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SAVANNAH RIVER NATIONAL LABORATORY
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SPECIATION OF METHYLMERCURY AND ETHYLMERCURY BY GAS CHROMATOGRAPHY COLD VAPOR ATOMIC FLURESENCE SPECTROSCOPY

Methylmercury speciation by ethylation and P&T-GC-CVAFS

Existing models and simulants of tank disposition media at SRS have presumed the presence of high concentrations of inorganic mercury. However, recent quarterly tank analyses show that mercury is present as organomercurial species at concentrations that may present challenges to remediation and disposition and may exceed the Saltstone Waste Acceptance Criteria (WAC). To-date, methylmercury analysis for Savannah River Remediation (SRR) has been performed off-site by Eurofins Scientific (Lancaster, PA). A series of optimization and validation experiments has been performed at SRNL, which has resulted in the development of on-site organomercury speciation capabilities using purge and trap gas chromatography coupled with thermal desorption cold vapor atomic fluorescence spectroscopy (P&T GC/CVAFS). Speciation has been achieved for methylmercury, with a method reporting limit (MRL) values of 1.42 pg for methylmercury. Results obtained by SRNL from the analysis of past quarterly samples from tanks 21, 40, and 50 have demonstrated statistically indistinguishable concentration values compared with the concentration data obtained from Eurofins, while the data from SRNL has demonstrated significantly improved precision and processing time.

Figure 1 demonstrates the separation of methylmercury from elemental mercury (Hg(0)) and the combined peak of ethylmercury and mercury^{++} following sample derivatization with sodium tetraethylborate.

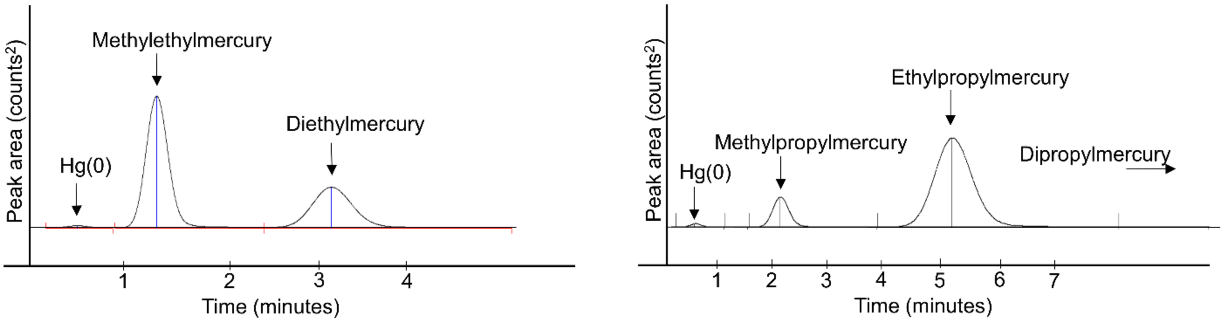

Figure 1: Demonstrating chromatographic resolution of methylmercury^{+} achieved using tetraethylborate derivatizing agent (left), and resolution of methylmercury and ethylmercury using tetra-n-propylborate (right)

Calibration of methylmercury using increasing concentrations of methylmercury spiked into deionized water produced a linear calibration curve of three orders between 0.025 and 25 ng/L concentrations. The mean relative standard deviation (%RSD) of the calibrant samples was 0.76%, with a mean accuracy across all calibration points of 99.7%

Determinations of method detection limit (MDL) and MRL were performed using equations 1 and 2 on n = 35 replicate measurements of laboratory blanks over a period of two weeks. An MDL was found to be 0.427 pg, with a MRL of 1.42 pg. At the MRL, a precision of 3.8 %RSD was achieved. In a 40 mL sample, this equates to 0.0355 pg/mL, or parts per trillion (ppt). The Saltstone WAC lists methylmercury limits in the order of parts per million (ppm). The significantly higher sensitivity afforded by this method allows for high dilution of waste samples, reducing the exposure received by an analyst.

$$\textit{Equation 1: } MDL = \sigma_{n} \times t_{99,n-1}$$

$$\textit{Equation 2: } MRL = MDL \times 3.33$$

$\sigma_{n} =$ standard deviation

Non-radioactive development testing of samples spiked with known concentrations of methylmercury allowed SRNL to validate the method performance. Figure 2 reports the results of cold testing of methylmercury analysis in water or tank simulant samples spiked with known concentrations of methylmercury.

