Determination of Mercury in Urine

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Existing methods for the determination of mercury in urine are rather time-consuming. A more rapid method has therefore been elaborated, involving a wet combustion with sulphuric acid and potassium permanganate. After reduction of the excess of permanganate with hydrogen peroxide and oxalic acid the mercury is extracted with dithizone solution at a pH value of between 0 and 1, where other metals do not interfere. The dithizone-mercury dithizonate solution formed is divided in two parts, one of which is extracted with sodium thiosulphate solution and then used as blank solution in the spectrophotometric determination of the dithizonate in the other part. The method has been used for some years as routine control for persons employed in the manufacture of methyl mercuric hydroxide and in steeping seed with mercury containing material.

The method most frequently used in Sweden for the determination of mercury in urine and biological material is that of Vesterberg and Sjöholm¹. According to this method the sample is subjected to wet combustion in an acidic solution of chlorine and, after evaporation of the chlorine, mercury is precipitated as sulphide with cadmium sulphide as collector. The mixture of sulphides is filtered off, dissolved in a solution of potassium chlorate and hydrochloric acid and reprecipitated. After treatment again with chlorine and hydrochloric acid, followed by evaporation of the chlorine, the mercury is extracted with a solution of dithizone and the excess of the reagent titrated with a silver nitrate solution. This method is time consuming and therefore not suited for routine analysis; the result of an analysis is only obtained after 4—5 days. For some years another more rapid method of analysis has been used in this laboratory with good results. It is much less laborious and the results are available after about 12 hours. In this method also the sample is subjected to wet combustion and the mercury determined by means of dithizone. The method does not contain anything essentially new but is the result of a critical review of available procedures. It has been used only for the routine control of persons, employed in the manufacture of methyl mercuric hydroxide and in steeping seed with mercury containing material. The method may possibly be useful in other cases and is published for that reason.

The determination of mercury in organic material involves mainly three problems, namely the oxidation of the organic material without the loss of mercury, reduction of the oxidising agents used and finally the actual determination of mercury. According to the proposed method the sample is oxidised in an acid potassium permanganate solution², which was found to be the most rapid method. No loss of mercury has been observed in any case with the types of mercury compounds used. For destruction of the excess permanganate many reducing agents have been proposed such as hydroxylamine and hydrazine. However when these substances are used, the mercury can not be extracted quantitatively with dithizone and the results of the analyses vary³. The best results have been obtained by reduction of the permanganate with a slight excess of hydrogen peroxide followed by the addition of a small amount of permanganate and finally decolourising with oxalic acid. Using this procedure it very occasionally happens that the dithizone is destroyed, probably by oxidation. If the sample solution after oxidation is left until the next day this phenomenon usually disappears. In order to avoid the loss of the whole analysis a test extraction is made with a small part of the sample solution with dithizone solution. If this solution turns a pale green or yellow green colour distinctly different from the usual green to olive green colour of the dithizone-dithizonate mixture or if the dithizone solution fades completely, the sample solution is boiled with some hydrogen peroxide after oxidation of the excess oxalic acid by the addition of permanganate. The oxalic acid must be destroyed to avoid the loss of mercury during boiling.

The solution of inorganic mercury thus obtained is extracted with a solution of dithizone in chloroform. To avoid interference from other metals that may be present, the extraction is carried out at a pH-value of between 0 and 1^{4,5}, which is most easily obtained by diluting the solution of the sample. The spectrophotometric determination of the mercury may be done in two ways, either by determining the decrease in the extinction of the dithizone-solution at 520 m μ , the highest absorption maximum of the dithizone, or by determining the increase of the extinction at 490 m μ , the absorption maximum of the mercury dithizonate. The latter method seems to be the best for the small quantities of mercury usually occurring in urine. The original dithizone solution may be used as reference solution. A slightly more exact result will be obtained by extracting the mercury from part of the solution to be determined, and using the dithizone solution thus obtained as a reference solution. In this way an automatic correction for the eventual fading of the dithizone will be obtained and as well for other heavy metals that may occasionally be present.

Solutions of potassium iodide⁵ or of sodium thiosulphate², both giving good results, have been suggested for the extraction of mercury.

PROCEDURE

Reagents

- 1) Concentrated sulphuric acid
- 2) Potassium permanganate
- 3) Hydrogen peroxide 15 % (1 volume hydrogen peroxide 30 % + 1 volume water).
- 4) Potassium permanganate solution in water (4 %).
- 5) Oxalic acid solution 0.5 M.

