THE PENNSYLVANIA STATE UNIVERSITY

SCHEYER HONORS COLLEGE

DEPARTMENT OF CHEMISTRY

DEVELOPING A RELIABLE PROTOCOL FOR THE SIMULTANEOUS ANALYSIS OF SELENIUM AND MERCURY IN LAKE ERIE FISH

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ABSTRACT

The presence of mercury in the human diet, commonly found in seafood, is a health concern for people of all ages. According to the EPA, introducing excessive amounts of mercury to the gastrointestinal tract can lead to skin rashes, dermatitis, mood swings, memory loss, mental disturbances and muscle weakness. Exposure to mercury has increased types of tumors found in rats, and in some cases, ingesting it has resulted in kidney failure. Recently, scientists have studied the relationship between selenium and mercury, as selenium has the potential to reduce health risks associated with mercury. Selenium irreversibly binds with mercury and prevents the toxic element from crossing the blood brain barrier and affecting the brain. The objective of this project was to develop a protocol for determining the individual concentrations of mercury and selenium from fish commonly caught and consumed in Presque Isle Bay in Erie, Pennsylvania. Although previous research has been completed in other freshwater and marine environments, no research has focused on the Presque Isle watershed to date.

Complications with selenium and mercury standards, including volatility, was shown to affect the calculated concentrations of unknown samples. A NIST standard reference material was therefore utilized in order to optimize the digestion of the fish, the matrix for the calibration standards for both mercury and selenium in order to obtain accurate measurements using inductively coupled plasma mass spectrometry (ICP-MS). Using the protocols developed, one could assess possible health risk in the fish consumed from Presque Isle Bay and the relationship between selenium and mercury within these fish.

TABLE OF CONTENTS

Chapter 1: Mercury, Health and Its Relation to Selenium

Mercury (Hg) has multiple forms, all of which are toxic. The three main forms are elemental, inorganic and organic. Elemental mercury is commonly found in "thermometers, dental amalgams, fluorescent light bulbs, electrical switches, mining and industrial processes" (*Mercury*, 2009). This type of mercury is also emitted when fossil fuels, such as coal are burned. Humans become exposed to elemental mercury through vapors, which can cause extensive lung damage (*Mercury*, 2009). Inorganic mercury is commonly found as salts, where mercury is bonded with sulfur or oxygen. Mercury salts are used to make other chemicals, and in some countries, it is used in makeup. When humans consume inorganic mercury over time, they are at risk for digestive issues. Also, repetitive skin application of this type of mercury leads to neurological disorders, kidney dysfunction, skin rashes and memory problems (*Mercury*, 2009).

Organic mercury is a neurotoxin produced by microscopic organisms in water and soil, where they bind elemental or inorganic mercury with carbon. Organic mercury moves up the food chain from these organisms to fish and humans (*Mercury*, 2009). The consumption of organic mercury, commonly ingested in seafood, is a great concern for people of all ages (Raymond & Ralston, 2004). Mercury moving through the food chain is known to cause mercury poisoning and has killed individuals in Minamata and Iraq (Lui et al., *2012*). Additionally, while long term exposure to mercury has led to cardiovascular issues in rats, studies of human exposure to high doses of mercury have been inconclusive (Park & Mozzaffarian, 2010). Methylmercury is the most common form of organic mercury and consists of a methyl group attached to the Hg^{+} ion. If a pregnant woman consumes methylmercury, it passes through the placenta (*Mercury*, 2009) and affects the development of fetal brain cells and nervous tissue (Raymond & Ralston, 2004).

Selenium (Se) is an essential element required in the human diet. Selenium is a crucial base for selenoproteins, which play a crucial role in reproduction, thyroid hormone metabolism, DNA synthesis, and protection against oxidative damage. There are two forms of selenium: organic and inorganic. While both are essential, excess intake of either form can be toxic and lead to hair or nail brittleness and/or loss (*Selenium*, 2020). Most selenium is found in human and animal tissues in the form of selenomethionine. Approximately 25-46% of selenomethionine is stored in the skeletal muscle. Studies have suggested that selenium concentrations play a role in cancer, cardiovascular disease, thyroid disease, and cognitive decline (*Selenium,* 2020).