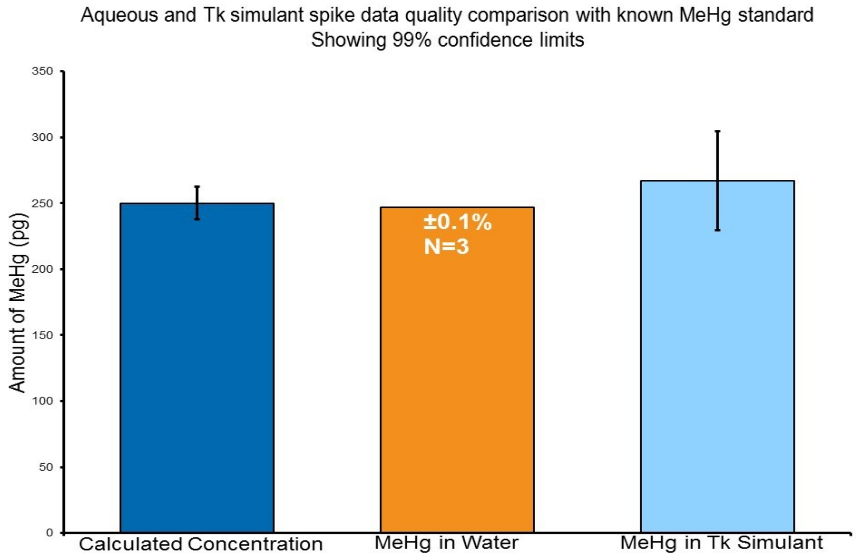

Figure 2: Cold testing of the speciation of methylmercury in deionized water and simulated tank waste

Comparison testing was performed on existing quarter tank samples: fourth quarter 2016 tank 50, first quarter 2017 tank 50, tank 40 – F3, and a tank 21 quarterly sample. Mean results from this analysis are summarized in figure 3. The mean results obtained by SRNL were negatively biased by 6.7% compared with data obtained on the same samples analyzed by Eurofins. Mean precision obtained by SRNL analyses was 0.933 %RSD, compared with 8.87 %RSD obtained by Eurofins. Calibration verification samples run concurrently with quarterly tank sample batches were prepared at 250 pg calculated concentrations. The mean accuracy of calibration verifications run with each quarterly tank sample was 99.8% accuracy.

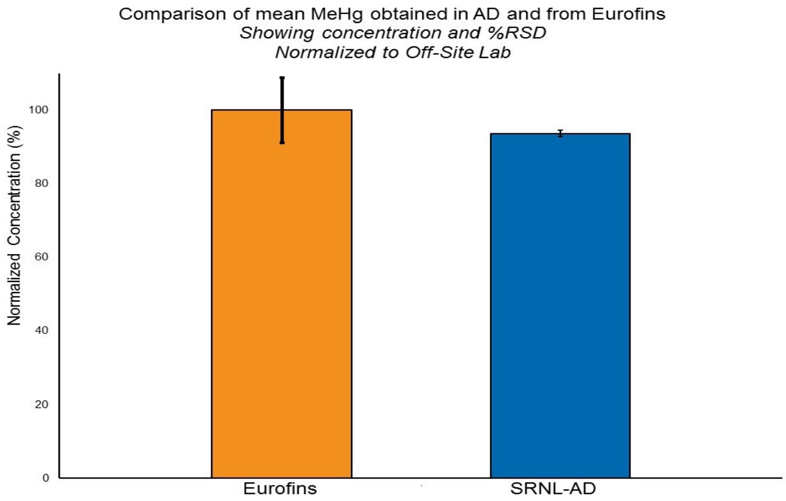

Figure 3: Comparison of mean analytical performance of SRNL laboratory compared with results obtained from the same samples analyzed by Eurofins

Matrix spikes were generated by the addition of a known concentration of methylmercury to tank samples. Following the analysis of the tank sample, a calculation was performed to quantify the known methylmercury spike. This calculation would demonstrate the presence of an interfering compound present in the matrix, potentially producing biased results. Matrix spike analyses produced a mean accuracy (recovery) across all samples of 100.2%. Improvement in recovery observed between distillation and direct analysis is shown in figure 4. Radioactive tank waste was not analyzed using distillation to avoid unnecessary fouling of laboratory equipment with contamination. All blank measurement results reported in the analysis of quarterly tank samples produced chromatographic peaks for methylmercury below the MDL. Specific data is provided in table 1.

