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NATIONAL REACTOR TESTING STATION
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ABSTRACT

A method is presented for the determination of mercury in a variety of inorganic and organic samples. After selected pretreatments, the mercury dithizonate complex is extracted from an EDTA-citrate medium into chloroform, the absorbance of which is measured at 495 mμ.

The effects of 35 metal ions and 8 nonmetal anions have been studied. Only silver(I) interferes at less than a 50 to 1 molar ratio.

SUMMARY

Three different pretreatments followed by a spectrophotometric method have been developed to determine mercury at microgram levels in a variety of inorganic and organic solutions and solids. Inorganic solutions are reacted with hot nitric acid to oxidize mercury to the (II) oxidation state. Inorganic solids are dissolved in acids or fused with potassium pyrosulfate. Organic materials or inorganic materials that contain organic matter are digested with a nitric-sulfuric acid mixture.

In the spectrophotometric method, the mercury(II) dithizone complex is extracted into chloroform and measured at 495 mμ. A single extraction of the complex from an EDTA-citrate masking medium is sufficient when the concentrations of copper and silver are below established limits. A double extraction increases the tolerance for copper. In this double extraction procedure, the complex is extracted into chloroform from either an acidic ($\leq 0.75\ \underline{N}$) medium or the EDTA-citrate masking medium at pH 2.85±0.35. Mercury is stripped from the chloroform phase with an acidic nitrite solution, the nitrite is destroyed with hydroxylamine and aniline, and mercury(II) dithizonate is extracted a second time into chloroform.

The method is highly selective. Of 35 metal ions and 8 nonmetal anions studied in the double extraction procedure, only silver(I) interferes at a molar ratio to mercury below 50 to 1.

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INTRODUCTION

In a processing plant for the recovery of uranium from irradiated nuclear fuels, a variety of samples which contain mercury is analyzed. The mercury usually is introduced as mercury(II) nitrate to catalyze the nitric acid dissolution of aluminum clad fuels. Samples include the dissolution reagent, the dissolved fuel at various stages in the plant process, waste streams, and streams from the calcination process.[1] In the calcination process, the liquid waste is converted to micron-size solid particles by fluidized bed evaporation. During this process, mercury tends to volatilize to the environs. Vegetation samples often are analyzed for mercury to monitor for the release of mercury. In addition, miscellaneous samples of paints, inks, cardboards, and plastics are analyzed.

EXPERIMENTAL AND DISCUSSION

In general, the extraction-spectrophotometric method is patterned after a British method.[2] Some of the apparatus and several details of the method have been changed. The major effort was the development of pretreatment procedures for various inorganic and organic samples to increase versatility. The pretreatments and the extraction-spectrophotometric method are schematically diagrammed in Figure I and the detailed procedure is given in the Appendix.

Inorganic solutions are heated with nitric acid to make certain all the mercury is in the (II) oxidation state. The oxidation step is done in a test tube heated in a hot water bath.

Inorganic solids are fused with potassium pyrosulfate in a quartz tube with a water-condenser reflux section. During the fusion, much of the mercury volatilizes as free mercury or as mercury compounds into the condenser and must be returned to the tube. To accomplish this, the condenser water is turned off until the level of refluxing nitric acid nearly reaches the top of the condenser at which time it is turned on and the heating is continued until the melt dissolves.

Organic materials and aqueous solutions which contain organic materials are digested with a mixture of nitric acid and sulfuric acid in the apparatus pictured in Figure A-1 in the Appendix. With this apparatus, the nitric acid which distills from the digestion flask may be returned to the flask as required.

In all three pretreatment procedures, precautions must be taken to prevent the loss of mercury compounds, many of which volatilize from heated solutions. Using a Hg-203 tracer, losses of about 8% were obtained when organic materials were digested in the apparatus described by the British workers.[2] The addition of a vapor trap, shown in Figure A-1 of the Appendix, gives quantitative recovery of mercury.

