KJELDAHL
BENCHNOTES

Determination of Arsenic in Urine, Hair and Nail Samples with Micro Digestor

Principle: *After concentration and oxidation of the sample, arsenic is liberated as arsine (AsH_3) by zinc in acid solution. The generated arsine is then passed through a roll of glass wool moistened with lead acetate solution to remove H_2S and collected in pyridine solution of silver diethyl-dithiocarbamate. The change of color is proportional to the amount of arsenic in the sample.*

Specimen

Urine: 50 ml aliquot of a 24 hour urine. Minimum sample: 25 ml urine.

Hair: One gram of hair cut from the back of the head. A bunch of hair the thickness of a pencil and 4 inches long should weigh about one gram. Minimum sample: 0.5 g hair.

Nails: One gram of fingernails/toenails. Minimum sample: 0.5 g nails.

Hair, nails or urine may be used to assess chronic toxicity; however, it may be difficult to obtain sufficient sample of nails. In the case of acute poisoning, blood (20 ml), stomach washing (50 ml) or vomitus (50 ml) may be used.

Reagents

1. Sulfuric Acid, concentrated A.R.
2. Nitric Acid, concentrated A.R.
3. Hydrochloric Acid, concentrated A.R.
4. Potassium Iodide (KI), 15% W/V, J.T. Baker #3156. Dissolve 15 g KI in 100 ml distilled water.
5. Stannous Chloride ($SnCl_2 \cdot 2H_2O$), 40% W/V, J.T. Baker, #3980. Dissolve 40 g of $SnCl_2 \cdot 2H_2O$ in 100 ml of 1 + 1 HCl.
6. Pyridine - J.T. Baker #3348.
7. Silver diethyl-dithiocarbamate, J.T. Baker #H739. Dissolve 1.0 g of silver diethyl-dithiocarbamate in 200 ml of Pyridine. Store in an amber bottle in the refrigerator. Solution is stable indefinitely.
8. Zinc Granular, 20 mesh. J.T. Baker #4244.
9. Ammonium Oxalate, saturated solution, J.T. Baker #0746. Add 2.7 g Ammonium Oxalate monohydrate to 100 ml distilled water. Mix. Allow to come to equilibrium. Store in a glass bottle.
10. Lead Acetate, 10% W/W - $(CHCOO)_2 Pb \cdot 3H_2O$), J.T. Baker #2271. Weigh 20 g lead acetate and add to a beaker containing 180 ml distilled water.
11. Lead Acetate on Glass Wool. A wad of glass wool is inserted in the adapter that sits above the reaction flask where arsine is generated. Ten drops of 10% lead acetate is added to the glass wool.

Calibration

a) Stock Arsenic Standard (1 mg As/ml)

1. Weigh out 1.3204 g of Arsenic Trioxide (AS_2O_3, Primary Standard) J.T. Baker #0061.
2. Dissolve in approximately 10 ml 6N NaOH.
3. Add 90 ml of distilled water. Mix.
4. Acidify with concentrated HCl (about 5 ml).
5. Transfer to a 1 liter volumetric flask with distilled water and dilute to the mark. This solution is stable indefinitely.

LABCONCO

Labconco Corporation ■ 8811 Prospect Avenue ■ Kansas City, MO ■ 64132-2696
800-821-5525 ■ 816-333-8811 ■ FAX 816-363-0130 ■ E-MAIL labconco@labconco.com

6-24-A-5/96-100-R2

b) Dilute Arsenic Standard (50 μg As/ml)

1. Transfer 10 ml of Stock Standard to a 200 ml volumetric.
2. Add 2 ml concentrated HCl.
3. Make up to volume with distilled water. Prepare fresh.

c) Working Standard (1μg As/ml)

1. Transfer 5 ml of Dilute Standard (50μg/ml) to a 250 ml volumetric flask.
2. Add 2 ml of concentrated HCl.
3. Make up to volume with distilled water (1μg/ml). Prepare fresh.

The 5 μg Standard is carried through each run. It is prepared by transferring 5.0 ml of the Working Standard (1μg/ml) to a digestion flask.

Both the 5μg standard and a blank of 5 ml of deionized water are carried through the procedure starting with C2.

A standard curve is not prepared with each run. The one on file is used as long as the 5μg Standard that is carried through with each run compares with the previous reading.

When necessary, prepare a standard curve as follows:

1. Transfer the following volumes of Working Standard (1 μg As/ml) to digestion flasks of 0.5, 1.0, 2.5, 5.0, 10.0, 15.0 and 20.0 ml which corresponds to 0.5, 1.0, 2.5, 5.0, 10.0, 15.0 and 20.0 μg of arsenic.
2. Add 25 ml of urine from a common pool to each digestion flask.
3. Add 25 ml of urine to a digestion flask without any standard to serve as a blank.
4. All are carried through the procedure in the usual way.
5. Construct a curve of concentration vs absorbance on squared graph paper.
6. Retain curve for future use.

Quality Control

A control consisting of 5.0 ml of this Working Standard is carried through the complete procedure. This contains 5μg arsenic.

Procedure

A. Glass Preparation

All the glassware should be washed in nitric acid following the normal washing procedure. Use concentrated HNO_3 to rinse the Kjeldahl flasks (or 200 ml round bottom long neck boiling flasks), the Erlenmeyer flasks and the cuvettes. The gas dispersion tubes are soaked in 1 + 1 HCl overnight then rinsed with deionized water. The glass wool holders are washed by suction with concentrated HCl and then with deionized water.

