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# Simultaneous sub-picogram speciation of monomethylmercury and monoethylmercury in caustic nuclear tank waste using aqueous propylation

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## Abstract

Monoalkylmercury species have been measured in nuclear waste tanks at Savannah River Site, a superfund nuclear site in South Carolina. Common and standard methods for organomercury speciation could not be implemented within the context of the radioanalytical facilities in Savannah River National Laboratory (SRNL) in a safe, cost-efficient manner to facilitate data-driven regulatory action. SRNL conducted development, optimization, and validation work focused primarily on combining monomethylmercury and monoethylmercury speciation into one analysis to reduce method runtime, limit analyst radiation exposure, and improve the instrumental footprint area. Sodium tetra-n-propylborate was used as a derivatizing agent to enable simultaneous chromatographic resolution of monomethylmercury and monoethylmercury. Linear calibration of monomethylmercury and monoethylmercury ranged five orders of magnitude, generating detection limits of 0.033 pg and 7.50 pg, respectively. Calibration verifications maintained 101% and 99.1% accuracy, respectively, with mean recoveries from waste samples of 98.8% and 98.4%. Dilution volume was optimized to eliminate sample distillation, reduce sample radioactivity by 100% and 99.99992% for alpha and beta/gamma radiation, improve method runtime by 337%, and decrease total instrumentation footprint by 60.4%, compared with current standard methods. This method was validated internally against certified standards and externally via interlaboratory comparison. Compared with standard methods, this work represents a significant improvement in safety, efficiency, and sensitivity of industrial organomercury speciation, particularly in industrial nuclear analysis.

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Mercury is ubiquitous as a global pollutant, arising from natural and anthropogenic sources in nearly all types of environmental samples. The detrimental biological and environmental effects of mercury are well established,[1] and the worldwide threat of mercury pollution has been the focus of recent international efforts.[2] Among anthropogenic sources of mercury, fossil fuel processing and mining are significant contributors due to their inherent mercury content.[3] Some industrial process, however, use reagent mercury for catalytic purposes; these processes, thus, produce mercury-contaminated waste.[4] Savannah River Site (SRS) is a Department of Energy (DOE) nuclear facility and superfund site in Aiken, South Carolina that produced tritium, plutonium, and other special nuclear materials for national defense. Today, SRS houses two large tank farms for storing high-level nuclear waste and sludge, as well as waste processing facilities, environmental monitoring laboratories, and Savannah River National Laboratory (SRNL).

Liquid waste stored in tanks at SRS undergoes processing and remediation by separation, stabilization, and storage.[5] The high-activity waste is vitrified and storage in steel casks, while lower-level waste is transformed into a cement.[6] For five decades, elemental mercury has been used to aid in catalytic dissolution of aluminum present in the tanks from numerous sources. This ongoing process has resulted in the deposition of over 60,000 kg of mercury into the tank farms.[7] Optimization of the vitrification process has required tank-waste simulants and models to investigate long-term stability and leaching potential of vitrified waste. These tests presumed the presence of elemental mercury, mercuric oxide, and mercuric nitrate. However, organic mercury species have been discovered in recent routine analyses, primarily in the form of alkylmercury species like methylmercury and ethylmercury.[7] While the formation mechanisms of these species in tank waste has not been fully elucidated, the ability of vitrified waste to prevent leaching of mercury species has been investigated by researchers.[8] Vitrified waste leachate was found to contain predominately organomercury species, indicating that the vitrified waste form may not adequately remediate organomercury from the environment. Quantification of organomercury in the tanks prior to vitrification is necessary to ensure long-term stability and adherence to all environmental regulatory requirements.

The U.S. Environmental Protection Agency (EPA) has produced standard methods for the analysis of organomercury species in aqueous environments.[9,10] In these methods, and other

commonly used techniques, mercury species are analyzed by gas chromatography (GC) interfaced with one of several types of detectors.[11,12] Species are isolated from potential interferents using carbon trapping or distillation, [9,13,14] then derivatized to the fully alkylated forms to increase volatility. Derivatizing agents alkylate the charged mercury species using ethyl, phenyl, and, less commonly, propyl functional groups. Once volatile, the alkylmercury species can be purged from an aqueous solution using gas bubblers and trapped prior to thermal desorption into a carrier gas for GC analysis. Common detectors used for this work include inductively coupled plasma mass spectrometry (ICP-MS) and atomic fluorescence spectroscopy (AFS). [10,15] Quantitative limits in modern organomercury speciation typically range between 0.10 pg/g – 100 pg/g, dependent upon the analytical techniques employed.[12,16-19]

In a nuclear environment, these common techniques for mercury speciation create several drawbacks: runtime, laboratory space, and method efficacy. The increased sample preparation time resulting from serial (i.e. not simultaneous) analysis of species, as well as carbon trapping or distillation processes, can prevent regulatory data being received within an actionable timeframe. Instrumentation for the analysis of radioactive samples must be fully contained within a certified radiological fume hood, creating a priority for instrumentation with reduced footprint areas. Researchers have shown that, in high concentrations, chloride present in samples can reduce the effectiveness of methylmercury derivatization to less than 10%.[12] In addition, researchers have demonstrated that, under some conditions, substantial amounts of methylmercury may be formed during distillation in samples containing $Hg^{2+}$ ions.[20]