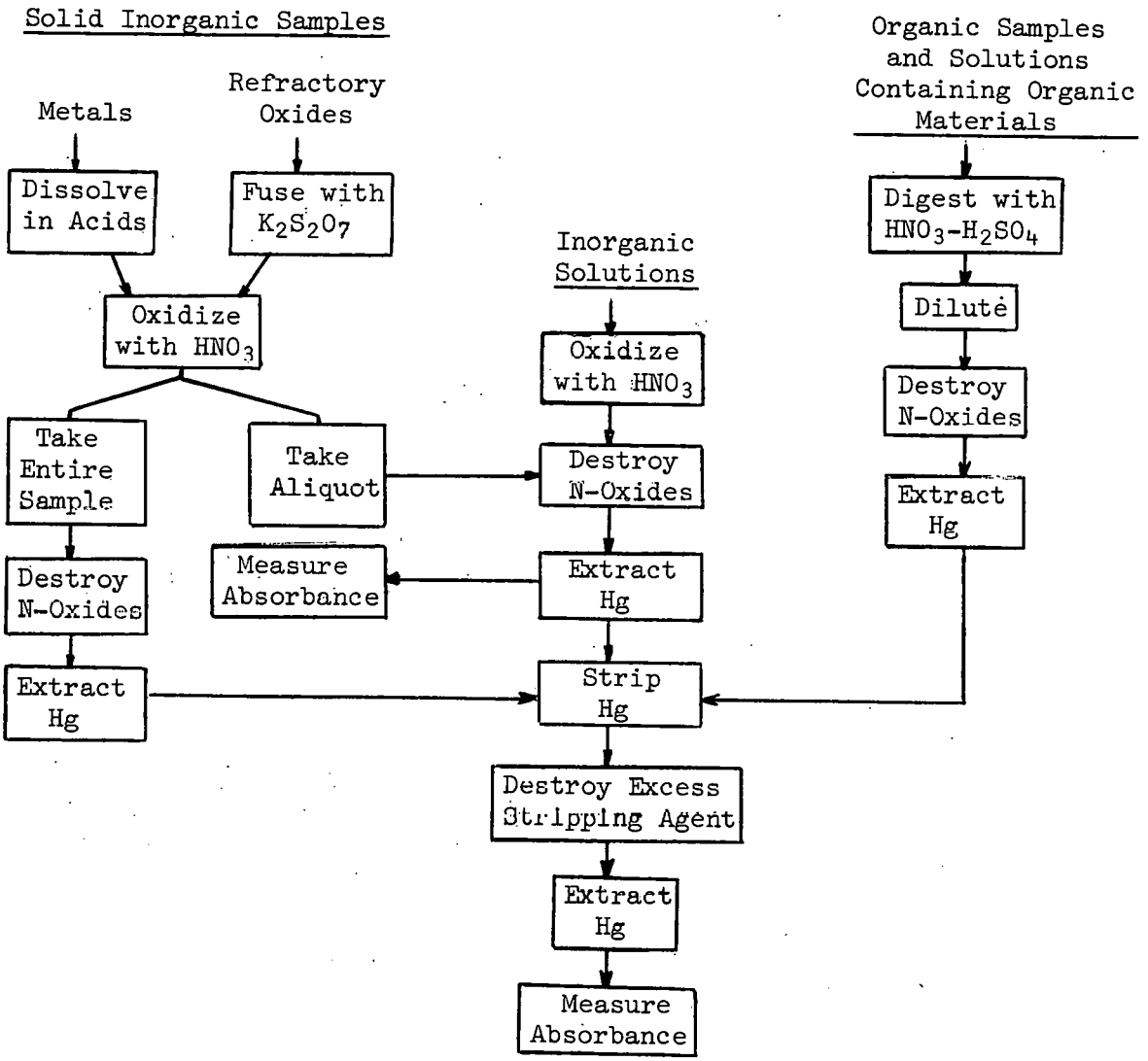

Figure 1. Analysis Scheme

The resulting solutions from all the pretreatment procedures are analyzed by an extraction-spectrophotometric method based on the extraction of mercury(II) dithizonate into chloroform. A single extraction satisfactorily separates mercury from all the cations studied except copper and silver at molar ratios to mercury of 150 to 1 and 0.1 to 1, respectively.

In the single extraction procedure, hydroxylamine is added to the solution from the sample pretreatment to destroy nitrite. Aniline then is added to destroy the last traces of nitrite including any nitrite that forms from nitric oxide. An EDTA-citrate buffer-complexer solution is added, and the pH is adjusted to 2.85±0.35 with concentrated ammonium hydroxide and a chloroform solution of dithizone is added. After extraction, the absorbance of the complex in the separated chloroform phase is measured at 495 mμ.

In the double extraction procedure, the mercury first is separated from most other cations by extraction into chloroform as the dithizone complex from an acidic solution (≤0.75N). The mercury is stripped from the chloroform phase with sodium nitrite and hydrochloric acid. Most of the excess nitrite then is destroyed with hydroxylamine. Aniline is added to destroy the last traces of nitrite and EDTA-citrate solution is added. The pH is adjusted to 2.85±0.35 with sodium hydroxide, the dithizone-chloroform solution is added, and the mercury(II) dithizone complex is extracted into chloroform for measurement at 495 mμ.

Variables in the Spectrophotometric Method

Extraction Acidity. To extract mercury quantitatively from acidic solutions, the maximum acidity is 0.75N. When an EDTA solution is used, the practical pH range is 2.5 to 3.2. Below pH 2.5, EDTA precipitates. Above pH 3.2, the extraction of mercury is incomplete.

