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EVALUATION OF MERCURY STANDARD STABILITY FOR ANALYZING HUMAN URINE USING INDUCTIVELY COUPLED PLASMA SPECTROMETRY

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EVALUATION OF MERCURY STANDARD STABILITY FOR ANALYZING HUMAN URINE USING INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY

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DEDICATION

To my inspiring parents, and brother,

for being the cheerleading squad,

and their endless love, support and encouragement.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

EVALUATION OF MERCURY STANDARD STABILITY
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Quantitation of total mercury has been of great interest not only in environmental fields, but also in medical research. This is because mercury (Hg) is one of the most toxic elements, and is also a well–known global pollutant. Researchers strive to understand how toxic elements bioaccumulate in both aquatic ecosystems and terrestrial systems, along with these effect on human health. Numerous studies and analytical methods have been published for the assessment of mercury in many different sample matrices. Accurate analysis of these complex matrices is dependent on accurate and reliable analytical standards. However, the standards preparation step is time consuming. This stability study is to extend the storage lifetime of the mercury intermediate standards, provide high recoveries, and minimize the mercury memory effect which will improve the applicability of biomonitoring studies for human health risk assessment related to mercury exposure. This approach can save time, and reduce cost if the storage lifetime of the standards can be sufficiently predicted. Three preservative solution combinations were investigated: 1% (v/v) nitric acid with 1% (v/v) sulfamic acid and 1% hydrochloric acid; 2% (v/v) nitric acid with 1% (v/v) sulfamic acid; and 5% (v/v) nitric acid with 1% (v/v) sulfamic acid. Standards were stored in sealed polypropylene containers at 3 temperatures (-28°C, 1°C, and room temperature). The use of 1% (v/v) nitric acid, 1% (v/v) sulfamic acid preservative solution, and 1% (v/v) hydrochloric acid in mercury intermediate standards stored in the refrigerator provided the best performance for up to 90 days. The results were in good agreement with the certified quality control values.

CHAPTER I: INTRODUCTION

1.1 Mercury

Mercury (Hg), is one of the most toxic elements, found in nature. Mercury has been used for many purposes for centuries, including medicine, commercial purposes, ritual practices, and even used as a poison [1]. Mercury, is a well-known global pollutant and is an environmental threat to living organisms due to its toxicity, its property to bioaccumulate, and the bioavailability of the chemical forms of the element.

Mercury is categorized as a nephrotoxin and neurotoxin. Mercury is commonly found in fluorescent light bulbs, electronics, fungicides, and medical equipment [2]. The main reason for measuring mercury in body fluids is because large segments of the population are commonly exposed to dental amalgams [3], vaccines (but though today, mercury has been virtually eliminated from vaccines) [4], seafood [5-7], and mercury is released into the atmosphere from geologic deposits.

In human biomonitoring projects, especially those involving the Centers for Disease Control and Prevention (CDC) and analytical testing laboratories, toxicologists and scientists use quantitative methods to assess human exposure to mercury. Human biomonitoring is a method to quantitate the amounts of toxic chemicals in humans' bodies and determine how exposure affects the body over time. These biomonitoring studies provide information which helps in identify potential health risks.

Humans can be exposed to three forms of mercury: elemental, inorganic, and organic [2, 8-12]. **Elemental mercury** (Hg^0), also known as metallic mercury, exists as a silver liquid at room temperature. Humans are exposed to elemental mercury in the following ways: mercury filled thermometers (historically speaking; but today, most thermometers no longer contain

elemental mercury), dental fillings, and fluorescent lightbulbs [2]. Other pathways of human exposure to elemental mercury are through human activity, including the burning of fossil fuels which releases elemental mercury into the atmosphere [8]. Mercury is also released into the atmosphere via the natural occurrence of mercury degassing from volcanic activity [9].The major route of human exposure to elemental mercury is through inhalation of vapors [10]. When elemental mercury enters the bloodstream, it is oxidized to Hg^2. The half-life of elemental mercury in the body is 60 days [8].

Inorganic mercury has two oxidation states: Hg_2^{2+} (Hg (I) or mercurous ion) and Hg^{2+} (Hg (II) or mercuric ion). Mercury salts have been found in fungicides [9] and other disinfectants [11]. Inorganic mercury is also found in cosmetic products [12], batteries, and is used in the synthesis of organic compounds [11]. The major route of absorption of inorganic mercury salts in humans is through ingestion. Another route of inorganic mercury exposure is absorption through the skin. Although only 10% of mercuric ions are actually absorbed in the gastrointestinal (GI) tract, inorganic mercury ions can cause decay and corrosive injury in the GI tract. The greatest absorption of mercuric ions occurs in the gut and kidneys. The half-life of inorganic mercury compounds is approximately 40 days in the body [8].

Organic Mercury: The most common alkyl groups present in organomercury compounds are methyl, ethyl, and phenyl. Methylmercury is biotransformed from inorganic mercury, and is considered to be the most neurotoxic organo-mercury compound [2]. The biotransformation process, “methylation”, takes place in the ecosystem via microorganisms and plants. When large quantities of inorganic mercury are deposited in an aquatic environment, microbes take up the “unwanted inorganic mercury products” produced from both natural and anthropogenic sources, and metabolize them into organomercury compounds [7]. Once the

methyl group is attached, organomercury compounds are bioaccumulated along the aquatic food chain. Therefore, top predatory mammals and fish such as tuna, sharks, and whales have the highest concentrations of methylmercury. Methylmercury is absorbed in the gastrointestinal tract after ingestion. It takes 48 hours for methyl mercury to spread to other tissues upon entry into the bloodstream. Approximately 90% of the absorbed methylmercury is excreted via the bile into the feces [2]. The half-life of methylmercury in human blood is from 40 to 70 days [8].

Depending on the chemical form of the mercury and the route of exposure, the notable target organs for toxic effects are the brain, the central nervous system, and the kidneys. Chronic exposure to either elemental mercury vapors or inorganic mercury salts can lead to emotional disturbances. Patients diagnosed with mercury poisoning may develop renal disease, paresthesia, and ocular lesions [13]. Paresthesia means patients experience a sensation of tickling and numbness on the skin [14]. Long term exposure to mercury may result in hallucinations, muscular seizures, delirium, and death.

1.2 Laboratory Testing and Analytical Methods:

It is very important to develop accurate and efficient methods to quantitate the mercury levels in biological matrices for medical purposes. Blood and urine are the most common biological matrices that are analyzed. A great deal of research has been devoted to the development of simple, sensitive, and robust methods for quantitating mercury in biological specimens. Many analytical tools have been developed for quantitating total mercury. The techniques and applications that have been employed for trace metal analysis and metal speciation include cold vapor atomic absorption spectrometry (CVAAS) [15-17], inductively coupled plasma atomic emission spectrometry (ICP-AES) [17-20], and inductively coupled plasma mass spectrometry (ICP-MS) [23-27]. ICP-MS is the most widely recommended

analytical technique for ultra-trace metals analysis due to the capabilities and advantages it provides.

One of the advantages of ICP-MS is the capability for rapid multi-element analysis. With this powerful analytical tool, the detection limit can be as low as parts per trillion (ppt). For trace metal analysis, ICP-MS provides much lower detection limits compared to atomic spectrometries such as inductively coupled plasma atomic emission spectrometry (ICPAES) [17-20], and graphite furnace atomic absorption spectrometry (GFAAS) [21, 22].

In the early development of ICP-MS, it was known for monitoring elements, including mercury, mostly in environment samples. In the past decades, public health concerns have gradually increased. Recently, several methods for the determination of total mercury in human urine have utilized ICP-MS [13, 23-26]. Early work by Kalamegham and Ash [23] proposed a simple procedure for measuring total mercury, both inorganic and organic, in whole blood and urine using 25% (v/v) hydrochloric acid with cysteine. This approach was important because no procedure had been described for mercury quantitation in clinical specimens at that time.

However, in later studies, many researches have experienced losses in mercury during either sample preparation or storage [42-45, 58, 59]. Therefore, samples that contained mercury must be preserved with strong acids and strong oxidizing agents prior to analysis to reduce mercury memory effect (carry over). Later developments in the use of ICP-MS by Nixon studied the effects of using gold and dichromate in hydrochloric acid as agents to reduce carry over [24]. This approach found that dichromate with hydrochloric acid provided effective reduction of mercury. A recent study by Jones and Pirkle [13] evaluated the measurement of total mercury and iodine in human urine. This procedure used 10 % (v/v) hydrochloric acid to preserve mercury in solution. The method by Jones and Pirkle was published by the Centers of Disease

Control and Prevention (CDC), and has become the approved instrumental method for the analysis of total mercury in human urine. A study by Parsons showed that mercury solution can be also preserved with 1% (v/v) nitric acid and 1% (v/v) sulfamic acid preservative solution [26]. In addition, ICP-MS can be used in combination with other techniques. For instances, ICP-MS can also be coupled with liquid chromatography (LC) for more advanced research such as speciation of mercury compounds. Tsoi proposed a LC-ICP-MS method for mercury speciation using headspace solid phase microextraction [27].

1.3 Principle of ICP-MS Operation:

An inductively coupled plasma mass spectrometer (ICP-MS) is an instrument that utilizes a high-temperature ICP source combined with a mass spectrometer. The inductively coupled plasma, ICP, is the main sample introduction component of the ICP-MS instrument. A plasma, which is the heat source, is created by coupling radio frequency (RF) power into a flowing stream of argon gas seeded with electrons. The ICP converts the atoms of the elements in the sample to ions, which are then separated and detected by the mass spectrometer [28].

1.3.1 Sample Introduction System Overview

The purpose of the sample introduction system is to transfer samples into the plasma and convert samples to an atomic and ionized state (Figure 1.1). The sample introduction system of the ICP-MS, contains the following parts:

(1) Auto-sampler

(2) Peristaltic pump

(3) Nebulizer

(4) Spray chamber

(5) Injector

(6) ICP torch

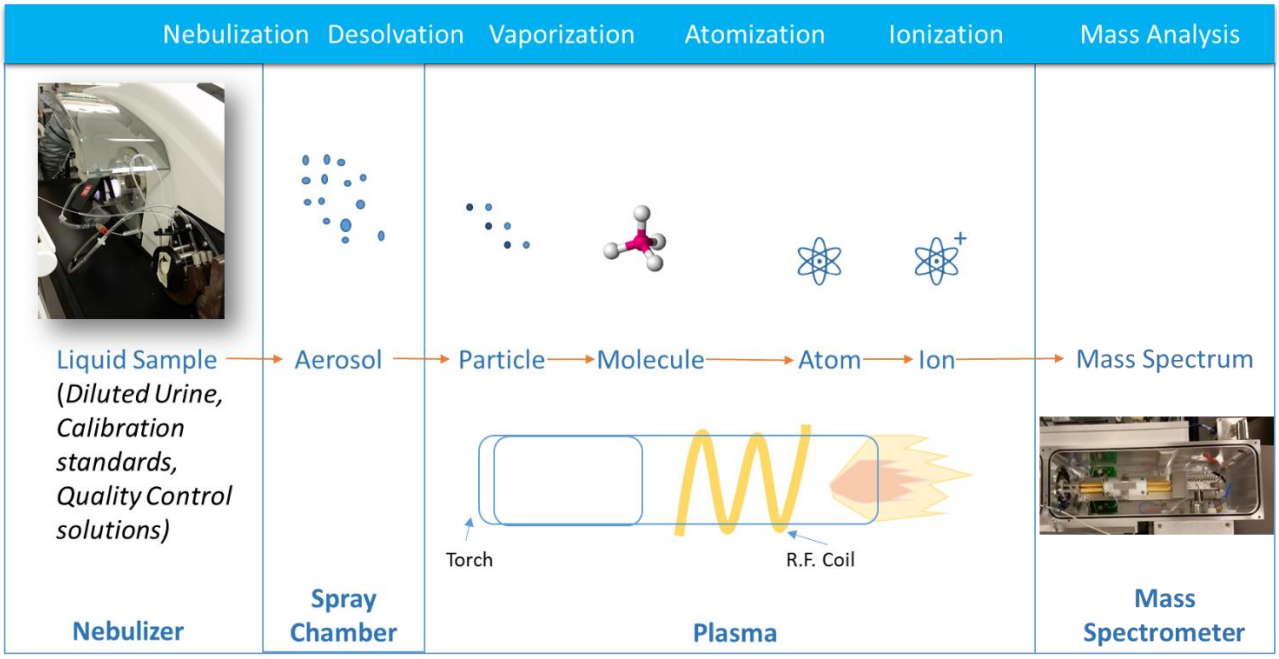

Figure 1.1: The ICP-MS sample introduction and ion formation system
Figure adapted by Agilent Technologies from ICP-MS Primer.

A diluted urine sample is pumped through the peristaltic pump and then into the nebulizer. Samples are then converted into an aerosol and transported from the nebulizer to the spray chamber. A portion of the aerosol is carried through the spray chamber, and then introduced into the ICP torch by the nebulizer gas flow. This is done through an injector tube. The injector tube is connected directly to the spray chamber. Once the sample aerosol is exposed to the torch, where the plasma is formed, the sample aerosol is desolvated completely and the components in the aerosol are atomized and ionized.

The torch and radio frequency (RF) load coil are located inside the ICP region. The torch is made of two concentric quartz tubes, so the argon flows at different rates. [30]. The end of the outer tube is surrounded by the RF load coil. The RF load coil is connected to the RF generator.

The purpose of the RF generator is to sustain the argon plasma. The electro-magnetic field is generated at the end of the outer tube when the power is supplied to the RF load coil from the RF generator. Free electrons are introduced into the torch and are excited by the RF magnetic field where they bombard the argon atoms [30]. This happens continually, releasing more and more electrons to form argon ions. When this process becomes self-sustaining argon is completely ionized, and the argon plasma forms. This plasma is used to atomize and ionize the sample for analysis [30]. The plasma is formed in the tube of the torch near the end surrounded by the RF load coil. The temperature of this plasma can be up to 7000 K.

The ions from the sample and the argon ions pass onto the mass spectrometer (MS) via the interface cones.

1.3.2 Interface Region Overview

In an ICP-MS, the ICP region and the mass spectrometer region of the instrument operate at different pressures. Therefore, the interface region is needed to help the ions to be transported efficiently in the argon sample stream of high pressure into the low pressure region of the mass spectrometer. This interface region consists of two interface cones, the sample and the skimmer cones, and a vacuum pump. These cones are used to center the ion beam coming from the torch. The ions from the ICP are then focused by the cylinder lens in the system. The purpose of the cylinder lens is to collimate the broad ion beam coming from the skimmer cone into a narrower beam and center it into the entrance slit of the MS [28].

1.3.3 Mass Spectrometer Overview

The mass spectrometer is the main analytical component of the instrument which provides separation and identification of the ions. Once the ions are inside the MS, the beam of ions first travels through the ion optics. The ion optics are used to focus the ion beam. The ions

next enter the dynamic reaction cell (DRC), and then are transmitted through the mass-analyzing quadrupole, where they are separated, before being detected. The dynamic reaction cell (DRC) technology is used to eliminate interferences and provides additional control of the ICP-MS sensitivity. The DRC uses a reaction gas (i.e., hydrogen, methane, or helium) to remove interfering species from the ion beam. Once the interfering species are bombarded with the reaction gas, they are converted into non-interfering reaction products which can be rejected either by the DRC or by the analyzer quadrupole [24, 29]. Since there are no isobaric or polyatomic interferences involved in this single element analysis, the DRC was not utilized for this experiment since interference corrections or removal of interfering species are not needed.

Once the ions enter the MS, they are separated by their mass-to-charge ratio. In this system, a quadrupole mass filter is used. In a quadrupole mass filter, AC and DC voltages are applied to opposite pairs of the rods to produce a hyperbolic electric field [28, 30]. The ions of a single mass-to-charge ratio can pass through the rods to the detector by tuning the voltages on the mass filter.

1.4 Method Validation

Method validation is an important step of the method development process, because instruments, analysts, ambient conditions, and other variables can affect the repeatability of the test results [31, 32]. Method validation assures that the method is reproducible, and confirms the adapted method performs in this study as expected [26]. Understanding the application and limitations of the adapted analytical method will provide accurate quantitation of sample information [32]. Therefore, the instrument must be qualified to be used and the analytical method must be validated prior to performing any experimental laboratory research [31, 32]. Otherwise, it is unknown if the method provides accurate results.

The guidelines for validation requirements vary widely for different organizations, however, the goal of validation is always to produce valid analytical test results [31]. The analytical methods must be proven to provide accurate and reliable results for the material being measured or analyzed [32]. Organizations which provide regulations and guidelines for validation include the United States Food and Drug Administration (FDA), Pharmaceutical Inspection Cooperation Scheme (PIC/S), International Conference for Harmonization (ICH), Unites States Pharmacopeia (USP), and International Organization for Standardization/International Electrotechnical Commission (ISO/IEC 17025). The general requirement of validation parameters are the following, [31-34]

- Accuracy
- Precision (Reproducibility, Repeatability, and Intermediate Precision)
- Selectivity
- Specificity
- Linearity
- Limit of Detection
- Limit of Quantitation
- Robustness
- Ruggedness
- Stability

However, the requirements for specific validation criteria vary between guidelines from different organizations, because different organizations may have different definitions for the specific terms. An analytical test method must meet the validation criteria by evaluating in-house testing

results. If modifications are made to a validated method, all changes should be documented, and if appropriate, a new validation should be carried out [31, 35, 36].

1.5 Validation of the ICP-MS Instrument and Analytical Method for Quantitating Total Mercury in Human Urine

This study was conducted at the New Hampshire Public Health Laboratories (NHPHL) in Concord, NH. NHPHL, is a member of the Laboratory Response Network (LRN), which was developed and is under the supervision of the Centers for Disease Control and Prevention (CDC). LRN provides rapid response to bioterrorism, chemical emergencies, or other public health outbreaks [37]. In addition, these laboratories are capable of operating 24/7 for an extended period of time [37].

The first objective of this study was to validate an ICP-MS method for quantitating total mercury in human urine [26]. For most medical and clinical biomonitoring research purposes, the concentration of total mercury in human urine is used as a biomeasure of long-term exposure to both elemental and inorganic mercury [26, 38]. Pooled urine samples were used for this investigation. The ISO/IEC17025, the CDC Laboratory Response Network – Chemical (LRN-C) analytical method validation guidelines, and the Food Emergency Response Network (FERN) validation guidelines were followed for this method validation [33, 35, 39]. These three guidelines provided procedures to demonstrate the specific performance characteristics that define and quantify method performance [33, 35, 39, 40].

According to the ISO/IEC 17025 guidelines, the definition of method validation is,

> "*The confirmation by examination and the provision of objective evidence that the particular requirements for a specific intended use are fulfilled.*" [35]

The Food Emergency Response Network (FERN) also defines the purpose of validation.

"*Method validation is a process by which a laboratory confirms by examination and provision of objective evidence that the particular requirements for specific uses are filled."*[33]

These three documents, the ISO/IEC17025, the CDC Laboratory Response Network – Chemical (LRN-C) analytical method validation guidelines, and the Food Emergency Response Network (FERN) validation guidelines provide a well-written detailed guidelines which can be used to validate an analytical method [33, 35, 39]. The validation parameters assessed during this validation process were the following: (1) Repeatability (precision); (2) accuracy (Trueness); (3) limits of detection (LOD), (4) limits of quantitation (LOQ); and (5) recovery was assessed during this validation process.

1.6 Comparison of Chemical Additive Preservation Solutions for Total Mercury Analysis in Human Urine

Accurate analysis of mercury in human urine is dependent on accurate and reliable analytical standards. Some studies have determined that mercury standard solutions are stable from a week to months at room temperature [13, 26, 42, 43]. Under the conditions studied, the standards were found not to be stable for as long as necessary. The preservation, stability, and storage condition of standards must be investigated for the following reasons:

1. The requirement for high sample throughput when responding to an emergency.
2. Minimizing the generation of hazardous wastes.
3. Reducing standard preparation time.
4. Improving the cost effectiveness of the analysis.

The second objective of this study was to find solution conditions which prolong the storage lifetime of the intermediate working standards by exploring the effect of using several

potential acid – preservative solution combinations. Many preservatives and storage containers have been evaluated and reported to solve the problems associated with the storage of samples for mercury analysis [44, 45]. Recent research by Feldman indicated that increasing the percentage of nitric acid in standards minimizes mercury volatilization and absorption onto plastic containers [43]. Another documented method developed by the CDC indicates that the storage lifetime for the standards can be extended for up to 6 months with the addition of hydrochloride acid to standards stored in glass at 2 to 4 °C [13]. Containers made from polypropylene (PP) were used for this study because they are free from trace metals contamination and are less expensive. Furthermore, changing the storage condition from room temperature to a lower temperature may help prevent of mercury loss or slow down mercury evaporation.

Mercury intermediate standards were prepared in the low parts per billion concentration range. Three preservative solution combinations were investigated: (A) 2% (v/v) of concentrated nitric acid with 1%(v/v) of sulfamic acid; (B) 5% (v/v) of concentrated nitric acid with 1%(v/v) of sulfamic acid; and (C) 1%(v/v) of concentrated nitric acid with 1%(v/v) sulfamic acid and 1%(v/v) of concentrated hydrochloric acid; [13, 42, 43] These three standard solutions were stored at three different temperatures: at room temperature (19 to 23 °C), in the freezer (between -28 to -20 °C), and in the refrigerator (1 to 2 °C) for comparison. These mercury intermediate standards were stored for three months under these conditions, to evaluate the stability of these standards.

1.7 Evaluate Validated Method with Improved Standards using Proficient Testing and Spiked Mercury Urine Samples

The ultimate goals for this study are: (1) validate method conditions for ICP-MS analysis of mercury in urine samples; (2) improve the stability of the mercury intermediate standards;

and, (3) evaluate the validated method with the improved standards using proficiency testing and quality control samples. Urine samples provided by staff members containing unknown mercury levels were pooled. Proficiency testing samples provided by the New York State Public Health laboratories were also examined. The pooled urine analysis was conducted to investigate and assess the total mercury level utilizing the validated method and the most stable standards as previously established.

CHAPTER II:

Method Validation for Determination of Total Mercury in Human Urine by ICP-MS

2.1 Introduction:

Accurate measurement of total mercury in human urine is dependent on the analytical method and the instrument performance. It is very important and necessary to ensure that the analytical method is validated, and the instrument is qualified [33]. The validation of a method for determining total mercury in human urine by inductively coupled plasma mass spectrometry at the New Hampshire Public Health Laboratories is described in this chapter.

2.2 Instrumentation:

All analyses were performed on a PerkinElmer Sciex ELAN DRCII 6000 ICP-MS (PerkinElmer Sciex, Thornhill, ON, Canada) instrument equipped with an ASX520 autosampler (CETAC Technologies, Omaha, Nebraska ,USA), and a glass nebulizer (PerkinElmer Sciex, Thornhill, ON, Canada). A cyclonic spray chamber (PerkinElmer Sciex, Thorhill, ON, Canada) was used to reduce the mercury memory effect. The mercury memory effect is caused by impurities accumulating in the sample introduction system which affects the efficiency and reliability of the analytical procedure. Nickel sampler and skimmer cones were employed.

2.3 Method Validation

2.3.1 Method

The total mercury analysis method was adapted from the Laboratory of Inorganic and Nuclear Chemistry, Division of Environmental Health Sciences, Wadsworth Center Department of Health of the State of New York [26].

2.3.2 Instrument settings

Before the analysis, a daily performance solution is used to complete a daily performance check of the instrument. A daily performance solution, also called the SmartTune Solution standard ELAN/DRC/PLUS/II (Perkin Elmer, Shelton, CT, USA), contains the following: 10 µg/L of barium, and 1 µg/L each of beryllium, cesium, cobalt, iron, indium, lead, magnesium, thorium and uranium, in 0.5 % (v/v) HNO_3. The nebulizer gas flow and the lens voltage were adjusted based on the response of the daily performance check to obtain the minimum counts for the cerium oxide level (the CeO/Ce ratio), doubly charged ions, and the maximum number of counts for ^{115}Indium. The purpose for measuring the CeO/Ce ratio is to determine the plasma robustness or effective plasma temperature. The lower the CeO/Ce ratio, the more robust the plasma is [54]. Robustness means the instrument's ability to effectively perform. The acceptable levels of each parameter for the daily performance check are listed in **Table 2.1** [26]. The operating conditions for the ICP-MS instrument are listed in **Tables 2.2A and 2.2B**. The integrated peristaltic pump ensures a constant flow rate for the transfer of liquid from the sample container to the nebulizer [30].