Many previously ubiquitous techniques for mercury analysis in industrial application have been shown to provide insufficient control over interferents and species conversion.[12,20-22] While acknowledging the shortcomings of past techniques, sensitive and modern methods for the accurate speciation of alkylmercury species have been developed in recent years;[15-19,23] but similar research has yet to be dedicated to optimize and validate mercury speciation for complex and caustic industrial applications. This research sought to build upon work on simultaneous speciation of monoalkylmercury compounds, while conforming to the unique safety and security practices of a nuclear facility.[13] Outside of nuclear waste, this research may be applicable to mercury speciation in other industrial analyses of highly complex or caustic matrices. This research hypothesized that a fast, safe, sensitive, and accurate method could be developed to

quantify monomethylmercury and monoethylmercury simultaneously in caustic nuclear waste tank samples, while decreasing radiation exposure, diluting interfering compounds, removing analytically harmful preparatory steps, and reducing the instrumental footprint.

## Experimental

### Safety and security

The work described herein is not intended to provide a thorough review of all safety and security protocols in place at SRNL and DOE, nor all necessary precautions that should be taken for safe and secure analysis of radioactive samples. SRNL has in place numerous administrative controls that limit access and grant accountability to secure and limited areas. The purpose of these protocols prescribed by DOE is to control introduction of individuals and materials into limited areas, handling and disposal of low- and high-activity radioactive samples and waste streams, nuclear criticality and safety awareness, and many other aspects of handling, processing, and storage of materials within SRNL to ensure the safety and security of analysts, laboratories, and instrumentation.

The analysts performing this work were registered on radiological workers permits that govern the radiological exposure limits of specific laboratory work. All analysts working with radioactive materials were required to have completed a radiological workers training courses, with periodic recertification. This research was performed inside of a laboratory designated as a radiological buffer area (RBA), and inside of a hood certified for radiological work, designated as a potentially contaminated area work area (CA). The RBA dose rate limits were 0.05 mSv/hr (at 30 cm) and transferable contamination limits were 0.02 Bq/100 $cm^2$ α and 0.20 Bq/100 $cm^2$ βγ. The CA hood dose rate limits were 0.05 mSv/hr at 30 cm and transferable contamination of 3.33 Bq/100 $cm^2$ α and 166 Bq/100 $cm^2$ βγ. No airborne contamination was permitted in the RBA or CA. The CA hood was surveyed periodically for whole body and skin dose rates as well as transferable contamination and airflow. The CA hood demonstrated acceptable whole body and skin dose rates, as well as airflow velocity and volume.

**Tank sample collection and storage**

Prior to arrival at SRNL, 250 mL variable depth samples were collected by Savannah River Remediation (SRR, Aiken SC) from a selected tank following approved tank sample collection protocols. Typical waste samples are highly caustic aqueous solutions containing high concentrations of salts and various organic compounds. The waste sample was transported to SRNL high-activity lead-lined shielded cells for apportionment and dilution. Using remote-operated robotic arms in the shielded cells, one 30 mL aliquot was transferred to a Teflon bottle without headspace and one 15 mL aliquot was portioned into a glass vial without headspace and sealed with a Teflon-lined cap. These samples were stored in the dark once removed from the cells. The 15 mL allotment was set aside for unrelated analysis. The 30 mL aliquot underwent a 1:2500 dilution with deionized water and 1.2 mL concentrated HCl preservative in a chemical fume hood rated for radioactive work in preparation for mercury speciation. Amber glass bottle containing 250 mL portions of this diluted sample were stored in SRNL refrigerators at 4 – 6 °C until analysis.

**Sample analysis and quality control**

Prior to speciation, the 250 mL bottle containing the 1:2500 tank sample dilution and preservative was removed from the refrigerator and underwent further dilution, derivatization, purge & trap (P&T), gas chromatography (GC), pyrolysis, and cold vapor atomic fluorescence spectroscopy (CVAFS). The analytical method used for this analysis was based on Methods 1630 and 1631 from the U.S. Environmental Protection Agency (EPA), prescribing the standard methods for the analysis of mercury and methylmercury in water by P&T-GC-CVAFS.[9,10] Sample batches for methylmercury analysis, ethylmercury analysis, and simultaneous methylmercury and ethylmercury analysis were prepared following the same procedure- with modification to only the identity of standards used, the derivatizing agent used, and the instrumental parameters.

