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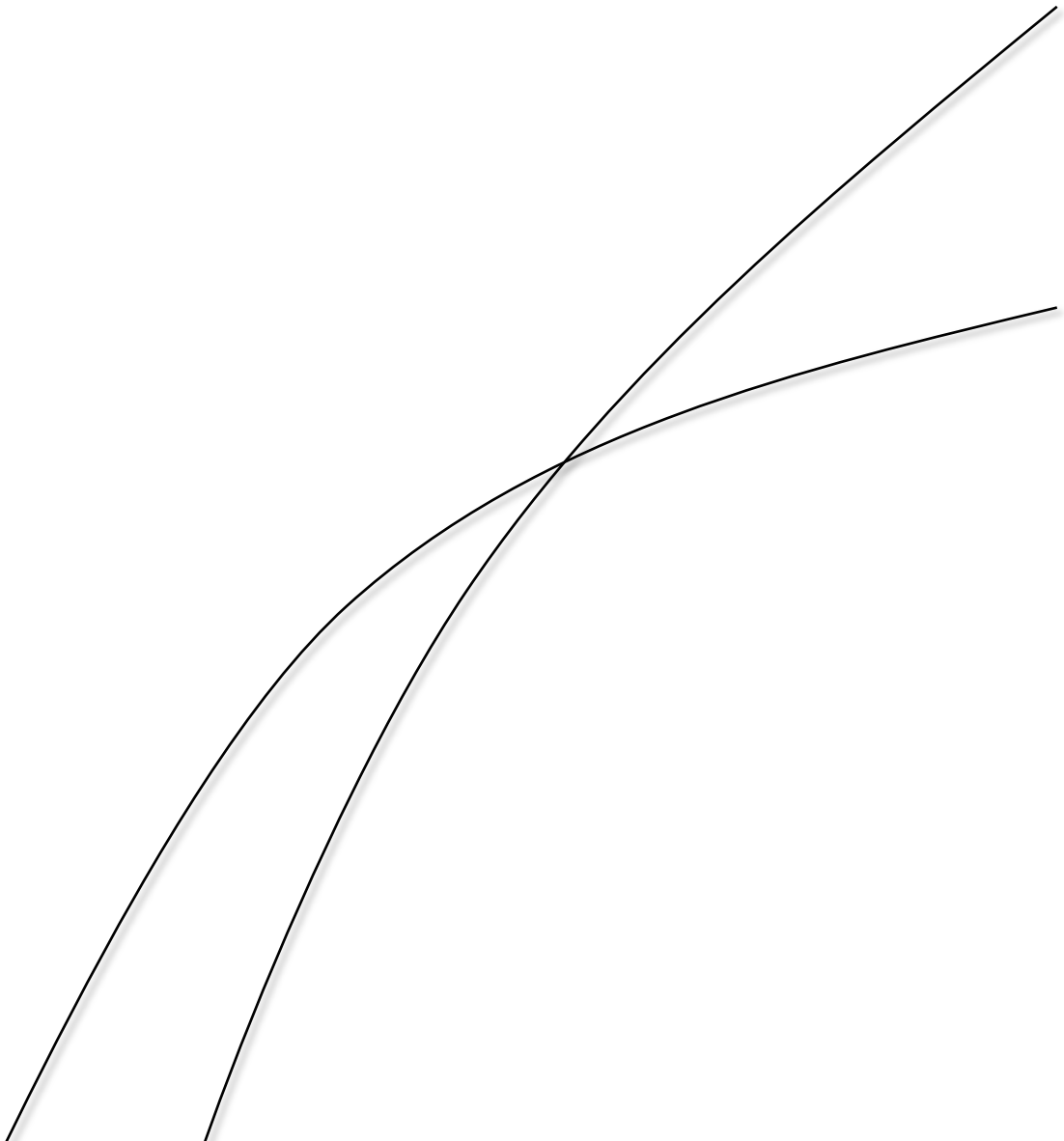
STANDARD OPERATING PROCEDURES FOR THE DETERMINATION OF TOTAL MERCURY IN HAIR, BLOOD AND URINE BY THE ALTERNATIVE METHOD



Abstract

The alternative standard operating procedures are described for laboratories that have access to instruments with flow injection analysis and gold amalgamation followed by either cold vapour atomic absorption spectrophotometry (CV-AAS) or cold vapour atomic fluorescence (CV-AFS) detection. The standard operating procedure describes the digestion procedure. Mercury present in digested samples can then be determined either by a flow injection procedure or by gold amalgamation CV-AFS (or CV-AAS).

Keywords

Mercury – analysis
Methylmercury compounds – analysis
Fetal blood – chemistry
Umbilical cord – chemistry
Hair – chemistry
Urine – chemistry
Biomarkers – analysis
Flow injection analysis
Spectrophotometry, atomic
Environmental exposure

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Abbreviations

BrCl	bromine chloride
CV-AAS	cold vapour atomic absorption spectrophotometry
CV-AFS	cold vapour atomic fluorescence
HCl	hydrochloric acid
$HgCl_2$	mercuric chloride
HNO_3	nitric acid
KBr	potassium bromide
$KBrO_3$	potassium bromate
$K_2Cr_2O_7$	potassium dichromate
$KMnO_4$	potassium permanganate
$SnCl_2$	stannous chloride
v/v	volume/volume
V_2O_5	vanadium pentoxide
w/v	weight/volume

Acid digestion of the biological samples

SCOPE OF THE METHOD

The method described is intended to determine the total mercury (Hg) in biological samples.

