

Mercury in Beverages Using Green Chemistry, Microwave Extraction Sample Preparation and Detection by the QuickTrace® M-7600 CVAA Mercury Analyzer

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INTRODUCTION

Mercury (Hg) is a naturally occurring element, known to cause a wide variety of human health problems depending on dose, duration and route of exposure. Ongoing exposure can lead to neurological symptoms such as tremors, muscle weakness, motor and speech impairment and a decline of cognitive abilities. The most common means by which people in the United States are exposed to mercury is by ingesting contaminated food and water.¹

Consequently, the testing of food and beverage samples for Hg is essential to minimize exposure to this hazardous element. Methods approved by government agencies such as US FDA EAM 4.7, which was written for ICP-MS, can reliably quantitate the amount of mercury in samples, but may not have the level of sensitivity desired.² By combining this method with analytical techniques such as cold vapor atomic absorption (CVAA), or cold vapor atomic fluorescence (CVAf), the sensitivity of the method can be improved. The downside of this approach is an increase of stannous chloride reagent waste generated by the CVAA technique, which is hazardous as well as expensive.

In recent years, green chemistry has been of increasing importance to the scientific community, and the United States Environmental Protection Agency (US EPA) and American Chemical Society (ACS) have partnered to create a "Green Chemistry Challenge". This challenge encourages scientific solutions and promotes the use of green chemistry principles, the most important of which is waste prevention, and includes the reduction of the analytical reagents.^{3,4}

To further green chemistry principles, Teledyne Leeman Labs has previously studied configuring the M-7600's peristaltic pump with reduced internal diameter (ID) tubing to minimize reagent use and waste production. The use of reduced ID tubing when performing US EPA 7471B is described and successfully demonstrated in application note [AN1905](#).⁵

The study presented in this application note was challenged with not only creating a modification to a method traditionally used within the ICP-MS community, but to do so in coordination with green chemistry methodology. This adaptive application note will demonstrate that by coupling a clean microwave digestion process with a Teledyne Leeman Labs QuickTrace® M-7600 mercury analyzer configured with reduced ID tubing, that accurate results can be achieved while simultaneously decreasing hazardous waste production and reagent cost.

INSTRUMENTATION

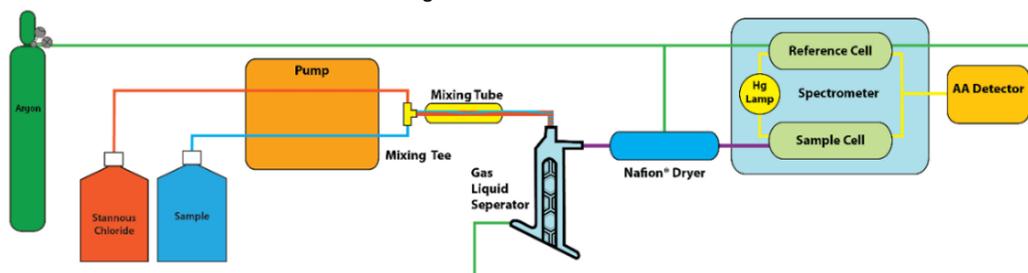
The QuickTrace® M-7600 is an independent stand-alone analyzer that uses CVAA spectrometry to obtain reliable quantitative data from simple to complex sample matrices. The working range for the QuickTrace® M-7600 mercury analyzer is from <0.5 ng/L to >700 µg/L. This dynamic quantitative range allows mercury concentrations to be determined in a broad range of sample substrates without dilution or pre-concentration.

Figure 1 QuickTrace® M-7600 CVAA Mercury Analyzer and CETAC ASX-560 Autosampler



The QuickTrace® M-7600 has a four-channel peristaltic pump that ensures consistent sample uptake to the analyzer and allows for sample/reagent reduction online in a closed system. The reduced sample then flows into the non-foaming gas-liquid separator (GLS), where the sample is purged with argon as elemental mercury is liberated. The mercury then passes through the Nafion® drying cartridge and into the sample cell where it is measured at 253.7 nm. Refer to Figure 2. The analyzer was also equipped with an optional ASX-560 autosampler for automated analysis.

