A COMPARISON OF METHODS FOR THE DETERMINATION OF ARSENIC IN BIOLOGICAL MATERIALS AND IN ORGANIC COMPOUNDS [1] [2]

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The need of a reliable method for the determination of arsenic became apparent early in the study of the selenium-arsenic antagonism. This paper reviews the various arsenic methods tested in this laboratory during the past year.

Experimental and Results

The arsenic method used in this laboratory consisted of a digestion of the material in 200 ml. of a 2:1 HNO_3—H_2SO_4 mixture. The solution was heated, taking care to prevent charring by adding more HNO_3 when necessary, until a clear solution was secured plus the evolution of SO_3. The digested mixture was then treated with HBr—Br_2 mixture and distilled. The distillate was reduced with SO_2, filtered and the filtrate made to a definite volume. An aliquot was treated with HNO_3, evaporated to dryness, heated in an oven at 130° C. for 1 hour and dissolved in 25 ml. of hydrazine sulfate—ammonium molybdate solution. The maximum color developed within 30 minutes when the sample was heated in a 75—80° C. constant temperature water bath. The sample was then made up to 25 ml. with H_2O and read at 660 mu in the colorimeter.

The method is time consuming for it requires at least two days for a 12 sample series. The macroquantities of reagents are quite expensive and great care must be taken to remove all nitrates. Many steps in the procedure require special precautions to prevent contamination especially with

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iron compounds whose presence prevents color development. However, an advantage of the method is that the arsenic concentration in the unknown may be checked by a preliminary determination and a second aliquot can be used for an accurate determination.

TABLE I
Arsenic Recoveries from Liver Homogenates

	Micrograms of Arsenic
25 ug. sample	24.0
50 ug. sample	51.0

Recoveries were good as shown in Table I but the accuracy is poor below 15 micrograms per sample. The working range is from 20—75 micrograms. The interference of the selenium fixative, HgO, prevented the use of this method with the distillates from selenium samples. For these reasons other methods were attempted.

Method of Magnuson and Watson

The micro method of Magnuson and Watson (1) as modified by Maren (2) depends on distillation of pentavalent arsenic as such to give a distillate of pentavalent arsenic without the use of oxidizing agents. Since the molybdenum blue colorimetric procedure is based upon pentavalent arsenic and thus the use of nitric acid (as the oxidizing agent) is not required in this procedure, the method looked promising.

The sample is digested by a 2:1 mixture of HNO_3 and H_2SO_4 and after the digest is colorless it is heated until strong fumes of SO_3 appear. The sample is distilled in a microstill (commercially available) and the distillate is treated with the molybdate—hydrazine SO_4 mixture. The solution is then diluted to 35 ml. and read at 660 mu in the Evelyn colorimeter. After several trials a good standard curve was obtained with K=175. This agreed fairly well with the K=170 of the previous method.

The method of Sultzaberger (3) was also used. Digestion was accomplished, using a 125 ml. Erlenmeyer flask as

outlined above, and the arsenic was distilled from the same flask. In this method, arsenic trichloride is distilled in a stream of hydrogen chloride and trapped in dilute HNO_3. The distillate (or an aliquot) was evaporated to dryness and the color developed with 10 ml. of a molybdate—hydrazine SO_4 solution. A standard curve was established and used to determine the amount of arsenic in the unknown samples. Recoveries of arsenic from organic matter by two methods are compared in Table II.

TABLE II

Arsenic Recovery Experiments

Micrograms of Arsenic added	Micrograms recovered by Magnuson and Watson Method	Microgram recovered by Sultzaberger Method
2	5.5	2.1
5	4.9	5.2
5	5.1	4.6
*5	3.4	4.4
8	7.8	8.2
10	9.1	10.1
15	16.7	
20	17.5	18.4
*20	20.2	18.9
25	24.0	24.2
35	35.0	32.2
50	52.0	46.0

*Ammonium oxalate was added to the samples at the final stages of digestion to facilitate removal of oxides of nitrogen.

The Sultzaberger method, though more time consuming, has proved in this laboratory to be more reliable and sensitive than the Magnuson and Watson method.

Brief mention is made of the bromate titration method by Banks et al (4). Digestion was accomplished as above and titration with $KBrO_3$ gave excellent results on 180—200 mg. samples of neoarsphenamine. In attempting to reduce the quantities of arsenic to the 10 to 100 microgram range, the method proved so erratic that it was discarded.

It appears that extensive treatment is necessary to separate the arsenic from the interfering substances. It is especially necessary to remove any oxides of nitrogen before

the color development step. Regardless of these and other precautions, the accurate micro-determination of arsenic in biological materials is difficult to achieve.

Discussion

The literature classifies the many methods of arsenic determination into the two most commonly used—the Gutzeit test and the molybdenum blue colorimetric method. The Gutzeit method is employed by the A.O.A.C. (5) and is well presented by How (6) and Goldstone (7). The colorimetric methods of Deniges (8) was improved by Truog and Meyer (9), Zinzadze (10) and others (7) and today the methods of Sultzaberger, and Magnuson and Watson appear to be the most popular for biological materials.

Summary

Several methods for micro determination of arsenic in biological materials are presented and compared. The methods of Magnuson and Watson (as modified by Maren), and Sultzaberger appear at this time to be the better micro-methods.

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