Table 2.1: Acceptable criteria for the daily performance check.

Parameter	Acceptable level
Intensity of Mg	> 1000 cps*
Intensity of In, U	> 10000 cps
Oxide CeO/Ce	≤ 0.03
Lens Voltage	< 10 volts

*Note: cps is counts per second

Table 2.2A: Instrument settings.

Parameter	**Setting**
RF power	1100 – 1400 W
Nebulizer gas flow rate	0.67 – 0.99 L/min
Sweeps/reading	30
Readings/replicate	1
Replicates	3
Dwell time	50 ms
Curve type	Simple linear
Sample units	µg/L

Table 2.2.B: Integrated peristaltic pump speed and duration for sample analysis and sample rinse-out.

Action	**Pump Speed (rpm)**	**Duration (seconds)**
Read Delay and Analysis	-18	30
Sample Flush	-18	90
Wash	-24	0 to 240

Note: The negative sign indicates the direction of rotation is counterclockwise.

2.3.3 Reference Materials

The instrument was validated at the New Hampshire Public Health Laboratory (Concord, NH, USA) by running calibration standards and quality control (QC) materials. Quality control materials were used to ensure the accuracy of the analysis. Three quality control solutions were purchased from New York's State Public Health Laboratory, Wadsworth Center, Albany, NY, containing known concentrations of mercury in human pooled urine. A second set of QC samples included Standard Reference Materials (SRM) purchased from the National Institute of Standards and Technology (NIST). There are two levels of mercury in the SRM 3668 package. These certified solutions are prepared in pooled human urine. The certified mercury concentrations values for the quality control solutions are presented in **Tables 2.3A-B**.

Table 2.3A:Certified mercury concentration values for Quality Control Solutions from the Wadsworth Center, Department of Health, New York state laboratory.

Quality Control Solutions From Wadsworth Center	**Low Level I (L)**	**Medium Level II (M)**	**High Level III (H)**
Lot number:	*LRNC2013L*	*LRN2013M*	*LRN2013H*
Mercury Concentration (ppb)	Endogenous	3.59	31.0

Note: These QCs solution also referred as "*Urine Quality Assurance Materials for LRN-C Trace* Elements in Human Urine".

Table 2.3B: Certified mercury concentration values for the NIST Quality Control Solutions

NIST SRM 3668	**Level I**	**Level II**
Mercury Concentration (ppb)	0.910 ± 0.055	6.38 ± 0.46

Note: The lot number is 1504905.

2.3.4 Westgard QC rules

To evaluate whether the quality control (QC) solutions were within acceptable limits, Levey-Jennings and Westgard QC rules were applied to the data to establish acceptance and rejection. The Levey-Jennings rule uses a single set of control limits to evaluate whether the measurement is within the mean ± 2 standard deviations or the mean ± 3 standard deviations [26]. The Westgard QC rules evaluate data patterns to decide whether the quality control samples are in-control or need to be rejected.

Westgard rules, also known as the Westgard multirule QC procedure, were applied for this method to ensure that the quality control results meet its criteria for acceptability. The goal was to ensure the stability of this method and the instrument system on a routine basis [47, 48]. To achieve a satisfactory performance, each individual result for the 20 analytical runs must remain within two standard deviations of the calculated mean of the satisfied 20 analytical runs, and also must remain within the accuracy percentage range of the certified target value between 80 to 120 %. Each individual data point must be within plus or minus two standard deviations of the certified target value. This Levey-Jennings rule was applied to monitor on-going routine performance [47].

The Westgard approach uses 5 different control guidelines to evaluate if the result of an individual measurement is satisfactory.

The rules state [49]:

1. A run is rejected if the data point exceeds the mean ± three standard derivations. (1_{3S} rule)
2. The run is rejected when two repeated data points surpass the same mean ±two standard derivations. (2_{2S} rule)
3. The run is rejected if a data point goes beyond the mean plus two standard derivations and the next measurement exceeds the mean minus two standard derivations. (R_{4s} rule)
4. The run is rejected if 10 data points continuously fall on the same side of the mean. (10_x rule)

Further collection of data is needed to replace the failed quality control analytical runs, until a minimum of 20 in-control analytical runs are observed. Once 20 in control runs are obtained, the mean and standard deviations of the QCs results are then calculated [47]. The calculated mean of the QCs are compared with the true value (Table 2.3A and B) to evaluate the accuracy (recovery) of these QCs results (Section 2.5.2).

The method is considered to be validated when the results have fulfilled the requirements of the Westgard QC rules.

2.4 Reagents

All reagents were prepared under class 100 clean room conditions using the following:

(1) Resistivity ≥ 18 MΩ.cm double distilled deionized water (Barnstead Nanopure; Millipore Corporation, Bedford, MA)

(2) Double distilled nitric acid (67-69% (v/v), Optima grade, Fisher Scientific, Pittsburgh, PA)

(3) Sulfamic acid (99.3% (w/v) ACS reagent grade; Sigma-Aldrich Company, St. Louis, MO).

(4) Certified and periodically calibrated pipettes were used at all times.

(5) All samples and standards were prepared in a certified biohazard safety cabinet (BSC).

2.4.1 Preparation of standards for the working curve

The mercury stock standard solution was a NIST traceable aqueous solution of 1000 mg/L of mercury as $HgCl_2$, CertiPrep (SPEX CertiPrep, Metuchen, NJ). This mercury stock standard was used to prepare the mercury intermediate stock standard (which contained 8 mg/L Hg stock solution). The mercury intermediate stock standard was used to prepare the mercury intermediate standards. The preparation scheme for the mercury intermediate standards are illustrated on Figure 2.1.

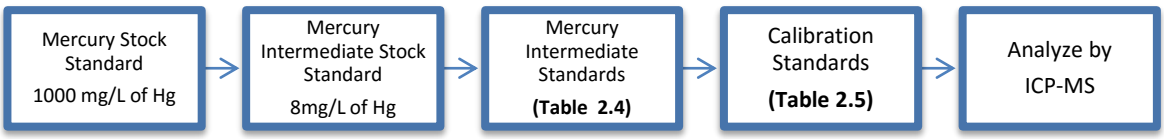

Figure 2.1: Preparation scheme for mercury standards

A series of aqueous mercury standards were prepared by dilution of the 8 mg/L Hg stock solution to give the following concentrations: 0 µg/L, 2 µg/L, 5 µg/L, 10 µg/L, 20 µg/L, 30 µg/L, 40 µg/L of mercury. (Table 2.4)

Table 2.4: Concentrations of the mercury intermediate standards

	Standard 1	**Standard 2**	**Standard 3**	**Standard 4**	**Standard 5**	**Standard 6**
Concentration (µg/L)	2.00	5.00	10.0	20.0	30.0	40.0

All aqueous standard solutions, mercury intermediate stock standard and mercury intermediate standards, were prepared from double-distilled deionized water with a resistivity of greater than 18 MΩ.cm (Barnstead Nanopure; Millipore Corporation, Bedford, MA), and each contained both of the following stabilizing and preservative agents: 1% (v/v) double distilled HNO_3, and 1% (v/v) sulfamic acid preservative solution [26].

2.4.2 Procedures for preparation of base urine

All urine samples used in this experiment were pooled urine collected from donors at the New Hampshire Public Health Laboratory (NHPHL). These samples were then acidified with 1% (v/v) double-distilled nitric acid, and 1% (v/v) sulfamic acid preservative solution. The acidified urine samples are referred to as "Base Urine". All acidified base urine samples were proportionally dispensed into 15 mL polypropylene centrifuge tubes and stored at ≤ -20˚C until analysis or up to one year. It is reported that the urine samples are stable for one year under these conditions [26]. For short term storage, base urine samples were stored at 2 – 4 ˚C for one month. Prior to the analysis, frozen base urine samples were placed on the rocker at room temperature to defrost. The defrosted urine was used to prepare calibration standards, spiked samples, and quality control reference materials [26].

2.4.3 Sulfamic Acid Preservative Solution

An acid rinsed 100 mL glass graduated cylinder was used to transfer 90 mL of ≥18 MΩ.cm water to a 125 mL Teflon™ container. 20 g of sulfamic acid (99.3% (w/v) ACS reagent grade; Sigma-Aldrich Company, St. Louis, MO) was weighed into a weigh boat (Hexagonal Polystyrene weighing dishes, Fisherbrand™, Fisher Scientific, Pittsburg, PA) and transferred to the Teflon™ container. The capped container was set on a rocker and agitated overnight until the solid was completely dissolved. Then, 10 mL of concentrated Triton X-100 (Integra, Kent, Washiongton) was added. This solution was labelled as "Sulfamic Acid Preservative Solution". Sulfamic acid preservative solution was stored at room temperature. The storage lifetime of the sulfamic acid preservative solution was stated from the adapted method (Section 2.3.1) to be 1 month [26].

2.4.4 Diluent

The diluent solution was used to prepare the 1:19 dilution of all reagents, base urine, standards and QC materials. This solution contained the following: 1% (v/v) of double-distilled nitric acid, 1% (v/v) of sulfamic acid preservative solution, 20 µL of 1000 mg/L iridium (SPEX CertiPrep, Metuchen, NJ), 200 µL of 10000 mg/L gold ($AuCl_3$ Stock solution which is $AuCl_3$ dissolved in 5%(v/v) HCl) (SPEX CertiPrep, Metuchen, NJ), and an additional 1 mL of 10% (v/v) Triton X-100™; diluted to 2 L with ≥18 MΩ.cm double-distilled deionized water in a 2 L Teflon™ container. The diluent solution was stored at room temperature. Iridium was used as an internal standard. The storage lifetime of the diluent was stated from the adapted method (Section 2.3.1) to be 1 month [26].

2.4.5 ICP-MS Rinse Solution

For this method, a rinse solution was used to minimize the mercury memory effect (carry-over) between samples. The rinse solution was prepared in a 2 L Teflon™ container by adding 40 mL of double-distilled nitric acid, 1mL of 10% (v/v) Triton X-100™, 200 µL of 10000 mg/L $AuCl_3$ stock solution (SPEX CertiPrep, Metuchen, NJ), and diluted to 2 L with double distilled deionized water (resistivity, ≥18 MΩ.cm).

2.5 Experimental

2.5.1 Evaluation of mercury content in pooled base urine samples

All pooled urine samples were examined to screen for mercury content, which must be ≤ 0.11 µg/L of mercury, prior to urine acidification (Chapter 2.4.2). Two batches of pooled urine samples were obtained in August 2014 and May 2015. The results for mercury from the ICP-MS indicated that for both the 2014 and 2015 batches of pooled urine, there was no significant mercury content in the pooled based urine since the signals for mercury were below the limits of detection of this assay.

Samples including calibration standards, urine blank, and QC materials were diluted to a predetermined volume and mixed before analysis. The reagent blank solution contained 1000 µL of 1% (v/v) double-distilled concentrated nitric acid, 1% (v/v) sulfamic acid preservative solution, and 9000 µL of diluent. This reagent blank was used as the blank for urine-based QC samples, and external reference samples such as NIST SRM 3668 (Chapter 2.3.3).The urine blank was prepared by combining 500 µL of based urine, 500 µL of reagent blank, and 9000 µL of diluent. This urine blank was used as the blank for the calibration standards. The preparation scheme for these different solutions is presented in **Table 2.5**.

Table 2.5: Summary of solution preparation process.

	Volume (µL)				
	Mercury Intermediate Standards	**Base Urine**	**1% HNO_3 / 1% Sulfamic Acid Preservative Solution**	**Samples/ QCs**	**Diluent**
Reagent Blank	**-**	**-**	**1000**	**-**	**9000**
Calibration Standards	**500**	**500**	**-**	**-**	**9000**
Urine Blank	**-**	**500**	**500**	**-**	**9000**
Samples/ QCs	**-**	**-**	**500**	**500**	**9000**

2.5.2 Quality Control Solutions - Accuracy

Quality Control solutions were purchased from the New York State Public Health Laboratory (Wadsworth Center, Albany, NY). Three different concentration levels of quality control (QC) solutions were employed: QC level I – (L), QC level II – (M), and QC level III– (H). The letters represent low (L), medium (M), and high (H) concentrations of Hg. These certified mercury concentrations were used as references to estimate whether the control measurement is within the acceptable range. The concentrations of each of the quality control materials are listed in **Tables 2.3A and 2.3B**.

The mean of each quality control solution was calculated from the 20 in-control data points collected. The calculated means were then compared with the actual value, and the % accuracy (Equation 2.1) was required to be within 90% to 110% of the actual value [33, 39-41].

$$\%\ Accuracy = \frac{|(Calculated\ Mean)|}{Actual\ Value} \times 100\% \qquad \text{Eq. (2.1)}$$

2.5.3 Acceptance criteria for the calibration curve

Six different concentrations of mercury intermediate standards were analyzed to prepare a calibration curve. These standards were analyzed in triplicate. The correlation coefficient for fitted line must be ≥ 0.99 (Figure 2.2) [33, 39].

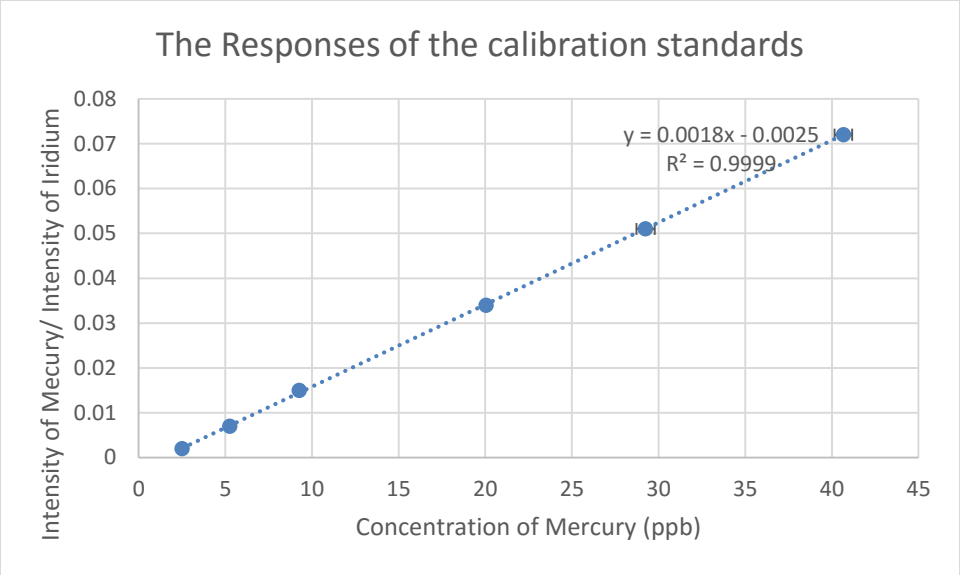

Figure 2.2: This is an example of a calibration curve of the intensity ratio between mercury and the internal standard iridium vs. the mercury concentration. The correlation coefficient R^2 is 0.9999. Note: ppb is also expressed as µg/L.

2.5.4 Recovery

Spiked urine samples were prepared at two different concentrations: 5 ppb and 30 ppb of mercury. Two replicates were prepared and analyzed at each concentration. The mean of the % recoveries (Equation 2.2) must be within 90 to 100% of the theoretical value [33, 39-41]

$$\%\ Recovery = \frac{Result}{Theoretical\ Value} \times 100\% \qquad \text{Eq. (2.2)}$$

2.5.5 Calculation of the Limits of Detection and Quantitation for mercury

According to the CDC LRN-C and FERN validation guidelines, the limit of detection (LOD) is the lowest concentration of an analyte that can be detected at a specified level of confidence but not necessarily quantitated as an exact measurement [28, 33, 50]. The limit of quantification (LOQ) is the lowest concentration of analyte that can be quantitatively measured with an acceptable level of uncertainty [33]. The lowest concentration of the mercury intermediate standard solution, 2 ppb, was prepared in four solutions from the 2ppb standard

solutions and were analyzed in triplicate from this solution. A total of 20 runs of the 2 ppb mercury standards were employed to calculate the limits of detection and quantitation for this method. To obtain values for the LOD and LOQ, the formulas shown in Equation 2.3 and 2.4, respectively, were used.

$$LOD = 3 \times SD_{20\ runs\ of\ 2\ ppb\ mercury\ standards} \quad \text{Eq. (2.3)}$$

$$LOQ = 10 \times SD_{20\ runs\ of\ 2\ ppb\ mercury\ standards} \quad \text{Eq. (2.4)}$$

2.5.6 Repeatability – Precision

Spiked urine samples and quality control reference materials were employed to measure the repeatability of the measurement. A series of replicates were prepared and analyzed. During the method validation process, requirements for acceptable precision were followed according to the ISO 17025 and FERN guidelines [33, 35]. Based on the guidelines, the requirements for the acceptable relative standard derivation (RSD) should be within ± 2% (Equation 2.5) [33, 35]. Therefore, all measurements for standards and samples must have an RSD be within ± 2%.

$$\%\ Relative\ standard\ deviation\ (RSD) = \left(\frac{Standard\ deviation}{Mean}\right) \times 100\ \% \quad \text{Eq. (2.5)}$$

2.6 Result and Discussion:

2.6.1 Mercury memory effect:

Routine quantitation of mercury by ICP-MS can be affected by a memory effect in the sample introduction system. The “mercury memory effect”, also known as “carry-over”, is observed when the residual mercury signal fails to return to the baseline [25]. Mercury can remain as a vapor in the spray chamber, and/or adsorb onto the spray chamber walls [25]. This results in long washout times for the mercury, which results in longer analysis times. Addition of acids may only prevent mercury from adhering to the spray chamber walls, however mercury vapor may still exist in the spray chamber [25]. Therefore, gold was added to both the diluent

and rinse solutions to prevent loss of mercury to volatilization and adsorption [51]. Also, to help overcome this issue, the washout time from the adapted method [26] was modified from 240 seconds to 300 seconds.

2.6.2 Purpose of adding preservative agents and oxidizing agents to samples and standards:

The main purpose for adding the oxidizing or preservative agents to the samples and standards is to prevent loss of mercury prior to analysis [15, 52]. Oxidizing agents are used to prevent the reduction of Hg^{2+} to the volatile, uncharged Hg^0 state. Oxidizing agents that have been used include HNO_3, Au^{3+}, and sulfamic acid [52, 53].

Sulfamic acid, H_3NSO_3, is a strong inorganic acid [54], and is recommended for use as a primary standard in acid-base titrations [55, 56]. Sulfamic acid in aqueous solution is stable at room temperature [57]. Sulfamic acid forms sulfuric acid and nitrous oxide by the following reaction with concentrated nitric acid:

$$HSO_3NH_2 + HNO_3 \rightarrow H_2SO_4 + H_2O + N_2O \quad (1)$$

Sulfamic acid reacts with mercuric ion and forms mercuric sulfamates which keep the mercuric ion from being vaporized:

$$Hg^{2+} + 2HSO_3NH_2 \rightarrow Hg(SO_3NH_2)_2 + 2H^+ \quad (2)$$

Triton X- 100 is a nonionic surfactant that solubilizes proteins, and is used commonly as a detergent in the laboratory. Auric ion (Au^{3+}) is a powerful oxidizing agent which keeps mercury (II) in solution by the following reaction [58],

$$Hg_2^{2+} + Au^{3+} \rightarrow 2Hg^{2+} + Au^+ \quad (3)$$

Since the 1960's, it has been known that micro-organisms might be involved in the volatilization of mercury [59]. Addition of sulfamic acid and Triton X-100 to the mercury standards, as a preservative, inhibit bacterial growth in the solutions [60], and also prevent losses

during storage [15]. Bacterial growth, such as by chemolithoautotrophic sulfur bacteria, in the solution can convert mercury into volatile mercury compounds via methylation [60, 61]. Researchers proposed that the addition of Triton X-100, which acts as a surfactant, also improves sample transport efficiency by reducing the build-up of mercury vapors in the nebulization spray chamber [15, 52]. Studies have also shown that adding sulfamic acid and Triton X-100 as preservative agents keeps the mercury stable in urine samples at room temperature for up to one month [15, 60]. The US Environmental Protection Agency (EPA) mercury preservation protocol states that the addition of gold chloride ($AuCl_3$) in a 5 % (v/v) HNO_3 solution can prevent mercury precipitation in the sample introduction system and avoid carryover. This is due to the presence of a strong oxidizing agent, auric ion (Au^{3+}), which keeps the mercury as mercuric ion in solution [62].

2.6.3 Early Studies

The method adapted from the New York's State Public Health Laboratory indicated that the mercury intermediate standard solutions are stable at room temperature for one week. However, the mercury response measured for the quality control solutions were found to increase over the first two days, observation numbers 3, 4, and 6. (**Figure 2.3** – labeled as solid line). If the intermediate standard solutions were analyzed either immediately after being prepared or within the next two days, the QC concentrations were within the $\pm$ 20% of the certified concentration (**Figure 2.3**). The results are shown in **Figure 2.3**, observations number 1, 2, 5 and 7.

Variations in the responses for mercury in the intermediate standard solutions could be caused by the loss of mercury, such as if Hg^{2+} is reduced or adsorbed on the walls of the

container. To resolve this problem, the concentration of mercury in each standard was measured on the day of preparation, and then monitored over the next five days to evaluate the stability of the standards over time. These data show that the intensity of signal for the mercury intermediate standards declined over time which resulted in, an apparent increase in the concentration of mercury in the QC solutions (**Figure 2.4**). A second set of QC data were then collected and evaluated using with freshly made standards. This resulted in the QC concentrations being within $\pm$20% of the certified concentration (**Figure 2.3** – labeled in dash line).

The combined initial data for the QC solutions, Level III (H), of the reproducibility chart are presented in **Figure 2.3**. The solid line shows the trend, observations numbered 1 to 7, for the data collected when the standards were being prepared fresh every week. Control observations numbered 8 to 15, following the ★ and the grey dashed line in **Figure 2.3** for the data were collected when the standards were being prepared fresh daily.

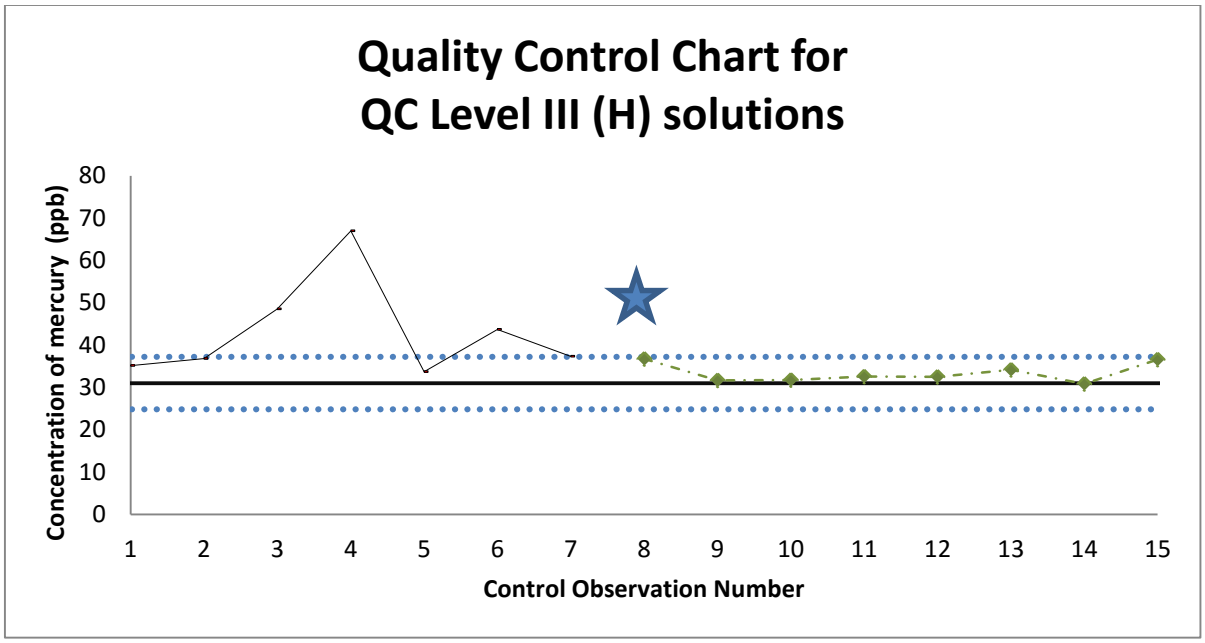

Figure 2.3: Quality Control – QC III (H) concentration chart. (Dotted lines are ± 20% of the certified concentration, and the solid line is the actual value of the certified concentration. (Table 2.3A – 31.0 ppb of mercury) Control observations numbered 8 to 15, following the ★ and grey dash line, for the data were collected when the standards were being prepared fresh daily.

