12-1999

Trace-Level Determination of Fluorescently Labeled Phenoxy Acid Herbicides and the Study of Their Crop Uptake and Soil Accumulation

Noura Saeed Al Nuaimi

Follow this and additional works at: http://scholarworks.uaeu.ac.ae/all_theses

Part of the Environmental Sciences Commons

Recommended Citation
TRACE-LEVEL DETERMINATION OF FLUORESCENTLY LABELED PHENOXY ACID HERBICIDES AND THE STUDY OF THEIR CROP UPTAKE AND SOIL ACCUMULATION

A thesis

Submitted to the Faculty of Science the United Arab Emirates University in partial fulfillment of the requirement for the Degree of Master of Science

By

Noura Saeed Al Nuaimi
B.Sc. in Science, Major Chemistry
Faculty of Science, UAE University (1997)

United Arab Emirates University
Faculty of Science
December 1999
Title: Trace-level determination of Fluorescently labeled phenoxy acid herbicides and the study of their crop uptake and soil accumulation

Name: Noura Saeed Al Nuaimi

SUPERVISORS

<table>
<thead>
<tr>
<th>Name</th>
<th>Position</th>
<th>Signature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prof. Mustafa M. Kamal</td>
<td>Professor of analytical Chemistry, Department of chemistry, Faculty of Science, United Arab Emirates University</td>
<td></td>
</tr>
<tr>
<td>Dr. Yehia S. Mechref</td>
<td>Lecturer of Analytical Chemistry, Department of Chemistry, Faculty of Science, United Arab Emirates University</td>
<td></td>
</tr>
<tr>
<td>Dr. Wajdi M. Zoghaib</td>
<td>Lecturer of organic Chemistry, Department of Chemistry, Faculty of Science, United Arab Emirates University</td>
<td></td>
</tr>
</tbody>
</table>
The Thesis of Noura Saeed S.D. Al-Naimi for the Degree of Master of Science in Environmental Sciences is Approved.

Chair of Committee, Prof. Mustafa M. Kamal

Examining Committee Member, Dr. Meshgan M. Al-Awar

Examining Committee Member, Dr. M. S. Ibrahim

Dean of The Faculty of Science, Prof. A. S. Al-Sharhan

United Arab Emirates University
1999
TABLE OF CONTENTS

CHAPTER 1
1.1 INTRODUCTION
1.2 EXPERIMENTAL METHODS
1.3 RESULTS AND DISCUSSION
1.4 CONCLUSIONS

CHAPTER 2
2.1 EXPERIMENTAL METHODS
2.2 RESULTS AND DISCUSSION
2.3 CONCLUSIONS

APPENDIX A
A.1 SUPPLEMENTARY MATERIAL

INDEX

ABBREVIATIONS

LIST OF FIGURES

LIST OF TABLES
# TABEL OF CONTENTS

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGMENT</td>
<td>i</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>ii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>vi</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>vii</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>x</td>
</tr>
</tbody>
</table>

## CHAPTER I (INTRODUCTION)

1.1. MODE OF ACTION OF HERBICIDES ........................................ 5
1.2. DEGRADATION OF PHENOXY ACID HERBICIDES ...................... 6
1.3. SORPTION/DESORPTION OF HERBICIDES ......................... 8
1.4. METHOD OF ANALYSIS OF PHENOXY ACID HERBICIDES ............. 12
   1.4.1. Gas Chromatography (GC) .................................. 12
   1.4.2. Liquid Chromatography (LC) ............................. 15
   1.4.3. High Performance Liquid Chromatography (HPLC) ..... 17
   1.4.4. High Performance Capillary Electrophoresis ......... 20
   1.4.5. Immunoassay ........................................... 21
1.5. DERIVATIZATION .................................................. 21
   1.5.1. Derivatization for GC .................................. 21
   1.5.2. Derivatization for HPLC ................................ 22
   1.5.3. Derivatization for CE .................................. 20
1.6 AIM OF THE WORK ............................................... 24

## CHAPTER II (EXPERIMENTAL METHOD)

2.1. REAGENTS AND MATERIALS ....................................... 26
2.2. INSTRUMENTATION ............................................. 30
2.3. PROCEDURE .................................................. 31
   2.3.1. Derivatization (general procedure) .................... 31
   2.3.2. Soil Treatment ......................................... 31
   2.3.3. Plant Treatment ........................................ 32
   2.3.4. Separation Conditions ................................ 32
## Chapter III (Results and Discussions) .................................................. 35

### 3.1. UV-Absorption of Phenoxo Acid Herbicides ................................................. 35

### 3.2. HPLC Separation of Underivatized Phenoxo Acid Herbicides ...................... 35
   - 3.2.1. Isocratic separation conditions of phenoxo acid herbicides ..................... 35
   - 3.2.2. Gradient separation conditions of phenoxo Acid herbicides ..................... 41

### 3.3. Derivatization .......................................................... 45
   - 3.3.1. Optimization of derivatization conditions .................................. 47
   - 3.3.2. Assessment of derivatization .................................................. 51
   - 3.3.3. Mass Spectrometry ................................................................. 51
   - 3.3.4. Monitoring the progress of the reaction ........................................ 53
   - 3.3.5. Optimization of separation conditions of 2-aminobenzamide labeled phenoxo acid herbicides .......................................................... 57
   - 3.3.6. Using different derivatizing agents ............................................ 63

### 3.4. Applications of the Derivatization Method .............................................. 69
   - 3.4.1. Determination of Phenoxo acid herbicides movement through soil .......... 69
   - 3.4.2. Determinations of phenoxo acid herbicides in roots and leaves .......... 77

### Conclusions .................................................................................. 80

### References ................................................................................... 83

### Arabic Summary
ACKNOWLEDGEMENT

This study is the result of the generous help of many individuals. I would like to extend a special thanks to Dr. I. A. Al-Shamsi, Head of the Department of Science and Mathematics, for allowing me the chance to join the Department of Science and Mathematics. I also appreciate the valuable help and support from the Laboratory Manager, M. Kamal, and the rest of the students, especially M. Hashim. I would like to thank my family and friends for their understanding and support during the research.
Acknowledgement

This study is the result of dedicated effort of many individuals, several of whom deserve special mention: I thank Prof. Dr. Abdul Rahman S. Al-Sharhan, Dean of the Faculty of Science, UAE university, for offering the chance to join the Environmental Science Master Program. I greatly appreciate my supervisors Prof. Mustafa M. Kamal, Dr. Yehia S. Mechref and Dr. Wajdi M. Zoghaib Faculty of Science, Chemistry Department, UAE University for their effort in the completion of this study.

I am extremely grateful to Dr. Yehia S. Mechref for his suggestion, continuous guidance, and supervision throughout the study. I thank my colloquies in chemistry labs, Faculty of Science, UAE University for their valuable cooperation.

I also appreciate the valuable input and support from the Center Laboratories Unit (CLU). I especially thank Mr. Mohamed F. Bedair for his help and support throughout the study.

I thank my family and friends for their understanding, support, and encouragement.
ABSTRACT

A challenging problem facing the determination of phenoxy acid herbicides in samples is their low UV absorptivity and the interference associated with the matrix in which these analytes exist. This thesis is concerned with improving the detection limits of this group of herbicid as well as reduce or eliminate interference associated with the sample matrix.

Native phenoxy acid herbicides could be analyzed by high performance liquid chromatography (HPLC) with ultraviolet detection. Phenoxy herbicides were analyzed by HPLC employing a gradient conditions that allowed baseline resolution of all analytes used in this study in approximately 20 min. However, the average detection limit of these herbicides using such conditions and a UV detector was only $0.5 \times 10^{-6}$ M. This detection limit was higher than the concentration at which these herbicides exit environmentally.

The procedure developed in this study for improving the detection limit of the studied herbicides involved the derivatization of analytes with a fluorescing derivatization reagents such as 2-aminobenzamide and 1-aminonaphthol-4-sulfonic acid. The procedure was based on the coupling between the carboxylic acid group of the analytes and the amine group of the derivatization reagent. As a result of this coupling an amide bond was formed between the analytes and the derivatization reagents.
procedure required the utility of a condensation agent such as benzatriazol-1-yloxy tris (dimethylamino) phosphonium hexafluorophosphate (BOP). The utility of this condensation reagent allowed the completion of the labelling in approximately 40-50 min for all analytes. The labelling with these derivatization reagent allowed better detection limit as well as separation. Initially, 2-aminobenzamide was used as a derivatization reagent and the derivatization for all analytes was determined to be efficient as was deduced from HPLC and mass spectrometric analysis. However, the derivatization with this derivatization reagent reduced the resolution of the separation and varying the separation conditions did not result in attaining baseline resolution of all analytes in a single analysis. Therefore, l-aminonaphthol-4-sulfonic acid was used instead as a derivatization reagent and it proved to be more effective in providing baseline resolution for all analytes. l-aminonaphthol-4-sulfonic acid derivatized herbicides were baseline resolved by HPLC and the average detection limits of all analytes was approximately 1 x 10^{-8} M. This is a two order of magnitudes improvement over the detection limit of native phenoxy acid herbicides by UV detector. Moreover, the separation of those labelled phenoxy acid herbicides was attained in shorter time. The separation of labelled herbicides was achieved in less than 12 min relative to the 20 min needed for the separation of native phenoxy acid herbicides.
This developed procedure was utilized for studying the uptake of crops and soil accumulation for phenoxy acid herbicides. First, the movement of phenoxy acid herbicides through the UAE soil was conducted employing this developed procedure. The method allowed the sensitive monitoring of the movement of these phenoxy in the UAE soil. The study was conducted to monitor the movement of these phenoxy acid herbicides under field conditions. The method demonstrated the weak interaction between the analytes and the UAE soil since the highest concentration of these herbicides were determined to exist in the bottom layer, thus supporting the expected weak interaction between the analytes and the soil.

Second, the method was also employed to study the uptake of plants for these phenoxy acid herbicides. The uptake of plants for these herbicides could not be monitored by UV detectors due to high interference associated with the matrix in which the final sample existed. Using the developed method substantially eliminated the interference and allowed the determination of herbicides in the roots and leaves of plants treated with these herbicides. The study demonstrated differential uptake of plants to these herbicides as was dictated by the amount determined in the leaves and roots.