Figure 2 QuickTrace® M-7600 Process Diagram



The M-7600's QuickTrace® software instrument controls include, but are not limited to: argon flow, lamp, pump control, smart rinse threshold and over-range protection. Parameter optimization allows for the quantitation of mercury in the trace ng/L to µg/L range within a single calibration plot.

This application note will demonstrate the use of reduced ID system tubing to deliver accurate results. The stannous chloride was added using flared orange/yellow 0.51 mm tubing (TLL PN 15-4309-102). The sample and waste lines were both white/white 1.02 mm tubing (TLL PN 15-1308-102). To verify the detection limit of the instrument with the reduced ID tubing, a matrix matched method detection limit (MDL) was performed using CFR 40 136 Part B.⁶

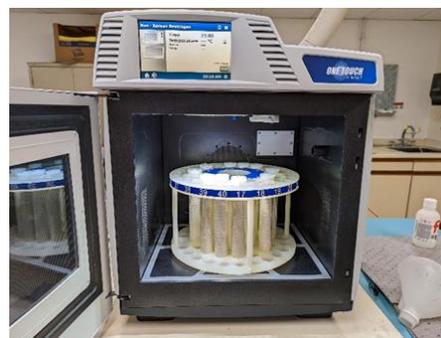
To demonstrate the effectiveness of the reduced ID tubing in the presence of a complex acidic matrix, six commonly consumed beverage types were subjected to microwave digestion using a microwave accelerated reaction system (MARS 6) by the CEM Corporation (Matthews, North Carolina), followed by detection using the QuickTrace® M-7600 (CVAA) mercury analyzer. Inorganic mercury in standards and digested samples was reduced to elemental mercury by excess online addition of 10% stannous chloride in 7% hydrochloric acid (HCl). US FDA EMA 4.7 specifies the use of nitric acid, hydrogen peroxide and post-digestion preservation with hydrochloric acid.² However, hydrogen peroxide was replaced with sulfuric acid due to a potential kinetics issue in the reduction of oxidized mercury to elemental mercury.

EXPERIMENTAL

Laboratory Apparatus

- 50 mL Polypropylene capped sample tubes
- 21-Position, tube autosampler rack
- Disposable, single use, plastic transfer pipettes
- Volume-dependent air displacement pipettes and tips: 10 mL, 5 mL and 1 mL
- 100 mL Polypropylene graduated cylinder
- "CEM MARS 6 Microwave Digestion System with MARSXpress™ Carousel and 55 mL PFA liner and cap (CEM PN 574125) (or similar)
- Vessel capping station or hand capping torque device
- Digestion vessel cleaning brush (CEM PN 302030)
- 100 mL Volumetric flasks (10 to 20)
- 2000 mL Volumetric flask
- Funnels
- DI Water wash/squirt bottles
- Analytical balance for checking pipette volume accuracy

Figure 3 CEM Mars 6™ Microwave



Reagents

- A source of either distilled or deionized (DI) water (DI water should be >18.2 MΩ-cm resistivity)
- ACS Grade concentrated nitric acid (HNO₃)
- ACS Grade concentrated sulfuric acid (H₂SO₄)
- ACS Grade hydrochloric acid (HCl)
- Instrument specific solutions, reducing and rinse

Digestion Vessel Preparation

1. Double rinse MARSXpress™ digestion vessel, vessel plug and vessel cap with DI water.
2. Prepare a 10% Contrex® “CA” Acidic Cleaner and Detergent (V:V DI water) (or equivalent cleaning solution). Acidic, phosphate-free liquid detergent works for descaling, mineral removal and oxide removal.
3. Wet digestion vessel cleaning brush with 10% Contrex CA solution and clean the inside of the digestion vessel using CEM cleaning brush
4. Triple rinse the vessel, vessel plug and vessel cap with DI water.
5. Add 10 mL of 10% HNO₃ (V:V) to the vessel.
6. Number/label the vessel, vessel plug and vessel cap.

