ANALYSIS OF ULTRA-TRACE LEVEL METHYL MERCURY IN WATER BY AQUEOUS PHASE ETHYLATION

FLORIDA DEPARTMENT OF ENVIRONMENTAL PROTECTION

BUREAU OF LABORATORIES

2600 BLAIR STONE ROAD

TALLAHASSEE, FLORIDA 32399

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TABLE OF CONTENTS

1. SCOPE AND APPLICATION 3
2. SUMMARY OF METHOD 3
3. APPARATUS AND EQUIPMENT 4
4. REAGENTS AND STANDARDS 5
5. CALIBRATION 10
6. SAMPLE COLLECTION AND PRESERVATION 11
7. SAMPLE PREPARATION 11
8. SAMPLE ANALYSIS/QUANTIFICATION 12
9. QUALITY CONTROL MEASURES, ACCEPTANCE LIMITS, AND CORRECTIVE ACTIONS 14
10. SAFETY/WASTE MANAGEMENT 15
11. CONTAMINATION PREVENTION 16
12. INTERFERENCES 16
13. MAINTENANCE AND TROUBLESHOOTING 18
14. REFERENCES 19
APPENDIX OF SIGNIFICANT CHANGES 20
APPENDIX A Tekran 2700 Instrument Settings 21

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ANALYSIS OF ULTRA-TRACE LEVEL METHYL MERCURY IN WATER BY AQUEOUS PHASE ETHYLATION

1. SCOPE AND APPLICATION

This method is for the determination of methyl mercury in water samples including surface water, rainwater, and ground water. Most natural waters contain trace levels of methyl mercury on the order of non-detect to a few hundred pg/L. There is a need to monitor methyl mercury at these levels due to its high toxicity, bioaccumulation and biomagnification in the environment. The method detection limit (MDL) for this method is 0.02 ng/L 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 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.[1-4] The modified method incorporates the following steps: direct aqueous ethylation, purge & trap concentration, gas chromatography separation, pyrolysis, and cold-vapor 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. All-together, the stream-lined sample preparation, automated analysis, and use of a surrogate provides for a fast, low cost, and reliable analysis.

This SOP is used to analyze samples having the LIMS Test Identifiers (Test ID) W-CH3HG and W-CH3HG-F.

2. SUMMARY OF THE METHOD

2.1. The analytical procedures used in this method are based on EPA Draft Method 1630, but incorporates significant modifications in the sample preparation to simplify the process and enhance method performance. Side-by-side comparisons between this method and EPA Draft Method 1630 have been performed. The modifications from 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

2.2. The modified method consists of three major steps: matrix modification, derivatization and analysis. Almost all surface waters have small quantities of dissolved organic matter, or dissolved organic carbon (DOC). This organic matter primarily consists of humic and fulvic acids and often impart color to the water. Water samples from the Florida Everglades typically have high loading of DOC, and are often deeply colored. The DOC constitutes a serious interferent in the determination of methyl mercury because of inhibition during the derivatization step and foaming caused by DOC during the sample purge procedure. The interference can be ether inhibited by matrix modification or

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eliminated by sample cleanup. The matrix modification is achieved by adding citrate buffer and antifoaming reagents, and in most cases works extremely well. The matrix modification step is simple and fast to perform. Most importantly, it greatly reduces the chances of destroying or creating methyl mercury during sample preparation, and thus has higher method precision over sample cleanup approaches involving distillation as prescribed in Method 1630.

2.3. Following matrix modification, the sample is then analyzed using a combination of chemical derivatization, sample concentration, chromatographic separation, pyrolysis, and atomic fluorescence detection. The derivatization step transforms the methyl mercury into a volatile compound, methyl ethyl mercury, which may be 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.

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 to convert the organomercury compounds to atomic mercury prior to detection. The atomic mercury is detected with great sensitivity and selectivity using a fluorescence detector.

3. APPARATUS AND EQUIPMENT

3.1. Sampling Containers – Methyl mercury samples may be collected in amber glass or Teflon bottles. Due to cost and convenience considerations, glass bottles are preferred. A bottle study has been performed demonstrating equivalency and is available for review. The I-Chem 500 mL bottles used for sampling are cleaned by rinsing two times with deionized (DI) water and are then baked overnight in a clean oven at 160 °C. The Teflon-lined caps are similarly rinsed two times with DI water and baked overnight in a clean oven at 105 °C. Teflon sampling bottles, 500 mL, are cleaned by soaking in 6 % aqua regia overnight, followed by 3 rinses with laboratory DI water. The sampling containers can be cleaned in batches of up to 120 bottles. Two percent of the cleaned bottles are filled with DI water and are analyzed for quality control (QC) bottle checks. The criterion for the QC bottle check requires that no analytes of interest be present at a concentration greater than the reporting limit.