EDTA-Citrate Level. Neither the extraction nor the color intensity of the mercury(II) dithizone complex is adversely affected when up to 15 ml of the EDTA-citrate masking solution is used in the aqueous phase.

Stripping Conditions. Mercury is stripped quantitatively from the chloroform phase with 0.5 to 1.5 mmole of sodium nitrate at 1N acidity. In the procedure, sodium nitrite is held at 0.75 mmole and, to facilitate pH adjustment for the second extraction, 10 ml of 1N HCl is used.

Destruction of Nitrite and Nitrogen Oxides. Nitrite and gaseous N-oxides are present in sample solutions after pretreatment steps. In the double extraction procedure, nitrite and N-oxides are introduced in the stripping step. These must be destroyed to avoid oxidizing the dithizone in the subsequent extraction. Hydroxylamine and aniline are used for this destruction. Hydroxylamine is the principal reductant, while aniline destroys the last traces. Aniline has been found to be a more efficient scavenger than urea. It also effectively destroys nitrite generated in solution from nitric acid.

Twenty-five mmole of hydroxylamine and 1 mmole of aniline adequately destroy nitrite before high acidity extractions. Five mmole of hydroxylamine and 0.5 mmole of aniline are sufficient to destroy the excess nitrite left after the stripping step. In both cases, 5 minutes is sufficient for the hydroxylamine to react. The aniline reacts instantaneously. Neither the extraction nor the color intensity of the mercury dithizonate is affected at these levels of hydroxylamine and aniline.

Diverse Ion Effects

The effects of 35 metal ions and 8 anions on the single extraction procedure are summarized in Table I. Only silver(I) interferes at less than a 50:1 molar ratio to mercury.

Sensitivity

With the sample sizes specified in the procedure, the sensitivity of the method is 0.5 µg/g for solid organic samples, 10 µg/g for solid inorganic samples, and 0.2 µg/ml for aqueous inorganic solutions. The range of the spectrophotometric method is 1 to 35 µg of mercury.

Precision

Sixty aqueous standards, equally split between the single extraction and double extraction procedures, were analyzed by six analysts. The pretreatment used was nitric acid oxidation. The percent standard deviation ranged approximately linearly from 17% at 1 µg of mercury to 5% at 25 to 35 µg.

Table I. DIVERSE ION EFFECTS ON THE SINGLE EXTRACTION PROCEDURE

Ion or Mixture Investigated	Tolerance Level, Ion to Hg Molar Ratio[a,b]
Be(II), Bi(III), Ca(II), Cd(II), Ce(III), Co(II), Cr(III), Cs(I), Fe(III), Ge(IV), Ho(III), In(III), La(III), Mg(II), Mn(II), Mo(VI), Ni(II), Pb(II), Sn(II,IV), Sr(II), Th(IV), Ti(IV), U(VI), V(V), Y(III), Zn(II), Zr(IV)	Each ion individually at 1000
Ag(I)	0.1[c]
Au(III), Pd(II), Pt(IV)	Each ion individually at 50
Cu(II)	150[c], 750[d]
Borate	2000
Bromide	1000
Chloride	7.5×10^5
Iodide, thiosulfate	50[c]
Phosphate	3.0×10^4
Sulfate	1.3×10^5
Synthetic aluminum-zirconium matrix	
Al(III), Zr(IV)	Each at 2000
Cr(VI), U(VI)	Each at 50
Cl^{-1} plus NO_3^{-1}	1×10^4
Simulated aluminum fuel dissolver product	
Al(III)	4×10^4
Cu(II)	150[c]
Cr(III), U(VI)	Each at 50
Fe(III)	200
Mn(II), Ni(II)	Each at 100
NO_3^{-1}	12×10^4

TABLE I (Cont'd)

Ion or Mixture Investigated	Tolerance Level, Ion to Hg Molar Ratio[a,b]
Simulated calciner product fused with potassium pyrosulfate	
Al(III), Zr(IV)	Each at 2000
Cr(VI)	50
K(I)	2.4×10^5
SO_4^{-2}	1.3×10^5

(a) Mercury level maintained at 20 μg (1×10^{-4} mmole).

(b) Except where noted otherwise, the tolerance level listed is the highest level studied and does not represent the maximum permissible level.

(c) Maximum tolerance level.

(d) Double extraction procedure.

REFERENCES

1. Lakey, L. T., Bower, J. R., IDO-14620, Rev. 1, December, 1963.

2. Analytical Methods Committee, Analyst, 90, 515 (1965).

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APPENDIX

DETAILS OF PROCEDURE

APPARATUS AND REAGENTS

A. Apparatus

NOTE: All glassware should be boiled in 4M HNO_3 and rinsed with distilled water before use.

