A RAPID METHOD FOR THE MICRO-ESTIMATION OF LEAD IN URINE

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A method is described for the determination of microgram quantities of lead in small volumes of urine. Recoveries from urine samples of added lead show that the mean results of triplicate analyses will, 19 times out of 20, lie within $\pm 12.5 \mu\text{g.}$ per litre of the true concentration. The determination of lead in a single specimen of urine may be carried out in three hours.

In the literature on the estimation of urinary lead, it is usually recommended that not less than 100 ml. aliquots of the urine sample be taken for analysis. This has certain disadvantages, not the least of which is the time required to ash the organic content of the urine. In addition, spot samples of urine collected from groups of workmen are often less than the 200 ml. volume required for duplicate estimations. For these reasons, a rapid and accurate method was sought for the determination of total lead in small volumes of urine. The method finally devised is based on the dithizone mono-colour method described by Fischer and Leopoldi (1940).

The organic matter in a 20 ml. sample of urine is destroyed by ashing with nitric acid and the residue is dissolved in water. Bismuth is detected and removed by washing at pH 3.0-3.4 with a solution of dithizone in chloroform which is then discarded. Cyanide solution, buffered to pH 10, and an alkaline solution of dithizone are added and lead is extracted as the dithizonate into chloroform. Excess dithizone is washed out of the organic phase with potassium cyanide solution. The optical density of the washed chloroform extract is directly proportional to the quantity of lead present.

The advantages claimed for the method are as follows:

1. The small volume of urine needed for an accurate determination considerably reduces the time required to ash the sample and hence the time taken for the determination.

2. The ashing and extraction of lead with dithizone are carried out in the same vessel, thus reducing contamination from external sources.

Apparatus

The ashing and first extraction are carried out in a 50 ml. "Pyrex" glass conical flask fitted with a B19 socket. This flask is fitted with a B19 cone carrying a 2 mm. bore stopcock and 4 cm. stem (Fig. 1).

Excess dithizone is removed in a 150 ml. "Pyrex" separating funnel.

In order to render it lead-free, all glassware is treated with hot 50% nitric acid and rinsed with distilled water followed by lead-free water. The flasks should be kept covered at all times except during acid digestion, or when additions are made. Reagents are stored in "Polythene" wash bottles from which dropwise additions may conveniently be made. Where more accurate additions are required, the reagents are best stored in "Polythene" and borosilicate glass automatic dispensers. Spectrophotometric measurements are carried out on a "Unicam" SP600 spectrophotometer using 2 cm. glass cells.

Reagents

For details of methods for the preparation of lead-free reagents see the Report of the Analytical Methods Committee (1954).

Lead-free water.—Distilled water re-distilled in an all "Pyrex" glass apparatus.

Nitric Acid 0.5N.—Dilute 31 ml. concentrated nitric acid (B.D.H., low in lead) to 1 litre with lead-free water.

Hydrochloric Acid 1N.—Dilute 86 ml. concentrated hydrochloric acid (B.D.H., low in lead) to 1 litre with lead-free water.

Ammonium Hydroxide 5N.—Dilute 355 ml. concentrated 0.880 ammonium hydroxide (B.D.H., low in lead) to 1 litre with lead-free water.

FIG. 1.—The ashing and extraction flask.

Chloroform.—Laboratory reagent chloroform stabilized by the addition of about 1% by weight of ethyl alcohol.

Dithizone Solution A.—Make up fresh each day. Dissolve 0.02 g. of pure diphenylthiocarbazone in 40 ml. of chloroform. To this solution add 4 ml. of 5N ammonium hydroxide, 36 ml. lead-free water, and shake for 30 seconds. Allow the phases to separate and reject the chloroform layer. The aqueous layer is dithizone solution A.

Dithizone Solution B.—Take 30 ml. of dithizone solution A and acidify with 1N hydrochloric acid until a permanent precipitate just forms. Add 20 ml. chloroform and shake for 30 seconds. Allow the phases to separate and reject the aqueous layer. The chloroform layer is dithizone solution B.

Ammonium Citrate Solution.—Dissolve 400 g. citric acid crystals in water and add sufficient reagent ammonia to make the solution alkaline to phenol red. Dilute the solution to 1 litre with water and make lead-free.

Potassium Cyanide 10% W/V Solution.—Dissolve 50 g. of potassium cyanide in sufficient water to make 100 ml., remove the lead content, and dilute the concentrated solution to 500 ml. with lead-free water.

Ammonium Citrate/Potassium Cyanide Solution.—Mix five parts of ammonium citrate solution with two parts of potassium cyanide solution. This solution is freshly prepared each day.

Methyl Orange Indicator Solution.—0.1% solution in lead-free water.

Phenolphthalein.—Dissolve 1 g. of phenolphthalein in 50 ml. of alcohol and add 50 ml. of lead-free water.

Antifoam.—Midland Silicone Antifoam "A"; 2% solution in carbon tetrachloride.

Standard Lead Solutions.—Dissolve 1.5984 g. of dried recrystallized lead nitrate in 1 litre of 1% nitric acid (10 ml. of nitric acid diluted to 1 litre with lead-free water).

One millilitre = 1.0 mg. Pb.

This solution keeps indefinitely in "Pyrex" glass or "Polythene" flasks.

Prepare a standard solution containing 1 µg. Pb per ml. by dilution of stock solution with 1% nitric acid. This dilute solution should be prepared as required.