In recent years, scientists have shown interest in the relationship between selenium and mercury in fish. Raymond and Ralston suggested that selenium and mercury affect each other's bioavailability, meaning the interactions between the two make them unusable and non-harmful to the body (Raymond & Ralston, 2004). They also suggest that humans keep free selenium in their body in order to combat the mercury ingested (Raymond & Ralston, 2004). Research in Poplar Creek and Clinch River in Tennessee show that most fish had a selenium to mercury ratio greater than 1, which would be expected to minimize the danger of mercury consumption (Burger et al., 2012). To date, research concerning the amount of mercury and selenium of fish from Presque Isle Bay, Erie Pennsylvania has never been investigated.

The original goal for this work was to utilize inductively coupled plasma mass spectrometry (ICP-MS) to simultaneously measure both Hg and Se in digested perch and walleye fillets. However, in that pursuit, a reliable protocol had to be developed, which proved tougher than anticipated. First, there were complications with selenium and mercury standards, including high ionization potential and high volatility, respectively. Second, microwave digestion of organic matter provides its own unique obstacles with regards to measuring these two elements.

Third, ICP-MS does have the potential to suffer from spectral interferences at the mass of these two elements. These three challenges were addressed by utilizing a NIST Standard Reference Material of frozen Lake Michigan fish tissue homogenate (SRM #1947). This thesis will present the development of an optimized protocol to use in future measurements of actual fish samples that are currently being stored at -80 ^{o}C at Penn State Behrend.

Chapter 2: Methods

2.1: Lake Erie Fish

To date, there has yet to be a study that focuses on the mercury and selenium concentration in fish from Lake Erie. Fish were collected from various areas around Presque Isle Bay including the Lake Erie locations of Shorewood and Shades Beach. Walleye and Perch were chosen because they are the most common fish that are caught and consumed in this region. A depth of 13 meters has proven to be beneficial in catching perch and walleye. A total of 20 fish were captured, where half of the samples were perch and the other half walleye. After capture, the location, age, sex, and weight were recorded to determine if any physical characteristics play a role in the selenium to mercury levels. The fish were euthanized upon capture with an electric net. The fish were filleted directly after euthanasia. These fillets were wrapped in aluminum foil and plastic freezer bags and stored at -80 ^{o}C until the day of digestion.

2.2 Protocol for Making Standards and Samples

Before making standards, all containers were acid washed for 24 hours using 2% nitric acid with 0.5% hydrochloric acid. For this experiment, 50-mL plastic vials were utilized for standards and samples, while plastic 1-L bottles were used to store the solvent d all containers were thoroughly rinsed with ultrapure water prior to use. A total of 5 standards were prepared from stock standard solutions of 1000 mg/L mercury for ICP (Sigma Aldrich) and either a 1000 mg/L selenium standard (Sigma Aldrich) or an Environmental Calibration Standard (Agilent) that contained 10 mg/L of selenium. Through serial dilutions, the following standards were prepared using mass to determine the concentration in parts per billion (ppb): 10, 5, 2, 1 and 0.5. A solution containing 2% ultrapure nitric acid, 0.5% hydrochloric acid, 5% acetic acid and 2 ppm of gold was used to dilute standards, as discussed in the results section.

2.3 Mechanics and Protocol for Microwave Digestion of Fish Tissue

Since ICP-MS is only able to measure concentrations of solutions, fish samples were digested using a high-powered microwave. For this experiment the MARS 6 Microwave Digestion System (CEM Corporation, Mathews, NC) was utilized. This instrument utilizes specialized PTFE vessels that increase pressure while the temperature is increased, effectively liquefying the samples. For this work, a 0.2-0.25 g aliquot of SRM-1947 fish sample was placed in the specialized vessel with 5 mL of reagent grad nitric acid (70%), and eventually an additional 2 mL of 30% hydrogen peroxide. This was repeated twice to produce 3 unknown samples. Pre-existing digestion settings, including a preset temperature and time profile, for fish tissue from the digestor were used to properly digest samples. After digestion, the samples were placed in a labeled acid-washed container and diluted to a total mass of 50 g with the same solution used to dilute standards. In the present research, time constraints prevented the analysis of actual Lake Erie fish samples and future work will utilize the above process for future studies.

2.4 Mechanics and Protocols for Using Inductively Coupled Plasma Mass Spectrometry

All solutions were analyzed using a 7900 Series Inductively Coupled Plasma Mass Spectrometer (ICP-MS; Agilent Technologies, Inc., Santa Clara, CA). Within this instrument, samples were introduced to argon and aerosolized with a nebulizer. As the sample entered and neared the ICP Torch, ionization occurred through a high temperature 27-megahertz argon plasma. Desired positive ions were sent to the octupole reaction cell. Here, helium atoms collide with any polyatomic ions to decrease possible interference. Highly charged elemental ions passed into the hyperbolic quadrupole mass analyzer, where they were separated based on their mass:charge ratio and sent to the detector to receive the signal (*The Principle of ICP MS*, 2016).

