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Method Development and Validation of Total Mercury Content in Effluent Wastewater by Cold Vapor Atomic Fluorescence Spectroscopy

Sandra N. Ogedengbe
Buffalo State University, snj1986@gmail.com

Advisor
Dr. Heo, Dr. Kim
First Reader
Jinseok Heo, Ph.D.
Second Reader
Joonyeong Kim, Ph.D.
Third Reader
Sourav Biswas, Ph.D.
Department Chair
M. Scott Goodman

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An Abstract of a Project
in
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ABSTRACT

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The presence and concentration of ambient mercury contamination in our natural environment and workplaces will continue to be closely monitored and regulated as it imposes grave implications and serious risks to human health. Ongoing quantitative analysis has already become a routine part of industrial chemical plants' in-process and end-stage testing. Mercury contamination in waste generated by these chemical processes can present substantial operational hurdles, as compliance must be demonstrated by treatment, accurate measurement, and timely reporting of waste materials against stringently low limits before release into natural bodies of water or the municipal water supply.

An accurate and reliable low-level method of analysis for the chemical detection and quantitation of total mercury content in effluent wastewater from an industrial chemical plant has been validated and deemed suitable for its intended use. The method validation parameters included an assessment of selectivity, linearity (range), accuracy, precision, and robustness. Acceptable system suitability and sample results for all experiments were demonstrated according to current acceptable practices and limits laid forth in a pre-determined validation plan.

Buffalo State University
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Approved by

Jinseok Heo, Ph.D.
Associate Professor of Chemistry
Project Advisor, First Reader

Joonyeong Kim, Ph.D.
Professor of Chemistry
Second Reader

Sourav Biswas, Ph.D.
Assistant Professor of Chemistry
Third Reader

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I. Introduction

1.1 Project Objective

To develop, optimize, and validate an accurate and precise method for the speedy determination of mercury in treated wastewater at the parts per trillion (ppt) level for application in an industrial chemistry quality control laboratory. A series of parameters will be adjusted and evaluated to determine suitability in instrument method settings, the most efficient sample preparation procedures, and the rigor (robustness) of the methodology. The overall test method will be optimized to develop a cost-effective, user-friendly, rapid means of ppt level determination without jeopardizing the analytical integrity of the operation.

1.2 History of Mercury Proliferation

The historical uses of mercury in the United States are varied and its toxicity, environmental impact, and harmful health effects on humans, animals, and marine life, have been well documented. This has resulted in justified federally regulated limits around users and producers in what has been termed "cradle to grave" regulations governing storage, treatment, and disposal of hazardous waste, such as mercury, in the 1976 congressional act of Research Conservation and Recovery (RCRA) [1]. Since this time the Environmental Protection Agency (EPA), along with other governing regulatory bodies including the U.S. Food and Drug Administration (FDA) and Occupational Safety and Health Administration (OSHA), in addition to local and state agencies, namely the New York State Department of Environmental Conservation (NYSDEC), have passed a web of laws and recommendations around exposure limits, permissible emission limits released into the environment, and allowable limits in drinking water and food. These agencies have sought to monitor and restrict, and in doing so reduce, remediate, and mitigate mercury waste and its effects on surrounding populations [2]. They have collectively advanced the mercury agenda in a number of policies, most notably in: the Persistent Bioaccumulative and Toxic (PBT) Pollutants Program, the White House Clean Water Action Plan, the Mercury Report to Congress, and the US-Canada Bilateral Toxics Agreement, just to name a few of many

[3]. As a result of these efforts and policies, the NYSDEC has implemented a practice of placing strict site-specific limits on individual manufacturers and plants. In fact, proposed ecologically protective mercury standards are typically well below drinking water standards, a trend that has resulted from this overall agenda and has sustained into present day [3]. A local and well-established manufacturing facility in Western New York, situated along the Niagara River has been no exception to this governmental oversight. This nearly one hundred-year-old plant, one of the single largest producers of active oxygens in the Western hemisphere, has been mandated by NYSDEC to ensure all water released back into the river is at or below their site-specific permitted limit of 50 ppt. This has resulted in the need for an in-house chemical analysis method of mercury measurement in treated wastewater produced by the plant, this method would need to be highly sensitive, selective, and reliable. This analytical method is to be performed in-house by (non-scientist) plant operators, five days a week, at least twice a day, and as such it must also be cost-effective and user-friendly.

Mercury is a highly toxic metal that is naturally found in the environment. Some of its natural sources include volcanoes, rocks, and sediments [4]. In the US the use of mercury in research and industry has a long history and continued reach into society today, with inordinately high levels of the more biologically potent, methylmercury, in natural bodies of water well beyond the FDA's highest allowable limit in fish per serving of 0.15 μg/g [5]. The Northeast Tri-State area of the US, which includes New Jersey, New York, and Delaware reports "legacy" pollution levels of mercury in some of its fish populations well over 0.30 μg/g, twice the allowable limit [6]. These high levels of residual mercury pollutants are thought to have been caused by past and present industries such as chloro-alkali plants, petroleum refineries, steel mills, coal-fired power plants, wastewater treatment, and other chemical manufacturers [6]. Between 1940 and 1970 large quantities of mercury, thought to be into the millions of kilograms, were used in the United States federal government nuclear defense programs. Specifically, the Aircraft Nuclear Propulsion Program, which used mercury in its molten salt reactors for lithium isotope separation [7]. The lithium isotope Li-6 (^{6}Li), which naturally occurs at 7.4%, proved critical to the

federal government's defense programs and was in short supply at the time. Li-6 readily dissolves in mercury at optimal conditions and mercury was the main solvent used in various separation techniques [7]. Although this defense project, and others like it, eventually came to an end and/or were defunded, the use of mercury continues—the total number of mercury contamination and waste from these defense projects is thought to be into the hundreds of thousands of kilograms, and is mostly unaccounted for, and thought to be lost to the environment under and around the facilities [7].

Although mercury mitigation efforts were taken, mercury releases from equipment failure, spills, or leaks still occurred, and recovery efforts were not always 100% effective [7]. Even in these cases, some businesses, specifically the chloro-alkali industry, report losses of more than 50% [7] [6]. Also, dated methods of mercury detection and sampling that were in place during the first half of the 20th century are now thought to have underestimated the actual quantities, specifically in water; for example, Brooks notes, "The proportional autosampler in use in the early 1950s skimmed the surface of the creek and would not have sampled Hg associated with larger particles carried by the stream." [7].

Mercury exposure has deleterious effects on humans even at minute doses. The toxic effects of exposure depend on the form which dictates the bioactivity; methylmercury is the most lethal form [4] [8]. Mercury has the ability to bioaccumulate in the tissue and organs of living organisms and is thus passed on in animal-based food diets, which over time results in irreversible and serious health effects including kidney renal failure, liver failure, cardiovascular diseases, neurological and psychological impairment, and eventual death [9]. Mercury has also been linked to carcinogenesis and is known to also affect the gastrointestinal, respiratory, and reproductive organ systems [9]. Mercury is also known as a gonadotoxic, embryotoxic, and teratogenic agent [9]. Its ability to cross the placental blood barrier results in developmental effects on the unborn, up to loss of pregnancy.

The health effects of mercury exposure were well-known by the 1940s. However, in recent years because of the uptick in published research and findings on the harmful health effects of mercury exposure, federal and state regulators have enforced tightened limits and have more closely monitored

and regulated the release of mercury. Generally, occupational safety efforts are undertaken to regularly screen workers for exceedances of mercury content in their blood or urine. The main occupational exposure route of mercury to a worker is in the vaporous form, and mercury levels in workplace indoor air are often higher than ambient air [2]. Scrubbers, fans, and personal protective respiratory equipment are used to reduce exposure to workers. However, with many of these efforts that are effective at preventing personnel exposure, do not effectively mitigate and reduce the amount of released contaminates into the environment, and mercury loss to the air and ground can be swept away for distances, especially in extreme weather events resulting in high standing water and overflow of storm drain networks [6]. In the past, responses to sites such as these were often a lagging factor due to a lack of regulatory monitoring and oversight of plants' industrial hygiene practices that impact their surrounding natural environments. In 1980 the US instituted a process of monitoring and treating specifically high-level contaminated sites by assignment to the US National Priorities List as a Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) [1]. This act would appropriate funds and resources to specific hazardous waste sites that would allow for ongoing monitoring, treatment, and prevention/reduction of mercury exposure.

