

REINSCH ANALYSIS FOR ARSENIC, ANTIMONY, BISMUTH AND MERCURY IN FOODSTUFFS

Introduction:

While the human intake of sub-milligram amounts of metals such as arsenic, mercury, antimony and bismuth are common to a normal diet, suicidal as well as homicidal fatalities have occurred from the misuse of their toxic salts. The use of arsenic as a chemical murder weapon has historical origins in the odorless and nearly tasteless qualities of its trioxide salt. Human fatality from the ingestion of "white arsenic" (arsenic trioxide) can occur from as little as 200-300 mgs while the average lethal dose for an inorganic mercury salt is about one gram.

The following procedure describes the testing of common foodstuffs for the presence of these metals. It employs a well established analytical test commonly known as the REINSCH, which is simple and rapid to perform.

Principle:

The analysis is based on the fact that metallic arsenic, antimony, bismuth and mercury will deposit on a copper wire placed within a sample matrix that is acidified and heated. This deposition is visually recognized as a black or silvery staining of the copper wire. The analysis is followed by a confirmation technique utilizing XRF analysis for the particular element.

Specimen:

Sample matrices can be comprised of common foodstuffs (e.g cakes, candy and beverages) and/or liquids.

Instrumentation, Reagent and Materials:

Note* All chemicals are analytical reagent grade and stored at room temperature unless otherwise specified.

- a) Homogenizer and/or mortar and pestle
- b) Erlenmeyer flask (50 ml)
- c) Serological pipets (5 ml)
- d) Use one of the following:
 - 1) Copper spiral of #20 gauge wire wrapped around a glass rod 10 times
 - 2) Copper foil (0.1 mm width; 5x10 mm rectangle)
- e) Graduated cylinder (25 ml)
- f) Dilute Nitric Acid
Prepared by dissolving 5 ml of concentrated nitric acid in 10 ml of distilled water.
Prepare fresh in a test tube.
- g) Concentrated Hydrochloric Acid
- h) 1N Hydrochloric Acid
Prepared by dissolving 16.6ml of concentrated hydrochloric acid in 50ml of distilled water contained within a 200ml volumetric flask. The solution is then brought to the 200 ml volume mark using distilled water. It is stored in a clear glass reagent bottle at room temperature and is stable for an extended period of time.
- i) Ethanol
- j) Steam bath or equivalent
- k) Arsenic trioxide
- l) Magnetic stirrer

Preparation of Standards and Controls:

a) Analyte standard solution:

A 0.1 mg/ml standard solution of arsenic trioxide is prepared by adding 10 mgs (0.01 grams) of arsenic trioxide to a 100 ml volumetric flask. This is diluted with 1N HCl to the 100 ml mark. Dissolution takes place with magnetic stirring (1-2 hours). Prepare annually and store at room temperature.

b) Negative Control:

A distilled water blank or a matrix similar to the submitted specimen (if known and available) is used as a negative control.

c) Positive Control:

A portion (10 mls) of the analyte standard solution is used as a positive control. Alternatively, if the test sample amount permits, the positive control can be generated by mixing 10 mls of the analyte standard solution with 10 grams of the specimen.

Procedure:

1. If no standard solution is prepared, begin by making 1N HCl. Then prepare the analyte standard solution. This solution can be stored and used for one year after preparation date.
2. Prepare copper wires (one for positive control, one for negative control, and one for each sample). Label each with piece of tape.
3. Put large pyrex dish filled with water on hot plate. Heat until it is boiling.
4. Get large test tubes (one for each sample and the 2 controls). Add dilute nitric acid to each tube (5 ml of concentrated nitric acid in 10 ml distilled water).
5. Place each copper wire in each test tube. Observe the effervescence for about 30 seconds. Terminate chemical reaction by rinsing the wire with distilled water, then ethanol, then distilled water. The clean coil should have a shiny appearance. If not, repeat procedure.
6. Place each copper wire in separate 50 ml erlynmeyer flasks containing 4 ml of concentrated HCl.
7. Thoroughly homogenize the submitted specimen to ensure a representative aliquot is sampled. This may be accomplished by grinding in a mortar and pestle (candy), grinding by hand (cakes, cookies), or vigorously shaking the specimen beverages).
8. Add 10 grams or mls of uniform specimen to the flask followed by 10 mls of distilled water. A negative and positive control are similarly processed.

Note (a) - If a specimen was initially homogenized with an equal volume of distilled water, use 20 grams of homogenate as the sample with no further addition of distilled water.

Note (b) - A positive control that is generated in the specimen matrix also uses 20 grams of sample (10 grams specimen/10 mls analyte standard solution) with no further addition of distilled water.

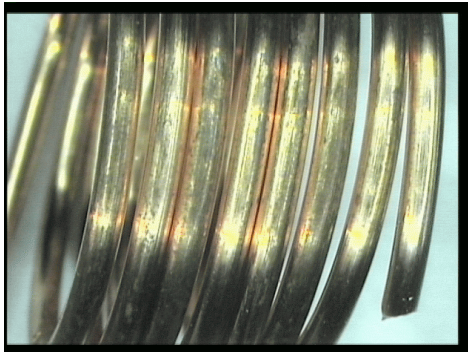
9. Cap the flask with aluminum foil and swirl the contents to ensure a uniform distribution of the acid.
10. Partially submerge the Erlynmeyer flask in a steam bath or equivalent boiling water bath for one hour. Stir the contents by occasional swirling of the flask.
11. After the elapsed time, remove the wire and rinse with distilled water. Visually inspect the wire for a deposited black or silvery coating. Photographs may be used to document negative or positive results.

Control:

1. The test is considered negative if the copper wire is not coated with a black or silvery stain.
2. A distilled water negative controls yields no black or silvery deposit on the copper wire (see photograph).
3. Arsenic trioxide used as the positive control will stain the copper wire black. Antimony and bismuth yield a similar black stain while mercury coats the wire with a silvery deposit. These stains are fixed and should not rinse off with distilled water (see photograph).

Sensitivity and Specificity:

1. This procedure readily detects the metal cations (arsenic, antimony, bismuth and mercury) at concentrations of about 0.01% (w/w) in food. The primary value of this test is exclusionary and false negatives are unlikely. However, if a false negative is suspected and specimen amount permits, a positive control should be generated in the specimen matrix (see preparation of standards and controls) followed by a reassay of the sample to ensure staining of the copper wire.
2. The deposit of a fixed (water insoluble) black or silvery stain on the copper wire only indicates and does not identify the presence of arsenic, antimony, bismuth or mercury. Tarnishing of the copper wire may occur from the presence of other metals such as selenium or tellurium. XRF will be used to target the individual metal for qualitative data. See XRF procedure:



Blank	As, Sb, Bi

-100 us/mL

XRF PROCEDURE

The copper samples of the positive and negative control along with the question samples are placed in the XRF chamber and subjected to the following conditions:

Source: Zirconium Target
KV 24 - 25
Ma .5 - 3.3

The K alpha line for arsenic will appear at 10.54keV the K beta line will be at 11.72keV.

Conclusions:

Positive - Examination of the questions sample(s) revealed the presence of Arsenic.

Negative - Examination of the questions sample(s) did not reveal the presence of Arsenic.

Safety:

- 1) Take standard precautions for the handling of chemicals and biological materials.
- 2) Metals are cumulative in the body and care should be taken to avoid skin contact with standard solutions.