Standards were prepared from certified stock solutions. A 10 µg/mL methylmercury or ethylmercury calibration standard (referred to herein as methyl/ethylmercury) was prepared using 0.1 mL of a 1 mg/mL Me/EtHgOH stock solution and 9.9 mL of HPLC reagent water into an acid-washed glass vial. The vial was capped and shaken thoroughly. A 1 µg/mL working standard was prepared by pipetting 1 mL of the calibration standard and 9.0 mL of reagent water

into an acid-washed glass vial and shaken thoroughly. A 10 µg/mL and 1 µg/mL quality control (QC) standard pair was prepared from the Me/EtHgCl solution as above. Calibration standards were prepared at 10, 50 250, 500, and 1000 pg from the 1 µg/mL working standard. Blanks were prepared using 0.3 mL of 2M acetate buffer and 39.7 mL reagent water. Calibration verification samples were prepared at 250 pg using 0.3 mL of 2M acetate buffer, 0.25 mL of the working standard, and 39.45 mL of reagent water. To prevent fouling of analytical equipment, tank samples attained approximately 1,000,000-fold dilution before introduction into the MERX-M. To prepare the tank samples for analysis, 0.1 mL of each 250 mL amber glass bottle was diluted to an appropriate dilution factor via serial dilution to effect a 1,000,000-fold dilution (2500-fold, 100-fold dilution, 100-fold dilution, then 0.4:1). For experimental purposes, a 2,000,000-fold dilution was also performed on all tank samples by changing the final step of the serial preparation to a 0.2:1 dilution. These final vials were spiked with 0.3 mL of 2M acetate buffer, 0.05 mL of derivatizing agent, filled to the top with reagent water to eliminate headspace, and sealed with a Teflon-lined cap before being inverted to effect mixing.

CVAFS detector was calibrated to achieve maximum peak height by adjusting the photomultiplier tube voltage such that a 25 pg standard of methylmercury produced a peak height of approximately 12,000 counts. Before the start of each batch of samples, the detector was “zeroed” such that the baseline background reading would be subtracted, producing a baseline of zero counts. The qualitative and quantitative software would automatically measure baseline noise before the start of each batch of samples. The noise measured must produce a standard deviation less than 100 counts to for the analysis to continue. A quality assurance / quality control (QA/QC) template was used as part of an SRNL measurement control system for each batch of sample to ensure the proper rinses, blanks, calibrants, calibration verifications, sample sets, and closing blanks were run with each batch. This measurement control system was designed as a method to monitor the performance of the GC-CVAFS measurement system and to provide a graded approach to establish appropriate quality of the data for the task requirements. Prior to GC-CVAFS, each sample is held on one of three Tenax TA trap (Buchem BV, Apeldoorn, The Netherlands). Thus, each QA/QC vial-type was run in triplicate. This template can be found in table 1.

Table 1: Tank sample batch template that was followed to ensure QA/QC

| Vial Number | Vial Type | Sample Description | Matrix |
|---|---|---|---|
| 1 | 3x Rinse | Non-analytical opening blank to flush system | Deionized water |
| 2 | 3x Blank | Reagent blank to establish baseline | Deionized water |
| 3 | 5x Calibration | Calibration curve standards | Deionized water |
| 4 | 3x Calibration verification | Prepared as the mid-point calibration standard | Deionized water |
| 5 | 3x Blank | Reagent blank to establish baseline | Deionized water |
| 6 | Sample | 1,000,000- fold dilution of the received sample | Tank sample |
| 7 | Sample 2x dilution | 2,000,000-fold dilution of the received sample | Tank sample |
| 8 | Matrix spike | Tank sample spiked with calibrant standard | Tank sample |
| 9 | 3x Calibration verification | Prepared as the mid-point calibration standard | Deionized water |
| 10 | 3x Closing blank | Reagent blank to establish baseline | Deionized water |

Criteria for acceptance of each QA/QC vial type were defined following consultation with the vendor, the guiding EPA methods, and optimized analytical parameters. Blank and rinse vials must produce no peaks exceeding the method limit of detection (LOD). The mean accuracy of the calibration standards, defined by how accurately the experimentally determined concentration of each standard matches the prepared concentration, must be 80 – 120%. The matrix spike must be recovered at a value 65 – 135%. Precision of the calibration verification samples must be < 31% relative standard deviation (%RSD). For internal and external validation work, %RSD and 95% confidence intervals (where indicated) were calculated as a standard for comparison with calculated concentrations. Likewise, a p-value cutoff of p=0.05 was used for hypothesis testing. Method and reporting limits of detection (LOD) and quantification (LOQ) were determined by analysis of replicate blanks (n=15) and calculated as follows, where $\sigma_n$ is standard deviation of n samples:

$$\textit{Equation 1: } LOD = \sigma_n \times t_{99,n-1}$$

$$\textit{Equation 2: } LOQ = LOD \times 3.33$$

**Instrumentation and analytical parameters**

A MERX-M system for the analysis of total mercury and organomercury (Brooks Rand Instruments) was used for the analytical aspect of this work. Samples containing organomercury were derivatized to induce volatility, distilled to isolate the analytes of interest, purged of volatile compounds, separated via isothermal gas chromatography, reduced to Hg(0) via pyrolysis, and detected by CVAFS. The analytical instrument consisted of a 72-position MERX Autosampler tray, a Hg Speciation P&T module, three Tenax TA traps, a Hg Speciation GC and Pyrolysis

module containing a mini-column GC (operated isothermally at 36 °C) and pyrolysis trap (held at a stable temperature of 700 °C), and Model III CVAFS photomultiplier tube detector (peak emission wavelength of 253.7 nm). For testing and optimization, a 10-position Methylmercury Distillation System (Brooks Rand Instruments), including a heated sample-holding block rack and chilled Teflon tube disposition reservoir rack with accompanying 10 rotameters to control gas flow, was used to isolate methylmercury from solution and potential interferences. Mercury Guru Software was used for instrument control and data analysis. Derivatizing agent-dependent analytical parameters can be found in table 2. The purging gas, drying gas, and GC carrier gas flow rates were 50, 40, and 35 PSI, respectively. Gas flow rates were controlled using instrument rotameters.