1. Absorbance cells, Pyrex, matched pairs, 1-cm and 5-cm, with covers.
2. Beakers, assorted sizes.
3. Centrifuge tube, 50-ml.
4. Culture tube, with Teflon-lined screw cap.
5. Digestion apparatus for organic samples (Fig. A-1).
6. Filter paper, Whatman 41.
7. Fisher Filtrator.
8. Funnels, assorted sizes.
9. Graduated cylinder, 10-ml.
10. Hot plate.
11. Magnetic stirrer, with Teflon-coated stirring bars.
12. Meker burner.
13. Membrane filter, 0.45-μ pore size, with Millipore filtering apparatus.
14. Micro burner.
15. pH meter, Leeds and Northrup or Beckman, with capillary glass-calomel electrodes or a single-probe glass-calomel electrode.
16. Pipets, macro, volumetric, assorted sizes, with suction bulb.
17. Pipets, micro, assorted sizes, with control syringe.
18. Pipets, Mohr, 5- and 10-ml.
19. Quartz fusion apparatus for inorganic solids. Use a 100-ml round bottom quartz flask or a 35-mm diam x 6-in. quartz test tube with a 24/40 outer joint in conjunction with the condenser illustrated in Fig. A-1.
20. Separatory funnels, 60-ml, with Teflon stopcocks.

21. Separatory funnels, 500-ml, with ground glass stoppers and Teflon stopcocks.

22. Spectrophotometer, Beckman Model DU, DK, or B, or Cary Model 14.

23. Variac.

B. REAGENTS

NOTE: Use Analytical Reagent Grade chemicals and distilled water for the preparation of all reagents and throughout the procedure.

1. Ammonium hydroxide, conc.

2. Aniline hydrochloride solution, 0.5M. Add 45 ml of conc HCl to 500 ml of water. Add 46 ml of freshly distilled aniline SLOWLY with efficient stirring. Transfer to a 1-liter volumetric flask and dilute to volume with water. The final solution should be colorless or have only a faint pink or yellow tinge. Store in the dark and prepare a fresh solution every month.

   NOTE: The aniline must be added slowly to dilute HCl solution to avoid excessive coloration of the solution.

3. Buffer-complexer reagent. Dissolve 37.22 g of $Na_2EDTA \cdot 2H_2O$, 94.50 g of monochloroacetic acid, and 90.48 g of ammonium citrate in a 2-liter beaker with 1500 ml of distilled water. Use sufficient conc NH_4OH to insure complete dissolution (pH ≈3.5). Dilute to 2 liters with water.

4. Chloroform.

5. Dithizone stock solution, 0.0105 (w/v)% (4×10^{-5}M). Dissolve 0.0525 g of dithizone in chloroform and dilute to 500 ml with chloroform. Store in a refrigerator when not in use.

6. Dithizone solution, 0.00105 (w/v)% (4×10^{-6}M). Pipet 50.00 ml of the 0.0105% stock solution into a 500-ml volumetric flask and dilute to 500 ml with chloroform. Store in a refrigerator when not in use.

7. Hydrochloric acid, 1.0M.

8. Hydroxylamine hydrochloride, 2.5M. Dissolve 174 g of $NH_2OH \cdot HCl$ in 800 ml of distilled water. Filter the solution through a 0.45-μ membrane filter and dilute to 1 liter with water.

9. Mercuric nitrate stock solution, 500 μg/ml. Dissolve 0.5000 g of redistilled mercury metal in 20 ml of 7.5M HNO_3 with heating. Boil gently for 10 min, cool, then dilute to 1 liter with water. Store as 40-ml units in sealed glass ampoules.

