Optimizing Methods for ICP-MS Analysis of Mercury in Fish: An Upper-division Analytical Chemistry Laboratory Class

Wonhyeuk Jung[1#*], Christopher S. Dunham[1*], Katie A. Perrotta[1*], Yu Chen[1], James K. Gimzewski[1,2], Joseph A. Loo[1,3]

[1]Department of Chemistry and Biochemistry, University of California, Los Angeles, California 90095, United States

[2]California NanoSystems Institute, University of California, Los Angeles, California, 90095, United States

[3]Department of Biological Chemistry, David Geffen School of Medicine, University of California, Los Angeles, California 90095, United States

[#]Current address: Department of Cell Biology, Yale University, New Haven, Connecticut, 06516, United States

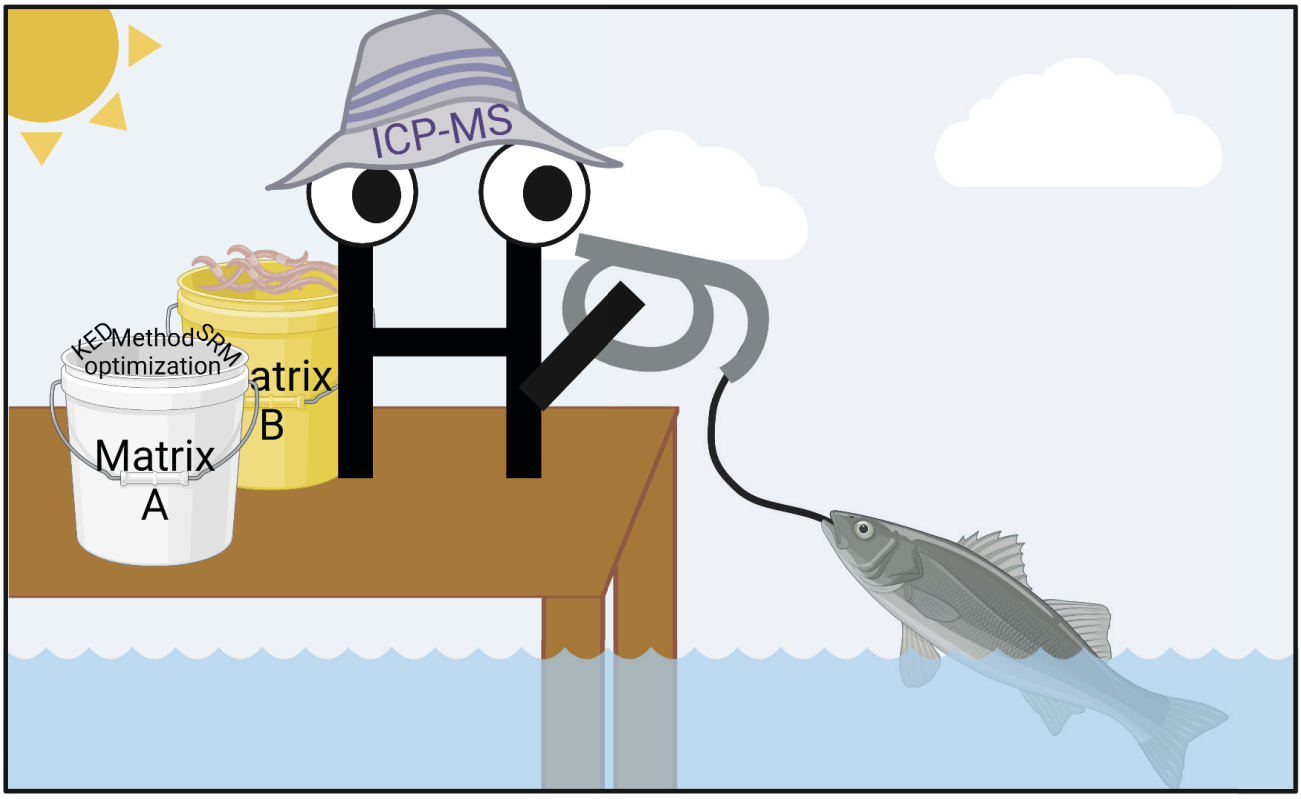
[*]These authors contributed equally to this work.

ABSTRACT

Food safety science is an important field due to its practical applications in maintaining public safety and confidence in consumer goods. A significant component of food safety science is the detection and regulation of heavy metals in food. Heavy metals such as mercury (Hg) are of particular concern because of their potential to damage the nervous system, gastrointestinal tract, and other organ systems in humans and other organisms. The stringent standards and practices for the analysis of Hg in fish, as implemented by institutions such as the Food and Drug Administration (FDA), require both skilled analytical chemists and sensitive quantitative techniques, e.g. inductively coupled plasma mass spectrometry (ICP-MS). These needs inspired the development of an upper-division, undergraduate analytical chemistry experiment that is designed to teach students how to quantify mercury in commercial fish products via ICP-MS analysis. In this hands-on laboratory exercise, students were taught how to use a standard reference material (SRM) for method validation and to understand how different matrices can affect the accuracy of the analysis. Students also learned how to optimize ICP-MS instrument parameters such as kinetic energy discrimination (KED) voltage. Students worked in small groups and across lab sections to analyze their data and to identify the best parameter set for

their experimental conditions. This lab exercise provides a rigorous, practical, and challenging experience for aspiring analytical chemists and can be readily adapted to the needs and interests of any institution with access to an ICP-MS instrument.

