

Selected Chlorinated Acid Herbicides on the Sewanee Domain

Introduction

The objective of the research is to identify any unknown pesticide present in the soil samples we collect from the Sewanee domain by analyzing the soil samples using liquid chromatography (LC) coupled with mass spectrometry (LC/MS and LC/MS/MS). When we started the research, the plan was to look for organophosphorus, chlorinated acid and carbamate pesticides in the soil sample. We did not have the instruments required to extract carbamate pesticides from the soil sample so we decided to look for organophosphorus and chlorinated acid pesticides. However, we limited our research to the identification of seven chlorinated acid herbicides using LC/MS and LC/MS/MS in the soil samples. We initiated our research by reading some research papers based on pesticides and their identification with the aid of liquid chromatography and mass spectrometer (MS). We applied the methods for extraction from soil described in the literature and made some changes as required for our research. We finalized the soil sample collection method, the extraction procedure for chlorinated acid herbicides and the methods for High Performance Liquid Chromatography (HPLC) and MS to identify the herbicides in the soil samples. Though the objective of the research was not fully met, the future interns could use the methods we developed to collect soil samples, extract the herbicides, and analyze with LC and MS.

The seven chlorinated acid herbicides

We purchased standards consisting of a mixture of organophosphorus, chlorinated acid and carbamate pesticides separately. We ran the standards in HPLC but there was no way to relate the peaks to the pesticides in the mixture so we decided to buy standard with only one pesticide. During this period, we also decided that we would work on chlorinated acid herbicides only. We researched the crops that were grown in Tennessee and the pesticides that were used for those crops. In addition, we also ordered standards of some persistent chlorinated acid herbicides. The seven pesticides are dieldrin, picloram, mirex, heptachlor epoxide (isomer B), lindane, 2,4-dichlorophenoxyacetic acid (2,4-D) and dichlorodiphenyltrichloroethane (DDT). The standards of each pesticide are run through HPLC and the retention time is noted. The standards are then analyzed through LC/MS to obtain an MS spectrum for each pesticide.

Soil Sample Collection

The location from where the soil is collected is recorded using GPS. The time, weather conditions and a short description of the location are noted. The condition of streams or other water resources is noted. A clean shovel is used to collect the soil sample. The soil sample is collected 4-6' inch deep and stored in a plastic box. This procedure is followed for all of our trials. We have not yet decided how to store soil samples. We need to decide how to keep records of the soil samples and how to ensure that soil samples are not contaminated or lose moisture and so on. The best way to store soil samples we have decided for now is storing them in the glass bottles washed in a base bath.

Extraction

We followed the following extraction procedure. The extracting solution and sulfuric acid are corrosive so they should be used under the hood.

1. 20.0 ± 0.1 g of soil is weighed.
2. 50 ml of 0.5N KOH in 10% KCl extracting solution (10% KCl is the solvent) is added to each soil sample and mixed thoroughly by shaking.
3. The sample is placed in a boiling water bath for 15 mins.
4. The sample is transferred to a centrifuge test-tube.
5. The sample is placed on a horizontal shaker for 15 mins.
6. The sample is centrifuged at 1300 rpm for 15 mins.
7. 3.0ml aliquot is transferred to a small centrifuge test-tube and 150 μ l of 12N sulfuric acid is added.
8. The sample is vortexed for 30s.
9. To confirm the pH is < 1.5 , pH paper is used. If pH is not below 1.5, additional acid is added.

Cleaning the extract

We modified the cleaning procedure by using 1:1 acetonitrile and 0.04% glacial acetic acid instead of HPLC grade water. We also used methylene chloride and acetonitrile (ACN) only. However, the mixture of ACN and glacial acetic acid gives good results in the HPLC run.

1. 2 ml of chloroform is added to the extract. The chloroform should be used under the hood.
2. The extract is vortexed and centrifuged at 3000 rpm for 2 minutes. We can see three layers on the test-tube. The upper layer is yellow, the middle layer is very thin and the lower layer is clear. The herbicides if present dissolve in chloroform.

3. The lower chloroform layer is removed into another centrifuge tube. This step is repeated two more times.
4. The chloroform extract is evaporated to just dryness.
5. 4 ml of 1:1 acetonitrile and 0.04% glacial acetic acid is added immediately, vortexed briefly, sonicated for 5 mins and briefly vortexed again.