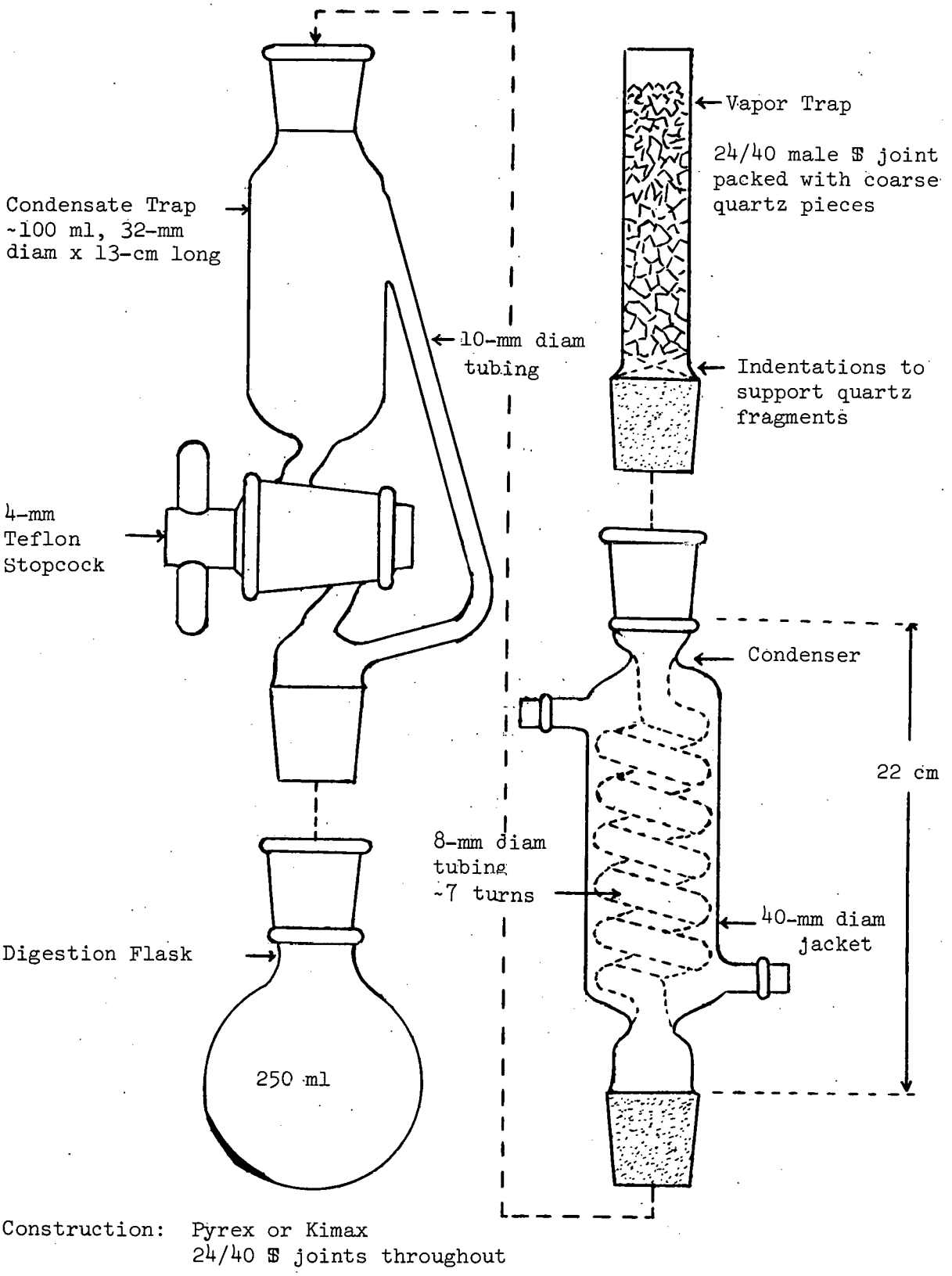

Figure A-1. Digestion Apparatus for Organic Samples

10. Mercury calibration standard solutions.

    a. Standard I, 30.00 µg Hg/ml. Dilute 15.00 ml of the mercury stock solution to 250 ml with 0.25M HNO_3.

    b. Standard II, 20.00 µg Hg/ml. Dilute 10.00 ml of the mercury stock solution to 250 ml with 0.25M HNO_3.

    c. Standard III, 8.00 µg Hg/ml. Dilute 4.00 ml of the mercury stock solution to 250 ml with 0.25M HNO_3.

    d. Standard IV, 6.00 µg Hg/ml. Dilute 3.00 ml of the mercury stock solution to 250 ml with 0.25M HNO_3.

11. Nitric acid, conc and 1M.

12. Nitric acid-sulfuric acid digestion mixture. Mix, as needed, conc H_2SO_4 and conc HNO_3 in the ratio of 1:5.

13. Potassium pyrosulfate, $K_2S_2O_7$.

14. Sodium hydroxide, 0.80M. Dissolve 32.0 g of NaOH in water and dilute to 1 liter with water.

15. Sodium nitrite solution, 0.75M. Dissolve 51.76 g of $NaNO_2$ in 500 ml of water and dilute to 1 liter with water. Prepare a fresh solution every month. Store in the dark.

PROCEDURE

NOTE: Use distilled water throughout the procedure.

Mercury(II) reduces to mercury(I) or (0) by reaction with hydroxylamine, especially in weakly acidic or basic solution. Also, it may precipitate as the hydroxide from weakly acidic or neutral solutions. For these reasons, the procedure, once begun, may be interrupted only at those points where the acidity is high and hydroxylamine is absent. These points are at the end of each pretreatment procedure before any dilution or pH adjustment (steps C-2, D-9, E-2, F-9, and G-2) and at the end of the stripping step (C-12) when the hydrochloric acid concentration is about 0.9M

A. Blank

Process a reagent blank with each set of samples per the procedure selected for the analysis of samples. For aqueous samples, use 3 ml of distilled water in place of the sample. With inorganic solids and organic samples, omit the addition of a sample substitute, but introduce all reagents used for the samples in amounts equal to those used for the samples.