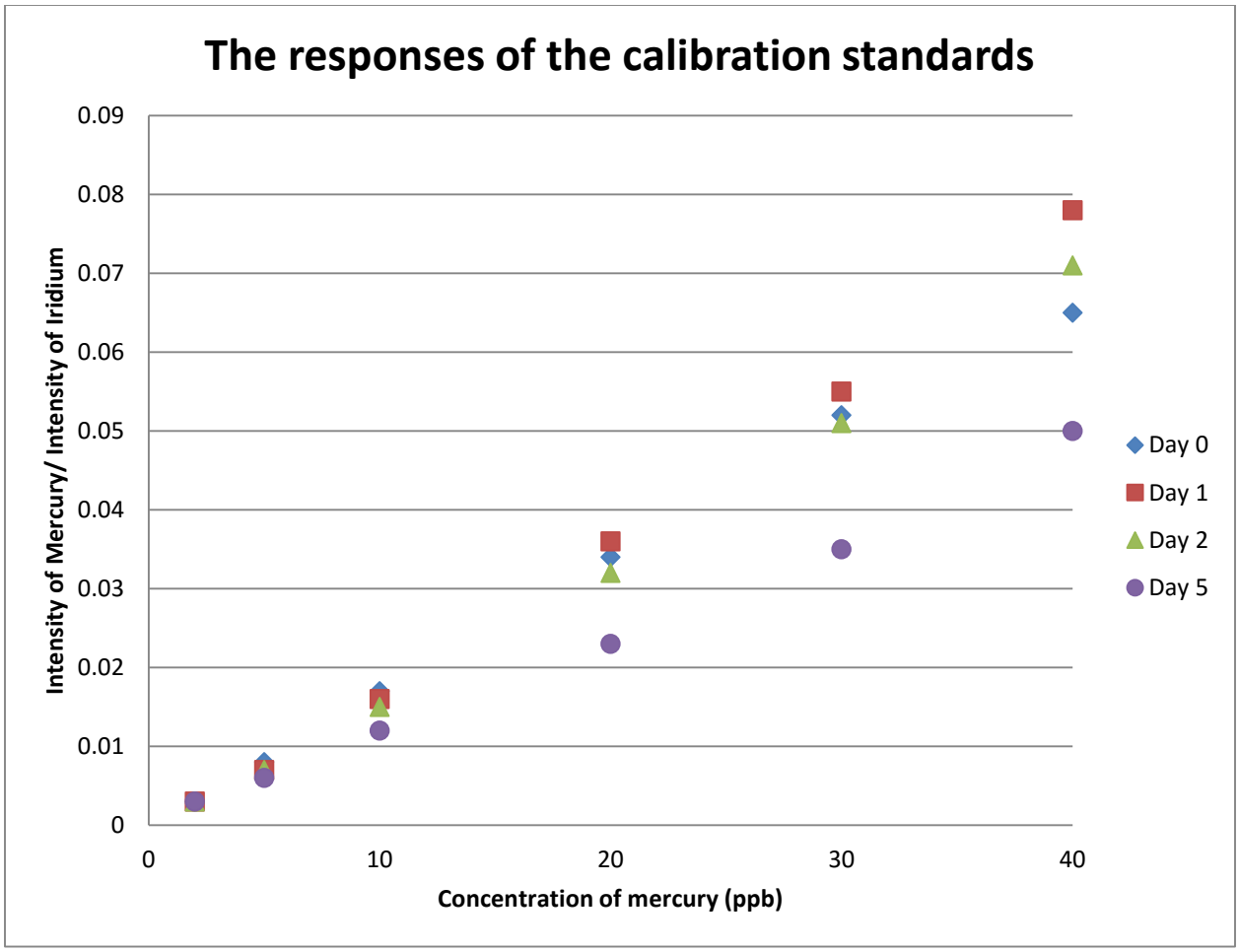

Figure 2.4: The responses of the standards were measured over 5-days to monitor response stability. Each standards were done in three replicates.

Based on the results from the response stability of the mercury intermediate standard solutions (**Figure 2.4**), the standards were stable within 20% for the first two days after preparation. However, a loss of signal intensity appeared on day 5 of the mercury intermediate standard solution. This leads to a response intensity decreased which resulted in increased QC response. Therefore, the mercury intermediate standard solutions were not as stable as required under the storage conditions being used [14]. To overcome this issues the intermediate standards were freshly prepared prior to analysis.

2.6.4 Evaluation of Criteria for the Acceptance of the Quality Control Results

A total of 27 days of QC Level III (H) data were acquired. In the overall QC Level III (H) reproducibility chart (**Figure 2.5**), control observations numbered 1, 9, 13, and 17, indicated by the ★, violated the Westgard QC rules. These measurements exceeded the plus 20% of the certified target mean requirement.

The same pattern was observed in the reproducibility chart for QC Level II (M) (**Figure 2.6**) where some of the measurement were classified as being out-of-control according to the Westgard QC rules. Control observations numbered 3, 8, and 13, following the ★, needed to be rejected. The control observations numbered 8 to 10, exceeded plus 20% of the certified target mean. A group of measurements, control observations numbered 12 to 14, continuously fell on the same side of the mean.

Since some of the results did not meet the criteria of the Westgard rules, further collection of data was required to replace those failed measurements. Therefore, additional measurement of QC Level II (M) and Level III (H) were made to replace the out-of-control results. At least 20 analytical runs in total were required to validate this method. The final corrected in-control results for the QC Level III (H) standard were within two standard deviations of the calculated mean, and are displayed in **Figure 2.7**. All 20 of these analytical runs are in compliance with the Westgard rules. The corrected in-control data for the QC II (M) standard are shown in **Figure 2.8**.

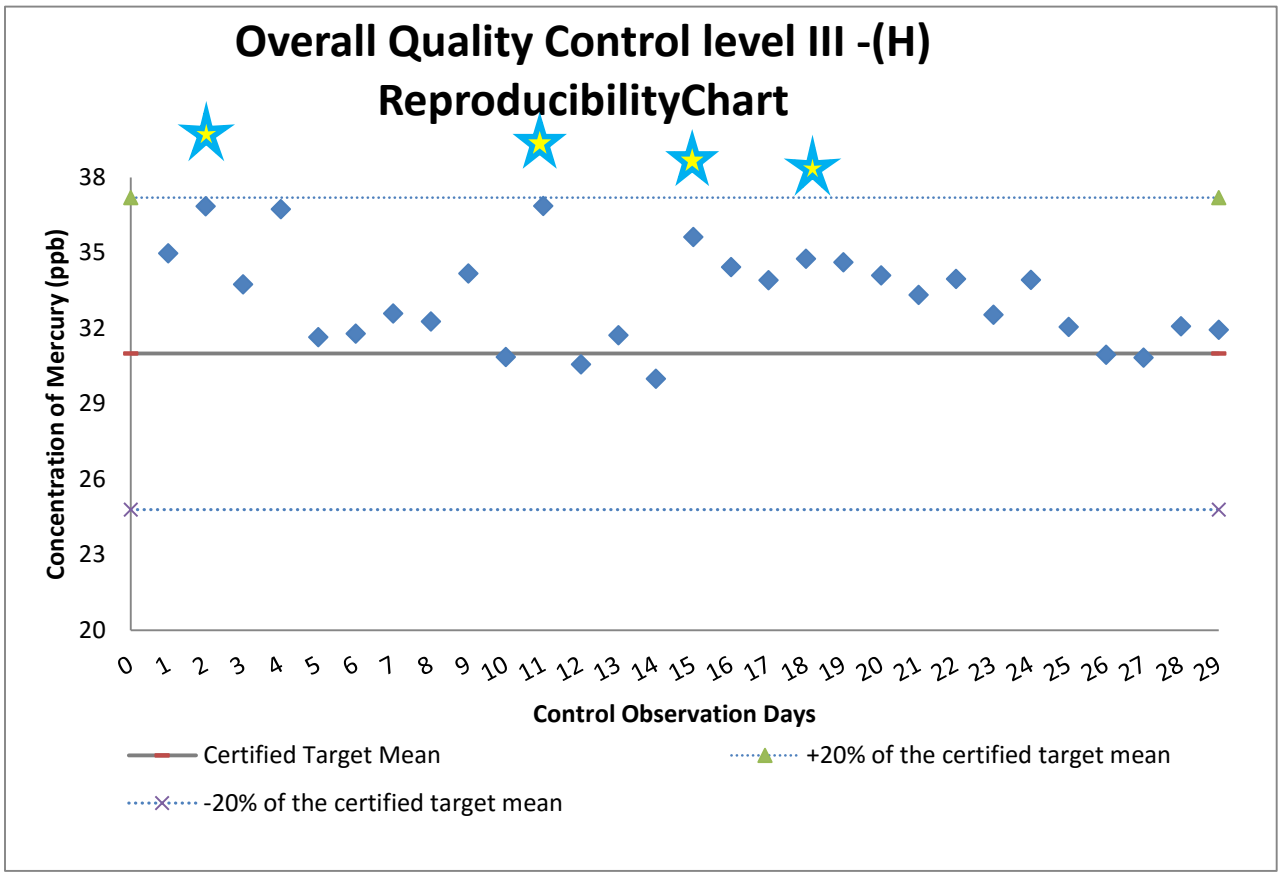

Figure 2.5: This is the overall reproducibility chart for quality control reference sample level III (H). Data were acquired for a total of 29 days. The solid line is the certified target mean. The triangle-dashed line represents plus 20% of the certified target mean. The cross-dotted line represents minus 20% of the certified target mean. The certified target mean of QC level III (H) contained 31.0 µg/L of mercury (**Table 2.3 A** Control observations numbered 1, 9, 13, and 17 following the ★, needed to be rejected due to some of the measurement were classified as being out-of-control according to the Westgard QC rules.

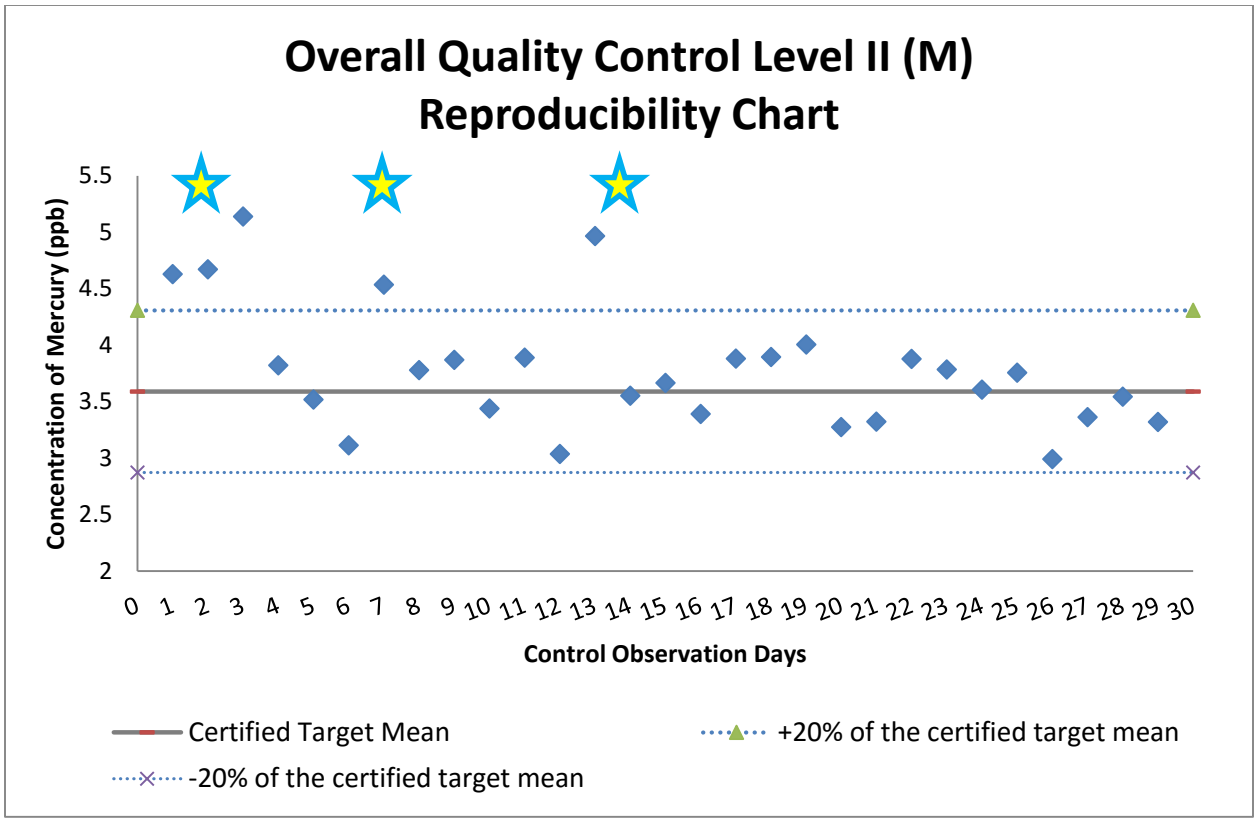

Figure 2.6: This is the overall reproducibility chart for quality control reference sample level II (M). Measurements were taken over 30 days. The solid line is the certified target mean. The triangle-dashed line represents plus 20% of the certified target mean. The cross-dotted line represents minus 20% of the certified target mean. The certified target mean of QC level II (M) contained 3.59 µg/L of mercury (**Table 2.3 A**). Control observations numbered 3, 8, and 13, following the ★, needed to be rejected due to some of the measurement were classified as being out-of-control according to the Westgard QC rules.

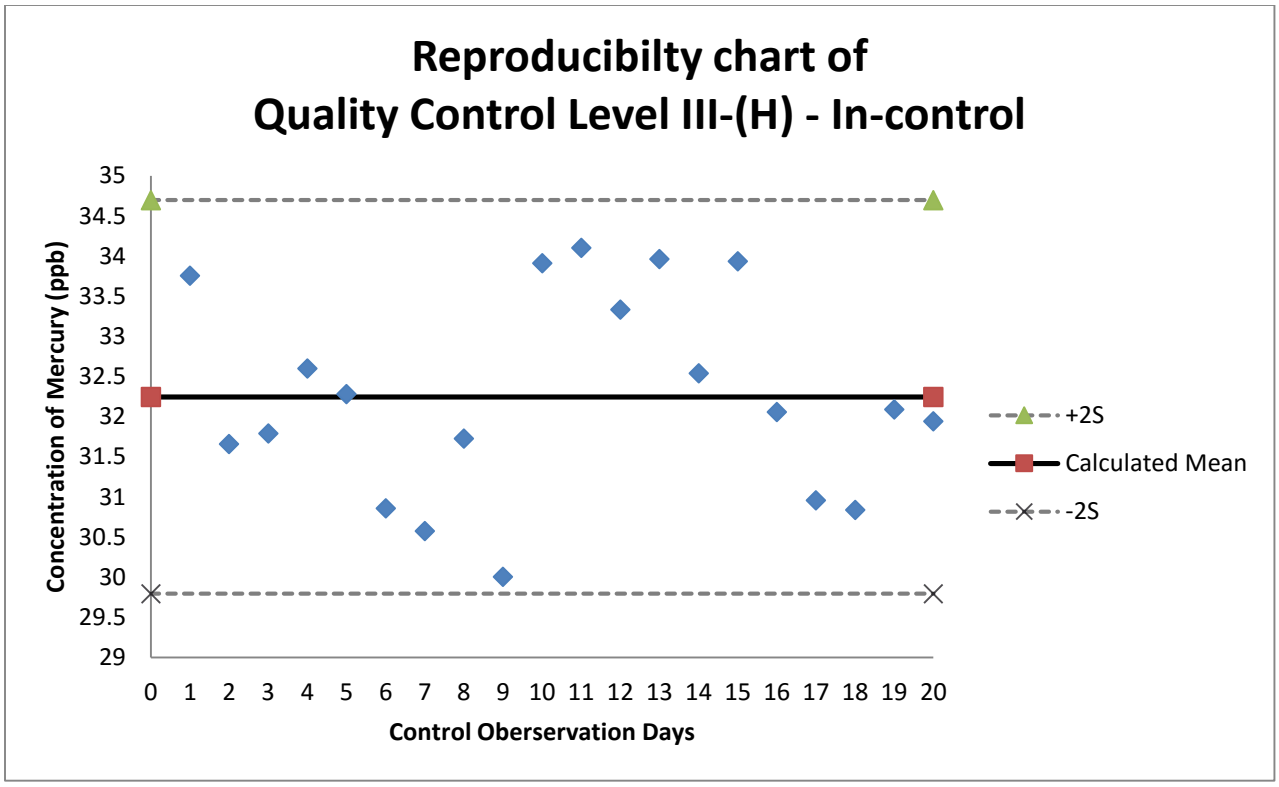

Figure 2.7: This is the in-control reproducibility chart for quality control reference sample level III (H) with the out-of-control measurements in **Figure 2.5** replaced. All of the measurements in this figure meet the Westgard QC rules. The black solid line represents the average measured mercury concentration for the 20 measurements of the quality control level III (H) solution. The triangle-dashed line represents plus two standard deviations of the calculated mean. The cross-dashed line represents minus two standard deviations of the calculated mean.

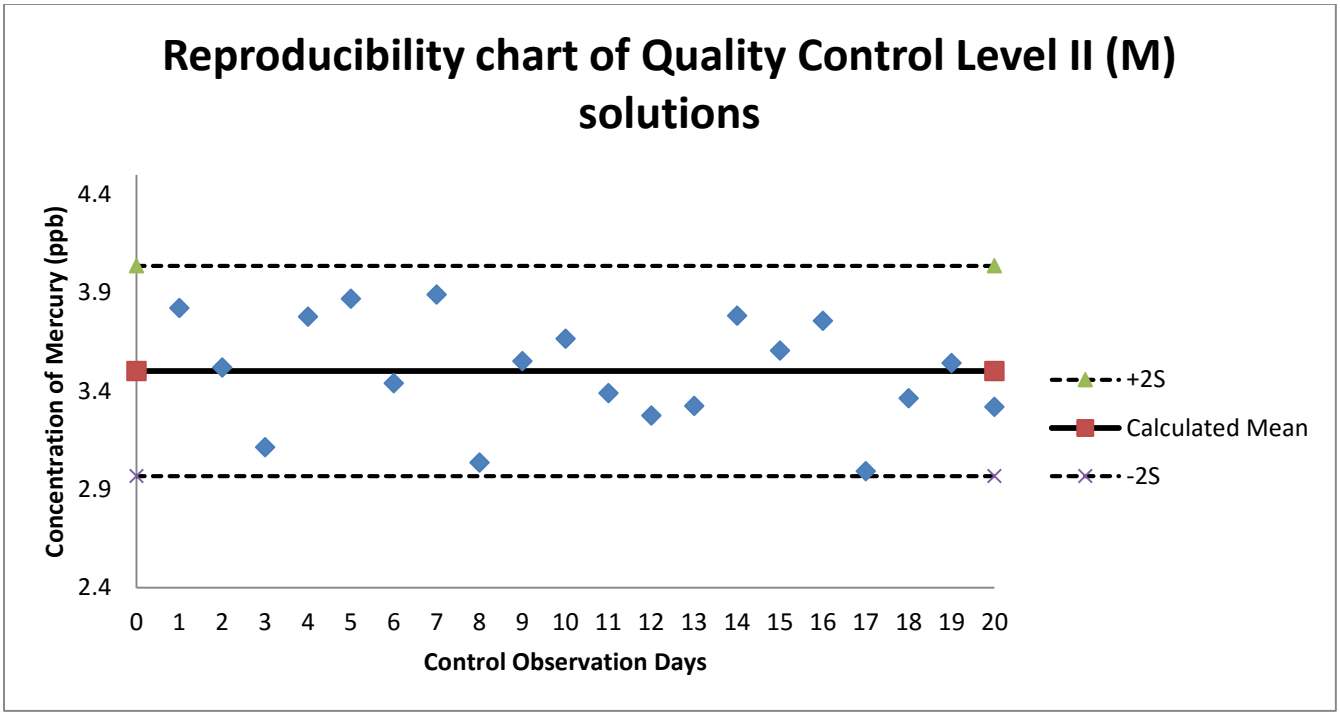

Figure 2.8: Quality control chart for the Level II (M) solution showing a total of 20 in-control results. The black solid line represents the average measured mercury concentration for the 20 measurements of the quality control level II (M) solution. The triangle-dashed line represents plus two standard deviations of the calculated mean. The cross-dashed line represents minus two standard deviations of the calculated mean.

Control measurements in the QC Level I (L) reproducibility chart are not reported because the obtained results were below the LOQ of the instrument. The results cannot be considered reliably quantifiable.

The accuracy, recalculated mean, and standard deviation for each QC level standard results are summarized in **Table 2.6**. The percent accuracy for QC Level II (M) and Level III (H) were 93.0 % and 105%, respectively, which are within the acceptable range of 90% - 110%. However, QC Level I (L) was below the quantitation limit.

Table 2.6: Calculated mean and standard deviation of QCs solution

Quality Control	**Certified Value (µg/L)**	**Calculated Mean (µg/L)**	**Standard Deviation (S)**						**% Accuracy (Section 2.5.2)**
			-1 S	**+1S**	**-2S**	**+2S**	**-3S**	**+3S**	
Level I (L)									***Below LOQ***
Level II (M)	3.59	3.56	3.31	3.81	3.06	4.06	2.81	4.31	88.9 to 111%
Level III (H)	31.0	32.7	31.4	34.0	30.1	35.3	28.8	36.6	93.0 to 105%

2.6.5 Recovery Percentage, Precision, Limit of Detection and Quantitation:

The range of % recoveries for the QC Level II– M and QC Level III– H standard were from 87.2% to 108%, and 99.5% to 112% respectively. The recovery for spiked urine samples, 5.0 µg/L and 30.0 µg/L, were 93% and 102 % respectively.

Additionally, a total of 20 runs of the lowest concentration mercury standard, 2 µg/L, were employed to calculate the limit of detection for this method. The limit of detection for the total mercury content by this method was calculated to be 0.258 µg/L, and the limit of quantitation of the total mercury content found to be 0.861 µg/L.

A second set of quality control samples were also analyzed to validate the method. The second set of QC samples included Standard Reference Material (SRM) purchased from the National Institute of Standards and Technology (NIST). There are two levels of mercury in the SRM 3668 package which are prepared in pooled urine. The certified values are given in **Table 2.7**. The results of the analysis of these SRM materials are listed in **Table 2.8**.

Table 2.7: Certified mercury concentration values of Quality Control Solutions.

NIST SRM 3668	**Level I**	**Level II**
Mercury Concentration (µg/L**)**	0.910 ± 0.055	6.38 ± 0.46

Table 2.8: Results obtained for the analysis of SRM 3668 Level I and Level II.

Measured Data	**Level I**	**Level II**
Mercury Concentration (µg/L**)**	0.890 ± 0.370	6.83 ± 0.52
Standard Deviation		

Fresh intermediate standard solutions were used to prepare the calibration plot to measure the mercury concentrations of the SRM 3668 solutions. Comparison between the results obtain and the SRM 3668 certified concentration values, showed the data were within the reference material range. The percent relative standard deviation (%RSD) of the results for Level I and Level II were 41.26 % and 7.65 %, respectively. Since the Level I concentration is below the

lowest calibration standards – 2 µg/L, but above the calculated limit of quantitation, these results indicated that a lower concentration calibration standard, such as 0.5 µg/L could be utilized to improve the accuracy of quantitation for QC solutions that were at such a low range.

