

3

AAS Analysis of Total Mercury Content in Fish Flesh

Quantitation by Atomic Absorption Spectroscopy (AAS)

Experiment Overview

In this 7-session experiment you will utilize atomic absorption spectroscopy (AAS) to quantify the total mercury bioaccumulated in a fish flesh sample.

Significant components of this experiment, as expected for most any quantitative experiment, are method validation and sample preparation. While guidance for the method validation is presented below, it is your responsibility to consult published literature to produce the sample preparation process and plan from the following three publications:

- ‘A Comparison of Techniques for Preparing Fish Fillet for ICP-AES Multielemental Analysis and the Microwave Digestion of Whole Fish’
- ‘Optimization of a Microwave-Pseudo-Digestion Procedure by Experimental Designs for the Determination of Trace Elements in Seafood Products by Atomic Absorption Spectrometry’
- ‘Field Sampling, Preconcentration and Determination of Mercury Species in River Waters’.

which are all posted on the D2L site. It is intended that the first two references be consulted for guidance in developing a sample digestion protocol (or analyte leaching protocol – i.e. pseudo-digestion). The third reference should be consulted specifically to aid you in constructing a pre-concentration protocol. As the extracted analyte will be at a concentration well below the methods lower limit of detection (LOD), a pre-concentration process will need to be employed. This third reference should help direct your protocol development for mercury complexation (sodium diethyldithiocarbamate (SDDC) is the complexing agent used in this experiment), C18 solid phase extraction (SPE) column conditioning, concentration/enrichment of complexed mercury species with C18 SPE columns, and subsequent elution of complexed species from SPE columns. In other words, these references should guide you in your protocol to step through the sample preparation “flow diagram” illustrated in Figure 3.2. You will not analyze the total mercury via the techniques discussed in these references, you will use these references to guide your sample preparation, and then perform the quantitative analysis via AAS.

This experiment requires significant sample preparation due to the facts that i) samples can only be introduced to the AAS as aqueous, fully-dissolved solutions (which is quite different state than the provided ‘native sample’)

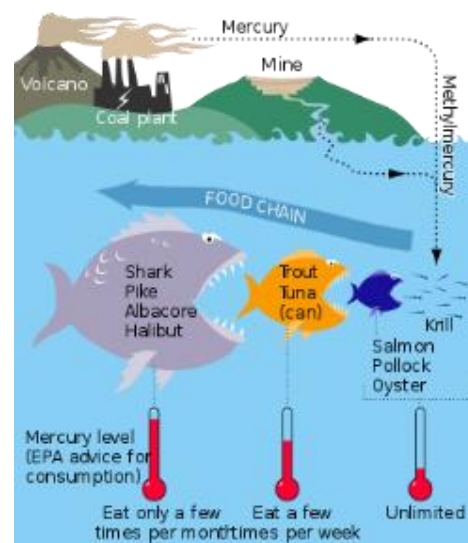


Figure 3.1: Model for biomagnification of mercury in the food chain. Image courtesy of Wikipedia: https://en.wikipedia.org/wiki/Mercury_in_fish#/media/File:MercuryFoodChain.svg

and ii) the total mercury concentration in the digested fish solutions will be below the method/instrument detection limit. As the concentration of mercury in the digested fish solutions will be lower than the detection limit for AAS, the mercury species must be pre-concentrated to enable quantitative analysis; procedures for such are discussed in the second reference listed above.

