ANALYSIS OF METHYL MERCURY IN SEDIMENT AND TISSUE BY KOH/CH_3OH DIGESTION FOLLOWED BY AQUEOUS PHASE ETHYLATION

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1. SCOPE AND APPLICATION

This method is applicable to the determination of methyl mercury in tissue, sediment, and some types of waste samples. In the environment, there is a need to monitor methyl mercury across a large range of solid matrix types and concentration levels due to bioaccumulation and biomagnification of this highly toxicity compound. The analytical approach used in this method is designed for trace-level analysis, where the detection limit is usually limited by the matrix or background rather than by instrumental limitations. The method detection limit (MDL) for this method is 0.02 ng/L (0.0001 – 0.2 mg/kg) of methyl mercury as Hg^{o}, where minimal background or matrix interference is present. The practical quantitation limit (PQL) for this method is 0.08 ng/L (0.0004 – 0.8 mg/kg) of methyl mercury as Hg^{o}.

The Florida Department of Environmental Protection (DEP), Bureau of Laboratories developed a direct-ethylation sample preparation and automated analysis technique for the determination of methyl mercury, based on modifications to the EPA draft method 1630 (see sections 12.1, 12.2, 12.3 and 12.4). The modified method uses a KOH digestion followed by aqueous ethylation, purge and trap, pyrolysis and cold-vapor atomic fluorescence spectrometry (CVAFS) detection. Addition of the surrogate compound, n-propyl mercury, is also another modification from the original method that provides for enhanced monitoring and assessment of the analytical process.

This SOP is used to analyze samples having the laboratory information management system (LIMS) test identifiers (Test ID) T-CH3HG and S-CH3HG.

2. SUMMARY OF METHOD

2.1. This method consists of three major steps: sample digestion, derivatization, and analysis. Methyl mercury in solid samples, such as tissue and sediment, is usually bound to organic matter. In the case of animal and fish tissue it is usually bound to sulfur containing amino acids or proteins. It is necessary to chemically dissociate or extract the methyl mercury from its organic matrix before it can be analyzed. This must be accomplished without significantly degrading or producing methyl mercury in the process of extraction. A solution of 25 % potassium hydroxide in methanol is used to extract the methyl mercury from its host matrix. Once the sample is digested, the extract solution is processed similarly to water samples in DEP SOP **HG-005**, which is a modification of EPA Draft Method 1630.

2.2. The analytical methodology used in this procedure is a modification of the draft EPA method 1630. The modifications to the original draft method include:

- Full automation of the sample analysis
- Direct sample ethylation, without distillation
- Use of a citrate buffer instead of an acetate buffer
- Use of a silicone-based anti-foaming agent
- Inclusion of a surrogate compound
- Use of a *Tenax* ™ trapping media instead of a VOCARB carbon trap

2.3. The derivatization step transforms the methyl mercury into a volatile derivative, methyl ethyl mercury, which is purged from the sample and concentrated in a *Tenax* ™ trap. The derivatization also converts the inorganic mercury species Hg^{+2} and the surrogate compound n-propyl mercury chloride into the volatile derivatives diethyl mercury and n-propyl ethyl mercury, respectively. Small amounts of atomic mercury (Hg°) and dimethyl mercury may also be present and concentrated in the trap.

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2.4. In the analysis step, the volatile mercury components are collectively desorbed from the *Tenax* ™ trap, separated by gas chromatography, and detected using a fluorescence detector. As the separated components emerge from the gas chromatograph, they pass through a pyrolysis furnace at 720 °C which converts the organomercury compounds to elemental mercury prior to detection. The elemental mercury is detected with great sensitivity and selectivity using an atomic fluorescence detector.

3. APPARATUS AND EQUIPMENT

3.1. *Digestion tubes* - Sample digestions are performed in 75 mL Pyrex ™ glass tubes. A 50 mL calibration mark and unique ID has been placed on each tube. The calibration volume must be verified to within ± 1 % on new tubes prior to use and verified on a rotational/annual basis thereafter. Digestion tubes outside the 50 mL ± 1 % (0.50 mL) requirement must be discarded. Results are recorded on the Digestion Tube Volume Calibration Check Form.

The tubes are cleaned by brushing with soap water and immersion in 6 % aqua regia with BrCl overnight, followed by copious rinsing with deionized (DI) water. The glassware is then baked in a clean oven at 105 °C overnight before use. The clean tubes are capped with new septa and double bagged in new polyethylene zipper bags until needed. The digestion tubes use the same septa and caps as used with the 40 mL VOC vials.

3.2. *Class A Volumetric Flasks* - Only Class A volumetric flasks may be used. The graduated glassware used for sample preparation must be specially prepared, and for this reason many glassware items are dedicated for only one purpose. Seven 100 mL volumetric flasks are dedicated to the preparation of calibration standards. Additionally, 100 mL volumetric flasks are dedicated to the preparation of second-source QC check sample (DORM-3). All flasks are soaked in aqua regia overnight and rinsed with DI water 6 times prior to use.

3.3. *Fisher Dry Bath Incubator* - The incubator is an 18-well heating block used for the digestion of solid samples. Each block accommodates 75 mL glass culture tubes with a maximum outer diameter of 25 mm and small hole for a thermometer or thermocouple.

3.4. *Labconco Freezer Dryer* - The Labconco 12 L freezer dryer is only suitable for aqueous-based samples. The system is programmable for walk-away operation. Sediment samples from customer Ambient with percent solids less than 25 % must go through the freeze-drying process to increase the percent solids. Refer to DEP SOP **CM-030** for operation of the freeze dryer.

3.5. *Tekran Model 2700 Methylmercury Analyzer* - Serial Number 007, purchased in 2011:

3.5.1. *GC Oven* – Built in oven, programmable for either isothermal or temperature ramp chromatography with a wide-bore capillary GC column.

3.5.2. *Pyrolyzer* – Located downstream of the GC oven is a programmable pyrolyzer used to reduce all gas-phase mercury compounds to gas-phase elemental mercury. The normal pyrolyzer temperature is 720 °C.

3.5.3. *Tekran Model 2700 Cold Vapor Atomic Fluorescence Detector* – Features a temperature controlled, analytical grade mercury lamp, optical feedback electronics to ensure constant lamp intensity, and argon purging of the entire optical path to eliminate baseline shifts from ultraviolet (UV) light absorbing compounds in the laboratory ambient air.