GRAPHICAL ABSTRACT



KEYWORDS

Upper-Division Undergraduate, Analytical Chemistry, Instrumental Methods, Quantitative Analysis, Mass Spectrometry, Hands-On Learning/Manipulatives, Inquiry-Based/Discovery Learning, Laboratory Instruction, Food Science

INTRODUCTION

Government regulatory bodies such as the Food and Drug Administration (FDA) and the Environmental Protection Agency (EPA) routinely oversee the administration and enforcement of regulations governing hazardous compounds that can enter the food supply.[1] Screening mercury (Hg) in commercial fish products is of particular importance because seafood is the main source of this toxin for the general public.[2] Mercury toxicity can lead to brain damage including psychological

disturbance, impaired hearing, loss of sight, ataxia, loss of motor control, and general debilitation.[3] Moreover, Hg exposure during the embryonic phase can lead to severe abnormalities in psychomotor development.[4]

Thus, the FDA closely monitors total Hg levels in commercial fish, provides guidelines on its consumption, and prohibits the sale of fish that have mercury levels higher than an action level of 1 ppm.[5-9] Action levels represent the limit at which the FDA will take legal action to remove products from the market. The FDA regularly updates the elemental analysis manual for food and related products and inductively coupled plasma-mass spectrometry (ICP-MS) is one of the primary methods for the quantification of total Hg in fish.[10]

ICP-MS is a sensitive tool for elemental quantification with diverse applications, e.g. environmental sample analysis, water quality control, and food analysis.[11-14] This technique utilizes a plasma to achieve ionization and atomization of molecular species into their elemental components and is well suited for sensitive quantitative analysis, as it can detect analytes at concentrations well into the parts-per-trillion (ppt) range.[15] These traits make ICP-MS a particularly powerful technique for trace metal analysis in commercial products.

The prevalence of ICP-MS in real-world analytical chemistry applications has led to a number of proposed student lab exercises using ICP-MS over the last decade.[16-20] Such protocols are of great pedagogical value and introduce students to foundational concepts of ICP-MS and the practical application of the technique. However, important analytical chemistry concepts such as the impact of matrix effects on the accuracy of ICP-MS analysis and parameter optimization via the usage of a standard reference material (SRM), which should closely mimic the composition of the sample , have not been extensively explored in previously published student-led ICP-MS experiments.[17-20] Here, we introduce a hands-on lab exercise for an upper-division, undergraduate analytical chemistry course that mimics industry- and government-level ICP-MS protocols.

This undergraduate lab exercise was tested during the Winter 2020 quarter and fully implemented during the Winter 2022 quarter at the University of California, Los Angeles (UCLA) for a chemical instrumentation/analytical chemistry class. Students were tasked with optimizing experimental and instrument parameters with fish samples that were digested with two different acid matrices,

Digestion A (5.6% HNO_3 and 1.2%H_2O_2) or Digestion B (5.6% HNO_3, 0.74%HCl and 1.2%H_2O_2). In order to analyze these samples in an acid composition appropriate for the ICP-MS analysis, students diluted these samples 3.5-fold with water. The resulting two matrices for the samples were denoted as Matrix A (1.62% HNO_3 and 0.34% H_2O_2) and Matrix B (1.62% HNO_3,0.21% HCl, and 0.34% H_2O_2).

Furthermore, students were introduced to the concept of kinetic energy discrimination (KED) as a means of suppressing polyatomic interfering species.[21] Polyatomic interference is caused by polyatomic species that are isobaric with the target element. KED takes advantage of the fact that isobaric polyatomic species will lose more kinetic energy relative to the target ions as they travel through a cell filled with a non-reactive gas such as helium (Figure 1, Figure S1).