Table 2: Derivatization agent-dependent instrumental parameters

| **Ethylation Batch** | | **Propylation Batch** | |
|---|---|---|---|
| *Parameter* | *Time (minutes)* | *Parameter* | *Time (minutes)* |
| Run Duration | 5.0 | Run Duration | 10 |
| Heat Duration | 9.9 | Heat Duration | 9.9 |
| Retention Start Time | 1.1 | Retention Start Time | 4.0 |
| Retention Stop Time | 1.5 | Retention Stop Time | 5.0 |
| Drying Duration | 3.0 | Drying Duration | 4.0 |
| Purge Duration | 5.0 | Purge Duration | 9.0 |

Two derivatizing techniques were used: tetraethylborate (so-called “ethylating” agent) and tetra-n-propylborate (“propylating” agent). Both agents act as reducing agents to convert cationic mercury species, such as $CH_3Hg^+$, $C_2H_5Hg^+$, and $Hg^{2+}$, into fully-alkylated organomercury. Following derivatization with sodium tetraethylborate, these three cations will have become only two new compounds upon the addition of one or two ethyl- groups: methylethylmercury ($C_3H_8Hg$), and diethylmercury ($C_4H_{10}Hg$). To achieve speciation of ethylmercury and its separation from $Hg^{2+}$, sodum tetra-n-propylborate must be used. Following derivatization with sodium tetra-n-propylborate, these three cations will become methylpropylmercury ($C_4H_{10}Hg$), and ethylpropylmercury ($C_5H_{12}Hg$), and dipropylmercury ($C_6H_{14}Hg$). The process of reductive derivatization by sodium tetraethylborate has been thoroughly explored in other research, particularly in its application to methylmercury. However, sodium tetra-n-propylborate has not been extensively explored as a viable alternative, specifically in its ability to allow for simultaneous quantification of methyl- and ethylmercury.

The mechanisms of derivatization of organometallics using organoborates have been described previously.[24]

Blind interlaboratory comparison was performed in a commercial laboratory certified in ISO/IEC 17025:2005 to performed radiological measurements. Tank samples were prepared by SRR, with one aliquot sent for analysis externally and one aliquot stored for analysis by SRNL. The analytical procedure used by the external laboratory remains proprietary, though the method differs from the method developed in this work in that sequential purging and trapping steps were employed to retain a solution of volatilized organomercury from the initial purging step for potential reanalysis.

Radioactivity was measured for both laboratory safety and to assess the effect of dilution on radiation using Ludlum Series 10 Model 12 (Ludlum Measurements Inc., Sweetwater, TX) with a Model 44-9 "pancake probe" detector for assessing counts per minute (cpm) of beta and gamma radioactivity and a Model 43-136 probe detector for assessing cpm of alpha particles. The cpm measurement must be converted into disintegrations per minute (dpm), where 1 dpm is equal to 60 becquerel. This conversion is shown in equation 3, where $cpm_s$ is the counts per minute measured at a sample following radioactivity field monitoring protocols and $cpm_b$ is the background cpm measured following facility-specific field monitoring protocols.

$$\textit{Equation 3: } dpm = (cpm_s - cpm_b) \times 10$$

Alpha and beta / gamma were assessed on a routine tank sample in a glass bottle destined for cementious remediation prior to sample preparation or dilution. Alpha and beta / gamma were then measured in cpm on the sample following a 4,000,000x serial dilution in a glass vial. Background measurements were taken at the hood sash and vial measurements were taken ~1 cm distance from the vial. These measurements were performed by a certified radiological worker using verified and calibrated field-monitoring instruments. The measurements of radioactivity were not made by radiochemical methods nor with specialized high-sensitivity radioanalytical scintillation devices. The dpm results obtained using the handheld field monitors must be considered approximate.

