

6. The variation in strength of reagents may give different results. Some workers in making up Froehde's reagent use 0.05 gm., others 0.1 gm. up as high as 1.0 gm. for every 10 cc. of sulphuric acid.

7. The actual amount taken of the compound being tested, the amount of the reagent applied, and the proportion of reagent to the compound in certain cases cause a variation. Occasionally where the weight of the compound is directed, it is so much larger than it is possible to get in commercial analysis, the test is of but little value.

8. The order of mixing, whether the reagent is added to the compound or the compound to the reagent, which is in excess, may cause some variation.

9. On adding a reagent the color produced may be permanent, or it may change slowly requiring several minutes or even hours, or it may change very rapidly. The amount of substance being tested will often cause a variation in the time required for the change. Different workers may catch these colors at different stages of change. In analytical work it is impossible to use the same amount of substance each time or as the original test called for.

10. Some tests require the application of heat. The degree of heat and the rapidity with which it is applied in some cases causes a variation.

Since we must depend so largely on color tests for the identification of alkaloids and some other organic compounds, it is unfortunate that so many factors must be considered. To eliminate these as far as possible I would suggest to those who report results from color reagents: That they test the reagents used; that they state the strength of the reagent; that the reagent be added to the compound being tested, or if any other order be followed, it be so stated; that the amount of substance being tested be small, approximating what might be expected in making an analytical examination; that the amount of reagent added be one or two drops from a small stirring rod or dropper, unless otherwise stated; that if heat is to be used, the mixture of substance and reagent be placed on a bath in which the water is already boiling; that the name of the manufacturer of the alkaloid be given, where there is any doubt as to its purity.

ESTIMATION OF ATOXYL.*

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Quite recently we have had occasion to examine tablets for the amount of atoxyl present. Several methods for estimating atoxyl have been proposed and in order to find out the most reliable one the following assay methods were tried.

I—Sulphurous acid method.¹

II—The method proposed by Norton and Koch for estimating arsenic in presence of organic matter in a modified form.²

III—The method of the German Pharmacopœia.

IV—Method of the German Pharmacopœia modified.³

* Read in Scientific Section A. Ph. A., San Francisco meeting.

¹ Puckner and Clark (Journ. Amer. Med. Assn. 1907, p. 1041).

² Ibid, Norton and Koch (Jour. Amer. Chem. Sec. 1905, p. 1247).

³ Rupp and Lehman (Apoth. Zeit. 1911, p. 203).

V—W. Dullière's method.⁴

VI—Bougeault's hypophosphite method.⁵

The methods are carried out as follows:

Method I—A weighed quantity of atoxyl is placed in a strong flask of about 150 cc. capacity, 50 cc. of a freshly prepared saturated solution of sulphur dioxide added, and the flask stoppered with a sound cork tied down firmly. The flask and contents are heated in a water-bath for one hour, allowed to cool, the contents transferred to an Erlenmeyer flask and evaporated on a water-bath until all sulphur dioxide is expelled. After cooling the solution is made nearly neutral with sodium hydroxide, 2 gms. sodium bicarbonate added and the arsenic titrated with N/10 iodine V.S. using starch as an indicator.

When applying the method to a sample of atoxyl the following results were obtained:

Method I—	26.58	per cent	arsenic
	22.61	“	“
	25.70	“	“
	25.60	“	“

Method II—A weighed quantity of atoxyl is placed in a Kjeldahl flask and digested with 20 cc. of concentrated sulphuric acid until clear and nearly colorless. The mixture is transferred to a flask, using about 100 cc. of water to complete the transfer, made neutral with sodium hydroxide, 2 gms. sodium bicarbonate added and the arsenic titrated with N/10 iodine V.S.

The following results were obtained:

Method II—	24.7	per cent	arsenic
	24.5	“	“

This method is rather tedious and cumbersome. It takes almost six hours to oxidize the atoxyl.

Method III—2 gm. of atoxyl is mixed in a flask with 10 cc. of sulphuric acid and one cc. of fuming nitric acid and the mixture heated to boiling and boiled for one hour. After cooling two successive portions of each 50 cc. of water are carefully added and each portion is again removed by boiling. The residue is then diluted with 10 cc. of water, allowed to cool and mixed with 2 gms. of potassium iodide and 5 cc. of water, and finally sufficient water is added to dissolve any precipitate formed. After allowing the mixtures to stand for one half hour the iodine is titrated with N/10 sodium thiosulphate solution, each cubic centimeter of the latter corresponding to .003748 gms. of arsenic.

The following results were obtained:

Method III—	27.3	per cent	arsenic
	27.18	“	“
	26.80	“	“

Method IV—2 gm. of atoxyl is mixed in a 200 cc. flask with 10 cc. of concentrated sulphuric acid and the mixture heated on a water-bath at about 70°. One gram of crystallized potassium permanganate is then added in small portion shaking well after the addition of each portion, additional permanganate being added only when the evolution of gas has ceased. To the mixture, drop by drop 5 to 10 cc. of hydrogen peroxide solution is added until the brown color has disappeared and the solution has become limpid: The liquid is diluted with

⁴W. Dullière (Journ. Pharm. et Chim. 1912, p. 567).

⁵Bougeault (Journ. pharm. et chim. 1903, p. 97 and 1907, p. 13).