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3.6. *Tekran Model 2621 Auto-Sampler* - 63 positions. Includes a dual-bore needle capable of piercing the sample vial septa, injecting the purge gas, and extracting the eluted methylmercury for delivery to the analyzer.

3.6.1. *Autosampler Vials and Septa* – Thermo Scientific 200 Series, 40 mL. Amber, borosilicate glass VOA vials with 0.125" silicone/PTFE septa and white polypropylene cap.

3.7. *Pipettes:*

3.7.1. 10 – 100 µL adjustable Eppendorf

3.7.2. 100 µL non-adjustable Eppendorf

3.7.3. 100 – 1000 µL adjustable Eppendorf

3.7.4. 2 – 10 mL adjustable Finnpipette

3.8. *Balance* - Sartorius513-1S.

3.9. *Thermometer* - A NIST-traceable thermometer must be used to document the dry bath incubator during sample digestion.

4. REAGENTS, STANDARDS, AND CHEMICALS

All bottles of reagents in the laboratory are required to have unique identifiers. This is accomplished by logging purchased and prepared reagents into the LIMS standard prep tracker. A label, which includes a serial number, must be printed and placed on the bottle of reagent. See DEP SOP **MT-069** for labeling and documentation requirements as well as expiration date information for reagents. The serial numbers of reagents that are used during the preparation of samples are documented in the LIMS prep worksheet manager. The serial number of reagents used during analysis and standard preparation are documented on Excel reagent forms. Reagent forms for standard preparation and sample analysis are located on the Division's website.

4.1. *DI Water* – Type 1 DI water is prepared in the laboratory from the municipal water supply. The criterion for laboratory DI water requires that no analytes of interest be present at a concentration greater than the MDL. Other non-target compounds may be detected in the laboratory DI water, but must not interfere with the analysis of the target compounds.

The DI water is purified with a Solution 2000 Water Purification System that uses activated carbon cartridges and ion-exchange cartridges to remove impurities. The laboratory DI water is evaluated every day, when sample data is collected, as a method blank sample.

4.2. *Methanol (CH_3OH)* - Purge & Trap grade methanol is purchased in 500 mL amber bottles from Fisher Scientific.

4.3. *Gas Cylinders:*

4.3.1. Argon (UHP grade) used to purge the fluorescence detector as a sheath gas and as make-up gas.

4.3.2. Nitrogen (UHP grade) used as a general purpose gas to flush gas/water lines or dry tubing.

4.4. *Methionine* - Methionine is purchased from Sigma as 99 % DL-Methionine. The method blank and LFBs are prepared by including reagent grade methionine as a solid matrix.

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Since there are no commercially available clean biological tissues for mercury, the compound methionine is used as a biological matrix for blank and LFB samples. The methionine matrix will continue to be used until a suitable biological matrix is identified.

4.5. *25 % KOH/Methanol (weight %) Solution* - This solution is used for the digestion of tissue and other solids to aid in the release of methyl mercury from the sample matrix. It is prepared by dissolving 25 g of reagent grade KOH pellets (Aldrich) into 100 g of purge & trap grade methanol. Stir the final solution for a few minutes until all the pellets have dissolved to yield a clear solution. Store the solution in a Teflon bottle.

4.6. *Citrate Buffer + Anti-Foam Solution* - The buffer solution is used to adjust the pH of the samples to 4.5 before ethylation. The solution is prepared by weighing 107.9 g of reagent grade citric acid monohydrate (Aldrich), 146.79 g of reagent grade sodium citrate (Aldrich) or 167.1 g of reagent grade sodium citrate dehydrate and 125 mL of Antifoaming Agent-A (Sigma) into a 1 L volumetric flask and then dissolved into 500 mL of DI water. After the solid has dissolved, bring the solution up to the graduation mark using DI water, mix well, and transfer to a clean 1 L Teflon bottle. Normally no additional purification is required.

The buffer solution may be purified, with respect to methyl mercury, by filtering it through a cleanup trap consisting of *Tenax*^{TM} absorbent (30 cm) and granular charcoal (20 cm). The cleanup trap is prepared by pouring the trapping materials into a clean glass pipet. Place a glass wool plug into the pipet before adding the trap absorbents. When adding the trap media to the pipet, make sure that voids in the trap are removed by tapping the side of the pipet gently and then passing some DI water through the trap. After the trap is prepared, pour the citrate buffer solution into the top of the trap and collect the filtrate into a 500 mL small mouth Teflon bottle. The citrate buffer solution will pass through the filter slowly by gravity feed, but can be accelerated, if necessary, by using about 3-5 psi from a UPC N_2 gas source. The filtered citrate buffer solution is stored in the Teflon bottle used to collect the filtrate.

The buffer solution may also be UV light-treated in a UV digestion chamber to reduce methyl mercury levels by converting it to Hg^{+2}. The UV light treatment also has the added benefit of removing algae that may grow in the buffer solution over time.

The solute in the buffer may crystallize out from solution during storage and be visible as a slight to severe turbidity in the solution. Warm the bottle in a 90 °C sonicating water bath until the suspended solids have dissolved to yield a clear solution before use. The expiration date for the buffer solution is 360 days from preparation.

4.7. *Sodium tetraethyl borate ($NaBEt_4$) solution (2 %)* - Sodium tetraethyl borate (98 % purity from Strem Chemicals) is the ethylating reagent used to derivatize the methyl mercury chloride to volatile methyl ethyl mercury and n-propyl mercury to n-propyl ethyl mercury. The preparation of this reagent requires great care and should only be performed by an experienced chemist. The ethylating reagent is prepared by following the steps outlined below:

4.7.1. *Vial Preparation* - Fifty 2 mL amber GC vials with Teflon^{TM} lined caps are needed for storing the reagent. The vials (without cap) are first baked at 175 °C overnight. After baking, cool the vials to room temperature. Fill each vial with argon gas and cap immediately. Place the capped vials in a rack. The rack of vials must be protected from dust by placing it in a plastic zip-lock bag. The bagged rack is then placed in a freezer for at least 30 minutes or until needed.