**Reagents and consumables**

Helium carrier gas was used in the GC-CVAFS system (99.99% purity). Argon was used as the purging gas for the P&T system (99.99% purity). All reagent water was deionized water or HPLC grade reagent water from Fisher Scientific (Hampton, NH), where indicated. Buffering of samples was performed with 0.3 mL of 2M acetate buffer (Brooks Rand Instruments), certified free of methylmercury and suitable for application to EPA Method 1630. Preservation of samples was performed using 1.2 mL of 12.1 molar hydrochloric acid (Fisher Scientific). Preparation of sodium tetraethylborate and sodium tetran-propyl borate required potassium hydroxide (KOH, Fisher Scientific). Ethylmercury chloride was purchased from AccuStandard (New Haven, CT) at 100 μg/mL in methanol, methylmercury chloride (Brooks Rand Instruments) was purchased at 1 μg/mL in 0.5% (v/v) acetic acid and 0.2% (v/v) hydrochloric acid, and methylmercury hydroxide (Brooks Rand Instruments) was purchased at 1 μg/mL in 0.5% (v/v) acetic acid and 0.2% (v/v) hydrochloric acid. Methylmercury chloride and methylmercury hydroxide were certified and traceable to NIST 1641D, and ethylmercury chloride was certified by a laboratory holding ISO/IEC 17025:2005 certification. SRR prepared non-radioactive tank waste simulant, which matched the ionic, organic, and alkaline characteristics of the radioactive tank waste. This simulant was used for non-radioactive optimization work where noted.

The two derivatizing agents were prepared in-house using a 2% KOH solution and sodium tetraethylborate or sodium tetra-n-propylborate in tetrahydrofuran. The solutions were mixed, gently inverted, and distributed into separate 4 mL Teflon bottles before being transferred to a -20 °C freezer. One Teflon bottle was removed from the freezer at the start of sample preparation for one batch of samples. The bottle and any remaining derivatizing agent were discarded following a single use. This solution has a vendor-listed expiration of 3 hours once thawed. All monoethylmercury results were obtained using the n-propyl derivatizing agent. Monomethylmercury results are indicated as being obtained using either agent.

**Contamination and interferences**

Standardized methods for the analysis of methylmercury in aqueous solutions emphasize the importance of separating the methylmercury cation from possible interferences and contaminants. To this end, distillation on the front-end of sample prep is often required, along with acutely precise acidification for the analysis of environmentally sourced samples. In

particular, gold and iodide are known interferences, causing recovery of mercury to be reduced from 100% to 0% with increasing interferent concentration. Another known, but under-studied, interferent is sulfur-containing compounds (particularly from organic sources) that may be present in municipal water supplies in areas with highly humic soil. [9]

Contamination the sample with laboratory mercury or interferents, and contamination of the environment or laboratory with mercury from the sample were the primary concerns in contamination control. As prescribed by standardized methods, several contamination control steps were employed in this research. The use of metal-free laboratory apparatus and sampling equipment, performing sample preparation and analysis in environments known to be free of contamination, using disposable apparatus or covering and cleaning non-disposable apparatus, and avoiding sources of contamination, were part of the guiding philosophy of cross-contamination prevention. Wide-mouth fluorinated polyethylene bottles (Brooks Rand Instruments) certified for use in EPA Methods 1630 and 1631 were used for storage of reagent water. Sources of contamination were avoided by performing well-designed carryover studies, removing unused samples and waste, and preventing airborne contamination as dust or aerosol.

## Results and Discussion

### Chromatography, Calibration, and Derivatization

Chromatographic resolution of ethylated monomethylmercury was achieved using sodium tetraethylborate; while, propylated monomethylmercury and monoethylmercury were resolved using sodium tetra-n-propylborate. As seen in figure 1 (left), the ethylating agent produced resolved peaks for elemental mercury (peak 1), derivatized methylmercury (peak 2), and a combined peak containing derivatized ethylmercury and $Hg^{2+}$ (peak 3). Figure 1 (right) shows the propylating agent effected separation for elemental mercury (peak 1), methylmercury (peak 3), ethylmercury (peak 4), and $Hg^{2+}$ (peak 5, not shown).

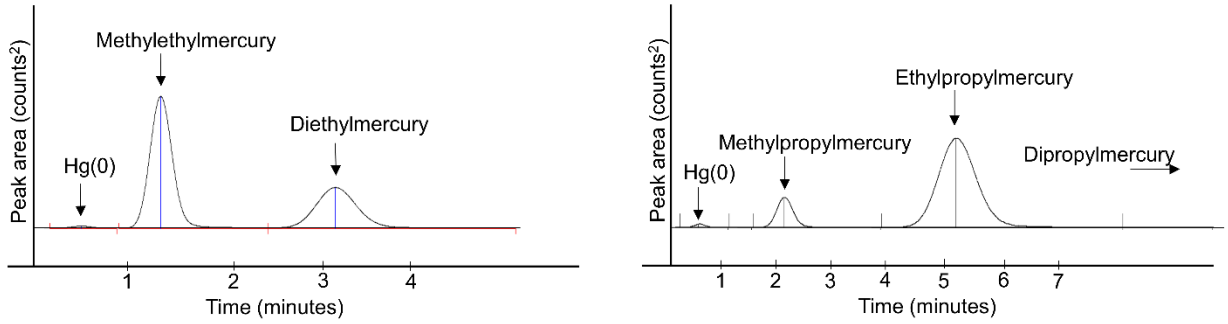


**Figure 1:** Chromatographic separation was achieved for both target organomercury analytes from the inorganic mercury species using both derivatization agents. Showing derivatives formed by sodium tetra-n-propylborate (right), and sodium tetraethylborate (left)

Linear and sensitive calibration of methylmercury and ethylmercury were achieved using the propylating agent in a solution containing a mixture of methylmercury chloride and ethylmercury chloride in deionized water. Calibration of methylmercury was also achieved using the ethylating agent, but ethylmercury was unable to be separated from $Hg^{2+}$. Monomethylmercury produced similar peak area, recovery, and %RSD under both derivatization schemes. Methylmercury calibration verifications demonstrated statistically similar mean accuracy (99.8% ±0.93% vs. 98.4% ±1.2%, p=0.115) using either derivatizing agent. This research was unable to produce calibration for elemental or cationic inorganic mercury peaks using either derivatizing agent.