The developed method improved detection limits by two orders of magnitudes, eliminated interference associated with sample matrix, allowed
the studying of movement of phenoxy acid herbicides through UAE soil, and permitted the studying of plant uptake of the phenoxy herbicides.
LIST OF TABLES, FIGURES AND ABBREVIATIONS
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Types Of Herbicides</td>
</tr>
<tr>
<td>1.2</td>
<td>Class Of Phenxoyalkanoic Acid Herbicides</td>
</tr>
<tr>
<td>3.1</td>
<td>Retention times of the analytes depicted in Figure 3.2</td>
</tr>
<tr>
<td>3.2</td>
<td>Retention times of the analytes depicted in Figure 3.3</td>
</tr>
<tr>
<td>3.3</td>
<td>Gradient separation conditions</td>
</tr>
<tr>
<td>3.4</td>
<td>Gradient separation conditions of Fig. 3.12</td>
</tr>
<tr>
<td>3.5</td>
<td>Gradient separation conditions of Fig. 3.13</td>
</tr>
<tr>
<td>3.6</td>
<td>Gradient separation conditions of Fig. 3.14</td>
</tr>
<tr>
<td>3.7</td>
<td>Gradient separation conditions of Fig. 3.17</td>
</tr>
<tr>
<td>3.8</td>
<td>Gradient separation conditions of Fig. 3.18</td>
</tr>
<tr>
<td>3.9</td>
<td>Gradient separation conditions of Fig. 3.19</td>
</tr>
<tr>
<td>3.10</td>
<td>The concentration of herbicides in different layers of soil</td>
</tr>
<tr>
<td>3.11</td>
<td>Concentration of herbicides in leave and root extract with the herbicides</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Structure of phenoxy acid herbicides</td>
<td>27</td>
</tr>
<tr>
<td>2.2</td>
<td>The structure of derivatizing agent</td>
<td>28</td>
</tr>
<tr>
<td>2.3</td>
<td>The structure of BOP reagent</td>
<td>29</td>
</tr>
<tr>
<td>2.4</td>
<td>Derivatization pathway</td>
<td>34</td>
</tr>
<tr>
<td>3.1</td>
<td>UV-Spectrum of 2,4-D</td>
<td>36</td>
</tr>
<tr>
<td>3.2</td>
<td>Chromatograms of phenoxy acid herbicides separated isocratically utilizing a 75% acetonitrile and 25% water mobile phase</td>
<td>38</td>
</tr>
<tr>
<td>3.3</td>
<td>Chromatograms of phenoxy acid herbicides separated isocratically utilizing a 50% acetonitrile and 50% water mobile phase</td>
<td>39</td>
</tr>
<tr>
<td>2.4</td>
<td>Chromatograms of herbicides and 2-aminobenzamide reagent separation conditions as in Figure 3.3</td>
<td>40</td>
</tr>
<tr>
<td>3.5</td>
<td>Chromatogram of underivatized phenoxy acid herbicides separated using the gradient program summarized in Table 3.3</td>
<td>44</td>
</tr>
<tr>
<td>3.6a</td>
<td>Chromatogram of 2-(4-chlorophenoxy)propionic acid derivatized with 2-aminobenzamide in acetonitril and triethylamine (TEA)</td>
<td>48</td>
</tr>
<tr>
<td>3.6b</td>
<td>Chromatogram of 2-(4-chlorophenoxy)propionic acid derivatized with 2-aminobenzamide in acetonitril and triethanolamine (TOHA)</td>
<td>48</td>
</tr>
<tr>
<td>3.7a</td>
<td>Chromatogram of 2-(4-chlorophenoxy)propionic acid derivatized with 2-aminobenzamide in methanol and TEA</td>
<td>50</td>
</tr>
<tr>
<td>Figure</td>
<td>Page</td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>------</td>
<td></td>
</tr>
<tr>
<td>3.7b</td>
<td>Chromatogram of 2-(4-chlorophenoxy)propionic acid derivatized with 2-aminobenzamide in dichloromethane and TEA</td>
<td>50</td>
</tr>
<tr>
<td>3.8</td>
<td>Mass spectrum of 4-(2,4-dichlorophenoxy)butyric acid derivatized with 2-aminobenzamide</td>
<td>52</td>
</tr>
<tr>
<td>3.9a</td>
<td>Chromatogram of 2-(4-phenoxy)propionic acid derivatized with 2-aminobenzamide</td>
<td>55</td>
</tr>
<tr>
<td>3.9b</td>
<td>Chromatogram of dichlorprop derivatized with 2-aminobenzamide</td>
<td>55</td>
</tr>
<tr>
<td>3.10</td>
<td>Chromatogram of phenoxy acid herbicides labeled with 2-aminobenzamide</td>
<td>56</td>
</tr>
<tr>
<td>3.11</td>
<td>Chromatogram of 2-AB labeled phenoxy acid herbicides separated isocratically</td>
<td>58</td>
</tr>
<tr>
<td>3.12</td>
<td>Chromatogram of 2-AB labeled phenoxy acid herbicides separated using different gradient system</td>
<td>59</td>
</tr>
<tr>
<td>3.13</td>
<td>Chromatogram of 2-AB labeled phenoxy acid herbicides utilizing non-linear gradient</td>
<td>60</td>
</tr>
<tr>
<td>3.14</td>
<td>Chromatogram of 2-AB labeled phenoxy acid herbicides utilizing gradient program</td>
<td>61</td>
</tr>
<tr>
<td>3.15</td>
<td>Chromatogram of 2-AB labeled phenoxy acid herbicides utilizing different C18 column</td>
<td>62</td>
</tr>
<tr>
<td>3.16a</td>
<td>Chromatogram of 2-(4-chlorophenoxy)propionic acid derivatized with 8-aminonapthalene-1-sulfonic acid</td>
<td>65</td>
</tr>
<tr>
<td>3.16b</td>
<td>Chromatogram of 2-(4-chlorophenoxy)propionic acid derivatized with 1-aminonapthalol-4-sulfonic acid</td>
<td>65</td>
</tr>
<tr>
<td>3.17</td>
<td>Chromatogram of 1-aminonapthalol-4-sulfonic acid derivatized phenoxy acid herbicides</td>
<td>66</td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
<td>Page</td>
</tr>
<tr>
<td>--------</td>
<td>-----------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>3.18</td>
<td>Chromatogram of ANOHS labeled phenoxy acid herbicides</td>
<td>67</td>
</tr>
<tr>
<td>3.19</td>
<td>Chromatogram of ANOHS labeled phenoxy acid herbicides under different separation conditions</td>
<td>68</td>
</tr>
<tr>
<td>3.20a</td>
<td>Chromatogram of soil extracted from layer No.1</td>
<td>71</td>
</tr>
<tr>
<td>3.20b</td>
<td>Chromatogram of soil extracted from layer No.2</td>
<td>71</td>
</tr>
<tr>
<td>3.20c</td>
<td>Chromatogram of soil extracted from layer No.3</td>
<td>72</td>
</tr>
<tr>
<td>3.20d</td>
<td>Chromatogram of soil extracted from layer No.4</td>
<td>72</td>
</tr>
<tr>
<td>3.20e</td>
<td>Chromatogram of soil extracted from layer No.5</td>
<td>73</td>
</tr>
<tr>
<td>3.20f</td>
<td>Chromatogram of soil extracted from layer No.6</td>
<td>73</td>
</tr>
<tr>
<td>3.21</td>
<td>Graph illustrating the change in the amount of phenoxy acid herbicides in soil layers</td>
<td>75</td>
</tr>
<tr>
<td>3.22</td>
<td>Chromatogram of soil extracted from layer No.6 derivatized and obtained with fluorescence detector</td>
<td>76</td>
</tr>
<tr>
<td>3.23a</td>
<td>Chromatogram of root extract, derivatized and obtained with fluorescence detector</td>
<td>78</td>
</tr>
<tr>
<td>3.24b</td>
<td>Chromatogram of leaves extract, derivatized and obtained with fluorescence detector</td>
<td>78</td>
</tr>
</tbody>
</table>
LIST OF ABBREVIATION

2,4-D: [(2,4-dichlorophenoxy)acetic acid]

2,4,5-T: [2,4,5-trichlorophenoxy]acetic acid]

2,4-DB: [2,4-dichlorophenoxy]butyric acid]

Dichlorprop: [2-(2,4-dichlorophenoxy)propionic acid]

Silvex: [2-(2,4,5-trichlorophenoxy)propionic acid]

2-AB: 2-aminobenzamide

ANOHS: 1-amino-2-napthol-4-sulphonic acid

BOP: Benzotriazolyloxytris(dimethylamino)phosphonium hexafluorophosphate

TEA: Triethyleamine

TFA: Trifluoroacetic acid

TEOHA: Triethanolamine

2,4-DP: 2-(2,4-dichlorophenoxy)propionic acid

2,4,6-T: [2,4,6-trichlorophenoxy]acetic acid]

2-(4-CP)PA: 2-(4-chlorophenoxy)propionic acid

4-(2,4-DCP)BA: 4-(2,4-dichlorophenoxy)butyric acid

MCPA: 4-chloro-2-methylphenoxy acetic acid

Mecoprop: 2-(4-chloro-2-methylphenoxy)propionic acid

2,4,5-TP: 2-(2,4,5-trichlorophenoxy)propionic acid
MCPP: 2-(4-chloro-2-methylphenoxy)propionic acid

MCPB: 4-(4-chloro-2-methylphenoxy)butyric acid

ADAM: 9-antryldiazomethane

ANDSA: 7-aminonaphthalene-1,3-disulfonic acid

DT$_{50}$'s: Estimated time for disappearance of 50% of initial concentration

SOM: Soil organic matter

DOM: Dissolved organic matter

GC: Gas chromatography

LC: Liquid chromatography

HPLC: High performance liquid chromatography

HPEC: High performance capillary electrophoresis

EPA: Environmental protection agency

ECD: Electron-capture detector

ELCD: Electrolytic conductivity detector

ITD: Ion trap detection

SPE: Soil phase extraction

GC-MS: Gas chromatography/mass spectrometry

SIM: Selected ion monitoring

EDIST: Electrodialysis sample treatment
**RPLC-DAD-PB-MS:** Reverse phase liquid chromatography-diode array detection particle beam mass spectrometry

**OSP2:** On line solid phase extraction

**ESP-MS:** Electro spray mass spectrometry

**TSP-MS:** Thermospray mass spectrometry

**LC/MS:** Liquid chromatography/mass spectrometry

**ES:** Electrospray

**CCFA:** Completely continuous-flow analysis

**SAX:** Strong anion exchanger

**PLRP-S:** Polymeric precolumn

**MECC:** Micellar electrokinetic capillary chromatography

**CZE:** Capillary zone electrophoresis

**CCE-LIF:** Capillary electrophoresis/laser-induced fluorescence
CHAPTER I
INTRODUCTION

Advocates can be defined in several ways. These include

The introduction of this chapter will be focused on the common name. Advocates are usually defined as

1. How they are used or consumed. 2. Where they are found. 3. How they are

characteristics. This chapter will be focused on the common name. Advocates are usually defined as

or more accurately is classified under classification or sub-group in listed in Table 1. They are grouped in different ways, which

helping to in understanding the regulations and/or from the point of

practice. Practically, this property of translation allows the use of the

low volume method to kill and systems of pest and plant effects. This

application (Ashton et al. 1998).
CHAPTER I
INTRODUCTION

Herbicides can be classified in several ways. These include classification based on the common name, functional groups of compound, and how they are used to produce optimum results (or according to physiological characteristics) (Ashton F.M. & Crafts A.S., 1981). Herbicides according to their functional group are summarized in Table 1.1 (Ashton F.M. & Crafts A.S., 1981).

