limit a very small decrease in fluorescence intensity occurs due to dilution of the reaction medium.

Temperature was observed to be a key factor in the derivatization reaction. The effect of temperature in range of 25-100 °C was investigated and fluorescence intensity was found to increase linearly with increase in temperature. Maximum fluorescence intensity was observed when the reaction mixture was heated at 100 °C for 15 minutes (Fig. 3.5.8). It is not possible to study the effect of temperature beyond 100 °C because the aqueous solvent system will get evaporated below the minimum volume necessary for the reaction. Also the fluorophore may not be stable at higher temperature.

The effect of heating time on the reaction was investigated by following the reaction procedure without heating and with heating for different time intervals keeping all the remaining conditions constant. It was found that without heating the reaction does not take place because the fluorophore formation involves complicated bond conversions which need high temperature. The fluorescence intensity of the fluorophore increases as the heating time is increased till 15 min, beyond which heating time has no substantial effect on the reaction. Therefore, for the sake of time and energy savings 15 min was selected as optimum heating time (Fig. 3.5.9).

The stability of the fluorophore was followed for 80 minutes and no substantial variation in the fluorescence intensity was observed which confirms the absence of any side reaction when the product is once formed (Fig. 3.5.10).

The fluorescence intensity of the product was found to increase linearly with increase in concentration of isoproturon over a concentration range of 0.5-20.0 μg mL^{-1} under optimum experimental conditions of the proposed method (Table 3.5.1 & Fig. 3.5.11). Also a calibration plot was constructed for determination of the unknown concentration of IPU in real samples (Fig. 3.5.12). The linear regression equation, slope, intercept, correlation coefficient, standard deviation and relative standard deviation of the response factors were determined. The limit of detection, LOD, (3S/b) and limit of quantification, LOQ, (10S/b) values were calculated at lowest quantifiable concentration of IPU (0.5 μg mL^{-1}) and were found to be 0.01 and 0.032 μg mL^{-1} respectively. Analytical characteristics of the proposed method are given in table 3.5.2.

A variety of compounds like urea and several pesticides were screened as possible interferents in the determination of isoproturon by the proposed method. The effect of

concentration of the possible interferents in different ratios to the analyte on fluorescence intensity was studied applying the same experimental conditions and results were reported in terms of percent fluorescence intensity (Table 3.5.3 & Fig. 3.5.13). It is evident from the figure that only glyphosate has a quenching effect on the fluorescence intensity of the fluorophore while other compounds have no substantial effect as interferents. However, glyphosate being completely soluble in water can be removed from isoproturon which is only slightly soluble in water.

The proposed method has been successfully applied for the determination of isoproturon in various spiked grains and seeds samples following different extraction strategies i.e. microwave extraction, extraction with shaking and extraction with shaking and separation with sintered glass cruisible fitted to metal water jet. Aliquots of the extracts from barley grains, wheat grains, poppy seeds, mustard seeds and beans were analysed by the proposed method and percentage recoveries of IPU achieved were 95.0 ± 2.0 %, 93.0 ± 4.0 %, 96.0 ± 0.5 %, 85.0 ± 1.2 % and 94 ± 1.0 for barley, wheat, poppy seeds, mustard seeds, and beans respectively. The results are given in Table 3.5.4. Percent recovery from mustard seeds is low because the oily fraction present in the seeds being co-extracted with the analyte trap the analyte molecules and as a result their percent recovery is affected. The results of percent recoveries indicate that the proposed method can be effectively applied for residue analysis of isoproturon in different cereals and grains.

Reference

1. T. Kovalczuk, J. Poustka, J. Hajslova, "HPLC-MS/MS method for analysis of isoproturon in difficult matrix: poppy seeds", Czech Journal of Food Sciences, 26, 146-152 (2008).

CONCLUSIONS

A spectrophotometric method using batch analysis, a flow injection spectrophotometric methods and a spectrofluorimetric method have been developed for determination of locally used herbicides like glyphosate, fenoxaprop-p-ethyl, pinoxaden and isoproturon and were applied for the analysis of samples of environmental importance.

The method developed for determination of glyphosate herbicide is simple and sensitive and is based on the formation of dithiocarbamic acid from glyphosate followed by the reaction of dithiocarbamic acid with copper (II) to form a yellow coloured complex. The method is applicable to environmental samples and is comparable in terms of sensitivity and reproducibility with literature methods.

The flow injection spectrophotometric method developed for glyphosate herbicide is based on formation of a yellow coloured condensation adduct in alkaline media. The method has low detection limit and high sample throughput. The method is applicable for determination of glyphosate in wheat grains with recoveries in the range of 90.0 ± 0.65 % to 93.0 ± 0.66 %. The proposed method is fast, simple and reproducible as compared to gas chromatographic and liquid chromatographic methods.

Flow injection spectrophotometric method developed for determination of fenoxaprop-p-ethyl herbicide is based on the formation of hydroxamate salt from fenoxaprop-p-ethyl and its subsequent reaction with iron (III) to form a red coloured complex in acidic medium. The method has low detection limit and high sample throughput and is applicable to environmental samples and has been applied to wheat and barley grains with recoveries in the range of 88.0 ± 1.7 % to 92.0 ± 1.0 %. The proposed method is fast, simple, sensitive and reproducible.

The FI spectrophotometric method developed for determination of PXD herbicide is based on conversion of PXD to hydroxamate and further reaction of hydroxamate with iron (III) for coloured complex formation. The method is applicable to environmental samples and applied to soil, wheat and tap water. The proposed method for the

determination and quantification of PXD herbicide is simple, sensitive and reproducible and can be applied in routine laboratories for analysis of PXD.

Spectrofluorimetric method developed for determination of isoproturon is based on the formation of a fluorescent product from isoproturon through derivatization with 2-CAA in the presence of ammonia. The method has been applied to cereals and seeds of cash crops with recoveries in the range of 85.0 ± 1.2 % - 96.0 ± 0.5 %. Interference effects of various related compounds have been investigated on the determination of IPU and the method was found to be free of interferences.

The investigated methods involve basic chemical reactions for which the proposed reaction mechanisms have been discussed. This opens a door especially for the community of organic chemists to isolate, identify and characterize the intermediates and the final products.

The methods can also be employed with certain modifications to analyze pesticides and drugs having the same or similar chemistry.