A Simplified Method for Determination of Urinary Lead

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A modification for simplifying the quantitative determination of urinary lead by reaction with dithizone is described. The detailed procedure is presented and data on recoveries are given. The method takes less time than the original procedure with no loss in accuracy.

The inhalation of lead and its salts, in the form of fumes or dust, has long been a problem in the industrial usage of lead. Awareness of this problem has led to several methods of determining lead exposure in suspected persons (4). One method which has become widely used is the determination of urinary lead levels.

A method which is most suitable was described by Cholak et al. (2, 3), and the procedure has been used with success in this laboratory. Because of the large number of samples received by our laboratory, modifications were undertaken to perform the assays in shorter time intervals. Contamination is a major problem and all glassware and equipment which comes in contact with the sample must be scrupulously cleaned prior to use. We therefore felt that elimination of as many transfers as possible would shorten the time for the determination, lessen the possibility of loss due to handling, as well as prevent any outside contamination. This report gives details of a modification which confines the entire procedure, up to the spectrophotometric reading, to the original specimen bottle.

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Materials and Methods

Reagents

All reagents are made up in deionized distilled water using glassware which has been rinsed twice with 8% nitric acid, 3 times with deionized distilled water, and oven dried.

1. Precipitating solution To 2.5 gm. of calcium carbonate, add just sufficient hydrochloric acid to effect solution. Then add 2 gm. of diammonium phosphate and make up to 100 ml. with deionized water. If phosphate precipitates, add hydrochloric acid drop by drop until solution clears.

2. Ammonia water solution 1% (W/V)

3. Hydrochloric acid 0.65% (W/V)

4. Buffer solution To 400 gm. of citric acid, add 280 ml. of de-ionized water. Add concentrated ammonia water until solution is just alkaline to phenol red. Cool, add 20 gm. of potassium cyanide and 10 gm. of sodium sulfite and dilute to 1 L. with deionized water. Store in refrigerator and make up fresh each month.

5. Dithizone solution For 24 samples (including one blank and two standards), place 12.1 mg. dithizone in 520 ml. of ammonia water (sp. gr., 0.90). Shake well and, within 15 min., filter the dithizone solution through cotton into 260 ml. of buffer solution. Stir periodically. This solution must be made fresh prior to each use.

6. Stock lead standard Dissolve 120 mg. lead nitrate in deionized water to make 1 L. of solution. This solution contains 75 mg. Pb per liter.

7. Working standard Solution contains 150 μg. of lead per liter. One milliliter of the stock solution is diluted to 500 ml. with deionized water to give the working standard.

8. Chloroform Reagent grade.

Specimens

Urine specimens are submitted in screw-cap, wide-mouth, round, 130-ml. bottles. Caps are fitted with washable plastic liners. These bottles are washed 2-3 times with 8% nitric acid, followed by 2-3 rinses of deionized water, and dried in an oven. These bottles are supplied by the laboratory to hospitals and dispensaries requesting urinary lead determinations. The entire quantitative procedure is carried out in this specimen bottle. The patient is instructed to void directly into the bottle, taking care to avoid touching any part of the bottle or the cap, except the outside.
Procedure

1. Measure out 50 ml. of deionized water into one bottle (blank).
2. Measure out 50 ml. of working lead standard into two different bottles (standard in duplicate).
3. To each bottle, add 1 ml. of lead precipitating reagent.
4. To each bottle, add 1 ml. of concentrated ammonia water (sp. gr., 0.90) and let stand 10 min.
5. Centrifuge for 5 min. at 2000 rpm. (Centrifuge used is an International, Size 2.)
6. After centrifugation, decant supernatant liquid into 100-ml cylinders and record the volume. Discard supernatant.
7. To the precipitate add 25 ml. of 1% ammonia water solution and shake for a few seconds.
8. Centrifuge for 5 min. at 2000 rpm.
9. Decant and discard supernatant.
10. Add 25 ml. of 0.65% HCl and mix until precipitate dissolves.
11. Add 10 ml. of chloroform.
13. Centrifuge for 3 min. at 2000 rpm.
14. Draw off supernatant with suction and discard. Transfer chloroform layer to Coleman spectrophotometer cuvette (19 × 105 mm).
15. Read absorbance at 520 mµ, using Coleman spectrophotometer.
16. Calculate lead concentration using following formula:

\[
\frac{\text{O.D. unknown}}{\text{O.D. standard}} \times \frac{7.5 \text{ g.}}{\text{unknown vol.}} \times 1000 = \mu g. \text{ Pb/L.}
\]

The following pretreatment is used when the ingestion or exposure to organic lead compounds is suspected. The urine sample is first treated by the addition of 1.5 ml. of an acid solution of potassium iodide: iodine (150 mg. potassium iodide, acidify with nitric acid, 75 gm. iodine, and make up to 250 ml. with delead distilled water) for each 50 ml. of urine sample. The digestion is accomplished by heating at 80° for 20 min. After cooling, 1 ml. of sodium sulfite (20%) is added to reduce any of the unreacted iodine. The sample is then carried on as the procedure is outlined.

Results and Discussion

The outlined procedure given above has been used for several years in this laboratory. The major advantage is the confining of the entire
procedure to the original sample bottle. This eliminates the use of rather large numbers of separatory funnels, centrifuge tubes, and other glassware as recommended in the original procedure and prevents the loss of material in transfer. It does not eliminate contamination of the original urine sample, a problem which is beyond the control of the laboratory.

Figure 1 shows a typical standard curve. The method follows Beer's law from 30–150 μg. Pb per liter. Below 30 μg./L. of lead, no correlation between concentration and absorbance was obtained. However, this was not considered to be a serious objection since values below the concentration of 30 μg./L. would certainly fall within the accepted normal excretion.

Recovery experiments were performed employing normal urine samples and adding known quantities of inorganic lead. These results are recorded in Table 1. Good recoveries were obtained in the range

![Standard curve for colorimetric determination of lead.](image)

**Table 1. Recovery of Lead in Urine**

<table>
<thead>
<tr>
<th>Expected concentration (μg. Pb/L.)</th>
<th>Calculated concentration* (μg. Pb/L.)</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>22.8</td>
<td>152</td>
</tr>
<tr>
<td>30</td>
<td>49.1</td>
<td>164</td>
</tr>
<tr>
<td>60</td>
<td>60.4</td>
<td>101</td>
</tr>
<tr>
<td>90</td>
<td>103</td>
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<td>120</td>
<td>112</td>
<td>93</td>
</tr>
<tr>
<td>150</td>
<td>142</td>
<td>95</td>
</tr>
</tbody>
</table>

*Figures represent the mean of three different determinations for each level of lead added to urine.
of 60–150 μg. Pb per liter. It has been a practice of this laboratory to report the exact lead concentration of any sample containing 50 μg./L. or more. Because of the discrepancy in the literature as to the normal urinary lead content, we have assumed that any urine containing more than 50 μg. lead per liter is worthy of notation and suggest additional samples be submitted for confirmation (1).

With the above procedure, we have found it quite easy to perform the analysis of 21 unknown samples plus the one blank and two standards in a relatively short period of time. This has greatly simplified the method and resulted in a substantial saving of time, one person being able to run 20–40 samples per day.

Summary

A modification of the urinary lead procedure is presented. The entire analytical method is confined to the original sample bottle, thus eliminating excessive glassware and possible outside contamination and providing a substantial saving of time. Recovery studies indicated no loss of accuracy.

References