

Mercury Determination in Tea Leaves, INCT-TL-1, USEPA Method 7473, Using the Teledyne Leeman Labs Hydra II_C Combustion CVAAS

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Introduction

Tea (*Camellia sinensis*) is one of the world's most popular non-alcoholic beverages and is consumed by over two-thirds of the earth's population. Experimental studies have demonstrated that the accumulation of significant amounts of excess non-essential trace elements in tea leaves may increase the metal body burden in humans over a period of prolonged tea consumption. The presence of heavy metals, often accumulated in the kidneys and liver, are widely reported to be toxic to the human body, disrupting numerous biochemical processes and potentially leading to cardiovascular, nervous, kidney and bone diseases.

In particular, the heavy metal mercury (both organic and inorganic) is known to be one of several widespread environmental toxins capable of causing a variety of negative health effects in humans, and its presence in tea is of increasing concern.¹

This application note will demonstrate the ability of the Teledyne Leeman Labs Hydra II_C Mercury Analyzer to determine total mercury using USEPA Method 7473. USEPA Method 7473 is approved for both laboratory and field analysis for mercury in solids, semi-solids and solutions using Thermal Decomposition, Amalgamation and Atomic Absorption Spectroscopy.² The Certified Reference Material (CRM), Tea Leaves (INCT-TL-1), produced and certified by the Institute of Nuclear Chemistry and Technology in Warsaw, Poland was used for this analysis.

Instrumentation

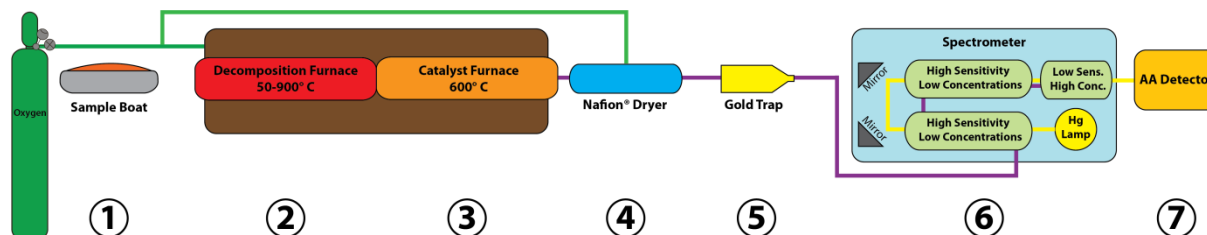
The Teledyne Leeman Labs Hydra II_C is a fully automated mercury analyzer that measures mercury in diverse sample matrices directly with little to no sample preparation. Instead, it employs sample combustion (thermal decomposition), mercury concentration by gold amalgamation and detection by Cold Vapor Atomic Absorption Spectroscopy (CVAAS). The instrument operates using a universal power supply compatible with main power input 110/220 V, 50/60 Hz power outlet and oxygen supplied at 15 to 20 psig. All instrument operating parameters (including furnace/catalyst temperature, gas flows, autosampler control) and sample cycle stages are computer controlled for ease-of-use. Through proper selection of the instrument's operational parameters, mercury determination can be performed on a diverse sample set across a dynamic range consisting of absolute per sample mass of mercury from 0.001 ng to 1500 ng. The Teledyne Leeman Labs *Hydra II_C Mercury Analyzer Operator's Manual* provides extensive guidance on parameter optimization.

Figure 1 depicts the analytical process with gas flowing from left to right. The Hydra II_C mercury analyzer employs combustion of a sample at high temperatures with oxygen. The gases resulting from this decomposition are carried through a heated catalyst to remove halogens, nitrogen oxides, and sulfur oxides. The remaining combustion products, including elemental mercury (Hg⁰), are swept through a dryer and then to a gold amalgamation tube which captures the mercury while allowing the other gases to pass through.



The amalgamator is then heated to release the accumulated Hg^0 into a carrier gas which transports it into the Cold Vapor Atomic Absorption Spectrometer. The transient signal is measured in series by a high-sensitivity cell followed by a low-sensitivity cell. The two peaks are integrated and reported against the best calibration of the two cells available. The use of two cells provides the best detection limit with a wider dynamic range than that provided by a single optical cell path length. Waste gases exiting the system are chemically “scrubbed” with a carbon trap or exhausted safely out of the lab at the end of the process.

Figure 1 Hydra II_C Mercury Analyzer Principle of Operation



Experimental

The Hydra II_C is operated by the Teledyne Leeman Labs Envoy software that provides sample specific control of the system. The software’s parameters can be optimized for sample drying and decomposition (combustion is customizable for temperature and duration) for each individual sample to facilitate analysis of mercury in various sample matrices. For this application note, the system was calibrated up to 4 ng. The operating conditions used during sample analyses are shown in Figure 2 and Table I.