Indeed, mercury concentrations at or above the permissible limits in the river or lake water can lead to troubling and deadly outcomes if left untreated. As a result of this, monitoring and oversight of producers and users of heavy metals, specifically mercury, is a necessity to the community and environment at large due to the potential impact on human health. Hence this study sought to develop, optimize and validate a method so that lay operators may quickly, easily, and relatively affordably obtain accurate mercury measurements down to the ppt level in wastewater produced by a local manufacturing plant before it is reintroduced back into the Niagara River. These efforts were challenged, in that the most common techniques currently employed for this level of detection are typically esoteric in nature, and relatively costly in time and resources.

1.3 Mercury Sampling and Testing

Reliable and precise mercury analysis was crucial to operations given the generator, an industrial chemistry plant, is situated along the Niagara River between two federally protected and ecologically critical Great Lakes: Lake Ontario and Lake Erie. It was required that the methodology be proven to be accurate due to the ongoing second-party monitoring and site-specific regulatory guidelines set by the EPA, and enforced and monitored by NYSDEC, for mercury levels in waters released from the plant into the river. Citations from governmental regulatory bodies such as the DEC, EPA, and other local municipalities including the city and county, could jeopardize operations at the plant, up to costing it its legal right to operate. For this reason, method development and validation were critical next steps to ensure the plants future. The validation of the developed methodology was designed to systematically prove the analytical integrity of the employed methodology by demonstrating accuracy, precision, and reliability (fitness) for its intended use. This effort was of great importance to the business continuity of the organization and its relevant stakeholders as it is documented in this report.

Considerations were taken due to the complexity of the aqueous-based sample matrix which included residual potassium and sodium sulfate generated from the proprietary electro-catalytic process of persulfate production employed by the industrial chemical plant. Before any initial testing, during the last stages of the persulfate process in the generation of this wastewater, the water is chemically treated with a chelating agent to reduce the levels of mercury and other heavy metals. Then, the wastewater is transferred to a retention tank where it is sampled and tested by operators to ensure mercury is at or below the allowable limit before it is released back into the Niagara River. The expected range of analyte concentrations spanned from trace levels (ppm to ppb) to ultra-trace (ppt). An ample supply of aqueous test samples would be readily available daily from one main source, so a non-destructive methodology was not a requirement of this project, and sampling technique (top, middle, bottom) was not employed. However, special consideration was given to the possible introduction of residual mercury contamination, and for this reason, purified trace-level acids and specialized low-leachable test containers were used. Due to the nature of the persulfate process where salts are continually produced in a non-batched process,

mercury results would be needed within hours of sample generation, and with budgetary constraints in mind, offsite testing was also not a practical option. Ease of use was an additional requirement for the instrument and method of choice since non-chemist operators would be required to perform this testing as a routine part of their duties, at least once to twice daily. For this reason, the method validation was designed to also prove reliability and accuracy, while accounting for minor fluctuations in the reagent recipes to demonstrate method ruggedness against these small but expected differences between multidisciplinary, non-scientist, chemical operators (See Figure 1.1).

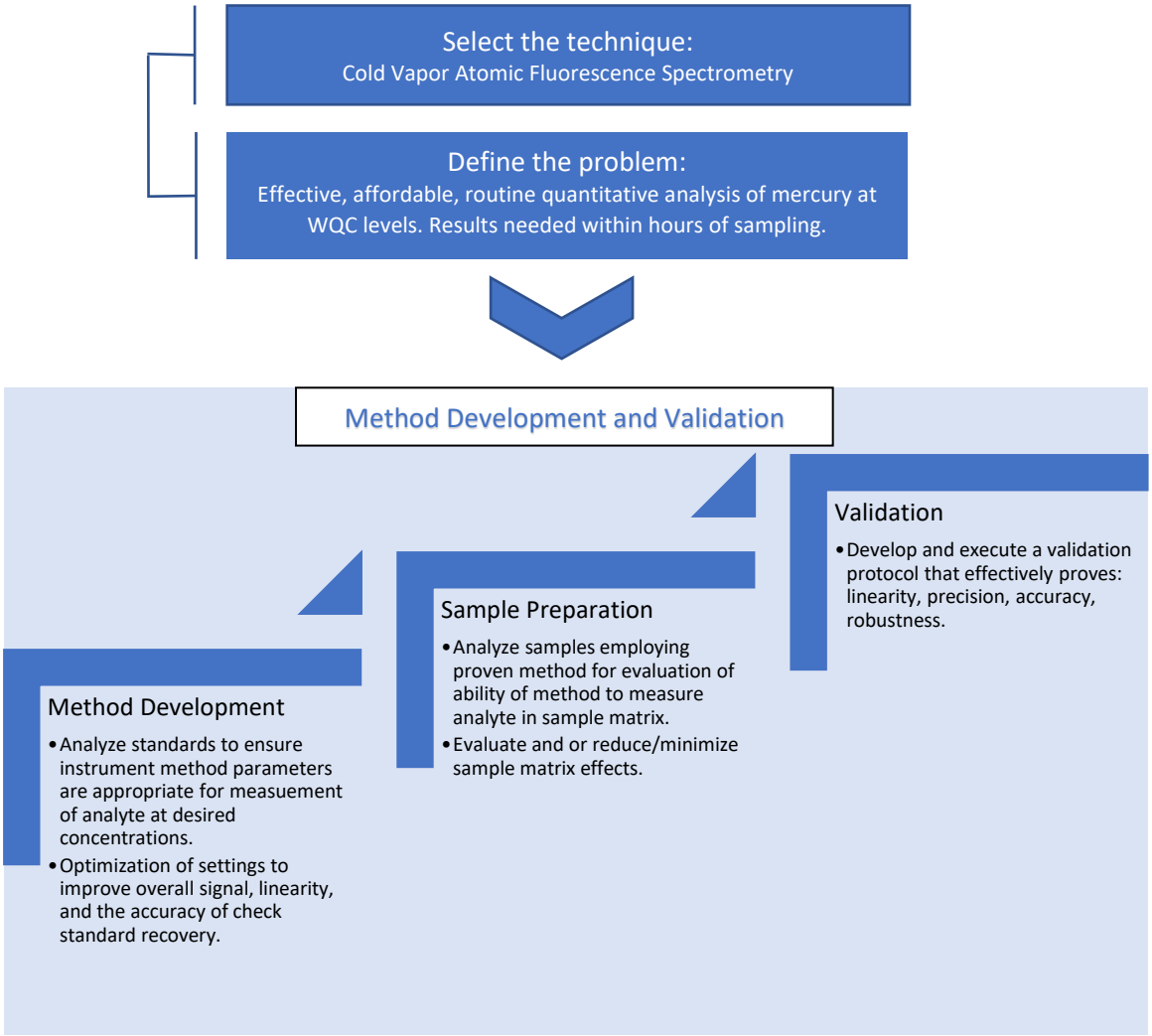


Figure 1.1 The schematic diagram of the overall process from instrument selection through method development for the validation of a chemical mercury detection method of analysis.

1.4 Common Methods of Elemental Analysis

Atomic spectroscopy is one of the most widely used methods of elemental metal determination and covers a diverse range of analytical instruments and techniques that rely on electromagnetic absorption and emission of an atomic state sample [11]. This optical atomic spectroscopy includes various techniques such as optical emission, flame atomic absorption, electrothermal atomic absorption, and atomic fluorescence [11]. Mass spectrometry is widely used to identify elements and compounds based on mass-to-charge ratio. In particular, inductively coupled plasma mass spectrometry (ICP-MS) is one of the essential elemental analysis methods [12].