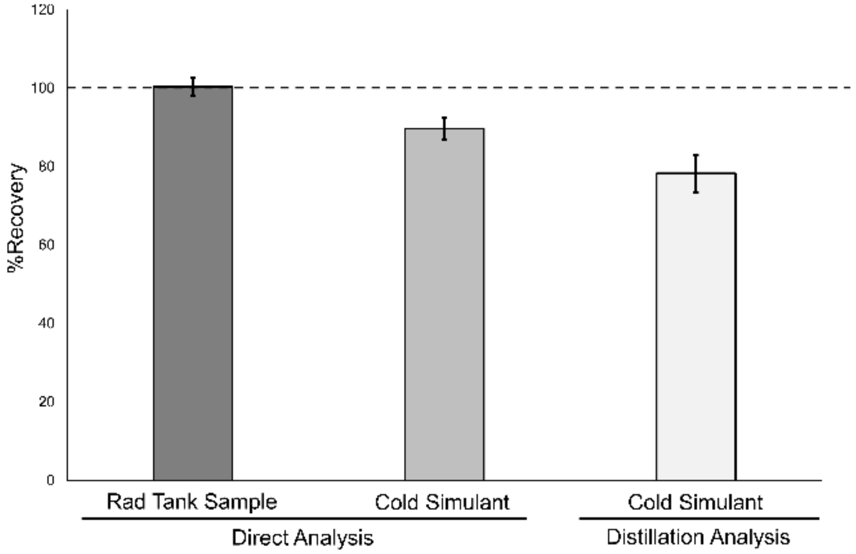

Figure 4: Recovery attained for methylmercury in the analysis of spiked methylmercury in simulant and radioactive tank samples, compared using distillation and direct analysis. Showing 95% confidence bars and indicating the 100% recovery level (dashed)

Table 1: Results obtained by SRNL for quarterly tank samples selected for interlaboratory comparison

4Q16 Tank 50				**Precision**		
4000x dilution	44.5	ppm	n=3	+/-	0.6	%
2000x dilution	45.5	ppm	n=3	+/-	0.4	%
ICV/CCV	103	%	n=4	+/-	3	%
Cal Curve	100	%				
Blanks	0.29	pg		+/-	49	%
4Q16 Blank	0.131	pg		+/-	63	%
Matrix Spike	101	%				
SRNL Value	44.1	ppm	n=6	+/-	1.3	%
1Q17 Tank 50				**Precision**		
4000x dilution	33.4	ppm	n=3	+/-	3.4	%
2000x dilution	33.1	ppm	n=3	+/-	6.2	%
ICV/CCV	99.7	%	n=4	+/-	7.2	%
Cal Curve	100	%				
Blanks	0.345	pg		+/-	39	%
4Q16 Blank	0.314	pg		+/-	25	%
Matrix Spike	98.5	%				
SRNL Value	33.1	ppm	n=6	+/-	0.6	%
Tank 40 - F3				**Precision**		
4000x dilution	< 13.8	ppm	n=3	+/-		%
2000x dilution	< 13.8	ppm	n=3	+/-		%
ICV/CCV	99.7	%	n=4	+/-	1.5	%
Cal Curve	100	%				
Blanks	0.507	pg		+/-	31	%
Tk40-F3 Blank	< 13.8	ppm		+/-		%
Matrix Spike	98.5	%				
SRNL Value	< 13.8	ppm	n=6	+/-		%
Tank 21				**Precision**		
4000x dilution	151	ppm	n=3	+/-	1.8	%
2000x dilution	157	ppm	n=3	+/-	4.5	%
Tk21 0x dilution	153	ppm	n=3		6.4	
ICV/CCV	96.6	%			4.9	
Cal Curve	103	%		+/-	3.8	%
Blanks	0.208	pg		+/-	71	%
Tk21 Blank	<0.334	pg				
Matrix Spike	103	%	n=6	+/-	1.4	%
SRNL Value	153	ppm			3.7	%

Testing was performed to determine the effect that dilution may have on the final quantified result of methylmercury. All quarterly tank samples were analyzed with 2000x and 4000x dilution. No significant difference was found between the set of results obtained at 4000x and the set of results obtained at 2000x (p=0.969). Tank 21 was analyzed at 4000x, 2000x, and 0x dilution levels and results were obtained of 151 ± 2.64 ppm, 157 ± 7.00 ppm, and 153 ± 9.82 ppm. It was determined that dilution had no effect on quality or accuracy of methylmercury quantification.