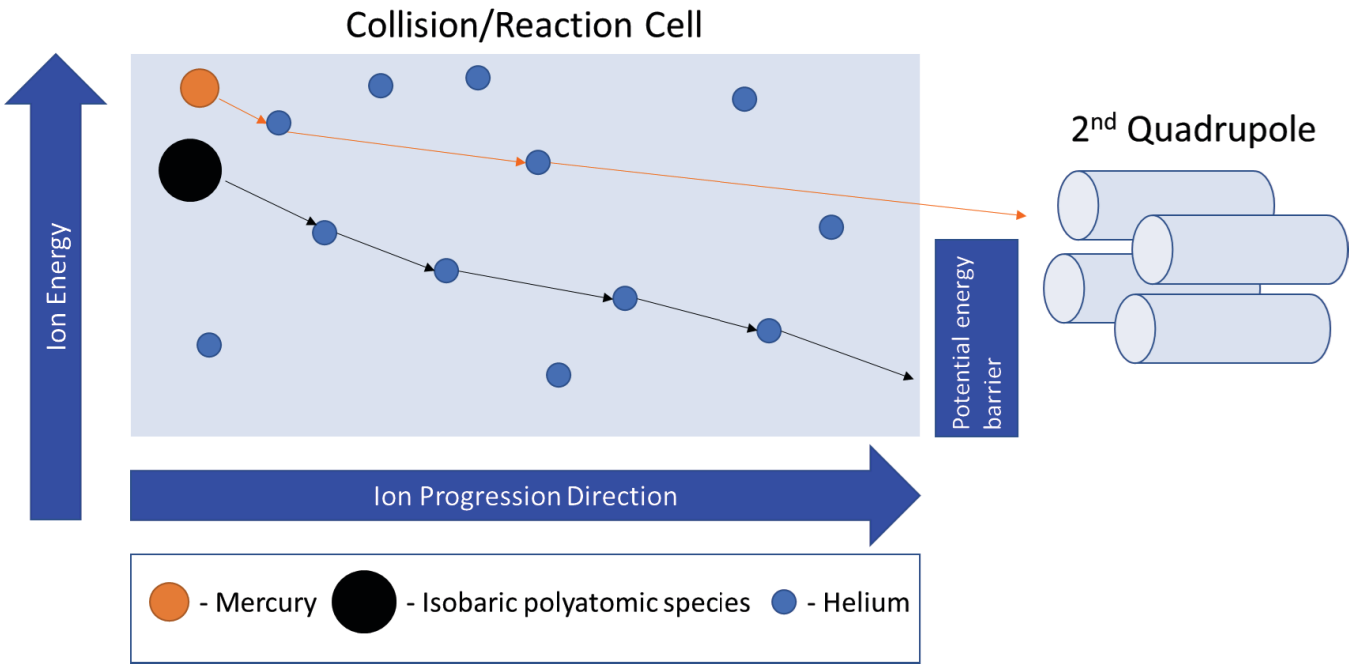


Figure 1. Kinetic energy discrimination can be used to suppress polyatomic interference (or isobaric interference). Isobaric polyatomic species have larger collision cross sectional areas compared to the target molecule (^{201}Hg, orange spheres) and experience more collisions as they travel through the helium (blue spheres)-filled collision/reaction cell. The resulting kinetic energy difference between the target atoms of interest and the isobaric polyatomic species (black spheres) can be taken advantage of by utilizing an energy filter between the cell and the 2nd quadrupole, ensuring that only the analytes enter the quadrupole analyzer. By adjusting the energy difference between the cell and the quadrupole, the potential energy barrier that ions need to overcome (depicted as KED voltages in this experiment) can be varied.

The SRM 1947 (Lake Michigan Fish Tissue) from the National Institute of Standards and Technology (NIST) was used for method validation.[22] Students analyzed each sample/matrix

combination across multiple lab periods. Each lab section investigated a different KED voltage. Students pooled their data across lab sections together into a centralized cloud repository for analysis. Using their data, students determined an optimal matrix and KED voltage combination for their analysis. Finally, students used the optimized parameter dataset to assess the safety of the fish sample for potential human consumption.

This experiment aimed to achieve the following learning outcomes:

1. Students will learn how to validate a method with standard reference material (SRM)
2. Students will investigate how different acid matrix compositions affect accuracy of the analysis.
3. Students will learn how to optimize instrument parameters. In this case students observed how varying the KED voltage affected the analysis.

EXPERIMENTAL PROCEDURE

Reagents

Hydrochloric acid (37%, ≥99.999% trace metal grade, Sigma-Aldrich, Cat. no. 339253), nitric acid (70%, ≥99.999% trace metal grade, Sigma-Aldrich, Cat. no. 225711), hydrogen peroxide 30% (trace metal grade, Sigma-Aldrich, Cat. no. 95321), and water (high performance liquid chromatography (HPLC)-grade, Fisher, Cat. no. W9-1) were used to generate matrices for digestion and analysis. Scandium stock solution (100 μg/mL, 7% HNO_3, Inorganic Ventures, part no. CGSC10-125ML) and yttrium stock solution (100 μg/mL, 2% HNO_3, Inorganic Ventures, part no. MSY-100PPM-125ML) were diluted to 8 ng/mL and 4 ng/mL, respectively, with either Matrix A (1.62% HNO_3 and 0.34% H_2O_2) or Matrix B (1.62% HNO_3,0.21% HCl, and 0.34% H_2O_2) to generate internal standard (ISTD) solutions. Mercury standard (10 μg/mL, 5% HNO_3, Agilent, part no. 5190-8575) was diluted with either Matrix A or Matrix B to generate a 10 ng/mL stock solution, which was used to make calibration solutions. ICP-MS tuning solution (10 mg/mL Ce, Co, Li, Mg, Tl, and Y, Agilent, part no. 5190-0465) was diluted to 1 μg/L with 2% HNO_3 to conduct instrument warm-up and auto-tuning. SRM 1947 (Lake Michigan Fish Tissue, certification date 9/3/2020, expiration date 12/31/2026) was purchased from NIST.[21] Various fish (canned Skipjack tuna, Chilean sea bass filet, swordfish filet) were purchased from local supermarkets. Following digestion (see the Microwave Digestion & Sample Preparation section for

details), the samples were stored in acid-resistant Nalgene™ Narrow-Mouth Bottles Made of Teflon™ PFA (Fisher, Cat. no. DS1630-0001).