B. Calibration and Bench Standard

Four standards are recommended for this method - two for the high range (to be measured in 1-cm cells) and two for the low range (to be measured in 5-cm cells). If only one type of sample is to be analyzed, two appropriate standards will suffice. However, if the mercury concentrations of the samples vary over a wide range, the use of all four calibration standards will minimize repeat analyses.

Process calibration standards with each run by the same procedure as that used to analyze the samples. Use 1.00-ml aliquots of the appropriate standards. Divide the micrograms of mercury in the standard by the absorbance to obtain the conversion factor. For each of the two groups of standards, the difference between the two factors should not exceed established limits and the average of the two factors should agree with the established conversion factor within specified limits. If either of these requirements is not met, reprocess the pair or pairs of calibration standards. Contact your supervisor if difficulties are still experienced.

C. Analysis of Aqueous Inorganic Samples of Unknown Composition

NOTE: If the levels of diverse ions are known not to exceed the tolerance limits, the shorter single extraction procedure (Procedure G) may be used.

1. Pipet an aliquot, 5 ml or less, containing 1 to 35 µg of mercury into a 50-ml centrifuge tube.	The use of a wide-mouth test tube such as a 50-ml centrifuge tube facilitates pH adjustment in step 6. Refer to Table I for information on tolerance levels for diverse ions.
2. Add 1 ml of conc HNO_3 and immerse the centrifuge tube in a boiling water bath for 5 min.	Nitric acid oxidizes Hg(0,I) to Hg(II). Prolonged digestion will lead to loss of mercury by volatilization. If brown NO_2 fumes are observed, aspirate the fumes with mild suction before adding the hydroxylamine hydrochloride.
3. Cool, dilute to about 15 ml with water, add a small stirring bar and 2 ml of 2.5M $NH_2OH \cdot HCl$, then let stand for 5 min with intermittent stirring.	Mercury(II) reduces with standing times longer than 10 min.
4. Add 1 ml of 0.5M aniline hydrochloride.	

5.	Add 5 ml of the buffer-complexer solution.	
6.	With the aid of a pH meter, adjust the pH to 2.85±0.35 with conc NH_4OH.	
7.	Transfer the sample quantitatively to a 60-ml separatory funnel with water rinses.	
8.	Add approximately 15 ml of 0.00105% dithizone-chloroform solution and shake vigorously for 30 sec. Let the two layers separate, then swirl the separatory funnel to settle the floating droplets of chloroform.	Use polyethylene stoppers.
9.	Drain the lower chloroform layer into a clean 60-ml separatory funnel.	Do not transfer any of the aqueous phase. A small amount of the chloroform layer left in the separatory funnel will be recovered in step 10. Observe the color of the chloroform solution. If it is golden-yellow or nearly so with very little blue-green color of free dithizone, extract the residual aqueous phase in the original separatory funnel with 10 ml of the 0.00105% chloroform-dithizone solution and drain the chloroform extract into the clean separatory funnel. If the second extract still lacks the blue-green color of free dithizone, discard the sample and process a new, smaller aliquot.
10.	Extract the residual aqueous phase in the original separatory funnel for 15 sec with 5 ml of chloroform and drain the chloroform into the clean separatory funnel.	Do not transfer any of the aqueous phase. Discard the aqueous phase. The two organic phases (steps 9 and 10) are combined.
11.	To the dithizone-chloroform extract, pipet 10 ml of 1.0M HCl with a volumetric pipet and 1 ml of 0.75M $NaNO_2$ solution. Stopper and shake for 30 sec.	The nitrite destroys the dithizone and returns the mercury to the aqueous phase.