B. Cleaning Hair and Nails Before Analysis

1. Weigh sample as received to ensure there is enough.
2. Transfer it to a 100 ml beaker.
3. Wash with a few drops of commercial shampoo which was previously checked and found not to contain arsenic and about 50 ml warm distilled water (Johnson's Baby Shampoo is acceptable.)
4. Stir well with a stirring rod and then decant the wash water.
5. Rinse two or three times with about 50 ml distilled water.
6. Add about 50 ml distilled water and about two drops of concentrated Ammonium Hydroxide.
7. Stir well and decant.
8. Add about 50 ml distilled ethyl alcohol and two drops concentrated NH_4OH.
9. Stir well and decant.
10. Rinse with about 25 ml ethyl alcohol and decant.
11. Rinse with about 50 ml ethyl ether and decant.
12. Allow to dry, then proceed with analysis.

C. Oxidation

1. Transfer a measured sample to a 200 ml round bottom Kjeldahl flask. Use 25 ml urine, 0.5 - 1 g washed and dried hair or nails, 20 ml blood or 50 ml of stomach washing or vomitus. If the desired amount is not available, record the exact amount used. See Note 1 when the sample is blood or tissue.
2. Add 5 ml concentrated H_2SO_4.
3. Add 30 ml concentrated HNO_3.
4. Prepare a blank using 5 ml concentrated H_2SO_4 and 30 ml concentrated HNO_3 and a control consisting of 5 ml of Working Standard and the acids as used in the blank.
5. In a fume hood, boil on Labconco Micro Digestor until the volume is reduced to 20-25 ml and then reduce the heat. See Note 2.
6. Add several drops of concentrated HNO_3 from a Pasteur pipet. Continue heating until all the brown fumes are driven off and the digestate is water white and fumes of H_2SO_4 evolve. See Note 3.
7. Cool for about 5 minutes.

8. Add 5 ml saturated ammonium oxalate and heat again until fumes appear.
9. Continue heating to produce fumes for 5 to 10 minutes longer.

D. Liberation of Arsenic

1. Allow the oxidized mixture to cool for about 20 minutes.
2. Transfer the digested sample to a 125 ml Erlenmeyer with ground glass neck 24/40. Wash the flask carefully with about 25 ml distilled water and add to the Erlenmeyer flask. Add water to make a volume of about 100 ml.
3. Add 20 ml concentrated HCl to each bottle.
4. Add 4 ml of 15% potassium iodide.
5. Add 1 ml of 40% stannous chloride.
6. Swirl contents in flask and allow to stand 15 minutes to ensure complete reduction to the trivalent state.
7. Into a 17 x 150 mm glass tube, transfer 4.0 ml of the pyridine containing silver diethyl-dithiocarbamate.
8. Add about 11 g (8 ml) of zinc metal, granular 20 mesh, to each flask and immediately stopper with the delivery tube. Make sure the tip of the delivery tube is near the bottom of the tube containing the pyridine. See Note 4.
9. Allow 30 minutes for the complete evolution of arsenic.
10. Transfer pyridine solution to a spectrophotometer cuvettes.
11. Read pyridine solution in the spectrophotometer at 545 nm and record absorbance for each tube.

Calculations

Read unknowns from the permanent calibration curve. The standard 5 µg arsenic which was carried through the procedure serves as a quality control sample. It should read 5 µg ±1 µg.

Calculate the amount of arsenic per 100 grams, or per dl, taking into account the amount and kind of sample used.

To convert to SI units as follows:

1. µg/dl ÷ 0.0075 = n mol/L
2. µg/100 g ÷ 7.5 = n mol/g

If the total volume of urine is known, it is reported as n mol/day, otherwise as n mol/L.

Background or Normal Values:

Hair: <13 nmol/g or <100 µg/100 g
Urine: <650 nmol/d or <5 µg/dl

Notes

1. When using blood or tissue, oxidize as for urine. If this is not suitable, a mixture of acids may be used as follows: 48 ml of concentrated HNO_3, 42 ml of concentrated H_2SO_4 and 12 ml of 70% perchloric acid ($HCl\ O_4$). If the sample is tissue (e.g. liver), homogenize 10 g and transfer to a 100 ml volumetric flask. Pipette out the equivalent of 2 g of tissue for analysis.
2. The sample turns brown as it starts to become more concentrated. Do not allow the solution to become very dark (add more HNO_3) or a reducing condition may develop and the arsenic will become volatile and be lost.
3. It may be necessary to add a few drops of concentrated HNO_3 several times before a clear digestate is obtained. If it is not water white by the time H_2SO_4 fumes are given off, cool the flask for about 10 minutes and then add 2 to 3 ml concentrated HNO_3 and boil again. Repeat if necessary.
4. The Erlenmeyer flask is fitted with ground glass joint (24/40) units. Into the flask is fitted a unit that has a fritted disc which holds the glass wool carrying the lead acetate. It has a male ground glass fitting on the bottom and the female one at the top. The delivery tube has a male ground glass fitting which goes into the glass wool holder and the other end has a fritted gas disperser which is completely immersed into the pyridine solution.

References

1. Jacobs, R. N., *The Analytical Toxicology of Industrial Inorganic Poisons,* p. 377 (1967)
2. Curry, A., *Poison Detection in Human Organs,* p 154-160 (1969)
3. Dubois, L., Teichmant, and Monkman, J.L., *A Study of the Determination of Arsenic by the Method of Vasak and Sedivec.*
4. Victoria General Hospital, Department of Toxicology, Nova Scotia, Canada