Chapter III

Comparison of Chemical Additive Preservation in Mercury Intermediate Standards for Total Mercury Analysis in Human Urine by ICP-MS

3.1 Introduction

Accurate analysis of mercury in urine is depends on accurate and reliable analytical standards. The experience in our laboratory indicated the standards have much shorter stability times than the stated storage lifetime for a method previously developed by New York State Laboratory [26] (**Chapter II**) requiring in the fresh preparation of standards for each analysis. However, some studies have determined that mercury standard solutions are stable from a week to months at room temperature using various additives [13, 42, 43, 63]. The purpose of this study was to evaluate by ICP-MS the stability of the mercury intermediate standard solutions under various preparation and storage condition. The ultimate goal of this study was to find conditions which produce stable mercury intermediate standard solutions for at least one month.

3.2 Materials and Methods

3.2.1 Additive Studies

Mercury is one of the most volatile metals. Since mercury volatilization persists, addition of acids or oxidizing agents may prevent volatilization of mercury, thereby acting as preservative agents. Other possible reasons for instability could be due to mercury adsorbing onto the walls of containers or precipitating. Acids such as nitric acid [26, 43, 64], sulfuric acid [63, 64], and hydrochloric acid [13, 65]; oxidizing agents such as potassium permanganate [43, 64, 66], gold (III) [58, 62], and potassium dichromate [25, 43, 64] have been used as preservative agents. Thus, choosing the right preservative reagents may provide help achieve the following benefits:

1) Reduce sample and standards preparation time

2) Minimize the generation of hazardous waste

3) Improve the cost effectiveness of the analysis

4) Support high sample throughput when responding to an emergency

3.2.2 Standard Stability Studies

Various preservatives and storage containers have been evaluated to address problems associated with the storage of standards for mercury analysis [44, 45]. A recent study by Feldman [43] indicated that increasing the percentage of nitric acid can minimize mercury volatilization and adsorption onto plastic containers. Another documented method developed by the CDC indicates that the storage lifetime of standards can be extended for up to 6 months by the addition of hydrochloric acid to standards stored in glass at 2 to 4 °C [13]. Furthermore, changing the storage condition from room temperature to a lower temperature may help prevent mercury loss. Therefore, all three standard solutions studied here were stored at three different temperatures: at room temperature (19 to 23 °C), in the refrigerator (1 to 2 °C), and in the freezer (-25 to -28 °C) in sealed polypropylene containers (15 mL and 50 mL sterile conical; Sarstedt, Newton, NC) for comparison.

The Wadsworth Center quality control solutions, (Chapter 2.3.3), were analyzed to determine the stability of the standards. A set of freshly made standard solutions was also analyzed to determine the concentrations of the standard solutions being evaluated.

3.2.3 Composition of mercury intermediate standard solutions

The aim of the second part of this project was to prolong the storage lifetime of the intermediate working standards by exploring the use of several potential acid – preservative solution combinations. Three different acids were chosen for evaluation in this study: 1) nitric

acid, sulfamic acid preservative solution, and hydrochloric acid. The three different combinations tested are listed in Table 3.1.

Table 3.1: The acid-preservative solution combinations investigated for extending the intermediate standard storage lifetimes.

Solution	***A***	***B***	***C***
Nitric Acid	0.314 mol/L	0.785 mol/L	0.157 mol/L
Sulfamic Acid Preservative Solution	0.021 mol/L	0.021 mol/L	0.021 mol/L
Hydrochloride Acid	-	-	0.116 mol/L
Reference	[42, 62]	[43]	[13, 31]

3.3 Experimental

3.3.1 Instrumentation:

All analyses were performed on a PerkinElmer Sciex Elan DRCII 6000 ICP-MS (PerkinElmer Sciex, Thornhill, ON, Canada) instrument. The instrumentation was described in Chapter 2.2.

3.3.2 Method

The method utilized was adopted from the Laboratory of Inorganic and Nuclear Chemistry, Division of Environmental Health Sciences, Wadsworth Center Department of Health of the State of New York [26]. The instrument settings are the same as listed in Table 2.2A. Modification of the method was based on the previous study (Chapter 2). The wash time was changed from 240 seconds to 300 seconds to avoid mercury memory effect. The integrated peristaltic pump speed and duration of sample analysis and sample rinse-out are listed in Table 3.2.

Table 3.2: Integrated peristaltic pump speed, and duration of sample analysis and sample rinse-out times.

Action	Pump Speed (rpm)	Duration (seconds)
Sample Flush	-18	90
Read Delay and Analysis	-18	30
Wash	-24	300

Note: The negative sign indicates the direction of rotation is counterclockwise.

3.3.3 Preparation of the mercury intermediate standards

All reagents were prepared using double distilled deionized water having resistivity ≥ 18MΩ.cm (Barnstead Nanopure; Millipore Corporation, Bedford, MA). Three high purity acids were used to prepare the mercury intermediate standard solutions: double distilled nitric acid (67-69% (v/v), Optima grade, Fisher Scientific, Pittsburgh, PA), sulfamic acid (99.3% (w/v) ACS reagent grade; Sigma-Aldrich Company, St. Louis, MO); and hydrochloric acid (30-35% (v/v), Veritas®, GFS Chemicals, Columbia, OH). Certified and periodically calibrated pipettes were used at all times. All base urine, standards, and preservative solutions were prepared under the same conditions as described in Chapter 2. All sample preparation was performed in certified BSC. The preparation procedures for the stock standard solution, and the intermediate stock standard were described in Chapter 2.4.1. A series of aqueous mercury standards were prepared by dilution of the 8mg/L Hg stock solution to give the following mercury concentrations: 2 µg/L, 5 µg/L, 10 µg/L, 20 µg/L, 30 µg/L, 40 µg/L (**Table 2.4**). The blank was an aqueous solution contained 1% (v/v) nitric acid and 1% (v/v) sulfamic acid preservative solution. The preparation scheme for the mercury intermediate standards is given in Figure 2.1. Procedures for preparation of base urine, sulfamic acid preservative solution, and diluent are as described in Chapter 2.4.2, 2.4.3, and 2.4.4, respectively. Each set of mercury intermediate standard solutions were aliquoted into smaller polypropylene containers (15 mL and 50 mL conical polypropylene

containers) and stored at three different temperatures: room temperature (19 to 23 °C), in the refrigerator (1 to 2 °C), and in the freezer (-25 to -28 °C).

3.3.3.1 Preparation of mercury intermediate standard solution set A

Each mercury intermediate standard of set A contained 2% (v/v) double distilled nitric acid (0.314 mol/L), and 1% (v/v) sulfamic acid preservative solution (0.021 mol/L). Each polypropylene volumetric flask was partially filled with double distilled deionized water (resistivity, ≥ 18MΩ.cm), followed by addition of the appropriate volume of the mercury intermediate stock standard. Two milliliters of double distilled nitric acid, and one milliliter of sulfamic acid preservative solution were added to the flask, then diluted to volume with double distilled deionized water. The final concentration of mercury in each standard is listed in Table 2.4.

3.3.3.2 Preparation of mercury intermediate standard solution set B

Each mercury intermediate standard of set B contained 5% (v/v) double distilled nitric acid (0.785 mol/L), and 1% (v/v) sulfamic acid preservative solution (0.021 mol/L). Each polypropylene volumetric flask was partially filled with double distilled deionized water (resistivity, ≥ 18MΩ.cm), followed by addition of the appropriate volume of the mercury intermediate stock standard. Five milliliters of double distilled nitric acid, and one milliliter of sulfamic acid preservative solution were added to the flask, then diluted to volume with double distilled deionized water. The final concentration of mercury in each standard is listed in Table 2.4.

3.3.3.3 Preparation of mercury intermediate standard solution set C

Each mercury intermediate standard of set C contained 1% (v/v) double distilled nitric acid (0.157 mol/L), 1% (v/v) hydrochloric acid (0.116 mol/L), and 1% (v/v) sulfamic acid

preservative solution (0.021 mol/L). Each polypropylene volumetric flask was partially filled with double distilled deionized water, (resistivity, ≥ 18MΩ.cm), followed by addition of the appropriate volume of the mercury intermediate stock standard. One milliliter of double distilled nitric acid, one milliliter of hydrochloric acid, and one milliliter of sulfamic acid preservative solution were added to the flask followed by diluting to volume with double distilled deionized water. The final concentration of mercury in each standard is listed in Table 2.4.

3.3.4 Preparation of calibration standards and quality control solutions for analysis

For ICP-MS analysis, calibration standards for constructing the working curve were prepared by transferring a 500 µL aliquot of the appropriate aqueous intermediate standard, a 500 µL aliquot of base urine, and 9000 µL of diluent to a 15 mL of conical polypropylene container (Sarstedt, Newton, NC). The stability of stored mercury intermediate standards was monitored by running quality control standard solutions (Chapter 2.3.3.). Any changes in stability were evaluated by analyzing freshly prepared mercury intermediate standards. The preparation scheme for the standard solutions for ICP-MS analysis are shown in Table 2.5.

3.3.5 Definition of stability

To evaluate the stability of the mercury intermediate standards, quality control solutions were employed. The definition of stability is that the quality control results are desired to have a percent accuracies in the range of 90 to 110% of the certified value of the target (Equation 3.1). The acceptable range of percent accuracies are between 80 to 120% of the target certified value. Another term, the allowable percent error must be within 25 percent to be considered stable (Equation 3.2) [39, 40]. Mercury loss percentages were also calculated to determine how much mercury was lost over time at each concentration for the mercury intermediated standards for

each set (Equation 3.3). The percent mercury loss must be within 20% to be considered reasonable [33, 39-41].

$$\%\ \boldsymbol{Accuracy} = \frac{\boldsymbol{Measured\ Quality\ Control\ Value}}{\boldsymbol{Actual\ Quality\ Control\ Value}} \times \mathbf{100}\% \qquad \text{Eq. (3.1)}$$

$$\%\ \boldsymbol{Error} = \frac{|\boldsymbol{Measured\ Value - Actual\ Value}|}{\boldsymbol{Actual\ Value}} \times \mathbf{100}\ \% \qquad \text{Eq. (3.2)}$$

$$\%\ \boldsymbol{Mercury\ Loss} = \left(\mathbf{1} - \frac{\boldsymbol{Measured\ Value}}{\boldsymbol{Actual\ Value}}\right) \times \mathbf{100}\% \qquad \text{Eq. (3.3)}$$

3.4 Results and Discussion

Mercury, a volatile element, is also easily adsorbed onto the walls of the container, which will result in inaccurate quantitation. Many researchers and authors have faced the challenge of finding the most suitable additive preservative conditions to preserve mercury in the solution. In the low ppb (µg/L) range, Litman *et al.* stated that high adsorption rates of mercury were found on glass, polytetrafluoroethylene (PTFE), and polyethylene (PE) [67]. This could be due to mercury having high mobility, and is likely to react with and adsorb on its surroundings [68]. The problem could be reduced or eliminated by adding an oxidizing agent to the calibration solutions. Research has shown addition of acids may also reduce mercury adsorption on the walls. Crompton stated that mercury in aqueous solution tends to be stabilized at low pHs, high ionic strengths, and with the addition oxidizing reagents [69]. Feldman found noticeable amounts of mercury are lost even with the presence of various reagents, including nitric acid, dichromate, andsulfuric acid [43]. Therefore, to overcome this problem, the types of storage materials and the choice of acid preservative solutions were parts of this study.

Polypropylene (PP) and polyethylene (PE) containers are usually preferred over glass because they are less expensive and trace metal free. PE containers have high resistance against HCl but are less resistant to nitric acid. PP is resistant to dilute nitric acid. However, PP is less

resistant to concentrated HCl and will turn yellow or brown with prolonged exposure [70]. Since nitric acid is preferred for trace metal analysis by ICP-MS due to its oxidizing ability as well as producing less polyatomic or isobaric interferences, polypropylene is an ideal container for storage of solutions [71]. All solutions in this study were stored in polypropylene (PP) containers instead of glass or polyethylene containers.

As discussed in Chapter 2.6.2, the purpose of adding oxidizing agents is to help stabilize mercury in solution. Most researchers found that the appropriate amount of nitric acid to preserve mercury are between 1 to 5% (v/v) [69]. However, there is no standardized method, and the storage lifetime of standards varied. For instance, in methods published by government agencies are included the United States Environmental Protection Agency (US EPA) methods, such as EPA 200.8 and EPA 6020A, and, the Centers of Disease Control and Prevention (CDC) method, such as 3002.1. Louie and Wong indicated 1% (v/v) HNO_3 with 0.01% (v/v) HCl is the ideal acid combination for preservation of mercury and other elements for simultaneous multi-element quantitation by ICP-MS [42]. Bornhorst studied the effects of chemical additives and storage conditions on quantitation of 16 trace elements in urine by ICP-MS, and suggested that refrigeration of samples is recommended to prevent bacterial growth [72]. Parikh and Mahmoud found that samples prepared solely with 5% HNO_3 were unstable over time. However, samples treated with 2% HCl and Au^{3+} were all well within control limits with good recoveries over time [73]. Parikh and Mahmoud proposed that there could be a possible mechanism for the stabilizing effect of gold and hydrochloric acid on mercury is they make the reduction of Hg^{2+} unfavorable. This can be explained by comparing the redox potentials of mercury in the presence of either hydrochloric acid or Au^{3+} [73]. As discussed in Chapter 2.6.2, Au^{3+} is a strong oxidizing agent which keeps the Hg^{2+} in solution. Comparing the standard reduction potentials for mercury

(Equation 4.1) and gold (Equation 4.3), the addition of gold (Au^{3+} reduction; Equation 4.3) is more favorable [73]. Addition of HCl, can provide a chloride ligand to mercury and form tetracloro-mercurate (II) in solution [73]. The reduction potential of tetracloro-mercurate (II) (Equation 4.2) is lower than the Hg^{2+} (Equation 4.1), which results in limiting the formation of Hg^0. The formation of $HgCl_4^{-2}$ complex ions is also likely to prevent the adsorption of mercury onto the inner walls of the PP container [42].

$$Hg^{2+} + 2e^- \leftrightarrow Hg^0 \qquad E^0 = 0.85 \qquad \text{Eq. 4.1}$$

$$[HgCl_4]^{2-} + 2e^- \leftrightarrow Hg^0 + 4Cl^- \qquad E^0 = 0.41 \qquad \text{Eq. 4.2}$$

$$AuCl_4^- + 3e^- \leftrightarrow Au + 4Cl^- \qquad E^0 = 1 \qquad \text{Eq. 4.3}$$

3.4.1 Mercury intermediate standard solution set A

For mercury intermediate standards solution set A, 2% (v/v) of double distilled nitric acid and 1% (v/v) of sulfamic acid preservative solution were added to the Hg intermediate standards to improve the storage lifetime. These standard solutions were stored at three temperatures room temperature (19 to 23 °C), in the refrigerator (1 to 2 °C), and in the freezer (-25 to -28 °C). Set A mercury intermediate standards stored at room temperature were found to be not stable with mercury concentrations decreasing dramatically within two weeks (Figure 3.1). The mercury concentration fell by almost 50% during the first week, and approached 100% loss during the second week (Figure 3.2). The true concentration of the intermediate standards could not be accurately quantitate at the lower concentration range because it reached the limit of quantitation of the instrument, resulting in 100% loss of mercury. Therefore, the conclusion is that the Set A mercury intermediate standards are unstable when stored at room temperature.

For Set A mercury intermediate standards stored at lower temperatures, the results are presented in Figures 3.3 and 3.4. For the set A standards, the percentage mercury loss when

stored at refrigerator temperature were within 20% during the first week and the second weeks. However, the percentage of mercury lost for the set A standards stored at refrigerator temperature was nearly 80% after the week 2.

Set A mercury intermediate standards were also stored in the freezer, with the results shown in Figures 3.5 and 3.6. Mercury intermediate standards were found to be stable for two weeks, the percentage of mercury lost was within 20% during this time. But, after two weeks, the percentage of mercury loss for standards number 1 (2ppb) and 4 (20ppb) exceeded 60%. This could be possibly be caused by freezing and thawing of the standard solutions.

Though storage at lower temperature resulted in reduced mercury loss, yet none of the storage conditions in set A mercury intermediate standards provided stability for up to the one month. These experiments show that the Set A mercury intermediate standards must be prepared fresh prior to analysis, or prepared fresh weekly if stored at a lower temperature such as in the refrigerator or freezer.

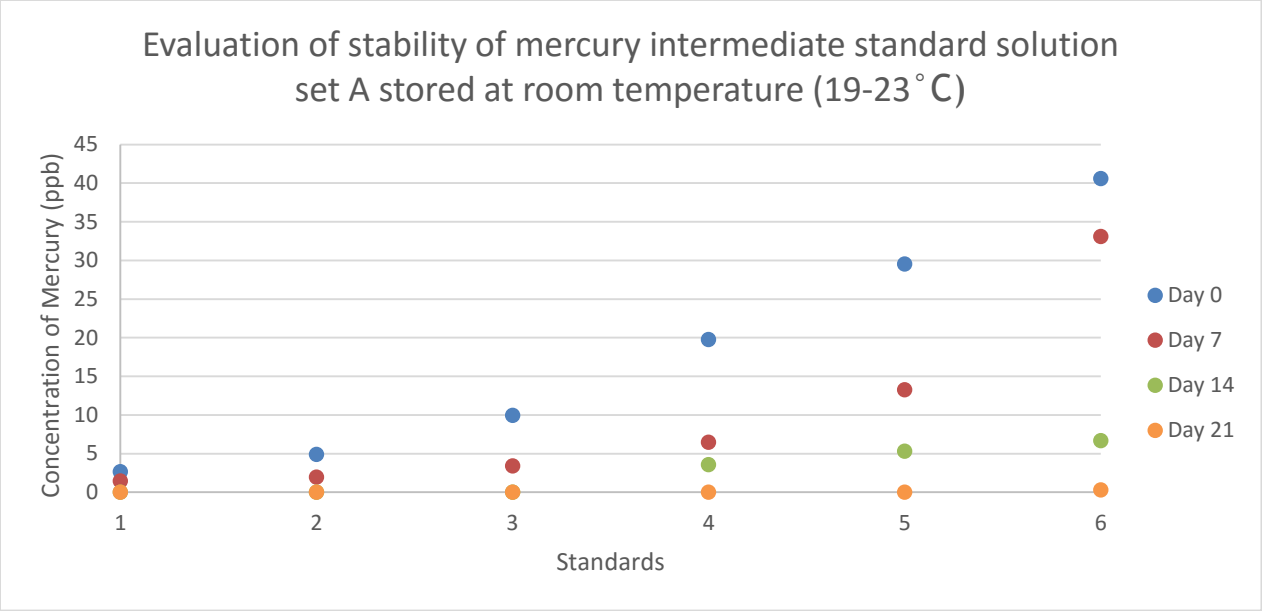

Figure 3.1: Evaluation of mercury intermediate standard solution (set A) stored at room temperature (19-23°C). The responses of the Hg intermediate standards were measured for three weeks to monitor response stability. Standards numbered 1 through 6 corresponded to concentrations ranging from 2µg/L to 40µg/L of mercury as given in **Table 2.4**. The concentration of mercury decreased rapidly by day 14. The following week, the concentration of all mercury intermediate standard almost reached near to zero. This could be due to mercury adsorbing onto the container walls or volatilization.

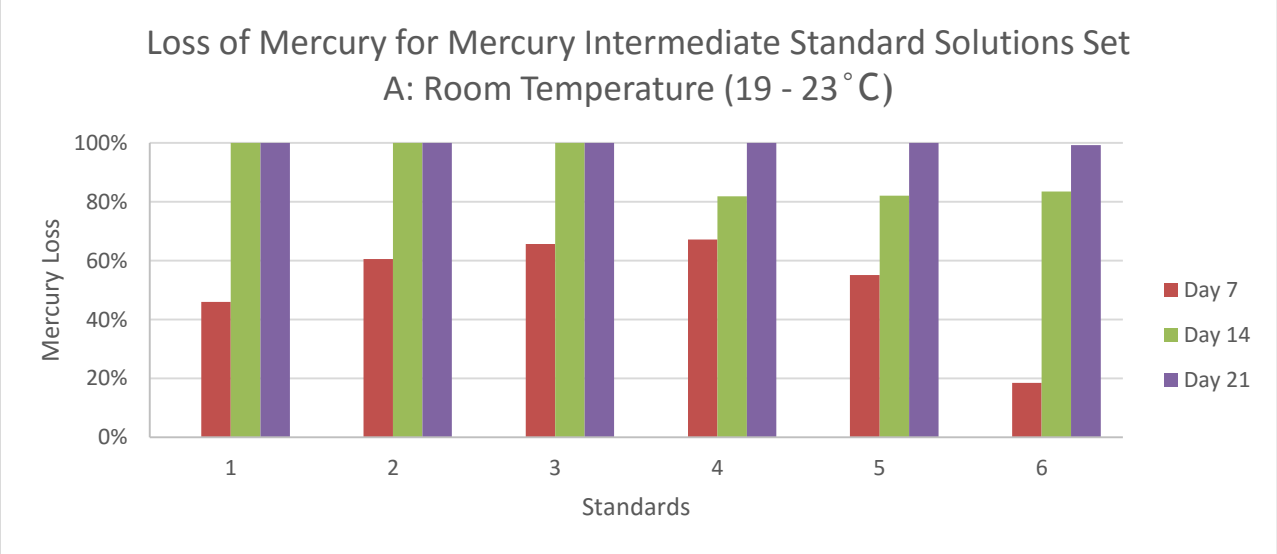

Figure 3.2: Percentage loss for mercury intermediate standard solution set A, stored at room temperature (19-23 °C) for three weeks. Standards numbered 1 through 6 corresponded to concentrations ranging from 2µg/L to 40µg/L of mercury as given in **Table 2.4**.

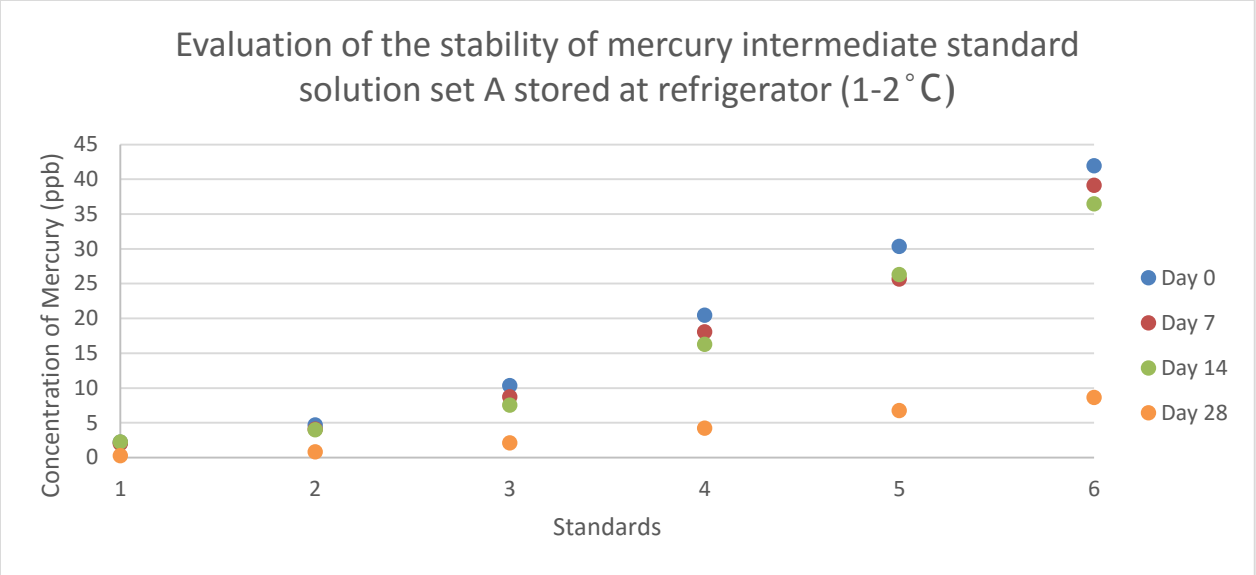

Figure 3.3: Evaluation of the mercury intermediate standard solution (set A) stored at refrigerator temperature (1-2°C). The responses of the Hg intermediate standards were measured for four weeks to monitor response stability. The mercury concentration was found to decrease further after two weeks (Measured on Day 28). Standards numbered 1 through 6 corresponded to concentrations ranging from 2µg/L to 40µg/L of mercury as given in **Table 2.4**.