A five-point calibration was performed using prepared standards of methylmercury chloride and ethylmercury chloride spiked together into deionized water and analyzed using tetra-n-propylborate and P&T-GC-CVAFS. The coefficient of determination for the calibration of methylmercury was 0.9999 with a linear range of 10 pg – 1000 pg. Methylmercury achieved a LOD of 0.0330 pg and LOQ of 1.11 pg, corresponding to an absolute LOQ of 0.0278 parts-per-trillion (ppt) in a 40 mL sample. At the low calibration point (10 pg), methylmercury was quantified with an accuracy of 94.7 ± 3.8%. Mean accuracy across all calibration points was 99.9%%. Calibration verification samples were conducted with methylmercury hydroxide, producing an accuracy of 101% ± 3.0%. Mean recovery of methylmercury in this mixed solution was 98.8% ±0.15%.

Ethylmercury was quantified using a five-point calibration curve at values identical to methylmercury. A coefficient of determination was achieved for this calibration at 0.9998 with a

linear range of 10 pg – 1000 pg. Ethylmercury achieved a LOD of 7.50 pg and LOQ of 22.4 pg, corresponding to an absolute LOQ of 0.560 ppt in a 40 mL sample. At the low calibration point (10 pg), ethylmercury was quantified with an accuracy of 96.4 ± 15%. Mean accuracy across all calibration points was 98.4% ± 6.8%. Calibration verification samples were conducted with ethylmercury hydroxide, producing an accuracy of 99.1% ± 0.90%. Mean recovery of ethylmercury in this mixed solution was 98.4% ± 2.3%. The experimentally determined LOQ values in this work meet or exceed LOQ values obtained by researchers utilizing commonly cited and standard methods.[17-19]

**Dilution Factors, Distillation, and Storage Time**

Given the comparatively restrictive regulations governing methylmercury, optimization of dilution factors, distillation, and storage parameters prioritized maintaining methylmercury analytical quality over ethylmercury. Dilution was used to reduce analyst radiation exposure. With an assumed mercury concentration in the tank samples between 10 – 1000 μg/mL organomercury, three dilution factors were analyzed: 2,000,000x, 1,000,000, and 2,500x, resulting in 0.20 ng, 0.40 ng, and 160 ng organomercury from a typical waste sample. Dilution was examined in a routine tank waste sample (n=3 per dilution). Accuracy (bias) was assessed by comparison of experimentally obtained concentrations values for methylmercury with known values generated by external analysis. These results are summarized in table 3. No significant difference in bias was observed in quantitative accuracy between the 2,000,000x and 2,500x ($p=0.671$). Expected organomercury concentrations in tank samples were permitted to dictate varying dilution levels to maintain adherence to the developed calibration curve.

Table 3: Methylmercury quantified in routing tank sample, compared with concentration data obtained externally

| Dilution Factor | Exp. Conc. (pg) | %RSD | %Bias |
|---|---|---|---|
| 2,000,000x | 151 | 1.75 | 0.667 |
| 1,000,000x | 157 | 4.46 | 4.67 |
| 2,500x | 153 | 6.42 | 2.00 |

Analyses were performed with distillation and without distillation (direct analysis) to assess effect on analytical quality. Given known tank concentrations, interferents present in a sample would be diluted to a level of ineffectiveness at 2,000,000x.[21] Figure 2 demonstrates the improved recovery when comparing distillation to direct analysis. Distillation and direct analyses were compared using non-radioactive ("cold") tank simulant samples (n=5). Cold direct analysis

achieved a mean recovery of 89.6% ± 2.7%, significantly greater than cold distillation analysis at 78.3% ± 4.8 (p=0.000860). Tank waste direct analysis demonstrated a mean recovery of 100.3% ±2.3%, statistically greater than cold simulant using direct analysis (p=0.000397). Results are shown from recovery studies performed in radioactive ("hot") tank samples for direct analysis only, as distilled tank waste was not expected to exceed the recovered of non-distilled (100.3%) and processing tank waste would have rendered the distillation system as high-level radioactive waste. In keeping with the DOE and ES effort to reduce the generation of nuclear laboratory waste, no distillation analyses were performed on tank waste samples.

