3. From the mother liquor of the crystals was obtained a resinous substance of high toxicity.

The investigation is being continued.

The authors are indebted to Mr. Claude Huggins of Springfield, Oregon, who provided most of the rhododendron leaves for this investigation, and to Mr. L. C. Britt, Chemist of the Oregon State Board of Pharmacy, who suggested several of the procedures used.

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AN ASSAY METHOD FOR TABLETS OF BELLADONNA EXTRACT.*

BY DALE T. WILSON.

Because of manipulative difficulties, the U. S. P. XI assay method for Extract of Belladonna is not applicable to tablets containing this extract. A modification of the U. S. P. XI assay method, involving the preliminary extraction of the alkaloids according to the shake-out method of the U. S. P. X, is somewhat more workable. However, the nature of tablet mixtures is such that rather stable emulsions are formed during the shake-out, with the result that assays are tedious and time-consuming. Furthermore, the final end-point in the titration is usually masked by the presence of chlorophyl and other colored extractive matter.

In an attempt to overcome these objectionable features the author has developed a method which appears to be quite satisfactory for the estimation of total alkaloids in Tablets of Belladonna Extract. The details of the procedure are given as follows.

Weigh not less than twenty tablets and determine the average weight. Powder and weigh accurately a sample equivalent to 25 grains of belladonna extract and transfer it to an extraction thimble having approximately a length of 94 mm. and an internal diameter of 33 mm. Place the extraction thimble in a medium size Soxhlet extractor and add about 125 cc. of ether in

* Chemical Control Laboratories, Eli Lilly and Company, March 10, 1938.

which has been dissolved 5 drops of hydrochloric acid. Extract for one hour, then discard this ether extract.

Now pour directly into the thimble in the Soxhlet extractor about 100 cc. of ether, which has been previously mixed with 7 cc. of stronger ammonia water. Stir the belladonna extract in the extraction thimble so that the ammonia comes in contact with all of the alkaloid. Add about 50 cc. of ether and extract for at least four hours, then apply a test to insure complete extraction.

Transfer the ether extract to a suitable separator and rinse the Soxhlet receiving flask with two 5-cc. portions of 2 per cent sulfuric acid, adding these acid washings to the separator. Extract the ether extract in the separator with four 10-cc. portions of 2 per cent sulfuric acid and collect these acid extracts in a second separator. Add an excess of stronger ammonia water to neutralize the acid extractions in the second separator and extract with 5 to 6 portions of chloroform or until the aqueous solution shows no test for alkaloids with Mayer's reagent. Filter the chloroform extracts into a 150-cc. beaker, evaporate to dryness on a water-bath and maintain at this temperature for fifteen minutes. Dissolve the residue in chloroform, evaporate to dryness on a water-bath, and continue the heating for fifteen minutes. Repeat this treatment for a third time. Dissolve the resulting residue in chloroform, add 5 cc. of $N/50$ acid, remove the chloroform by evaporation and titrate the excess acid with fiftieth normal sodium hydroxide, using a micro burette and methyl red as the indicator.

Each cc. $N/50$ acid = 0.00578 Gm. Belladonna Alkaloids.

DATA.

The results obtained in the assay of fourteen samples taken from three lots of belladonna extract tablets are given below. Percentages of the theoretical amount found are caculated on the basis of 1.25 per cent alkaloids of belladonna in Extract of Belladonna.

TABLE I.—TABLETS OF BELLADONNA EXTRACT, 1/8 GRAIN.

Assay Method Applied.	Sample in Grains of Belladonna Extract.	Number of Cc. $N/50$ Acid Required.	Percentage of the Theoretical Amount Found.
Proposed	25 grains	3.63 cc.	103.6%
Proposed	25 grains	3.65 cc.	104.1%
Proposed	25 grains	3.65 cc.	104.1%
Modified method of the U. S. P. X and XI	30 grains	4.36 cc.	103.7%
Modified method of the U. S. P. X and XI	30 grains	4.34 cc.	103.2%

TABLE II.—TABLETS OF BELLADONNA EXTRACT, 1/4 GRAIN.

Assay Method Applied.	Sample in Grains of Belladonna Extract.	Number of Cc. $N/50$ Acid Required.	Percentage of the Theoretical Amount Found
Proposed	25 grains	3.46 cc.	98.76%
Proposed	25 grains	3.49 cc.	99.62%
Proposed	25 grains	3.47 cc.	99.04%
Modified method of the U. S. P. X and XI	40 grains	5.61 cc.	100.10%
Modified method of the U. S. P. X and XI	40 grains	5.57 cc.	99.37%

TABLE III.—DISPENSING TABLETS OF BELLADONNA EXTRACT, 1 GRAIN.

Assay Method Applied.	Sample in Grains of Belladonna Extract.	Number of Cc. $N/50$ Acid Required.	Percentage of the Theoretical Amount Found.
Proposed	25 grains	3.61 cc.	103.0%
Proposed	25 grains	3.66 cc.	104.4%
Proposed	25 grains	3.63 cc.	103.6%
Modified method of the U. S. P. X and XI	40 grains	5.77 cc.	102.9%

COMMENTS.

By reducing the size of the assay sample to correspond with 25 grains of powdered extract the filtration during extraction of the alkaloids is considerably facilitated.

The first ether extraction, which is made in an acid medium, removes all of the available ether-soluble matter from the tablet sample, including much of the chlorophyl. Unless this material is removed the color of the indicator will be so masked in the titration as to make it difficult to determine the final end-point. This discarded ether extract has been found to yield negative results in the test for alkaloids. The alkaloids are then removed by ammoniacal ether extraction until complete exhaustion of the assay sample is obtained. The tablet filler remains behind with the exhausted drug and presents no difficulty in making subsequent extractions.

A distinctive advantage in the method is found in the fact that it requires a minimum amount of working time on the part of the analyst.

CONCLUSIONS.

1. A method is proposed for the assay of Tablets of Belladonna Extract which appears to be distinctly superior to a modified procedure based upon a combination of the U. S. P. X and the U. S. P. XI assay methods for Extract of Belladonna.

2. In principle this method may be advantageously applied to the determination of mydriatic alkaloids in other related pharmaceutical products.

I wish to acknowledge my indebtedness to Edward J. Hughes for his assistance in the preparation of this paper.

NOTE ON ASSAY OF CHLOROFORM LINIMENT, UNITED STATES PHARMACOPŒIA XI.*

BY SAMUEL W. GOLDSTEIN AND WILLIAM F. REINDOLLAR.

The method adopted by the Revision Committee of the United States Pharmacopœia XI for the determination of chloroform in chloroform liniment appears to be unnecessarily complicated, and in the hands of an inexperienced operator it is doubtful whether even approximate results would be obtained. Simpler procedures have been reported by Kunke (1) and Beal and Szalkowski (2).

Moraw (3) and Willgerodt (4) found that distillation methods applied to the determination of chloroform yielded low results. Beal and Szalkowski (2) used both the distillation and pressure bottle procedures and reported slightly lower results with the distillation method.

We found that, according to results obtained using the United States Pharmacopœia XI assay procedure, many of the commercial samples tested ran below the lower limit[1] prescribed for chloroform in chloroform liniment by the United States Pharmacopœia XI; therefore the following experiments were carried out.

* From the laboratories of the Bureau of Chemistry of the State of Maryland Department of Health.

[1] Since this work was completed the lower limit for chloroform in chloroform liniment was changed in the First Supplement of the United States Pharmacopœia XI from 40 Gm. to 35 Gm. of chloroform in 100 cc. of the liniment.