NOTE: Maximum volume for 55 mL CEM digestion vessel is 25 mL.

7. Cap vessel and place in microwave digestion system carousel in accordance with the manufacture’s loading instructions. For 10 vessels, CEM recommends using positions: 1, 3, 5, 7, 8, 9, 11, 13, 15 and 16.
8. Load filled carousel into the microwave digestion system and ensure it is correctly placed on the turntable drive lug.
9. Create a vessel cleaning method “CEM MARS 6”:
 - Sample type “water”
 - Power 1000 W
 - Ramp time 10 minutes
 - Hold time 10 minutes
 - Temperature 180 °C
 - Temp guard 200 °C
10. Run the vessel cleaning method.⁷

Volumetric Flask Preparation

1. Double rinse flasks with DI water.
2. Add 10 to 20 mL of 10% HNO₃.
3. Swirl, soak and swirl.
4. Triple rinse flasks with DI water.

Funnel Preparation

1. Soak in 10% Contrex solution.
2. Rinse with copious amounts of DI water.

Figure 4 Labeled MARSXpress™ Vessels

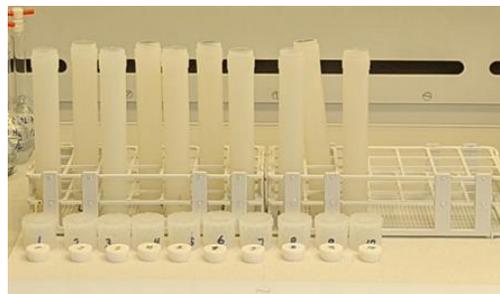


Figure 5 MARSXpress Carousel Loaded



Digestion Analytical Baseline

Perform a mock transfer of digestion solution into the 100 mL volumetric flasks to assist in orienting the funnels, flasks and digestion vessels for an orderly transfer of digested solution.

1. Stage cleaned funnels and volumetric flasks for use.
2. In a fume hood, slowly vent cleaned digestion vessels prepared in “Digestion Vessel Preparation”.
3. Rinse vessel plug with DI wash bottle, rinse into vessel
4. Place cleaned funnel into flask and slowly pour vessel solution into a cleaned flask.
5. Rinse vessel, partially fill with DI water and pour rinse into flask.
6. Rinse funnel.
7. Fill flask to the mark with DI water, invert and swirl six times to sufficiently mix.

Figure 6 Cleaned 100 mL Flasks



Create an M-7600 CVAA mercury analyzer method to analyze the vessel cleaning digest solution. The method doesn't need to be perfect, but should be well thought out to give an indication of contamination levels in the digestion process. Two reference calibration points can be used for calibration standards preparation, US FDA EAM 4.7 (0 to 2.5 µg/L calibration), and AOAC 2015-01 (0 to 5.0 µg/L calibration).^{2,8} After the vessel cleaning solution has been analyzed, assess the data set, and repeat cleaning for elevated levels of Hg.

Sample Preparation

Table I Sample Preparation			
Sequential Additions	mL	Wait Time (Minutes)	Notes
DI Water	5		If no reaction is observed after 10 minutes, continue with next addition of acid.
Sample	5		
HCl	2	5	
HNO ₃	2	15	
HNO ₃	2	15	
HNO ₃	2	15	
HNO ₃	2	15	
H ₂ SO ₄	2	15	
Cap and Digest			

Caution: To gauge increase and/or decrease in wait times, perform mock additions in a 50 mL test tube