To gather data, a new batch file was created for every set of solutions, saved as the date and a corresponding letter to signal at what point of the day the sample was analyzed. For example, 20200304a was the first batch performed on March 4th, 2020. All isotopes for mercury and selenium were selected as the analytes, while tungsten and germanium were utilized as internal standards. To prevent contamination from polyatomic ions, the collision mode was enabled so all solutions could be measured in the presence of helium. The flow rate of helium was set at 4.5 mL/min. Rinse times between samples were also increased to minimize mercury memory effects from the peristaltic pump tubing. There was a total of 3 rinse bottles, sequentially containing 2% nitric acid, deionized water and 1% hydrochloric acid, respectively. The helium flow rate and rinse time were the only settings that deviated from the preset protocols. To minimize contamination, 3 vials containing the calibration blank solution were placed in between the standard solutions and samples to provide additional rinses before the samples were tested. Additionally, a sample blank solution, which was digested with the samples, was remeasured after the calibration blank vials.

The concentrations for mercury was based on the most common isotope that has minimal interferences, Hg^{201}. Because of argon dimers, Se^{78} was used to determine the concentration of selenium. When calculating the concentrations in the standard reference material, it was important to subtract the counts per second obtained after remeasuring the blank. This provided accurate concentrations for standard reference material.

Chapter 3: Results

In order to develop a protocol for determining the amount of mercury and selenium in fish, the preparation of standards had to be optimized, including both their stability and ability to be prepared in the same sample matrix. First, the stability of the standards was tested to determine that the theoretical concentration matched the experimental concentration. Mercury is a volatile element, meaning it is easily lost as a gas and since the standards are open to air when the ICP-MS is collecting samples, mercury can be lost in standards or samples. Because the ICP-MS bases the experimental concentrations on the theoretical concentrations, less mercury in the standard could inflate the results of an unknown sample. An increase of exposure to air leads to an increase in mercury lost. Thus, keeping standards or samples over many runs could lead to less accurate results. It was hypothesized that gold could added to a solution to bind to the mercury to stabilize it and prevent it from leaving the standard as a gas. Figure 1 shows the ICP-MS response to mercury and selenium standards with and without the presence of gold. The mercury signal remains relatively constant in the presence of gold over the 10-day span. The counts per second increase seen in the mercury standard without gold is because the instrument tubing was extensively cleaned between day 3 and 10. Thus, fresh standards and clean tubing are recommended before collecting data for mercury.

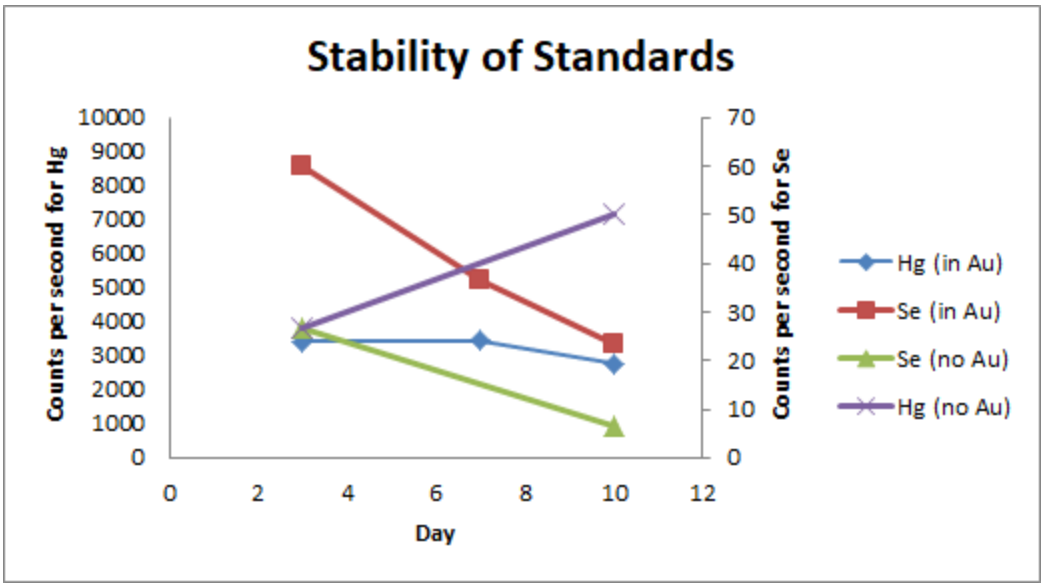

Figure 1: The counts per second, measured using ICP-MS, of selenium and mercury of one ppb standard over a two-week period to show how stable the standards remained overtime.