3.2. Tekran Model 2700 Methylmercury Analyzer – Serial Number 007, purchased in 2011.

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

3.2.2. 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.

3.2.3. Tekran Model 2700 Cold Vapor Atomic Fluorescence Spectroscopy Detector – Features a temperature controlled, analytical grade mercury lamp, optical

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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.

3.3. 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.

3.3.1. 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. The autosampler vials are cleaned prior to use with 6 % aqua regia. Fill the vial ¾ full with 6 % aqua regia and shake. Empty the aqua regia into the sink while flushing the drain with water. Rinse the autosampler vial 6 times with DI water. Allow the vials to dry by placing them upside down on a lint free laboratory wipe.

3.4. Pipettes:

3.4.1. 10 – 100 µL adjustable Eppendorf

3.4.2. 100 – 1000 µL adjustable Eppendorf

3.4.3. 2 – 10 mL adjustable Finnpipette

3.5. Balances - Sartorius Practum 513-1S analytical balance and Mettler Toledo NewClassic MF top-loading balance.

3.6. Class A volumetric flasks, assorted volumes.

3.7. Sonication Bath – Branson 5510 Ultrasonic Bath.

4. REAGENTS AND STANDARDS

All purchased and prepared reagents and standards must be logged into the Laboratory's standard prep tracker module in the LIMS. A label printed from the standard prep tracker must be placed on each bottle of purchased or prepared reagent and standard. See DEP SOP MT-069 for documentation requirements and expiration dates. Reagents and standards used for preparation are documented in the prep worksheet module in the LIMS. Reagents and standards used for analysis are documented on Excel forms located on the Laboratory's computer network at \\FLDEP1\DEAR\LABS\LAB5\Chemistry\InorganicPrep\Mercury\W-CH3HG & W-CH3HG-F.

4.1. Laboratory DI Water - Type-1 laboratory DI water is prepared in the laboratory from the municipal water supply. The Type 1 DI water maintains an approximate 18 Megohm-cm specific resistance.

The municipal 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. Purge & Trap Grade Methanol (CH_3OH) - Purge & Trap methanol is purchased from Fisher Scientific. Stored at room temperature in the fume hood cabinet with other flammable chemicals. Do not store with acids. The expiration date is provided by the manufacturer.

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4.3. 12 M Hydrochloric Acid (HCl) - Trace Metal Grade from Fisher Scientific. Stored at room temperature in the fume hood cabinet. The expiration date is provided by the manufacturer.

4.4. Gas Cylinders - Argon (UHP grade) used in the purge and trap, gas chromatography carrier, to purge the fluorescence detector, and as make-up gas.

4.5. Citric Acid Monohydrate – Purchased from Fisher Scientific. Stored at room temperature. If the manufacturer assigns an expiration date, it shall be used. If the manufacturer does not assign an expiration date, none is required.

4.6. Sodium Citrate – Purchased from Fisher Scientific. Stored at room temperature. If the manufacturer assigns an expiration date, it shall be used. If the manufacturer does not assign an expiration date, none is required.

4.7. Sodium Citrate Dihydrate – Purchased from Fisher Scientific. Stored at room temperature. If the manufacturer assigns an expiration date, it shall be used. If the manufacturer does not assign an expiration date, none is required.

4.8. Antifoaming Agent A – Purchased from Sigma-Aldrich. Stored at room temperature. If the manufacturer assigns an expiration date, it shall be used. If the manufacturer does not assign an expiration date, none is required.

4.9. Tenax Absorbent – Purchased from Sigma-Aldrich. Stored at room temperature. If the manufacturer assigns an expiration date, it shall be used. If the manufacturer does not assign an expiration date, none is required.

4.10. Granular Charcoal – Purchased from Sigma-Aldrich. Stored at room temperature. If the manufacturer assigns an expiration date, it shall be used. If the manufacturer does not assign an expiration date, none is required.