This thesis is concerned with phenoxy acid herbicides which comprise a family of phytotoxic substances that are used in the form of the parent acids, or more commonly as salt and ester (classification of phenoxy acid herbicides is listed in Table 1.2). They are growth regulators with hormone-like activity. That is, at relatively low concentration dosage, they bring about growth responses in regions distant from the point of application. Practically, this property of translocation allows their use by the low volume method to kill root systems of perennial weeds by foliar application (Ashton F.M. & Crafts A.S., 1981).
<table>
<thead>
<tr>
<th>Herbicide</th>
<th>Example</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inorganic compounds</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Halokanoic acids</td>
<td>Copper Sulfate</td>
<td>This type is very active against grasses inhibiting growth and causing chlorosis and necrosis of the leaves</td>
</tr>
<tr>
<td></td>
<td>Dalapon</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aromatic acids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amides</td>
<td>Dalapon</td>
<td>This type is primarily soil acting especially against annual grasses</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitriles</td>
<td></td>
<td>This herbicide is soil acting where it controls germinating annual weeds and buds of perennial weeds, it also may be used for aquatic weed control</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anilides</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitrophenols</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitrophenyl Ethers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitroanilines,</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbamates</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ureas</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heterocyclic Nitrogen compounds-Triazines</td>
<td>Atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine)</td>
<td>This herbicide is both foliar and soil acting being taken up both by leaves of emergent weeds and by the roots of weed seedlings emerging after spraying</td>
</tr>
<tr>
<td>Heterocyclic Nitrogen compounds - Pyridines</td>
<td>Paraquat (1,1’-dimethyl-4,4’-bipyridinium ion normally formulated as the dichloride)</td>
<td>Destroys photosynthetic tissues and is used for variety of purposes including stubble cleaning, inter-row weed control, desiccation of various crops and killing out old pastures which can then be resown without ploughing</td>
</tr>
<tr>
<td>Heterocyclic Nitrogen compounds - Pyridazines</td>
<td>Norflurazon (4-chloro-5-methylamino-2-(α,α,α-trifluorom-tolyl) pyridazin-3-one)</td>
<td>Weed control in cotton stone fruits and cranberries being particularly active, against grasses and sedges</td>
</tr>
<tr>
<td>Heterocyclic Nitrogen compounds - Pyrimidines (uracils)</td>
<td>Bromacil (5-bromo-3-s-nutyl-6-methyluracil)</td>
<td>They are applied to the soil and absorbed via the roots but eventually inhibit the Hill reaction of photosynthesis causing chlorosis and death</td>
</tr>
</tbody>
</table>

**Continued**
<table>
<thead>
<tr>
<th>Herbicide</th>
<th>Example</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heterocyclic Nitrogen compounds Unclassified</td>
<td>Aminotrizole</td>
<td>Active against annual and perennial weeds where it inhibits regulators from buds, this group of compounds has a variety of chemical structures and type of herbicidal activity</td>
</tr>
<tr>
<td>Heterocyclic compounds Other Heteroatoms</td>
<td>Endothal (7-oxabicyclo(2,2,1)-heptone-2,3-dicarboxylic acid)</td>
<td>used as pre- and post-emergence for control of weeds beet and spinach</td>
</tr>
<tr>
<td>Organoarsenic compound,</td>
<td>Cacodylic acid (dimethyl arsinic acid)</td>
<td>Used for non selective post-emergence on non crop areas, for pasture renovation, as a desiccant and defoliant for cotton and for killing trees by injection,</td>
</tr>
<tr>
<td>Organophosphorus compounds</td>
<td>Bensulide (O,O-di-isopropyl-S-2-phenyl-sulphonylaminoethyl phosphorodithioate)</td>
<td>used as pre-emergence control of annual grasses and broad leave weeds in lawns</td>
</tr>
<tr>
<td>Unclassified compounds</td>
<td>Allyl alcohol</td>
<td>Used for weeding forestry nursery beds</td>
</tr>
<tr>
<td>Phenoxyalkanoic Acid</td>
<td>2,4-D [(2,4-dichlorophenoxy )acetic acid]</td>
<td>They are subdivided into several classes, as illustrated in Table II</td>
</tr>
<tr>
<td>Class</td>
<td>Example</td>
<td>Remarks</td>
</tr>
<tr>
<td>-----------------------</td>
<td>----------------------------------------------</td>
<td>-------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Phenoxy Acetic</td>
<td>2,4-D [(2,4-dichlorophenoxy)acetic acid] and 2,4,5-T [(2,4,5-trichlorophenoxy)acetic acid]</td>
<td>2,4-D: Used for selective herbicides in fields such as charlock, annual nettle and corn butter. 2,4,5-T: Used against woody species.</td>
</tr>
<tr>
<td>Phenoxy Butyric</td>
<td>2,4-DB [(2,4-dichlorophenoxy) butyric acid]</td>
<td>Treatment of many varieties of lucerne especially at seedling stages.</td>
</tr>
<tr>
<td>Phenoxy propionic</td>
<td>Dichloroprop [(2,4-dichlorophenoxy)propionic acid] and Silvex [(2,4,5-trichlorophenoxy)propionic acid]</td>
<td>Dichloroprop: Used as post-emergence, translocated herbicide for use in cereals for the control of a number of weeds such as chick weed, cleavers and other broad-leaved weeds.</td>
</tr>
</tbody>
</table>
1.1 MODE OF ACTION OF HERBICIDES

Numerous biochemical and metabolic changes in plants are induced by phenoxy acid herbicides. These changes effect plant structure and growth. One of these changes involves dedifferentiation and initiation of cell division in certain mature cells and inhibition of cell division in primary meristems (Ashton F.M. & Crafts A.S., 1981).

The growth response suggests that nucleic acid metabolism and the metabolic aspect of cell wall plasticity are most relevant to the mechanism of action of phenoxy herbicide. For example, the primary effect of low levels of 2,4-D on nucleic acid synthesis appears to be the stimulation of RNA polymerase and the subsequent stimulation of RNA and protein synthesis. Low levels of 2,4-D induce cell enlargement by increasing the activity of the autolytic and synthetic enzymes responsible for cell wall loosening and synthesis of new cell wall material. The abnormal stimulation of these processes by low levels of 2,4-D leads to uncontrolled growth, however the high levels of 2,4-D inhibit growth (Ashton F.M. & Crafts A.S., 1981).
1.2 DEGRADATION OF PHENOXY ACID HERBICIDES

Degradations of phenoxy acid herbicides in plant and soil are different. The degradation of phenoxy acid herbicides in plant involves: (1) degradation of the acetic acid side chain, (2) hydroxylation of the aromatic ring, and (3) conjugation with plant constituent (Flectcher W.W. & Kirkwood R. C., 1982).

Side chain degradation appears to be widespread in plants, but may be of major importance in only a few species in which it is sufficiently rapid and extensive to influence selectivity. Such species include red currant. Ring hydroxylation occurs generally at position (4) if it is unsubstituted (e.g. 2,6-CPA) or at position (3) if position (4) is already substituted (e.g. 2,4,6-T) (Flectcher W.W. & Kirkwood R. C., 1982).

Degradation is one of the main pathways by which pesticides are removed from soils (Bolan N. S. & Baskaran S., 1996). It has been established that the breakdown of phenoxy herbicides is the result of microbiological mechanisms. The bacteria *chromobacter*, *Alcaligenes*, *Arthrobacter*, *Bordetella*, *Flavobacterium*, *Pseudomonas*, and *Xanthobacter* have been isolated from soils and shown to degrade 2,4-D and MCPA in liquid nutrient media (Smith E. et al., 1994).
An increase of soil microbial activity is likely to increase the rate of degradation of pesticides. However, the importance of microbial aspects in the degradation of many pesticides remains to be elucidated because soil microbial indication, such as biomass and activity, have rarely been used regularly in studies involving pesticides transformation in soil (Bolan N. S. & Baskaran S., 1996). It was also concluded from research by several investigators that, the quantity of phenoxy herbicides reaching the soil from weed control would probably not have a serious effect on most soil microorganisms (Bovey R. W. & Young A. L., 1980).

Johnson and co-workers studied the degradation of 2,4-D in addition to its sorption and mobility on surface soils and two sub-soil layers from rice-producing areas of Arkansas (Johnson W. G. et al., 1995). They are reported that the dissipation rate constant for 2,4-D depended on time of incubation, nature of soil, temperature, and water potential. The dissipation of 2,4-D was more rapid under wet than dry soil conditions. They also found that, the dissipation rate was slower in sub soils than in surface soils. Moreover, the degradation of 2,4-D was faster by sunlight with DT50 's (Estimated time for disappearance of 50% of the initial concentration) of 1 to 12 h in aqueous solutions (Johnson W. G. et al., 1995).
Sorption and desorption are key processes controlling herbicide dissipation and efficiency in soil as well as the potential for contamination of ground and surface waters. Sorption refers to surface induced removal from solution, whether by adsorption, absorption, or precipitation. While desorption refers to the attraction and accumulation of molecules at the soil-water or soil-air interface resulting in molecules layers on the surface of soil particles (Harper S. S., 1994). The bioavailability of pesticide in soil is a function of the sorption desorption equilibria, because sorbed material can only be metabolized after desorption, when it becomes dissolved in soil solution (Carton A. et al., 1997).

There are different factors which affect the sorption desorption of pesticides. Soil organic matter (SOM) is one of the most important soil components in the sorption of pesticides and other organic pollutants. However, the mechanisms involved in sorption have not been clearly demonstrated, because it was difficult to isolate unaltered SOM and characterizing its chemical and physical properties (Benoit P. et al., 1996).

Sorption also affects the chemical's spatial distribution and availability to microorganisms of a chemical substance and is highly correlated to organic content and clay percentages in soil profile. The formation of slowly reversible or restricted sites within the soil system
limits the availability of the chemical or biological degradation, especially in cases where biodgradation occurs in the solution phase (Workman S. R. & Nokes S. E., 1998).

Sorption of organic pollutants on humic substances may involve several mechanisms simultaneously. The type of interaction depends on the molecular properties of the pollutant, i.e. polarity, ionizability, hydrophobicity, and on the characteristics of the humic substance, i.e. macromolecular structure, density, distribution and accessibility of different functional groups. Humic macromolecules have polar constituents with carboxylic, phenolic and amino groups, and hydrophobic constituents with aromatic and alkyl chains. Both constituents are potential sites for the sorption of organic matter (or pollutants) at the surface or inside the humic macromolecules, depending on the structural arrangement and the physicochemical environment, i.e. pH and ionic strength (Benoit P. et al., 1996).

In soils both adsorption of organic pesticides and the biological activity increase with an increase in the organic matter content. These two factors have opposite effects on the degradation of pesticides. Adsorption has often been shown to decrease the degradation rate of organic chemicals by reducing their availability to microbial attack (Bolan N. S. & Baskaran S., 1996).
The structure of soil has recently been shown to be a significant factor in herbicide sorption and subsequent leaching to ground water. In addition to the chemistry and structure of soil itself, the soil environment affects the sorption of herbicides. It is often assumed that adsorption is an exothermic process, whereby an increase in temperature would lead to decreased adsorption and increased desorption. However, thermodynamic studies of sorption have shown a highly variable relationship to temperature due to the complexity of the soil environment. An increase in temperature may cause an increase, a decrease or no change in sorption (Harper S. S., 1994).

In plants, herbicide interaction with plant residue has practical implications in terms of herbicide release to soil for target plant uptake. Herbicide binding can be weak or strong depending on the type and degree of decomposition of plant cover residue. Increased herbicide retention by plant residue not only reduces the amount reaching the soil, but also prolongs herbicide persisting. The latter could provide season long weed control from herbicide desorbed from plant residues over a period of time. However, the residue persistent beyond the crop season can potentially injure rotational crops including vegetables. Herbicide retention by plant residue can also minimize herbicide runoff and leaching losses (Reddy K. N. et al., 1995).
Adsorption/desorption characteristics are important in predicting pesticides mobility and fate in aquatic ecosystems. Pesticide sorption affects other processes that determine the final fate of these compounds in the aquatic environment (transport, degradation, volatilization, bioaccumulation, etc.) and also controls the persistence and irreversible binding of the pesticide. All these processes influence the possible effects of pesticides on non-target organisms in the aquatic environment. Moreover, sediments are saturated with pore-water which acts as a transport medium and distributing phase for pesticides. In addition, pore-water contains dissolved organic matter (DOM), colloids, and other macroorganic molecules, which can bind pesticides resulting in facilitated transport and enhanced bioavailability of these chemicals (GAO J. P., 1998).