Figure 7 Mock Wine Test



Figure 8 Prepared Sample Set

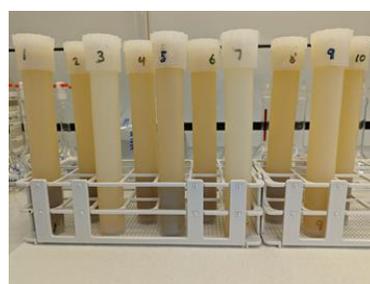


Table II QuickTrace® M-7600 Instrument Parameters and Conditions		
Autosampler Parameters		
Probe Depth (mm)	150	
Rinse Pump (%)	50	
Sample Uptake Time (s)	50	
Rinse Time (s)	100	
M-7600 Mercury Analyzer Parameters		
Gas Flow (mL/min)	100	
Pump Speed (%)	70	
Read Delay Time (s)	~67	
Replicate Read Time (s)	1	
Replicates	4	
Baseline Drift Correction (s)	24/28	
Analytical Parameters		
Calibration Type	Normal	
Quantitation Mode	Height	
Calibration Standards (No. and Conc.)	7	0, 0.05, 0.2, 0.5, 1, 2, and 5
Units	ppb (µg/L)	
Calibration Algorithm	Linear	Force Through Blank

Calibration

Calibration standards were matrix matched at half acid concentration of the final digestion volume of 100 mL: 1% HCl, 4% HNO₃, and 1% H₂SO₄. The % acid assumes that ~50% of the original acid volume was consumed during the digestion process. The instrument was calibrated using standards and settings shown in [Table II](#).