4.11. Citrate Buffer - 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 (Fisher Scientific), 146.79 g of reagent grade sodium citrate (Fisher Scientific) or 167.1 g of reagent grade sodium citrate dihydrate and 250 µL 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 and mix well. The citrate buffer solution should be evaluated for purity in a test blank and must have no analytes of interest present at a concentration greater than the reporting limit. Normally no additional purification is required. If necessary, the citrate buffer solution may be purified, with respect to methyl mercury, by filtering it through a cleanup trap consisting of Tenax™ absorbent (30 cm) and granular charcoal (20 cm) or by exposing the solution to UV radiation.

A 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 1 L wide 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 gas source. The filtered citrate buffer solution is stored in the Teflon bottle used to collect the filtrate.

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UV radiation may be used to decompose $[CH_3Hg]^+$ in the buffer solution. Using UV treatment is very simple and has the added benefit of killing any algae that may be present in the solution. Visible strands of algae have been observed in non-radiated buffer solution if it is not consumed within a few weeks.

The solute in the buffer may crystallize out from solution during storage and is visible as a slight to severe turbidity in the solution. Warm the bottle in a 90 °C sonication water bath until the suspended solids have dissolved to yield a clear solution before use. In a sterile Teflon™ bottle, the solution may be stored as long as one year without expiring.

4.12. 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 to volatile methylethylmercury and Hg^{+2} to diethyl mercury. The ethylating reagent is prepared by following the steps outlined below:

4.12.1. Vial Preparation - Fifty 2 mL amber GC vials with Teflon™ 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. Please 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 low temperature freezer (< -10 °C) for at least 30 minutes or until needed.

4.12.2. Potassium Hydroxide (KOH) Solution 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 (2 – 4 °C) by placing the bottle in a low temperature refrigerator 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.

4.12.3. Sodium tetraethyl borate ($NaBEt_4$) Solution (2 %) Preparation - Solid sodium tetraethyl borate ($NaBEt_4$) is purchased in small (1.0 g) quantities from Strem Chemicals (Newburyport, MA). The compound is received in small glass ampoules under argon gas, since it is air-sensitive. The original ampoule containing the compound is stored in a freezer until needed. To prepare the reagent, carefully clean the outside of the ampoule, removing any sealing wax that may be present. Open the 1.0 g ampoule in a laboratory hood and immediately pour and mix the entire contents of the bottle into the Teflon™ bottle containing 50 mL of chilled KOH. 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. Cap and label the filled vials. Place the vials into the freezer (< -10 °C) for storage until needed.

Note: $NaBEt_4$ is toxic, gives off toxic gases (triethylboron), and is spontaneously combustible. Exercise caution and always wear a lab coat, gloves, and safety glasses while working in the laboratory. To discard unused portions of the ethylating reagent, place the closed vial in a waste container labeled $NaBEt_4$ waste and keep the waste container in the fume hood. Once full, transfer the entire waste container to a shelf in the hazardous waste room.

4.12.4. Working with the $NaBEt_4$ Solution - Remove the prepared 2 mL vials of $NaBEt_4$

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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. The same vial may be used a maximum of two times (three freeze/thaw cycles) before being discarded. In the frozen state, the solution may be stored as long as one year without expiring. Do not use $NaBEt_4$ solid or solutions if they have a yellow color – notify supervisor.

4.13. Methyl Mercury Standards Preparation:

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, and eye protection while handling these solutions. If a spill should occur, change your gloves immediately and wash your hands with copious amounts of water.

4.13.1. Purchased Methyl Mercury Standard (neat powder) - The primary methyl mercury standard consists of neat methyl mercury chloride powder obtained from Strem Chemicals, Alfa Johnson Matthey, or Crescent Chemicals. If the manufacturer provides an expiration date, it shall be used. Store the bottle of neat powder away from light in a standard freezer at < -10 °C. Place the primary standard in a Ziploc bag or jar as added protection against bottle breakage or cross-contamination.

4.13.2. Methyl Mercury Standard MHG-A (400 mg/L CH_3HgCl as Hg) - Weigh 100.1 mg of CH_3HgCl (equivalent to 80 mg CH_3HgCl as Hg) on an analytical balance, and transfer the solid to a 200 mL volumetric flask containing 150 mL of methanol. Swirl and sonicate (if necessary) the flask until the solid dissolves, and then dilute the solution to the mark with methanol. Mix the solution in the flask thoroughly before transferring the solution into amber bottles with Teflon caps. Cap the bottles and place them in a freezer at < –10 °C for storage. This standard has a 2-year expiration.