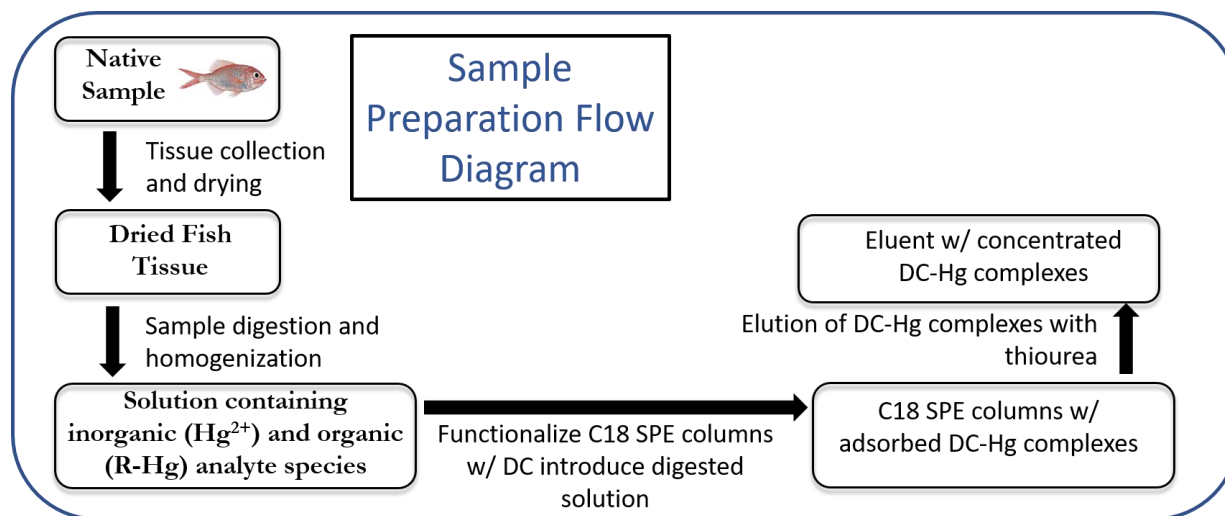


Figure 3.2 Flow diagram for the sample preparation necessary for this experiment

For your initial calculations and method development plans, you may presume the concentration limit of detection for mercuric aqueous solutions with AAS to be on the order of 5 ppm (by mass). You should also expect to find on the order of 1000 ng of mercury per gram of dried fish in the samples. This experiment is divided into three discrete milestones as detailed below.

Suggested Timeline

Session	Action
Before Lab Session 1	complete Milestone 1 pre-laboratory assignment
Lab Session 1	complete experimental for Milestone 1
Before Lab Session 2	complete Milestone 2 pre-laboratory assignment
Lab Session 2-4	perform experimental for Milestone 2
Before Lab Session 5	complete Milestone 3 pre-laboratory assignment
Lab Session 5-7	perform experimental for Milestone 3

Milestone 1: Instrument and Sample Quantity Validation

Milestone 1 Assessment

- Milestone 1 Pre-laboratory assignment : 39 points
- Milestone 1 Experimental and Technical Report : 45 points

Milestone 1 Overview

Proper and thorough experimental design requires recognition that any time a new analytical technique or instrument is enlisted, such as flame AAS, some reference standard should initially be analyzed before attempting analysis of a real and unknown sample. This initial analysis enables one to:

- 1) become familiar with new instruments and software
- 2) develop protocols and determine optimal instrument and software settings
- 3) estimate detection limits, sensitivity, and resolution for the technique
- 4) establish rules-of-thumb for volumes or masses of sample required for analysis
- 5) measure a known system to verify proper functionality

As this atomic absorption (AA) spectrometer and corresponding software are likely new to you, for this first milestone you will prepare a series of aqueous mercury standards (as detailed in the experimental section) and analyze them via AAS in order to accomplish the above goals. Both standard and “blank” solutions, you will determine estimates for the lower concentration limit of detection, the resolution, and the sensitivity for the method – and also confirm that a linear response (or determine the model for the response) to concentration (i.e. Beer’s Law) is attained that can be used for interpolation.

One drawback for employing AAS as a quantitative chemical analysis technique is that the method typically requires a relatively large volume of sample solution (on the order of 10 mL), and thus a relatively large quantity of analyte, for analysis. This can be problematic since the analytical chemist is often tasked with quantifying trace quantities of analyte; quantities of analyte which can be unwittingly diluted to concentrations below the instrument’s detection limit. Thus two more key outcomes for this first milestone are development of a procedure for analysis via AAS that utilizes minimal sample volume and determination of what this minimum volume is. Developing such protocol is critical for your subsequent analysis of the fish sample in that it will dictate the amount of fish tissue and extent of pre-concentration required for analysis.

Milestone 1 Pre-lab Assignment

Recall that it is mandatory that your pre-lab assignment for the specific milestone be approved by your TA before you begin performing any experimental work.