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4.7.2. *Solvent Preparation* - One gram of reagent grade KOH pellets is dissolved in 50 mL of DI water in a pre-cleaned 125 mL Teflon bottle. Add argon gas to the bottle to exclude air. The KOH solution is then chilled to near freezing by placing the bottle in a freezer for at least 30 minutes. The KOH solution is ready to use when it starts to form a slush, but do not freeze the solution.

4.7.3. *Sodium tetraethyl borate ($NaBEt_4$) Solution (2 %) Preparation* - Solid sodium tetraethylborate ($NaBEt_4$) is purchased in small (1.0 g) quantities from Strem Chemicals (Newburyport, MA). The compound is received in small amber glass bottles under argon gas, since it is air-sensitive. The original bottle containing the compound is stored in a freezer until needed. To prepare the reagent, carefully clean the outside of the bottle, removing any sealing wax that may be present. Open the 1.0 g bottle in a laboratory hood and immediately pour and mix the entire contents of the bottle into the Teflon bottle containing the 50 mL of chilled KOH solution. Remove the chilled rack of 2 mL vials from the freezer and swiftly fill each of the vials with 1 ml of the $NaBEt_4$ solution. The transfer process into the smaller vials goes faster and gives better results when two people work together. You must work fast in order to assure the $NaBEt_4$ reagent does not degrade. Cap and label the filled vials. Place the vials into the freezer for storage until needed.

4.7.4. *Working with the $NaBEt_4$ Solution* - Remove the prepared 2 mL vials of $NaBEt_4$ solution from the freezer as needed and thaw slightly until a small layer of liquid collects on top. To prevent the reagent from thawing completely during use, an ice-filled cooling jar is used to hold the vial. This reagent is very sensitive to any oxidant, including oxygen. Therefore, it is imperative to minimize the length of time the reagent is exposed to air. The vial must be capped tightly immediately after use, and returned to the freezer. Typically the $NaBEt_4$ vials are used to prepare only 1 batch of samples, but may be used a maximum of two times (three freeze/thaw cycles) before being discarded. The expiration date for the 2 % $NaBEt_4$ solution is 360 days from preparation.

4.8. *Methyl Mercury Standard Preparation* - All bottles of standard in the laboratory are required to have unique identifiers. This is accomplished by logging purchased and prepared standards into the LIMS standard prep tracker. A label, which includes a serial number, must be printed and placed on the bottle of standard. See DEP SOP **MT-069** for labeling and documentation requirements as well as expiration date information for standards. Standards used during sample preparation shall be documented in the LIMS prep worksheet manager. Standards that are used during the analysis of samples must be documented in the lab report. Standards used for analysis are documented on Excel standard forms. Standard forms for analysis are located on the Division's website.

Note: *Concentrated solutions of CH_3HgCl are highly toxic. Care must be taken while working with these solutions. Always wear gloves, preferably plastic-laminate Silver Shield™ gloves, while handling these solutions. If a spill should occur, change your gloves immediately and wash your hands with copious amounts of water.*

4.8.1. *Purchased Methyl Mercury Standard (Neat powder)* - The purchased methylmercury standard consists of neat methylmercury chloride powder obtained from Strem Chemicals or Alfa Johnson Matthey. If the manufacturer assigns an expiration date, it shall be used. If the manufacturer does not assign an expiration date, none is required. Store the neat powder in an amber bottle

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away from light in a freezer at < -10 °C. Place the purchased standard in a jar as added protection against bottle breakage or cross-contamination.

4.8.2. *Methyl Mercury Standard MHG-A (400 mg/L Hg)* - Weigh 250.4 mg of CH_3HgCl (equivalent to 200 mg Hg) on an analytical balance, and transfer the solid to a 500 mL volumetric flask containing 250 mL of purge & trap grade methanol. Swirl and sonicate the flask until the solid dissolves, and then dilute the solution to the mark with methanol. Mix the standard in the flask thoroughly before transferring the solution into an amber bottle with Teflon cap. Cap the labeled bottles and place them in a freezer at < -10 °C for storage. The expiration date for MHG-A is 360 days from preparation.

4.8.3. *Methyl Mercury Standard MHG-B (400 µg/L)* - Dilute 100 µL of MHG-A with purge & trap grade methanol to a final volume of 100 mL in a pre-cleaned glass volumetric flask. Mix well and transfer the standard into 125 mL amber, glass bottle. Store the labeled bottle in a freezer at < -10 °C. The expiration date for MHG-B is 360 days from preparation.

4.8.4. *Methyl Mercury Standard MHG-C (2µg/L)* - Dilute 500 µL of MHG-B with purge & trap grade methanol to a final volume of 100 mL in a volumetric flask. Mix well and transfer the standard into an amber, glass bottle. Store the labeled bottle in a freezer at < -10 °C. The expiration date for MHG-C is 360 days from preparation.

4.8.5. *Methyl Mercury Standard MHG-D (200 ng/L)* - Dilute 100 µL of MHG-B with purge & trap grade methanol to a final volume of 200 mL in a volumetric flask. Mix well and transfer the standard into five labeled 40 mL VOC vials. Store the vials in a freezer at < -10 °C. The expiration date for MHG-D is 360 days from preparation.

4.9. *Calibration Standard Preparation*. When preparing calibration standards take precaution not to expose the instrument system to mercury levels above 10 ng/L, otherwise there may be contamination or carryover problems. The instrument is designed for trace level work only. Exposing the instrument to ppb levels, or higher, may result in serious damage.

4.9.1. Calibration standards, with concentrations of 0.020 - 5.0 ng/L, are prepared using the working standards MHG-C and MHG-D. Continuing calibration verification (CCV) standards [STD-0.4 (0.4 ng/L) or STD-1 (1.0 ng/L)] are analyzed every 10 samples.

4.9.2. A new calibration curve must be prepared if any of the following situations occur: 1) An initial instrument calibration needs to be performed; 2) a CCV standard is repeatedly not within acceptance limits; 3) significant instrument maintenance is performed.

4.9.3. Calibration standards are prepared in 100 mL volumetric flasks from the two working stock solutions MHG-C (2 µg/L) and MHG-D (200 ng/L). The volumes and standard required to make the calibration standards are shown in Table 1. 4.0 mL of citric buffer is added to each 100 mL flask to maintain the proper pH.