Table 1.1 summarized the comparison of several detection methods for heavy metals. Although numerous methods exist for the determination of heavy metals, several factors must be considered in choosing the most suitable technique for mercury detection: instrument price, the limit of detection, running and maintenance costs, and required skill level of the operator. Various methods of analysis, detectors, and instruments have been employed over the years to measure low-level mercury in effluent wastewater. Although methylmercury has higher levels of toxicity, all forms of mercury have adverse human effects; therefore, the technique employed did not require the speciation of mercury at the active oxygen manufacturing plant. Since, the limit requirements imposed upon the site were placed on total mercury concentration, without making distinctions between specific forms of mercury. Although ICP-MS allows for total mercury determination and speciation within the desired linear range, it was eliminated as the preferred instrument for this line of testing due to the advanced skillset and training required for operation and upkeep, and the high purchase price. Of the optical spectroscopic options, the cold vapor technique is highly sensitive in mercury detection, relatively easy to operate, and less costly than ICP-MS. It is generally less complex which allows for inhouse troubleshooting and maintenance, in place of costly service contracts.

Table 1.1 Comparison of instrumentation commonly used in heavy metal detection and quantitation.

	Inductively coupled plasma mass spectrometry (ICP-MS)	Inductively coupled plasma optical emission spectroscopy (ICP-OES)	Flame atomic absorption spectroscopy (FAA)	Cold vapor atomic fluorescence spectroscopy (AFS)
Methodology	Multielement plasma based mass spectrometry	Multielement plasma based spectroscopy	Single element atomic spectroscopy technique	Single element atomic fluorescence spectroscopy
Detection range	Lower ppt	Less than 1 ppb	Lower ppb	Lower ppt
Complexity	HIGH	MEDIUM	LOW	LOW
Drawback(s)	Sensitive to sample matrix interference (TDS – total dissolved solids) [13].	Sensitive to sample matrix interference, and TDS [14].	Poor detection of rare earth metals and halogens [14] .	Requires heavy metal analyte to be volatile at room temperature.
Price	$50,000 – 500,000	$10,000 – 60,000	$10,000 – 50,000	$2,000 – 10,000
Advantage(s)	Multielement technique. Allows for speciation.	Multielement technique.	Can handle high TDS load (>10%).	Extremely selective with high sensitivity.

1.5 Cold Vapor Atomic Fluorescence Spectroscopy (CVAFS)

The decision to purchase a cold trap mercury analyzer for development of a quantitative method of mercury analysis was based on very specific conditions and needs of the business and its stakeholders. With governmental agency oversight in the form of exceedance limits down to trace levels levied on the site and the additional requirement of demonstrated accuracy and precision in corroborating results with an outside accredited test lab, the reliability of the reported results were critical to the plant's continued operation in the region. Although the manufacturing facility houses QC and R&D laboratories, lay operators routinely and independently perform the testing without the oversight of trained chemists or

laboratory technicians due to workflow issues. This presented a unique challenge of finding an accurate and reproducible, yet highly sensitive, but user-friendly approach, with low maintenance at an affordable unit cost for the detection of mercury.

CVAFS proved advantageous to our expressed goals for several reasons. It is specific to mercury in that the detector is highly sensitive and attuned to the fluorescent wavelength of mercury. Should there be any interference it would be easily detected in a change to the peak shape, and the methodology is accurate and reproducible at the water-quality criteria levels published by EPA. Additionally, it was more affordable and comparatively easier to operate and maintain than other elemental analysis methods. The Millennium Merlin mercury analyzer relies on CVAFS, which is comprised of a three-stage design: sample intake, mercury extraction, mercury detection. The sample intake process consists of two multi-channel peristaltic pumps for the independent transfer of reductant reagent and sample solution into the gas-liquid separator. The flow rate (speed of rotation) of the pump coupled with the bore size of the tubing allows for the ratio of sample to reagent to always be at the desired 2:1 which controls the rate of reaction while suppressing instrument noise which directly results in increased signal [15] [16]. The sample and blank streams coupled with the stream of reductant reagent all feed into an automated sample valve which alternates between blank-to-waste and sample-to-detector, or sample-to-waste and blank-to-detector (See Fig. 1.2). The reductant along with either the blank or sample are mixed in the valve where the reaction is first initiated before passing to the detector (by way of the gas-liquid separator and dryer tube). During the sample analysis, the sample and reductant streams are open between sample runs. During blank injections, the blank and reductant streams are open.

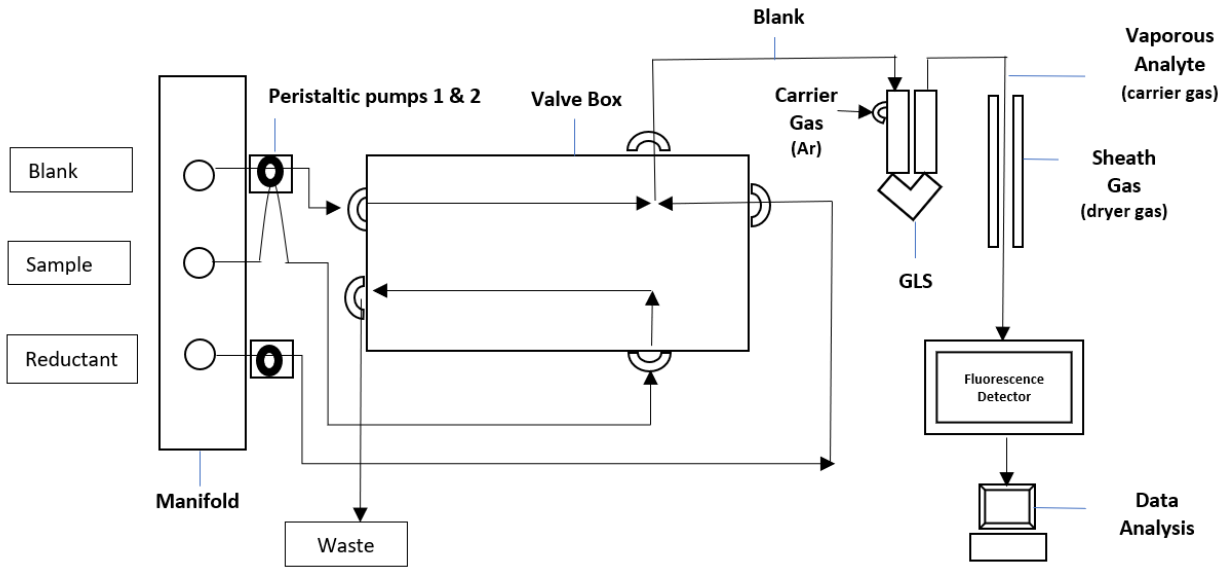
Initiation of the reaction to convert and extract mercury gas Hg^0 (gaseous elemental mercury) takes place in the valve when the reductant, tin(II) chloride, reduces compositional mercury(II) to elemental gaseous mercury as shown in eq. (1.1) [15]. Once these streams converge and mix, the solution feeds into one side of the U-shaped gas-liquid separator (GLS), where the dissolved vaporous mercury is sparged out by a stream of argon (Ar) gas bubbled in through the top of the separator. The excess

supernatant is then pushed out the opposite side of the separator by a pressure differential created inside the U-shape.

$$HgCl_2\ (aq) + Sn^{2+}(aq) \rightarrow Hg^0(g) + 2Cl^-(aq) + Sn^{4+}(aq) \quad \text{(Eq. 1.1)}$$

As the purge gas bubbles into the GLS, the converted mercury vapor is released from solution and travels up through a dryer tube and towards the detector by the Argon (Ar) carrier gas. A patented Perma Pure drying membrane acts as the drying tube where a laminar flow of Ar gas removes excess water vapor from the inner stream of extracted gaseous mercury [16]. The carrier gas then moves the sample stream up through a chimney into the detector unit. The detection process consists of a mercury vapor lamp which acts as the ultraviolet (UV) light source and a photomultiplier tube (PMT), a photon counting module, both components are staged at right angles relative to each other [15]. The analyte gas enters the detector unit at the sample cell where the atomized mercury exits up out the chimney directly into the lamp's light path. The excited atoms re-radiate their absorbed energy in the form of fluorescence. Fig. 1.3 shows the energy diagram for the atomic fluorescence in comparison to atomic absorption and atomic emission. After passing through a 254 nm filter, the fluorescence emission is detected by the PMT [15]. The intensity of the fluorescence light is directly proportional to the concentration of mercury in the sample. The signal is transmitted to the accompanying computer.