TECHNICAL PRINCIPLE

This method is applicable to all biological samples with total Hg concentrations higher than 1 ng/g. The purpose of the strong acid digestion is to decompose the samples and oxidize and convert any organic forms of Hg into inorganic Hg.

SAFETY PRECAUTIONS

Follow universal precautions. Wear gloves, a laboratory coat and safety glasses while handling human blood, plasma, serum, urine or other bodily fluids or tissues. Place disposable plastic, glass and paper items (pipette tips, autosampler tubes and gloves) that come into contact with human biological fluids (such as urine) in a biohazard autoclave bag. Keep these bags in appropriate containers until they are sealed and autoclaved.

When the work is finished, wipe down all work surfaces where human biological fluids were handled with a 10% (volume/volume (v/v)) sodium hypochlorite solution or equivalent. The use of the foot pedal on the Micromedic Digiflex™ is recommended because it reduces the analyst's contact with working surfaces that have been in contact with human biological fluids and allows the hands to be free to hold specimen cups and autosampler tubes. Dispose of all biological samples and diluted specimens in a biohazard autoclave bag at the end of the analysis according to the guidelines for disposal of hazardous waste.

DIGESTION OF BIOLOGICAL MATERIAL

Equipment

- Glass bottle, 1 litre, cleaned according to the procedure for glassware.
- Volumetric flask, 500 ml (Class A), cleaned according to the procedure for glassware.
- Teflon vials with caps (60 ml), cleaned according to the procedure for Teflon.
- Polypropylene spatulas.
- Hot plate and aluminium block.
- Precision balance.

Cleaning glassware

Prior to use, wash all laboratory glassware thoroughly as follows.

- Allow the Teflon and glass vessels to soak overnight in 2% Micro-90 detergent solution.
- Rinse the vessels thoroughly first with tap water then with bidistilled water.
- Rinse with 0.5% potassium permanganate ($KMnO_4$) solution.
- Rinse with water until the colour of the $KMnO_4$ solution is no longer visible.
- Fill the vessels with 1% hydrochloric acid (HCl) solution and store in Hg-free storage facilities.
- Empty vials just before using them for sample processing and allow them to dry at 60 °C in a flow hood.

Cleaning Teflon

- Soak the vessels overnight in a plastic container in a soap solution (Micro solution 2% in tap water).
- Rinse thoroughly first with tap water and then with bidistilled water.
- Put the vessels in 50% (v/v) concentrated nitric acid (HNO_3) solution and heat at 60 °C for two days.
- Rinse thoroughly with bidistilled water (at least four times).
- Transfer the vessels into 10% (v/v) concentrated HCl solution for one day (at least) at room temperature.
- Rinse thoroughly with bidistilled water (at least four times).
- Store all vessels in polyethylene plastic bags. When possible (principally Teflon and glass bottles), fill the vessels with 1% HCl.

Reagents and chemicals

- HNO_3 (65%, analytical grade, low in Hg)
- HCl (30%)
- Vanadium pentoxide V_2O_5 (extra pure)
- Potassium dichromate ($K_2Cr_2O_7$)
- Potassium bromate ($KBrO_3$) (analytical grade)
- Potassium bromide (KBr) (analytical grade)
- bidistilled deionized water (>18 MQ cm)

There are two choices for oxidizing solutions.

K_2Cr2O_7 10% (weight/volume (w/v)) in bidistilled water

1. Weigh 50 g of $K_2Cr_2O_7$ into a clean 500 ml glass volumetric flask.
2. Add about 250 ml of bidistilled water and shake until the $K_2Cr_2O_7$ is dissolved.
3. Make up to the mark with bidistilled water.

BrCl oxidizing solution

1. Weigh accurately 11 g of $KBrO_3$ and 15 g of KBr into a clean 1 litre glass bottle.
2. Add 200 ml of bidistilled water.
3. Add carefully 800 ml of concentrated HCl; the dilution must be carried out in a well-ventilated fume hood to prevent exposure to toxic fumes released during dissolution of $KBrO_3$.
4. Keep the bottle wrapped with aluminium foil.

These two solutions can be kept for an unlimited time if stored in the dark at room temperature in a tightly closed Teflon or glass bottle in an Hg-free area.

Procedure

1. Shake the vials containing the samples for about two minutes for homogenization.
2. Wait a few minutes before opening the vials.
3. Weigh accurately from 0.5–1 ml of blood sample, 20–100 mg of hair sample or 1–2 ml of urine sample into Teflon vials (60 ml).
4. Weigh 45 mg of V_2O_5 into these vials.
5. Add 5 ml of concentrated HNO_3 (or more if necessary: the mixture must be liquid).
6. Close the caps and leave the vials to stand for at least one hour at room temperature. If the reaction is very strong, it may be safer to leave the samples at room temperature overnight before heating.
7. Put the tubes into an aluminium block on a hot plate at 90 °C and leave for three hours.