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Table 1: Calibration Standard Preparation Volumes and Concentrations

Calibration Standards	Name of the Stock	Volume of Stock used (µl)	Final Volume (ml)	Final Concentration (ng/L)
STD-0.02	Working Standard MHG-D (200 ng/L) Surrogate Working Solution (200 ng/L)	10 10	100	0.020
STD-0.10	Working Standard MHG-D (200 ng/L) Surrogate Working Solution (200 ng/L)	50 50	100	0.10
STD-0.2	Working Standard MHG-D (200 ng/L) Surrogate Working Solution (200 ng/L)	100 100	100	0.20
STD-0.4	Working Standard MHG-D (200 ng/L) Surrogate Working Solution (200 ng/L)	200 200	100	0.40
STD-1 (CCV-1)	Working Standard MHG-C (2.0 µg/L) Surrogate Working Solution (2 µg//L)	50 50	100	1.0
STD-2 (CCV-2)	Working Standard MHG-C (2.0 µg/L) Surrogate Working Solution (2 µg//L)	100 100	100	2.0
STD-5	Working Standard MHG-C (2.0 µg/L) Surrogate Working Solution (2 µg//L)	250 250	100	5.0

4.9.4. Ethylation of the calibration standards is accomplished by adding 60 µL of the ethylating reagent ($NaBEt_4$) to the 100 mL flasks. Add reagent water to bring the volume up to the graduation mark. Rotate the volumetric flask two times to mix the solution.

4.10. *SRM-QC Check Standards (DORM3-A and DORM3-B):*

4.10.1. Certified dogfish muscle tissue (DORM-3) from the National Research Council of Canada (NRCC) is used as an external QC check in this method. This material, which is a freeze-dried brown powder, contains 0.355 ± 0.056 mg/kg methyl mercury. The expiration date is provided by the manufacturer.

4.10.2. *Preparation of DORM3-A Standard (1.9 µg/L)* - Weigh 536 mg (accurately weighed to the nearest milligram) of the DORM-3 powder into a 75-mL digestion tube. Add 10 ml of 25 % KOH in methanol solution and digest the tissue SRM in the dry bath incubator at 90 °C for 60 minutes. The resulting digested solution has some residues. Transfer the DORM-3 solution to a clean 100 mL volumetric flask, and dilute to the mark with purge & trap methanol. This is DORM3-A. Transfer the solution to an amber glass bottle and keep in a

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freezer at < -10 °C. The expiration date for DORM-3A is 360 days from preparation.

4.10.3. *Preparation of DORM3-B Standard (190 ng/L)* - The DORM3-B standard is prepared by diluting the DORM3-A solution tenfold with purge & trap methanol. Add 10.0 mL of the DORM3-A solution to a 100 mL volumetric flask containing 50 mL of methanol. Fill the flask to the mark with methanol, cap the flask firmly and shake well. Transfer the standard to an amber glass bottle and store in a freezer at < -10 °C. The expiration date for DORM3-B is 360 days from preparation.

4.10.4. *Preparation of DORM-3 QC Check Sample (0.38 ng/L)* - To prepare the DORM-3 QC Check Sample, pipette 200 µL of DORM3-B into a 100 mL volumetric flask containing DI water and ethylate directly. Analyze at least one DORM-3 Check Sample standard along with every batch of 20 or fewer samples.

4.11. *Method Blank, Lab Fortified Blank (LFB), and Matrix Spike (MS) Samples:*

The method blanks, LFBs, and matrix spike samples are all prepared in a standard 75 mL digestion tube that has a calibrated 50 mL volume mark. Once the digestions are complete, the volume is brought up to the 50 mL mark with methanol. The final solution concentration for the samples is achieved by diluting the extract 1000X. Other dilution factors may be more suitable to a particular analytical situation, but will yield a different final concentration.

4.11.1. *Method Blank* - The sediment and tissue method blanks are prepared by weighing 0.10 g of Methionine and 1.0 g of Ottawa sand and adding it to 5.0 mL of 25 % KOH/CH_3OH in a clean digestion tube. The solution is then spiked with 25 µL of the 200 µg/L n-propyl mercury surrogate standard. The method blank is digested following the same preparation procedure as normal solid samples in a 75 mL digestion tube.

4.11.2. *Lab Fortified Blank (LFB)* - The LFB sample is prepared by weighing 0.10 g of Methionine and 1.0 g of Ottawa sand and adding it to 5.0 mL of 25 % KOH/CH_3OH in a clean digestion tube. The solution is then spiked with 62.5 µL of the standard MHG-B (400 µg/L) and 25 µL of the 200 µg/L n-propyl mercury surrogate standard. The LFB sample is digested following the same preparation procedure as for normal solid samples, yielding a final solution concentration of 1.0 ng/L after dilution.

4.11.3. *Matrix Spikes* - Weigh a sub-sample from a randomly selected sample. The amount of sample weighed is dependent on matrix type (see Section 5.5.1.). Record the weight to 3 significant figures in the prep worksheet. Add the sub-sample and 5.0 mL of 25 % KOH/CH_3OH into a clean 75 mL digestion tube. The solution is then spiked with 125 µL of MHG-B (400 µg/L) and 25 µL of the 200 µg/L n-propyl mercury surrogate standard. The matrix spike sample is digested following the same preparation procedure as for normal solid samples, yielding a final (added) solution concentration of 2.0 ng/L after dilution.

4.12. *n-Propyl Mercury Chloride Surrogate Spiking Solutions:*

4.12.1. *n-Propyl Mercury Chloride Surrogate Purchased Standard (10.0 mg/L)* - The n-propyl mercury chloride standard is custom prepared by ERA Chemical Corp. at a concentration of 10.0 mg/L in purge & trap grade methanol. The standard is stored at -10 °C and the manufacturer assigns a 360 days expiration date.

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4.12.2. *n-Propyl Mercury Chloride Surrogate Standard (200 µg/L)* - The surrogate standard is prepared by adding 1.0 mL of the purchased standard into a 50 mL volumetric flask. The volumetric flask is brought to volume with Purge & Trap grade methanol. After mixing, the standard is transferred to an amber bottle with a Teflon cap. The expiration date is 360 days from preparation and is stored at < -10 °C.