Sorption of herbicides to soil occurs through a number of mechanisms involving varying bond strengths and is dependent on the herbicide characteristics, soil surface characteristics, and competing solutes. The rate of sorption is controlled by the rate of herbicide reaction with the soil surface and by the rate of molecular diffusion into the soil particles (Harper S. S., 1994).
1.4 METHODS OF ANALYSIS OF PHENOXY ACID HERBICIDES

Because of the aforementioned facts, determination of phenoxy acid herbicides at low concentration is important. Several methods of analysis have been employed for the detection of phenoxy acid herbicides. These methods include, Gas Chromatography (GC), Liquid Chromatography (LC), High Performance Liquid Chromatography (HPLC), High Performance Capillary Electrophoresis (HPCE), and immunoassay.

1.4.1 Gas Chromatography (GC):

Gas chromatography and Liquid chromatography are the most used analytical techniques for the determination of phenoxy acid herbicide. Phenoxy acid herbicides are non-volatile and, thus can not be analyzed by GC without derivatization. The most used derivatization procedures in environmental applications are methylation of phenoxyalkanoic acid and the preparation of pentafluorobenzyl derivatives (Liska I. & Slobodnik J., 1996). Methylation of phenoxy acid is the method approved by the Environmental Protection Agency (EPA) (EPA 8150/8151) for determination of chlorinated phenoxy herbicides in water by GC with electron- capture detector (ECD) or Electrolytic conductivity detector (ELCD) (US EPA method 8150/8151). The derivitization of phenoxy acids with diazomethane was fast, complete, and required, apart from diethyl
ether removal and a filtration step, no further preparation prior to injection into the GC system (Bucheli T. D. et al., 1997). However, the use of diazomethane has several disadvantages including: its high toxicity and explosive properties, hence, requiring very careful treatment. Moreover, the response of electron-capture detector (ECD) to methyl derivatives is sometimes weak and varies from one chemical to another (Liska I. & Slobodnik J., 1996).

Analysis of several phenoxy acids in soil and cereals was carried out by gas chromatography with the highly sensitive ion trap detection (ITD) after esterification of the acids with BF_3-methanol (Sanchez-Brunete C. et al., 1994). Bucheli et al. employed identification and quantification of some phenoxy herbicide (2,4-D, Dichloroprop, MCPA, mecoprop, 2,4,5-T, and 2,4,5-TP) using GC/MS (Bucheli T. D. et al., 1997). Their method allows the routine and simultaneous determination of phenoxy acids in natural water at the concentration level of low nanogram per liter. Also, their use of isotope-labeled internal standards and internal calibrations allowed for the compensation of possible analyte losses, and permitted high precision and accuracy. A selective solid phase, which allowed excellent recoveries for a variety of polar pesticides, reduced matrix effects, and sequential elution of different pesticide classes was combined with a specific detector, thus permitting unambiguous identification and determination at the low nanogram per liter concentration level. As a result,
the method was able not only to serve as a routine screening tool for the assessment of some of the most widely used herbicides but also proved reliable and sensitive enough to study the fate and behavior of herbicides in various kinds of aquatic environments.

Liang and co-workers evaluated the determination of different herbicides (clopyralid, picloram, and silvex) particularly at 0.0100 μg/g, in Alberta soils of various types, using the Ca(OH)$_2$-water extraction method followed by measurement with GC/ECD (Tan L. K. et al., 1996). Use of Ca(OH)$_2$ and water to extract herbicides precipitates humic acid by the divalent Ca$^{2+}$ ion. Thus emulsion is not formed during the next partition process, which leads to a successful partitioning of the herbicides. However, this analytical method is not reliable for the determination of phenoxy herbicides at 0.0100 μg/g in soils that contain ≥ 5% organic matter (Tan L. K. et al., 1996).

A method for the analysis of more than 30 acidic herbicides in tap, ground, surface and sewage water at the low ng/l level was developed (Th. Heberer S. B. et al., 1993). In this method, water samples are extracted with solid-phase extraction (SPE) using carefully checked RP-C18 material. Recovery rates in this method were of more than 70%, and decent standard deviations for almost all compounds were obtained. Pentafluorobenzyl bromide is used for derivatization of these acidic
herbicides to form the pentafluorobenzylic esters of the acidic herbicides. Detection is performed with capillary gas chromatography/mass spectrometry (GC-MS) with electron impact ionization applying selected ion monitoring (SIM). This method makes recognition possible of 1 to 10 ng of the target compound in one liter of tap water with clear identification (Th. Heberer S. B. et al., 1993).

1.4.2 Liquid Chromatography (LC):

Liquid Chromatography is usually preferred for the analysis of phenoxy acid herbicides (phenoxyalkanoic acids and chlorophenols) in multiresidue on-line technique or off-line methods where no derivatization is required (GAO J. P. et al., 1998). However, analysis by HPLC is less sensitive than GC and requires gradient elution to achieve sufficient resolution.

Groenewegen and co-workers studied the effect of membrane pore size on the clean-up efficiency of electrodialysis sample treatment (EDIST) using several chlorinated phenoxy acids (Groenewegen M. G. M. et al., 1994). The study concluded that, EDIST of permanently charged compounds can be performed without major problems. Similar sample clean-up and analyte concentration can be achieved for weak acids and bases. However, several important aspects have to be taken into account. As the pH of acidified sample increase rapidly owing to proton transport to
the acceptor phase, high enrichment factors for basic compounds become neutral during EDIST.

Another analytical technique in LC involves the use of on-line enrichment reverse phase liquid chromatography –diode array detection particle beam mass spectroscopy (RPLC-DAD-PB-MS). The advantages of on-line trace enrichment procedures include better sensitivity, lower consumption of organic solvents, higher automation potential and simplicity of analysis compared with off-line procedure (Marce R. M. et al., 1995). Moreover, several acidic herbicide were determined using LC-high flow pneumatically assisted electrospray mass spectrometry with negative ionization (Chiron S. et al., 1995). The advantages of this system include low water requirement, the first time use of automated pre-concentration system-On-line Solid Phase Extraction (OSP2)- which is a powerful technique in environmental water analysis. In addition, the use of to Electro Spray Mass Spectrometry (ESP-MS) which provides much better structural information as compared to Thermospray Mass Spectrometry (TSP-MS). This method is useful technique in the identification of unknown acidic herbicides in estuarine water samples (Chiron S. et al., 1995). Micro-flow rate particle beam interface for capillary LC/MS was used also allowing greater sensitivity and much simpler operation (Cappiello A. et al., 1995). Crescenzi and co-workers applied a very sensitive and specific analytical procedure for determining 20 acidic
pesticide in aqueous environmental samples using pneumatically assisted electrospray (ES) LC/MS (Crescenzi C. et al., 1995).

An on-line extraction system with completely continuous-flow analysis (CCFA) prior to LC column was used for determination of chlorinated phenoxy acid herbicides (2,4-D, 2,4,5-T, and silvex) in water samples (Farran A. et al., 1990). Overall, LC is often a method of choice when polar, non-volatile or thermolabile compounds are analyzed (Liska I. & Slobodnik J., 1996).

1.4.3 High Performance Liquid Chromatography (HPLC):

High Performance Liquid Chromatography offers several advantages over aforementioned analytical methods and techniques, which explained previously. It is suitable for heat-sensitive compounds such as phenoxy acids. It is also well known that the potential of many chromatographic techniques is greatly enhanced when they are coupled with mass spectrometry. Unfortunately, the advantages gained by coupling HPLC and mass spectrometry are strongly obstructed by the inherent difficulties found in the ionization of chemically fragile solutes eluted in by a complex liquid mobile phase (Cappiello A.. et al., 1995).

Detection of phenoxy acid herbicides by HPLC has been based mainly on the poor selectivity of UV detection at 230 or 280 nm. This is
due to the fact that many of the compounds inherently present in the sample matrix absorb at these wavelengths. Therefore, in order to detect herbicides in water samples at concentrations lower than 1.0 μg/l, sample enrichment and purification by means of a selective solid-phase extraction cartridge or complicated HPLC system with on-line column-switching valves are needed. As the phenoxy acid herbicides contain carboxyl groups, precolumn fluorescence labeling must be taken into consideration for a sensitive and selective HPLC assay (Suzuki T. & Watanabe S., 1991).

Suzuki and Watanabe (Suzuki T. & Watanabe S., 1991) applied ADAM (9-anthryldiazomethane) to precolumn derivatization for the determination of 2,4-D, MCPA, MCPP, MCPB by reverse-phase HPLC. They evaluated a screening method utilizing ADAM for these herbicides in ground water samples. This method determined phenoxy acid herbicides at levels lower than 0.5 μg/l.

Di corcia and co-workers succeeded in developing a selective and specific HPLC assay for monitoring phenoxy acid herbicides in environmental aqueous samples (Di corcia A. et al., 1989). The extraction and purification of the sample were performed by a single miniaturized cartridge containing in the upper part carbopack and in the lower part a silica-based strong anion exchanger (SAX). Concentration factors higher than 500 were achieved by suitably sizing the trap. Direct injection of a large fraction of the final sample extract into the HPLC column operating
in the reverse-phase mode was possible, as the eluotropic strength of the solvent system for elution of phenoxy acids from the trap was lower than that of the mobile phase for HPLC.

Rene and co-workers applied a novel clean-up technique to a polymeric precolumn (PLRP-S) for the subsequent determination of eight phenoxy acid herbicides (MCPA, MCPP, 2,4-D, MCPB, 2,4,5-T, 2,4,5-TP, and 2,4-DP) in surface water (Rdink R. B. C. et al., 1991). The technique consists of a clean-up with 1000 of 0.1 mol sodium hydroxide solution (pH 12.5) and of a heatcut consisting of four precolumn bed volumes of eluent directed to waste followed by ten precolumn bed volumes of eluent directed to the analytical column (Rdink R.B. C. et al., 1991).

1.4.4 High Performance Capillary Electrophoresis (HPCE):

Both micellar electrokinetic capillary chromatography (MECC) and capillary zone electrophoresis (CZE) have been used to analyze acid herbicides (Mechref Y. & El Rassi Z., 1996) (Brumley W.C. & Brownrigg C.M., 1995) (Hsieh Y.Z. & Huang H. Y., 1997). MECC is preferred due to the similar charge-to-mass ratios of the different acid compounds (Brumley W.C. & Brownrigg C.M., 1995). For CZE, acetate buffers of high concentration (Hsieh Y.Z. & Huang H. Y., 1997) are preferred and have shown to generate reasonable efficiency separation.
Mechref & El Rassi (Mechref Y. & El Rassi Z., 1996), showed the possibilities of detection of phenoxy acid herbicides by a selective derivatization of these herbicides with 7-aminonaphthalene-1,3-disulfonic acid (ANDSA). They separated and detected the ANDSA-phenoxy herbicides by capillary electrophoresis/laser-induced fluorescence (CCE-LIF) at 0.2 ppb a concentration at which environmental herbicide samples are expected to exist. This study showed that, pre-column derivatization was quantitative (99.7% yield) and produced stable derivatives with no side products.