- 1) The prep-room provides you a 10% HNO_3 (by volume) calibrated standard solution with a Hg^{2+} concentration of 1000.0 ppm (by mass). Construct a detailed plan for how you will produce the series of four 50.0 mL mercuric standards as specified in the experimental section for this milestone. Note: you have access to 50.0 and 100.0 mL volumetric flasks, 0.5, 1.0, 2.0, 5.0, 10.0 mL volumetric transfer pipettes, and concentrated HNO_3 . Recognize, it is not essential that your standard be exactly equal to, for example, 1.0 ppm; it must only be near the desired value but, more importantly, a value that you know exactly. For ppm calculations for this question you can assume the density of solutions to be exactly 1.0 g/mL. Do not neglect the use of HNO_3 in your standards preparation – the matrix must be

the same for all of your solutions or your calibration curve would be meaningless due to matrix effects. For this experiment you will have access to both 20 – 100 μL and 100 – 1000 μL micropipettes in addition to the volumetric transfer pipettes. Note: if you use the micropipettes, you should verify their accuracy and precision using the density of water to verify that they are acceptable for the level of accuracy you desire.

- 2) Assume you have determined the lower concentration limit of detection (LOD) for mercury via your AAS protocol to be 5.0 ppm; i.e. the method cannot statistically distinguish solutions with mercury concentrations of 5.0 ppm or lower from that of a “blank” solution containing 0 ppm of mercury. In your protocol development you also determine that the minimum volume of sample solution that you can analyze via AAS is 20.0 mL. Assuming the dried fish sample contains 1000 ng of mercury per gram of dried tissue, what is the minimum mass of dried fish tissue that you would have to employ in order to prepare a solution with a mercury concentration above the LOD? For this question you can assume the density of all solutions to be 1.0 g/mL and all ppm concentrations are by mass – thus 1 μg / mL = 1 ppm.
- 3) While in the previous question you assumed a 5.0 ppm LOD, in this question you will utilize sample data provided to calculate the sensitivity, resolution, and LOD for the method; providing an opportunity to mirror the experimental and analysis process that you will employ to complete the first milestone. For this question you will need to download and analyze two .csv data files posted on the D2L site titled “Hg_AAS_Standards.csv” and “Hg_AAS_Blanks.csv”.

The “Hg_AAS_Standards.csv” file contains two columns of data. The first column is the concentration (in parts-per-million by mass) of prepared mercury standards. The second column is the measured absorbance from the AA spectrophotometer for each standard. Construct a calibration curve for this data and find the best-fit linear model for the response.

The “Hg_AAS_Standards.csv” file contains a single column of data; specifically 25 absorbance measurements, obtained from the AA spectrophotometer, for a mercury-free “blank” solution. You should use this set of data to determine the sample-to-sample measurement variation which is necessary for calculating the lower concentration LOD and resolution.

Utilize these two sets of data to calculate and report i) the method sensitivity – i.e. the slope of the calibration curve (include units!), ii) the lower concentration limit of detection (LOD) for this method – see your quantitative chemical analysis textbook for assistance, and iii) the resolution of the method. It is typically important to know the minimum difference in sample concentration that can be resolved with a certain method – i.e. the resolution of the method. For calculating the resolution, utilize:

Resolution $\sim$ (sample standard deviation of signal from “blank”) / (method sensitivity or slope) (Eq. 1)

- 4) Based on goals of this first milestone (consider the Overview, Pre-laboratory assignment, and Technical Report sections), construct a detailed list specifying the data that you must acquire in order to complete this first milestone – determine the sensitivity, resolution, and LOD. Make sure to use this list in the laboratory.

Milestone 1 Experimental

(Note: these experimental bullet-points are not necessarily in the order that they must be completed, they are just a list of requirements for the experiment and it is up to you to organize your time to complete them)

As there are multiple groups using the same instrument for this experiment, you will need to coordinate and cooperate effectively in this experiment – if you perceive any unfairness please bring this to the attention of your TA as soon as possible. Instead of viewing other groups as a barrier, observe differences in their protocols from your own to make improvements. As this is a 7-session experiment, there will be plenty of time for everyone to complete the analysis.