Figure 9 QuickTrace® M-7600, 5 µg/L Peak Profile Spectra

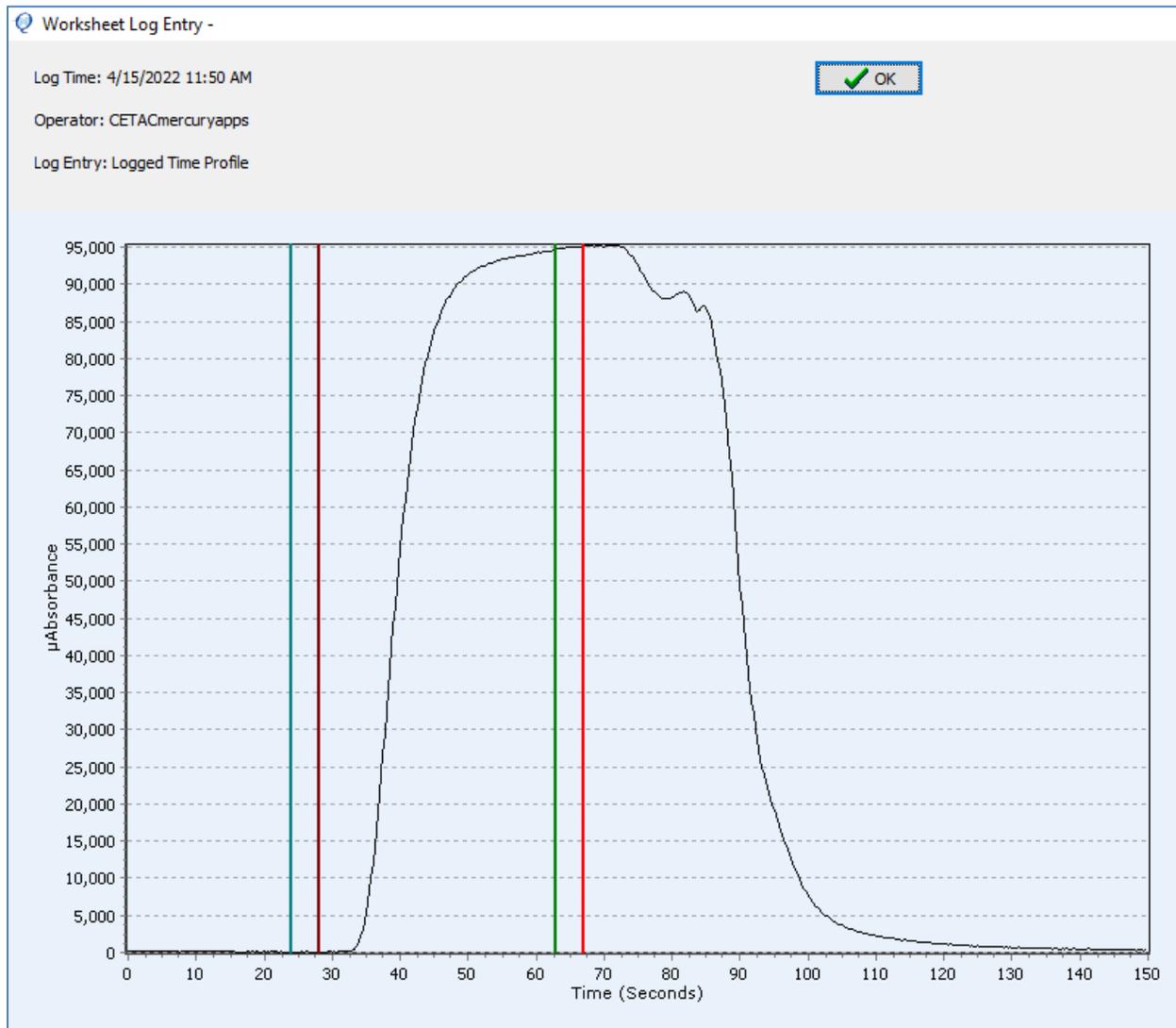
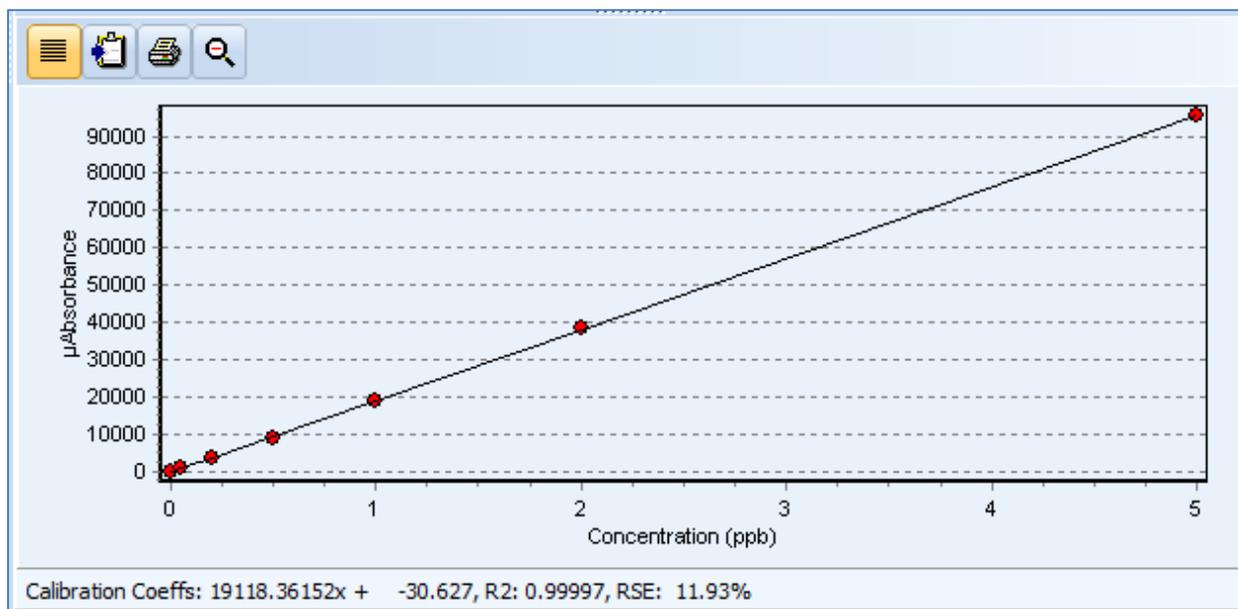