1.4.5 Immunoassay

Immunoassay is relatively new to the field of pesticide analysis. There has been considerable interest in exploring its role in the determination of chemicals. Richman and co-workers developed a rapid, low-cost screen at 10 ppb level for 2,4-dichlorophenoxyacetic acid (2,4-D) in apples, oranges, grapes, potatoes, peaches, and grapefruit. The immunoassay is carried out by diluting an acetonitrile extract produced in support of other analyses with aqueous buffer avoiding a separate extraction, derivatization, and cleanup necessary for GC (Richman S. J. et al., 1996).
1.5 DERIVATIZATION

Derivatization is an important step if sensitivity and selectivity requirements for the determination step are not met. It can be carried out in a pre-column or post-column depending on analysis required and instrumentation availability. Derivatization of phenoxy acid herbicides was achieved using different reagents for GC, HPLC and HPCE.

1.5.1 Derivatization For GC:

Derivatization is performed in gas chromatography (GC) to (Smyth M.R., 1992):

1. increase the volatility of the species of interest,
2. produce derivatives, which give better responses with more selective and sensitive detectors (e.g. ECD), and
3. improve the stability of the analytes.

Phenoxy acid herbicides were pre-column derivatized by using diazomethane as a derivatizing agent (US EPA method 8150/8151) (Bucheli T. D. et al., 1997). The methyl ester derivatives are then determined by GC with ECD or ELCD (US EPA method 8150/8151) or by GC/MS (Bucheli T.D., 1997). A pre-column derivatization of phenoxy acid herbicides using BF₃-methanol as a derivatizing agent was used for
analyses of several phenoxy acids in soil and cereals by gas chromatography with ion trap detection (Sanchez-Brunete C. et al., 1994).

1.5.2 Derivatization For HPLC:

In high performance liquid chromatography, derivatization is mainly used to improve the limit of detection, since polarity and stability of analytes are not as great a problem in HPLC as in GC (Smyth M.R., 1992).

Pre-column derivatization of phenoxy acid herbicides for HPLC is carried out using 9-anthryldiazomethane (ADAM) (Suzuki T. & Watanabe S., 1991).

1.5.3 Derivatization For Capillary Electrophoresis (CE):

Phenoxy acid herbicides were derivatized with 7-aminonaphtalene-1,3-disulfonic acid (ANDSA) and subsequently analyzed by HPCE (Mechref Y. & El Rassi Z., 1996). In CE the separation is based on differential electromigration of the analytes through a capillary filed with a background electrolyte. Therefore, a charged derivatizing agent is required to improve detection as well as separation. The derivatization of phenoxy acid herbicides with ANDSA replaced the weakly ionized carboxylic acid group with a strongly ionized sulfonic acid group, thus enhancing the separation of phenoxy acids. Moreover, derivatization introduced a
fluorescing tag to the analytes resulting in an enhancement of the detection (Smyth M.R., 1992).

On the other hand, Brumley and Brownrigg (Jung M. & Brumley, 1995) derivatized phenoxy acid herbicides for their separation by HPCE with 5-(aminoacetamido) fluorescein utilizing the same derivatization technique introduced by Mechref and El Rassi (Mechref Y. & El Rassi Z., 1994). However, derivatization with this tag resulted in the formation of many side products that interfered with separation.
1.6 AIM OF THE WORK

Chemicals of different properties have found their way into the field of chemical weed control. Food crops have suffered less damage and in particular the ravages of serious vector-born diseases have been lessened by chemical applications. One of these chemicals is phenoxy acid herbicides, which are used world wide in agriculture and forestry for controlling the presence of unwanted broad-leaf weed.

The adverse effects of these herbicides in human, health and environment has prompted an intensive research for finding solutions for this problem all over the world. The main goals of this study are:

1. The development of a fast, efficient and selective labeling procedure for the labeling of phenoxy acid herbicides.

2. Increasing the hydrophobicity of the phenoxy acid herbicides by the formation of an amide bond between the carboxylic acid group of the herbicides and the amine group and the hydrophobic labeling reagent, will facilitate the reverse-phase HPLC separation.

3. The amide bond formation will be achieved by the utility of BOP coupling reagent, which allow the completion of the reaction between 40-50 min.

4. The improved detection sensitivity of the herbicides will permit the accurate and sensitive determination of the crop uptake and soil accumulation of these herbicides.
These goals will be achieved through:

1. Optimizing the derivatization conditions by testing several solvents and reagents.

2. Optimizing the separation conditions by varying the separation conditions.

3. Testing the effectiveness of this technique through its application for the determination of the phenoxy acid herbicides in soil and plant.
CHAPTER II
EXPERIMENTAL METHODS
CHAPTER II
EXPERIMENTAL METHODS

2.1 REAGENTS AND MATERIALS

Phenoxy acid herbicides including (2,4-dichlorophenoxy) acetic acid (2,4-D), 2-(2,4,5-trichlorophenoxy) propionic acid, (2-(2,4-dichlorophenoxy) propionic acid (Dichloroprop) were purchased from Aldrich (Milwaukee, WI). While (2,4,5-trichlorophenoxy) acetic acid (2,4,5-T) and 2- (2,4,5-trichlorophenoxy) propionic acid (Silvex) were obtained from chem Service (West Chester, PA). The structures of these phenoxy acid herbicides are given in Figure 2.1.

The derivatizing agent 2-Aminobenzamide was obtained from Aldrich (Milwaukee, WI). 1-aminonapthol-2-sulphonic acid was purchased from BDH (Chemical limited people, England). The structures of these two derivatizing agent are given in Figure 2.2.

Benzotriazolyloxytris (dimethylamino) phosphonium hexafluorophosphate (BOP) which was used in derivatization reaction was purchased from Sigma Chemical Co (St.Louis, MO). The structure of BOP is given in Figure 2.3. Solvent including methanol, acetonitrile, and dichloromethane were obtained from Riedel-de Haen (Seelze,
Figure 2.1: Structure of Phenoxy Acid Herbicides, common names are between brackets.
**Figure 2.2:** The structure of the derivatizing agents

- 1-Aminonaphthol-4-sulfonic Acid
- 2-Aminobenzamide
Figure 2.3: The structure of BOP Reagent
Germany). Triethylamine (TEA) and trifluoroacetic acid (TFA) were purchased from (Milwaukee, WI).

2.2 INSTRUMENTATION

High Performance Liquid Chromatography (HPLC) was performed with a Waters-Alliance 2690, USA. Waters detectors, UV-Vis 486 and scanning fluorescence detector 474 were utilized for detection of phenoxy herbicides and derivatized phenoxy herbicides respectively. The analytes were chromatographed on Phenomenex (USA) C18-ODS(2) reversed phase packed column (250x4.6mm).

UV-Vis spectra were obtained on UV-Vis scanning spectrophotometer, UV-2101 PC double beam (Shimadzu). Fluorescence spectra were obtained on spectofluorometer, Rf 5000 (Shimadzu). Waterbath B-480, Rotabapor R-124 (Buchi- Switzerland) were utilized for the evaporation of the solvents. Other instruments, used in this thesis include, shaker, orbital Envirm, model 3527-1, and pH-meter model 691 (Metrohm).
2.3 PROCEDURE

2.3.1 Derivatization (general procedure)

Phenoxy herbicides were derivatized according to the following general procedure. 1 equivalent of phenoxy herbicide was dissolved in an appropriate solvent containing 3 equivalents TEA. 10 equivalents of derivatizing reagent and 2 equivalents of BOP were simultaneously added to the solution. The mixture was stirred at room temperature for 50 min. Several solvents were tested including acetonitrile, methanol and dichloromethane. The scheme of derivatization according to Le-Nguyen and co-workers (Le-Nguyen D. et al., 1987) is illustrated in Figure 2.4.

2.3.2 Soil Treatment

Soil was collected from Al kabesi an area in Al-Ain (Abu Dhabi-UAE). Soil treatment was conducted according to the following procedure. First, soil was sprayed with a mixture of all phenoxy herbicides in question at a concentration of 32.2 mg/l. This concentration was determined by calculating the surface area of the soil bed (Bovey R. W. & Young A. L., 1980). Irrigation was carried out once daily for 30 days. The soil was divided into six layers (the depth of soil was 30 cm.). 1 kg of each layer was collected, dried, and extracted with 1.5 L of methanol. The extracts were dried using rotary evaporator, then dissolved in 10 ml of acetonitrile.
Prior to HPLC analysis samples were filtered. A part of each soil layer extract has derivatized according to the derivatization procedure described above.

### 2.3.3 Plant Treatment

The onion plant (*Allium cepa* L.) was sprayed with a mixture of all herbicides in question at a concentration of 14mg/l. The plant was irrigated once daily for 30 days. Onion was harvested and divided into leaves, roots and bulb. Each of these parts was washed with water then homogenized separately with 300ml of 0.01 hydrochloric acid using a food processor. The aqueous solution was extracted with hexane, the organic layer removed on a rotary evaporator, and the residue was dissolved in 5 ml of acetonitrile. Prior to HPLC analysis samples were filtered. Part of the extract was derivatized according to the derivatization procedure described above.

### 2.3.4 Separation Conditions

Solvent A 0.1% TFA in water and solvent B was 0.1% TFA in acetonitrile. The initial composition of the mobile phase was 25% solvent B, which was increased linearly to 90% over 30 min. The column was increased then reconditioned with 75% A and 25% B for 15 min. The flow rate was set at 1ml/min. Separation was achieved by utilizing a gradient
system, samples were injected utilizing an auto injector with a 20 μl capacity.
Figure 2.4: Derivatization pathway
CHAPTER III

RESULTS AND DISCUSSION
CHAPTER III
RESULTS AND DISCUSSION

3.1 UV-ABSORPTION OF PHENOXY ACID HERBICIDES

Phenoxy acid herbicides differ from each other in the length of the alkyl chain as well as the type and degree of substitution on the benzene ring (see Figure 2.1). Therefore, their UV absorption profiles are similar and they all have absorption maximum at 230 and 280 nm.

Figure 3.1 illustrates the UV spectrum of 2,4-D as a representative of the phenoxy acid herbicides utilized in this study. As mentioned above and like any other phenoxy acid herbicides, 2,4-D exhibits UV absorption maximum around 230 and 280 nm. The absorption of 2,4-D at 280 nm is much weaker than that at 230 nm. Therefore, throughout this study underivatized phenoxy acid herbicides were monitored at 230 nm.

