DETERMINATION OF LEAD

A Modification of the Fischer - Leopoldi Method

Within the last few years dithizone (diphenylthiocarbazone) has found extensive use as a reagent in determining minute amounts of lead, particularly in biological material. The following method has been developed in our laboratory as a means of obtaining rapid as well as accurate analyses for amounts of lead, 30 gamma or more, and incorporates ideas from the methods of Fischer and Leopoldi (1), Wilkins, Willoughby, et al. (5), and Winter et al. (3).

Procedure

Glassware and Reagents. Pyrex glassware should be used throughout the analysis. Squibb type, separatory funnels of capacity 150 ml. and suitably graduated, are placed on an adjustable rack built to hold two rows of six each, placed directly one above the other. All glassware must be lead free when put into use. Hot nitric acid of about 30 volume per cent is used for rinsing, followed by distilled water.

Merck's blue label reagents are preferred. A high grade of chemical insures a low reagent blank. The special reagents required are as follows:

(1) Double distilled water produced by a Barnstead still.

(2) Grasselli C. P. reagent ammonia.

(3) Grasselli C. P. reagent nitric acid.

(4) Grasselli C. P. reagent hydrochloric acid.

(5) Special acid solution, lead free, containing 48 volume per cent of nitric acid, 4 volume per cent of hydrochloric acid and the balance as water.

The acids used for this solution must be obtained by the redistillation of reagents (3) and (4) through a silica still. The water used must be reagent (1) redistilled from a pyrex still.

(6) Sodium hydroxide solution - 25 per cent by weight. This solution is made by dissolving 10 lbs. of Merck's reagent NaOH (pellet form) in 13.65 liters of water. After standing, the clear solution is drawn off by means of a siphon.

(7) Citric acid solution - Dissolve 400 grams of citric acid C. P. (the monohydrate) in about 500 ml. of distilled water, neutralize with ammonia to pH 3.5-4.0 using 0.04 per cent bromphenol blue as an indicator, add a little copper salt, and pass in H_2S. Let stand over night and filter. Boil off every trace of H_2S and dilute to one liter with distilled water. This stock solution may be diluted ten times with distilled water such that 1 ml. = 0.04 gm. of citric acid. Add a small amount of chloroform as a preservative.

(8) Special ammonia for neutralization - Redistil reagent (2), passing the evolved gas into ice-cold redistilled water.

(9) Potassium cyanide solution - 10 per cent W/V.

(10) Merck's Reagent Chloroform.

(11) Dithizone purified. To 50 ml. of chloroform add one gram of commercial dithizone such as produced by the Eastman Kodak Company. Shake out with three consecutive 100 ml. portions of one volume per cent ammonia using redistilled, lead free ammonia. Acidify the ammonia extracts with dilute 30 volume per cent redistilled, lead free hydrochloric acid. Dissolve the precipitated dithizone with chloroform using three or four 20 ml. portions. Wash the chloroform extracts two or three times with redistilled water. Evaporate the chloroform with gentle heat and remove the last traces at 30°C. in vacuo.

(12) Dithizone solution No. 1. This solution contains 59 mg. of commercial dithizone (approximately 86 % purity) per liter of chloroform. Store in brown glass bottle. One ml. = approximately 10 gamma Pb.

(13) Dithizone solution No. 2. This solution contains 30 mgs. of reagent (11) per liter of chloroform. Store in brown glass bottle under a layer of 6 per cent sulphurous acid. One ml = approximately 20 gamma Pb.

(14) Special Dithizone Extraction Solution. Place in a large separatory funnel, one liter of an aqueous solution containing 10 ml. of reagent (9) and 10 ml. of reagent (2). Add 10 ml. of reagent (12) and shake vigorously for several minutes releasing pressure formed at the start. If the chloroform layer is red and the water layer colorless, draw off the chloroform layer and repeat the operation until the chloroform layer is either colorless or slightly green. No more dithizone should be added than is necessary to remove all of the lead. The water layer should have only a slightly brownish color, immediately after preparation, and should become colorless after standing several hours in the sunlight. The solution is then ready for use.