Figure 10 QuickTrace® M-7600 Calibration Plot



Calibrations must have a correlation coefficient of ≥ 0.9975 or better to be considered valid. Modified US FDA EAM 4.7 was validated using a second-source standard that was prepared at a concentration of 1 $\mu\text{g/L}$, and had an acceptance criteria of $\pm 10\%$.² For method quality control (QC), an initial calibration verification (ICV) standard was analyzed at the beginning of the sequence. A continuing calibration verification (CCV) standard and a continuing calibration blank (CCB) were analyzed every 10 samples, and at the end of the sequence. The CCV is required to be within 10% of its known value, while the CCB must be less than half of the lowest standard of the curve.

A typical calibration curve for this method is shown in Figure 10. A peak from a typical method validation profile, 5 $\mu\text{g/L}$ calibration standard is shown in Figure 9. The M-7600 mercury analyzer configured with the reduced ID tubing generated excellent calibration curves for all analytical batches and demonstrated excellent system linearity. It was observed that the green chemistry tubing configuration had no significant impact on peak shape.

Procedure

The scope of the method applies to commonly consumed beverages. The analysis principles apply to a sample of known volume that is pre-treated with HCl, HNO₃ and H₂SO₄. The pre-treatment solution is then subjected to microwave digestion for 40 minutes. The extraction solution is diluted to 100 mL. An aliquot of diluted solution is analyzed by chemical reduction using the CVAA analytical technique. Quantification of Hg in solution is calculated back to the original beverage and reported.

5 mL of DI water was added to each microwave digest vessel for “vessel wetting”, then 5 mL of beverage sample was added, followed by 2 mL HCl, 8 mL HNO₃ and 2 mL H₂O₂. It is highly recommended that a mock digest of previously unknown samples be performed in a secured 50 mL test tube to gauge severity of the reactions. The samples were then digested using the CEM MARS 6 microwave digestion system. It should be noted that CEM recommends a power setting of 800 W for 8 to 12 vessels.⁹ For this method, a slightly more aggressive power setting on the “ramp” portion and less aggressive setting on the “hold” portion was implemented. Reagents and microwave conditions for sample digestion are shown in Table III.

Table III CEM MARS 6 Microwave Digestion System Sample Digestion Parameters/Conditions			
Parameter	Minutes	Power (W)	°C
Ramp	25	1000	180
Hold Time	15	600	180
Control Temperature			180
Guard Temperature			200

As stated earlier, the calibration standards were matrix matched at half acid concentration of the final digestion volume of 100 mL: 1% HCl, 4% HNO₃, and 1% H₂SO₄. The % acid assumes that ~50% of the original acid volume was consumed during the digestion process. The analytical standards 0.05, 0.2, 0.5, 1.0, 2.5 and 5.0 µg/L were prepared from a 10 µ/L intermediate standard in 3% HCl.

Each of the six (6) analytical runs (Brewed Coffee, Orange Juice, Red Wine, Soda Pop, 1% Milk and Brewed Tea) consisted of: calibration, ICV, CCV, CCB, CCB, seven (7) Samples, Pre-digest Spike, Post-digest Spike, tap Water and a control Blank.

The “Analytical Experimental Variables” consist of digestion vessel cleaning and validation, volumetric vessel cleaning and validation, funnel cleaning and validation, and sample tube cleaning and validation. For this application, it is prudent to empirically test the sample’s reaction and timing to the acid addition.