Blank (diluent) to detector



Sample to detector

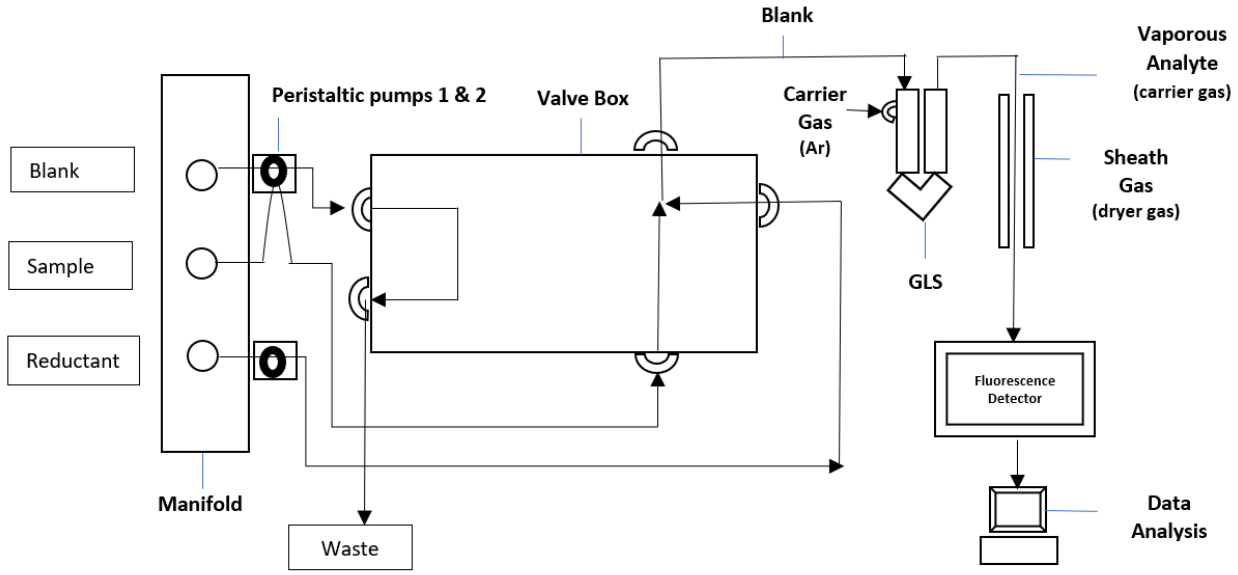


Figure 1.2 Instrument diagram in both blank solution to detector position in valve (top), and sample solution to the detector position in valve (bottom).

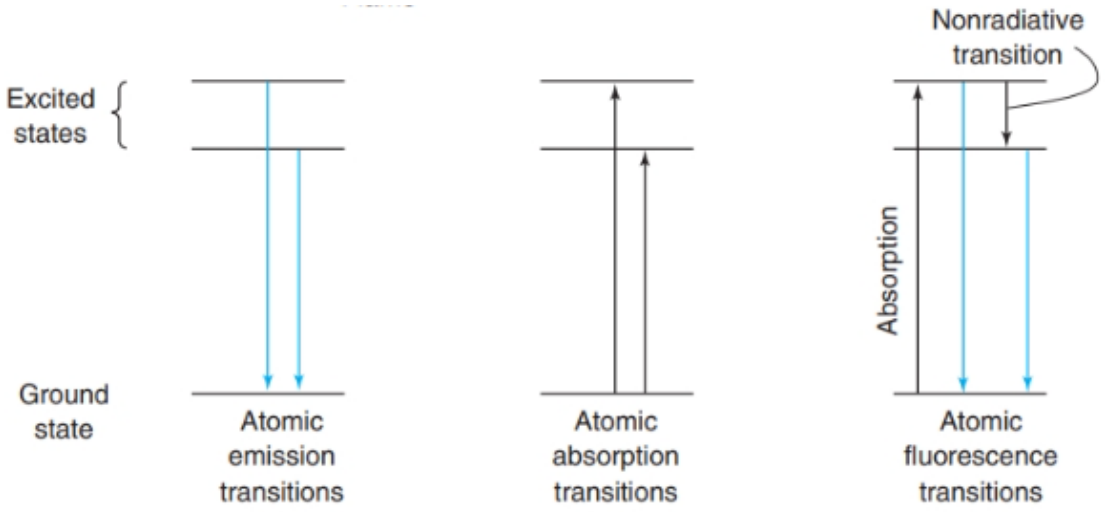


Figure 1.3 The energy diagram of atomic emission, atomic absorption, and atomic fluorescence.

II. Experimental Section

2.1 Materials

Hydrochloric acid 34 – 37% (ARISTAR PLUS for trace metal analysis) was purchased from VWR Chemicals BDH (Radnor, PA). Tin(II) chloride (anhydrous, > 99%, crystalline) and 0.1 N standardized solutions of potassium bromate and potassium bromide were purchased from Alfa Aesar (Ward Hill, MA). Mercury standard solution (1.000 ± 10 µg/mL in 5% (v/v) HNO_3) was obtained from Inorganic Ventures (Christiansburg, VA). Hydroxylamine hydrochloride was purchased from EMD Millipore Corporation (Billerica, MA). Low leaching, leak free, virgin polyethylene Class A sample digestion containers - DigiTubes® were purchased from SCP Science (Quebec, Canada). All solutions were prepared with inhouse purified water with a resistivity of ≥ 18 mega ohms.

2.2 Equipment

Mercury determination was obtained by a PSA Analytical 10.025 Millennium Mercury Analyzer cold trap vapor atomic fluorescence analyzer (Orpington, Kent, UK). Sample volumes were measured and delivered with Eppendorf Repeater Pipettor E3/E3x equipped with various sizes of Combitips (Hamburg, Germany). A method to quantitate mercury in treated wastewater has been developed and validated as

accurate and precise withstanding expected variations in solution preparations and operating parameters (ruggedness), and variations associated with different operators (robustness). The overall methodology was designed to develop the most cost-effective, user-friendly, and quickest means of ppt-level mercury determination without jeopardizing reliability and accuracy of the operation.

This method allows for the timely determination of mercury with just 3-minute runtimes and a limit of detection of 10 ppt for application in an industrial chemistry QC laboratory. A variety of parameters were evaluated to determine the instrument's final method settings, including adjustments to the gain, filter factor, and analysis time periods. The most efficient sample preparation procedures were also developed to minimize overall sample analysis time and reduce material costs since ultrapure reagents are needed in low-level metals analysis.

Method development began with the initial selection of instrument run parameters. The Merlin Mercury Analyzer allows for the adjustment of 18 different method parameters and 6 different instrument parameters to allow for precise fine tuning of run conditions. The method parameters that were evaluated along with their essential function, are summarized in Table 2.1. The method parameters that were adjusted between each trial run are detailed in Table 2.2, while the resultant run data for each method are summarized in Table 2.3. The optimization of parameter settings was critical in achieving consistent analysis results between runs and amplifying signal count, where a strong signal is more robust and less sensitive to small changes and thus performs better. Initial settings were determined by sheer trial and error and predominantly based off the functions as defined in the instrument's user manual. The primary initial objective in instrument method development was to maximize signal strength, resulting in a consistent and reliable signal response in order to produce an analytically sound standard curve. The response factors would be used to produce a calibration curve from which all analysis within an individual run would be calculated against. The linearity of the calibration curve is assessed by the square of correlation coefficient (r^2) value of the best-fit line across each of its points. An r^2 value less than 0.99 would indicate poor linearity—no results reported in this method validation were calculated against a

failing calibration curve. A failure to achieve linearity would result in any one standard solution, or all, being reprepared and analyzed, then reassessed for passing linearity. The accuracy of each curve is assessed against a separate check-standard solution, generally prepared around the midpoint of the curve. The check-standard is required to recover within 20% of its known concentration. During method development, each candidate method was assessed by the performance of its component method parameters with the calibration curve. Methods that increased signal response with a passing linearity and passed a check-standard percent recovery were retained. Parameters that worked against these goals were tuned and/or further adjusted. Of the 24 total adjustable parameters, 8 method parameters and all 6 instrument parameters were attuned to achieve a strong and reliable signal; the resultant standard curve consistently achieved passing linear regression, with a r^2 value of ≥ 0.999.