8. Allow the samples to cool to room temperature before opening the tubes. Leave the tubes to cool in a fume hood to avoid toxic acid fumes.
9. Add about 20 ml of bidistilled water.
10. Add 1 ml of $K_2Cr_2O_7$ solution (final concentration = 2% v/v), or 0.5 ml of BrCl solution (final concentration = 1% v/v).
11. Dilute to the mark with bidistilled water (dilution volume = 57.5 ml).
12. Shake the vials and wait for sedimentation of material before analysis.

These samples can be kept for a few days before analysis if they are stored in the refrigerator (+4 °C). The maximum storage time must be determined by experience for each kind of sample.

Reagent blanks

At least three blanks should be prepared for each batch of analysis. They are prepared in a similar manner as the samples, except that no sample is added to the digestion tubes.

Reference materials

At least one certified reference material should be used and prepared in triplicate for each batch of analysis. These digestions are prepared in a similar manner as the sample. Certified reference material should be of a similar composition and concentration of Hg as the samples.

Determination of total Hg using flow injection analysis and CV-AAS detection

PRINCIPLE AND APPLICATION

Biological samples are mineralized with strong acids. The inorganic Hg is reduced to its elemental form with stannous chloride using a flow injection principle. The cold Hg vapour is separated from the digested samples in a gas-liquid separator and the Hg vapour is then passed through the quartz absorption cell of an AAS where its concentration is measured. The light beam of the Hg hollow cathode lamp is directed through the quartz cell into a monochromator and on to a detector that measures the amount of light absorbed by the atomized vapour in the cell. The amount of energy absorbed at the characteristic wavelength is proportionate to the concentration of the element in the sample.

EQUIPMENT, MATERIALS AND SOLUTIONS

Equipment

AAS Varian-Spectra AA-10 and VGA-76 or any equivalent system based on the flow injection principle.

Materials

- Micropipettes.
- Teflon bottles 125 ml, cleaned according to the procedure for Teflon.
- Precision balance.
- Glass volumetric flasks from 50 ml to 1000 ml (Class A), cleaned according to the procedure for glassware.

Cleaning glassware

Prior to use, thoroughly wash all laboratory glassware as follows.

- Allow the Teflon and glass vessels to soak overnight in 2% Micro-90 detergent solution.
- Rinse the vessels thoroughly first with tap water then with bidistilled water.
- Rinse with 0.5% $KMnO_4$ solution.
- Rinse with water until the colour of the $KMnO_4$ solution is no longer visible.
- Fill the vessels with 1% HCl solution and store in Hg-free storage facilities.
- Empty vials just before sample processing and allow them to dry at 60 °C in a flow hood.

Cleaning Teflon

- Allow the vessels to soak overnight in a plastic container in a soap solution (Micro solution 2% in tap water).
- Rinse thoroughly first with tap water and then with bidistilled water.
- Put the vessels in 50% (v/v) concentrated HNO_3 solution and heat at 60 °C for two days.
- Rinse thoroughly with bidistilled water (at least four times).
- Transfer the vessels into 10% (v/v) concentrated HCl solution for one day (at least) at room temperature.
- Rinse thoroughly with bidistilled water (at least four times).
- Store all vessels in polyethylene plastic bags. When possible (principally Teflon and glass bottles), fill the vessels with 1% HCl.

Reagents and chemicals

- HNO_3 (65%, analytical grade, low in Hg)
- $K_2Cr_2O_7$ (analytical grade, low in Hg)
- KBr
- $KBrO_3$
- Stannous chloride ($SnCl_2$) (analytical grade, normal or low in Hg)
- HCl (30%)
- Mercuric chloride ($HgCl_2$) (salt) or standard Hg solution (1000 mg/L)
- bidistilled deionized water (>18 MQ cm)
- Argon (pure quality)

Reagent solutions

20% w/v $SnCl_2$ in 20% v/v HCl (200 ml)

1. Weigh accurately 40 g of $SnCl_2$ into a clean glass beaker using a plastic spatula (beaker and spatula are used only for $SnCl_2$).
2. Add 40 ml of concentrated HCl directly to the $SnCl_2$ and transfer to a 200 ml volumetric flask. Mix and wait for complete dissolution of $SnCl_2$.
3. Add bidistilled water to the mark (200 ml).
4. With older stock of $SnCl_2$ it may be necessary to warm up the solution on a hot plate to obtain complete dissolution of $SnCl_2$ (do not allow to boil).
5. In case of low concentration samples, if the $SnCl_2$ used is not "low in Hg", it should be purged with nitrogen for two hours before use.
6. This solution should be made fresh for each day of analysis.

Note: all glassware used for preparation of the $SnCl_2$ solution should be kept separate from the remaining laboratory ware in order to avoid cross-contamination of ware for trace element determination.