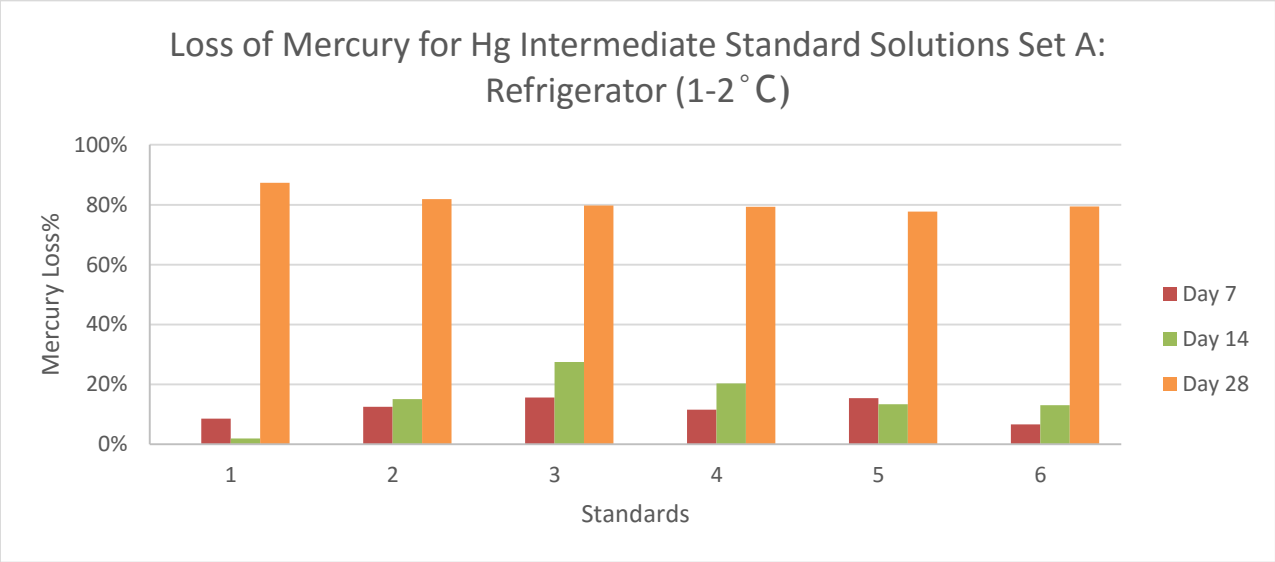

Figure 3.4: Percentage loss for mercury intermediate standard solution set A, stored in the refrigerator (1-2°C) for four weeks. Standards numbered 1 through 6 corresponded to concentrations ranging from 2µg/L to 40µg/L of mercury as given in **Table 2.4**.

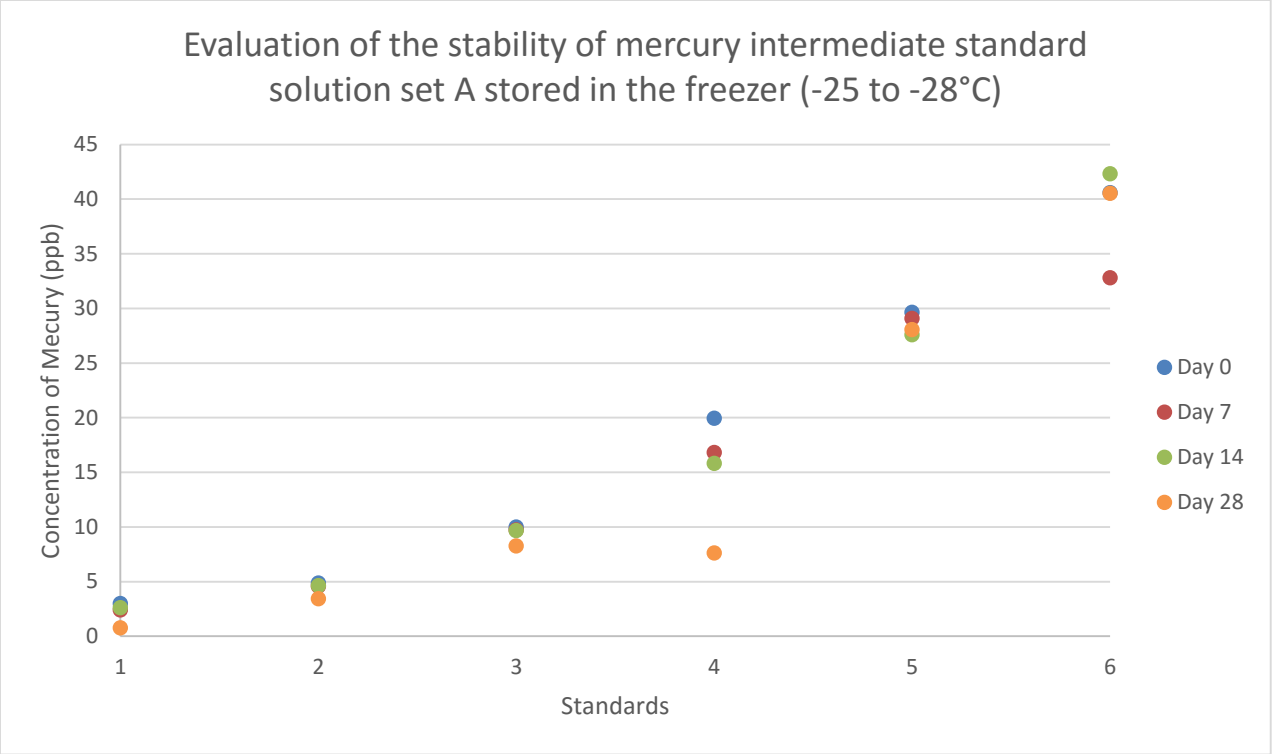

Figure 3.5: Evaluation of the mercury intermediate standard solution (set A) stored in the freezer at -25 to -28 °C. The responses of the Hg intermediate standards were measured for four weeks to monitor response stability. Standards numbered 1 through 6 corresponded to concentrations ranging from 2µg/L to 40µg/L of mercury as given in **Table 2.4**. The responses of the mercury intermediate standards were inconsistence throughout the monitor period.

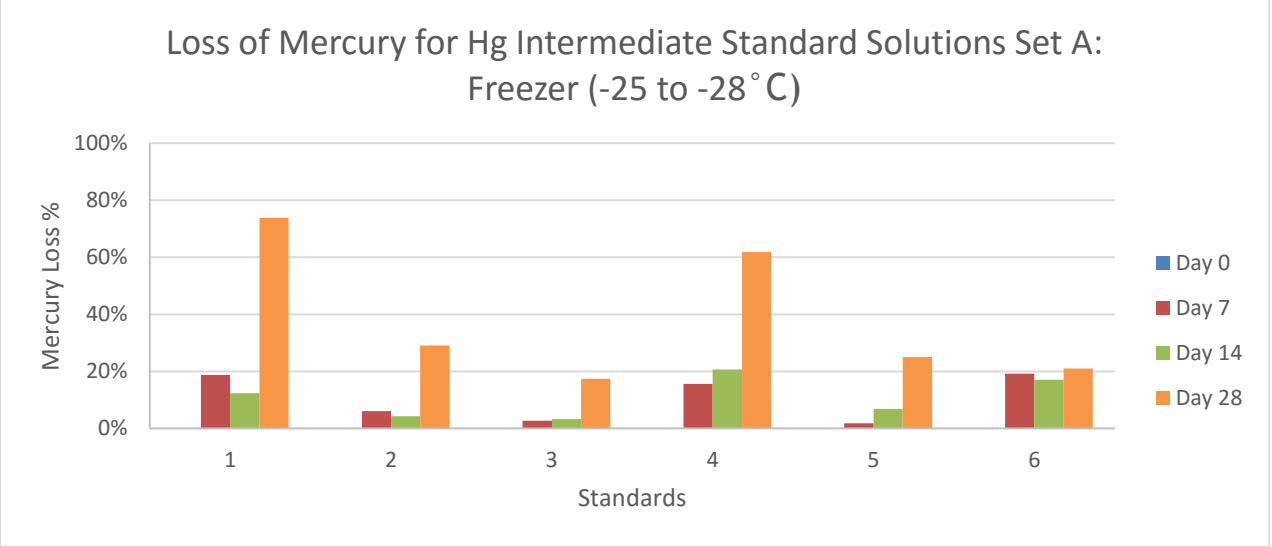

Figure 3.6: Percentage loss for mercury intermediate standard solution set A, stored in freezer (-25 to -28°C) for four weeks. Standards numbered 1 through 6 corresponded to concentrations ranging from 2µg/L to 40µg/L of mercury as given in **Table 2.4**.

Therefore, mercury intermediate standard solution set A did not achieve the goal of this research purpose. The mercury intermediate standard solutions were found to be stable for only one week with the addition of nitric acid and stored in lower temperature (1-2°C and -25 to -28°C). Mercury loss was greater than 60% after three weeks for storage at all temperatures.

3.4.2 Mercury intermediate standard solution set B

Mercury intermediate standard solution set B containing 5% (v/v) of double distilled nitric acid and 1% (v/v) of sulfamic acid preservative solution was evaluated to determine if these solution conditions improved storage lifetime. These standard solutions were stored at three temperatures: room temperature (19-23 °C), refrigerator temperature (1-2°C), and at freezer (-25 to -28°C).

For mercury intermediate standard solution set B stored at room temperature, it was found that the concentration of mercury decreased dramatically by one week after preparation (**Figure 3.7**). The percentage of mercury loss for the set B standards stored at room temperature are presented in Figure 3.8. The mercury concentration decreased by more than 60% for all standards except for standard numbered 1 (2ppb) during the first week of storage, and was greater than 80% for all standards at two weeks (Day 14).

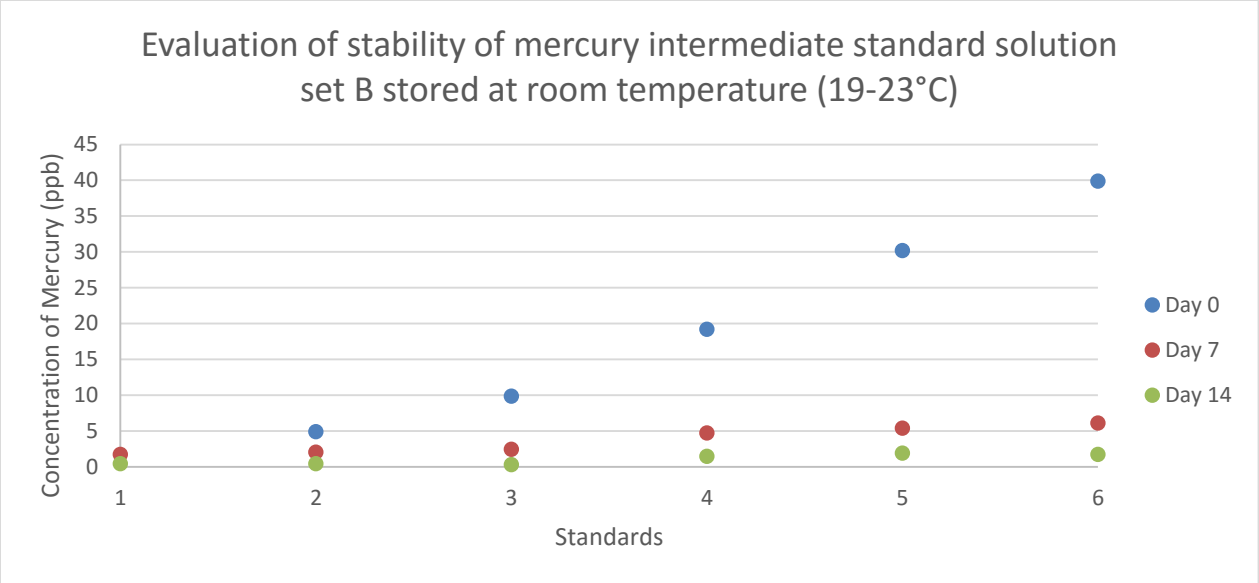

Figure 3.7: Concentration of mercury in mercury intermediate standard solutions set B stored at room temperature (19-23°C) and evaluated for two weeks. Standards numbered 1 through 6 corresponded to concentrations ranging from 2µg/L to 40µg/L of mercury as indicated in **Table 2.4**.

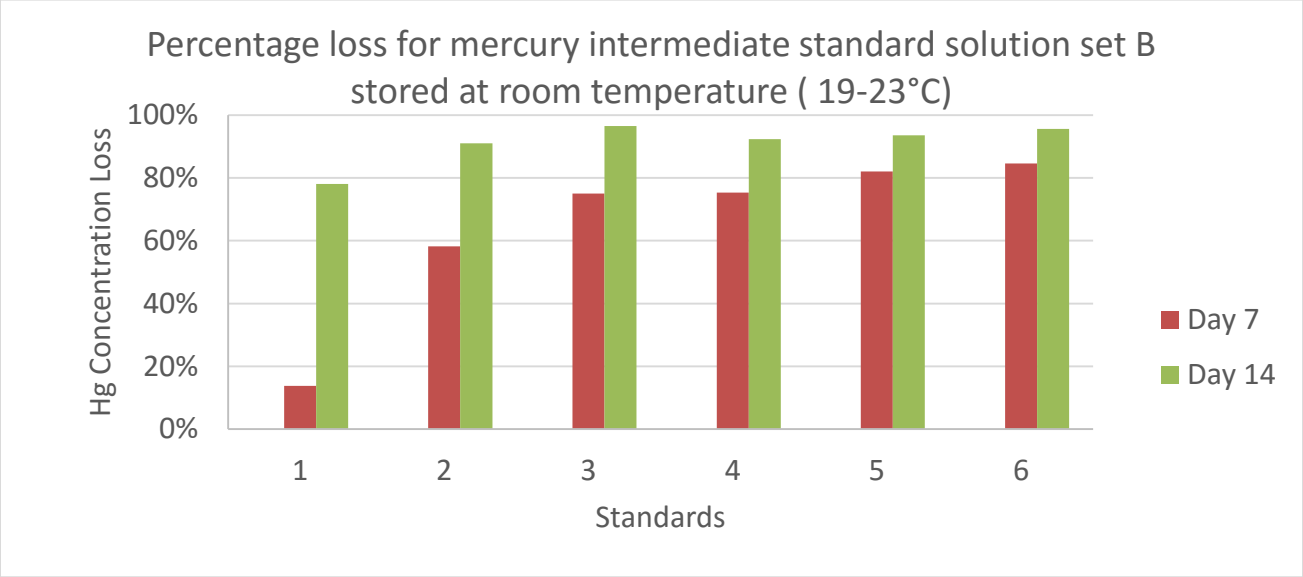

Figure 3.8: Percentage of mercury lost for mercury intermediate standards solution set B stored at room temperature (19-23°C). These standards were monitored for two weeks. Standards numbered 1 through 6 correspond to concentrations ranging from 2µg/L to 40µg/L of mercury (**Table 2.4**).

The results obtained for the mercury intermediate standard solution set B stored in the refrigerator at 1 to 2 °C for up to three weeks, the evaluation of these standards is presented in **Figure 3.9**. Set B standards were found to be stable within 25% for three weeks when stored at refrigerator temperature. After four weeks, almost all of the mercury standards decreased in concentration by 30% or more. For standards number 2 (5ppb) and 3 (10ppb) of set B stored in the refrigerator, the mercury loss reached 100% and 80 % after four weeks (measured on Day 28), respectively (**Figure 3.10**). As shown Figure 3.10, variation in mercury loss was observed among all the standards. As discussed in Chapter 2, ICP-MS is a very sensitive instrument which requires a daily performance check before performing any analysis. Therefore, the instrument parameters could be optimized. In order to accurately quantitate and measure the mercury intermediate standards, as mentioned in Chapter 3.3.4, new set of mercury intermediate standards were freshly prepared prior to analysis to measure the actual mercury concentration of each standard solutions. Consequently, this could have resulted in variation in mercury concentrations.

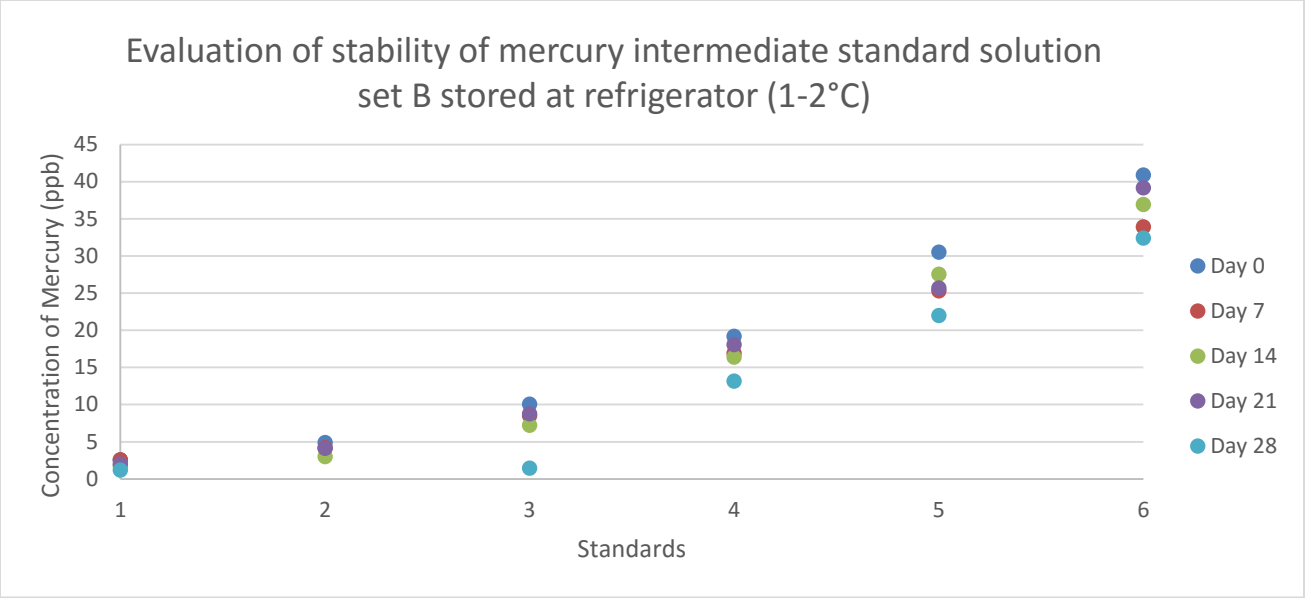

Figure 3.9: Results for mercury intermediate standards solution set B stored in the refrigerator (1-2°C) and monitored for mercury concentration over four weeks. Standards numbered 1 through 6 corresponded to concentrations ranging from 2µg/L to 40µg/L of mercury as indicated in **Table 2.4**.

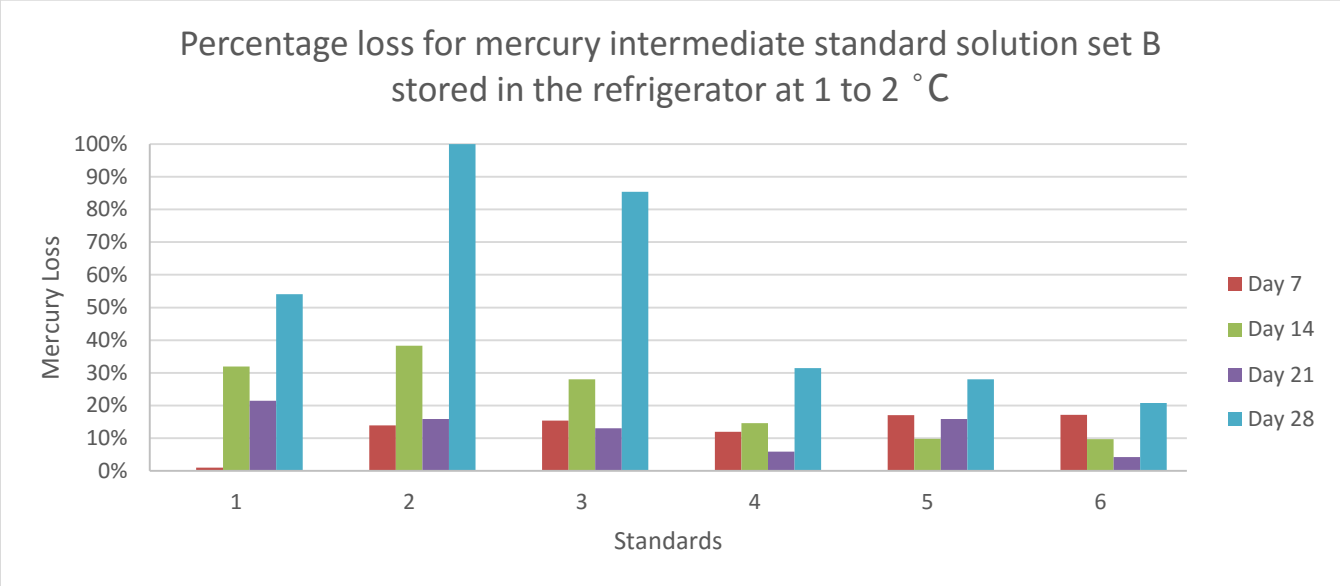

Figure 3.10: Percentage of mercury lost for mercury intermediate standards solution set B stored in the refrigerator (1-2°C). These standards were monitored for four weeks. Standards numbered 1 through 6 corresponded to concentrations ranging from 2µg/L to 40µg/L of mercury as indicated in **Table 2.4**.

The results obtained for the mercury intermediate standards solution set B stored in the freezer at -25 to -28°C, are presented in **Figures 3.11** and **3.12**. The mercury intermediate standard solution set B stored in the freezer were found to be stable for three weeks. Although the mercury loss for the set B standards were within 20 % for three weeks, the stabilities of the set B standards stored in the freezer were not consistent. This inconsistency could be due to the freezing and thawing cycle. Based on the results obtained, the set B mercury intermediate standards need to be prepared fresh every two weeks and stored in the freezer.

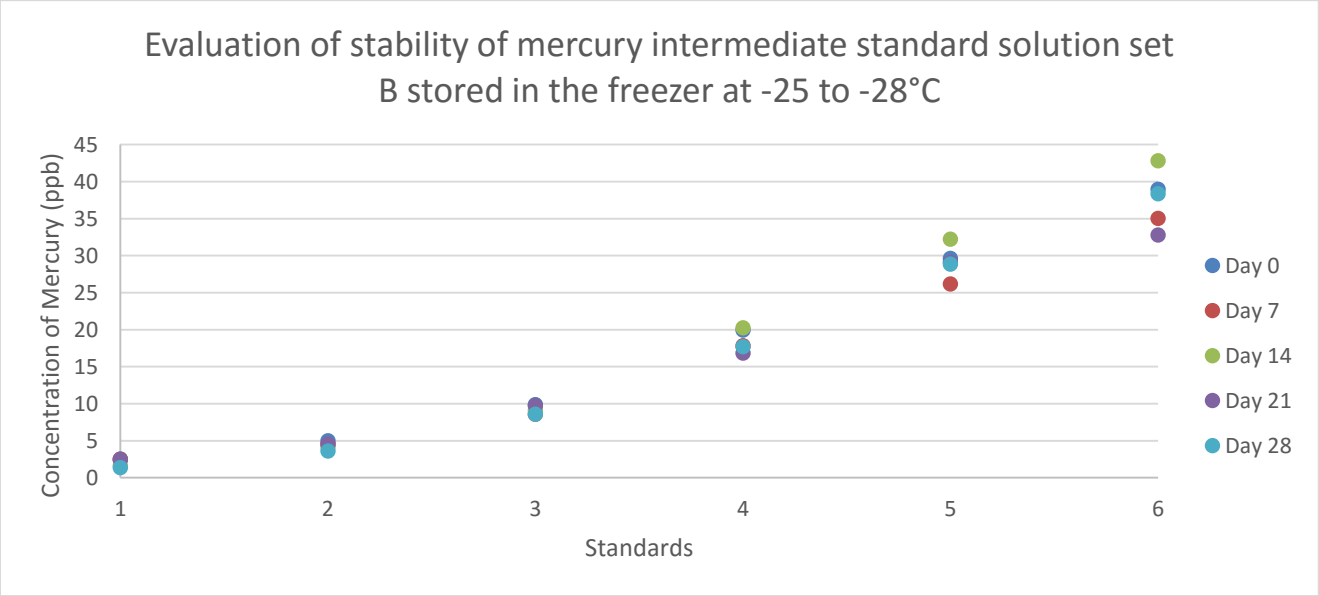

Figure 3.11: Results for mercury intermediate standards solution set B stored in the freezer (-25 to -28 °C) and monitored for mercury concentration over 4 weeks. Standards numbered 1 through 6 correspond to concentrations ranging from 2µg/L to 40µg/L of mercury (**Table 2.4**).