Figure 2 Operational Conditions

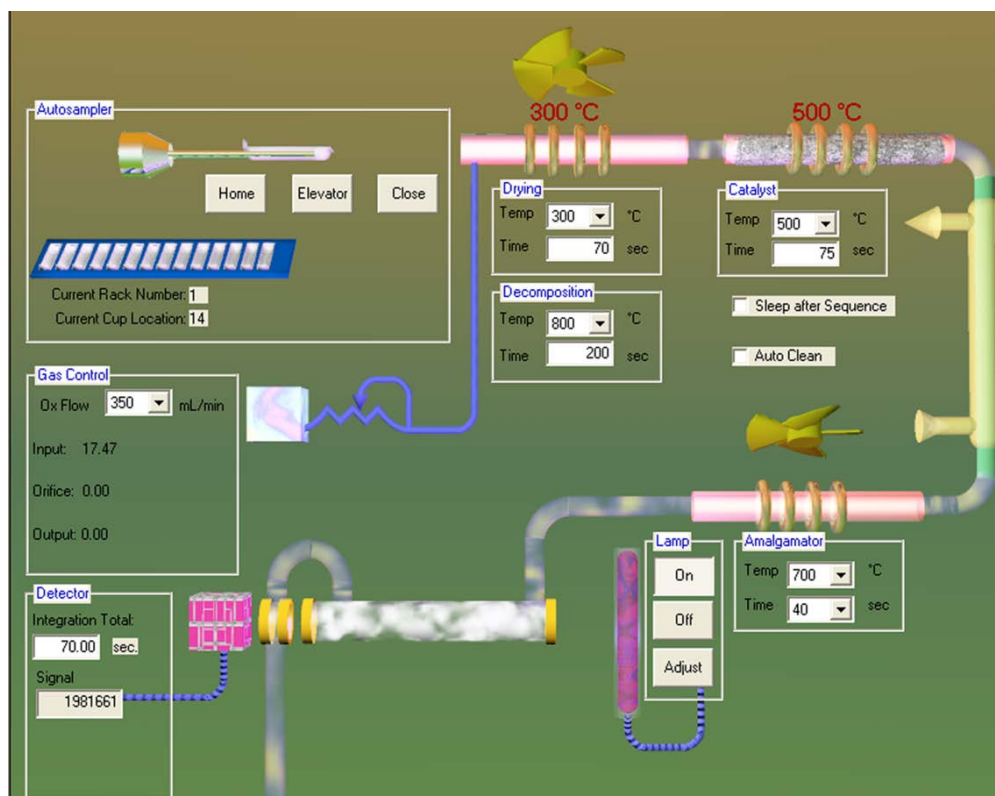


Table I Operational Conditions		
Sample	Method Parameter	Parameter Value
Tea Leaves (INCT-TL-1)	Drying Temperature	300 °C
	Drying Time	70 Seconds
	Decomposition Temperature	800 °C
	Decomposition Time	200 Seconds
	Catalyst Temperature	500 °C
	Wait Time	75 Seconds
	Amalgamator Temperature	700 °C
	Amalgamator Time	40 Seconds

Tea Leaves (INCT-TL-1) CRM was used for this analysis. According to the Institute of Nuclear Chemistry and Technology, the CRM was “prepared from black tea, usually packed in tea bags. Tea leaves collected and processed in Argentina were ground and sieved. All precautions were taken to avoid contamination of the material with metals. The next step was homogenization by mixing in a plastic drum. After preliminary homogeneity checking by X-ray fluorescence, the material was distributed into PP bottles and final homogeneity testing was performed by neutron activation analysis determining Br, Ce, Co, Cr, Cs, Fe, K, La, Mn, Na, Rb, Sc and Sm in subsamples (Fisher’s test). Good homogeneity of INCT-TL-1 at least for sample weight of 5 mg was confirmed. Long-term stability was assured by sterilization of the whole lot of the material by electron beam radiation and is being monitored during storage. Certification of the candidate reference material was done on the basis of world-wide interlaboratory comparison exercise in which 109 laboratories from 19 countries using various analytical methods, participated. Data evaluation was performed using the software package ACQS-1. Several criteria were employed to decide whether the overall mean can be given the status of recommended (certified) or information value. The material was prepared and certified by the staff of the Dept. of Anal. Chem., of INCT under the supervision of Prof. Dr R.Dybczyński.”³

Note: The only sample preparation involved with INCT-TL-1 was the thorough mixing of the bottle initially and between each sample weighing to assure and maintain homogeneity.