3.2 HPLC SEPARATION OF UNDERIVATIZED PHENOXY ACID HERBICIDES

3.2.1 Isocratic Separation Conditions of Phenoxy Acid Herbicides

As mentioned earlier phenoxy herbicides possess almost similar functional groups and their structural variation does not affect their relative hydrophobicity, thus rendering their separation by HPLC utilizing
Figure 3.1: UV-Spectrum of 2,4-D
an isocratic mode of analysis impossible. Figure 3.2 illustrates the chromatograms of the phenoxy herbicides, investigated in this study under isocratic conditions utilizing a 75% acetonitrile and 25% water mobile phase. The retention times of the various analytes are summarized in Table 3.1. Under these separation conditions, baseline resolution is only attained between three analytes at a time while 2,4,5-T, 2,4-D and 2-(4-chlorophenoxy)propionic acid as well as silvex and 2-(2,4-dichlorophenoxy)propionic acid coeluted. Decreasing the organic content of the mobile phase to 50% did not enhance resolution, rather resulted in the coelution of more analytes. The chromatograms of analytes separated isocratically utilizing 50% acetonitrile and 50% water as mobile phase are depicted in Figure 3.3 and retention times are summarized in Table 3.4. As in the case of the higher organic percentage mobile phase, only three analytes could be baseline resolved at a time, while 2,4,5-T, 2,4-D, 2-(2,4-dichlorophenoxy)propionic and 2-(4-chlorophenoxy)propionic acid coeluted. Moreover, the derivatizing reagent 2-aminobenzamide coeluted with silvex. Other mobile phase compositions were tested and all proved to be ineffective in providing better resolution. Therefore, it can be concluded that isocratic separation of the different phenoxy acids under investigation is unsuitable, and ineffective in monitoring the progress of derivatization.
Figure 3.2: Chromatograms of Phenoxy Acid Herbicides Separated Isocratically utilizing a 75% acetonitrile and 25% water mobile phase

Table 3.1: Retention times of the analytes depicted in Figure 3.2

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>Analyte</th>
<th>Retention Time (Min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2-(4-CP)PA</td>
<td>1.931</td>
</tr>
<tr>
<td>2</td>
<td>2,4-D</td>
<td>1.865</td>
</tr>
<tr>
<td>3</td>
<td>Dichlorprop</td>
<td>2.068</td>
</tr>
<tr>
<td>4</td>
<td>2,4,5-T</td>
<td>1.818</td>
</tr>
<tr>
<td>5</td>
<td>4-(2,4-DCP)BA</td>
<td>3.565</td>
</tr>
<tr>
<td>6</td>
<td>Silvex</td>
<td>2.025</td>
</tr>
</tbody>
</table>
Figure 3.3: Chromatograms of Phenoxy Acid Herbicides Separated Isocratically utilizing a 50% acetonitrile and 50% water mobile phase

Table 3.2: Retention times of the analytes depicted in Figure 3.3

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>Analyte</th>
<th>Retention Time (Min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2-(4-CP)PA</td>
<td>2.141</td>
</tr>
<tr>
<td>2</td>
<td>2,4-D</td>
<td>2.078</td>
</tr>
<tr>
<td>3</td>
<td>Dichlorprop</td>
<td>2.388</td>
</tr>
<tr>
<td>4</td>
<td>2,4,5-T</td>
<td>2.148</td>
</tr>
<tr>
<td>5</td>
<td>4-(2,4-DCP)BA</td>
<td>6.995</td>
</tr>
<tr>
<td>6</td>
<td>Silvex</td>
<td>2.708</td>
</tr>
<tr>
<td>R</td>
<td>2-AB</td>
<td>2.975</td>
</tr>
</tbody>
</table>
Figure 3.4: Chromatograms of Herbicides and 2-Aminobenzamide Reagent; separation conditions as in Figure 3.3.
since elution time of most analytes was similar to the elution time of 2-
aminobenzamide as is illustrated in Figure 3.4. This has prompted the need
to perform the separation under gradient conditions.

### 3.2.2 Gradient Separation Conditions of Phenoxy Acid Herbicides

Phenoxy acid herbicides possess the same functional group and their
molecular weights are comparatively the same, thus their hydrophobicity is
expected to be similar. The basis of HPLC separation on a C18 column is
slight differences in hydrophobicity of the analytes, hence, different
partitioning coefficients. Moreover, small hydrophobicity differences can
be attenuated by utilizing gradient elution which results in the resolution of
analytes with relatively similar partitioning coefficient.

As mentioned in the previous section, isocratic separation of
phenoxy acids did not provide the desired resolving power. Therefore,
gradient chromatography was resorted to in order to attain baseline
resolution of all the phenoxy acid herbicides in question. Initially, the
gradient program summarized in Table 3.3 was utilized.
Table 3.3: Gradient separation conditions of Figure 3.5

<table>
<thead>
<tr>
<th>Time Min.</th>
<th>Flow ml/min.</th>
<th>% A (HPLC grade water with 0.1% TFA)</th>
<th>% B (Acetonitrile with 0.1% TFA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>1.50</td>
<td>75</td>
<td>25</td>
</tr>
<tr>
<td>25.00</td>
<td>1.50</td>
<td>25</td>
<td>75</td>
</tr>
<tr>
<td>30.00</td>
<td>1.50</td>
<td>75</td>
<td>25</td>
</tr>
</tbody>
</table>

Although such gradient program provided the resolution desired, severe tailing was observed for all analytes hindering efficient separation and suggesting the presence of secondary interactions between the analytes and the stationary phase. It is believed that this interaction is between the carboxylic acid groups of the analytes and the hydroxyl groups of the silica based stationary phase. This interaction has not supposed to happen, especially, since the column used was end-capped. However, it appears that the end-capping of the column was not complete, thus leaving few hydroxyl groups available for interaction with acidic analytes or any other analytes capable of forming hydrogen bonds. Secondary interactions, especially caused by hydrogen bonding, are conquered by lowering the pH of mobile phase or the addition of suitable counter-ions (ion-pair chromatography). We chose to lower the pH of mobile phase through the addition of trifluroacetic acid (TFA) which is commonly used as a reagent for this purpose.
The addition of TFA at concentration of 0.01% substantially optimized the efficiency of separation and eliminated any tailing (see Figure 3.5). Under the gradient conditions summarized in Table 3.3 and after the addition of TFA to both A and B solvents, the order of elution of analytes was (1) 2-(4-chlorophenoxy)propionic acid, (2) 2,4-D, (3) 2(2,4-dichlorophenoxy)propionic acid, (4) 2,4,5-T, (5) 4-(2,4-dichlorophenoxy)butyric acid, and (6) silvex. The detection limit of the analytes under these separation conditions was $5 \times 10^{-6}$ M, which is higher than the concentrations at which they exit environmentally. Detection limit can be improved directly or indirectly. The later involves preconcentration step, which is lengthy and tides. On the other hand, direct improvement in detection limits involves precolumn derivatization of analytes with a fluorophore or a chromophore. Phenoxy acid herbicides are relatively good UV absorbing species, thus tagging them with a chromophore is not expected to improve their detection limit substantially. However, tagging the analytes with a fluorophore is expected to enhance detection limits by at least three orders of magnitude.
Figure 3.5: Chromatogram of underivatized phenoxy acid herbicides separated using the gradient program summarized in Table 3.3.
3.3 DERIVATIZATION

Analytes are generally tagged with a suitable chromophore or fluorophore to improve their detection limits. As the tagging process brings about dramatic changes in the structure of the analytes, it is essential to select the tag discreetly not only to allow the sensitive detection of the derivatized analytes but also to produce the changes needed for the subsequent separation step. In other words, it is preferred that the tag also augment selectivity by enhancing hydrophobicity differences between the analytes. Such an enhancement would ultimately prompt high resolution of the separation. Other criteria for a successful pre-column derivatization include (i) high derivatization yield, (ii) high reaction selectivity (iii) formation of a single product, (iv) absence of detectable side products, (v) minimum sample work-out and clean up, and (vi) no analyte degradation.

The carboxylic group of phenoxy acid herbicides is a good candidate site for the attachment of a fluorophore. Therefore, improving the detectability of phenoxy acid herbicides in this study is based on the attachment of a fluorescing species to the carboxylic acids available on all phenoxy acid herbicides. In general, carboxylic acids can be converted to either amide derivatives or acylhydrazides, or to esters. In this study, the formation of amide bonds was selected due to the higher stability of amide bonds over ester bonds. The amide bond formation is based on the
condensation reaction between the carboxylic acid group of the analyte and the amino group of the fluorophore utilizing benzotriazolyloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP) as a condensation reagent. Since its discovery in 1975 by Castro et al. (Castro B. et al., 1975), BOP reagent has been mainly utilized in the classical peptide syntheses in solution. In this study reagent is used to derivatize phenoxy acid herbicides with a fluorophore to enhance their detection. The reaction pathway is summarized in Figure 2.4. The reaction involves the deprotonation of carboxylic groups through the addition of TEA to solvent in which the analytes are dissolved. This step is followed by the formation of an ester bond between the deprotonated analytes and BOP reagent. Eventually, this ester bond is attacked by the amino group of the fluorophore to form an amide bond between the analyte and the fluorophore (i.e., 2-aminobenzamide or 1-amo-2-naphthol-4-sulfonic acid). The coupling reaction also produces hydroxybenzotriazole (HOBT), thus inducing an acid-base reaction with TEA as illustrated in Figure 2.4. This reaction prompted the use of TEA in excess since the derivatization reaction is optimum under basic conditions (Castro B. et al., 1975).
3.3.1 Optimization of Derivatization Conditions:

Although the derivatization procedure utilized in this study is based on a well established procedure that has proven to be very successful in peptide synthesis (Mechref Y. & El Rassi Z., 1994) (Le-Nguyen D. et al., 1987) (Castro B. et al., 1975) two parameters had to be optimized namely, the amine and the solvent in which derivatization was carried out.

Initially, two amines were tested to optimize the derivatization procedure. Figures 3.6a and 3.6b illustrate the effect of triethylamine (TEA) and triethanolamine (TEOHA) on the derivatization efficiency of 2-(4-chlorophenoxy)propionic acid, respectively. As could be concluded from the comparison of those two figures, derivatization efficiency is substantially higher with TEA than with TEOHA. Both figures were obtained for the derivatization of the same amount of analyte under the same conditions except for the amine employed. The scale for both figures is the same, yet the amount of 2-(4-chlorophenoxy)propionic acid derivatized when TEA was used is more than twice the amount derivatized utilizing TEOHA; as is suggested by the peak areas of both figures. Therefore, derivatization of all analytes were conducted employing TEA to obtain optimum reaction efficiency. This agrees with the suggested reaction mechanism which proposed the need for the reaction to be optimum in basic media. Triethylamine is a stronger base than TEOHA.
Figure 3.6a: Chromatogram of 2-(4-chlorophenoxy)propionic acid derivatized with 2-aminobenzamide in acetonitrile and triethylamine (TEA)

Figure 3.6b: Chromatogram of 2-(4-chlorophenoxy)propionic acid derivatized with 2-aminobenzamide in acetonitrile and triethanolamine (TEOHA)
The efficiency of derivatization was also tested for three solvents namely acetonitrile; methanol and dichloromethane. The efficiency of the derivatization in these solvents was determined; like in the case of the amine, by comparing the peak area for the derivatization of 2-(4-chlorophenoxy)propionic acid performed under the same conditions except for the solvent in which the derivatization is performed. Comparing figures 3.6a, 3.7a and 3.7b, the peak areas observed in Figure 3.6a is higher than that observed in figure 3.7a which in turn is higher than that observed in the other figures. Indicating that the acetonitrile solvent provides higher reaction efficiency.
Figure 3.7a: Chromatogram of 2-(4-chlorophenoxy)propionic acid derivatized with 2-aminobenzamide in methanol and TEA.

Figure 3.7b: Chromatogram of 2-(4-chlorophenoxy)propionic acid derivatized with 2-aminobenzamide in dichloromethane and TEA.
3.3.2 Assessment of Derivatization

3.3.3 Mass Spectrometry

Derivatization of phenoxy acid herbicides was initially assessed by mass spectrometry. The analyte 4-(2,4-dichlorophenoxy)butyric acid derivatized with 2-aminobenzamide was subjected to fast-atom bombardment mass spectrometry; the spectrum of this analysis is depicted in Figure 3.8. Underivatized 4-(2,4-dichlorophenoxy)butyric acid has a molecular weight of 249.10 g/mole, thus a signal for this underivatized herbicide would appear at 249.0 m/z. As can be seen in Figure 3.8, there is no signal at this m/z value suggesting a complete conversion of the analyte. Moreover, the 2-aminobenzamide derivative of this analyte would have an m/z value of 368.25 which appears in Figure 3.8. Mass spectrometry data suggest complete conversion of the analyte to reagent derivatives. Note the reaction was performed under the conditions described in Chapter II. The signal at m/z value of 387 corresponds to formation of the product potassium adduct (i.e. \([M+K]^+\)).
Figure 3.8: Mass spectrum of 4-(2,4-dichlorophenoxy)butyric acid derivatized with 2-amino benzamide
3.3.4 Monitoring the progress of derivatization

The progress of the reaction was also monitored with UV detector, where the analytes were labeled according to the procedure summarized earlier and the peak height of the analyte was determined with various times. The reaction rate was high to the extent that the underivatized analyte completely disappeared after less than 40 min of reaction. Figure 3.9a demonstrates the completion of derivatization of 2-(4-chlorophenoxy) propionic acid after 40 minutes of reaction. The elution time of the underivatized analyte is illustrated with an arrow in the figure, as can be seen the analyte has completely disappeared. On the other hand, a new peak at around 16 min appeared which corresponds to the labeled phenoxy acid herbicide. To conclusively a certain the derivatization monitoring the reaction with a fluorescence detector for the appearance of a new peak could be only due to the formation of fluorophore-analyte product (see Figure 3.9b).