Method

Acid Digestion.—Measure 20 ml. of urine into the 50 ml. flask, add two drops of Antifoam "A" and 1 ml. of concentrated nitric acid. If the urine collection contains a phosphate or carbonate deposit, add concentrated nitric acid (exactly 5% of the total urine volume), shake until the urine is clear, and withdraw 21 ml. aliquots for analysis. Insert the stopcock with the tap in the open position, and take the contents of the flask to dryness on the hot plate. Remove the flask from the hot plate in order to prevent ignition of the residue which will almost certainly produce a low result. Add 10 drops of nitric acid to the residue and again take to dryness. Repeat the operation once more so that a pure white residue is obtained. Dissolve the residue in 10 drops of acid and 10 ml. of lead-free water, bring the solution to the boil and allow to cool.

The solution should now be clear but occasionally it may be clouded due to the presence of silica which, however, does not interfere with the determination of lead.

Determination of Lead.—Add one drop of methyl orange indicator to the clear solution followed by sufficient 5N ammonia solution to produce a yellow colour. Adjust the pH of the solution to 3.0-3.4 by the addition of 0.5N nitric acid until a peach pink colour is produced. Add five drops of dithizone solution B and 5 ml. of chloroform. Shake the flask for 30 seconds, invert, and allow the contents to settle. The colour of the chloroform layer should be green showing excess dithizone, but if it is orange indicating the presence of bismuth, then add more dithizone solution B and repeat the process until the chloroform layer is coloured green. Discard the chloroform layer; this operation may be facilitated by warming the base of the flask with the hand.

Add three drops of phenolphthalein indicator to the solution followed by 1.4 ml. of ammonium citrate/potassium cyanide solution. Add 5N ammonium hydroxide until the pH of the solution reaches 10.0 as shown by the red colour of the indicator. Measure

carefully 10 ml. of chloroform into the flask followed by one drop of dithizone solution A. Shake the flask for 30 seconds and allow the layers to separate. If the chloroform layer is cherry red add another drop of dithizone solution A, shake the flask for 30 seconds, and again observe the colour of the chloroform layer. Repeat the addition of dithizone solution A until the colour of the chloroform layer is mauve, blue, or green showing the presence of excess dithizone.

Run the chloroform layer into a 150 ml. separating funnel containing 50 ml. of 0.5% potassium cyanide solution (2.5 ml. of 10% potassium cyanide solution made up to 50 ml. with lead-free water) and shake for 30 seconds. Dry the stem of the funnel with a rolled filter paper and plug lightly with cotton wool. Discard the first few drops of the chloroform layer and run the remainder of the layer into a 2 cm. spectrophotometer cell.

Measure the optical density of the chloroform solution at 520 $m\mu$ using chloroform as the reference solution. Carry out a blank determination with each series of tests.

Standard Curve.—Transfer aliquots of standard solution containing 0, 1, 2, 3, 4, and 5 micrograms of lead to 50 ml. conical flasks and make up the volumes to 20 ml. with lead-free water. Treat the solutions in exactly the same way as the urine samples for the development of colour and measure the optical density of the chloroform extracts at 520 $m\mu$. Plot a graph of optical density against lead concentration.

Results

In order to check the method, recoveries of added lead were made from urine samples. To 33 aliquot portions of the same urine collection, amounts of standard solution containing from 0 to 6 μg . lead were added accurately from a micrometer syringe. Over that part of the range in which the upper limit for normal urine is found, namely 1 to 2 μg . Pb per 20 ml. or 50 to 100 μg . Pb per litre, standard solution was added to the urine aliquots in increments of 0.1 μg . lead. The lead contents of the urine samples were then determined by the method just described. The results are shown in the form of a graph of lead added plotted against lead found (Fig. 2).

The straight line of unit slope which is the best fit to the points on the graph was calculated and the 95% confidence limits drawn. It may be seen that 32 out of the 33 lead recoveries fall within these limits. The intercept on the y axis indicates that the concentration of lead originally present in the urine amounted to 0.15 μg . per 20 ml.

FIG. 2.—Recovery of lead added to urine samples.

Confidence limits calculated for single, the mean of duplicate, and the mean of triplicate estimations show that 95% of results will fall within the range of ± 21.7 , ± 15.3 , and ± 12.5 μg . per litre, respectively, of the true concentration of urinary lead. The mean value of triplicate analyses is thus considered to be sufficiently precise for the method to be used as an aid in the diagnosis of lead absorption or intoxication.

Discussion

Stannous tin, bismuth, and thallium, as well as lead, are extracted from alkaline cyanide solution by dithizone. Although these metals are infrequently present in urine, the following precautions were taken to prevent interference: (1) the nitric acid digestion converts any stannous tin to the stannic form which, in amounts usually present in urine, does not interfere; (2) bismuth is removed by dithizone extraction at pH 3.0-3.4; (3) the 0.5% potassium cyanide solution used to wash the chloroform extract removes any thallium, along with excess dithizone, from the chloroform layer.

In order to standardize the error due to the slight solubility of chloroform in potassium cyanide

solution, the volume of the cyanide wash was carefully controlled.

Because of the minute amounts of lead involved in the estimation, the elimination of sources of contamination is of the utmost importance. This is largely achieved by the use of plastic "squeeze" wash bottles as reagent dispensers. In this way contamination from pipettes, burette taps, and flask stoppers is avoided. Moreover, a considerable saving in time is effected by the use of dispensers rather than pipettes and this, taken together with the comparatively short time required to ash a 20 ml. sample, means that triplicate analyses of a single

urine collection may be carried out in about three hours.

Parallel investigations of the mixed colour and reversion methods of lead estimation have shown that, under the conditions described above, the mono-colour method is to be preferred because of its higher sensitivity and lower variability.

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