(15) Standard lead solution. Dissolve 0.1598 gram of lead nitrate crystals in one volume per cent nitric acid so that the final volume is one liter. Dilute this stock solution ten times with one per cent nitric acid such that 1 ml = 10 gamma Pb. The one per cent nitric acid used for this solution must contain redistilled nitric acid and redistilled water.

(16) Indicators. 0.04 per cent bromphenol blue, 0.04 per cent phenol red and 0.04 per cent meta cresol purple, each made up according to Clark and Lubs. Methyl orange aqueous, made up as a saturated solution.

(17) Copper solution. Dissolve 1 gram of electrolytic copper in minimum HNO_3 and make up to 500 ml.

Preparation of Sample

a. Urine

Transfer the complete sample to a beaker of suitable size. Add nitric acid in the proportion of 100 ml. of acid per liter of urine.

Evaporate on the hot plate to a low volume, such that transfer may be made to a 500 ml. glass dish, rinsing the beaker with a small amount of hot water. Evaporate to dryness on the hot plate, char gradually at the mouth of an open electric muffle furnace and finally ignite in a closed muffle at 450°-500°C. to a white ash. Remove from the muffle and allow to cool.

Dissolve ash by treatment with 50 volume per cent nitric acid using for final solution 25 ml. of nitric acid per liter of original urine. Stir with heat until all soluble salts dissolve.

Filter into a 600 ml. beaker through a 12.5 cm. Whatman No. 40 filter paper, rinsing the glass dish and filter paper with hot water. Dilute the filtrate to approximately 400 ml. and neutralize with sodium hydroxide solution to pH 3.4-3.5 using a few drops of aqueous methyl orange as indicator. Should the end point be overstepped, a few drops of 10 volume per cent hydrochloric acid may be added to the faintest pink coloration.

Gas with H_2S and allow to stand over night. Filter on a 12.5 cm. Whatman No. 40 filter paper, washing the precipitate with freshly prepared hydrogen sulphide water, containing 0.1 per cent of its volume of hydrochloric acid, discard the filtrate, and dissolve the precipitated sulphides from the filter paper back to the original beaker using hot (lead free) special acid solution, followed by washing with hot water. Alternate this process until the sulphides are completely dissolved. Concentrate the final solution on the hot plate to about 20 ml. thereby removing traces of hydrogen sulphide and dissolving free sulphur. Should a clear solution not be obtained it is best to repeat the gassing operation.

Transfer to a 50 ml. glass stoppered cylinder and dilute to the mark at 20°C.

b. Faeces

Transfer the sample to a weighed 500 ml. vitreosil dish, rinsing the container with hot water. Evaporate to dryness on the hot plate. Weigh and record the dry weight when constancy is obtained.

Place in the electric muffle at 450°-500°overnight and when cool weigh again, recording the ash weight.

Treat the ash with 20 ml. of 50 volume per cent nitric acid and evaporate to dryness on the hot plate. Place in the muffle and ignite to a white ash. Remove and when cool treat the ash with 50 volume per cent nitric acid using just enough to dissolve soluble salts. The ash from faeces very often contains some carbon which is very difficult to remove by ignition.

Filter into a 600 ml. beaker using the same procedure as with urine. However, due to the presence of large amounts of tricalcium phosphate present in solution, the sample must be neutralized very carefully to prevent the precipitation of abnormal amounts of this salt prior to gassing. Sodium hydroxide must be added slowly with rapid stirring until the opalescence persists. The pH is now 5.4-5.5, and the solution should be barely acid to methyl orange when tested.

Gas with H_2S, again neutralize the final filtrate, after dilution, with sodium hydroxide solution, this time using methyl orange as an indicator. Gas again and dilute the final filtrate to 25 ml. in a glass stoppered cylinder.

C. Tissues

(1) Large Tissues

Transfer the tissue to a beaker of suitable size, rinsing the container as usual with water. Treat with an adequate amount of 50 volume per cent nitric acid. Allow to stand in the open beaker overnight, then cautiously heat on the hot plate until the tissue is partially digested. If fat predominates, remove to a 500 ml. vitreosil dish, cautiously ignite in the muffle at 450°-500°C., starting the ignition at the mouth of the muffle with the door open. Concentrate the contents remaining in the beaker and transfer with rinsing into the vitreosil dish containing the ash obtained from the ignited fat. Again cautiously ignite in the muffle.