- Utilize the concentrated mercury standard solution provided to prepare four different standards with concentrations of ~ 10., 20., 40., 60. ppm. Make sure to recognize the solution / matrix composition (e.g. 10 wt% nitric acid, etc.) of the standards - you must reproduce this matrix for all of your dilutions and “blank” in order to minimize matrix effects. After preparing your four standards in the appropriate matrix, you will likely need at least 100 mL more of this matrix-matching solution to function as the “blank”.
- Utilize the Quick Start Guide for the AA spectrometer to configure it for analysis and acquire the data necessary to complete this milestone.
- Hint: consider using test tubes or vials for introducing your samples to the AA spectrometer. The form factor of test tubes and vials may be optimal for transferring relatively small sample volumes to the spectrometer.

Milestone 1 Technical Report

Download and use the “Technical Report Template” to construct a concise, neat, and well formatted technical report for this Milestone 1. This Technical Report for Milestone 1 must be submitted no later than 7 days after the day that you begin the experimental laboratory component of Milestone 2. Your report must include:

- Abstract
- Figure illustrating the response / calibration curve from the standards you prepared. The figure should include the best-fit linear model for the response curve. Use the LINEST function in Excel to obtain standard uncertainty values in both the slope and y-intercept for this model.
- Table summarizing the method LOD, sensitivity, and resolution
- Short discussion on how the LOD, resolution, and sensitivity were determined
- Short description in the experimental section that prescribes the minimum sample volume that should be prepared (and what you are going to prepare in your subsequent) to quantitatively analyze via AAS. You should also describe the optimal vessel (e.g. beaker, etc.) for delivering the sample to the instrument.
- The carbon copies of your notebook pages used for this Milestone 1 should be attached to the back of the hard-copy of this report when you submit it

Milestone 2: Method Validation with Control Sample

Milestone 2 Assessment

- Milestone 2 Pre-laboratory assignment : 50 points
- Milestone 2 Experimental and Technical Report : 76 points

Milestone 2 Overview

Sample preparation (or “sample prep”) - referring to the ways in which a sample is treated prior to analysis - is typically a significant component of the experimental design and implementation for quantitative chemical analysis experiments. For example, when performing AAS, the instrument requires that the sample be introduced as a thoroughly dissolved and homogeneous aqueous solution, clearly conditions not satisfied with the provided fish sample. As you will discover, sample preparation is often the most time intensive, frustrating, challenging, interesting, and important aspect of chemical analysis. At first thought, the sample preparation required for analyzing fish with AAS probably seems rather trivial, for example : i) digest a fish sample in strong acid, ii) filter the subsequent digested solution, iii) concentrate the analyte using a solid phase extraction (SPE) column, iv) elute the column, and v) analyze this eluent solution via AAS to determine the concentration of mercury. This analysis would indeed enable quantitation of mercury **in the eluent**, but how does this correspond to the concentration of mercury **in the original fish tissue**? It is essential to recognize that during each of the sample preparation steps from above, some fraction of the mercury present in the fish tissue would likely be lost due to incomplete digestion, adsorption on the filters and SPE columns, etc. Thus a valid experimental design must include a process for quantifying the loss of analyte through the sample preparation process. For example, maybe the sample preparation process results in only 18.8% of the analyte present in the original sample (mercury in fish tissue for this experiment) making it through the sample preparation process and being dissolved in the final eluted solution; i.e. 81.2% of the initial analyte is lost in the sample preparation process. Recognize that it may not actually be problematic that 81.2% of the analyte was lost through the sample preparation process - as long as you know that 81.2% was lost; because if you quantify the loss you can mathematically correct for this in your data analysis. But this correction is only possible if you validate and quantify your sample preparation procedure; and this process is necessary for completing the second milestone of this experiment.