Microwave Digestion and Sample Preparation

All samples were digested via microwave digestion. Microwave digestion was performed by teaching assistants (TAs) and staff at the ICP-MS facility within the UC Center for Environmental Implications of Nanotechnology at UCLA. Samples (method blank, approx. 1 g SRM, approx. 1 g fish) were measured into clean Teflon vessels for acid digestion (the exact masses are reported in the Experimental Protocol document). The digestion was carried out using either a mixture of 4 mL 70% HNO_3, 2 mL 30% H_2O_2, and 1 mL H_2O (called Digestion A), or 4 mL 70% HNO_3, 2 mL 30% H_2O_2, and 1 mL 37% HCl (Digestion B) at 190℃ for 20 minutes in a microwave digestion system (Titan MPS, PerkinElmer). Once the samples were cooled to room temperature, they were subsequently diluted to the final volume of 50 mL by adding filtered deionized water. In later steps detailed in "Teaching Methods", Digestion A is further diluted to 1.62% HNO_3 and 0.34% H_2O_2 (matching the composition of "Matrix A"). Digestion B is diluted to 1.62% HNO_3, 0.21% HCl, and 0.34% H_2O_2 (matching the composition of "Matrix B").

ICP-MS Instrument Parameters

An 8800 QQQ ICP-MS system (Agilent) equipped with an SPS 4 Autosampler (Agilent) was used for the experiment. ^{201}Hg isotope was targeted for the analysis. MassHunter Workstation 4.1 software (Agilent) was used to control the system and to process the data. The key parameters for ICP-MS are listed in Table 1 and were consistent throughout all experiments.

Table 1. Instrument parameters.

RF Power	1550 W	*Nebulizer Pump*	0.10 revolutions per second
RF Matching	1.80 V	*S/C Temp*	2°C
Sampling Depth	8.0 mm	*Gas Switch*	Dilution Gas
Carrier Gas	1.00 L/min	*Makeup/Dilution Gas*	0.20 L/min
Option Gas	0.0%		

Teaching Assistant Responsibilities and Classroom Organization

The TAs held several responsibilities both preceding and following the lab period. Teaching assistants were tasked with preparing stock solutions of Matrix A and Matrix B solvents for calibration curves, preparing working solutions of internal standards, preparing sample microwave digestions, performing instrument warm-ups and pre-run maintenance (e.g. rinsing the probe, and sample and internal standard inlet tubes), and consolidating and uploading student data to the course's cloud storage repository. The average additional time commitment from the TAs for these tasks was approximately 6 hours per week.

This exercise was performed alongside and independent of several distinct mass spectrometry laboratory exercises during the Winter 2022 quarter of the Chemical Instrumentation class. The 42 students participating in the class either had limited or no prior analytical chemistry lab experience. The class maintained three distinct laboratory sections of approximately 14 students per section. Each section operated two distinct, four-hour lab periods per week (e.g. Tuesday/Thursday morning, Tuesday/Thursday afternoon, Wednesday/Friday afternoon) for 10 weeks (i.e. the duration of classroom instruction for the academic quarter). Students from each section worked in groups of 3-4 during the week in which they were assigned to this experiment to minimize crowding around the instrument while accommodating a relatively large laboratory class size. A full breakdown of the class structure is laid out in Figure 2.

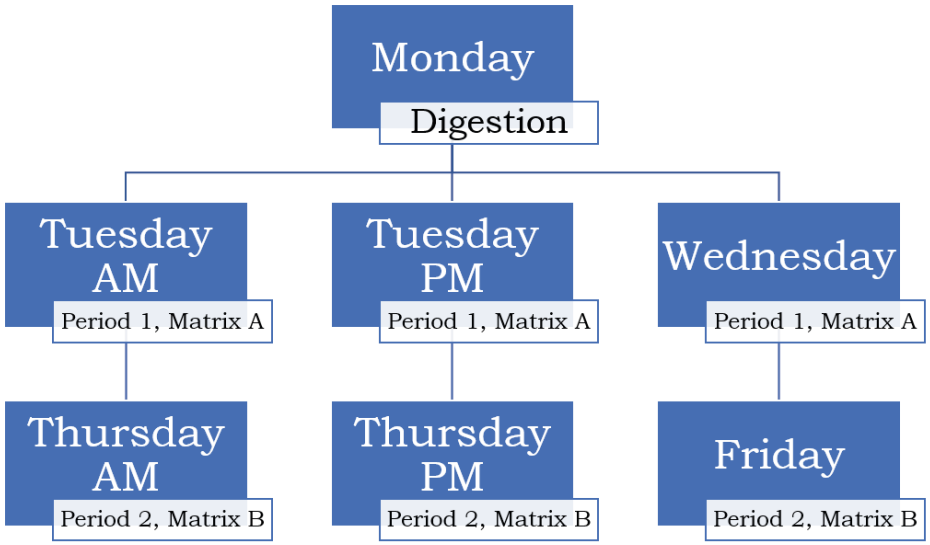


Figure 2. Laboratory section timelines. On Monday samples were delivered to the ICP-MS core at the California NanoSystems Institute by the TAs. These samples were digested following the methods described above by the core staff and picked up by the TAs the following morning prior to the Tuesday AM section. During Period 1 samples were analyzed using Matrix A. Period 2 followed a similar protocol to Period 1 but instead used Matrix B. Data from each of the three sections was compiled in a central repository to compare data across periods.