The ash now obtained generally contains traces of carbon and should be treated with a suitable amount of 50 volume per cent nitric acid. Upon evaporation to dryness and final ignition in the muffle, the ash obtained is generally white, containing only traces of carbon.

Treat the final ash with 50 volume per cent nitric acid using just enough to dissolve all soluble salts.

Filter, neutralize and gas, following the same procedure

as for faeces if the sample contains large amounts of calcium and phosphorous, otherwise follow the same procedure as used for urine.

(2) Small Tissues

Transfer the tissue to a clean vitreosil dish of suitable size, rinsing the container as usual with hot distilled water. Add nitric acid redistilled from a vitreosil still, approximately 1 ml. per gram of tissue. Heat on the hot plate until digestion is partially complete and excess moisture has been removed.

Place in mouth of open electric muffle furnace until charring is complete then ignite in the closed muffle at 450°-500°C.

Remove from furnace and when cool moisten the ash with a few drops of the redistilled nitric acid. Again cautiously heat in the mouth of the muffle and reignite to a white ash.

Remove from the muffle, allow to cool, and treat with hydrochloric acid, redistilled from a vitreosil still, using as small an amount as possible to complete solution, rinsing into a 25 ml. glass stoppered cylinder with distilled water and diluting to the mark at 20°C.

Separation of Lead

The separation of lead (and bismuth if present) from other metals is effected by extraction of the sample, prepared as described above, with dithizone solution No. 1. A proper aliquot of the entire sample must be taken to obtain an amount of lead ranging between 0 and 100 gamma. A suitable aliquot can only be ascertained by the method of trial and error.

The aliquot is removed by means of a clean government standard pipette and is transferred to the separatory funnel. Add 15 ml. of reagent (7) and 2 drops of Phenol Red indicator. Then add redistilled ammonia drop by drop, swirling the funnel for mixing, until a distinct yellow coloration develops.

Now add 2 ml. of reagent (9), follow by the addition of ammonia as before until a red color develops, indicating a pH of approximately 7.5 and then add 5 ml. of dithizone solution No.1. The stoppered funnel is shaken vigorously, releasing the pressure developed at the start. Depending upon the amount of lead present, the color of the chloroform phase will range from a brilliant red-orange, with appreciable amounts of lead, through purple to the unchanged green color of the dithizone solution. Extraction of the aqueous solution

is repeated with successive 5 ml. portions of the dithizone solution, delivering each after extraction, to another separatory funnel, until the color of a newly added portion of dithizone remains unchanged. The aqueous phase is extracted once more, as a precautionary measure, and is then discarded. The total volume of dithizone solution used is noted as it gives a preliminary indication of the amount of lead present, and is therefore of value as a guide in the final lead estimation.

In special cases such as the analysis of small tissues which does not entail a preliminary separation of lead as the sulphide, the chloroform solution of the lead-dithizone complex is first washed with 25 ml. of water containing one drop of reagent (9), quantitatively separated, and transferred to another separatory funnel. Contamination of either phase by the other is avoided by running out the chloroform layer until about 0.2 ml. remains. Small portions of chloroform are successively added and withdrawn, maintaining a chloroform trap at all times, until the separation of all chloroform-soluble components is complete.

The lead is removed from the organic complex by shaking the chloroform solution with 20 ml. of one volume per cent nitric acid; the dithizone remains in the chloroform phase, whereas the lead, in the form of the nitrate, passes into the aqueous phase.

After separation of the two phases, the aqueous phase is washed repeatedly with small portions of chloroform until a freshly added portion remains colorless. Draw off the chloroform phase completely, discarding, and adjust the aqueous phase to pH 7.5, adding special ammonium hydroxide drop by drop and using one drop of Phenol Red as an indicator. The sample is now ready for the final analysis.

Final Analysis for Lead.