Although selenium is not volatile like mercury, it is difficult to measure using ICP-MS because it has a high ionization potential (which leads to low signal intensity as shown in Figure 1) and the argon-argon dimer has the same mass, 80 Da, as the most abundant selenium isotope. The former issue was addressed by adjusting the sample matrix of the selenium standards to contain a comparable amount of carbon as in the fish samples by adding 5% acetic acid to the dilution solution. Carbon has been shown to increase the ionization efficiency of selenium in the plasma and lead to higher signal intensities. (Gammelgaard & Jons, 1999). Figure 2 compares the ICP-MS signal intensities between the selenium standards with and without added carbon in the matrix. Without a carbon-containing matrix, the counts per second for selenium were low, leading to an artificially high concentration value for the fish SRM samples, which naturally contain a higher level of carbon. However, when carbon, in the form of 5% acetic acid was added to the standards, the counts per second of selenium increased significantly.

The latter issue with selenium detection involving the Ar-Ar dimer was overcome by monitoring the selenium 78 isotope, which is the second most abundant Se isotope, and by incorporating a helium collision cell. The purpose of the collision cell was to break apart any multi-element species that will have the same mass of the intended isotope. By introducing acetic acid to the standard matrix, incorporating the helium collision cell, and monitoring the ^{78}Se isotope, the experimentally determined concentration of the SRM matched the cited value. These conditions will be utilized in future measurements of Lake Erie fish.

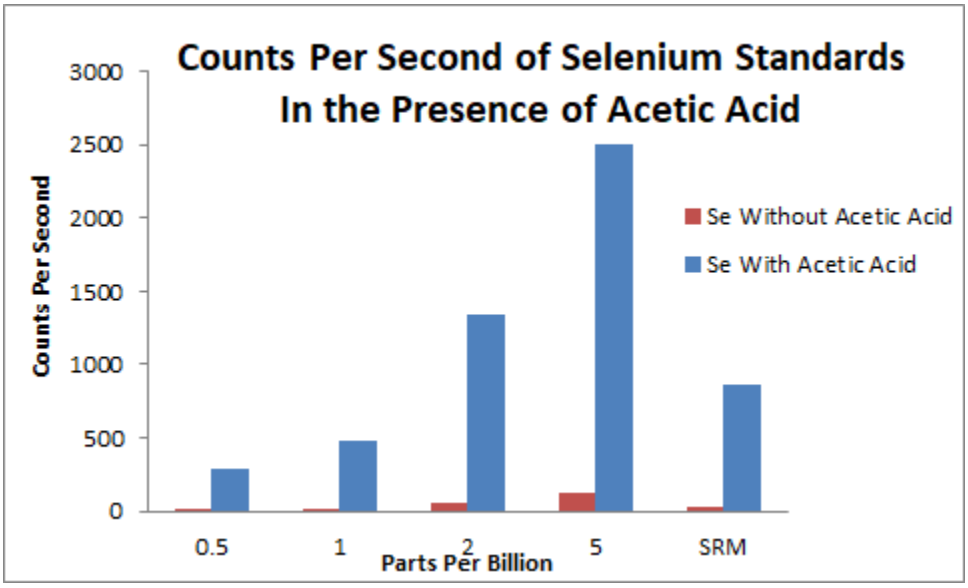

Figure 2: The counts per second of selenium standards with and without acetic acid to test if adding carbon to the matrix affects the signal of selenium determined using ICP-MS

One concern of this experiment was whether adding mercury and selenium in the same standards affected the measurement of either element. Standards of each individual element were compared to standards prepared containing both elements. It was determined that the presence of selenium in the standard did not affect the mercury counts, nor did the presence of mercury in the standard affect the selenium counts. This is shown in Figures 3 and 4, respectively. Normally,

running a set of standards and reference samples would take 45 minutes. Consequently, running each element separately, which is required in atomic absorption spectroscopy, another technique that could be employed for these measurements, would take twice the time. Therefore, a major advantage in this protocol is the ability to have multi-element standards containing, in this case, both mercury and selenium to save time and money.