HNO_3 10% v/v (500 ml)

1. Put about 400 ml or bidistilled water into a 500 ml volumetric flask.
2. Add carefully 50 ml of concentrated HNO_3.
3. Make up to the mark with bidistilled water.
4. Shake well.

This solution can be stored if kept in a tightly closed flask.

There are two choices for oxidizing solutions.

K_2Cr2O_7 10% (w/v) in bidistilled water

1. Weigh 50 g of $K_2Cr_2O_7$ into a clean 500 ml glass volumetric flask.
2. Add about 250 ml of bidistilled water and shake until the $K_2Cr_2O_7$ is dissolved.
3. Make up to the mark with bidistilled water.

BrCl oxidizing solution

1. Weigh accurately 11 g of $KBrO_3$ and 15 g of KBr into a clean 1 litre glass bottle.
2. Add 200 ml of bidistilled water.
3. Add carefully 800 ml of concentrated HCl; the dilution must be carried out in a well-ventilated fume hood to prevent exposure to toxic fumes released during dissolution of $KBrO_3$.
4. Keep the bottle wrapped with aluminium foil.

These two solutions can be kept for an unlimited time if stored in the dark at room temperature in a tightly closed Teflon or glass bottle in an Hg-free area.

Mercury standard solutions

Stock standard solution 1: 1 mg/ml Hg in 10% nitric acid

1. Weigh exactly 1.354 g of $HgCl_2$ into a 1 litre glass volumetric flask.
2. Add about 500 ml of bidistilled water.
3. Add 10 ml of concentrated HNO_3 (low in Hg).
4. Complete to the mark with bidistilled water.
5. Shake well until complete dissolution is achieved.
6. Transfer into a 1 litre Teflon bottle.
7. Close tightly with a torque wrench and keep in the refrigerator (+4 °C).

Stock standard solution 2: 1 µg/ml Hg in 4% HNO_3

1. Weigh 95 g of bidistilled water into a 125 ml Teflon bottle.
2. Add 4 ml of concentrated HNO_3 (low in Hg).
3. Add 1 ml of BrCl solution (or 2 ml of $K_2Cr_2O_7$ solution).
4. Add 100 µl of solution stock 1 (1 mg/ml Hg).
5. Shake well.
6. Close tightly with a wrench and keep in the refrigerator (+4 °C).

Calibration curve (at least three standards and zero calibration)

1. Put about 10 ml of bidistilled water into a clean 50 ml glass volumetric flask.
2. Add reagents as in the digested samples (HNO_3/H_2SO_4 2:1, or HNO_3).
3. Add an appropriate quantity of stock standard solution (stock 1or stock 2, depending on the concentrations of the samples) with a micropipette.
4. Add 1 ml of BrCl solution (or 2 ml of $K_2Cr_2O_7$ solution).
5. Dilute to the mark (50 ml) with bidistilled water.
6. Shake well.

These solutions should be prepared fresh for every day of analysis.

ANALYSIS BY CV-AAS

Calibration curve

Prepare standard solutions with at least three standard concentrations plus one zero. The zero calibration is prepared as the standard solutions, but without adding the Hg standard.

If samples are not within the calibration curve, dilute them in the same matrix or prepare a new calibration curve.

Instrument conditions

- Wavelength: 253.7 nm
- Lamp current: 4 mA
- Slit width: 0.5 nm
- Reducing agent (20% $SnCl_2$ in 20% HCl): 1 ml/min
- Bidistilled water: 1 ml/min
- Rinse solution (10% HNO_3) or sample: 6.5 ml/min
- Inert gas: argon.

Optimization of the AAS

The following instructions are applicable for AAS Varian-Spectra AA-10 and VGA-76 or equivalent system based on the flow injection principle. If another instrument is used, the manufacturer's instructions should be followed.

1. Make sure the flame disk is inserted in the instrument.
2. Switch on the printer then the AAS instrument.
3. Press INDEX.
4. Select PROGRAM DIRECTORY.
5. Select mercury program number and push RECALL PROGRAM.
6. METHOD parameter must be:
   element No.: 24
   instrument mode: ABS
   calibration: must be CONCENTRATION
   measurement: must be INTEGRATION.
7. INSTRUMENT PARAMETER must be:
   lamp position: coded lamp position is automatically recognized
   lamp current: 4 mA
   sample introduction: MANUAL
   delay time (seconds): 70
   measurement time (seconds): 5.0
   replicates: 3
   background correction: ON.
8. Install the Hg lamp in the right position.
9. Go to NOTE. On this page, the concentration giving a response of 0.2 ABS is indicated.
10. Select the correct slit width (0.5) and set the monochromator to the right wavelength (253.7 nm).
11. Go to OPTIMIZATION. This step is done without the absorption cell in the light path of the AAS. On the screen there are two bars: one shows the level of energy of the Hg lamp and the other that of the deuterium lamp. Make sure the burner does not obstruct the light. Bring the lamp energy to the maximum by optimizing wavelength and lamp position successively; make these adjustments twice. If the signal bar is too large, press RESCALE. After optimization, the energy of the two lamps should be similar. If the message TOO LOW DEUTERIUM LAMP (or TOO HIGH) appears, turn on (or off) the attenuation of the deuterium lamp.
12. Check the photomultiplier value (PMV about 294 mV) and record the value in the log-book.
12. Install the absorption cell on the burner head and check that the light beam crosses the cell close to the centre.
13. Go to STANDARDS and enter the standard concentrations for the calibration curve.