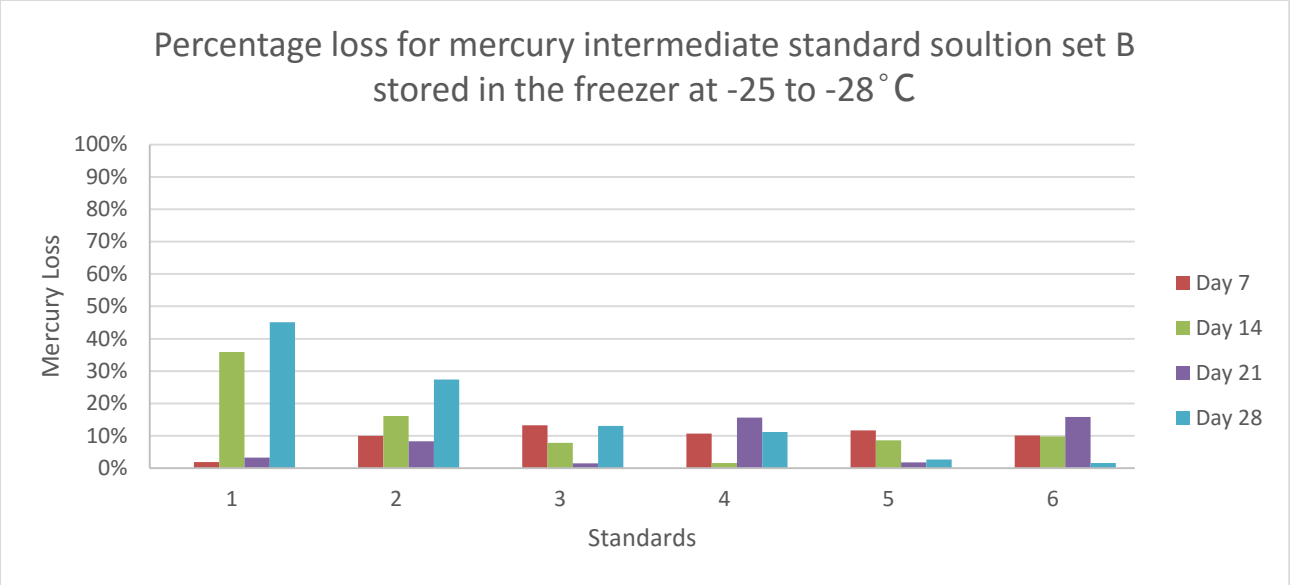

Figure 3.12: Percentage of mercury lost for mercury intermediate standards solution set B stored in the freezer at -25 to -28 °C and evaluated over 4 weeks. Standards numbered 1 through 6 correspond to concentrations ranging from 2µg/L to 40µg/L of mercury as indicated in **Table 2.4**.

3.4.3 Mercury intermediate standard solution set C

Mercury intermediate standards solution set C containing 1% (v/v) of double distilled nitric acid, 1% (v/v) hydrochloric acid, and 1% (v/v) of sulfamic acid preservative solution were evaluated to determine if these solution conditions improved storage lifetime. These standard solutions were stored at three temperatures: room temperature (19-23°C), refrigerator temperature (1-2°C), and in the freezer (-25 to -28°C).

Set C mercury intermediate standards were found to be stable within 20% loss for four weeks when stored at room temperature (Figure 3.13). As shown in Figure 3.14, most of the standards were stable for four weeks with mercury loss percentage was within 10%, except for standard numbered 1. Standard numbered 1 is the lowest concentrations studied, 2ppb. A 40% mercury loss was observed at the lower concentrations, which corresponds to a mercury loss of only 0.8 ppb in total. Therefore, a mercury loss of 20% was taken as being applicable to higher

concentration such as 5 ppb and above which shown to have a significant impact. For example, a 20% and 50% mercury loss at the highest concentration standard, 40 ppb, which corresponds to a mercury loss of 8 ppb and 20 ppb in total, respectively.

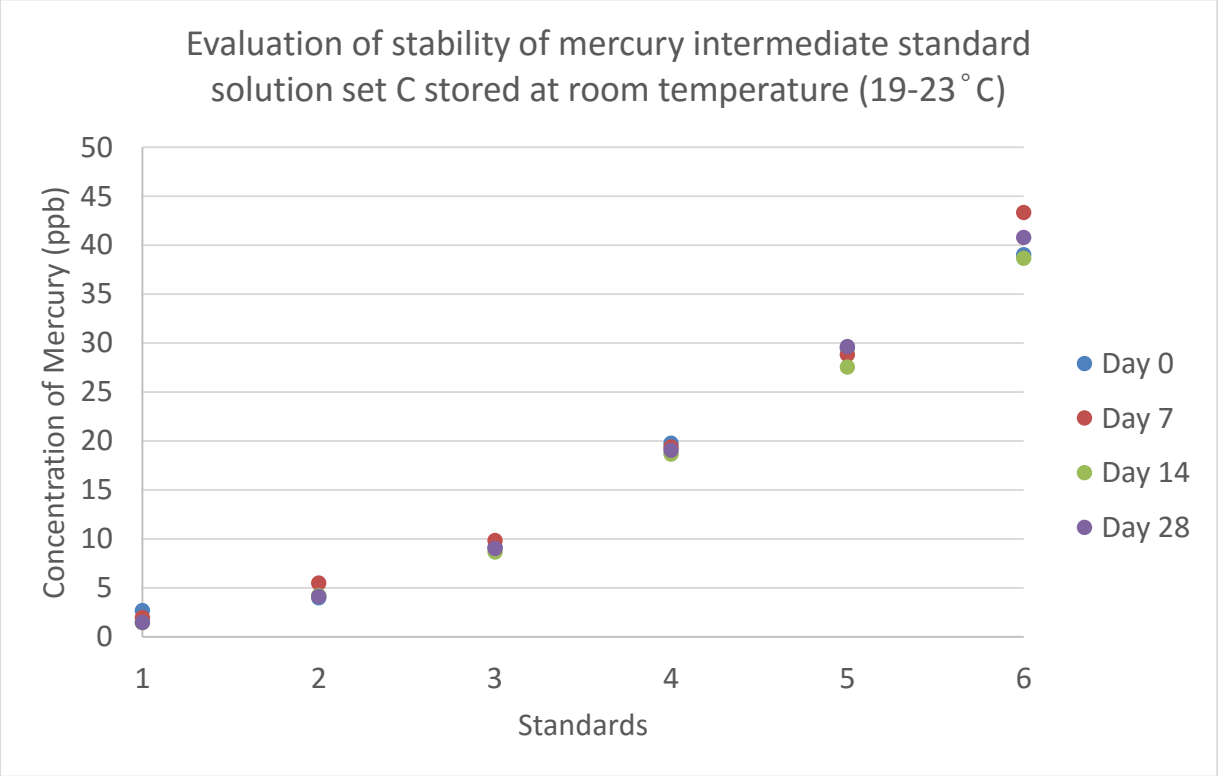

Figure 3.13: Results for mercury intermediate standard solution set C stored at room temperature (19 – 23 °C). These standards were found to be stable within 20% for four weeks. Standards numbered 1 through 6 correspond to concentrations ranging from 2µg/L to 40µg/L of mercury as indicated in **Table 2.4**.

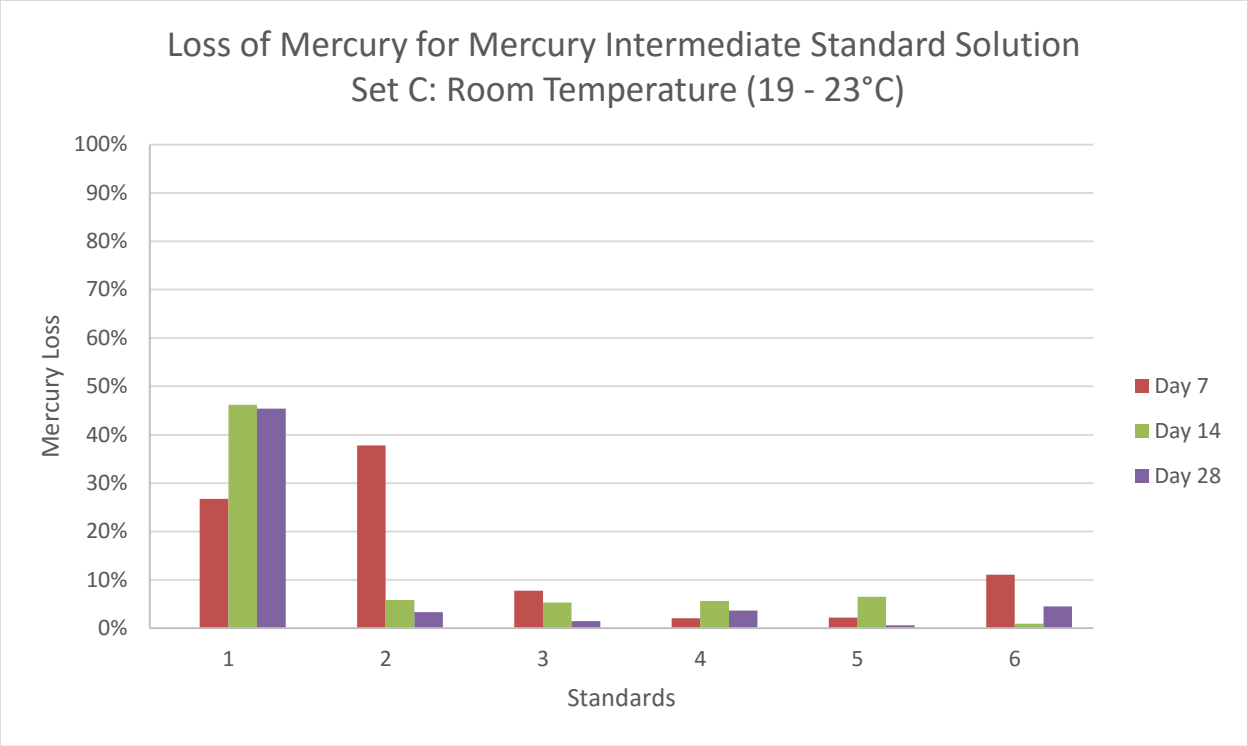

Figure 3.14: The percentages of mercury loss for mercury intermediate standard solution set C. Standards numbered 1 through 6 correspond to concentrations ranging from 2µg/L to 40µg/L of mercury as indicated in **Table 2.4**.These standards were stored at room temperature (19-23°C) and evaluated for four weeks. The percentages of mercury loss for the set C standards stored at room temperature were less than 10%, except for the lowest mercury concentration standards, numbered 1 and 2. The variation in mercury loss at the lower concentration range is likely due to concentrations approaching to the limit of the quantitation for the measurement.

Mercury intermediate standard solution set C stored in the refrigerator had similar trends as those stored at room temperature (**Figure 3.15**). The percentages of mercury loss for each standards over four weeks are presented in Figure 3.16. Most of the standards were stable for four weeks, with mercury loss percentages within 20%, except for standard numbered 2. Standard number 2 contained 5 ppb of mercury and was the second lowest concentration in the calibration standard range. At the concentration of 5ppb, a loss of 29% is reasonable since this corresponds to a loss of only 1 ppb for a 5ppb standard.

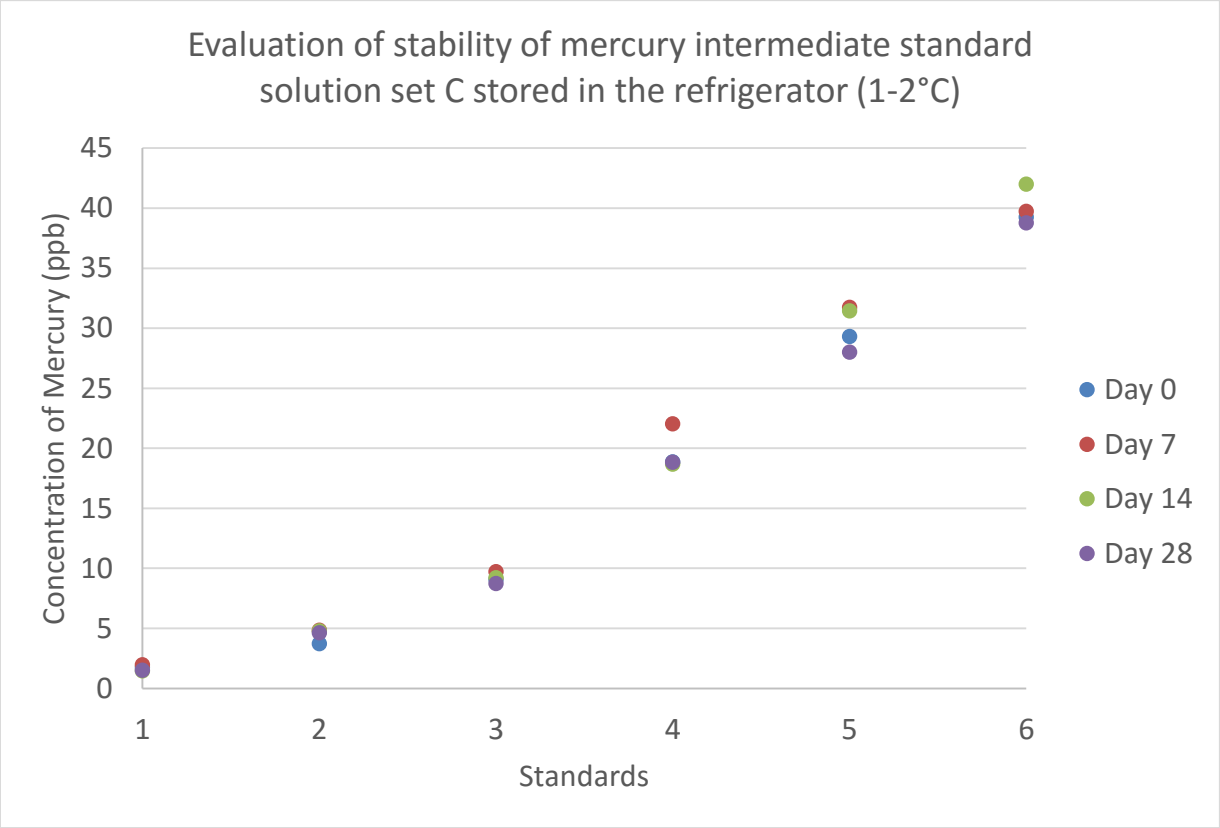

Figure 3.15: Evaluation of the mercury intermediate standard solution set C stored at refrigerator (1-2°C) were monitored for four weeks. Standards numbered 1 through 6 corresponded to concentrations ranging from 2µg/L to 40µg/L of mercury as indicated in **Table 2.4**.

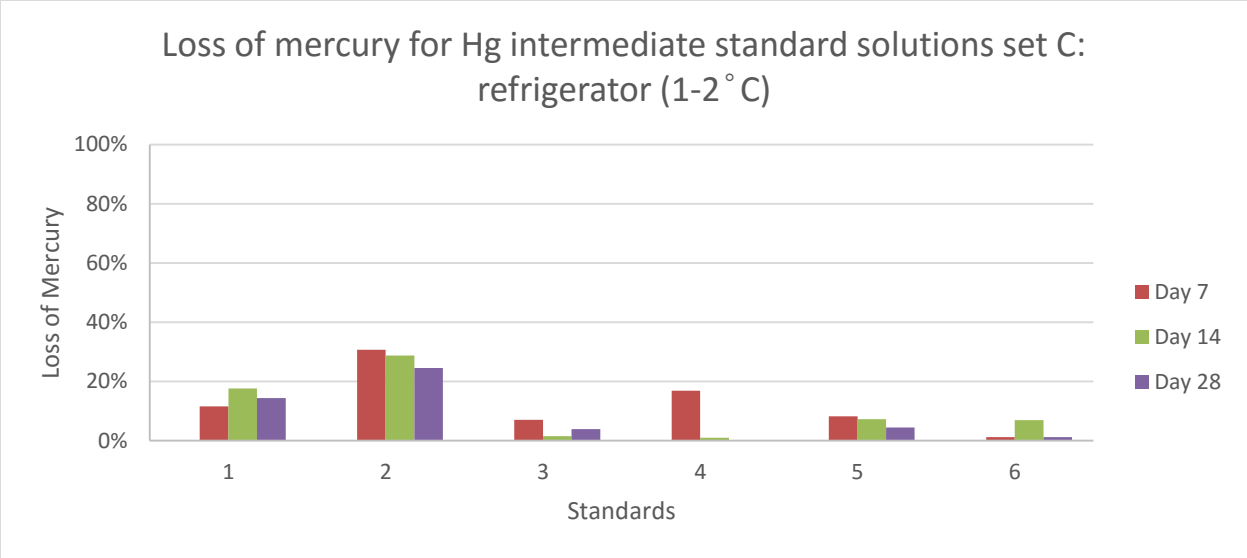

Figure 3.16: Percentage loss for the set C mercury intermediate standards, stored in the refrigerator (1-2°C) for four weeks. The percentage of mercury loss for the set C standards were less than 30% over four weeks. Standards numbered 1 through 6 correspond to concentrations ranging from 2µg/L to 40µg/L of mercury as indicated in **Table 2.4**.

Set C mercury intermediate standards were also stored in the freezer and were monitored over four weeks (**Figure 3.17**). The results showed when stored in the freezer, the set C standards were stable for one week, but after two weeks the mercury concentrations decreased substantially. After two weeks, the higher concentration standards, numbered 4 to 6, have reached a mercury loss of 30% (**Figure 3.18**). The loss in mercury could be due to mercury adhering to the walls of the container during the freezing and thawing cycle, or some other factor.

Based on the results, set C mercury intermediate standards were considered to be adequately stable for four weeks at both room and refrigerator temperatures. However, set C mercury intermediate standards stored in the freezer were stable for only one week. Based on this study, the recommendation for set C mercury intermediate standard solutions is to store the standards at either room temperature or refrigerator temperature.

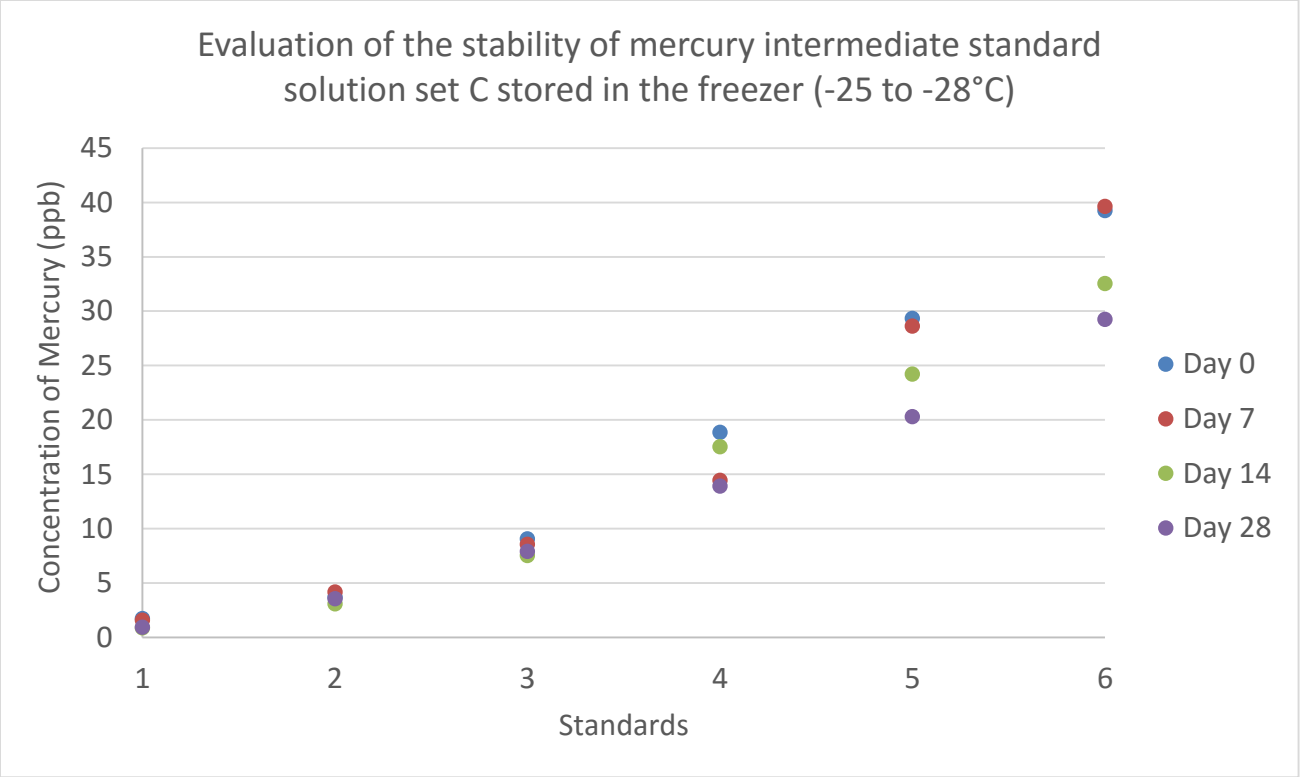

Figure 3.17: Evaluation of mercury intermediate standard solution set C stored in the freezer (-25 to -28°C). The responses of the Hg intermediate standards were measured for four weeks to monitor their stability. Standards numbered 1 through 6 correspond to concentrations ranging from 2µg/L to 40µg/L of mercury as indicated in **Table 2.4**.

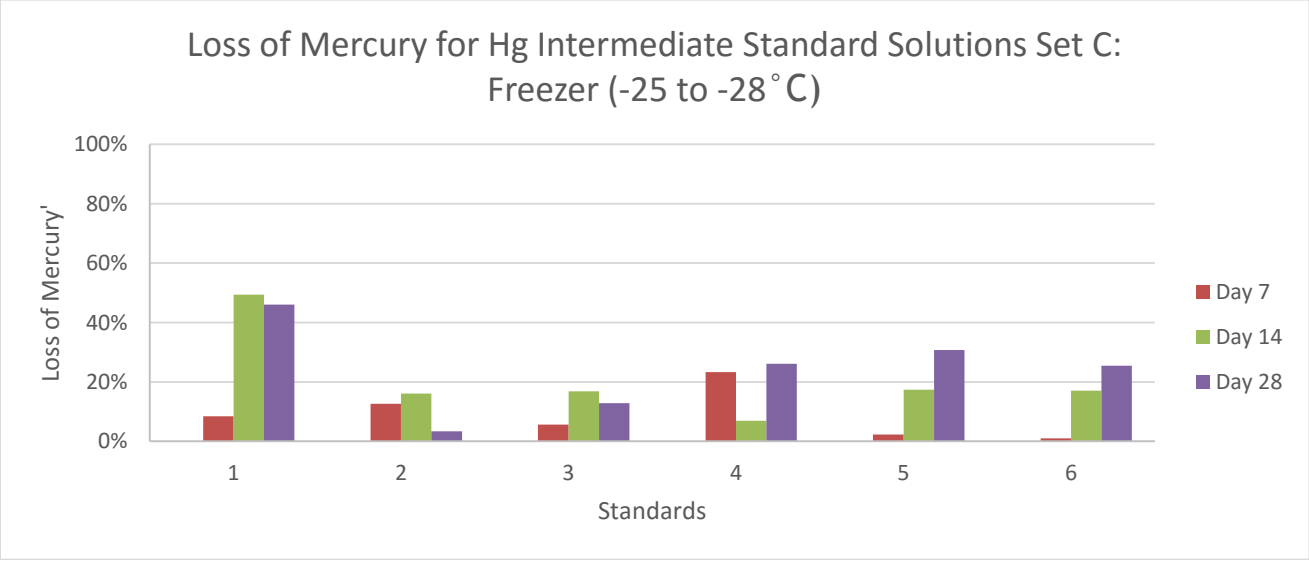

Figure 3.18: Percentage loss for mercury intermediate standards set C, stored in the freezer (-25 to -28°C) for four weeks. Standards numbered 1 through 6 corresponded to concentrations ranging from 2µg/L to 40µg/L of mercury as indicated in **Table 2.4**.