HPLC parameters

The parameters written below were determined after numerous trial and error. Changes were made in flow rate, injection volume and % B gradient before we decided the following parameters are to be used in further research.

Flow rate: 0.3 ml/min

UV range: 230nm

Injection Volume: 5-20 μ l

Column temperature: not controlled

Column: 2.1 X 150mm

Mobile phase A: 0.04% Glacial Acetic Acid in water

Mobile phase B: Acetonitrile

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| Time | B(%) |
|------|------|
| 0 | 0 |
| 1 | 40 |
| 2 | 52 |
| 3 | 60 |
| 4 | 100 |
| 8 | 100 |
| 9 | 0 |

Stop time: 10 – 15 mins

The extracts must be filtered to prevent contamination of the column. We used a 0.45 μm syringe and filter paper to clean the extracts before using them for HPLC and MS.

MS parameters

The following MS parameters were determined after numerous trials. Changes were made in injection volume, fragmentor and skimmer before confirming that these parameters are to be used in further research.

Injection Volume: 1 μl

ESI (Negative)

Mass range: 100-600 amu

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Drying Gas: 200 °C

Gas Flow: 9 L/min

Nebulizer: 40psi

Capillary: 3500V

Skimmer: 35 V

The MS parameters for Auto MS/MS and Targeted MS/MS are setup as per Agilent Q-TOF LC/MS Techniques and Operation Course Number R1904A Volume I&II, Student Manual.

Software programs used

We used Spartan to estimate the dipole moment of the pesticides. The dipole moment was based on the geometry optimized structure of the pesticides. We also used ChemDraw to predict the MS spectrum of the pesticides.

Figures of HPLC run and MS spectrum

The standard for each pesticide was run on HPLC and LC/MS. We also ran some extracts from soil samples we collected in central campus. We noted the retention time for the seven pesticides using HPLC. We also got MS spectrum for the seven pesticides from LC/MS. Though we got retention time for all the pesticides, we could get only MS spectrum for three pesticides only. They were picloram, 2,4- D and DDT. We also made the mixture of picloram and 2,4- D and ran through HPLC and LC/MS. Our future plan is to add the standard of each pesticide in soil sample separately, extract the pesticide and use HPLC and LC/MS to check if we get the same retention time and MS spectrum. We would further work on quantitation of the pesticide we could recover from the extraction procedure. We would further work on Auto MS/MS and Targeted MS/MS that deal with the fragmentation of the precursor ions.