Three trial methods were created and tested as summarized in Table 2.2. First, Trial Method 1 instrument parameters were set based on an assessment of the desired outcome and the functions of each setting. The resultant calibration curve produced the desired linearity and recovered the check-standard within specification. However, it was noted that the area counts, particularly in the standard solutions at the beginning of the curve (10 and 25 ppt) were low. This could allow for lower concentrations of standards to be more susceptible to small changes such as temperature change in the lab and aged reductant solution. These small fluctuations could cause an increase in the likelihood of calibration curve failure. Although the check-standard recovered within specification at 15% of its known concentration, it did not leave room for error considering the limit of ± 20%, therefore the method parameters were adjusted to improve these issues.

In Trial Method 2, to increase area count, which in theory could also improve check-standard recovery, the following instrument parameters were increased: gain, baseline check value, filter factor, and pump speed 2. In addition, the options of "allow negative results" and "valve flush" were turned on. The run time was also adjusted by doubling the delay period and increasing the analysis period. The former helps to achieve a more accurate baseline and the latter to ensure all the peak area was accounted

for. Although the area count increased substantially, by more than double for nearly all standards, the check-standard recovery suffered considerably in accuracy. The check-standard prepared at 50 ppt was calculated to be 98.02 ppt with Trial Method 2 run parameters. This was likely due to a combination of factors, but mainly due to the baseline check value being increased from 1 to 5, which allowed the instrument to begin the next run even if the baseline had not fully recovered back to a response of 1, in this case it could begin as high as a response of 5. It is thought the standard curve was built on an artificially high baseline, which decreased the pitch of the slope and caused the y-intercept value to be artificially inflated, leading to the high check-standard recovery. For this reason, in Trial Method 3, the baseline check value was brought back to its previous setting of 1, which allowed for longer run times, but a suitably recovered baseline; additionally the autozero function was turned on. The instruments "autozero" function allows for the calibration standard value assigned as "Blank" to be automatically set to zero, this essentially forces the y-intercept through zero and effectively acts to address unwanted skewing of the calibration curve as experienced in Trial Method 2. Minor adjustments were made to the filter factor and analysis period, and the valve flush feature was turned off. The valve flush feature allows for an automated flushing action to take place in the valve between runs. This feature increased the time between injections, and the overall analysis runtime, while consuming diluent solution. This feature was costly in time and materials. Sample run injections progress through three stages: delay, analysis, and memory. In the delay period the blank valve is open to the detector for a baseline reading while the sample is brought up through the probe to the sample valve, at which point the sample valve opens and the analysis of the sample solution begins. After the analysis period the valve switches back to blank solution and the baseline recovers as the mercury-containing sample or standard solution works its way out the instruments plumbing, which is termed as a memory period. Thus, the use of the additional valve flush feature was not necessary because the baseline recovery value was decreased, and further—an operator could react to an elevated baseline in the memory phase by siphoning diluent up through the sample line to flush out any carryover. Trial Method 3 produced linearity with a r^2=0.9997, a further increase in area counts, and the 95 % check-standard recovery (47.42 ppt). Trial Method 3 running

parameters were therefore adopted as the final method settings. All further method runs and subsequent method validation were analyzed with this final instrument method.

Table 2.1 Descriptions of adjusted instrument parameters.

Adjusted Instrument Parameter	Function
Pump Speed 1,2	Two variable speed multi-channel peristaltic pumps to deliver reagent and sample solutions.
Delay	The time required for the sample to reach the switching valve and establish a baseline value.
Analysis Period	The time in which measurements are taken.
Memory	The time for the sample signal to recover to the baseline value.
Baseline check type	Requires the software to check for baseline drift above a set threshold, unit will wait until the baseline recovers to previously defined limit.
Baseline check value	The value used (and the defined unit) when the baseline check option is turned on.
Gain	Sets the amplification on the detector.
Filter Factor	The number of points averaged to plot a single point.
Valve Flush	Specifies whether or not you wish to utilize the automated valve flush feature.
Auto Zero	Specifies whether you want the system to autozero between samples.
Allow negative peaks	Specifies whether or not you want to allow the calculation of negative results.

Table 2.2 Setup parameters for the three trial methods.

Parameter	Trial Method 1	Trial Method 2	Trial Method 3
Gain	1	100	100
Mode	Ratio	Ratio	Ratio
Baseline Check Type	None	Units	Units
Baseline Check Value	1	5	1
Filter Factor	1	52	32
Auto Zero	No	No	Yes
Allow Negative Results	No	Yes	Yes
Blank Subtraction	No	No	No
Delay Period, s	10	20	20
Analysis Period, s	30	50	40
Memory Period, s	60	60	60
Pump Speed 1, %	100	100	100
Pump Speed 2, %	50	100	100
Valve Flush	Off	On	Off

Table 2.3 Signal response, linear regression, and check sample recovery of each trial method.

Standard	**Trial Method 1**			**Trial Method 2**			**Trial Method 3**		
	Peak Height	Peak Area	r^2 ≥0.99	Peak Height	Peak Area	r^2 ≥0.999	Peak Height	Peak Area	r^2 ≥0.999
Blank (Diluent)	1.8278	23.115		1.119	38.24		0.5188	0.8828	
10 PPT Standard	7.8362	139.84		14.55	433.8		14.09	565.4	
25 PPT Standard	17.609	360.95		37.90	1607		36.01	1875	
50 PPT Standard	34.371	647.35	0.9992	73.77	3136	0.9996	71.81	3649	0.9997
100 PPT Standard	69.631	1297.4		151.9	6469		147.5	7758	
150 PPT Standard	107.34	1990.0		225.6	9654		215.9	11198	
50 PPT Check Standard	42.79			98.02			47.42		

Peak responses are displayed as peak height and area of injections of the calibration curve, along with the corresponding linear regression. Adjustments to the run method resulted in an overall improvement in signal response, check sample recovery and linear regression.

2.3 Standard Preparation

Linear regression across a series of 5 individually prepared standard solutions was used to establish a response curve proportional to the concentration of the target analyte. The standard calibration solutions were prepared in individual DigiTube containers by diluting the appropriate concentration and

volume of standard solution to the 50 mL mark with diluent solution (5% HCl) and then mixing them well.

Initially, the standards spanned a range of 10 – 150 ppt. With a neat sample analysis, the limit of quantitation (LOQ) in a sample solution matches that of the lowest point of the calibration curve at 10 ppt. However, with the variability of pH range in the wastewater and the need of acidification of the sample for preservation and mercurial complexation, an acid addition step was incorporated in the sample preparation procedure by requiring a dilution of 30 mL of sample to 50 mL with 5% HCl. Due to this sample dilution factor of 1.67, a sample with a concentration of 10 ppt would be diluted in the sample preparation step and would result in the final sample concentration being analyzed to be at an approximate 6 ppt, below the lowest calibrator of 10 ppt, and technically below the LOQ of the method. For this reason, the lowest calibrator was moved from 10 to 6 ppt to allow for a v/v LOQ of 10 ppt. This allowed for the method to be valid for sample results as low as 10 ppt, while taking into account the sample preparation dilution. Any final sample result below this concentration would be deemed invalid due to it being outside the range of the calibration curve.

2.4 Sample Preparation

Mercury naturally exists in three different oxidation states: a zero valent form known also as elemental mercury, a univalent form [Hg(I)], and its most abundant form-divalent mercury [Hg(II)] [3]. The divalent form is the most reactive and is typically present as a complex. Its stability varies depending on the composition of the sample matrix. For this reason, a 5% hydrochloric acid solution was used as a diluent to preserve the dissolved mercury and establish standard stability. It is known that dissolved mercury ions form complexes in HCl solution, which keeps it in solution as an ion, opposed to its gaseous elemental form—Hg^0, and helps prevents sample container adsorption until it is re-liberated by the reductant [17]. For mercury determination through fluorescence spectroscopy, typically two types of reduction chemistries are employed: stannous reagent ($SnCl_2$) and sodium borohydride. This methodology employs stannous reagent [18]. Li et. al. notes the conflicting data around stannous reagents efficacy as a

reductant in total mercury determination in natural waters containing sulfate ions, a known possible contaminate of the persulfate production plant [18] [19]. Our aqueous sample matrix may contain residual SO_4^{2-} and other oxidized sulfur containing compounds, a byproduct from persulfates production in our chemical plant site, which could potentially decrease the efficacy of the reaction between the stannous chloride and Hg(II) ions. Thus, a bromination digestion step was included before initiating the reduction of Hg(II) ions to Hg atoms by $SnCl_2$. This is typically employed in mercury detection by atomic fluorescence spectroscopy to reduce matrix interference caused by mercury complexes and will oxidize sulfide [20]. A mixture $KBrO_3$/KBr solution in hydrochloric acid liberates bromine into solution. This solution acts as a digestor in that free bromine, a strong electrophile, oxidizes methylmercury and other organic and inorganic mercuric compounds. To check the necessity of sample digestion step, the following set of experiments were performed: a mercury-containing sample was prepared and analyzed with or without the digestion step. In addition, a sample solution spiked with 50 ppt Hg was similarly tested with or without the digestion step. The results for this experiment are displayed in Table 2.4 – all samples were recovered within 20% of their known concentration. This proved that an additional $KbrO_3$/KBr oxidation step was not needed in the sample preparation procedure and that the reported mercury value was specific to mercury and reflected the total mercury content of the sample.