3.4.4 Results for Mercury Quality Control (QC) solutions

In the previous sections 3.4.1 to 3.4.3, the concentrations of mercury intermediate standards were being measured utilizing freshly made standards to evaluate and monitor the loss in mercury. In other words, the monitored set A, B, and C mercury intermediate standards were treated as samples. However, to truly reveal and check the accuracy of the analysis of the standards, an external standard must be employed. In this study, quality control solutions are used to evaluate the stabilities of the mercury intermediate standards. With that being said, the set A, B, and C mercury intermediate standards were used to construct the calibration curve, which was used for the analysis of the quality control solutions as samples. The quality control solutions were purchased from New York's State Public Health Laboratory, Wadsworth Center, Albany, NY, and contained known concentrations of mercury in human pooled urine. The % accuracy and % error results obtained are presented in the Tables 3.3 to 3.5. The % accuracy and % error results were calculated using equations 3.1 and 3.2. The definition of stability and the acceptable criteria were discussed in section 3.3.5. The desirable %accuracy is between 90 – 110%, and the acceptable % accuracy are ranging from 80 – 120%. The allowable % error should be within 25%.

The calculated %accuracy and %error for set A mercury intermediate standard solutions, are presented in Table 3.3. On the day of production at all temperatures, the %accuracy for most of the QC solutions level II (M) and level III (H) were within 10%, with the exception of the QC solution level III(H) at freezer temperature. The exception being off for the QC solution level III (H) at the freezer could be due to mercury which is vaporized or adheres to the walls of the container during sampling or the freeze-and-thaw cycle. The first week after the day of preparation, the % accuracy for QC level II (M) and level III (H) of set A standards stored at

room temperature, reached 277% and 168% respectively. After two weeks, the %error of QC level II (M) and level II (H) reached 290% and 537%, respectively, which indicated that the standards were not stable under these storage and solution conditions and needed to be prepared fresh prior to analysis. Set A mercury intermediate standards that are stored at refrigerator temperature were found to be stable within one week but unstable the following week. Similar results showed, poor % accuracy results were obtained when set C standard solutions were stored in the freezer. Lower % accuracy could be due to an increase in the concentrations in mercury intermediate standards or mercury memory effect during sampling; but, a high % accuracy indicated that the concentration in mercury intermediate standards decreased.

Table 3.3: Results from set A mercury intermediate standards obtained for the analysis of Quality Control level II (M) and level III (H) solutions.

Hg Intermediate Standard Solutions Set A: % Accuracy		**Day 0**	**Day 7**	**Day 14**	**After 14 days (Measured on Day 28)**
RT	**QC Level II (M)**	101%	277%	390%	
	QC Level III (H)	102%	168%	637%	
Refrigerator	**QC Level II (M)**	95.2%	79.4%	109%	116%
	QC Level III (H)	103%	115%	128%	826%
Freezer	**QC Level II (M)**	107%	147%	101%	117%
	QC Level III (H)	126%	136%	116 %	147%
Hg Intermediate Standard Solutions Set A: % Error		**Day 0**	**Day 7**	**Day 14**	**After 14 days (Measured on Day 28)**
RT	**QC Level II (M)**	1.56%	178%	290%	
	QC Level III (H)	1.92%	68.2%	537%	
Refrigerator	**QC Level II (M)**	4.76%	20.6%	9.48%	16.1%
	QC Level III (H)	3.26%	15.1%	28.0%	726%
Freezer	**QC Level II (M)**	6.92%	47.1%	1.57%	16.9%
	QC Level III (H)	25.9%	36.1%	16.3%	47.0%

Note: RT is room temperature.

Higher concentration of nitric acid may help to reduce the mercury volatilization during storage. For the set B mercury intermediate standards that were stored at room temperature, analysis of the QC solution resulted in extremely poor % accuracy and the %error for QC level II (M) which increased from 36.5% to 2690% within two weeks (Table 3.4). The results for set B mercury intermediate standards stored at room temperature were not stable for one week and therefore would need to be prepared fresh prior to any analysis. Stored at refrigerator temperature, set B mercury intermediate standards provided better % accuracy results than when stored at room temperature, however the results obtained for the QC solutions exceeded the acceptable %accuracy criteria after three weeks. The results obtained using set B standards, which were stored in the freezer, the analysis of the QC solutions resulted in acceptable % accuracy for two weeks, but was not acceptable at three weeks. The %error for the QC solutions

were within 20% but exceeded the acceptable criteria on day 21. The results obtained of this study indicate that the set B mercury intermediate standards must be made fresh weekly when stored at room temperature, and prepared freshly biweekly if stored in the freezer.

Table 3.4: Results of the analysis of Quality Control level II (M) and level III (H) solutions using the set B mercury intermediate standards.

Hg Intermediate Standard Solutions Set B: % Accuracy		**Day 0**	**Day 7**	**Day 14**	**Day 21**	**Day 28**
RT	**QC Level II (M)**	63.5%	429%	2790%		
	QC Level III (H)	98.9%	802 %	2840%		
Refrigerator	**QC Level II (M)**	74.5%	103%	72.4%	124%	663%
	QC Level III (H)	99.8%	112%	124%	167%	290%
Freezer	**QC Level II (M)**	88.4%	102%	67.8%	197%	
	QC Level III (H)	105 %	109%	108%	194%	
Hg Intermediate Standard Solutions Set B: % Error		**Day 0**	**Day 7**	**Day 14**	**Day 21**	**Day 28**
RT	**QC Level II (M)**	36.5%	329%	2690%		
	QC Level III (H)	1.06%	702%	2740%		
Refrigerator	**QC Level II (M)**	25.5%	3.06%	27.6%	24.0%	564%
	QC Level III (H)	0.129%	12.7%	24.1%	66.7%	190%
Freezer	**QC Level II (M)**	11.6%	1.91%	32.3%	97.2%	
	QC Level III (H)	5.52%	8.64%	8.42%	94.2%	

Note: RT is room temperature.

The % accuracy and % error for the QC solutions when using the Set C mercury intermediate standards are presented in Table 3.5. Set C mercury intermediate standards stored at room and refrigerator temperature, from the day of preparation to day 28, provided the best %accuracy results for the QC solutions. The %errors were also within 10% for the QC solutions. However, when using the set C mercury intermediate standards stored in the freezer, inconsistent % accuracy resulted which indicated the standards were unstable. Based on the results for the analysis of QC solutions using the set C standards, the recommendation would be to use set C

standards that are stored at room temperature, and in the refrigerator because these standards were stable for four weeks.

Table 3.5: Results of the analysis of Quality Control level II (M) and level III (H) solutions using the set C mercury intermediate standards. All analyses were carried out in triplicate.

Hg Intermediate Standard Solutions Set C: %Accuracy		**Day 0**	**Day 7**	**Day 14**	**Day 21**	**Day 28**
RT	**QC Level II (M)**	82.3%	106%	101%	103%	100%
	QC Level III (H)	104%	97.8%	109%	107%	108%
Refrigerator	**QC Level II (M)**		95.3%	102%	101%	86.4%
	QC Level III (H)		91.4%	103%	104%	107%
Freezer	**QC Level II (M)**		147%	110%	103%	60.5%
	QC Level III (H)		113%	129%	148%	168%
Hg Intermediate Standard Solutions Set C: % Error		**Day 0**	**Day 7**	**Day 14**	**Day 21**	**Day 28**
RT	**QC Level II (M)**	17.7%	5.76%	1.36%	3.34%	0.39%
	QC Level III (H)	3.88%	2.21%	9.43%	7.10%	8.62%
Refrigerator	**QC Level II (M)**		4.71%	2.73%	1.14%	13.6%
	QC Level III (H)		8.59%	3.17%	4.07%	7.32%
Freezer	**QC Level II (M)**		47.6%	9.91%	3.45%	39.5%
	QC Level III (H)		13.1%	29.3%	48.2%	67.8%

Note: RT abbreviates room temperature.

3.4.5 Long Term Monitoring Study for the Set C Mercury Intermediate Standards

Since the set C mercury intermediate standards was the only set to achieve the one-month storage lifetime goal, the storage lifetime study was extended to 3 months to further investigate the long term stability of these standards. The intensity responses of the mercury intermediate standard solutions set C at each temperature vs. time since preparation of the standard are presented in Figures 3.19 to 3.21. The loss of mercury for the set C mercury intermediate standards at each temperature is presented in Figures 3.22 to 3.24. At both room and refrigerator temperature, the correlation coefficients were greater than 0.99 for 13 weeks; however, for the intermediate standards stored at freezer temperature, the fit deviates from linearity after 3 weeks.

The percentage of mercury loss for set C standards, stored at both room temperature and in the refrigerator, showed similar results in Figures 3.22 and 3.23. Most of the standards stored at room temperature were found to be stable for 13 weeks with mercury losses within 20%. The exception was standard number 1 (Figure 3.22). Standard numbere1, though showing a 40% loss of mercury throughout the 13 weeks monitoring period, had an actual loss in concentration of mercury that was less than 1ppb for this 2ppb mercury intermediate standard. For intermediate standard solutions stored at refrigerator temperature, standards numbered 2 to 6, were found to have less than a 10% loss of mercury concentrations up to 13 weeks, except for standard number 1 (Figure 3.23). The ability to quantitate at the lower concentration range of the standards is more problematic as the limit of quantitation is approached. For the standards stored at freezer temperature, the concentrations mercury standards decreased dramatically and the mercury loss percentage exceeded 20% within two weeks (Figure 3.24). After two weeks, set C mercury intermediate standards that were stored in the freezer even reached 100% loss in mercury. Based on the results obtained, the set C mercury intermediate standards stored at room temperature and in the refrigerator are stable for longer than those stored in the freezer.

A set of quality control solutions, Level II (M) and Level III (H), were used to evaluate the stabilities of the mercury intermediate standards. To evaluate the results of this study, the % accuracy of the quality control results for the analysis for the QC solutions were evaluated. For the QC level II (M) solution, three different temperatures of set C mercury intermediate standards were compared over 13 weeks (Figure 3.25). The results for the set C mercury intermediate standards stored in the room temperature are illustrated in blue, orange represents the results for the standards stored in the refrigerator, and the grey dots are for the standards stored in the freezer. The %accuracy for the QC level II (M) solution when using intermediate

standards stored at freezer temperature increased week after week which indicated the concentration of mercury intermediate standards decreased over this period of time. For the other storage temperatures, room and refrigerator temperatures, the %accuracy were obtained within the range of 80% to 120% over 13 weeks. The %accuracy for the analysis of the QC level III (H) solutions using the intermediate standards stored at three different temperature are presented in Figure 3.26. The mercury intermediate standard stored at room temperature and in the refrigerator for the analysis of QC level III (H) solution, both produced %accuracies ranging from 90% to 110%. However, an increased %accuracy results were observed when using the standards stored in the freezer.

In conclusion, by applying a new set of freshly made standards to monitor the changes in mercury concentration of each mercury intermediate standards at different temperatures, losses in mercury were within 20% over 13 weeks when the set C mercury intermediate standards were stored at room temperature and in the refrigerator. QC solutions were analyzed and used to evaluate the stability of the mercury intermediate standards. The results proved that set C mercury intermediate standards were stable for 13 weeks when stored at room temperature and in the refrigerator. Based on the results obtained, the set C mercury intermediate standards, the storage lifetime was extended for up to 3 months if stored at room or refrigerator temperature.

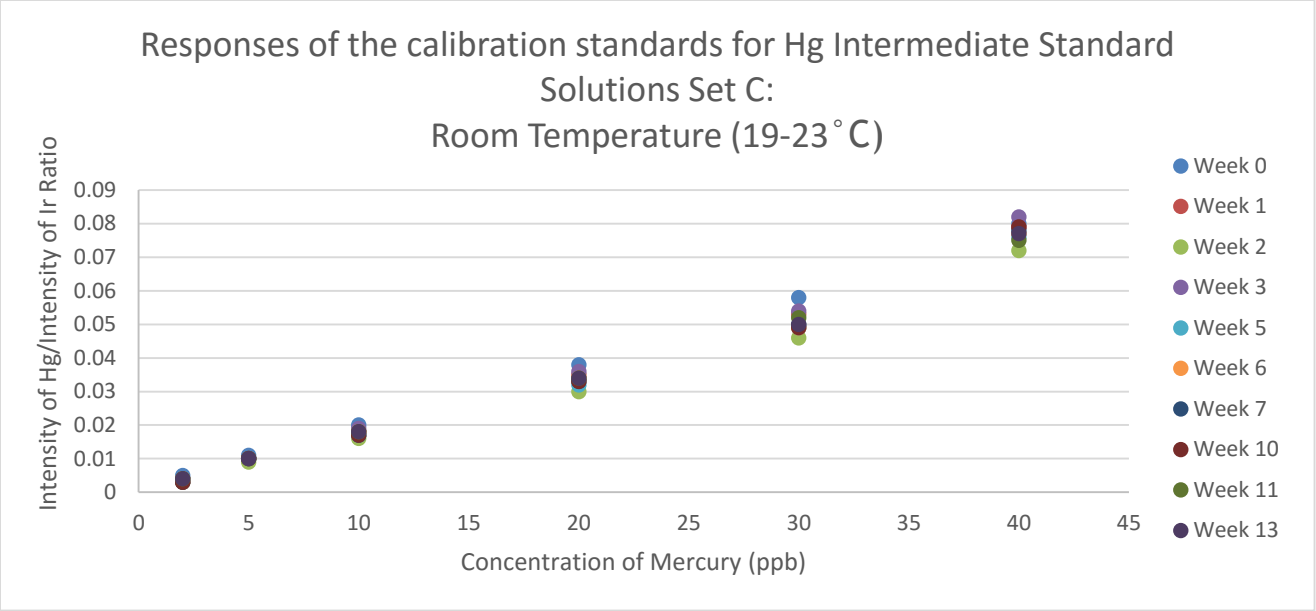

Figure 3.19: Responses of the calibration standards for mercury intermediate standard solution set C. This set C of standards was stored at room temperature and monitored for 13 weeks. Iridium was used as an internal standard.

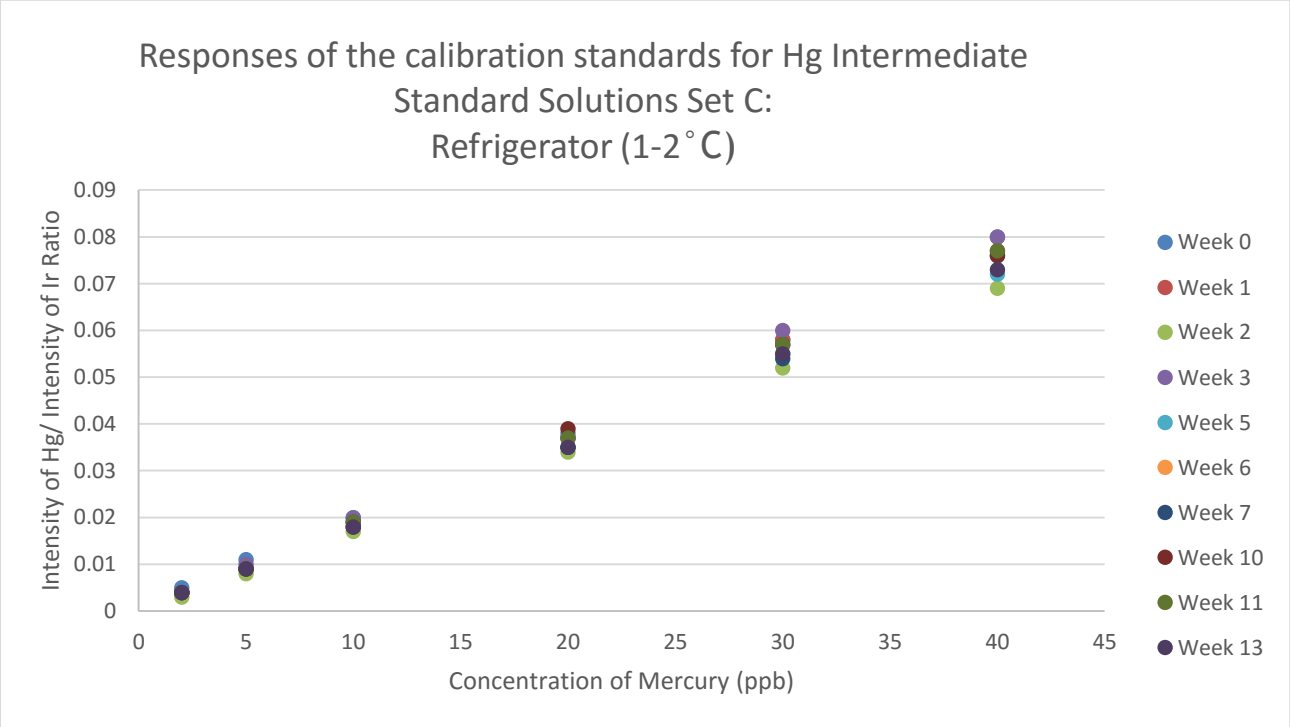

Figure 3.20: Responses of the calibration standards for mercury intermediate standard solution set C stored in the refrigerator. The responses of set C mercury intermediate standards were monitored for 13 weeks. Iridium was used as an internal standard.

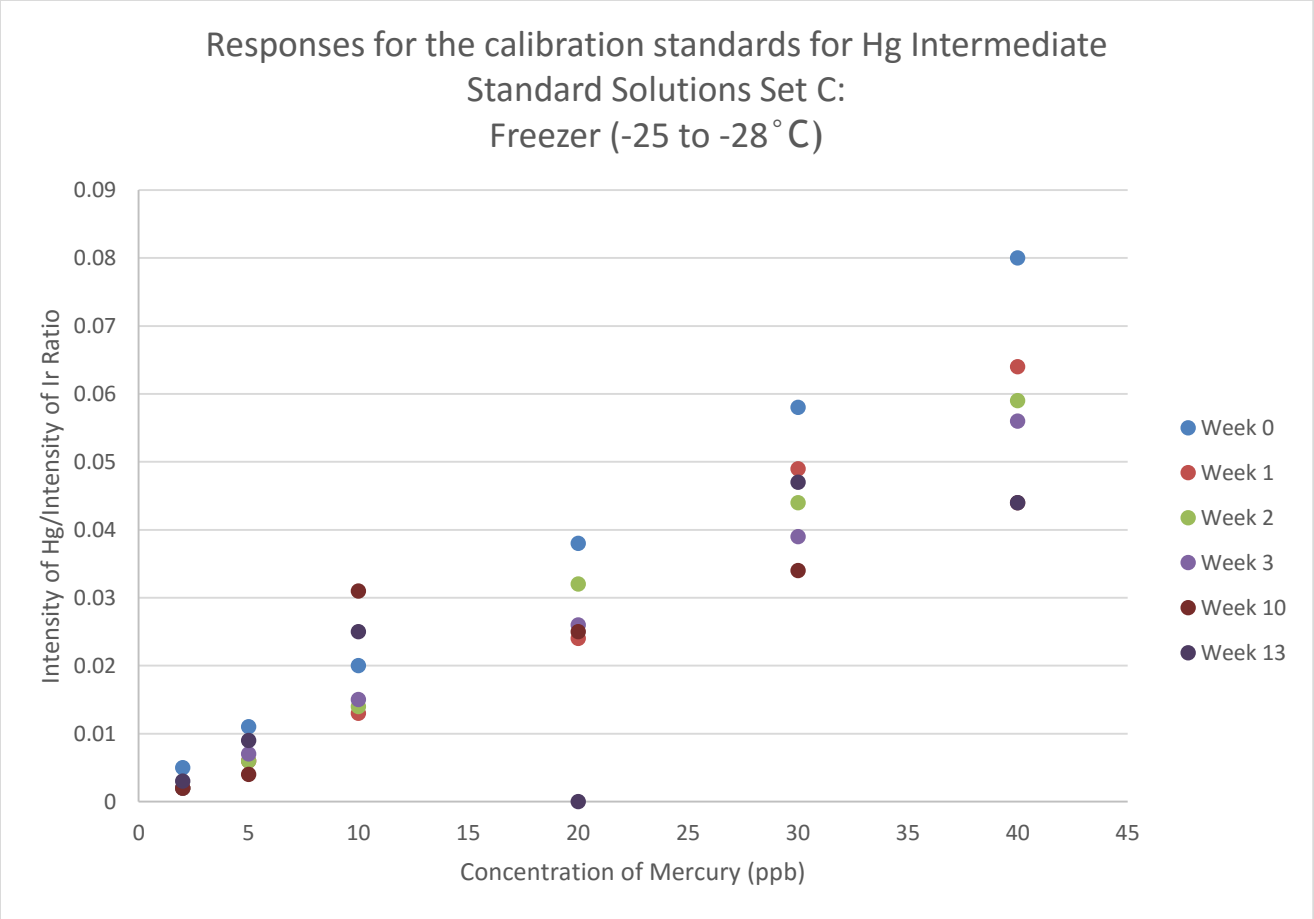

Figure 3.21: This chart showed the responses of mercury intermediate standard solution set C. This set C mercury intermediate standard solutions stored in the freezer and monitored for 13 weeks. Iridium was used as an internal standard.

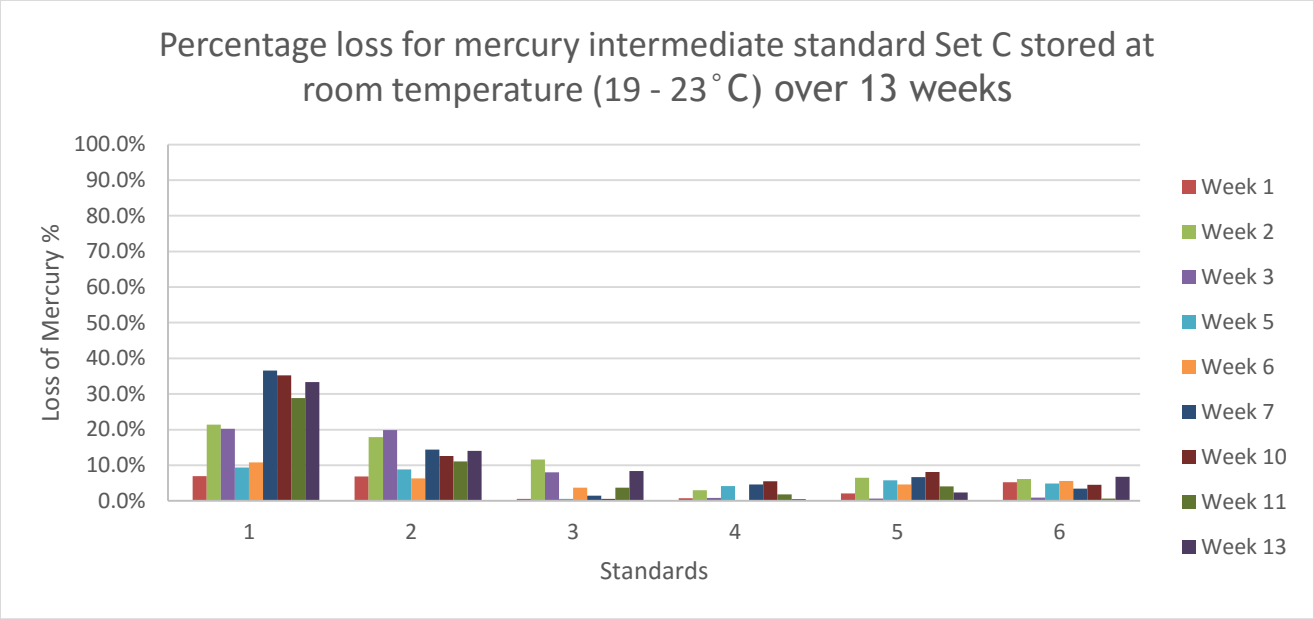

Figure 3.22: Percentage loss for mercury intermediate standard solution set C stored at room temperature for 13 weeks. For most of the intermediate standards, the percentage loss of mercury was less than 20% for 13 weeks, except for standard number 1 (2ppb mercury standard). Standards numbered 1 through 6 corresponded to concentrations ranging from 2µg/L to 40µg/L of mercury as given in **Table 2.4**.