In validating a sample preparation procedure (i.e. quantifying the loss of analyte in procedure and verifying efficacy), one might initially analyze a sample with a known analyte concentration. Since the mercury content in fish tissue is unknown, you will validate your procedure using a model substitute for fish tissue. For this process, the substitute will be mercury-free, textured vegetable protein (TVP) “nuggets”. TVP provides you a chemically similar matrix to fish flesh (but without the smell and mess) which can be spiked to contain any concentration of mercury that you so desire. More specifically, you will grind TVP nuggets to a fine and homogeneous powder for ease of digestion, and digest/leach with a minimum volume of digesting agents that you determine based on guidance from the posted references, “A Comparison of Techniques for Preparing Fish Fillet for ICP-AES Multielemental Analysis and the Microwave Digestion of Whole Fish” and ‘Optimization of a Microwave-Pseudo-Digestion Procedure by Experimental Designs for the Determination of Trace Elements in Seafood Products by Atomic Absorption Spectrometry’.

Due to the relatively high LOD for mercury using the AA spectrometer (~ 5.0 ppm) and the relatively low concentration range of mercury expected to be found in the fish sample (~ 1000 ng of mercury per gram of dried fish flesh), one can presume that some sort of pre-concentration step will be a necessary component of the overall sample preparation process. This comes from recognition that, based on the above rules-of-thumb, digesting 1 gram of dried fish in even 10 mL of nitric acid would result in a solution with a mercury concentration on the order of 100 ppb; well below the method detection limit, and thus a pre-concentration process will be necessary. For this experiment the pre-concentration procedure you employ should be guided by the reference posted on the D2L site and titled 'Field sampling, preconcentration and determination of mercury species in river waters'. Note: the digestion and preconcentration processes necessary to succeed at this are quite challenging; it is highly recommended that you allot sufficient time for this plan development and definitely consult your lab partner and/or TA to probe the details of your plan. Much time can be saved by proper preparation.

Milestone 2 Pre-lab Assignment

Recall that it is mandatory that your pre-lab assignment for the specific milestone be approved by your TA before you begin performing any experimental work.

- 1) You grind and homogenize a small sample of dried TVP in a coffee grinder which facilitates efficient digestion. You weigh out 100.0 grams of pulverized TVP, which you add to a small beaker. You have access to a 10 wt% HNO_3 stock solution with 1,000 ppm mercury; how much of this solution stock solution would you add to achieve a model system of fish tissue known to contain 1000 ng of mercury per gram of dried tissue? You may assume the density of all solutions to be 1g/mL. For this experiment you will have access to both 20 – 100 μL and 100 – 1000 μL micropipettes in addition to the volumetric transfer pipettes. Note: if you use the micropipettes, you should verify their accuracy and precision using the density of water to verify that they are acceptable for the level of accuracy you desire.
- 2) From a calibration / response curve (absorbance vs. concentration in ppm) you can utilize LINEST and determine the following best-fit linear model – including standard uncertainty associated with both the slope and y-intercept:

$$y = mx + b$$
$$\text{absorbance} = (\text{slope}) (\text{concentration [ppm]}) + b$$
$$\text{absorbance} = (0.012 \pm 0.004 \text{ ppm}^{-1}) (\text{concentration [ppm]}) - (0.004 \pm 0.003)$$

You perform a number of trials with a solution containing an unknown concentration of mercury for which you measure the absorbance to be 0.036 ± 0.007 . Utilize the above model to calculate and report the mercury concentration – INCLUDING UNCERTAINTY, you must propagate the uncertainty in the slope and measured absorbance – in the unknown solution.

- 3) For your result in the previous question – you probably reported something such as 'the concentration is 3.3 ± 0.8 ppm'. What does this quantity, with the uncertainty, mean to you? Specifically, what does the fact that there is the 0.8 ppm of uncertainty mean to you?
- 4) Utilizing the provided references, 'A Comparison of Techniques for Preparing Fish Fillet for ICP-AES Multielemental Analysis and the Microwave Digestion of Whole Fish' and 'Optimization of a Microwave-Pseudo-Digestion Procedure by Experimental Designs for the Determination of Trace Elements in

Seafood Products by Atomic Absorption Spectrometry' perform the following:

- a. Identify and summarily describe (in 50 words or less) **three** different techniques/methods, for the extraction of mercury from tissue, that were introduced in these references.
 - b. The second reference provided, 'Optimization of a Microwave-Pseudo-Digestion Procedure by Experimental Designs for the Determination of Trace Elements in Seafood Products by Atomic Absorption Spectrometry' describes the optimization of metal extraction via "pseudo-digestion procedures". In ~10-20 words, what is meant by "pseudo-digestion"?
- 5) Utilizing the provided references, 'A Comparison of Techniques for Preparing Fish Fillet for ICP-AES Multielemental Analysis and the Microwave Digestion of Whole Fish' and 'Optimization of a Microwave-Pseudo-Digestion Procedure by Experimental Designs for the Determination of Trace Elements in Seafood Products by Atomic Absorption Spectrometry' to guide you, select a method and **construct a detailed plan** for how you are going to perform the sample digestion/leaching process to quantify the total mercury in tissue (or specifically TVP which you'll be using in this Milestone 2). This plan is what you are going to employ during the laboratory session, so make sure it is sufficiently detailed. It must include an estimate for the time, the mass of dried tissue, reagents involved, temperatures, glassware, etc. You should probably consult your partner on this question. Note: this is a critical component of the pre-lab and you will NOT be allowed to enter the lab if your TA is not satisfied with your level of preparation as demonstrated by your response to this question.
- 6) Utilizing the provided reference 'Field Sampling, Preconcentration and Determination of Mercury Species in River Waters' to guide you, construct a detailed plan for how you are going to perform the pre-concentration process using sodium diethyldithiocarbamate (SDDC) chelating agent and C18 SPE columns. This task carries a significant fraction of the pre-lab assessment, so be thorough with your plan. This plan must include detailed plan for the incremental steps:
 - a. Description of how you will pre-condition the SPE columns (this must include specific masses and volumes to be employed)
 - b. Detailed description of how the SPE column will be modified with SDDC. This must include a description of how the SDDC solution will be prepared (volumes, masses, pH, etc.).
 - c. C18 SPE pre-concentration / enrichment and elution steps (see reference) – including estimates for volumes and any external conditions you may apply.
 - d. Description of what standard solutions and volumes will be employed to verify the pre-concentration and elution protocol
- 7) The final Experimental step in this Milestone 2 is to obtain fish samples and sufficiently dry them in the food dehydrator. Perform a literature search and construct a protocol (time and temperature) for using the food dehydrator to dry the fish samples.

Milestone 2 Experimental

(Note: these experimental bullet-points are not necessarily in the order that they must be completed, they are just a list of requirements for the experiment and it is up to you to organize your time to complete them)

- Make sure to read through the ‘Technical Report’ section of this handout when thinking about the experiment to ensure that your experimental plan includes all steps necessary to answer the questions for this experiment. In other words, the ‘Technical Report’ section of this handout also specifies experimental aspects you must perform.
- Before developing your digestion protocol with the TVP, it is suggested that you first focus your efforts on optimizing a pre-concentration protocol using aqueous Hg^{2+} standards and C18 SPE columns
 - Utilize aqueous Hg^{2+} standard solutions that you have prepared to verify that you can successfully concentrate Hg^{2+} in the C18 SPE columns and then elute the Hg^{2+} (you will verify success using the AAS).
 - After you are confident that you can both concentrate and elute the analyte, prepare a standard that can serve as a model for what you will have with the digested fish and TVP samples – a solution with a Hg^{2+} concentration in the parts-per-billion range. Make sure that you can effectively concentrate and then elute the analyte such that you obtain a solution with a Hg^{2+} concentration well above the technique LOD. Make sure you quantify the precision and efficiency of the concentration and extraction protocols you have optimized - this is a key component of this Milestone and the entire experiment.
 - The pre-concentration sample prep is key for success in this experiment and may require a number of laboratory sessions to complete. Once you have established a protocol, then move onto the subsequent tasks (utilizing the TVP) listed below.
- Homogenize portions of TVP with a coffee grinder and/or mortar and pestle.
- Weigh out a total of seven portions of finely ground TVP and place in labeled beakers
 - 1 of these samples will serve as a blank and will not be spiked with any mercury ever. This “blank” sample will likely need to be of greater volume because you will use it for determining the method LOD with the TVP matrix.
 - 4 of these samples will go through the digestion and pre-concentration process and THEN spiked with mercury such that the final solutions (to be injected into the AA spectrophotometer are of concentrations that correspond back to ~1000, 2000, 4000, and 6000 ng of mercury per gram of dried tissue/TVP. You are spiking them just before injection into the AA spectrometer such that you know exactly what the true concentration is for the given instrument response – allowing you to produce a calibration curve that sufficiently considers matrix effects.
 - 2 of these samples will be spiked with mercury BEFORE the digestion process to achieve mercury concentrations in the range of 1,000 – 10,000 ng of mercury per gram of dried tissue in order to determine the fraction of analyte lost through the sample preparation process.
- Based on the protocol you developed in your pre-lab, thoroughly digest all seven homogenized TVP samples using minimal volume of digesting agent. Make sure to produce sufficient notes about the procedure - the goal is to establish an optimal procedure for digesting your fish samples in the next milestone
- Utilize filter paper and Buchner funnel to filter your digested samples and pre-concentrate the samples using a protocol you developed in the pre-laboratory plan.
- Perform AAS on your seven samples
- While you will not be analyzing actual fish during this session, you must prepare (i.e. collect and dry) the fish tissue for analysis in the next milestone. Work collectively with the other groups also working on this project to determine a sufficient quantity of fish your entire laboratory section will need. I have