The Tea Leaves (INCT-TL-1) certificate included with the material did not list moisture content and instructed that it should be determined in-house on a separate portion of the material that would not be used for analyte determination. A cleaned and tared sample boat was loaded with ~0.100 grams (as-received weight), dried at 85 °C, and then weighed after 48 hours, per the certificate’s instructions. The percent moisture was determined to be 5.48%.

Calibration Standardization

Nickel boats loaded with diatomaceous earth were cleaned just prior to calibration by running them through the same method used for the analysis (unnecessary dry time was removed). While the boats were being cleaned, Intermediate Standards were prepared by serial dilutions of a 1000 mg/L certified primary standard purchased from LabChem® and shown in [Table II](#). The final analyzer Calibration Standards were prepared as shown in [Table III](#).

Laboratory 18.2 Mohm-cm resistivity DI water was used for the first Intermediate Standard dilution so that its resulting Nitric Acid (HNO₃) concentration would be 0.1%. Also, a 0.1% HNO₃ solution was made using 18.2 Mohm-cm resistivity DI water as diluent for the second Intermediate Standard and the final analyzer Calibration Standards. This protocol allowed for the same volume and concentration of HNO₃ preservative for each Calibration Standard as shown in Table III. The Intermediate Standards and Calibration Standards were prepared in the following manner:

- A 10,000 µg/L Intermediate Standard was made from the 1000 mg/L certified primary standard (preserved in 10% HNO₃) by performing a 100x dilution using 18.2 Mohm-cm resistivity DI water.
- A 1000 µg/L Intermediate Standard was made by performing a 10x serial dilution using the prepared 0.1% HNO₃ diluent solution (5 mL of the 10,000 ug/L standard into a total volume of 50 mL).
- Using a microliter pipettor for best accuracy, 40, 20, 10, 5 and 2.5 µg/L analyzer Calibration Standards were made by diluting 400 µl, 200 µl, 100 µl, 50 µl and 25 µl of the 1000 µg/L Intermediate Standard into a total volume of 10 mL in separate tubes using the prepared 0.1% HNO₃ diluent solution.
- 0.1% HNO₃ diluent solution was used for the analyzer Calibration Blank.

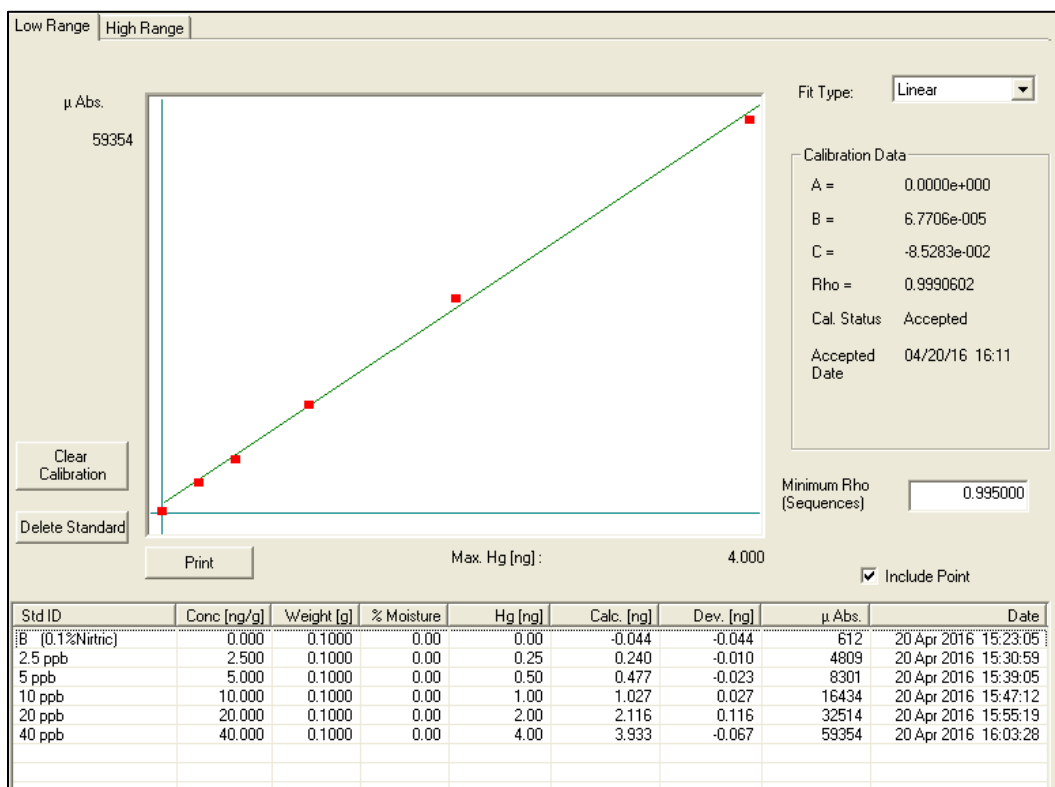
Table II Serial Dilutions for Intermediate Standards			
	Primary Standard	100x Dilution	10x Dilution
Hg Conc. in µg/L	1,000,000	10,000	1,000
HNO ₃ Conc. in %	10	0.1	0.1