20 cc. of water, boiled 10 to 15 minutes and again diluted with 50 cc. of water. After cooling 2 gms. of potassium iodide is added, the mixture allowed to stand in a well-closed flask for one hour and the liberated iodine titrated in the usual way with N/10 sodium thiosulphate solution. The results obtained were the following:

Method IV—	26.98	per cent	arsenic
	26.98	“	“
	26.98	“	“
	27.0	“	“
	27.0	“	“
	27.0	“	“

Method V—5 gm. of atoxyl is dissolved in 25 cc. of water, 20 cc. of N/10 silver nitrate solution are added and then sufficient water to make 50 cc. The liquid is mixed thoroughly and filtered. 25 cc. of the filtrate are mixed with ferric alum solution and a few drops of nitric acid and the excess of the silver nitrate is titrated back by ammonium sulphocyanide solution. The number of cubic centimeters of silver nitrate solution multiplied by .0075 would represent the amount of arsenic, if the silver atoxyl were completely insoluble in water. Since, however, this is not the case, the portion soluble in water should be taken into consideration and for this purpose .9 cc. should be added to the total amount of silver nitrate solution after subtracting the amount of sulphocyanide solution used for retitration.

The results were as follows:

Method V—	25.91	per cent	arsenic
	25.94	“	“
	27.24	“	“
	27.39	“	“
	27.39	“	“

Method VI—Atoxyl when treated with hypophosphorous acid in the cold is polymerized. This polymeric product is oxidized by iodine to amino-phenylarsenic acid. The reducing agent is obtained by dissolving 20 gms. of sodium hypophosphite in 20 cc. of water, adding to the solution 200 cc. of hydrochloric acid (1.17) and decanting the clear liquid from the separated sodium chloride. .15 to .2 gm. of atoxyl is dissolved in 1 or 2 cc. of water, the solution mixed with 15 to 20 cc. of the reducing agent and allowed to stand for 24 hours. The mixture is diluted with 15 to 20 cc. of water, the precipitate separated by filtration, washed well with diluted hydrochloric acid and is then treated for one-quarter hour with an excess of N/10 iodine solution. The excess of iodine is titrated back with N/10 sodium thiosulphate. We obtained the following results:

Method VI—	37.5	per cent	arsenic
	28.0	“	“
	17.26	“	“
	21.56	“	“

These results show that Method VI is almost worthless. When filtering and washing the yellow precipitate produced with hypophosphorous acid, at times a great amount of the precipitate goes through the filter.

We found that the modification of the method of the German Pharmacopœia works very well and gives satisfactory results.

The above figures further show that the salt used for the experiments which had been kept for about one year in a partly filled bottle, probably had lost some of its water of crystallization and contained only about two molecules of water.

Atoxyl with 3 molecules of water contains 25.59 percent of arsenic, with 2 molecules of water 27.3 percent of arsenic.

Considering the toxic character of atoxyl, great care should be taken in analyzing the product and arranging the dose according to the amount of arsenic.

It is interesting to note the varying composition of atoxyl in regard to water given in the literature. Thus Ehrlich and Bertheim⁶ report 4 molecules. Bechamp⁷ gives atoxyl with 5 molecules of water and when recrystallized from water with 6 molecules. When, however, recrystallized from an aqueous-alcoholic liquid the salt crystallized with only 2 molecules. Fournau⁸ claims that atoxyl crystallizes with 5 molecules and that it loses all the water but two molecules when exposed to the air. Moore, Nierenstein and Todd⁹ report 3 molecules of water of crystallization.

We then examined tablets of different grainage both by the method of the German Pharmacopœia and its modification and obtained the following results:

German Pharmacopœia.	German Pharmacopœia modified.
$\frac{1}{3}$ gr. 0.3114 gr. = 93.4 per cent	{ 0.323 gr. = 96.9 per cent 0.3126 " = 93.7 "
1 gr. 0.993 " = 99.3 "	{ 0.977 " = 97.9 " 1.0006 " = 100.06 "
3 gr. 2.83 " = 94.3 "	{ 2.79 " = 93 "
5 gr. 5.05 " = 101 "	{ 4.9 " = 98 " 4.99 " = 99.9 "

The calculation was based on atoxyl containing 3 molecules of water of crystallization.

Although this paper does not bring out anything new, we thought it might be interesting to compile the various methods offered for estimating atoxyl and scattered throughout the literature and give our experience with these methods. So far all experiments with estimating atoxyl in pills and tablets containing also Blaud's mass have failed, but we hope to report on a reliable method in the near future. The large amount of iron in the combination (5 grains Blaud's mass and $\frac{1}{3}$ grain atoxyl) greatly disturbs the estimation.

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DISCUSSION.

Dr. Dohme: I would like to ask whether any steps were taken to determine whether the tested atoxyl contained varying amounts of water of crystallization or not?

Dr. Engelhardt: The atoxyl which was taken for the estimation was an old sample which contained about two molecules of water of crystallization. We expect to continue the experiments with atoxyl containing varying amounts of water of crystallization in connection with the determination of atoxyl in the presence of Blaud's mass.

⁶ Berl. klin. Woch. 1901, 282.

⁷ Compt. rend. 411, 1172.

⁸ Journ. pharm. et chim. 1907, 530.

⁹ Biochem. Journ. 1907, 324.