4.13.3. Methyl Mercury Standard MHG-B (400 µg/L CH_3HgCl as Hg) - Dilute 100 µL of the MHG-A standard with methanol to 100 mL in a volumetric flask. Mix well and transfer the solution into an amber, glass bottle. Store the bottle in a freezer at < –10 °C. This standard has a 1-year expiration.

4.13.4. Methyl Mercury Standard MHG-C (2 µg/L CH_3HgCl as Hg) - Dilute 500 µL of the MHG-B standard with methanol to 100 mL in a volumetric flask. Mix well and transfer the solution into an amber, glass bottle. Store the bottle in a freezer at < –10 °C. This standard has a 1-year expiration.

4.13.5. Methyl Mercury Standard MHG-D (200 ng/L CH_3HgCl as Hg) - Dilute 100 µL of the MHG-B standard with methanol to 200 mL in a volumetric flask. Mix well and transfer the solution into an amber, glass bottle. Store the bottle in a freezer at < –10 °C. This standard has 1-year expiration.

4.14. Standard Reference Material – QC Check Standards (DORM3-A and DORM3-B):

4.14.1. Certified dogfish muscle tissue (DORM-3) from the National Research Council

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of Canada (NRCC) is used as an external standard reference material (SRM) 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.

4.14.2. Preparation of DORM3-A (1.9 µg/L) - Weigh 0.536 g (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 a heating block at 90 °C for 60 minutes (see DEP SOP HG-003). The resulting digested solution must be clear, but may have some undissolved residue. Transfer the DORM-3 digestate to a clean 100 mL volumetric flask, and dilute to the mark with methanol. This is DORM3-A. Transfer the solution to an amber glass bottle and keep in a freezer at < -10 °C. The expiration date for this prepared standard is one year from preparation.

4.14.3. Preparation of DORM3-B (190 ng/L) - The DORM3-B standard is prepared by diluting the DORM3-A solution tenfold with methanol. Add 10 mL of the DORM3-A solution to a 100 mL volumetric flask filled with 50 mL of methanol. Fill the flask to the mark with methanol, cap the flask firmly and shake well. Transfer the solution to an amber glass bottle and store in a freezer at < -10 °C. The expiration date for this prepared standard is 183 days from preparation.

4.14.4. Preparation of DORM3 QC Check Sample (0.38 ng/L) - To prepare the DORM3 QC check sample, add 50 mL of DI water to 100 mL volumetric flask. Add 0.2 mL of concentrated HCl, 0.2 mL of DORM3-B, 9 mL of the citrate buffer, and 0.2 mL of the 200 ng/L surrogate solution. Dilute to volume and mix well. Transfer 30 mL to a 40 mL vial and ethylate by adding 25 µL of $NaBEt_4$. Analyze at least one DORM3 standard along with every batch of 20 or fewer water samples.

4.15. Lab Fortified Blank and Matrix Spike Samples:

4.15.1. Lab fortified blank (LFB) (0.40 ng/L) - The LFB is analyzed as part of the quality control to ensure the efficacy of the method. To prepare an LFB, add 50 mL of DI water to a 100 mL volumetric flask. Add 0.2 mL of concentrated HCl, 9 mL of the citrate buffer, 0.2 mL MHG-D, and 0.2 mL of the 200 ng/L surrogate solution. Dilute to volume and mix well. Transfer 30 mL to a 40 mL vial and ethylate by adding 25 µL of $NaBEt_4$.

4.15.2. Matrix Spikes (1.0 ng/L) - Matrix spikes are analyzed as part of the quality control to ensure the efficacy of the method for the sample matrix and are normally prepared in duplicate unless there is insufficient sample. Randomly select a sample to spike. Add 50 mL of the chosen preserved sample to a 100 mL volumetric flask. Add 9 mL of the citrate buffer, 0.5 mL of MHG-D, and 0.2 mL of the 200 ng/L surrogate solution. Dilute to volume and mix well. Transfer 30 mL to a 40 mL vial and ethylate by adding 25 µL of $NaBEt_4$.

4.16. n-Propyl Mercury Chloride Surrogate Spiking Solution:

4.16.1. n-Propyl Mercury Chloride Surrogate Purchased Standard (10.0 mg/L) - The n-propyl mercury chloride primary stock solution is custom prepared by ERA Chemical Corp. at a concentration of 10.0 mg/L in methanol. The standard is

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stored at < -10 °C and the expiration date is provided by the manufacturer.