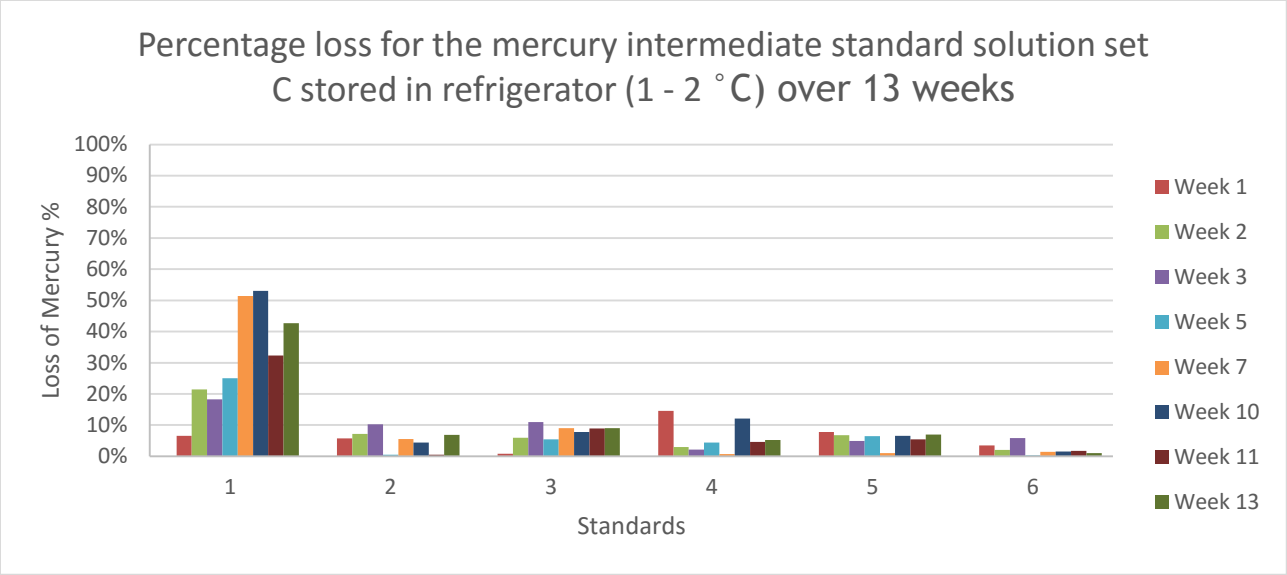

Figure 3.23: This chart shows the loss of mercury for set C mercury intermediate standards that were stored in the refrigerator over 13 weeks. Standards numbered 1 through 6 correspond to concentrations ranging from 2µg/L to 40µg/L of mercury as given in **Table 2.4**.

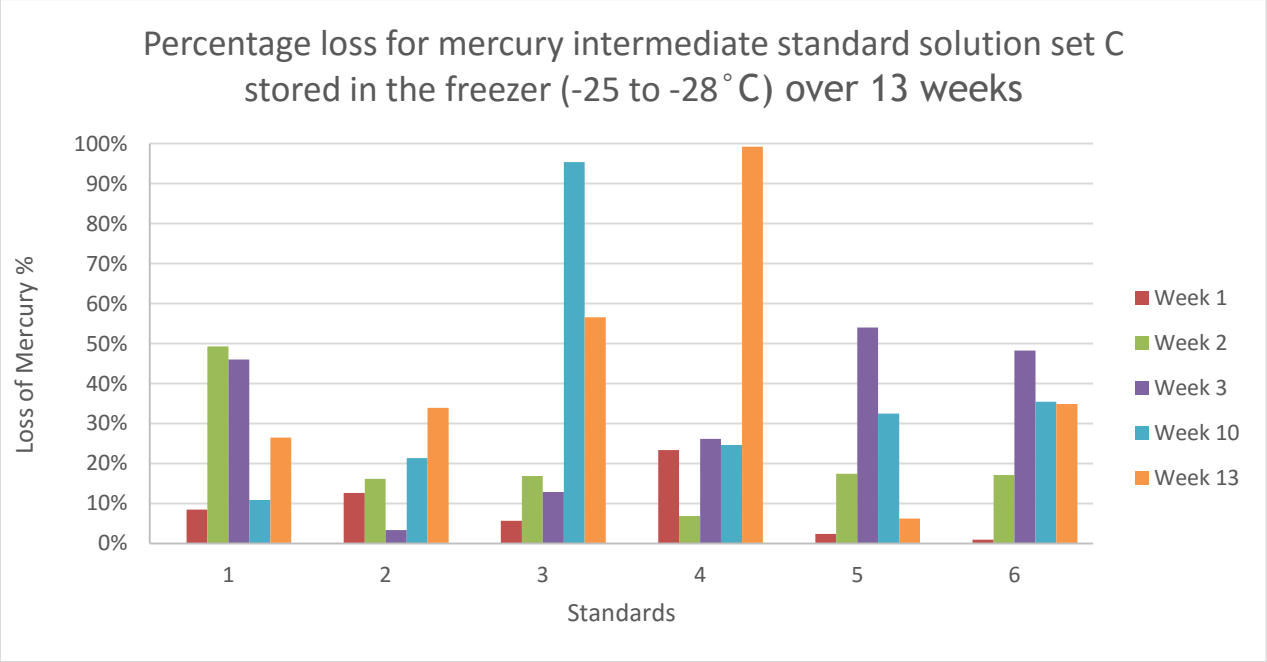

Figure 3.24: This chart shows the loss of mercury for the mercury intermediate standards set C stored in the freezer over 13 weeks. Set C mercury intermediate standards were found to be not sufficiently stable to be used as standards after two weeks. Standards numbered 1 through 6 correspond to concentrations ranging from 2µg/L to 40µg/L of mercury as given in **Table 2.4**.

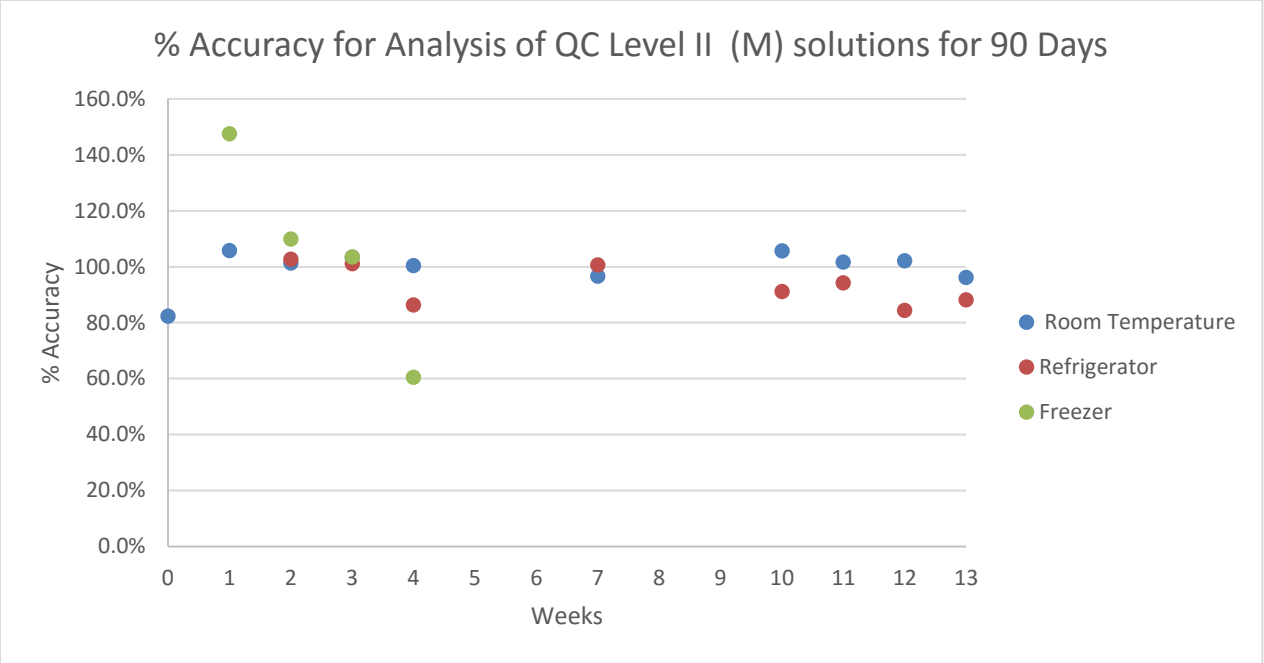

Figure 3.25: Results for the analysis of the QC Level II (M) solution using intermediate standard solution set C stored at room, refrigerator, and freezer temperatures for 90 days (13 weeks). Set C standards that were stored in the freezer showed a decreased % accuracy week after week which indicated the standards were found to be unstable. The three different temperatures are: at room temperature (19 to 23 °C), in the refrigerator (1 to 2 °C), and in the freezer (-25 to -28 °C)

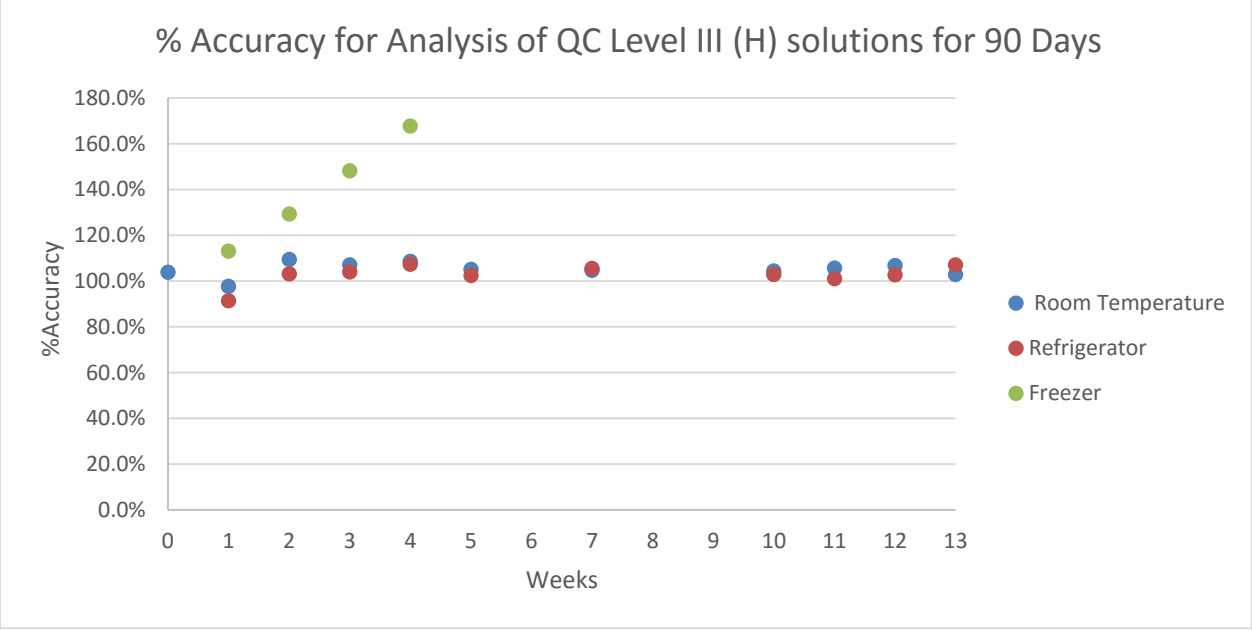

Figure 3.26: Results for the analysis of QC Level III (H) solution using intermediate standard solution set C stored at room, refrigerator, and freezer temperatures for up to 90 days (13 weeks). Set C standards that were stored in the freezer temperatures showed an increased % accuracy week after week which indicated the standards were not stable. The three storage temperatures are: at room temperature (19 to 23 °C), in the refrigerator (1 to 2 °C), and in the freezer (-25 to -28 °C).

3.5 Conclusion:

A combination of 1%(v/v) nitric acid, 1%(v/v) hydrochloric acid, and 1%(v/v) sulfamic acid preservative solution (Set C) is recommended for the preservation of intermediate standard solutions (calibration standard solutions) for quantitation of mercury by ICP-MS. The mercury intermediate standard solutions set C which were stored in polypropylene containers at both room (19 to 23 °C), and refrigerator temperatures (1 to 2 °C), were the most stable standards. These standards were all well within 20% of the certified target value with good recovery and accuracy. Less than 20% of mercury loss was detected in this acid combination for up to 90 days. These solution and storage conditions have met our goals for stability of the mercury intermediate standards for quantitate of mercury in human urine by ICP-MS.

Chapter IV: Quality Assurance

4.1 Introduction:

This chapter describes using the improved mercury intermediate standards described in previous chapter to analyze sets of quality controls materials to verify the method. The goal of this study is to evaluate the validated method (Chapter 2) and the improved mercury intermediate standards (Chapter 3) by analyzing sets of quality control solutions having known mercury concentrations.

4.1.2 Verification Process

For this study, an activity was conducted where the solutions containing unknown concentrations of mercury were analyzed for mercury content utilizing the validated method and the most stable standards established as described in Chapter 2 and 3. Two sets of unknown materials were acquired to analyze in this study: Proficiency testing samples, and urine samples containing an unknown quantity of mercury (spiked urine samples). The urine samples provided by internal staff members containing unknown mercury levels were pooled. Proficiency testing samples provided by the New York state public health laboratory were also examined. In the verification process, these solutions were treated as unknowns to assess the ability of the methodology to measure the mercury level in human urine samples. Results were compared to the analysis report issued by the NY state lab to ensure the results are within the accepted limit of 80 – 120%. Therefore, the certified values of these solutions were revealed after the analysis process is completed.

4.2 Materials

Mercury intermediate standard solution Set C stored at both room and refrigerator temperatures were chosen to evaluate the unknown mercury urine samples in this study. Two different sources of mercury urine samples were employed and examined in this study: (1) samples provided by the staff members at NHPHL, and (2) proficiency testing (PT) sample from the Wadsworth Center of New York state public health laboratory. The certified values of mercury in the PT samples are listed in Table 4.1.The target concentrations of mercury for the spiked pooled urine provided from staff members at NHPHL are shown in Table 4.2.

Table 4.1: Certified mercury concentration values of the proficiency testing samples provided by the Wadsworth Center of New York state public health laboratory [26].

Unknown Hg Urine ID number:	**Actual (µg/L) (n=4)**	**SD**
UM1503-21-30	17.2	0.8
UM1503-22-30	8.86	0.17
UM1503-23-30	24.9	0.7
UM1503-24-30	33.4	1.5
UM1503-25-30	12.9	0.3
UM1503-26-30	11.1	0.5
UM1503-27-30	22.1	0.3
UM1503-28-30	29.8	1
UM1503-29-30	42.6	0.6
UM1503-30-30	6.98	0.12

Note: SD abbreviates standard deviation.

Table 4.2: Target mercury concentration values of urine samples prepared from pooled urine samples provide by staff members at NHPHL.

Unknown #	Target Mercury Concentration (µg/L)
9	3.00
10	3.00
24	3.00
34	3.00
8	23.0
11	23.0
25	23.0
33	23.0
7	98.0
1	98.0
26	98.0
35	98.0

4.3 Results and Discussion

During the verification process, two sets of unknown samples were analyzed utilizing the improved set C mercury intermediate standards. These measurements of unknown samples prepared from staff members at NHPHL were performed on several days over a 90 day period (**Table 4.3**). Three different mercury concentrations for the spiked pooled urine samples were examined, 3.00 µg/L, 23.0 µg/L, and 98.0 µg/L of mercury. The percent accuracies were 88.7% to 114%, 90.4% to 108%, and 105% to 112%, for the 3.00 µg/L, 23.00 µg/L, and 98.00 µg/L respectively. The desired percent accuracy ranged from 90 to 110%, and acceptable percent accuracy ranged from 80 to 120% [33, 41]. The spiked pooled urine sample contained 23.0 µg/L were within the desired accuracy range, and the other two spiked pooled urine samples, 3.00 µg/L and 98.0 µg/L of mercury were in the acceptable accuracy range. This is due to the 3.00 µg/L pooled urine sample was closer to the lowest mercury standard, 2ppb. The spiked pooled

urine sample, 98.0 µg/L, was above the calibration range (2 µg/L to 40 µg/L), sample dilutions was made.

Table 4.3: Results and % accuracy of the unknown mercury samples prepared from staff members at NHPHL.

# Unknown	Result (µg/L) (n=3)	Target (µg/L)	Accuracy
9	3.19	3.00	106%
10	3.34	3.00	111%
24	3.43	3.00	114%
34	2.66	3.00	88.7%
8	24.7	23.0	108%
11	24.8	23.0	108%
25	24.2	23.0	105%
33	20.8	23.0	90.4%
7	104	98.0	106%
12	110	98.0	112%
26	103	98.0	105%
35	101	98.0	103%

For analysis of PT samples from the New York state public health laboratory set C mercury intermediate standards were used. The measurements was done by using standards that were stored for 90 days and the results are displayed in **Table 4.4**. The results for the PT sample measurement should be within the standard deviations of the certified target mean. The results obtained for the standards that were freshly prepared and stored at room temperature, were outside of the certified target mean range. The % accuracies were 103 to 109% for the standards that were freshly prepared. The % accuracies were 106 to 113% for the standards that were stored at room temperature. The results obtained using the standards stored at refrigerator temperature were within the range of the certified target mean. The % accuracies were 95 to 104%.

Table 4.4: Results for the analysis of PT samples for mercury using intermediate standards solution set C stored at room temperature (19-23°C), in the refrigerator (1-2°C), and freshly prepared. These measurements were taken within 90 days of initial preparation for stored standards. Three replicates were done on each measurement.

	Actual (n=4) (µg/L)	**SD**	**Fresh-made (µg/L)**	**Standard Stored at Room Temperature (µg/L)**	**Standard Stored in Refrigerator (µg/L)**
UM1503-21-30	17.2	0.8	18.4 ± 0.3	19.5 ± 0.1	17.3 ± 0.2
UM1503-22-30	8.86	0.17	9.58 ± 0.21	9.54 ± 0.13	8.47 ± 0.03
UM1503-23-30	24.9	0.7	27.2 ± 0.2	27.4 ± 0.2	25.9 ± 0.7
UM1503-24-30	33.4	1.5	35.3 ± 0.1	35.5 ± 0.4	33.7 ± 0.2
UM1503-25-30	12.9	0.3	13.4 ± 0.2	13.8 ± 0.2	12.8 ± 0.1
UM1503-26-30	11.1	0.5	11.6 ± 0.4		10.8 ± 0.2
UM1503-27-30	22.1	0.3	23.4 ± 0.2		22.1 ± 0.1
UM1503-28-30	29.8	1	31.3 ± 0.1		30.1 ± 0.8
UM1503-29-30	42.6	0.6	44.2 ± 0.5		43.9 ± 0.4
UM1503-30-30	6.98	0.12	7.46 ± 0.03		6.8 ± 0.25

A third set of quality control samples were also analyzed to assess the mercury intermediate standards stability. The third set of QC samples included Standard Reference Material (SRM) purchased from the National Institute of Standards and Technology (NIST). The certified values are given in **Table 2.7**.

Using the Set C of mercury intermediate standards stored at room temperature resulted in a value of 7.19 ± 0.27 µg/L for mercury in the SRM 3668 Level II standard. The % accuracy was 113%. When using the Set C mercury intermediate standards stored in the refrigerator, the result obtained was 6.41 ± 0.12 µg/L for mercury in the SRM 3668 Level II standard. The % accuracy was 100%. Comparison between the results obtained and the SRM 3668 Level II certified concentration, showed the results were within the reference material range. However, comparison between the % accuracies, the % accuracies showed the Set C mercury intermediate standards stored in the refrigerator produced a better result.

4.3.1 Reproducibility of Set C Mercury Intermediate Standards Comparison

A repeated experiment of Set C mercury intermediate standards was also conducted under the same conditions as described in Chapter 3 to see if the results obtained are reproducible. The results obtained for analyzing the PT sample for these Set C mercury intermediate standards are presented in **Table 4.5**. PT sample (UM1503-22-30) was chosen to evaluate this Set C mercury intermediate standards comparison. The UM1503-22-30 PT sample was reported to have a mercury concentration of 8.86 ± 0.17 µg/L.

Analysis of these PT sample using the initial set C of mercury intermediate standards stored at room and refrigerator temperatures resulted in a mercury concentration of 9.54 µg/L and 8.47 µg/L of mercury, respectively. The PT sample results for the repeated experiment of set C mercury intermediate standards stored at room and refrigerator temperatures were 9.77 µg/L and 8.93 µg/L of mercury, respectively. For the freshly prepared standards, the PT sample value was 9.51 µg/L of mercury.

The % accuracy for the initial and repeated set C of mercury intermediate standards stored at room temperature for 90 days were in the acceptable range of 107% and 110%, respectively. For the results obtained using set C mercury intermediate standards stored at refrigerator temperature, the initial result produced 95.6% accuracy, and the repeated experiment of standards obtained 101% accuracy. Freshly prepared standards produced 107.3% accuracy.

Based on the results obtained, all set C mercury intermediate standards stored at both room temperature and refrigerator are stable. For this study, although standards stored at room temperature and freshly prepared standards produced acceptable percentage accuracy, the results for the analysis of PT samples tended towards higher concentrations. This could be possibly due to mercury adhering on to the walls of the containers during the experiment. Both sets of Set C

mercury intermediate standards stored in the refrigerator produced results within the actual value range. One potential issue is that refrigeration of standards can inhibit bacterial growth compared to room temperature.

Table 4.5: Evaluated concentration of mercury and accuracy percentages of PT sample for mercury intermediate standards solution set C stored for 90 days.

			Room Temperature (19-23°C)		**Refrigerator (1-2°C)**		**Freshly made standard**
	Actual (µg/L)	**SD**	Initial **(µg/L)**	Repeated **(µg/L)**	Initial **(µg/L)**	Repeated **(µg/L)**	**(µg/L)**
UM1503-22-30	8.86	0.17	9.54±0.13	9.77±0.16	8.47±0.03	8.93±0.19	9.51±0.09
%Accuracy			107%	110%	95.6%	101%	107%

4.4 Conclusion:

Set C mercury intermediate standards stored at room temperature and refrigerator temperature provided acceptable percent accuracy results for the PT samples and unknown urine samples. However, this study showed that mercury intermediate standard solutions set C which was stored in the refrigerator (1-2°C) provided the best performance for 90 days.

Chapter V: Conclusions and Future Work

The ICP-MS is a powerful analytical instrument for detecting metals at the parts-per-billion (ppb) and parts-per-trillion (ppt) levels, and is capable of performing simultaneous multi-element analysis. The adapted ICP-MS method from the New York state public health laboratory for determining total mercury in human urine was validated in the NHPHL and found to provide rapid, routine monitoring of mercury in urine. During the method validation process in this laboratory, it was observed that concentration of mercury intermediate standards decreased over time. This decrease in concentrations could be possibly due to mercury adsorbing on the container walls, precipitation, or volatilization [59, 60, 75]. To achieve sufficiently accurate quantitation, a study was undertaken to prolong the storage lifetime of mercury intermediate standards with different acid preservative solutions. The results of this study showed that mercury intermediate standard solutions with 1% (v/v) hydrochloric acid, 1% (v/v) nitric acid, and 1% (v/v) sulfamic acid preservative solution which were stored in the refrigerator (1-2°C) provided the best performance for up to 90 days.

Though the mercury intermediate standards was considered as stable for 90 days, further extending the storage lifetime of the mercury intermediate standards can be investigated. In the future, with the multi-element capability of the ICP-MS, method can be explored and possibly identify other elements in the sample [72]. The method can also be extended to determination and speciation of mercury in human urine by coupling to other techniques such as liquid chromatography upon availability in the laboratory. However, the ability of the sample matrix to cause significant polyatomic interference in ICP-MS must always be taken into account. In addition, a more thorough study on standardizing the methods and reagents for analyzing

biological samples can be performed, because there is no universally standardized method on quantitating total mercury in human urine by ICP-MS.

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