posted an Instagram video link on the D2L site demonstrating how to effectively collect tissue from the fish. Make sure to record the initial mass that you acquire before drying as well. Place the fish parts in the dehydrator and dry them according to your plan you presented in the pre-laboratory assignment. Please don't start the dehydrator until the end of lab session because it is loud and annoying. Utilize the timer feature and then you will arrive to your next session to perfectly dried fish parts. Please do not work with the fish directly on uncovered laboratory benches! Lay down a layer of aluminum foil or butcher paper and dispose of all fishy garbage in the solid waste bucket labeled 'fish garbage' – let's minimize fishy smell in the lab. And please make an extra effort immediately use soap and water to clean up anything that comes into contact with fish throughout this entire experiment.

Milestone 2 Technical Report

Download and use the "Technical Report Template" to construct a concise, neat, and well formatted technical report for this Milestone 2. This Technical Report for Milestone 2 must be submitted no later than 7 days after the day that you begin the experimental laboratory component of Milestone 3. Your report must include:

- Abstract
- Experimental section detailing your optimized pre-concentration protocol
- Table/Figure illustrating the efficiency and precision of the optimized pre-concentration protocol obtained with mercury standards. There should also be Discussion that references this data as proof that an effective pre-concentration and elution protocol was developed.
- Figure illustrating the response / calibration curve from the four known mercury-spiked standards, plus the blank, that you prepared. This figure should include the best-fit linear model for the response curve – plus uncertainty. Use the LINEST function in Excel to obtain standard uncertainty values in both the slope and y-intercept for this model. Include the measured absorbance for the 2 TVP samples that were spiked before the sample preparation process – make the markers on the calibration curve corresponding for these two datapoints distinct from that of the standards. Make sure to describe the markers in the caption of the graph.
- Table summarizing the results for the two TVP solutions spiked before the sample preparation process. It should include i) the initial ppm of mercury for the TVP, ii) the ppm corresponding to the measured absorbance (before any correction), and iii) the percentage of mercury lost during the sample preparation procedure.
- Experimental section detailing the optimized digestion protocol for the TVP – this is what you will employ with the fish samples.
- The carbon copies of your notebook pages used for this Milestone 2 should be attached to the back of the hard-copy of this report when you submit it

Milestone 3: Quantitative Analysis of Fish Tissue

Milestone 3 Assessment

- Milestone 3 Pre-laboratory assignment : 32 points
- Milestone 3 Experimental and Technical Report : 61 points