Operation of the VGA

1. Switch on the argon. The gas flow has to be regulated to a minimum, with the orange light of the VGA off.
2. Put each of the three Teflon capillary tubes into the appropriate solutions:
   (i) $SnCl_2$ solution
   (ii) bidistilled water
   (iii) rinse solution (10% HNO_3).
3. Switch on the VGA and slowly tighten the pressure adjusting screw on the peristaltic pump until the liquids are pumped (do not overtighten the screw as this will shorten the life of the pump tubes).
4. Check that there are no leaks.
5. Leave the system running for about 10 minutes in order to clean the system. Disconnect the black tube from the quartz absorption cell if the system has not been running for a while (to prevent contamination of the cell).
6. Connect the tube between the gas-liquid separator and the absorption cell.

Calibration and samples measurement

At the top of the screen of the AAS, the solution that is going to be measured is indicated (blank; standard 1, 2, etc.; reslope; sample 1, 2, etc.). To choose the solution to be analysed, push SOLUTION TYPE. Always check that the solution that is going to be measured is the one asked for.

To measure a solution push READ.

The AAS and VGA should be operating at this stage.

1. Go to ANALYTICAL RESULTS.
2. Press INSTRUMENT ZERO with the rinse solution (HNO_3 10%).
3. Measure the rinse solution as a sample: this should give 0.000 ABS.
4. Measure the blank or the calibration curve as a sample. This should also be close to 0.000 ABS. If it is not, press INSTRUMENT ZERO again when aspirating the rinse solution.
5. Check the ABS value for one Hg standard (measure it as a sample). This gives the sensitivity of the instrument and should be recorded in the log-book.
6. Go to CALIBRATION.
7. Measure the calibration blank then the standards.
8. Aspirate the rinse solution for about one minute between each standard.
9. Check that the calibration curve is correct.
10. Measure first the reagent blanks then the reference materials. Calculate the concentration in µg/g of the reference material and check the accuracy of the result before continuing.
11. Run the samples.
12. Run the rinse solution for about one minute between each sample.
13. Measure a blank and reslope every four or five samples depending on the stability of the instrument.
14. Measure the same reference material at regular intervals during analysis.

Shut down procedure

1. Rinse all tubing with bidistilled water for about 20 minutes (make sure to keep the tube for the $SnCl_2$ solution separate from the other tubes).
2. Turn off the VGA system.
3. Release the tension from the tubing.
4. Turn off the argon.
5. Turn off the printer and AAS instrument.

Calculation

$$[C]\ (mg/kg) = \frac{(Cd - Cb) \times V}{W}$$

[C] = concentration of total Hg in dry sample (µg/g dry)
Cd = concentration of Hg in sample solution (µg/ml)
Cb = mean concentration of Hg in reagent blanks (µg/ml)
V = volume of dilution of digested samples (ml) =57.5 ml
W = dry weight of sample (g).

Determination of total Hg using double gold amalgamation and CV-AFS detection

PRINCIPLE OF THE METHOD

After decomposition of the samples in the presence of strong acids, Hg^{2+} is reduced to volatile elemental mercury Hg^0 with an excess of $SnCl_2$. Elemental mercury is concentrated on a gold trap and detected after desorption at 600 °C by cold vapour atomic fluorescence at 253.7 nm.

EQUIPMENT, MATERIALS AND SOLUTIONS

Equipment

- AFS detector (Brook Rand) or any other equivalent equipment.

Materials

- Volumetric flask, 100, 500 and 1000 ml (Class A).
- Glass bottles 1 litre, cleaned according to the procedure for cleaning glassware.
- Teflon bubblers (60 ml) (500 ml for water samples) cleaned according to the procedure for cleaning Teflon.
- Teflon tubing cleaned according to the procedure for cleaning Teflon.
- Teflon bottles, 125 ml and 1 litre, cleaned according to the procedure for cleaning Teflon.
- Quartz wool cleaned at 500 °C.
- Gold sand.
- Quartz columns for gold traps, cleaned according to the procedure for cleaning glassware.
- Drying columns (Teflon tube or quartz tube filled with soda lime), cleaned according to the procedure for cleaning glassware or Teflon.
- Heating system for gold traps (2 VARIAC, 6A and timer; Cr/Ni wire 0.5 mm).
- Flow meters.
- Integrator.
- Precision balance.

Cleaning glassware

Prior to use, wash all laboratory glassware thoroughly as follows.