RESULTS

Analytical results are shown in [Table IV](#). All calibration line correlations were well within an acceptable range of ≥ 0.9975 ; in fact, the worst correlation of 0.99973 easily met the acceptance criteria. All ICV and CCV % recoveries were also well within the required $\pm 10\%$. The blank corrected values for all sample batches was ≤ 0.005 µg/L. The digests were subjected to a simple MDL calculation of $STDEV \times 95\%$ (Student’s t-distribution, N=6). The MDL calculations ranged from 0.0012 to an upper value of 0.0088 µg/L. The Pre-digest Spikes indicated little to no analyte loss, with % recovery (% R) ranging from 98 to 102%. The Pre-digest Spike consisted of 0.5 mL of a 10 µg/L standard, with the final volume amounting to a spike value of 0.05 µg/L. The ability of the analytical system to measure and report at 0.05 µg/L was quite impressive. The Control Blank results demonstrate how difficult it is to achieve zero contamination, as they ranged from -0.003 to 0.01 µg/L. Each analytical run was blank corrected to the respective Control Blank for that specific analytical batch.

Not shown in [Table IV](#) are the Post-digest Spikes which were 0.048 µg/L (0.5 mL of a 1 µg/L into 10.5 mL). The average % R for all post-digest spikes was 95.8%. The passing recoveries for the matrix spike (MS), both pre and post digest at trace levels, demonstrate that the reduced ID tubing did not compromise analytical performance. Furthermore, the reduced ID tubing configuration showed significant benefit by only consuming ~2 mL of stannous chloride for each ~5.8 mL of sample dispensed into the analytical system.

Table IV Beverage Analysis for Mercury Concentration Results						
Date of Analysis	4/13/2022	4/15/2022	4/27/2022	4/29/2022	5/4/2022	5/13/2022
Type	Coffee	Orange Juice	Red Wine	Soda Pop	Milk 1%	Brewed Tea
R ²	0.99974	0.99997	0.99996	0.99994	0.99973	0.99989
ICB	-0.002	-0.002	-0.006	0.001	-0.001	0.002
ICV (1 µg/L)	0.984	0.967	0.963	0.967	0.997	0.992
ICV %R	98.4%	96.7%	96.3%	96.7%	99.7%	99.2%
CCB	-0.002	-0.003	-0.002	-0.001	-0.001	0.002
CCV (1 µg/L)	1.031	1.011	1.003	1.001	1.003	1.007
CCV % R	103.1%	101.1%	100.3%	100.1%	100.3%	100.7%
Blank Corrected Average Conc.	0.001	0.002	0.001	-0.002	0.005	-0.003
Blank Corrected Minimum Conc.	-0.001	-0.003	-0.001	-0.002	-0.001	-0.005
Blank Corrected Maximum Conc.	0.003	0.007	0.005	-0.001	0.009	-0.001
Blank Corrected Conc. STDEV	0.0015	0.0034	0.0021	0.0005	0.0036	0.0014
Uncertainty @ 95%	±0.0011	±0.0025	±0.0016	±0.0036	±0.0027	±0.0010
Beverage MDL @ 95%	0.0037	0.0083	0.0051	0.0012	0.0088	0.0034
Pre-Digest Spike (0.05 µg/L)	0.057	0.051	0.053	0.049	0.052	0.056
Blank Corrected Pre-Digest Spike	0.052	0.051	0.051	0.047	0.055	0.049
Pre-Spike % R ((SPC-SXC)/SP)	102%	98%	100%	98%	100%	98%
Tap Water	0.000	-0.002	0.000	0.001	-0.003	0.007
Control Blank	0.005	0.000	0.002	0.002	-0.003	0.010
CCB	-0.001	0.002	0.001	0.001	-0.002	0.001
CCV (1 µg/L)	1.053	1.022	1.024	1.005	0.998	1.004
CCV % R	105.3%	102.2%	102.4%	100.5%	99.8%	100.4%