4.16.2. n-Propyl Mercury Chloride Surrogate Standard (200 µg/L) - This standard is prepared by adding 2.0 mL of the purchased 10 mg/L n-propyl mercury chloride surrogate standard to a 100 mL volumetric flask containing 50 mL of methanol. Dilute to the mark with methanol. This standard is stored at < -10 °C and the expiration date is the same as the purchased standard from which it was made.

4.16.3. n-Propyl Mercury Chloride Surrogate Standard (2.0 µg/L) - This standard is prepared by adding 1.0 mL of the 200 µg/L n-propyl mercury chloride surrogate standard into a 100 mL volumetric flask. The volumetric flask is brought to volume with methanol. After mixing, the standard is transferred to an amber bottle with a Teflon cap. The expiration date for this standard is the same as the prepared standard from which it was made and is stored at < -10 °C.

4.16.4. n-Propyl Mercury Chloride Surrogate Standard (200 ng/L) - This standard is prepared by adding 100 µL of the 200 µg/L n-propyl mercury chloride surrogate standard to a 100 mL volumetric flask containing 50 mL of methanol. Dilute to the mark with methanol. After mixing, the standard is transferred to an amber glass bottle and stored in the freezer at < -10 °C. The expiration date is the same as the prepared standard from which it was made.

4.17. Aqua Regia (5 %) – To a 20 L polyethylene carboy half filled with DI water, slowly add 750 mL of HCl and 250 mL of HNO_3 in the hood. Dilute to the 20 L mark and mix thoroughly. Store at room temperature. The holding time for this cleaning solution is 1 year.

4.18. PQL check sample (0.08 ng/L) - The PQL check sample is prepared by adding 50 mL of DI water to a 100 mL volumetric flask. Add 0.2 mL of concentrated HCl, 9 mL of the citrate buffer, 40 µL of MHG-D, and 0.2 mL of the 200 ng/L surrogate solution. Dilute to volume and mix well. Transfer 30 mL to a 40 mL vial and ethylate by adding 25 µL of $NaBEt_4$.

5. CALIBRATION

5.1. Calibration standards ranging from 0.020 ng/L to 5.0 ng/L are prepared using the working standards MHG-D and MHG-C. Continuing Calibration Verification (CCV) standard (1.0 ng/L) is analyzed every 10 samples.

Table 1. Calibration Standard Preparation

Calibration Standards	Name of the Stock	Volume of Stock used (µl)	Final Volume (ml)	Final Concentration (ng/L)
STD-0.02	MHG-D (200 ng/L) Surrogate Standard (200 ng/L)	10 10	100	0.020
STD-0.10	MHG-D (200 ng/L) Surrogate Standard (200 ng/L)	50 50	100	0.10
STD-0.2	MHG-D (200 ng/L) Surrogate Standard (200 ng/L)	100 100	100	0.20
STD-0.4	MHG-D (200 ng/L)	200	100	0.40

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Calibration Standards	Name of the Stock	Volume of Stock used (µl)	Final Volume (ml)	Final Concentration (ng/L)
	Surrogate Standard (200 ng/L)	200		
STD-1	MHG-D (200 ng/L) Surrogate Standard (200 ng//L)	500 500	100	1.0
STD-2	MHG-C (2.0 µg/L) Surrogate Standard (2.0 µg//L)	100 100	100	2.0
STD-5	MHG-C (2.0 µg/L) Surrogate Standard (2.0 µg//L)	250 250	100	5.0
CCV	MHG-D (200 ng/L) Surrogate Standard (200 ng/L)	500 200	100	1

5.2. A new calibration curve should be prepared if any of the following situations occur, a CCV standard is not within acceptance limits or any instrument or operational maintenance is performed, including changing the Argon cylinder.

5.3. 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 type of stock solution required to make the standards are shown in Table 1. Before adding the standard and surrogate, add 0.2 mL of HCl and 9 ml of the citrate buffer.

5.4. Ethylation of the calibration standards is accomplished by adding 25 µL of the ethylating reagent ($NaBEt_4$) to the 40 mL vials containing 30 mL of standard (30 mL of calibration standard is transferred to 40 mL vials). Rotate the vials two times to mix the solution.

5.5. 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. If one of the QC check standards fails, then re-run the failed QC check sample for confirmation. If it fails again, then re-run the calibration.