Table 2.4 Digested and undigested peak responses with trial method 3.

Standard	**Peak Height**		**Peak Area**		**Digested**	**Undigested**
	Digested	*Undigested*	*Digested*	*Undigested*		
Blank (Diluent)	4.8033	4.5152	204.9	245.51	$r^2 = 0.9990$	$r^2 = 0.9997$
10 PPT Standard	41.779	23.477	2151.1	1282.1		
25 PPT Standard	68.489	68.102	3586.7	3584.8		

50 PPT Standard	133.90	136.44	6931.6	7210.8		
100 PPT Standard	266.09	267.22	13894	13887		
150 PPT Standard	400.43	398.79	20777	20694		
Sample A (df = 5.00)	190.08	192.04	9823.3	9606.5	[Hg] = 375 ppt (Height)	[Hg] = 355 ppt (Height)
Sample B – 50 ppt spike (df = 1.67)	41.105	39.791	2285.4	2061.4	99.8% (% Recovery)	90.5% (% Recovery)

The peak responses to two separate preparations of the standard curve, one set of standards and sample preparation was chemically digested, while the other set was not. Both standard preparations were analyzed with the same method of analysis along with a check sample. Peak responses demonstrate that sample digested vs. undigested is within the tolerable range of variance for check standards of ± 20%.

After a functional test method was developed, solution stability was assessed to establish the shelf-life of standard solutions in advance of method validation. Each standard of the calibration curve was prepared with the 5% hydrochloric acid diluent and analyzed within an hour of preparation. These standards were then later analyzed after 4-days. At the end of +4 days (after the first day they were prepared) a middle calibrator (50 ppt standard) was prepared fresh and measured against the aged standard curve and the middle calibrator of the aged solution was measured against a freshly prepared standard curve. The concentration based off peak height of the 50 ppt calibrator of the freshly prepared standard was calculated against the +4-day old standard calibration curve, and likewise the concentration of a +4-day old 50 ppt calibrator was determined against the freshly prepared calibration standard curve. The results of the standard curve freshly prepared versus the +4 day preparation are summarized in Table 2.5, while Table 2.6 includes +4 day standard stability results of the 50 ppt middle calibrator. The results

of both calculated values fell well within the ± 20% allowable range of recovery for check sample solutions and the response for each +4 day old standard solution recovered with a percent difference of ≤ 2%. This established a +4-day shelf-life for standard solutions prepared in a 5% hydrochloric acid solution. All sample and standard preparations presented in the method validation are prepared with this diluent and no standard solutions were used once they were past expiration date.

Table 2.5 Standard stability assessment, signal response after 4-days.

Standard Stability				**Percent difference** (after blank adjustment)
Name	Response	Name	Response	
Standards ≤1 day old		*Standards = +4 days*		
STANDARD	**PEAK HEIGHT**	**STANDARD**	**PEAK HEIGHT**	
Blank	*1.2632*	*Blank*	*3.8418*	-0.81
10 PPT	*26.029*	*10 PPT*	*28.808*	-0.76
25 PPT	*64.358*	*25 PPT*	*67.419*	-0.01
50 PPT	*128.23*	*50 PPT*	*130.82*	0.99
100 PPT	*256.54*	*100 PPT*	*256.59*	-1.67
150 PPT	*382.61*	*150 PPT*	*391.55*	-0.81
y = 2.5461 x + 1.0881		*y = 2.5728 x + 2.9656*		Linear regression

Standard solutions were analyzed immediately following preparation, and again 4-days later. Peak responses were compared, a minimal difference in peak height over time was observed.

Table 2.6 Results from +4-day standard stability experiment.

Standard Concentration level (ppt)	Fresh standard calculated against +4-day old calibration curve	+4-day old standard calculated against fresh calibration curve
50	50.95	48.81

Displays the calculated standard concentration of a fresh standard solution against a 4-day old standard curve, and a 4-day old standard solution against a fresh standard curve, both concentrations are within the allowable range of variable.

Validation experiments were assessed at one or all levels, which included 50% (25 ppt), 100% (50 ppt) , and 200% (100 ppt) of the allowable limit of 50 ppt. All solutions prepared in the execution of this validation are detailed in Table 2.7. Each sample was prepared by spiking it with an intermediate standard solution (ISS) prepared from a dilution of an externally certified mercury standard solution (SS) and analyzed using an identical method. In this way, the test procedure and instrument method were evaluated under identical test conditions with a matrix-matched sample solution that contained the analyte of interest.

Table 2.7 Concentrations of solutions prepared in execution of validation.

Calculation for Validation in Mercury

WS Solution Prep			*Sample Prep*		*Matrix Spike Solutions Prep*		
Soln. ID	**ISS (mL)**	**Total Vol**	**mL Sample**	**Total Vol mL**	**Spike - 50% (ISS spike vol., mL)**	**Spike - 100% (ISS spike vol., mL)**	**Spike - 200% (ISS spike vol., mL)**
WS1	0.6	100.0	30.0	50.0	0.750	1.50	3.00
WS2	1.5	100.0	**Sample ng/mL**		**Concen. in w/w ppt**		
WS3	6.0	100.0	600000000.000		25	50	100
WS4	12.0	100.0			**% of Limit**		
WS5	15.0	100.0			50	100	200

Element	**Specification** (ppt)	**Conc. in Stock** (ng/mL)	**Stock** (mL)	**Total Vol** (mL)	**Conc. in SS** (ng/mL)	**Vol. SS** (mL)	**Total Vol** (mL)	**Conc. in ISS** (ng/mL)
Mercury	50	1,000,000.00	0.100	100	1000	0.1	100	1.000

Concentration in Working Standards (ng/mL)					**Concentration in w/w ppt based on sample concentration**					**% of Limit**				
WS1	**WS2**	**WS3**	**WS4**	**WS5**	**WS1**	**WS2**	**WS3**	**WS4**	**WS5**	**WS1**	**WS2**	**WS3**	**WS4**	**WS5**
0.00600	0.01500	0.06000	0.12000	0.15000	10.0000	25.0000	100.0000	200.0000	250.0000	20	50	200	400	500

III. Results and Discussions

3.1 Method Validation Parameters

The critical next step following method development is analytical method validation. The International Organization for Standardization (ISO) describes method validation as, "confirmation through examination and provision of objective evidence that the requirements for a specified intended use or application are fulfilled" [21]. In respect to analytical methods used in commercial labs to release product, method validation is a means to assess the analytical integrity and performance of a test method to reliably, consistently, accurately, and precisely provide qualitative and quantitative results when performed within the laboratory. Validation protocols are typically executed to demonstrate equivalence between two methods when a new method is developed, or a method is revised [21]. The parameters and characteristics assessed in a method validation can vary depending on the instrumentation or technique, and purpose of the method of analysis, typically for quantitative analysis. Linearity, specificity, precision (ruggedness), accuracy, robustness, and stability are assessed; but no official guidelines present a required validation test schema. Guidance in method validation and system suitability have been published by several regulatory bodies, such as the U.S. Environmental Protection Agency (EPA), U.S. Food and Drug Administration (FDA), United States Pharmacopeia (USP), and the International Conference on Harmonization (ICH) [12]. Guidance in instrument suitability parameters and specific method validation criteria, such as acceptable range of percent recoveries, replicate sample preparations, and system suitability requirements have been adopted for the purposes of validating this method from USP General Chapter <233> and ICH Q2(R1) [22]. System suitability test and its establishment is an integral part of any method analysis and analytical run prior to generation of any test sample data. System suitability is based on the concept of grouping together equipment, instrument, electronics, software, operations, reagents, and test method into one all-encompassing system that is assessed for "suitability" by successful test injections (typically of the calibration curve and diluent) ahead of any live test or sample injections.