- Allow the Teflon and glass vessels to soak overnight in 2% Micro-90 detergent solution.
- Rinse the vessels thoroughly first with tap water then with bidistilled water.
- Rinse with 0.5% $KMnO_4$ solution.
- Rinse with water until the colour of the $KMnO_4$ solution is no longer visible.
- Fill the vessels with 1% HCl solution and store in Hg-free storage facilities.
- Empty vials just before use for sample processing and allow them to dry at 60 °C in a flow hood.

Cleaning Teflon

- Allow the vessels to soak overnight in a plastic container in a soap solution (Micro solution 2% in tap water).
- Rinse thoroughly first with tap water and then with bidistilled water.
- Put the vessels in 50% (v/v) concentrated HNO_3 solution and heat at 60 °C for two days.
- Rinse thoroughly with bidistilled water (at least four times).

- Transfer the vessels into 10% (v/v) concentrated HCl solution for one day (at least) at room temperature.
- Rinse thoroughly with bidistilled water (at least four times).
- Store all vessels in polyethylene plastic bags. When possible (principally Teflon and glass bottles), fill the vessels with 1% HCl.

Reagents and chemicals

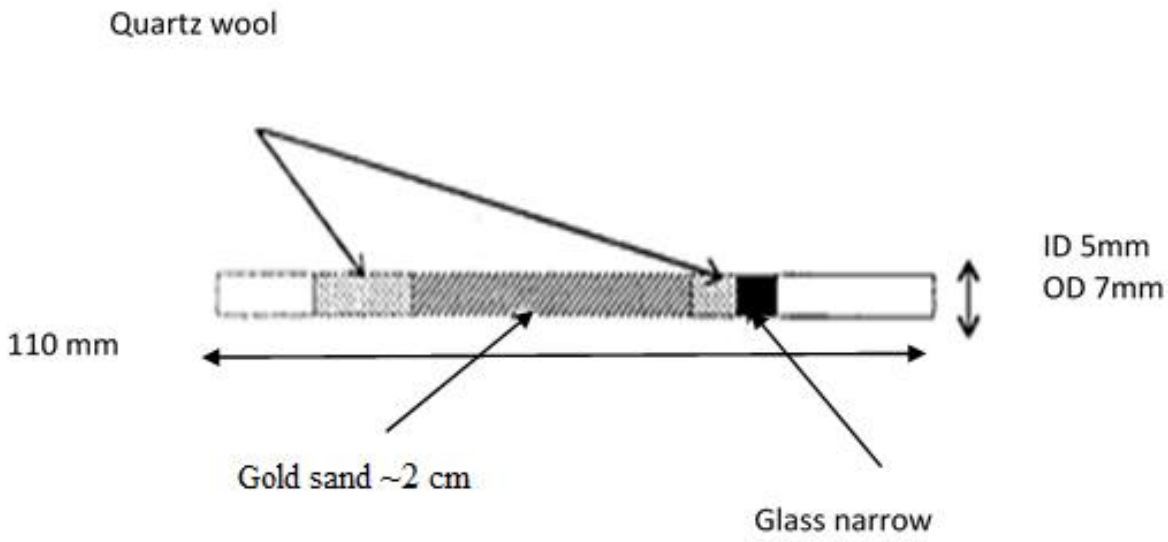
- $SnCl_2$ (analytical grade)
- $KBrO_3$
- KBr
- $HgCl_2$ (analytical grade, normal or low in Hg)
- HCl (30%)
- HNO_3 (65%)
- $K_2Cr_2O_7$ (analytical grade, low in mercury)
- Soda lime pellets (analytical grade)
- bidistilled deionized water (>18 MΩ cm)
- Argon (Hg-purified)

Preparation of gold trap (Fig. 1)

1. Put a small piece of quartz wool at the end of the longer part of the column. Settle it using a Pasteur pipette.
2. Insert about 2 cm of gold sand. It is better to weigh the sand put into the trap in order to obtain a better reproducibility between the traps.
3. Insert a larger piece of quartz wool using a Pasteur pipette. Try to settle all traps the same way.
4. Clean the new trap at least four times before use (see analytical procedure).

Note: when using new traps before starting with sample analysis, check the reproducibility of standard response for all traps.

Fig. 1. Gold trap



Reagent solutions

20% w/v $SnCl_2$ in 20% v/v HCl (100 ml)

1. Weigh accurately 20 g of $SnCl_2$ into a clean glass beaker using a plastic spatula (beaker and spatula are used only for $SnCl_2$).
2. Add 20 ml of concentrated HCl directly to the $SnCl_2$ and transfer to a 100 ml volumetric flask. Mix and wait for complete dissolution of $SnCl_2$.
3. Add bidistilled water to the mark (100 ml).
4. With older stock of $SnCl_2$ it may be necessary to warm the solution on a hot plate to obtain complete dissolution of $SnCl_2$ (do not allow to boil).
5. Purge the $SnCl_2$ solution with nitrogen for two hours in order to obtain an Hg-free solution.

There are two choices for oxidizing solutions.

