Determination of Mercury in Biological Media

1. SCOPE

By this method the total content of mercury – inorganic and organic – can be determined in any easily homogenated biological sample. Detection limit 0.02mg/kg for a one gram sample.

2. PRINCIPLES OF METHOD

When homogenated with an oxidising agent and acid the different forms of mercury in the sample are all converted into mercuric ion. Reducing agent then added in excess liberates elementary mercury, which by a stream of air is carried through the cell of a flameless atomic absorption instrument.

3. REAGENTS

3.1 Sulphuric acid, concentrated.

3.2 Potassium permanganate solution, 6% w/v.

3.3 Hydroxylamine chloride solution, 20% w/v.

3.4 Tin (II) chloride solution, 20% w/v.

3.5 Distilled water deionised by passing through mixed cation/anion exchange column.

4. APPARATUS

4.1 Spectrophotometer fitted with 10cm cylindrical gas cell, about 18mm ID, with quartz glass windows, and energy recording adaptor.

4.2 Recorder.

4.3 Source, mercury discharge lamp.

4.4 Macerator.

4.5 Digestion tubes, 100 x 16mm.

4.6 Burettes.

Note: Alternative to 4.1-4.3, an atomic absorption spectrophotometer with a hollow-cathode mercury lamp, and a recorder read-out can be used. The burner head is removed, and the gas cell placed in the light path.

5. PROCEDURE

5.1 *Cleaning of glassware*

Clean all glassware by washing in dilute HNO_3, followed by water, distilled water and deionized water.

5.2 *Instrument preparation*

Set up the apparatus as shown in the diagram. Switch on the photometer and allow the mercury lamp to warm up for 20min. With the aid of the potentiometer, adjust the sensitivity so that the full deflection of the recorder (20mV) corresponds to 100% light absorption. Control this sensitivity at intervals during analysis. The air is pumped through the apparatus by a respirator or electrical pump. Before entering the apparatus, the air is filtered through a tube containing active carbon and gold sand. Place a small plug of cotton wool between the sample tube and the gas cell to filter off any possible droplets carried by the air stream. Adjust the flow of air with the flow meter, (250ml/min) allowing the air to bubble through a digestion tube containing 3ml water.

5.3 *Instrument settings*

Wavelength: 253.7nm.
Slit width: 0.66mm.
Recorder chart speed: 1cm/min.
Air velocity: 250ml/min.

5.4 *Sample preparation*

Make duplicate tests on each sample. Include two water blanks and standard mercury solutions in each series of test.

5.4.1 Digestion of biological fluids

Pipette 1ml of the sample (e.g. urine, diluted blood) into a digestion tube, place the tube in a plastic covered wire test tube rack, and cool in a water bath. Add 0.2ml sulphuric acid from a burette and mix contents by swirling. Leave in water bath for a few minutes, and add 1.5ml potassium

permanganate solution from a burette. Shake carefully, and leave the tubes loosely stoppered overnight at room temperature. The following day, reduce the excess of permanganate with 0.3ml hydroxylammonium chloride solution from a burette. Determine the mercury content as below.

5.4.2 Digestion of biological tissue

Weigh 1g sample into a test tube large enough to accommodate the rotor of the macerator. Add 5(10)ml deionised water and homogenise. Rinse the rotor in several quantities of water before treating next sample. Weigh 1g homogenate into a 10ml bottle and digest as for the fluid samples, using 2ml sulphuric acid and 15ml permanganate solution. Reduce excess permanganate with 3ml hydroxylammonium chloride solution. Pipette 3ml into a digestion tube, and determine the mercury content.

5.4.3 Determination of mercury

Introduce 10 drops of tin (II) cloride solution into the small funnel over the air inlet tube (stopcock closed). Remove the digestion tube containing water, replace with a tube containing sample or standard; connect loosely to the glass joint. Introduce tin (II) chloride solution into the tube by opening the stopcock for a few seconds, then press the tube firmly against the joint. Air now bubbles through the solution, transferring the free mercury formed by reduction to the gas cell. The light absorption is registered by the recorder, rises rapidly to a maximum, and falls slowly. When the recorder pen has returned to the base line, remove the digestion tube, and rinse both sides of the gas inlet tube using a wash bottle. Place the digestion tube containing the next sample solution in position. If rapid speed of analysis is desired, the gas inlet tube can be rinsed before the recorder pen has reached the base line.

Note: An automatic method using an automatic sample changer has been devised by Lind and Skare (Analyst, 1971, 96 223-229), allowing analysis of 60 samples in two hours.

5.5 *Calibration graph*

Convert the peak heights obtained with standard mercury samples into absorbance units, and plot absorbance against mercury content, giving a calibration graph, which should be linear within the interval 0 to 0.3μg mercury. The reagent blank (2 to 5ng mercury) is included in the absorbance read on the calibration graph, and is automatically accounted for.

Convert peak heights obtained with the samples into absorbance units, and determine the mercury contents from the calibration graph. If necessary, dilute samples containing more than 0.3μg/ml mercury and repeat analysis.