Criteria are established around what is deemed acceptable and these criteria must be met for any following injections to be considered permissible or valid.

USP is an independent, not-for-profit, agency that sets quality standards for pharmaceuticals, dietary supplements, and herbal medicines enforceable by governmental agencies such as the FDA [23]. This agency's testing guidelines are used by industry pharmaceutical producers to test and produce within defined allowable limits. USP General Chapter <233> Elemental Impurities-Procedures specify the procedures and procedural limits for testing elemental impurities. USP suggests that for each analysis run sample suitability be assessed and established for the materials, methods, and instrument used along with a standardization solution during the analysis. For this reason, each analysis run was accompanied by a system suitability table comprised of a 5-point standardization curve with an r^2 value ≥ 0.999, and a separate check standard preparation at the mid-point of the curve. Also adopted from USP <233> is the established acceptability criteria about percent recovery for spiked samples and check standards: spiked samples for validation of quantitative procedures are required to recover within 70-150% of the spiked targeted value, with a relative standard deviation of ≤20% for precision, and ≤25% for intermediate precision [23]. Similarly, system suitability check standards require recovery at no more than 20% of the prepared value [13]. Although these strict guidelines were intended for pharmaceutical and medicinal applications, they have been adopted in this report as a strict and conservative approach to validation of this test method.

3.2 Accuracy

In this report, the trueness of the results produced is defined as an inherent characteristic that reflects the overall error (systematic and random) in the analytical test procedure and is comprised of two essential components: accuracy and precision [24]. Specifically, the accuracy of an analytical procedure expresses the closeness in value between the true amount and the measured amount. Spiked samples were prepared in triplicate at each level: 50%, 100, and 200% of the 50 ppt limit. All samples were prepared in class A - 50 mL Digitubes™ to a dilution factor of 1.67 by adding 30 mL of the wastewater sample. The

appropriate volume of 1 ppb ISS solution was used to spike each sample solution (except the matrix blank) after the addition of the wastewater; lastly, each tube was diluted to the mark with diluent (5% HCl solution). Matrix blank samples, used for background subtraction, were prepared by adding 30 mL of wastewater solution to a 50 mL Digitube™, then dilution to the mark with diluent. Each sample was analyzed against a calibration curve for the determination of achievable percent recoveries within the previously established range of 70-150%.

Table 3.1 Percent recovery results of spiked sample analysis for both accuracy (% recovery) and precision (%RSD).

Mercury Validation – Accuracy, Precision System Suitability		**Hg** (ppt)
Analyst 1	Std 1	6.000
	Std 2	15.00
	Std 3	60.00
	Std 4	120.0
	Std 5	150.0
	50 ppt Check Standard recovery, %	102
R (correlation coefficient)		1.00
Mercury Test Solution Results – Spike Recoveries		**Hg** (% Recoveries)
Analyst 1	Control	
	P-TS-50; A-TS-50 #1	116
	P-TS-50; A-TS-50 #2	118
	P-TS-50; A-TS-50 #3	111

	Avg.	117
	%RSD	3.1
	P-TS-100; A-TS-100 #1	90
	P-TS-100; A-TS-100 #2	98
	P-TS-100; A-TS-100 #3	99
	Avg.	96
	%RSD	5.2
	P-TS-200; A-TS-200 #1	88
	P-TS-200; A-TS-200 #2	92
	P-TS-200; A-TS-200 #3	111
	Avg.	97
	%RSD	12.7

3.3 Precision and Intermediate Precision (Ruggedness)

Precision, sometimes referred to as degree of scatter, expresses the closeness of measurements obtained from a particular sampling and or analysis. In this validation procedure, precision was assessed at two levels: repeatability and intermediate precision. The precision, as degree of scatter, is expressed as the coefficient of variation referred to as the percent relative standard deviation (%RSD) as shown in eq. 3.1, also referred to as percent variation coefficient (%CV) [25]. This measure of variance determines closeness of the data points around the mean. Precision was assessed along with accuracy as a measure of %RSD of triplicate preparations at each level: 50, 100, and 200% of the limit in Table 3.1.

$$RSD, \% = \frac{S}{\bar{x}} * 100 \qquad \text{(Eq. 3.1)}$$

where, s and x represent the standard deviation and the average of the measurements, respectively.

Intermediate precision, also sometimes referred to as inter-assay precision or ruggedness, allows for the assessment of the total precision under the variability of typical changes expected under normal run conditions. Intermediate precision experiments typically involve the pooling and assessment of data under normal conditions from lab to lab, instrument to instrument, and analyst to analyst to determine if the method of analysis is fit for its intended use [26]. A successful test method should be achievable on compatible systems between laboratories and trained personnel. A fragile test method, which is sensitive to these normal conditions, should be further improved. In this validation experiment, only one instrument was purchased for use in a sole QC laboratory, therefore ruggedness was assessed between trained analyst by calculation of the % RSD of six individual sample preparations spiked at the 100 % level of 50 ppt. A total of 12 sample recoveries were assessed against the USP requirement of ≤25% which allows for intermediate precision to be assessed through internal laboratory variations of runs performed on different days, by different analysts, or by different equipment [23]. Each precision test solution was also assessed for accuracy, as each solution's percent recovery was required to meet the acceptable limits established for accuracy 70 – 150%.

Table 3.2 Percent recovery results of spiked sample analysis in the assessment of precision and intermediate precision (%RSD).

Mercury Validation - Intermediate Precision System Suitability		**Hg** (PPT)
Analyst 1	Std 1	10.00
	Std 2	25.00
	Std 3	50.00
	Std 4	100.00

	Std 5	150.00
	50 ppt Check Standard recovery, %	94
R (correlation coefficient)		1.00
Test Solution Results – Spike Recoveries		**Hg** (% Recoveries)
Analyst 1; Analyst 2	ANALYST 1 – Int. P-TS-100 #1	90
	ANALYST 1 – Int. P-TS-100 #2	98
	ANALYST 1 – Int. P-TS-100 #3	99
	ANALYST 1 – Int. P-TS-100 #4	107
	ANALYST 1 – Int. P-TS-100 #5	101
	ANALYST 1 – Int. P-TS-100 #6	110
	ANALYST 2 – Int. P-TS-100 #1	111
	ANALYST 2 – Int. P-TS-100 #2	110
	ANALYST 2 – Int. P-TS-100 #3	110
	ANALYST 2 – Int. P-TS-100 #4	114
	ANALYST 2 – Int. P-TS-100 #5	112
	ANALYST 2 – Int. P-TS-100 #6	110
	Avg.	106
	%RSD	6.8

3.4 Robustness

The robustness is defined as a measure of the method's capacity to withstand small changes in the test procedure and/or run conditions. The robustness experiments were developed to mimic small variations that could be expected in the routine use of this method and measure its susceptibility to the variations. Since the instrument parameters could be locked through a feature of the software, instrument setting changes were not investigated. However, weekly to monthly preparations of reagent solutions, including the diluent (5% hydrochloric acid) and reductant (2% stannous chloride in 2% hydrochloric acid) are required, therefore a potential error in these reagent preparations does exist. Since the reductant reagent needs to be added in excess for the reaction to proceed to completion, and an increased concentration of acid helps to further stabilize and digest the sample, it was ideal to evaluate the effect of decreased concentrations of each reagent solution on the performance of the method. A deliberate 25% reduction in respective diluent acid and reductant concentrations were used to prepare three separate runs consisting of a set of standards and six sample solutions spiked to 50 ppt. The robustness experiments were designed to evaluate the stress of these reagents on the analysis: the first run was prepared with 3.75% hydrochloric acid diluent and ran with 2% stannous chloride in 2% hydrochloric acid, and the second run was prepared with 5% hydrochloric acid diluent and ran with 1.5% stannous chloride in 2% hydrochloric acid, and the last run was prepared with 3.75% hydrochloric acid diluent and ran with 1.5% stannous chloride in 2% hydrochloric acid. Each sample was evaluated against the previously established criteria of percent recovery withing the range of 70-150%. This experiment design was adapted from Youden's popular approach which required the identification of each influential factor, then for each identified factor, the nominal and extreme values were tested in a random order experimental design at the midpoint of the calibrated range [21] [27]. The results were summarized in Tables 3.3 – 3.5.