4.12.3. *n-Propyl Mercury Chloride Surrogate Standard (2 µg/L)* - The surrogate standard is prepared by adding 1 mL of the 200 *µg/L* standard into a 100 mL volumetric flask. The volumetric flask is brought to volume with purge & trap grade methanol. After mixing, the standard is transferred to an amber bottle with a Teflon cap. The expiration date is 360 days from preparation and is stored at < -10 °C.

4.12.4. *n-Propyl Mercury Chloride Surrogate Standard (200 ng/L)* - The surrogate standard is prepared by adding 100 µL of the 200 *µg/L* standard into a 100 mL volumetric flask. The volumetric flask is brought to volume with purge & trap grade methanol. After mixing, the standard is transferred to an amber bottle with a Teflon cap. The expiration date is 360 days from preparation and is stored at < -10 °C.

5. SAMPLE COLLECTION, PRESERVATION, AND PREPARATION

5.1. *Sample Collection* - The plant and animal tissue samples are collected in glass or Teflon large mouth containers. The containers are double bagged in opaque brown polyethylene bags and shipped for analysis.

5.2. *Sample Preservation* - The plant and animal tissue samples are preserved by freezing.

5.3. *Sample Holding Time* - The holding time for solid samples is 28 days from the time of sample collection.

5.4. *Sample Homogenization* - Solid samples must be homogenized prior to digestion to ensure representative sub-sampling and for particle size reduction. A description of representative matrix types and their respective homogenization procedure is given below:

5.4.1. *Feathers/Fur/Hair* – For small samples, no homogenization may be required. However, large samples may require size reduction for the digestion step. To aid in digestion, cut whole feathers, fur, and hair into small pieces and place into a clean agate mortar. The sample must then be cooled cryogenically in the mortar to aid in the grinding process. Pour liquid nitrogen, from a Dewar flask, into the mortar a small amount at a time so as to not rinse the sample out. At first the liquid nitrogen will quickly boil away, but after a minute, or so, of adding the liquid nitrogen it will begin to accumulate in the mortar. Once the sample is frozen it becomes fragile and is readily ground into a fine powder with the pestle.

5.4.2. *Other Solid Samples* – Samples such as fish tissue, bird organs, plant tissue, and sediment are homogenized using a mechanical bio-homogenizer, if required.

5.5. *Sample Digestion and Dilution.* Digestion is necessary to chemically dissociate methyl mercury from its host substrate before it can be analyzed. This must be done without destroying or creating methyl mercury itself in the process. In most solid samples, methyl mercury is bound to organic matter, proteins in tissue, and other sulfur containing

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species in sediments. A solution of 25 % potassium hydroxide in methanol is used to release the bound methyl mercury.

5.5.1. The precise amount of sample weighed for digestion will vary for different matrices, depending upon the expected methyl mercury level and the amount of sample available. Record the weight to 3 significant figures in the prep worksheet.

- For *Feathers/Fur/Hair, w*eigh 0.02 g to 0.05 g into a 75 mL digestion tube and add 5.0 mL of the 25 % KOH/Methanol solution.
- For *Other Solid Samples,* weigh 0.5 g to1 g into a 75 mL digestion tube and add 5.0 mL of the 25 % KOH/Methanol solution. The volume of KOH/methanol solution may need to be increased up to 20 mL to digest difficult samples.

5.5.2. A surrogate, n-propyl mercury, is added to each sample. The amount added depends somewhat on the dilution factor anticipated in the analysis of the extract. Typically, a solid sample is digested and diluted to 50 mL and is then diluted again by a factor of 100. Adding 25 µL of 200 µg/L n-propyl mercury standard to the sample will yield a final concentration of 1.0 ng/L in the sample. The final surrogate concentration will vary depending on the dilution factor used. The amount of surrogate solution added may need to vary depending on the level expected in the sample.

5.5.3. Cap the tube tightly, and place it in a *Dry Bath* heating block at 90 °C for one to four hours, or until the sample is liquefied. Samples consisting of protein tissue such as feather, muscle, or fish tissue must be dissolved completely to yield a clear yellowish solution at the end of the procedure. For sediment samples, the inorganic components of the sample will remain unaffected. Likewise, for plant samples, polymeric components such as lignin will also remain un-dissolved.

5.5.4. Read the heating block temperature with a NIST-traceable calibrated thermometer and record the heating block temperature in the prep worksheet. The temperature should be 90 ± 5 °C.

5.5.5. Add methanol to the digestate to bring the total volume up to the 50 mL graduation on the digestion tube. This is considered to be the nominal volume of the digested sample. Further dilution with methanol may be necessary to ensure that the concentration of the final solution is within the instrument's calibration range.

5.5.6. The methanol digestates must be diluted prior to derivatization and analysis. The dilution factor must be matched to the expected mercury level in the sample and the presence of matrix interference. Typical dilution factors range from 100 to 4000.

- *For LFB and Matrix Spike Samples* pipette 200 µL of the diluted methanol digestate into a 100 mL volumetric flask containing 50 mL of DI water. Proceed to the buffer addition step in Section 5.6.
- *For Animal Tissue and Sediment Samples* pipette 25 µL to 1.0 mL of the diluted methanol digestate into a 100 mL volumetric flask containing 50 mL of DI water. Sample volume depends on the anticipated level of methyl mercury in the sample, however using dilution factors less than 100 typically has poor surrogate recovery. Use smaller sample volumes

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for high-level samples. Record the volume to 3 significant figures in the prep worksheet. Proceed to the buffer addition step in Section 5.6. (In-vial process is also possible, but requires that all of the following volumes be adjusted accordingly for the standard VOA vial volume.)

- *For Plant Tissue Samples* pipette 1 mL of the diluted methanol digestate into a 100 mL volumetric flask containing 50 mL of DI water. Plant tissue samples normally have very low mercury levels and require the lowest dilution possible. Proceed to the buffer addition step in Section 5.6.

5.6. *Citrate Buffer Solution Addition* - Add 9.0 mL of citrate buffer to each 100 mL volumetric and bring the solution to the 100 mL mark with DI water. The citrate buffer serves to adjust the pH of the solution to the 4.1–4.5 range. The diluted sample solution is now ready for derivatization. Proceed to the sample derivitization step in section 5.7.

5.7. *Sample Derivatization*:

5.7.1. Add 60 µL of the ethylating reagent $NaBEt_4$ to the 100 mL flask containing the dilute sample digestion. (The derivatization step may alternatively be performed directly in a 40 mL VOA vial with the addition of 25 µL of the ethylating reagent.)