Using the pre-cleaned Nickel boats with diatomaceous earth, 0.100 mL aliquots from the mercury Calibration Standards in Table III were analyzed using the operating conditions shown in Table I to create a linear-fit calibration curve in the low-calibration (high-sensitivity) cell. The system developed a curve covering a range of 0.0 – 4.0 ng of mercury and is presented in Figure 3. The Envoy Software displays calibration plots as mass of mercury in nanograms versus micro absorbance of Hg.

Table III Prepared Analyzer Calibration Standards	
Calibration Point / Nitric Concentration	Aliquot
Blank (0.1%)	0.100 mL of 0.1% HNO ₃ diluent
0.25 ng (0.1%)	0.100 mL of the 2.5 µg/L standard
0.50 ng (0.1%)	0.100 mL of the 5.0 µg/L standard
1.00 ng (0.1%)	0.100 mL of the 10 µg/L standard
2.00 ng (0.1%)	0.100 mL of the 20 µg/L standard
4.00 ng (0.1%)	0.100 mL of the 40 µg/L standard

Note: As a general rule, when calibrating with, or analyzing liquids, a ratio of 70 seconds per .100 mL (at the typical temperature setting of 300 °C) is recommended for the Dry Time parameter.

Figure 3 Low-Range Calibration Curve



Procedure

The same procedure used to clean the Calibration Standard boats was used to clean sufficient empty nickel boats for sample analysis. With thorough mixing of the CRM bottle between each sampling, ~0.100 grams (the certification document accompanying the CRM listed a minimum sampling size of 0.100 grams for reasons of homogeneity) of sample was transferred into the pre-cleaned nickel boats. Exact weights for each individual sample were recorded and entered into the combustion method developed in the Envoy software. A total of seven samples were prepared in this manner and then loaded onto the boat shuttles for unattended analysis. The integrated cover over the shuttles was closed to prevent airborne particulates from contaminating the samples in the boats while they were waiting to be analyzed.

It is important to note that the Envoy software affords the time-saving ability to begin the analytical run (once sufficient samples have been weighed and the weights entered) while the remaining samples are weighed and then added to the end of the sequence. Alternatively, samples can be analyzed individually by loading the weighed sample boat directly onto the injector and entering the weight when prompted by the Envoy software.

Results

The Hydra II_C Mercury Analyzer's measurement of mercury in the CRM resulted in a successful correlation with the certified value for Tea Leaves (INCT-TL-1).

Seven replicates of the CRM were analyzed using the instrument operating conditions shown in [Table I](#). The results listed are corrected for the moisture content of 5.48%. The mean concentration and standard deviation were calculated and are listed in [Table IV](#). Individual analyses, giving a final result of 5.54 ug/kg ±0.46 μg/Kg (dry basis), are shown in [Figure 4](#). Tea Leaves (INCT-TL-1) has a certified concentration of 4.92 μg/Kg with an uncertainty of ±0.74 μg/Kg. A representative Tea Leaves (INCT-TL-1) sample peak is shown in [Figure 5](#).

Table IV Tea Leaves, INCT-TL-1, 4.92 µg/Kg ± 0.74		
Sample	µg/Kg	
1	5.419	
2	5.462	
3	5.477	
4	5.401	
5	5.665	
6	6.009	
7	5.320	
Mean = 5.536		
Uncertainty = 0.458		
n = 7 Replicates	STDEV = 0.234	RSD% = 4.223