12.	Let the two phases separate, then drain and discard the lower chloroform layer.	Take care that none of the aqueous layer is lost in the separation.
13.	To the residual aqueous phase, add 2 ml of 2.5M $NH_2OH \cdot HCl$. React for 5 min with intermittent swirling.	Mercury(II) reduces with standing times longer than 10 min.
14.	Rinse the separatory funnel with a little water, then add 1 ml of 0.5M aniline hydrochloride solution. Swirl to mix.	Rinse the stopper and the neck of the flask carefully to remove any traces of $NaNO_2$ present.
15.	Add 5 ml of the buffer-complexer solution, swirl to mix, then pipet 10 ml of 0.80M NaOH with a volumetric pipet to adjust the pH to the range 2.85±0.35.	The pH at this step is critical. Measure the pH of the blank with a pH meter to see if it is within the required range. If it is not, measure the pH of all samples. Adjust the pH, as necessary, to 2.85±0.35 with 1M HCl or 0.80M NaOH.
16.	Pipet precisely 15.00 ml of 0.00105% dithizone-chloroform solution, stopper, and extract for 15 sec.	The extraction time must be kept short to minimize the extraction of copper.
17.	Let the phases separate, then drain the lower chloroform phase into a 25-ml screw-cap culture tube. Measure the absorbance of the chloroform phase at 495 mμ against the reagent blank in 1-cm (5 to 35 μg of mercury) or 5-cm (1 to 10 μg of mercury) cells.	When in doubt, measure the absorbance with 5-cm cells first. This will permit subsequent measurements with 1-cm cells. The color of the chloroform-dithizone extract should show the presence of excess unreacted dithizone, i.e., a bluish-green cast. If not, discard the sample and process a new smaller aliquot. The walls of the tube adsorb water droplets which otherwise interfere. If necessary, centrifuge the tube. Under proper conditions, the color of the chloroform extract is stable for at least 2 hr. If slow but noticeable "bleaching" is observed, the nitrite probably was not destroyed adequately in steps 13 and 14. Discard the sample and process a new one.

D. Analysis of Inorganic Solids With Greater Than 100 µg/g Levels of Mercury

1. Transfer a 0.1-g sample containing greater than 10 µg of mercury to the quartz fusion apparatus.

This procedure is intended primarily for inorganic solids such as alumina and alumina-zirconia calcined materials that are difficult to dissolve. Dissolve metals and alloys under reflux in appropriate mineral acids and analyze per Procedure C.

2. Add 3.0 g of $K_2S_2O_7$.

3. Assemble the fusion apparatus. Turn on the cooling water and tilt the apparatus to about a 45° angle.

4. With a Meker burner, fuse the sample repeatedly until the melt clears.

5. Turn off the cooling water and return the apparatus to the upright position.

6. Add 6 ml of conc HNO_3 through the condenser and heat the mixture with a micro burner until the point of condensation just reaches the top of the condenser or until the start of bumping.

During fusion, mercury is volatilized into the condenser possibly as metallic mercury. The purpose of the HNO_3 reflux is to recover the volatilized mercury.

7. Cool slightly, turn on the cooling water, and rinse the condenser with 20 to 25 ml of 1M HNO_3.

8. Remove the fusion flask or tube and place it in a boiling water bath until the liquid clears and most of the solids dissolve.

It is not necessary to dissolve the solids completely.

9. Cool the solution and filter it through a 0.45-µ membrane filter directly into a 250-ml volumetric flask. Use at least three 10-ml portions of 1M HNO_3 for the transfer and washing and a Fisher

Filtrator-Millipore setup for the filtration.

10. Dilute to volume with water and mix well.

11. Pipet an aliquot, 25 ml or less, containing 1 to 35 µg of mercury into a 50-ml beaker.

12. Continue per Procedure C beginning at step 3.

If the observed net absorbance of the sample corresponds to less than 1 µg of mercury, select a larger aliquot (if this is permissible) in step 11. With a 25-ml aliquot of the diluted sample, the lower limit of determinability is 100 ppm of mercury in the original solid sample. If greater sensitivity is required, reanalyze the original solid sample per Procedure E.

E. Analysis of Inorganic Solids With Less Than 100 µg/g Levels of Mercury

1. Dissolve a 0.1-g sample per steps 1 through 8 of Procedure D.

2. Cool the solution, then filter the solution through a Whatman 41 filter paper into a 500-ml separatory funnel. Use three 10-ml portions of 1M HNO_3 to rinse the fusion flask and the filter paper.

3. Dilute to 250 ml with distilled water.

The acidity must be reduced to 0.75N or less.

4. Add 5 ml of 2.5M $NH_2OH \cdot HCl$ and let stand for 5 min with intermittent swirling.

Mercury(II) reduces with standing times longer than 10 min.

5. Rinse the separatory funnel with water, then add 2 ml of 0.5M aniline hydrochloride solution.

6. Extract for 30 sec with 10 ml of 0.00105% dithizone-chloroform solution. Drain the lower chloroform phase into a 60-ml separatory funnel.

   Considerable pressure is often built up within the separatory funnel. Cover the stopper with a tissue paper and remove the stopper carefully.