Table 3.3 Percent recovery results of spiked sample analysis in the assessment of robustness: decrease in reductant concentration.

Mercury Validation - Robustness : Reductant System Suitability		**Hg** (PPT)
Analyst 1	Std 1	10.00
	Std 2	25.00
	Std 3	50.00
	Std 4	100.0
	Std 5	150.0
	50 ppt Check Standard recovery, %	98
R (correlation coefficient)		1.00
Test Solution Results – Spike Recoveries		**Hg** (% Recoveries)
Analyst 1	R-R-TS-100 #1	97
	R-R-TS-100 #2	96
	R-R-TS-100 #3	98
	R-R-TS-100 #4	97
	R-R-TS-100 #5	96
	R-R-TS-100 #6	97
	Avg.	97
	%RSD	0.8

Table 3.4 Percent recovery results of spiked sample analysis in the assessment of robustness: decrease in diluent concentration.

Mercury Validation - Robustness : Reductant - Diluent System Suitability		**Hg** (PPT)
Analyst 1	Std 1	6.000
	Std 2	15.00
	Std 3	60.00
	Std 4	120.0
	Std 5	150.0
	50 ppt Check Standard recovery, %	95
R (correlation coefficient)		1
Test Solution Results – Spike Recoveries		**Hg** (% Recoveries)
Analyst 1	R-D-TS-100 #1	134
	R-D-TS-100 #2	120
	R-D-TS-100 #3	118
	R-D-TS-100 #4	121
	R-D-TS-100 #5	122
	R-D-TS-100 #6	122
	Avg.	123
	%RSD	4.7

Table 3.5 Percent recovery results of spiked sample analysis in the assessment of robustness: decrease in both of a reductant and a diluent concentrations.

Mercury Validation - Robustness : Reductant - Diluent **System Suitability**		**Hg** (PPT)
Analyst 1	Std 1	6.000
	Std 2	15.00
	Std 3	60.00
	Std 4	120.0
	Std 5	150.0
	50 ppt Check Standard recovery, %	93
R (correlation coefficient)		1.00
Test Solution Results – Spike Recoveries		**Hg** (% Recoveries)
Analyst 1	R-RD-TS-100 #1	129
	R-RD-TS-100 #2	129
	R-RD-TS-100 #3	128
	R-RD-TS-100 #4	128
	R-RD-TS-100 #5	131
	R-RD-TS-100 #6	126
	Avg.	128
	%RSD	1.1

Compared to production processes where raw materials go in as input, and after multiple steps the finished product is produced as the final output, methods of analysis are often considered similarly as smaller scale processes [26]. Within production environments, process capability index (Cpk) is often used to assess the ability of a process to perform within the defined specification. Cpk is a process index that statistically describes the potential capability of a process to perform within defined limits. It is essentially a measure of the likelihood of a process to randomly produce results outside of the specification limits, assuming the process is in control and normally distributed. The equation for this metric is included below as Eq. 3.2, and where the upper and lower specification limits are indicated as USL and LSL, respectively; standard deviation is indicated by σ, and $\bar{x}$ is the mean.

$$Cpk = \min\left\{\frac{USL-\bar{x}}{3\sigma}, \frac{\bar{x}-LSL}{3\sigma}\right\} \quad \text{(Eq. 3.2)}$$

The Cpk was used to evaluate all three levels assessed for accuracy to further prove the fitness of this method for its intended purpose in measuring mercury levels above and below the limit (50 ppt). The specification limits for percent recovery of 70 – 150% were used in calculations for the respective, LSL and USL values. Acceptable Cpk values demonstrate the dispersion of results and the set specification are within the methods range of capability. Generally accepted minimum levels of Cpk are included below: [26] [28].

- Cpk < 1.00, indicates method is not adequate to meet specifications
- 1.33 ≥ Cpk ≥ 1.00, indicates process is adequate, but will require close control
- 2.00 ≥ Cpk ≥ 1.33, indicates process is adequate
- Cpk > 2.00, indicates process excellence

The test results are summarized in Table 3.6.

Table 3.6 Calculated Cpk at each level from accuracy results.

Concentration level (ppt)	Cpk
25	7.61
50	4.98
100	3.22

IV. Conclusion

The analytical method for the detection of mercury in effluent wastewater from the persulfate manufacturing process using CVAFS was developed and validated based on previously established guidance from USP and ICH. System suitability was established at the start of each run, with subsequent evaluation of the following parameters: selectivity, accuracy, precision, intermediate precision (ruggedness), and robustness. The range of the test methods for LOQ has been established by the calibration curve (with a test solution dilution factor of 1.67) of 10 – 250 ppt.

Linearity of the standard solutions was assessed from the linear regression of intensity vs. concentration. The linearity results are included in the system suitability table for each experiment performed. All results met the acceptance criteria of $r \geq 0.99$. Standard longevity was established at no more than four days following initial preparation, by measuring a preparation of standards on day 1 and then again 4-days later. The peak heights were baseline-adjusted by subtracting the blank value determined at the start of each run. The percent difference of each solution at each standard level was then calculated and no solution was found to have a percent difference $\geq 2.0\%$. Selectivity was assessed and verified by analyzing neat sample preparations vs. digested and diluted sample preparations and diluent blank injections for the absence of interfering peaks within the retention time window of mercury. The identification of the mercury peak was obtained by comparative analysis of the sample peak to that of the reference standard solution. There were no interfering peaks observed in the diluent blank injection, and the retention time and peak shape of both the standard solutions and samples were a positive match for mercury, therefore suitable specificity can be claimed for the method.

In the assessment of accuracy and precision , a second analyst prepared 6 samples at the 100% level for intermediate precision (ruggedness). The concentrations of sample test solutions were 50, 100, and 200 % of the limit for mercury. All results met the acceptance criteria for accuracy and precision. Apart from linearity, selectivity, accuracy and precision, the robustness of the method was also assessed per ICH Q2 (R1) guidelines. Two parameters most susceptible to variation were evaluated as detailed in

the robustness section: diluent acid and reductant tin chloride. The concentration of sample test solutions used in the evaluation of robustness were prepared at the 100% level. All results recovered within the acceptable range of 70 -150%. Calculated recoveries for adjusted reductant and diluent experiments were biased high but still recovered within the acceptable range. This information is good to know in the occasion of future troubleshooting if similar patterns are demonstrated in the daily use of this method. Acceptable robustness results further prove that the methodology is analytically sound and resistant to the day-to-day human error and variability associated with QC laboratory analysis. The results of method validation are summarized in Table 4.1.

Table 4.1 Summary of method validation results

Accuracy (70-150% sample spiked recovery)	**Precision – Repeatability** (%RSD ≤25)	**3 preparations - 50% Level**	Accuracy **PASSED**
			Precision **PASSED**
		3 preparations - 100% Level	Accuracy **PASSED**
			Precision **PASSED**
		3 preparations - 150% Level	Accuracy **PASSED**
			Precision **PASSED**
Precision – Intermediate (%RSD ≤25)		**6 preparations by Analyst 1 - 100% Level**	**PASSED**
		6 preparations by Analyst 2 - 100% Level	
Robustness (70-150% sample spiked recovery) *Biased high		**6 preparations - Modified Diluent - 100% Level**	**PASSED***
		6 preparations - Modified Reductant - 100% Level	**PASSED**
		6 preparations - Modified Diluent and Reductant - 100% Level	**PASSED***

All other acceptable criteria set forth in the method and report were met. Therefore, the method is validated suitable for its intended use. As highlighted in table 4.1, the system biased high in the robustness experiments, specifically when the diluent was modified – further research into the mechanism and chemistry underlying this trend could provide improvement to the methodology.

V. References

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