Milestone 3 Overview

For this milestone you will utilize the protocols you developed and optimized in the first two milestones, plus the **method of standard additions**, to quantify the total mercury concentration (in ppm by mass) in your fish flesh sample. The method of standard additions is frequently employed in methods exhibiting matrix effects. Matrix effects reveal themselves as differences in a measured response due to differences in the matrix – not due to differences in the analyte concentration. AAS is a technique that is highly sensitive to error induced by matrix effects. Because of such matrix effects, it is completely invalid to employ a calibration curve obtained using external standards (standards produced in an aqueous solution of your manufacture and with different composition (matrix) than that of the digested fish solution) to interpolate analyte concentrations from a measured absorbance with a digested fish sample. You also cannot employ the normal procedure of just adding a known amount of analyte to a certain number of digested fish samples and constructing a calibration curve from these samples because there is already mercury present in these samples, and you don't know what that analyte concentration is. The typical calibration curve approach works when you know exactly what the concentrations of each external standard are. Thus an alternative method is required. The method of standard additions involves spiking the final “sample prepped” solution with a series of known amounts of analyte to obtain a standard addition response curve, and extrapolating to determine the analyte concentration in the un-spiked fish solution. Consult your Quantitative Chemical Analysis textbook for discussion and application of employing this method of standard additions.

Milestone 3 Pre-lab Assignment

Recall that it is mandatory that your pre-lab assignment for the specific milestone be approved by your TA before you begin performing any experimental work.

- As discussed in the Overview, due to the complex matrix involved, the method of standard additions will need to be employed for mercury quantitation via AAS. The table of data below is a sample set of data obtained from an AA spectrometer, similar to what you will acquire (and analyze) in the experiment. The data illustrates that this experiment began with seven 10 mL samples of digested and pre-concentrated fish solutions, which were individually spiked and analyzed. Use the provided data and the graphical method for standard additions (see your Quantitative Chemical Analysis textbook) to determine the concentration (in ppm by mass) of mercury in the un-spiked sample solution. Assume that each 10 mL sample was effectively constructed from 1.0 grams of dried fish flesh (i.e. the total 70 mL of all sample solutions came from 7.0 grams of dried fish tissue), calculate and report the total mercury content, in ppm by mass, in the original dried fish tissue. Make sure to include your standard addition plot that you constructed to perform this analysis in your pre-lab report. Hint: if you do not determine the concentration to be between 1.5 – 2.5 ppm, you have made a mistake!

Hg spike solution concentration: 100.0 ppm	
Density of all solutions: 1.00 g/mL	
Initial sample volume : 10.00 mL	
Measured Absorbance	Spike Volume (mL)
0.01271	0.00
0.01560	0.05
0.01831	0.10
0.02385	0.20
0.02929	0.30
0.03976	0.50
0.04983	0.70

2. Construct three detailed plans / procedures for completing this milestone:

- a. Fish sample digestion plan
- b. Pre-concentration plan
- c. Method of standard addition analysis using AAS plan – from Milestone 1, you know what sample volume is required for analysis. Your method of standard additions should employ at least 5 samples (4 spikes plus an un-spiked solution) – thus if you determined that 10 mL is the minimum volume needed for analysis, you would require at least 50 mL of total solution volume for performing the overall analysis via the method of standard additions. For selecting the concentration and volume of spike solution, it is suggested that your four spikes increase the Hg concentration by ~ 1, 2, 5, and 10 ppm respectively.

Milestone 3 Experimental

(Note: these experimental bullet-points are not necessarily in the order that they must be completed, they are just a list of requirements for the experiment and it is up to you to organize your time to complete them)

- Digest and pre-concentrate your fish sample using the plan you developed in the pre-laboratory component
- Utilize the method of standard additions, based on the plan you developed in the pre-laboratory component, with the AA spectrophotometer to analyze the total mercury concentration (in ppm by mass) for your fish sample

Milestone 3 Technical Report

Download and use the “Technical Report Template” to construct a concise, neat, and well formatted technical report for this Milestone 3. This Technical Report for Milestone 3 must be submitted no later than 7 days after the final laboratory session allotted for this experiment.

- Abstract that should include the total mercury content (in ppm) of the dried fish tissue that you analyzed – the 95% confidence interval should be reported for this value based on the standard uncertainties obtained from the best-fit slope and y-intercept from the graphical method of standard additions.
- Figure illustrating standard addition curve. Figure should include the best-fit linear model for the response curve. Use the LINEST function in Excel to obtain standard uncertainty values in both the slope and y-intercept for this model
- Experimental section explaining the optimal sample preparation procedure applied to the fish sample
- Experimental section explaining how you performed the method of standard additions.
- The carbon copies of your notebook pages used for this Milestone 2 should be attached to the back of the hard-copy of this report when you submit it