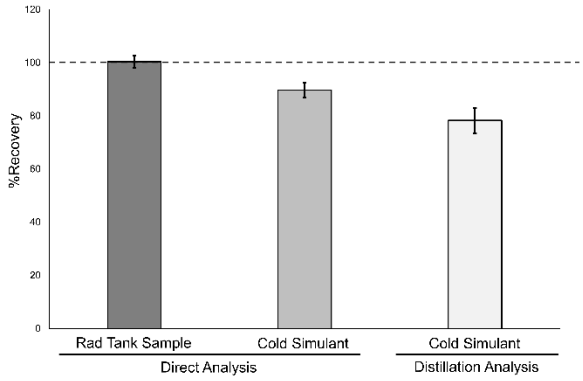


**Figure 2:** Differences in recovery of methylmercury observed between direct analysis and distillation analysis, and between radioactive and non-radioactive samples, showing %RSD. Dashed line 100% recovery

Internal research at SRNL has agreed with work published elsewhere in showing a degradation of stored methylmercury quantitative recovery over time, despite storage at optimal and preserved conditions. Methylmercury was optimized for storage time due to the extensive comparative research available for methylmercury relative to ethylmercury. Table 4 shows the bias observed between SRNL analysis of three radioactive tank samples and an external analytical laboratory. The external laboratory analyzed each tank sample with approximately the same time difference between sample collection and sample analysis (~120 days). SRNL analyzed the same samples (n=5) at time intervals of 135, 190, and 238 days between collection and analysis and compared the results with those achieved by the external lab. The samples analyzed at 136 and 190 days demonstrated no significant difference from the external lab results (p=0.000192, 0.05289), though interlaboratory bias increased from 6.0% absolute bias to 8.0%. At 238 day, SRNL demonstrated 28% bias compared with the external lab, which was significant

(p=0.1229). Pearson correlation between days of sample storage and the absolute bias in interlaboratory results was 0.7821, implying a strong correlation. These finding support research showing acid-preserved monomethylmercury storage limitations relative to non-preserved.[25,26] Further work is required to determine if reduction in analytical quality is due to analyte loss via absorption/adsorption to the glass or Teflon wall of the storage bottle or evaporative loss through the Teflon cap. Mass balance analyses must be performed, as well, to determine if the loss is due to organomercury species conversion. The maximum storage time for tank waste samples at SRNL was established at 180 days.

Table 4: Methylmercury analytical quality loss over time, compared with an external laboratory

| Days in storage | \|Interlaboratory Bias\| | SRNL %RSD | P-Value* |
|---|---|---|---|
| 135 | 6.0% | 1.0 | 0.000192 |
| 190 | 8.0% | 1.6 | 0.05289 |
| 238 | 28% | 0.2 | 0.1229 |

* Hypothesis test between results obtained by SRNL at the given time interval and those obtained by an external laboratory at ~120 days

Simultaneous chromatographic separation, speciation, and quantification of methylmercury and ethylmercury were achieved at low picogram levels. Removal of distillation improved method run time from 27 hours per batch to 8 hours per batch, a 337% improvement.

**Validation, application, and interlaboratory comparison**

Methylmercury and ethylmercury were analyzed at known concentrations as a mixture in deionized water and cold simulant by aqueous propylation derivatization. The analytical results were compared against the known concentration of the certified stock solution. Analytes were introduced into the analytical system via P&T; therefore, the validation parameters were calculated as analyte mass - not concentration. Solutions (n=5) were spiked with 250 pg of methylmercury chloride and 250 pg of ethylmercury chloride. Methylmercury was quantified in deionized water at 247 pg ± 0.247 pg and in tank simulant at 267 pg ± 10.68 pg. Ethylmercury was quantified in deionized water at 237 pg ±0.474 pg and cold tank simulant at 246 pg ± 2.58 pg. The positive bias of methylmercury in tank waste simulant and the negative bias of ethylmercury in deionized water compared with the certified standard were statistically significant (p=0.0495, 0.0486). In deionized water and tank simulant, ethylmercury was quantified with significant negative bias compared with methylmercury (p<0.00001, p=0.00145).

Matrix spike analyses demonstrated recovery for methylmercury and ethylmercury of 101% ±0.94% and 101% ±2.1%, respectively. Figure 3 demonstrates the results of the internal validation analyses.

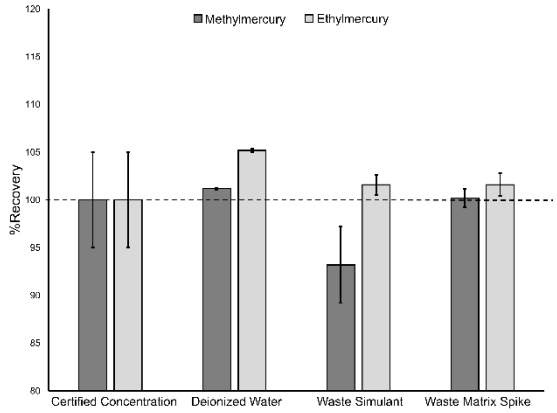


**Figure 3:** Methylmercury and ethylmercury solutions analyzed in deionized water and tank waste simulant, showing %RSD. Dashed line represents the known amount spiked into each matrix