A chromatogram of the six phenoxy acid herbicides detected fluorescently is illustrated in Figure 3.10. In this figure four analytes are base line resolved while two analytes have coeluted. Thus, the derivatization was complete, rapid, reproducible and efficient, yet the addition of the fluorophore to the analytes increased their hydrophobicity to an extent that their base line resolution that was attainable before
derivatization was hindered. Note that the separation conditions utilized for the separation of the derivatized phenoxy acid herbicides are the same as those employed for the separation of underivatized phenoxy acid herbicides (Fig. 3.5 and 3.10). Increasing hydrophobicity in this case negatively influenced the chromatographic separation of the phenoxy acid herbicides. This reduction in resolution, however, could be overcome by changing separation conditions. Several attempts were made to improve the resolution of the derivatized phenoxy acid herbicides. The results of these attempts are discussed next.
Figure 3.9a: Chromatogram of 2-(4-phenoxy)propionic acid derivatized with 2-aminobenzamide.

Figure 3.9b: Fluorescence chromatogram of dichloroprop derivatized with 2-aminobenzamide.
Figure 3.10: Chromatogram of Phenoxy Acid herbicides labeled with 2-aminobenzamide
3.3.5 Optimization of Separation conditions of 2-aminobenzamide labeled phenoxy acid herbicides

Different analytical conditions including isocratic and gradient separation were tested in order to attain base line resolution of 2-aminobenzamide labeled phenoxy acid herbicides. Figure 15 shows the isocratic separation utilizing 45% acetonitrile containing 0.1 TFA and 55% water containing 0.1 TFA of mobile phase. Base line resolution for only five analytes was attained.

Table 3.4 summarizes the gradient separation conditions for 2-aminobenzamide labeled phenoxy acid herbicides. The corresponding chromatogram is given in Figure 3.12. The resolution obtained was not as good as expected, and the separation time was long. Varying the separation conditions as shown in table 3.5, 3.6, and 3.7 resulted in the chromatograms in Figure 3.13, 3.14, and 3.15, respectively. In Figure 3.13 the resolution was poor since there was a coelution of three analytes.

Comparing the chromatograms in Figure 3.14 and 3.15, it could be concluded that, the separation conditions utilized in Figure 3.15 resulted in a more efficient separation with optimum resolution and relatively short retention time.

Despite all attempts, attaining base line resolution of 2-aminobenzamide labeled phenoxy acid herbicides was not accomplished. This prompted the use of different derivatizing agents.
Figure 3.11: Chromatogram of 2-AB labeled phenoxy acid herbicides separated isocratically.
Figure 3.12: Chromatogram of 2-AB labeled Phenoxy acid herbicides separated using different gradient system

Table 3.4: Gradient separation conditions of Figure 3.12

<table>
<thead>
<tr>
<th>Time (min.)</th>
<th>Flow (ml/min.)</th>
<th>% A (Acetonitrile with 0.1% TFA)</th>
<th>% B (HPLC grade water with 0.1% TFA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial (linear)</td>
<td>1.50</td>
<td>25</td>
<td>75</td>
</tr>
<tr>
<td>10.00</td>
<td>1.50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>20.00</td>
<td>1.50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>25.00</td>
<td>1.50</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>30.00</td>
<td>1.50</td>
<td>25</td>
<td>75</td>
</tr>
</tbody>
</table>
Figure 3.13: Chromatogram of 2-AB labeled phenoxy acid herbicides utilizing non-linear gradient.

Table 3.5: Gradient separation conditions of Figure 3.13

<table>
<thead>
<tr>
<th>Time (min.)</th>
<th>Flow (ml/min.)</th>
<th>% A (Acetonitrile with 0.1% TFA)</th>
<th>% B (HPLC grade water with 0.1% TFA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial (non-linear)</td>
<td>1.50</td>
<td>25</td>
<td>75</td>
</tr>
<tr>
<td>25.00</td>
<td>1.50</td>
<td>85</td>
<td>15</td>
</tr>
<tr>
<td>30.00</td>
<td>1.50</td>
<td>25</td>
<td>75</td>
</tr>
</tbody>
</table>
Figure 3.14: Chromatogram of 2-AB labeled phenoxy acid herbicides utilizing gradient program.

Table 3.6: Gradient separation conditions of Figure 3.14

<table>
<thead>
<tr>
<th>Time (min.)</th>
<th>Flow (ml/min.)</th>
<th>% A (Acetonitrile grade water with 0.1% TFA)</th>
<th>% B (HPLC grade water with 0.1% TFA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial (linear)</td>
<td>1.50</td>
<td>25</td>
<td>75</td>
</tr>
<tr>
<td>25.00</td>
<td>1.50</td>
<td>85</td>
<td>15</td>
</tr>
<tr>
<td>20.00</td>
<td>1.50</td>
<td>25</td>
<td>75</td>
</tr>
</tbody>
</table>
Figure 3.15: Chromatogram of 2-AB labeled phenoxy acid herbicides utilizing different C18 column.

Table 3.7: Gradient separation conditions of Figure 3.15

<table>
<thead>
<tr>
<th>Time (min.)</th>
<th>Flow (ml/min.)</th>
<th>% A (Acetonitrile)</th>
<th>% B (HPLC grade water)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial (linear)</td>
<td>1.50</td>
<td>25</td>
<td>75</td>
</tr>
<tr>
<td>10.00</td>
<td>1.50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>20.00</td>
<td>1.50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>25.00</td>
<td>1.50</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>30.00</td>
<td>1.50</td>
<td>25</td>
<td>75</td>
</tr>
</tbody>
</table>
### 3.3.6 Using Different Derivatizing Agent

As mentioned earlier, to attain better analytes resolution the use of other derivatizing agents was employed. This was prompted by the lack of high resolution of the 2-aminobenzamide derivatized phenoxy acid herbicides. In this contest, two fluorophores were used, namely 8-aminonapthalene-1-sulfonic acid, and 1-amino-2-naphthol-4-sulfonic acid (ANOHS).

2-(4-Chlorophenoxy) propionic acid was used as a representative analyte to evaluate the efficiency of derivatization of the two fluorophores. The corresponding chromatograms are illustrated in Figures 3.16a and 3.16b. Comparing the peak areas, the peak area was higher with 1-amino-2-naphthol-4-sulfonic acid, thus indicating a better efficiency of derivatization using this fluorophore.

As a result, this derivatizing agent was utilized, and optimum separation conditions were determined. Figure 3.17 depicts the isocratic separation of ANOHS utilizing 30% acetonitrile and 70% water containing 0.1% TFA of the mobile phase. The resolution is relatively good, however the retention time was long.

Better resolution and shorter separation time were obtained under different separation conditions. In Figure 3.18, separation was performed under gradient conditions, which are summarized in Table 3.8. On the other hand, Figure 3.19 represents gradient separation employing the separation conditions summarized in Table 3.9. Comparing Figure 3.18
and 3.19 we can see that under the separation conditions used in Figure 3.19 the time of separation becomes shorter and the resolution becomes better.

Finally, we can conclude that, using ANOHS was the best fluorophore to obtain maximum base line resolution and shorter separation time using the separation conditions summarized in Table 3.9.
Figure 3.16a: Chromatogram of 2-(4-Chlorophenoxy)propionic acid derivatized with 8-aminonapthalene-1-sulfonic acid

Figure 3.16b: Chromatogram of 2-(4-Chlorophenoxy)propionic acid derivatized with 1-amino-2-napthalene-4-sulfonic acid.
Figure 3.17: Chromatogram of 1-amino-2-naphthol-4-sulfonic acid derivatized phenoxy acid herbicides.
Figure 3.18: Chromatogram of ANOHS labeled phenoxy acid herbicides

Table 3.8: Gradient separation conditions of Figure 3.18

<table>
<thead>
<tr>
<th>Time (min.)</th>
<th>Flow (ml/min.)</th>
<th>% A (Acetonitrile with 0.1% TFA)</th>
<th>% B (HPLC grade water with 0.1% TFA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>1.50</td>
<td>25</td>
<td>75</td>
</tr>
<tr>
<td>15.00</td>
<td>1.50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>30.00</td>
<td>1.50</td>
<td>25</td>
<td>75</td>
</tr>
</tbody>
</table>
Figure 3.19: Chromatogram of ANOHS labeled phenoxy acid herbicides under different separation conditions.

Table 3.9: Gradient separation conditions of Figure 3.19

<table>
<thead>
<tr>
<th>Time (min.)</th>
<th>Flow (ml/min.)</th>
<th>% A (Acetonitrile with 0.1% TFA)</th>
<th>% B (HPLC grade water with 0.1% TFA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>1.50</td>
<td>25</td>
<td>75</td>
</tr>
<tr>
<td>15.00</td>
<td>1.50</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>30.00</td>
<td>1.50</td>
<td>25</td>
<td>75</td>
</tr>
</tbody>
</table>
3.4 APPLICATIONS OF THE DERIVATIZATION METHOD

3.4.1 Determination of the concentration of phenoxy acid movement through soil.

The applicability of the derivatization procedure developed, was tested by studying the sorption/desorption of phenoxy acid herbicides in UAE soil. Extracted soil samples treated with phenoxy acid herbicides according to the procedure discussed in Chapter II were labeled with the derivatizing agent (ANOHS). The sorption/desorption data was compared for underivatized and derivatized phenoxy acid herbicides.

From the obtained chromatograms of different layers it can be noticed that, in layer No.1 (Figure 3.20a) there was trace appearance of phenoxy acid herbicides in the soil as well as other peaks corresponding to other organic molecules existing in the soil. The movements of phenoxy acid herbicides through the soil was continuos, as there were small amount of herbicides in the first few layers. From Figures 3.20b, 3.20c, 3.20d, and 3.20e which represent the chromatograms of the soil extracted from layers 2,3,4,and 5, respectively, it can be concluded that trace amounts of herbicides are present. However, layer No. 6 (Figure 3.20f) showed the presence of test herbicides at high concentrations. The concentrations of the different phenoxy acids in the six layers are summarized in Table 3.10 and Figure 3.21. Thus, it can be concluded that the nature of soil in the
UAE allows the movement of phenoxy acid herbicides through. In other words, sorption of herbicides is very low in UAE soil.

The extent of sorption/desorption of phenoxy acid herbicides was different from one herbicide to another. This difference is based on the different hydrophobicity of individual herbicides, which could be explained by the low organic matter present in the soil.