6. SAMPLE COLLECTION AND PRESERVATION

6.1. Sample Collection - Water samples are collected in 500 mL amber glass bottles or Teflon™ bottles. The bottles are labeled, bagged in polyethylene bags and shipped for analysis.

6.2. Sample Preservation - The water samples must be immediately preserved upon receipt in the laboratory by acidifying the samples with 0.20 mL of 12 N HCl per 100 mL of sample. The samples are then stored in a sample refrigerator at 4.0 ± 2 °C away from light until analysis.

6.3. Sample Holding Time - The holding time for preserved water samples is 6 months from the time of sample collection.

7. SAMPLE PREPARATION

Samples are prepared in batches of up to 20 samples. The following quality control samples are

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prepared along with the samples using the same containers and reagents: method blank, DORM3, LFB, PQL, and duplicate matrix spikes. Samples are spiked at a frequency of 10 %. If more than 10 samples are selected for a batch, one sample is chosen for duplicate matrix spikes and another samples is chosen for duplicate matrix spikes.

7.1. Matrix Modification and Derivatization for samples:

7.1.1. Check the sample pH to ensure it is less than 2. If the samples were previously acidified to 0.2 % HCl, then do not add additional HCl. However, non-acidified samples must be acidified to 0.2 % HCl using 12 M HCl before proceeding.

7.1.2. Fill a 100 mL volumetric flask with 50 mL of sample.

7.1.3. Add 0.2 mL of the 200 ng/L n-propyl mercury surrogate solution to yield a final concentration of 0.40 ng/L.

7.1.4. Add 9 mL of the citrate buffer solution to the flask and mix well. The buffer solution already contains antifoaming reagent for high dissolved organic compound samples.

7.1.5. Dilute to the mark with sample. Transfer 30 mL of sample to a 40 mL vial.

7.1.6. Add 25 µL of the ethylating reagent $NaBEt_4$ to the 40 mL vial. Screw the Teflon cap on firmly and immediately mix the sample by inverting the vial.

Note: The importance of good mixing immediately upon addition of the ethylating reagent must be stressed. Low recoveries have been observed in cases where the sample was inadequately mixed during the ethylation step.

8. SAMPLE ANALYSIS/QUANTIFICATION

8.1. Analysis procedure for Tekran 2700 Methylmercury Analyzer:

8.1.1. 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, 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 30 – 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.

8.1.2. 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

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- 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 re-establish communication with the software.

8.1.3. 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.

8.1.4. 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.

8.1.4.1. Example analysis sequence:

Clean
Clean
Wash
Wash
Calblank
Calblank
Calblank
Std0.02
Std0.1
Std0.2
Std0.4
Std1
Std2
Std5
Wash
Wash
CCV
CCB
MBLK
MDL
PQL Check
DORM3
LFB
Sample A
Sample A Matrix Spike
Sample A Matrix Spike Duplicate
Samples…
CCV
CCB

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

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

8.1.7. 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.

8.1.8. 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: YYYYMMDDw1. As an example, consider 20140730w1. This name indicates that the file contains data from the first analytical run on water (w1) samples conducted on July 30th, 2014. This nomenclature identifies the day the data file is generated and the matrix analyzed. Since this SOP applies to waters only, the letter 'w' is used as the identifier. 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 is 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\LABS\Lab3\Chem_Data\Inorganic\Mercury\Tekran 2700 with the same naming format, YYYMMDDw1.

8.1.9. Calibration Equation -

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

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

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

The following samples must be analyzed with any set of samples to ensure proper quality assurance in the data collected during the sample batch. The various quality control measures and acceptance criteria are discussed. The necessary corrective actions for unacceptable data are also discussed. Details on reporting non-conforming situations may be found in DEP SOP LB-002. Calculations are summarized in DEP SOP MT-058. Corrective actions for out-of-control data and contingencies for handling unacceptable data are contained in this SOP as well as DEP SOP LB-027.

9.1. 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 acceptance criterion is 70 % to 125 %. A CCV must be analyzed at the beginning, every 10 samples, and at the end of the run. If one of the QC check standards fails, re-run the failed QC check sample for confirmation. If it fails again, re-run the calibration.

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9.2. Method blank - The method blank must have a value below the MDL (0.020 ng/L). At least one method blank is prepared and analyzed for every 20 samples. If the method blank fails, check for possible contamination from the glassware cleaning procedure and sample preparation procedure. There is no need to rerun the samples if the methyl mercury levels in the samples are less than the MDL or greater than ten times the method blank. Samples not satisfying this condition must be reanalyzed or appropriately qualified.