A blind interlaboratory comparison study was performed in SRNL and a radiologically and ISO-certified external laboratory. To eliminate the reduced precision effected by the n-propyl agent, tetraethylborate was used to compare interlaboratory results. Results of the comparison can be found in table 5. Results are shown as concentration, mg/L, and precision, as %RSD, of four separate tank waste samples prepared by SRR and sent through the established protocol to the external lab and SRNL. If a non-detect was reported, "< LOQ" was returned, where LOQ was the calculated limit of quantification. No tank samples included in the study contained quantifiable amounts of ethylmercury, which is typical for quarterly tank waste analysis. SRNL exhibited -6.35% mean bias compared with the external lab, driven primarily by sample A. These sets of quantitative results for methylmercury do not significantly differ ($p=0.806$). SRNL demonstrated significantly greater precision of replicate analyses, ±0.93%, compared with the external lab, ±8.9% ($p=0.00812$). SRNL matched the external lab in identifying non-quantifiable samples. The LOQ reported by SRNL for methylmercury (which differs from the LOQ from section 3.1 due to the inclusion of volume) was 28-times greater than the LOQ reported by the external laboratory. The LOQ reported by SRNL for ethylmercury was 5.6-times lower than the mean LOQ reported by the external laboratory.

Table 5: Interlaboratory comparison of blind analyses performed by SRNL Analytical Development (AD) and an external laboratory on tank samples labeled A – D.

| | | SRNL AD | | External Lab | |
|---|---|---|---|---|---|
| | | Concentration (mg/L) | | Concentration (mg/L) | |
| | **A** | 44.1 | ±0.0882 | 61.6 | ±6.0368 |
| | **B** | 33.1 | ±0.331 | 35.2 | ±2.0416 |
| | **C** | < 13.8 | - | < 0.49 | - |
| | **D** | 39.2 | ±0.6272 | 36.1 | ±3.971 |
| | **A** | < 0.163 | - | < 0.017 | - |
| | **B** | < 0.163 | - | < 1.8 | - |
| | **C** | < 0.163 | - | < 1.7 | - |
| | **D** | < 0.163 | - | < 0.17 | - |

### Instrument footprint and radiation exposure

The total footprint (surface area) of the P&T-GC-CVAFS system, with distillation system, was measured as 1.69 $m^2$. A standard liquid chromatography- ICP-MS system would measure 1.05 $m^2$.[27,28] Upon removal of the distillation system, the footprint area decreased to 0.67 $m^2$. Removal of the distillation system decreased the footprint area of the analytical system by 60.4% relative to standard methods with distillation and 37% relative to another common technique. This allowed sample preparation in the same hood as sample analysis, resulting in substantial potential savings in hood-cost and analyst hours, as well as decreasing the likelihood of radioactive contamination events.

Measured background alpha radiation at the hood face was 0 dpm. Measured background beta / gamma at the hood face was 1,000 dpm. Following background subtraction, the undiluted sample measured $1x10^5$ dpm / mL alpha (or $4x10^6$ dpm alpha for a 40 mL sample), $3.0x10^8$ dpm/ mL beta / gamma (or $1.2x10^9$ dpm beta / gamma for 40 mL sample). Following sample preparation, 4,000,000x dilution, and background subtraction, the resulting vial measured 0 dpm alpha activity and 1,000 dpm beta / gamma activity in 40 mL. This method reduced the measured radioactivity by 100% for alpha particles and by $1x10^6$-fold for beta / gamma activity, in comparison with the recommended sample preparation in standard methods.[9,10] While total vial beta / gamma radiation decreased linearly with dilution, dpm/ mL of tank waste appeared to scale linearly per volume of original sample. No significant difference was observed in dpm / mL measurements for beta / gamma before and after dilution. The complete reduction in measured

alpha dpm was likely partially attributable to the increased glass wall thickness of the final sample vial.

### Contamination and interferents

No organomercury contamination was discovered in any blanks analyzed as part of QA/QC for the tank waste batches. When the high and low calibration points were used as upper and lower limits, no carryover was discovered in the analyses of a 1000 pg calibration sample, followed by blank analyses.

Significant contamination of the analytical system was reported early in development. The cause was localized to the deionized water, sourced municipally in South Carolina. Researchers have reported similar mercury contamination and carryover issues in the analysis of samples containing sulfur.[28,29] The soil, and therefore the municipal water, local to SRNL is highly humic- thereby imparting minor sulfur concentrations to the facility water, even after filtration and deionization.[30] The system contamination issue was not observed following exchange of facility deionized water for HPLC-grade reagent water.

## Conclusion

This method may enable speciation of monomethylmercury and monoethylmercury in industrial applications in which mercury is present in relatively high levels, with increased safety and efficiency by reducing analyst exposure to a sample, decreasing instrument footprint area, and improving method runtime, while meeting or exceeding reported LOQ values obtained using standard and commonplace methods. In future work, the source of organomercury loss over time should be explored completely. This developed and externally validated method has been applied to quarterly tank samples from the Savannah River Site for the analysis of organomercury.

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