7. Repeat step 6.

8. Repeat step 6 using 10 ml of chloroform in place of the dithizone-chloroform solution. Discard the aqueous phase.

9. Continue per Procedure C beginning at step 11.

F. Analysis of Organic Samples and Inorganic Samples Containing Organic Matter

1. Weigh or pipet a sample containing 1 to 35 µg of mercury into the flask of the digestion apparatus (Fig. A-1).

   This procedure has been found to be satisfactory for 2-g samples of organic matter such as vegetation. If larger samples must be processed to reach the desired sensitivity, process separate 2-g samples per steps F-1 through F-15, combine the dithizone-chloroform and chloroform extracts of steps F-13 through F-15, then complete the determination per step 16.

2. Add 15 ml of freshly prepared HNO_3-H_2SO_4 digestion mixture.

3. Assemble the digestion apparatus per Fig. A-1, turn on the cooling water, and heat the sample with a heating mantle controlled by a Variac. Boil vigorously and collect the distillate in the condensate trap. Continue the digestion until only 2 to 3 ml of acid remains and H_2SO_4 fumes and charring is observed.

   A Variac setting of 105 to 110 is recommended.

7.	Return the condensate to the digestion flask, cool slightly, then admit about 50 ml of water through the top of the digestion apparatus.	Admit the water slowly.
8.	Reflux for 5 min, or longer if necessary, to dissolve precipitated sulfate salts.	
9.	Disassemble the digestion apparatus. Rinse each component with water and collect the rinses in the flask.	Rinse the side arm of the condensate trap also.
10.	Transfer the contents of the flask quantitatively to a 500-ml separatory funnel with water rinses and dilute to about 400 ml with water.	For quantitative extraction of the mercury, the acidity must be 0.75N or less.
11.	Add 10 ml of 2.5M $NH_2OH \cdot HCl$, mix well, then let stand for 5 min. Swirl intermittently during the 5-min period.	Mercury(II) reduces with standing times longer than 10 min.
12.	Add 2 ml of 0.5M aniline hydrochloride solution and mix well.	
13.	Extract the mercury with a 10-ml portion of 0.00105% dithizone-chloroform solution for 30 sec. Drain the lower chloroform layer into a 60-ml separatory funnel.	Considerable pressure is often built up during the extraction. Cover the stopper with a tissue paper and remove the stopper carefully.
14.	Repeat step 13.	Combine the organic phases.
15.	Repeat step 13 using 10 ml of chloroform in place of the dithizone-chloroform solution. Discard the aqueous phase.	Combine this organic phase with the two previous organic phases in the 60-ml separatory funnel.
16.	Continue per Procedure C beginning at step 11.	

G. Analysis of Aqueous Inorganic Samples of Known Composition (Single Extraction Procedure)

NOTE: If the composition of the sample is unknown or is known to contain copper at concentrations that exceed the tolerance limits, Procedure C must be used.

1. Pipet an aliquot, 5 ml or less, that contains 1 to 35 µg of mercury into a 50-ml centrifuge tube.	The sample must not contain more than 4 mmole of aluminum nor more than 0.03 mmole of copper(II). See Table I for the tolerance levels of other ions.
2. Add 1 ml of conc HNO_3 and immerse the centrifuge tube in a boiling water bath for 5 min.	Nitric acid oxidizes Hg(0,I) to Hg(II). Prolonged digestion will lead to loss of mercury by volatilization.
3. Cool, dilute to about 25 ml with water, add 2 ml of 2.5M $NH_2OH \cdot HCl$, then let stand 5 min with intermittent swirling.	If much brown NO_2 fumes are observed, aspirate the fumes with mild suction before adding the $NH_2OH \cdot HCl$. Mercury(II) reduces with standing times longer than 10 min.
4. Add 1 ml of 0.5M aniline hydrochloride.	
5. Add 10 ml of the complexer-buffer solution and adjust the pH to 2.85±0.35 with conc NH_4OH.	
6. Transfer the sample quantitatively to a 60-ml separatory funnel with water rinses.	
7. Add exactly 15.0 ml of 0.00105% dithizone-chloroform solution and extract for exactly 15 sec.	The extraction time must be kept short to minimize the extraction of copper.
8. Drain the lower chloroform layer into a 50-ml culture tube.	The walls of the culture tube adsorb water droplets which otherwise interfere. If necessary, centrifuge the tube.

9. Measure the absorbance of chloroform extract against the reagent blank at 495 mμ in 1-cm (5 to 35 μg of mercury) or 5-cm (1 to 10 μg of mercury) cells.

When in doubt, measure the absorbance with 5-cm cells first. This will permit subsequent measurements with 1-cm cells.

The color of the chloroform-dithizone extract should show the presence of excess unreacted dithizone, i.e., a bluish-green cast. If not, discard the sample and process a new smaller aliquot.

Under proper conditions, the color of the chloroform extract is stable for at least 2 hr. If slow but noticeable "bleaching" is observed, the nitrite probably was not destroyed adequately in steps 3 and 4.