A standard is prepared by introducing into a separatory funnel, 3 ml. of standard lead solution, reagent (15), and adding 17 ml. of one volume per cent nitric acid. Adjust to pH 7.5 in the usual manner. Now placed in the supporting rack are the sample and the standard. To each, add 2 ml. of reagent (9). Both solutions are now distinctly alkaline.

Add dithizone solution No. 2 from a burette in 1 ml. portions, shaking the separatory funnel after each addition, and allowing the chloroform phase to separate until a slight purplish tinge is produced. At this point dilute the chloroform phase to 20 ml. by the addition of chloroform, shake well and allow a final separation. Withdraw the chloroform phase into another separatory funnel containing 20 ml. of reagent (14). Shake well and repeat this operation four times, until the red chloroform soluble dithizone complex remains.

Treat with 10 ml. of 10 volume per cent hydrochloric acid and shake to liberate the lead in the aqueous phase as lead chloride, leaving the dithizone as chloroform soluble to be measured by colorimetry. The operation just mentioned must be carried out in the dark room. A Duboscq colorimeter is used, equipped with a suitable blue glass filter and electrical illumination. Remove moisture from the stem of the separatory funnel by means of a cotton swab. Draw off the bluish-green dithizone solution into a colorimeter cup. This applies to the standard as well as the unknown. Set the unknown at 31 and use the standard for making readings. Each division corresponds to one gamma of lead.

Removal of Bismuth Interference.

Bismuth, if present, seriously interferes, inasmuch as it reacts with the dithizone reagent under the same conditions as specified for lead. This element may be present as a result of previous medication.

Therefore it is imperative to test for the presence of bismuth. To the nitric acid extract containing lead and bismuth (if present) as the corresponding nitrates is added two drops of meta cresol purple. The solution is adjusted to pH 2.0 by the addition of special ammonia diluted to 10 volume per cent. Five ml. of dithizone solution No. 1 is added. If upon shaking the stoppered separatory there is no change in color, bismuth is not present. The chloroform dithizone containing phase is removed in the manner as described in the initial separation of lead and the final analysis for lead is continued after readjusting to pH 7.5

If bismuth is present in large amounts the concentration of dithizone solution No.1 may be increased to facilitate separation. However, the solution must not be so concentrated as to mask the color changes during extraction. A solution containing 0.20 gram of dithizone per liter will

be found suitable. This method of separation has been described by Willoughby et al (4).

Discussion

Assuming Beer's Law to hold, a separate blank determination was made consisting of all reagents used in the final determination of lead, that is, following the initial separation. This blank was found to be 1 gamma. Therefore the colorimeter must be set at 31 instead of 30. Likewise a blank run upon all reagents used in the analysis, including the initial separation and final determination showed an analysis of 3 gamma.

Therefore in calculating the final analysis it is necessary to subtract 3 gamma from the colorimeter reading obtained, multiply this result by the factor of aliquotion and subtract a reagent blank for the amount of nitric acid and sodium hydroxide solution used in preparation of the sample. Nitric acid may be analyzed spectrographically. The analysis of nitric acid is expressed as gamma Pb per liter and the analysis of sodium hydroxide as gamma Pb per 100 ml. The amount of Pb contained in all other reagents used for preparation is so small that it can be neglected, considering the sensitivity of this method of analysis.

Constant care must be exercised throughout the entire procedure to avoid lead contamination. Separatory funnel stopcocks may be lubricated with pure white vaseline. Glass stoppers may be anchored to the tops of the separatory funnels with stopper ties made of rubber, which permit of easy removal of the stoppers, but do not allow them to hang in contact with the sides of the funnels. Dithizone solution No.1 and 2 as well as chloroform may be drawn from graduated dispensing burettes, the solutions themselves being stored in brown glass bottles. Pyrex glass is not necessary for these particular containers. No lubrication of stopcocks is necessary in this instance, as the liquid furnishes its own lubrication.

A dark room has been found necessary for the final colorimetric reading, since day light, particularly when the sun is shining, often causes the dithizone to partially oxidize. Even a trace of the yellow oxidized dithizone leads to imperfect color matching, as well as incorrect colorimeter readings. When using a dark room it is possible to make eleven consecutive analyses using one standard.

Literature Cited

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