5.7.2. Cap the flask and rotated gently 2-3 times to mix the sample. Immediately transfer the sample into a 40 mL VOA vial and cap firmly, allowing no headspace. Flip the vial upside down and tap it with a finger to check for bubbles inside the vial. If there are bubbles add more sample and recap the vial.

5.7.3. The ethylating reaction takes about 15 minutes to reach completion. It is therefore critical that the sample preparation steps proceed rapidly once the ethylating reagent is added to the sample. Also, there is a risk of incomplete reaction if the sample is analyzed prior to completion of the 15 minute time period. Make sure the samples are allotted at least 15 minutes to ethylate before proceeding with the analysis.

5.8. *Sediment samples freeze-drying:*

5.8.1. Sediment samples from customer Ambient with percent solids less than 25 % must be processed using the freeze-dryer. Refer to DEP SOP **CM-030** for instructions.

5.8.2. Weight about 100 g of sample and pre-freeze the aliquot.

5.8.3. Place samples onto one of the freeze-drier trays and place the thermocouple sensor near the samples.

5.8.4. Start the Freezer Dryer Program and perform freeze-drying at -10 °C for 96 hours.

5.8.5. Refer to DEP SOP **CM-030** for shut down, defrosting, and cleaning.

6. SAMPLE ANALYSIS AND QUANTITATION

6.1. *System start up from shutdown* – If the instrument was scheduled to not be used for several days, it may have been placed in shutdown mode. First, check the argon supply and make sure there is enough for the day's planned analysis. To turn the instrument on,

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flip the switch on the rear panel of the instrument. Allow 1 hour for instrument warm up and stabilization. Remember to turn on the carrier gas (Ar) supply to the instrument whenever the system is powered up (the pressure delivered to the instrument should be 60 PSI). Argon flow should always be on whenever the system is powered up to keep the system as clean as possible and prevent possible fogging of the cuvette and lamp.

6.2. *Front LCD panel* – By pressing the two arrow buttons under the display simultaneously following power up, two LCD status screens can be viewed. To switch between the screens press one of the up/down arrow buttons. The primary screen features a useful summary of the system's status. The secondary screen deals mainly with water detector alarm settings and information used during the set up at the factory. See the Model 2700 User Manual for details on the system status indicators. For normal operation, the status icons should have the following statuses:

- General System Status Yellow
- Tekran USB Status Green
- Detector Status Green
- Syringe Status Blinking red (no syringe)
- Liquid Detector Alarm Should *not* be visible.

If any of these signals is not in the above status or any of them appear in a different color, the instrument should be restarted to reestablish communication with the software.

6.3. Locate the Tek-MDS icon on the computer desktop and double click to open the software. If the instrument is communicating properly with the software, the message 'Online' should appear in the status bar at the bottom of the screen.

6.4. *Create (or retrieve) a worksheet* - Sample names along with autosampler vial locations can be entered as the system warms up and stabilizes. The worksheet should always start with one or two 'Clean' cycles, followed by several washes. Click File → Save to save the worksheet.

Example analysis sequence:

Clean
Clean
Wash
Wash
Calblank
Calblank
Calblank
Std0.02
Std0.1
Std0.2
Std0.4
Std1
Std2
Std5
CCV
CCB
PQL Check
DORM3

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LFB
Sample A
Sample A Matrix Spike
Sample A Matrix Spike Duplicate
Samples…
CCV
CCB

6.5. Use the 'Run Worksheet' button to specify the first and last sample in the worksheet.

6.6. After pressing the 'Start' button the system will start analyzing the first sample in the worksheet.

6.7. When all samples in the worksheet have been analyzed, the autosampler will automatically park itself in the wash station and the system's settings will revert to their 'Idle' state. The argon supply must be left on when the instrument is in the 'Idle' state.

6.8. *Exporting Results* – After the run is completed, print the data and 'export file' to Tek Data. The file should be stored in the directory 'Tek Data'. The naming of the data file should follow the following convention: YYYYMMDDs1. As an example, consider 20130730s1. This name indicates that the file contains data from the first analytical run on sediment (s1) samples conducted on July 30th, 2013. This nomenclature identifies the day the data file is generated and the matrix analyzed. If more than one run is conducted in any given day, then the numbers 1, 2, … may be used to number successive runs in the day. Check to make sure the file saved correctly before exiting the program. Then, the worksheet needs to be saved as an Excel and text file. The Excel and text file are saved to \\FLDEP1\DEAR\Labs\Lab3\Chem_Data\Inorganic\Mercury\Tekran 2700 with the same naming format, YYYYMMDDs1.

6.9. *Calibration Equation* -

Concentration (ng/L) = (Peak Area – Blank Subtraction) / Calibration Factor

The software determined the value for Blank Subtraction as the average of the Calblank peak areas. The Calibration Factor is determined using an EPA-style slope-average.

7. DATA PROCESSING AND ARCHIVAL

Refer to the records maintenance and storage DEP SOP **LB-013** for data archival procedures.

8. QUALITY CONTROL MEASURES, ACCEPTANCE LIMITS, AND CORRECTIVE ACTIONS

The following QC samples must be analyzed with all sample batches to ensure proper quality assurance in the data collected. The various quality control measures and acceptance criteria are provided and set within the limits as established by EPA Method 1630. Corrective actions for unacceptable data are also discussed. Details on reporting non-conforming situations may be found in DEP SOP **LB-002**.

8.1. *Calibration Curve and Continuing Calibration Verification (CCV) Sample* - The calibration curve must be linear and have a correlation coefficient greater than or equal to 0.995. Prepare a new calibration curve in the event of a correlation coefficient failure or QC check standard failures. The CCV recovery must lie between 70 % and 125 % of its expected value. If the check standard does not pass the QC criteria, then re-run the failed

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QC check sample. If the QC check fails again, then make instrument repairs and re-run the calibration.

8.2. *Method Blank* - The method blank must have a value < MDL and > -MDL. At least one method blank must be analyzed for every 20 samples. If the method blank fails, check for any possible contamination from the glassware cleaning procedures and sample preparation procedures. There is no need to rerun the samples if the methyl mercury levels in the samples are either greater than ten times the method blank or below the detection limit. Samples not satisfying this condition must be reanalyzed, re-ethylated, re-digested, or results reported with appropriate qualification.