9.3. PQL Check Sample - The PQL is verified in every batch by analyzing a DI water sample spiked at the PQL level (0.08 ng/L) using the MHG-D (200 ng/L) standard. The PQL sample must be reanalyzed if it does not pass QC requirements. If the PQL does not pass when re-analyzed, the batch may need to be re-prepared or the results must be appropriately qualified.

9.4. QC Check Standard Reference Sample - An external QC SRM is analyzed to check the calibration curve. Dogfish muscle tissue (DORM-3) is used as the SRM for this analysis. The DORM3 recovery must lie between 70 % and 130 % of its expected value. In case of DORM3 failure, re-run the DORM3 standard. If the DORM3 standard sample fails a second time, then prepare a new calibration curve.

9.5. Matrix Spikes - Duplicate matrix spikes are analyzed to check the efficacy of the analysis. The matrix spikes are prepared by spiking the sample with methylmercury stock to 1.0 ng/L in final solution. The recovery of each matrix spike must lie between 70 % and 130 %. The RPD between the duplicate spikes must be below 30 %. In case of matrix spike recovery failure, re-run samples. One matrix spike and one matrix spike duplicate are prepared at a frequency of 10 %. For a batch of 10 samples or less, one matrix spike and one matrix spike duplicate are required. For a batch with 11 – 20 samples, two samples are spiked in duplicate.

9.6. LFB - One LFB is prepared for every 20 samples by spiking a method blank before digestion with methyl mercury stock to 0.40 ng/L in final solution. The LFB recovery must be between 70 % and 130 % of its expected value. In case of LFB failure, re-run samples or qualify sample results.

9.7. 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 re-analyzed if the surrogate recovery limits are exceeded.

9.8. If for any reason the quality control results do not fall within the acceptance limits, then the samples must be appropriately commented and/or qualified as described in DEP SOP LB-027. This SOP describes the laboratory's policy on qualifying data.

10. SAFETY/WASTE MANAGEMENT

10.1. Review the Health and Safety Plan located on the Division of Environmental Assessment and Restoration (DEAR) intranet site.

10.2. Always wear protective eyewear and lab coat while in the laboratory.

10.3. Gloves must be worn when handling analytical standards, samples, reagents, and waste materials.

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

10.5. Safety equipment must be maintained in good operating condition and tested periodically as required by regulation.

10.6. Organo-mercury compounds such as methyl 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.

10.7. 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 only under a hood. These compounds are handled safely using normal laboratory protocols by wearing safety glasses, lab coat, and gloves at all times, and using a laboratory hood when appropriate. Dispose of sodium tetraethyl borate waste (closed vials) in the $NaBEt_4$ waste container located in the hood. Once the waste container is full, transfer the entire waste container to one of the shelves in the hazardous waste room located on the loading dock. Make sure the waste container is labeled '$NaBEt_4$ Sodium Tetraethylborate Waste'.

11. CONTAMINATION PREVENTION

11.1. To prevent contamination of the work environment it is essential that all waste generated from this test be treated according to good laboratory waste management practices. This is particularly true when dealing with potentially lethal compounds such as methyl mercury and other mercury-based compounds.

11.2. To prevent sample cross contamination it is essential to store stock solutions and samples in separate refrigeration units.

11.3. 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.

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

11.5. 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.

11.6. Maintain clean working surfaces free of debris and dust. Ensure that all equipment is properly cleaned following each use.

11.7. Change work gloves regularly when handling samples and reagents.

12. 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

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interferences due to contamination and artifacts, and (2) negative interferences due to loss of analyte and signal quenching. Different interferences may occur during different stages of the analysis. The interferences, their sources, and solutions to overcoming them are discussed below.

12.1. Positive Interferences:

12.1.1. Methyl mercury is not a common contaminant. This is fortuitous, since it allows one to conduct ultra-trace analysis in a conventional laboratory setting. In contrast, total mercury analysis at these levels would require a Class 100 Clean Bench or Room. However, all precautions should be taken to ensure that the analysis is conducted in a lab that is as mercury-free as possible. This includes precautions such as not allowing mercury thermometers in the premises of 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. We have determined that a citrate buffer is significantly cleaner than an acetate buffer with respect to methylmercury. Even without additional cleanup, the citrate buffer tends to be essentially free of methylmercury to well below our reporting limit.