Sample age was found to affect the comparative results obtained from SRNL and Eurofins. The relative error in analytical results, relative to Eurofins, obtained from the SRNL analysis of quarterly tank samples is demonstrated in figure 4 as a function of the number of days between the sampling process and the analysis by SRNL. Each tank sample was analyzed in triplicate. Tank 40 – F3 results fell below reporting limits and was excluded from this calculation. At the p = 0.05 level, the bias present in each sample, relative to Eurofins, was statistically different from the other two, and increased with increasing sample age. With the accumulation of larger amounts of data, this effect can be investigated further. With the data currently available, it is reasonable to suggest that all quarterly samples be analyzed within 180 days to retain appropriate analytical accuracy.

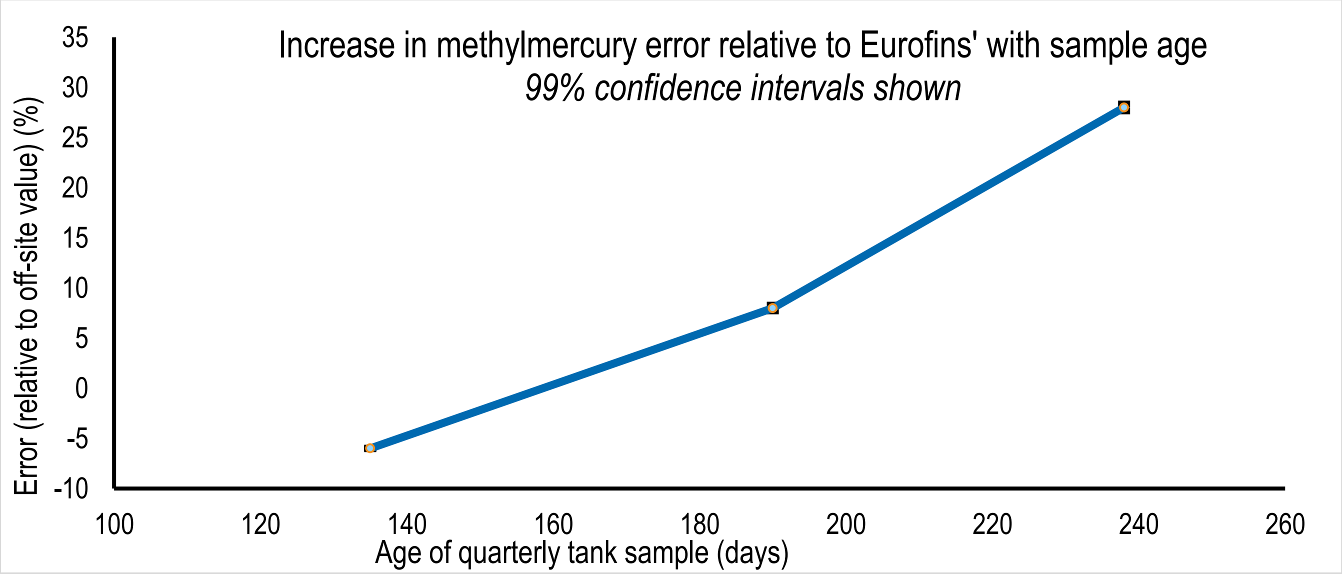

Figure 4: Increase in relative error observed between methylmercury concentration determined by SRNL and Eurofins, compared with sample age

A method for methylmercury speciation and quantification has been developed, optimized, and validated at SRNL. The sensitivity achieved by this method affords the high precision and accuracy required by existing and future WAC limits, and allows for high dilution of tank samples to comply with ALARA practices. Analytical parameters have been determined in cold samples, in both water and simulated tank waste, and in radioactive quarterly tank samples. The method performance in both non-radioactive and radioactive samples has been compared with either the analysis of known standards (for non-radioactive samples) or by interlaboratory comparison (for radioactive samples). SRNL has produced a valid method for the determination of methylmercury concentrations in quarterly tank samples.

Ethylmercury speciation by propylation and P&T-GC-CVAFS

For speciation and quantification of ethylmercury, several method parameters were modified. Notably, the derivatizing agent tetraethylborate used for methylmercury speciation was exchanged for the derivatizing agent tetra-n-propylborate. This derivatizing agent enabled purging of both methyl- and ethylmercury from solution, as well as chromatographic resolution of ethylmercury from Hg^{2+} by the addition of a propyl functional ground to the charged monoalkylmercury species. The optimized and modified instrumental parameters used for ethylmercury speciation can be found in table 2 (right), compared with the analytical parameters used for methylmercury analysis (left).