8.3. *PQL Check Sample* - The PQL is verified in every batch by analyzing a DI water sample spiked at the PQL level (0.080 ng/L) using the DORM-3 standard. A PQL check sample must be analyzed for every batch of samples. The PQL sample must be reanalyzed if it does not pass QC requirements. If the PQL does not pass when reanalyzed, the batch of samples needs to be re-ethylated or re-digested.

8.4. *QC Check Standard Reference Sample* - An external QC standard reference material (SRM) is analyzed to check the calibration curve. Dogfish muscle tissue (DORM-3) is used as the SRM for this analysis. The DORM-3 recovery must lie between 70 % and 125 % of its expected value. If the DORM-3 standard does not pass the QC criteria, then re-analyze the standard. If the DORM-3 standard sample fails a second time, then prepare a new calibration curve. If the DORM-3 standard fails after preparing a new calibration curve, then the batch of samples, including the DORM-3 should be re-ethylated or re-digested.

8.5. *Matrix Spikes* - Duplicate matrix spikes are analyzed to check the efficacy of the digestion and ethylating procedures. The matrix spikes are prepared by spiking a randomly selected sample with methyl mercury at an added level of 2.0 ng/L in the final solution (see section 4.11.3.). The recovery of each matrix spike must be between 70 % and 130 % of its expected value. The RPD between the duplicate spikes must be ≤ 30 %. In case of matrix spike recovery failure, re-run, re-ethylate, or re-digest the samples.

8.6. *Lab Fortified Blank (LFB)* - One LFB is prepared for every 20 samples by spiking a method blank before digestion with methyl mercury at a level of 1.0 ng/L in the final solution (see section 4.11.2.). The LFB recovery must be between 70 % and 130 % of its expected value. In case of LFB failure, re-run, re-ethylate, or re-digest the samples.

8.7. *n-Propyl Mercury Surrogate Recoveries* - The compound n-propyl mercury chloride is spiked into all samples to monitor the sample preparation, derivatization, and analysis steps. The recovery of the surrogate must lie between 50 % and 150 % of its expected value. The sample should be reanalyzed, re-ethylated, or re-digested if the surrogate recovery limits are exceeded.

8.8. If for any reason the quality control results do not fall within the acceptance limits, then the associated samples must be appropriately commented and/or qualified as described in DEP SOP **LB-027**.

9. SAFETY AND WASTE MANAGEMENT

9.1. Review the Laboratory Health and Safety Plan, https://www.fldepnet.org/content/dear/bureau-laboratories-safety-documents.

9.2. Always wear protective eyewear and lab coats while in the laboratory.

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9.3. Gloves must be worn when handling analytical standards and waste materials.

9.4. Laboratory waste containers must be labeled. When full, the laboratory waste containers must be emptied into designated waste drums located within the Waste Room in the Laboratory Complex.

9.5. Organo-mercury compounds such as methyl mercury chloride and n-propyl mercury chloride are known to be highly potent neurotoxins. Solutions containing high levels of methyl mercury chloride such as standards and stock solutions must be handled with great care. Excess or expired methyl mercury standards or stock solutions must be disposed as hazardous wastes. The CH_3Hg^+ waste container is located in the hood.

9.6. Sodium tetraethyl borate ($NaBEt_4$), the ethylating reagent used in this analysis, is a highly toxic compound, is air sensitive, and potentially pyrophoric. Sodium tetraethyl borate must be handled under a hood. These compounds are handled safely using normal laboratory protocols by wearing safety glasses and gloves at all times, and using a laboratory hood when appropriate. Dispose of sodium tetraethyl borate waste in the $NaBEt_4$ waste container in the hood.

10. CONTAMINATION PREVENTION

10.1. To prevent lab contamination of methyl mercury and other mercury species, all waste generated from laboratory activities must be treated carefully, following the waste management procedure outlined above.

10.2. To prevent potential sample cross contamination it is essential to store standards, water samples, and solid samples in different refrigeration units.

10.3. All samples must be bagged at all times. Dispose of samples according to laboratory policy in a timely fashion to avoid build-up of older samples.

10.4. Do not use standard reagent glassware to measure or handle samples. Dedicate separate glassware for this purpose.

10.5. Inorganic mercury contamination is frequently encountered in laboratory environments due to broken thermometers and other mercury-containing devices. Diligence and proper disposal must be exercised to minimize the amount of mercury in the work environment since this is a common interference for the methyl mercury analysis.

10.6. Hair, dander, and common dust may contain elevated levels of methyl mercury. Thus, good hygiene and laboratory cleaning practices need to be in place.

11. INTERFERENCES

The methyl mercury method is generally interference free when procedures are performed correctly. Possible interferences may be divided into two major categories, (1) positive interference due to contamination and artifacts, and (2) negative interference due to loss of analyte and signal quenching. The interferences, their sources, and solutions to overcoming them are discussed below.

11.1. *Positive Interferences*:

11.1.1. Methyl mercury is not a particularly common laboratory contaminant at elevated levels, however, all precautions must be taken to ensure that the analysis is conducted in a lab that is as mercury-free as possible. This includes precautions

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such as not allowing mercury thermometers in the lab, and keeping away highly contaminated samples (both inorganic and organic) from the lab.

The steps outlined previously for the cleaning of glassware must be rigorously followed to minimize contamination from a previous batch of samples. Reagents are another potential source of contamination. The citrate buffer used to adjust sample pH has been found to be significantly cleaner than the method-specified acetate buffer, with respect to methyl mercury. The citrate buffer tends to contain methyl mercury at levels well below the MDL.

11.1.2. A positive interference may also be caused by species such as aromatics (toluene, benzene etc.) that fluoresce strongly in the UV at 254 nm. These aromatics have occasionally been detected in sodium tetraethyl borate. When this happens, the manufacturer needs to be notified of the problem, and a fresh batch of the reagent (with a different lot number) must be procured. The fluorescence from aromatics can be very strong and masks the methyl mercury signal, potentially saturating the detector.

11.2. *Negative Interferences:* (Negative interference are much more common in this analysis.)