HAZARDS

The handling of mercury samples described in this protocol was approved by and in accordance with UCLA Environmental Health and Safety (EH&S) guidelines. Students should always wear protective goggles, gloves, and lab coats during the experiment. 70% HNO_3 and 37% HCl used for mercury extraction are caustic and should be handled with care. The calibration solutions that contain HNO_3, HCl, and mercury should be generated with care in a well-ventilated fume hood and should be disposed of properly following data acquisition. Finally, it is highly recommended that the autosampler chamber be ventilated during and after the experiment to minimize build-up of mercury-containing vapor.

RESULTS AND DISCUSSION

Students were instructed on the fundamental concepts of quantitative analytical chemistry and selected concepts of ICP-MS (instrumentation, application) during the lecture, in addition to assigned reading for the course. For the laboratory exercise, students were provided with the protocol accessible in the Experimental Protocol. During the ICP-MS experiment, each of the three sections were assigned their own KED voltage to investigate (ranging from 0-12 V). Students then compiled their raw data into the central data repository to compare KED and matrix effects across the 3 sections.

Each “Lab Period 1” began with a TA-guided question-and-answer session to ensure students completed the pre-lab exercises (see Experimental Protocol), followed by guided inquiry and a discussion of experimental methods. These discussions covered: conical tube rinse protocols (see Experimental Protocol), sample preparation and dilution, calibration curve preparation, and kinetic energy discrimination voltage. During this period, students were instructed on how to use serological- and/or micro-pipettes. The TAs prepared the 10 ng/mL working stock Hg solution, from which students generated their calibration curves in Matrix A. Students calculated the calibration curve concentrations in the pre-lab assignment. Water was then used to dilute the method blank, SRM, and

fish sample 3.5-fold to match the matrix composition of Matrix A. After the completion of solution preparation, students vortexed all containers and loaded their samples into the autosampler.

Next, students were instructed on how to use the instrument software and received guidance on basic principles of sample batch design. The final batch parameters were approved by the TAs before batch submission to the instrument queue for analysis. Representative calibration curves, produced by the MassHunter software using student data, are shown in Figure 3. All student data presented herein are done with the explicit consent of the students who produced the data.