$K_2Cr_2O_7$ 10% (w/v) in bidistilled water

1. Weigh 50 g of $K_2Cr_2O_7$ into a clean 500 ml glass volumetric flask.
2. Add about 250 ml of bidistilled water and shake until the $K_2Cr_2O_7$ is dissolved.
3. Make up to the mark with bidistilled water.

BrCl oxidizing solution

1. Weigh accurately 11 g of $KBrO_3$ and 15 g of KBr into a clean 1 litre glass bottle.
2. Add 200 ml of bidistilled water.
3. Add carefully 800 ml of concentrated HCl; the dilution must be carried out in a well-ventilated fume hood to prevent exposure to toxic fumes released during dissolution of $KBrO_3$.
4. Keep the bottle wrapped with aluminium foil.

These two solutions can be kept for an unlimited time if stored in the dark at room temperature in a tightly closed Teflon or glass bottle in an Hg-free area.

Mercury standard solutions

Standard stock solution: 1 mg/ml Hg in 10% HNO_3

1. Weigh exactly 1.354 g of $HgCl_2$ into a 1 litre glass volumetric flask.
2. Add about 500 ml of bidistilled water.
3. Add 10 ml of concentrated HNO_3 (low in Hg).
4. Complete to the mark with bidistilled water.
5. Shake well until complete dissolution is achieved.
6. Transfer into a 1 litre Teflon bottle.
7. Close tightly with a torque wrench and keep in the refrigerator (+4 °C).

Intermediate standard solution: 1 µg/ml Hg in 4% HNO_3

1. Weigh 95 g of bidistilled water into a 125 ml Teflon bottle.
2. Add 4 ml of concentrated HNO_3 (low in Hg).
3. Add 1 ml of BrCl solution (or 2 ml of $K_2Cr_2O_7$ solution).
4. Add 100 µl of solution stock 1 (1 mg/ml Hg).
5. Shake well.

If a more dilute solution is needed, dilute stock 2 solution by following the above procedure. The bottles of standard solutions should be closed tightly with a wrench and kept in the refrigerator (+4 °C).

ANALYTICAL PROCEDURE

1. Prepare the samples as described above.
2. Gold traps must be cleaned before use by heating to 600 °C without being connected to the AFS detector. Then check if they are free from residual Hg by measuring released Hg after heating them again.
3. Clean the bubbler (once, or several times if the system has not been used for some time) following the procedure below.
4. Measure bubbler blank by following the procedure below and verify the absence of contamination of the system. If the values of the bubbler blank are too high, continue to clean the system until the bubbler blank values are correct and stable.
5. Calibrate the system by following the "calibration curve" procedure below. This calibration must be done at least twice during the day.
6. Measure reagent blank and reference material solutions (digested at the same time as the samples) following the procedure below ("reagent blank analysis" and "sample analysis"). Verify the absence of Hg contamination and the accuracy of measurements before starting to analyse the sample.
7. Start to measure the sample as described below (sample analysis). During the run for quality control purposes, the reference material and the reagent blanks must be measured at least twice for each calibration curve.

Cleaning of the bubbler

1. Rinse and fill the bubbler (3/4) with bidistilled water.
2. Add 500 µl of $SnCl_2$ solution.
3. Purge with argon for 15 minutes.

Bubbler blank

1. Rinse and fill the bubbler (3/4) with bidistilled water.
2. Add 500 µl of $SnCl_2$ solution.
3. Fix the gold trap and purge with argon for 15 minutes.
4. Analyse the trap.

Calibration curve

1. Rinse and fill the bubbler (3/4) with bidistilled water.
2. Add standard solution (50–150 µL of 1 ng/ml stock solution, equivalent to 50–150 pg of Hg).
3. Add 500 µl of $SnCl_2$ solution.
4. Fix gold trap and purge with argon for 15 minutes.
5. Remove the trap and analyse it.

The calibration curve must be prepared at the level of the sample concentrations. If necessary, more concentrated standards than indicated can be used.

Reagent blank analysis

1. Rinse and fill the bubbler with bidistilled water (the quantity of bidistilled water depends on the volume of reagent blank to be added).
2. Add the blank solution (reagent blank). The volume must be at least equal to the volume of the sample to be used for analyses (that is, if 10 ml of sample are necessary for analysis, at least 10 ml of reagent blank must be analysed). If the level of Hg in the reagent blank is very low, larger volumes of the blank solution can be used for analysis.
3. Add 500 µl of the $SnCl_2$ solution.