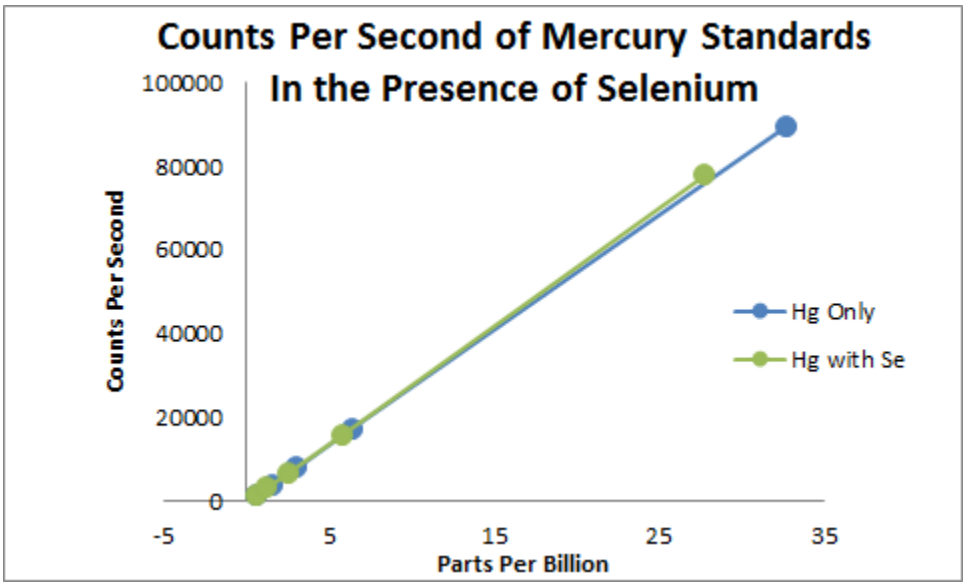

Figure 3: The counts per second of mercury standards with and without selenium in the solution to prove that selenium in the standard does not affect the mercury signal measured using ICP-MS

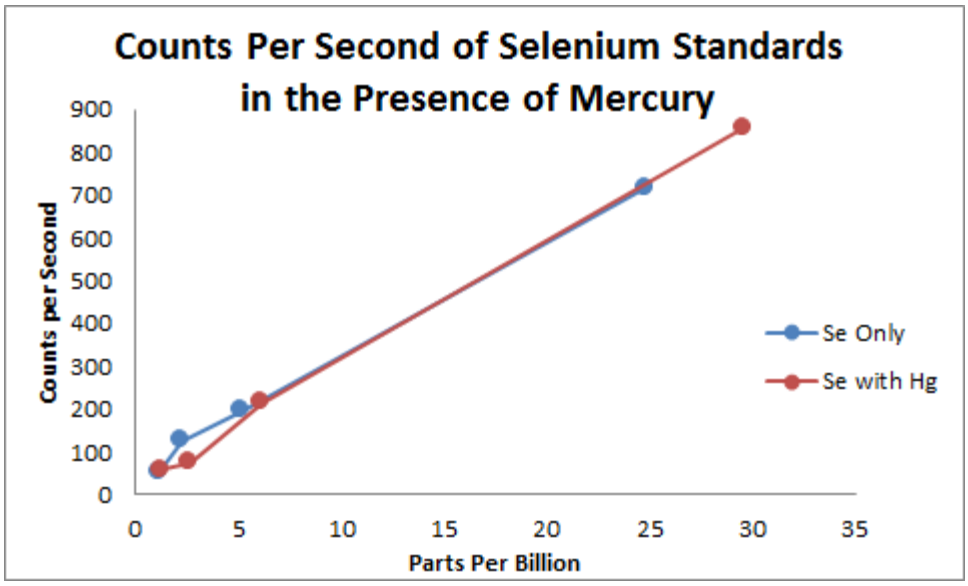

Figure 4: The counts per second of selenium standards with and without mercury to prove that mercury in the standard does not affect the selenium signal measured using ICP-MS

The optimized standards were then used to verify the concentrations of each element in the NIST SRM-1947 samples to confirm that these standards would be accurate in determining an unknown. The known reference samples consisted of homogenized fish tissue from Lake Michigan with mercury and selenium concentrations of 254 (+/- 5) ppb and 475 (+/- 80) ppb, respectively. In a final study, two sample aliquots of 0.2 g were massed, and microwave digested in a solution containing 5 mL HNO_3 and 2 mL H_2O_2. In one of the samples (sample B), about 15-20 μL of Au stock solution was also added to see if it was possible that volatized Hg was being lost in the digestion. Both digestions were then diluted to 50 g of total mass using the 5% acetic acid/HCl/HNO_3/Au solution described in Chapter 2. Using standards prepared with the same solution, the experimental concentrations of the reference samples were determined as shown in Table 1.