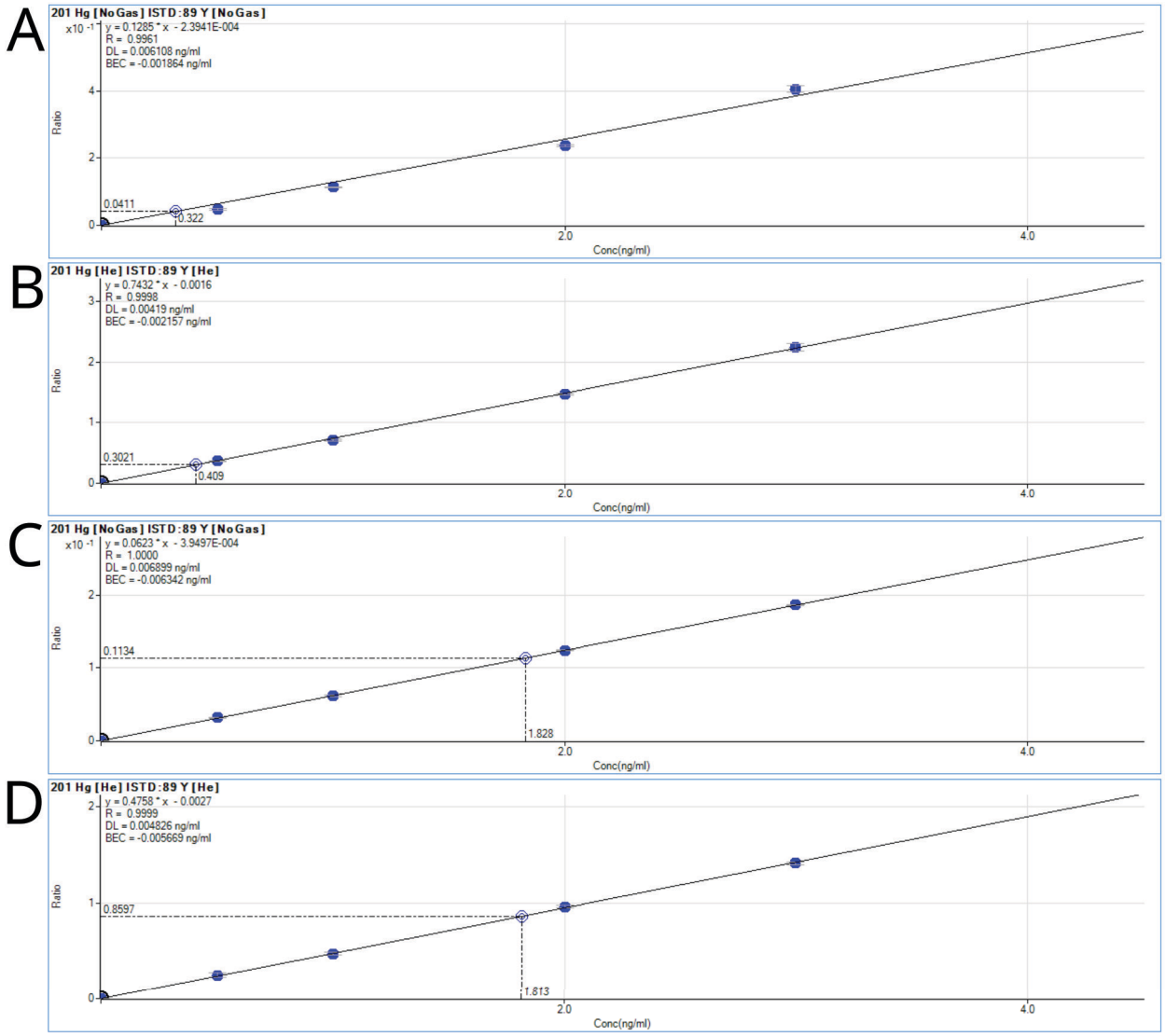


Figure 3. Calibration curves produced from student data. The y-axis is expressed as the ratio between analyte signal (Hg) to internal standard (Y). A) Matrix A in no gas mode, B) Matrix A in helium mode, C) Matrix B in no gas mode, and D) Matrix B in helium mode. Overall linearity was excellent with R^2 values ranging from 0.980 to 1.000.

During "Lab Period 2", students repeated the procedures followed during lab period 1, except now using Matrix B. As above, calibration standards were made in Matrix B from the 10 ng/mL working stock Hg solution. Water was used to dilute the method blank, SRM, and fish sample 3.5-fold to match the composition of Matrix B. Following sample preparation and batch submission to the instrument software queue, students engaged in a TA-led discussion about data analysis. Students examined data collected during the previous lab period by both themselves and the groups in other lab sections. They were asked to first calculate the percent error of the average SRM concentrations of each run (Equation 1). Sample and SRM masses from the microwave digestion were provided in a shared README file. Using this data, students calculated the expected mercury concentration for the SRM in each matrix and compared these values to the acquired data.

Equation 1: ((expected [SRM] - observed [SRM]) / expected [SRM]) * 100

A review of recently published literature that involves ICP-MS-based analysis of Hg shows that recovery rates around 90 to 95% are accepted as sufficient for method validation.[23-25] Students validated the method by checking whether the percent error calculated from Equation 1 is within 10%. Students were asked to determine which matrix performed better in combination with other parameters by comparing expected and observed SRM mercury concentrations. Then, students assessed which KED voltage was the most optimal by comparing the datasets from several different KED voltages (e.g. 0V, 2V, 6V). For the final phase of the experiment, students were asked to use the data obtained from the optimal set of method parameters to evaluate whether their fish sample would be safe to consume according to FDA guidelines.

Educational outcomes for this exercise were assessed through a combination of pre- and post-lab surveys and a written lab report assignment. All students were provided with a short list of questions to address in their report concerning method validation and optimization, results, and interpretation. Students from all groups determined that Matrix B, with its hydrochloric acid component, served as a superior matrix for mercury analysis. Students determined that the choice in optimal gas mode could

vary based on the applied KED. In many cases, students found that the no gas mode and helium gas mode with a KED of 6V to be the optimal method based on SRM error (%). An example of parameter optimization analysis as performed by the students is shown in Table 2.

Table 2. Student-generated summary of data for determining the optimal parameter combination.

Matrix	*Mode*	*KED (V)*	*Fish (ng/mL)*	*Mean SRM (ng/mL)*	*SRM error (%)*	*Error within 10% Y/N*
A	No Gas	0V	0.180	2.140	21.64	NO
A	He Gas	0V	0.179	2.060	17.12	NO
A	No Gas	2V	0.180	2.051	16.60	NO
A	He Gas	2V	0.179	1.873	6.453	YES
A	No Gas	6V	0.194	2.233	26.93	NO
A	He Gas	6V	0.230	2.024	15.03	NO
B	No Gas	0V	0.284	1.959	7.423	YES
B	He Gas	0V	0.274	1.912	4.849	YES
B	No Gas	2V	0.266	1.976	8.367	YES
B	He Gas	2V	0.280	1.955	7.236	YES
B	No Gas	6V	0.268	1.852	1.589	YES
B	He Gas	6V	0.237	1.784	-2.147	YES

PEDAGOGICAL EVALUATION AND STUDENT OUTCOMES

The pedagogical benefits of this experiment were evaluated through student laboratory reports, student surveys, and anecdotal communication from students throughout and following the exercise.