4. Fix the gold trap and purge with argon for 15 minutes (Fig. 2).
5. Remove the gold trap and analyse it (Fig. 3).

Fig. 2. Bubbler system for total Hg analysis

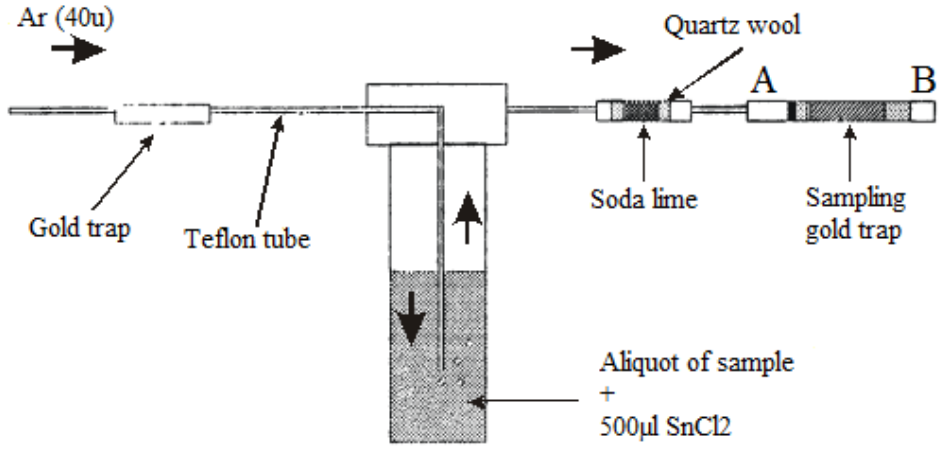
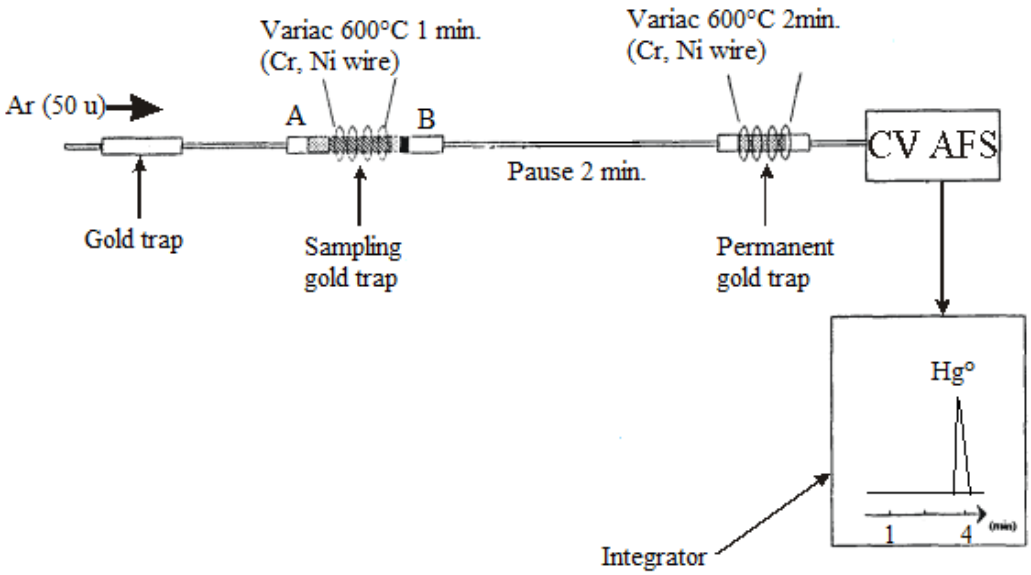


Fig. 3. Analytical system



Sample analysis

1. Rinse and fill the bubbler with bidistilled water (the quantity of bidistilled water depends on the volume of the sample to be added, from 0 ml for low concentration samples such as urine and blood to three quarters of the bubbler volume for higher concentration samples).
2. Add the sample solution. The volume of sample to be added depends on the concentration of the sample, from a few microlitres for hair samples to a few millilitres for blood and urine samples. The response of the sample should be within the calibration curve.
3. Add 500 µl of the $SnCl_2$ solution.
4. Fix the gold trap and purge with argon (nitrogen or air) for 15 minutes (Fig. 2).
5. Remove the gold trap and analyse it (Fig. 3).

Double amalgamation analysis (Fig. 3)

1. Place the sampling gold trap at measuring (analytical) system in a flow of argon.
2. Release Hg by heating the sampling gold trap at 600 °C for one minute.
3. Wait for two minutes for mercury to amalgamate on permanent gold trap.
4. Release mercury from the permanent gold trap by heating it at 600 °C for two minutes.
5. Detect by CV-AFS.

CALCULATION

Plot the calibration curve using:
X = pg. of Hg^{2+} in added standard
Y = response of integrator (peak area in arbitrary units).
Calculate the calibration curve using linear regression of all standard points (at least three standards) and the mean of bubbler blanks (unit) for zero value:

$$y = b + ax.$$

Reagent blank:

$$[B](pg/ml) = \frac{(Ab - b)}{a \times V}$$

[B] = concentration of methyl mercury in reagent blank (pg/ml)
Ab = response obtained for aliquot of reagent blank analysed (peak area in arbitrary units)
V = volume of reagent blank analysed (ml)

Samples:

$$[S](pg/g) = \frac{\left[\left[\frac{As - b}{a \times Va}\right] - [B]\right] \times Vs}{W}$$

[S] = concentration of Hg in dry sample (pg/g dry)
As = response obtained for the aliquot of sample analysed (peak area in arbitrary units)
Va = aliquot of sample analysed (ml)
Vs = total sample volume (ml)
W = dry weight of sample (g)
[B] = concentration of methyl mercury in reagent blank (pg/ml).