Figure 4 Results with Uncertainties

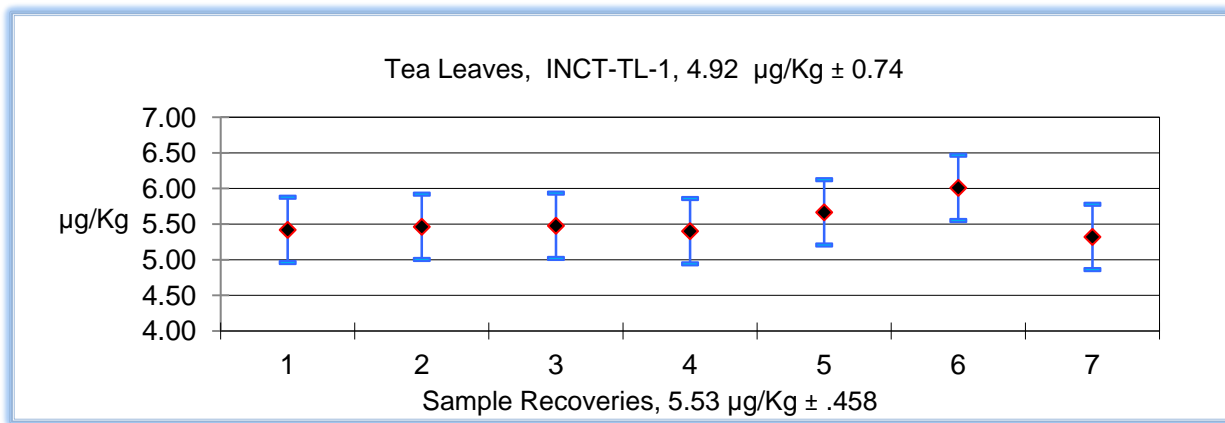
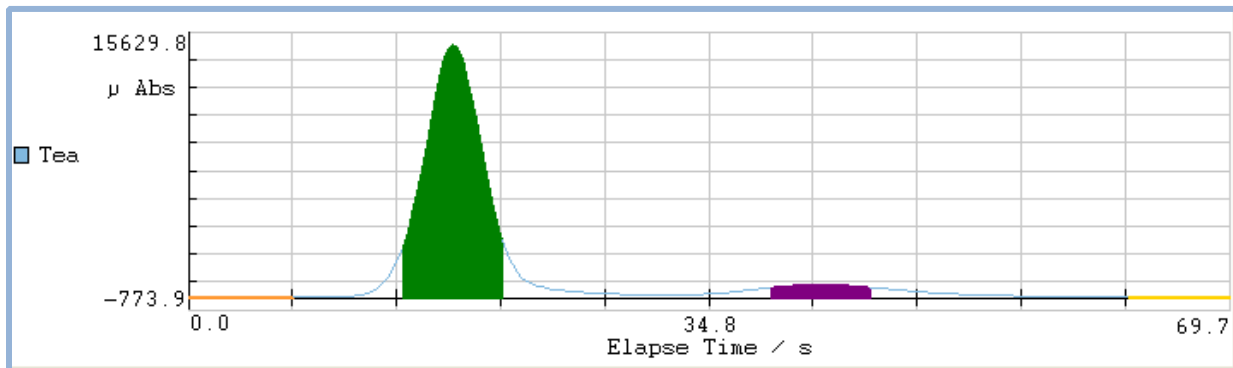


Figure 5 Representative INCT-TL-1 Sample Peak



The Quality Control (QC) Standards and Matrix Spikes are listed in Table V with their recoveries. A Laboratory Control Sample (LCS), as required by EPA 7473, was prepared from a second-source commercial standard, and analyzed before and after the samples. After the initial seven replicates, a Spike and Spike Duplicate were also analyzed by addition of .100 mL of the 5 ug/L solution used during the calibration step.

Table V Mercury Determination in Tea Leaves Quality Control		
Quality Control (in µg/L) Standards	µg/Kg	% recovery
5 µg/Kg (0.5 ng) LCS - pre sample	4.34	86.7
5 µg/Kg (0.5 ng) LCS - post sample	4.26	85.2
Tea (INCT-TL-1) Spk (0.5ng)		121.0
Tea (INCT-TL-1) SDup (0.5ng)		101.0

Conclusion

The Hydra II_C Combustion CVAAS Mercury Analyzer is capable of analyzing and determining total elemental mercury (Hg⁰) concentrations in Tea Leaves (INCT-TL-1) using the guidance in EPA Method 7473 and the operating conditions in Table I. Additionally, the integrated autosampler provides a fast, simple and convenient approach for the analysis of mercury. The use of combustion (decomposition) virtually eliminates sample preparation, as well as the production of hazardous chemical wastes resulting in reduced technician time and operating expenses.

References

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2. United States Environmental Protection Agency (USEPA). Mercury in solids and solutions by thermal decomposition, amalgamation, and atomic absorption spectrophotometry - EPA Method 7473-2007 - February 2007 Revision 0. [Online] <https://www.epa.gov/sites/production/files/2015-12/documents/7473.pdf> (accessed May 24, 2016)
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