Detection Limits

To demonstrate system precision when using the reduced ID tubing configuration, seven aliquots of two ultra-trace standards, 0.005 µg/L (5 ng/L) and 0.003 µg/L (3 ng/L), were digested and analyzed with the same method used for analysis of the beverage samples. The MDL study was conducted using the US EPA guidelines set forth in CFR 40 Part 136 Appendix B, Rev 1.⁶

Because the template for revision 1 has historical significance in our application's laboratory, and mathematically all the calculations are imbedded in the template calculations, it was the preferred choice for the MDL study. Two ultra-trace 0.005 µg/L and 0.003 µg/L standards were processed through the entire analytical procedure and resulted in a 0.0026 µg/L (2.6 ng/L) MDL. To simplify the results, the MDL was rounded to ≤0.003 µg/L (3 ng/L). The lower limit of quantitation (LOQ), thus became a simple multiple of the MDL (3 X MDL), thus ≤ 0.009 µg/L (≤ 9 ng/L). MDL results are present in [Figure 11](#).

Figure 11 MDL Study

MDL Study		Hg Microwave Beverage Digestion (2022)	
40 CFR Ch.1 (7-1-91) Part. 136, App B			
	Run 1 @ 5 ng/L		Run 2 @ 3 ng/L
	6.6		5.4
	5.4		4.0
	5.8		2.7
	6.5		2.7
	4.6		4.6
	5.5		4.6
	6.7		2.5
Average	5.9		3.8
	0.77398 = Run 1 STDEV		1.15388 = Run 2 STDEV
	0.599048 = STDEV ²		1.331429 = STDEV ²
	2.223 = F ratio		
	1.93047619 Sum STDEV ² (Run 1 and 2)		
	0.9652381 Sum STDEV ² / 2 ((Run 1 and 2) / 2)		
	0.98246532 STDEV Pooled		
	2.634 = MDL		

Conclusion

The MDL of 0.0026 µg/L (2.6 ng/L) demonstrates that the complete system capability exceeds many other analyzers when working in the ultra-trace ranges. With the data set in hand, and using the LOQ calculation of 0.009 µg/L based off of the MDL study, all actual digest MDL data fell within the calculated LOQ of ≤0.009 µg/L.

Because the sample was 5 mL into a final volume of 100 mL (20 X), a reported value of ≤0.180 µg/L Hg would be an acceptable concentration to fall below for Coffee, Orange Juice, Red Wine, Soda Pop, Milk, and Brewed Tea. All calibration line correlations are well within an acceptable range. All ICV % recoveries were well within ±10%. All pre and post digestion spikes were ±5% of actual concentration, providing assurance that the entire analytical process was extremely sound and in control from start to finish. The "Uncertainty" values correspond to a level of confidence of 95% and were calculated for the seven replicates for each beverage analyzed. Results are shown in [Table IV](#).

The microwave digestion and analysis of the six beverage types tested was not difficult, once the process of trial and error determined a reliable method. While both digestion and analysis for a sample set can be completed in one day, the true challenge lies in the labor-intensive cleaning between sample sets. Pre-digestion spike recovery was very good amongst all beverage types tested, and ranged from 98% to 102%.

With so many fluid pieces, strict good laboratory practices (GLP) should be employed to obtain meaningful data. By subjecting the analyzer to gas flow, pump rate and uptake rate modifications, it can be anticipated that MDL's of ≤1 ng/L can be achieved (thus lowering the LOQ to <3 ng/L). The lower MDL would then give a theoretical dilution reported value of ≤0.060 µg/L (60 ng/L). The microwave extraction method yielded a final average result for all beverages of ≤0.180 µg/L (180 ng/L).

In conclusion, the Teledyne Leeman Labs QuickTrace® M-7600 mercury analyzer combined with a microwave digestion system demonstrated the ability to report values in the trace Hg range, and performed well as a complex analytical system. It should be reiterated that performing this method was relatively straight-forward, but cleanliness of all laboratory apparatus is of the utmost importance for the data integrity.

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