Employing fluorescence detector in the case of derivatized phenoxy acid herbicides extracted from the layer allowed better detection and eliminated other interfering peaks originating from organic molecules, (See Figure 3.22).
Figure 3.20a: Chromatogram of Soil extracted from layer No. 1

Figure 3.20b: Chromatogram of Soil extracted from layer No. 2
Figure 3.20c: Chromatogram of Soil extracted from layer No. 3

Figure 3.20d: Chromatogram of Soil extracted from layer No. 4
Figure 3.20c: Chromatogram of Soil extracted from layer No. 5

Figure 3.20f: Chromatogram of Soil extracted from layer No. 6
Table 3.10: The Concentration (ppb) of herbicides in different layers of soil

<table>
<thead>
<tr>
<th>Herbicide/Layer (Conc.)</th>
<th>L.1</th>
<th>L.2</th>
<th>L.3</th>
<th>L.4</th>
<th>L.5</th>
<th>L.6</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,4,5-T</td>
<td>19.93</td>
<td>13.92</td>
<td>45.88</td>
<td>104.11</td>
<td>18.59</td>
<td>326.79</td>
</tr>
<tr>
<td>SILVEX</td>
<td>100.7</td>
<td>26.2</td>
<td>90.35</td>
<td>181.76</td>
<td>62.72</td>
<td>261.4</td>
</tr>
<tr>
<td>2,4-D</td>
<td>2.06</td>
<td>2.05</td>
<td>11.04</td>
<td>36.72</td>
<td>7.34</td>
<td>226.7</td>
</tr>
<tr>
<td>2-(4-CP)PA</td>
<td>36.3</td>
<td>22.36</td>
<td>57.88</td>
<td>11.29</td>
<td>50.59</td>
<td>532.8</td>
</tr>
<tr>
<td>4-(2,4-DCP)BA</td>
<td>0</td>
<td>10.04</td>
<td>27.28</td>
<td>93.46</td>
<td>56.9</td>
<td>256.44</td>
</tr>
<tr>
<td>Dichlororrop</td>
<td>5.11</td>
<td>2.85</td>
<td>18.22</td>
<td>53.8</td>
<td>40.38</td>
<td>297.23</td>
</tr>
</tbody>
</table>
Figure 3.21: Graph illustrating change in the amount of phenoxy Acid herbicides in soil layers
Figure 3.22: Chromatogram of Soil extracted from layer No. 6 with Fluorescence Detector
3.4.2 Determinations of phenoxy acid herbicides in roots and leaves.

When a UV-detector was employed to determine the amount of phenoxy acid herbicides uptake by the crop, many interfering peaks appeared. That made it impossible to quantitatively determine the amount of herbicide uptake by the plant. However this interference was much lower when the derivatization procedure was utilized, (see Figure 3.23).

The uptake of tested herbicides by roots and leaves was different. In roots, the herbicides taken in high concentration were silvex and 4-(2,4-DCP)BA, in addition of trace amounts of other tested herbicides (Figure 3.23a). On the other hand, the herbicides taken in high concentration in leaves was 4-(2,4-DCP)BA and trace amounts of other tested herbicides (Figure 3.23b). The amount of herbicides accumulated in roots and leaves are summarized in Table 3.11.

All in all, the study of the crop uptake of herbicides gave as evidence that there is a movement of tested phenoxy acid herbicides from roots to leaves. This movement differs from one herbicides to another depending on the properties of individual herbicides.
Figure 3.23a: Chromatogram of root extract, derivatized and obtained with fluorescence detector

Figure 3.23b: Chromatogram of leaves extract, derivatized and obtained with fluorescence detector
Table 3.11: Concentration of herbicides in leave and root extracts of onion plant treated with the herbicides

<table>
<thead>
<tr>
<th>Herbicide</th>
<th>In Leave Extract (ppm)</th>
<th>In Root Extract (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-(4-CP)PA</td>
<td>5.71</td>
<td>0.78</td>
</tr>
<tr>
<td>2,4-D</td>
<td>10.2</td>
<td>7.26</td>
</tr>
<tr>
<td>Dichlorprop</td>
<td>0.44</td>
<td>0</td>
</tr>
<tr>
<td>4-(2,4-DCP)BA</td>
<td>13</td>
<td>8.13</td>
</tr>
<tr>
<td>2,4,5-T</td>
<td>3.5</td>
<td>7.33</td>
</tr>
<tr>
<td>Silvex</td>
<td>2.13</td>
<td>11.4</td>
</tr>
</tbody>
</table>
CONCLUSIONS
CONCLUSION

Phenoxy acid herbicides are ionic herbicides that could be separated using reversed-phase columns; however, the ionic nature of these herbicides may cause the efficiency of separation on such column to be low. This is especially become an issue when reversed-phase columns are not end-caped, thus exposing siloxane groups and permitting secondary interaction. This problem could be rectified either by using a well end-caped column or using an acidic mobile phase. Both strategies decrease secondary interactions between the analyte and the stationary phase. In this study, the second strategy was employed. TFA was added at 0.1 % to both solvent A and B of the chromatographic system. TFA is an acidic reagent that insures the protonation of both phenoxy acid herbicides and siloxane groups of the stationary phase, thus eliminating any secondary interactions. Accordingly, optimum separation efficiency of phenoxy acid herbicides was attained utilizing a gradient separation system in which the composition of the mobile phase was varied linearly from 25 % acetonitrile and 0.1% TFA to 75% acetonitrile and 0.1% TFA in 25 min. Baseline resolution of all analytes tested in this study was attained in ca. 15 min. However, the detection limit was only ca. 1 x 10-5 M at 230 nm detection wavelength.
Although the separation conditions aforementioned were sufficient to attain efficient and rapid separation, the detection limits of these herbicides was higher than the concentration at which they could accumulate. Therefore, derivatization of these phenoxy acids with a suitable fluorophore, which would subsequently allow their determination by the higher sensitivity fluorescence detector, was pursued. Derivatization of these herbicides was attained through condensation reaction between the carboxylic acid group of the herbicides and an amino group of a suitable fluorophore in the presence of a condensation reagent such as BOP reagent. This derivatization procedure has been proven very effective in the production of synthetic polypeptides. Derivatization conditions were optimized with respect to amount of analyte, amine, solvent and derivatization reagent. It was concluded that optimum yield of derivatization was accomplished using acetonitrile and triethylamine at the same concentrations utilized in the production of synthetic peptides. The reaction was completed in 40-55 min as was determined by mass spectrometry and HPLC analysis. Initially, 2-aminobenzamide was used as a derivatizing agent; and exhibited high reaction efficiency and three orders of magnitude improvement in the detection limit. However, resolution of 2-aminobenzamide labeled phenoxy acid herbicides separated on reversed-phase C18 column was less than that of the native analytes. Two herbicides coeluted and their separation was not possible under any separation
conditions tested. Accordingly, it was believed that a solution to this problem could be accomplished by employing different derivatizing agents. Two additional derivatizing agents were tested and 1-amino-2-naphthol-4-sulfonic acid exhibited higher derivatization efficiency and moderate separation time. Baseline resolution 1-amino-2-naphthol-4-sulfonic acid labeled herbicides was attained and the detection limit was ca. 1 x 10^-8 M using a fluorescence detector.

The effectiveness of this technique was not only limited to standard sample, but was also effective in the determination of phenoxy acid herbicides accumulation in soil as well as plant uptake of these herbicides. The very low detection limit of labeled phenoxy acid herbicides allowed their determination in soil treated at ppb concentrations (2.05-532.8 ppb). Moreover, the derivatization reduced interference from organic species that exit in soil.

The technique was also very effective in monitoring the uptake of plants for these herbicides. The method allowed the determination of the differential uptake of plants to the herbicides when they were treated. The method substantially reduced interference arisen from species existing in plants. The study showed that herbicide uptake in roots and leaves were not to the same extent.
REFERENCES
REFERENCES


Chiron S., Papilloud S., Haerdi W., and Barcelo D. (1995), "Automated on-line liquid-solid extraction followed by liquid chromatography-
high-flow pneumatically assisted electrospray mass spectrometry for the determination of acidic herbicides in environmental waters", Anal. Chem., Vol.67, pp. 1637-1643.


US EPA method 8150/8151.

ARABIC SUMMARY
كما إن فصل هذه المواد يحتاج إلى إضافة أحماض تعمل على إلغاء الشحن السالبة التي يمكن أن تواجدها على هذا النوع من المبيدات بحيث تعمل هذه على تقليل إمتصاص المركبات على سطح الطور الصلب (stationary phase) في عملية الفصل الكروماتوغرافي.

إن تفاعل المواد المفلورة مع هذه المبيدات مكن من فصل وتحديد هذه المبيدات عند تراكيز منخفضة بلغت جزء من المليوني (ppb). وساعدت هذه الطريقة التحليلية على فصل وتحديد تراكيز منخفضة لهذه المبيدات في التربة كما أسهمت في التخلص من تداخل الشروائيد في عملية التحليل الكمي. وللسبب، ثبت صلاحية هذه الطريقة لتحديد كميات المبيدات التي تتمسها النباتات.
المخصص العربي

Phenoxy Acid) مشكلتان تواجه تحديد مجموعة مربّعات الأعشاب تعرف بـ

(Herbicides)؛ ألا وهما ضعف امتصاص هذه المجموعة للضوء؛ إضافة إلى امتصاص بعض

الشواbies التي تتواجد مع هذه المجموعة للضوء عند نفس طول الموجة الضوئية المنعكس.

الناتجة عن القياسات. في هذه الأطروحة سوف نقدم حلاً هائلاً للمشكلتيتين من خلال تفاعلك

(Fluorescence) (Fluorophore) حيث تستخدم الفلورة.

هذه المبيدات مع مركبات مفلورة في الناتجة عن المركب المتكون للتحديد الدقيق لتراكيز قليلة من هذه المبيدات وبدون تأثير

الشواbies. وسوف نعرض استخدام هذه الطريقة مع الفصل الكروماتوجرافي لفصل وتحديد

تراكيز مجموعة من هذه المبيدات العشبية. وقد تم استخدام هذه الطريقة في تجديد نسب

تواجد المبيدات في التربة. وقد تبين أن أفضل فصل لهذه المبيدات قبل تفاعلها مع المركبات

المفلورة يحدث باستخدام الفصل الكروماتوجرافي المعمد على تغيير تركيز المذيبات المستعملة

وذلك خلال عملية الفصل.
جامعة الإمارات العربية المتحدة
كلية العلوم

عنوان الرسالة: التحليل الكمي الكروماتوغرافي لعينات من مبيدات الحشائش في النباتات والتربة

اسم الطالبة: نورة سعید النعيمي

لجنة الإشراف

<table>
<thead>
<tr>
<th>التوقيع</th>
<th>الوظيفة</th>
<th>الاسم</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>أ.د. مصطفى محمد كمال</td>
<td></td>
</tr>
<tr>
<td></td>
<td>أ.د. يحيى سالم مشرف</td>
<td></td>
</tr>
<tr>
<td></td>
<td>د. وحيد ميشيل زعيب</td>
<td></td>
</tr>
</tbody>
</table>
التحليل الكيمي الكروموباتوجرافي لعينات من مبيدات الحشائش في النترة والنبات

رسالة مقدمة من الطالبة
نورة سعيد النعيمي
بكالوريوس في العلوم/كيمياء
استكمالاً لمتطلبات الحصول على درجة الماجستير في العلوم
(علوم البيئة)

جامعة الإمارات العربية المتحدة
مكتبة العلوم

ديسمبر 1999

Shreem
2016.11.27
15:14:45
+04'00'