12.1.2. A positive interference may also be caused by species such as aromatics (toluene, benzene etc.) which 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) should be procured. The fluorescence from aromatics is usually very strong, and masks the methylmercury signal, usually saturating the detector.

12.2. Negative Interferences:

12.2.1. 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. An 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.

12.2.2. 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. Additional sample clean-up, such as the distillation technique in EPA method 1630, may be required in extreme cases.

12.2.3 Suspended Solids - Unfiltered suspended solids may interfere with the derivatization step. The presence of particulates is normally apparent by the sample appearance. Filtering or diluting the sample may remove the interference.

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12.2.4. 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.

13. MAINTENANCE AND TROUBLESHOOTING

The Tekran Model 2700 Methylmercury Analyzer is capable of operation for extended periods without maintenance. Several checks should be performed on a daily basis to ensure that the instrument is operating properly. Document all maintenance in the Tekran 2700 Instrument Maintenance Logbook.

13.1. Look at the instrument LCD panel and S/W applications for warning indicators or messages.

13.2. Examine the peak shapes to ensure that the GC components are working properly.

13.3. Check the 'row details' sheet for one or more samples to see if lamp voltages are in range and stable.

13.4. Check the latest calibration to look for sudden changes in sensitivity.

13.5. Maintenance Schedule – The replacement/service intervals below are estimates and depend on the number of samples analyzed, the sample matrix, and the concentration of methylmercury found in the samples.

Table 2. Maintenance Schedule

Maintenance Item	Check Interval	Maintenance Interval
Carrier gas pressure check	Daily	As required
Scrubber replacement	Yearly	Several Years
Cuvette cleaning	6 months	As required
Lamp replacement	Each calibration	~12 months
Tenax® Trap	Daily	~12 months
Trap Oven UV Heat Lamp	Daily	~12 months
GC column	Daily	~24 months
Pyrolyzer Insert	Daily	~12 months
Guard column	Daily	~12 months

Note: See section 4 of the Model 2700 Methylmercury Analyzer user manual for details on maintenance.

13.6. Argon gas cylinder replacement - Replace the carrier gas cylinder when the pressure drops to the point that the system no longer functions properly (approximately 100 psi).

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13.7. Troubleshooting - See 'Model 2700 Methylmercury Analyzer User Manual' section 5 or contact Tekran Instruments Corporation; phone number 1-416-449-3084; email: lab-air-info@tekran.com.

13.7.1. Suspected hardware problems - Ensure that the power is available and connected to all modules. Ensure that all program parameters are correctly set. Refer to the 'Model 2700 Quick Start Guide' for more set-up related information.

13.7.2. Suspected analytical problems:

13.7.2.1. Ensure that standards and reagents are still good.

13.7.2.2. See Tek-MeHgDS 2.7 'Lamp Wizard' function under the System tab to check to see if the lamp needs servicing or replacement.

13.7.2.3. Ensure that there are no gas leaks. Make sure fittings are tightened and run a pressure test.

13.7.2.4. Verify that the correct temperatures are being reached in the Tenax trap and GC oven.

14. REFERENCES

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

14.2. 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)

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

14.4. SOP from Frontier GeoScience, FGS 013: Method for Ultra-Trace Level Determination of Methyl Hg in Water.15.5

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

14.6. DEP SOP Hg-003, Analysis of Methyl Mercury in Sediment and Tissue by KOH/CH3OH Digestion Followed by Aqueous Phase Ethylation

14.7. DEP SOP LB-002, Non-Conformance Reporting System

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

14.9. DEP SOP LB-027, SOP for Reporting Qualified Data.

14.10. DEP SOP LB-031, Limit of Detection Verification.

14.11. DEP SOP MT-058, Procedure for Metals Data Review and Authorization

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

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

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

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intranet.

APPENDIX OF SIGNIFICANT CHANGES

Summary of SOP Version Edits:

1) Updated Chemical Reagent section.
2) Updated version number and effective date for SOP.
3) Updated concentration levels in intermediate and working standards.
4) Updated references.

11/21/2013 The Tekran 2700 Methylmercury Analyzer was validated and the analysis procedure was added to this SOP (08/19/2013). The SOP was moved from the VOC group to the Metals group on 21-Nov-2013.

11/06/2014 Section 6.3 was edited to conform to the method (EPA method 1630) specified holding time.

12/03/2014 HCl is now added to QC standards at the same concentration as the sample preservation. The same volume of citrate buffer is now added to QC standards and samples.

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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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