11.2.1. *Fluorescence quenching* - One form of negative interference may be caused by impurities that decrease or quench atomic fluorescence. These include traces of oxygen and water vapor in the carrier gas stream. Using UHP grade argon as the carrier and makeup gases minimize the problem of negative interference. Inline oxygen and hydrocarbon trap also minimizes chances for interference from the carrier gas. The purge and trap is equipped with a dry purge cycle that also helps prevent introduction of moisture into the sample path from the trap during the desorb step.

11.2.2. *Dissolved Organic Carbon (DOC)* - Elevated levels of DOC can interfere with the derivatization step. The presence of elevated levels of DOC in the sample matrix may be visually apparent as color, typically yellow or brown, in the water sample. This interference may require sample dilution. It has been observed that suspended solids, caused by the precipitation of organic material, cause more interference than DOC. Since DOC tends to precipitate over time, it is advantageous to analyze the sample as soon as possible to minimize DOC-related interference. Additional sample clean-up, such as the distillation technique in EPA method 1630, may be required in extreme cases.

11.2.3. *Suspended Solids* - Unfiltered suspended solids may interfere with the derivatization step. The presence of particulates is normally visually apparent. Sample dilution may be required to reduce the interference to an acceptable level. The methyl mercury concentration in a filtered sample should be the same or lower than for a non-filtered sample collected for the same site (assuming both sample types are collected). Investigate for possible matrix interference if the methyl mercury concentration in a non-filtered sample is significantly lower than a filtered sample collected for the same site. The interference should also be apparent in the surrogate compound recovery.

11.2.4. *Sulfides* - Elevated levels of sulfide in the sample matrix may interfere with the derivatization step. The presence of sulfides in the sample may be indicated by a “rotten egg” odor. This interference may be removed by allowing the sulfides in the sample adequate time to oxidize or may otherwise require sample dilution. Additional sample clean-up, such as the distillation technique in EPA method 1630, may be required in extreme cases.

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12. REFERENCES

12.1. Draft EPA Method 1630, "Methyl Mercury in Water by Distillation, Aqueous Ethylation, Purge and Trap, and CVAFS", USEPA Office of Water, January, 2001.

12.2. Bloom, N. S. "Determination of Picogram Levels of Methyl Mercury by Aqueous Phase Ethylation, Followed by Cryogenic Gas Chromatography with Cold Vapor Atomic Fluorescence Detection". Can. J. Fish. Aq. Sci. **46**, 1131 (1989).

12.3. Bloom, N.S. and Crecelius, E.A. "Determination of Mercury in Seawater at Subnanogram per liter Levels", Mar. Chem. **14**, 49 (1983).

12.4. SOP from Frontier GeoScience, FGS-010: Method for Ultra-Trace Level Determination of Methyl Hg in High Level Solids by KOH/Methanol Digestion Followed by Aqueous Phase Ethylation and CVAFS Detection.

12.5. Quality Manual, Bureau of Laboratories, FDEP, located on the DEP Bureau of Laboratories intranet.

12.6. DEP SOP **CM-018**, Laboratory Policy Regarding Manual Chromatographic Peak Integration.

12.7. DEP SOP **CM-030**, Operating Instructions for the Labconco Freeze Dryer System and Stoppering Tray Dryer.

12.8. DEP SOP **HG-005**, Analysis of Ultra-Trace Level Methyl Mercury in Water by Aqueous Phase Ethylation.

12.9. DEP SOP **LB-002**, Non-Conformance Reporting System.

12.10. DEP SOP **LB-006**, Standard Operating Procedure for Records Maintenance and Storage.

12.11. DEP SOP **LB-013**, Standard Operating Procedure for Archiving/Retrieving Documents.

12.12. DEP SOP **LB-024**, Significant Figures: Policy of the DEP Bureau of Laboratories.

12.13. DEP SOP **LB-027**, Standard Operating Procedure for Reporting Qualified Data.

12.14. DEP SOP **LB-031**, Limit of Detection Verification.

12.15. DEP SOP **MT-069**, Metals Standard and Reagent Preparation Procedure.

12.16. DEP SOP **NU-039**, Calibration of Auto-Pipettes and Dispensers.

12.17. DEP SOP **OG-001**, Safety and Waste Management Practices in the Organic Analysis Groups.

13. APPENDIX OF SIGNIFICANT CHANGES

13.1. Added revision summary.

13.2. Updated references.

13.3. Updated calibration standards and levels

13.4. Added language for use of the freeze dryer.

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13.5. The Tekran 2700 Methylmercury Analyzer was validated and the analysis procedure was added to this SOP (09/04/2013). This SOP was also moved from the VOC group to the Metals/Mercury Group.

APPENDIX A

Tekran 2700 Instrument Settings

Command	Parameters	Start	Duration	Notes
TrapOvenTemp	32.0 °C	1	3000	Trap Oven Temperature Set
GCOvenTemp	220.0 °C	1	190	GC Oven Temperature Set
PyroTemp	720.0 °C	1	3000	Pyrolyzer @ 720 °C
V5	ON	2	7	
A/S Up		2	3	A/S up
A/S Move	-2	6	3	A/S Move to sample
A/S Down		10	5	A/S Down to sample
V4	ON	16	419	
V7	ON	16	419	Trap load vent
V5	ON	180	540	
V6	ON	180	255	V6: Sparger gas bypass
A/S Up		180	3	A/S
A/S Move	0	186	3	A/S
GCOvenTemp	55.0 °C	191	293	Oven Idle
A/S Down		192	4	A/S
TrapOvenTemp	172.0 °C	436	15	Trap: Desorb preheat mode, 172 °C, no flow
Process Value Sca		440	1	Read Instrument
TrapOven Temp	180.0 °C	449	60	Trap 180 Desorb
V10	ON	449	60	Desorb V10
V8	ON	450	270	V8: TrapBackflushHPR supply
V9	ON	450	270	V9: Trap Backflush Vent
GCOvenTemp	92.0 °C	509	300	GC Desorb
AD24 Start	0	509	211	Capture during 300 sec
TrapOvenTemp	203.0 °C	510	193	Trap: Bake @ 200
GCOvenTemp	105.0 °C	640	49	GC Oven Temp 150 to Clean
GCOvenTemp	220.0 °C	650	120	
TrapOvenTemp	32.0 °C	670	68	Trap idle temp
Done		750	0	Done

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