Table 1. Recovery of added amounts of mercury. A comparison of the amount (in μg) of mercury added with that found by analysis.

a) Methyl mercuric hydroxide			c) Mercuric chloride		
Added	Found	Difference	Added	Found	Difference
10	10	0	0	0	0
10	10	0	0	0	0
20	17	-3	20	19	-1
20	21	+1	30	29	-1
30	31	+1	0	0	0
30	32	+2	0	0	0
40	42	+2	0	0	0
40	42	+2	19	20	+1
50	45	-5	30	31	+1
50	45	-5	25	25	0
b) Phenyl mercuric acetate			14	13	-1
Added	Found	Difference	20	19	-1
20	23	+3	0	0	0
20	24	+4	0	0	0
			20	20	0
			10	9	-1
			10	9	-1
			15	16	+1

- 6) Hydrogen peroxide 5 % (1 volume hydrogen peroxide 30 % + 5 volumes water).
- 7) Dithizone solution (10 mg of dithizone are dissolved in chloroform and diluted to 1 000 ml).
- 8) Sulphuric acid 0.1 N.
- 9) Sodium thiosulphate solution in water (1.5 %).
- 10) Mercury standard solution I (100 μg Hg^{2+} per ml). Mercuric chloride (135.7 mg) is dissolved in water and diluted to 1 000 ml.
- 11) Mercury standard solution II (2 μg Hg^{2+} per ml). Mercury standard solution I (5.00 ml) is diluted with water to 250 ml. This must be made immediately before use.

Destruction of organic matter. Shake the sample of urine vigorously in order to disperse any sediment and pour 500 ml into a 2 000 ml flask. Add 50 ml of conc. sulphuric acid in portions and then 25 g of solid potassium permanganate and shake the flask gently a couple of times. Heat over a flame under reflux, until boiling, and continue boiling for 8 hrs. If all the potassium permanganate is consumed during this period, cool the sample to room temperature and add about 10 g of solid potassium permanganate. Continue the boiling for a total time of 8 hrs. Cool to about 20° C. Wash the condenser down into the flask with about 15 ml of dist. water. Add hydrogen peroxide, 15 % in portions of 2–3 ml, each time shaking the flask several times rather vigorously until the sample is decolourised. Add a small excess of potassium permanganate solution and decolourise again, but this time with oxalic acid solution, and then add an excess of 2–3 ml of the oxalic acid solution.

Preliminary test extraction. Measure the volume of the decolourised sample in a cylinder and then transfer 20 ml to a 50 ml separating funnel. Add 1 ml dithizone solution and shake vigorously several times. Examine the dithizone layer by a diffuse light. If the dithizone solution fades, pour the main solution back into the flask and add a small excess of potassium permanganate solution and then decolourise with hydrogen peroxide solution, 5 %. After addition of an excess (2 ml) of hydrogen peroxide, reflux the sample for an hour and it will after cooling, be ready for further treatment. If the dithizone did not fade in the preliminary test extraction, this reheating is unnecessary.

Extraction of the mercury. After dilution of the sample solution to 1.6 l, pour it into a 2 l separating funnel and add 25 ml dithizone solution with a pipette. Shake the separating funnel vigorously 100 times, and separate the chloroform layer and filter it through glass wool into a 50 ml flask. Use part of this solution as the sample for the measurement of the extinction while the rest is poured into a 100 ml separating funnel and shaken with a mixture of 50 ml 0.1 *N* sulphuric acid and 10 ml 1.5 % sodium thiosulphate. This mixture is previously saturated by shaking with some chloroform which is then carefully separated. After shaking with the thiosulphate solution the dithizone solution should have a clearly bluish green colour and after filtration through glass wool it is used as the blank solution for the absorption measurement. This is made at a wave length of 490 $m\mu$ and the value obtained is compared with a standard curve, made using solutions of known concentration. In the calculation of the quantity of mercury in the sample correction must be made for traces of mercury in the chemicals used and for the quantity of the sample consumed by the preliminary test extraction.

Standard curve. A standard curve is made by mixing 1.6 l water and 50 ml conc. sulphuric acid with different known quantities of mercury standard II. It is convenient to use 0, 5, 10, 15, 20 and 25 ml equivalent to 0, 10, 20, 30, 40 and 50 micrograms of mercury. The samples are shaken with 25 ml dithizone solution which is then treated in the same way as before, and the standard curve is made with the extinction values obtained.

Test analyses. As well as the analyses done during the elaboration of this method, check analyses have also been carried out later while the method has been in use. To urine or in some cases to water has been added known quantities of mercuric chloride, methyl mercuric hydroxide or phenyl mercuric acetate and these samples have been mixed with the routine analyses. In Table I all the samples are collected that have been analysed during one period of time. As is clear from the table the difference between the mercury added and that found amounts to only a few micrograms, a fact that must be considered satisfying.

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