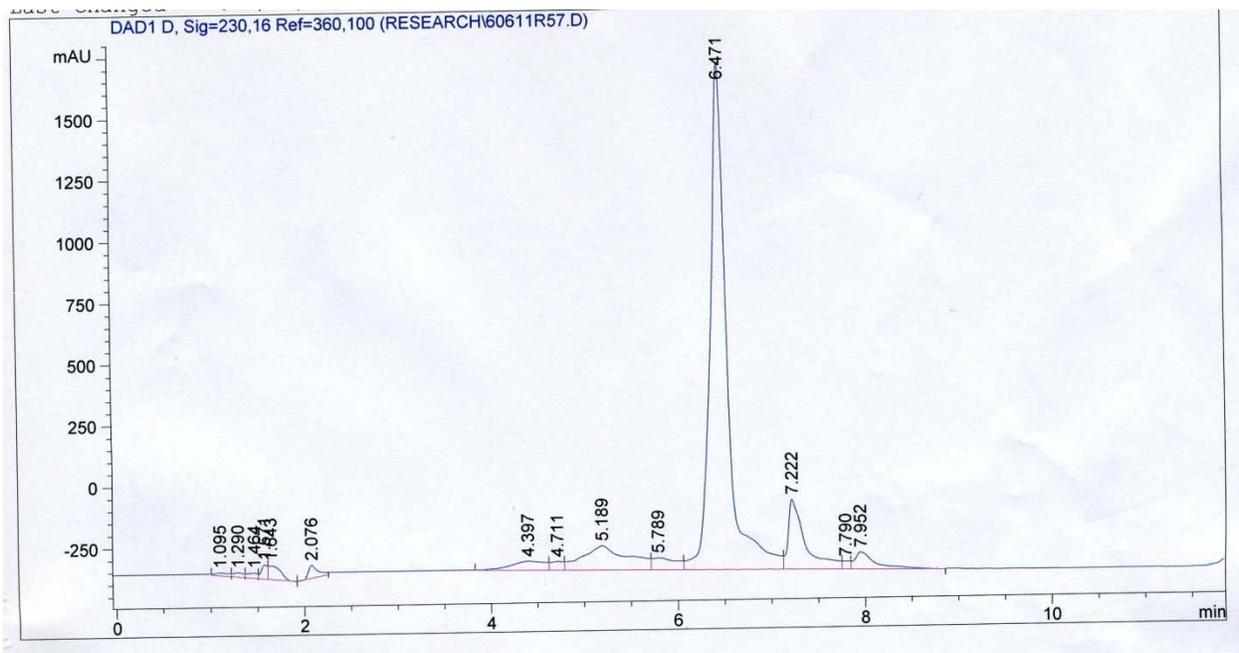


Figure 1: HPLC run on 2,4- D. The retention time of 2,4 – D is 6.47 mins.

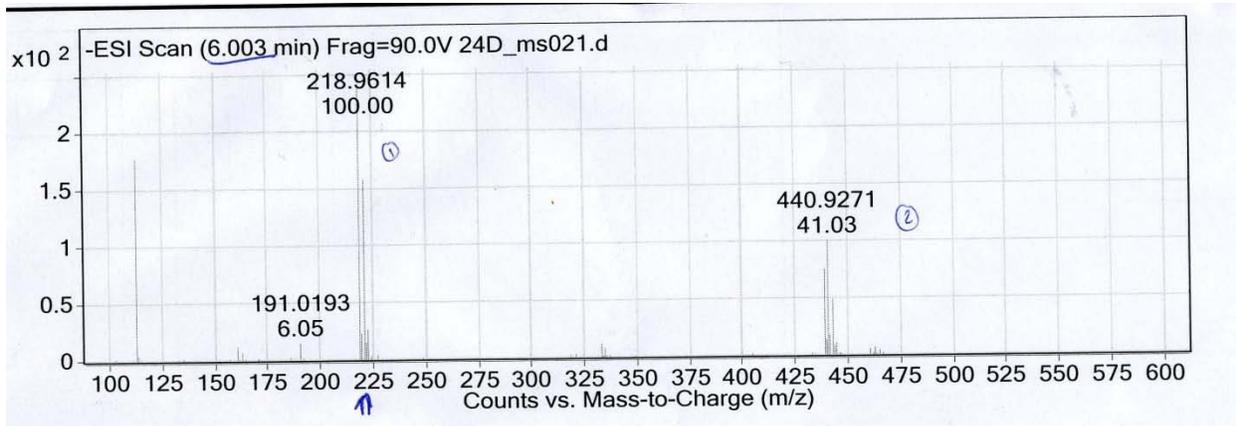


Figure 2: MS spectrum of 2,4- D. The molar mass of 2,4- D is 221.04 g/mol.

We started our research project from the very beginning by collecting the instruments and chemicals we would require. We were not continuing what earlier interns had accomplished. We read some research papers that gave ideas on how to begin and what things we would require. I learned how much time and effort is required to start research. I would just imagine how much time and effort the researchers put in to publish their papers. It would take months and years to put all the things together and to obtain publishable results.

However, our results were not always consistent with the graphs and peaks that we see in research papers and books. It requires numerous experiments to get a decent peak. For example, while working on the HPLC during the first two weeks, we were getting different peaks every time from the same sample. The retention times were nowhere near to what we thought them to be. The peaks in other research papers and books are sharp, clean and presentable to be published in papers. After learning more about HPLC, we got similar peaks in each trial from the same sample. We then made changes to some parameters to deal with tailing, negative peaks and sharpness. During times when the instrument was not working, we used the time on literature research and software programs to learn more about the pesticides. Patience is utmost required when the results are inconclusive and the research is moving at a slow pace.

When we start a new research project, we should do our own research on the topic. This helps to generate ideas as to how to approach the project. The information we gain helps us solve the problems we might face during our research and explain the results. Many times we asked ourselves why we are getting such results. By consulting other research papers on the topic, we may obtain some insight. If we start our research with no knowledge on the topic, there are many options to follow. But with some prior knowledge, we could eliminate some of the options that we don't need to try.

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Working on this project made me take the initiative to become more independent. I would plan what I will do before coming for work and tell Dr. Shibata. Whenever I am confused and don't have any idea how to proceed, Dr. Shibata would direct me. I have always wanted to work in lab so Dr. Shibata gave me the opportunity to work not only in lab but use the HPLC and the MS that I have only read about in books.

Reference

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