Student lab reports were structured similar to scientific papers in the Journal of Analytical Chemistry. A detailed rubric was used by the TAs to grade each report. This rubric outlined strict

criteria outlined with the aforementioned learning outcomes that must be met by the students in order to achieve a high grade. The discussion and conclusion section of the lab report required students to successfully address the following items:

- Is the method valid? Justify your answer. What does having a valid method allow you to do?
- Which matrix performs best? Explain your selection.
- Do you notice any effects of KED? Is there a KED voltage that performs best?
- Which combination of parameters would you use to achieve optimal results? How did you come to this conclusion?

The discussion and conclusion section was worth a total of 27 points and the scores were distributed as shown in Figure 4. The majority of students performed exceptionally well, scoring above 25 points out of 27 (above an A-). Only 5 students out of the 38 who submitted the report scored below a C. These lab report scores indicate that this laboratory exercise successfully taught the students the desired learning goals.

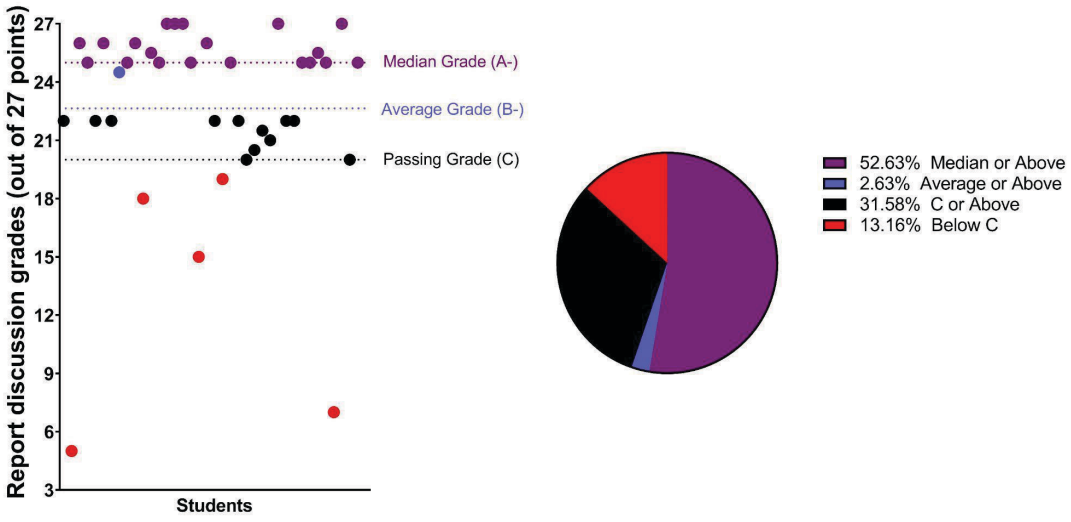


Figure 4. The score distribution of the discussion section of the reports. Each point represents an individual student. This section specifically evaluates whether the learning objectives stated above were achieved.

Below is an example conclusion from a student lab report that demonstrates student understanding of the laboratory exercise.

> "With this study, an accurate measurement was able to be made of store-bought Skipjack tuna. Utilizing varying experimental conditions such as different matrices, KED, and gas modes, an optimal condition was able to be found and used. Matrix B, containing 1.6% HNO_3, 0.34% H_2O_2, and 0.21% HCl, 6V of KED and a No Gas mode yielded the lowest deviations from a NIST-Certified SRM at roughly 1.59%. The concentration of mercury in this sample was found to be roughly 43ppb in comparison the FDA reported 95% confidence intervals of 120 ppb to 140 ppb. Therefore, this method of analysis can verify that the mercury contents of the store-bought Skipjack tuna is within safe ranges for human consumption which is essential due to mercury being a potent toxin in high quantities."

Anecdotally, students expressed a significant amount of enthusiasm and engagement throughout the lab exercise. Several students provided unprompted feedback expressing appreciation for the overall rigor, robustness, and practical nature of the exercise. Perhaps most importantly, a number of students informed the TAs that this exercise significantly improved their perception of analytical chemistry and led to a greater interest in the field.

Surveys were administered prior to and after the conclusion of the lab exercise to gauge the educational benefits of the class. Students were asked to rate their understanding on a scale of 1-10, 10 being the greatest understanding. The surveys assessed students' general understanding of ICP-MS, how a SRM is used for method verification, polyatomic interference, the purpose of using different gas modes, and the application of KED (Figure 5). The students' understanding increased after the lab in all areas. The class also resulted in the students feeling more confident in their ability to explain the concept of KED to a colleague. In the post-lab survey, there was an additional question that asked, "Did this lab help to improve your understanding of ICP-MS and undergraduate-level analytical chemistry techniques and data analysis?", to which 98% of students responded "yes".