Table 2: Analytical parameters used in two derivatization schemes for the speciation of methylmercury and ethylmercury

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

Linear and sensitive calibration of ethylmercury was achieved using the propylating agent in a solution containing a mixture of methylmercury chloride and ethylmercury chloride in deionized water. Ethylmercury was quantified using a five-point calibration curve at calibration points 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 MDL of 7.50 pg and MRL of 22.4 pg, corresponding to an absolute MRL 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 MRL values in this work meet or exceed MRL values obtained by researchers utilizing commonly cited and standard methods.

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 5 demonstrates the results of the internal validation analyses.

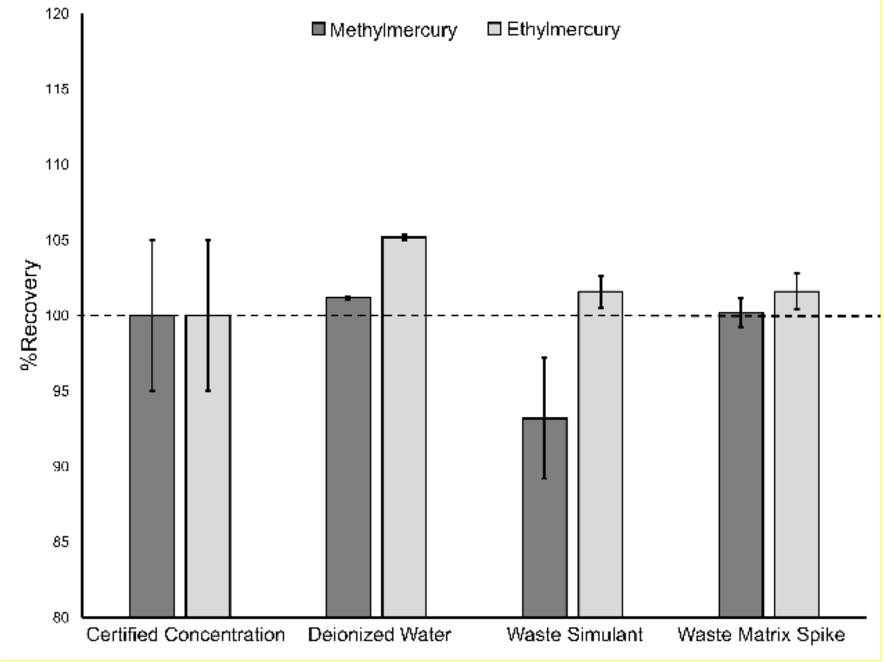

Figure 5: Methylmercury and ethylmercury solutions analyzed in deionized water and tank waste simulant, showing %RSD. Dashed line represents the known amount spiked into each matrix

A blind comparison was performed in SRNL and the ISO-certified external laboratory. Results of the comparison can be found in table 5, showing mean results for methylmercury and ethylmercury quantified in routine tank samples (n=3). 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 Tank 504Q16. 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)**	
	Tk504Q16	44.1	±0.0882	61.6	±6.0368
	Tk501Q17	33.1	±0.331	35.2	±2.0416
	Tk40-F3	< 13.8	-	< 0.49	-
	Tk21	39.2	±0.6272	36.1	±3.971
	Tk504Q16	< 0.163	-	< 0.017	-
	Tk501Q17	< 0.163	-	< 1.8	-
	Tk40-F3	< 0.163	-	< 1.7	-
	Tk21	< 0.163	-	< 0.17	-

No organomercury carryover 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 carryover in 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 carryover and carryover issues in the analysis of samples containing sulfur. 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. The system contamination issue was not observed following exchange of facility deionized water for HPLC-grade reagent water.

This method has enabled speciation of monomethylmercury and monoethylmercury in radioactive tank waste 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 MRL values obtained using standard and commonplace methods. This method has been validated by interlaboratory comparison with the external laboratory currently performing mercury speciation for SRR. The internal validation performed assessing recovery, accuracy, and sensitivity of ethylmercury, combined with the interlaboratory comparison, while returning <MRL, demonstrated the ability to match typical results returned by the external laboratory for ethylmercury. This addendum for quantification of ethylmercury has demonstrated a quantitative accuracy of ±5%, closely matching the internal accuracy achieved in methylmercury quantification. Additionally, this work has resulted in a method with a consistent MRL, in contrast with the variable (by up to ± 2 orders of magnitude) MRL provided by the external lab.