Table 1: The experimentally determined concentration of SRM-1947 samples. SRM-1947 was reported to contain 475 ppb selenium and 254 ppb mercury.

Reference Sample	Experimental Selenium Concentration (ppb)	Experimental Mercury Concentration (ppb)
A	421	227
B	471	245

While this is an extremely small sample set, the data agrees well with the literature values of the SRM sample. This suggests that both, gold and acetic acid should be included in the matrix. The values determined in Sample B were even closer to the literature values than that for Sample A. This may suggest that adding a small amount of gold to the digestion solution may be beneficial to the analysis, however more tests need to be performed in order to confirm this.

Chapter 4: Conclusion

Mercury is a toxic element that can lead to health problems, including neurological disorders, lung and kidney damage, rashes, birth defects, and cardio vasculature disease. Selenium, an essential element, is thought to bond with mercury and prevent it from furthering damage to the body. Raymond and Ralston advise at least a one to one ratio between the two elements in food, such as fish, however a higher level of selenium compared to mercury is more desirable. Although no fish samples from Lake Erie were tested, the protocol for how to determine the ratio between mercury and selenium was developed. Mercury and selenium standards, as well as the digested fish samples, should be prepared with a sample matrix containing 2% nitric acid, 0.5% gold, 2% hydrochloric acid and 5% acetic acid. Fish tissue collected from Walleye and Perch must be digested in 5 mL of concentrated nitric acid and 2 mL of 30% hydrogen peroxide and then diluted with the same sample matrix. There is a possibility that adding a small amount of gold solution to the digestion may also be advisable, but more tests must be performed. The concentration of each element can then be determined using an ICP-MS. Following this protocol resulted in data that reasonably agreed with that published for NIST SRM-1947. Future work will focus on applying this protocol to examining selenium and mercury levels in the fish from Presque Isle, Lake Erie and make future recommendations about the safety of their consumption. Additional research may also focus on differentiating the different types of mercury (e.g., inorganic vs. organic) in the fish tissue.

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Education

The Pennsylvania State University, Erie PA Graduation May 2020
Bachelor of Science in Biology
Minor in Chemistry and Trauma Studies Certificate Scheyers and Behrend Honors Scholar

Relevant Courses

Organic Chemistry I & II Biochemistry I & II Comparative and Human Anatomy Physiology
Quantitative Analysis Microbiology Instrumental Analysis Physics I & II

Experience

The Pennsylvania State University - Erie, PA - *Research Assistant* *September 2018- Present*

- Researching the concentrations of selenium and mercury in Lake Erie Fish
- Operated Inductively Coupled Plasma Mass Spectrometry, High Pressure Microwaves, and Graphite Furnace technology

Clinical Observation Hours *March 2017-Present*

- Butler eye Care (12 hours)
- Wigton Eye Care (6 Hours)
- Pearle Vision (18 Hours)
- Molnar and Associates Vision Care (8 Hours)
- Lee Eye Center (12 Hours)

The Pennsylvania State University - Erie, PA - *Resident Assistant* *August 2017 - Present*

- Provide residents with paraprofessional advising regarding academics and campus resources
- Coordinate educational programs to help increase resident awareness and quality of life
- Enforce and promote university policies and values
- Work in teams to supervise residence halls and residents

Ponderosa - Butler, PA - *Waitress and Cashier* *April 2014 - Present*

- Gained interpersonal and customer service skills
- Opened and closed the restaurant

Technical Skills

Graphite Furnace Atomic Absorption IR Spectroscopy NMR Spectroscopy
Gel Electrophoresis Thin Layer Chromatography Column Chromatography
Inductively Coupled Plasma Mass Spectrometry Chemical Standards and Buffers

Activities and Community Involvement

Scrubs Club - *Vice President and Founding Board Member* *August 2018 - Present*

- Volunteer at Habitat for Humanity
- Assist students in finding future vocations in healthcare

Lion Ambassadors - *Membership Committee Chair* *September 2016 - Present*

- Conduct campus tours for university open houses and promote school pride and involvement
- Recruit, interview and integrate new club members