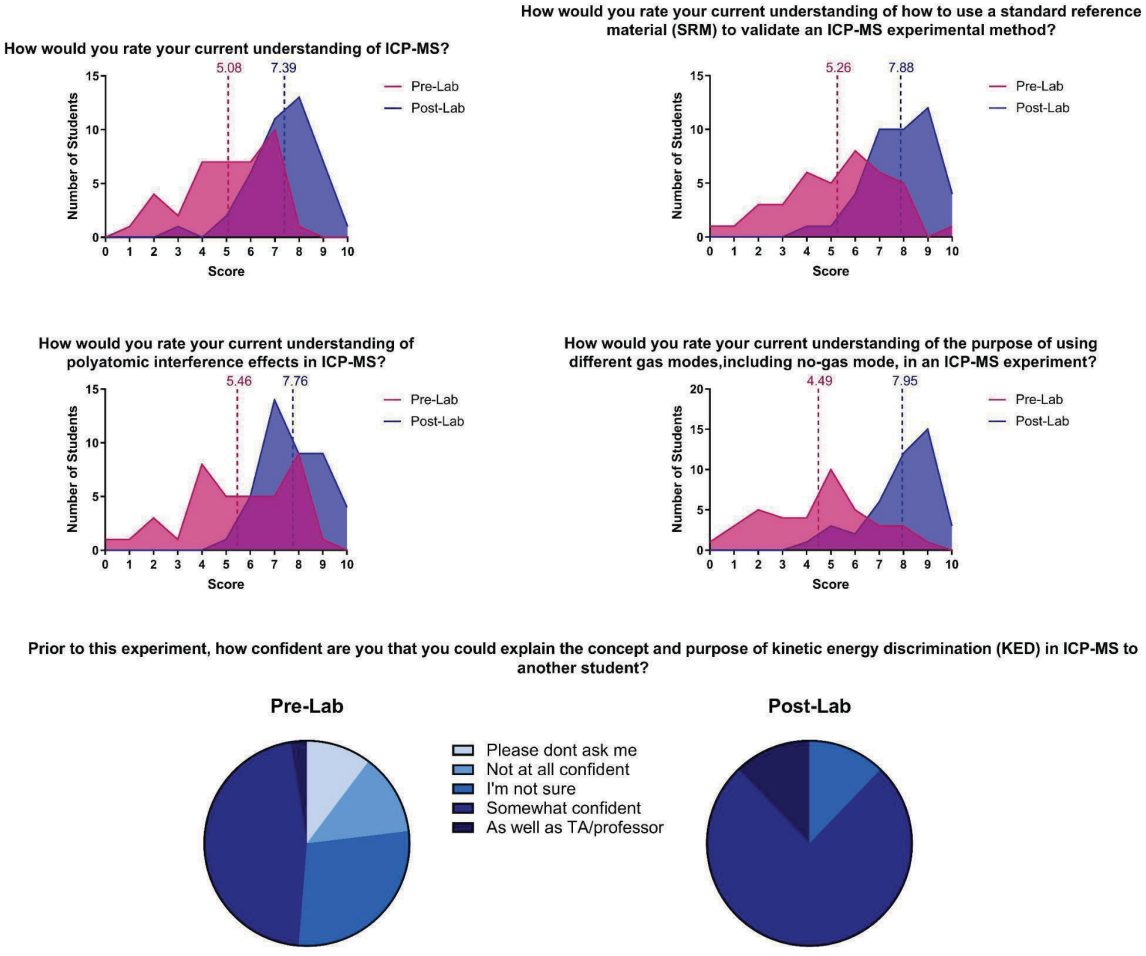


Figure 5. Student responses to pre- and post-lab surveys. The numbers listed above the dashed lines are the mean of the probability distributions.

Our objective in this lab exercise was to use the methodology employed by the FDA to teach advanced method development and parameter optimization skills. Students were able to successfully select the most optimal ICP-MS method by comparing the experimentally determined and expected values of mercury in the SRM. Using this information, students could inform the report reader about whether the fish was safe for human consumption according to FDA standards. Overall, the real-world relevance of the experiment kept students engaged and interested in the lab exercise.

CONCLUSION

This laboratory exercise was successfully incorporated into an analytical chemistry class at UCLA. For this exercise, mercury concentration determination with ICP-MS was performed on consumer-

grade fish samples. However, this exercise can be adjusted accordingly to be implemented into any course curriculum that aims to teach the concept of optimizing instrument parameters for measuring real world samples. The utilization of FDA-approved practices introduces students to real-life applications of government-level analytical chemistry techniques for food safety analysis. The class provides students with hands-on practical lab work experience and discussion-focused data interpretation which promotes development of analytical skills essential for aspiring analytical chemists. The pre- and post- lab surveys and laboratory reports highlight student growth in ICP-MS data acquisition, method optimization, and data analysis.

ASSOCIATED CONTENT

Supporting Information

The Experimental Protocol is available on the ACS Publications website at DOI: 10.1021/acs.jchemed.XXXXXXX. [to be filled in by ACS.]

Experimental Protocol

AUTHOR INFORMATION

Corresponding Author

*E-mail: jloo@chem.ucla.edu

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors wish to thank Dr. Chonghyun Chang at the ICP-MS facility, UC Center for Environmental Implications of Nanotechnology, for his support for this project. The authors are thankful for the wonderful contributions made by the students of the Chemical Instrumentation class. The authors wish to thank Kevin Koshiro for being the ultimate team player while assisting in troubleshooting and operational exercises. We acknowledge Biorender as the software used for figure generation. Finally, support from the US National Science Foundation (CHE-1808492 to JAL) and UCLA educational improvement grant (IIP #20-12) is acknowledged.

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