

## SAMPLE 2.

Determination 1.	14.11	per cent of sulphur combined as sulphides.
Determination 2.	14.12	per cent of sulphur combined as sulphides.
Determination 3.	14.19	per cent of sulphur combined as sulphides.
Determination 4.	14.44	per cent of sulphur combined as sulphides.
Determination 5.	14.17	per cent of sulphur combined as sulphides.
Determination 6.	14.29	per cent of sulphur combined as sulphides.
Determination 7.	14.35	per cent of sulphur combined as sulphides.
Determination 8.	14.39	per cent of sulphur combined as sulphides.

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### A SOURCE OF ERROR IN THE ASSAY OF BELLADONNA PLASTER.\*

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Assays of belladonna plasters having yielded low results, samples of the same lot were submitted to me for analysis and the results showed that the material fully met the U. S. P. standard of "not less than 0.25 per cent and not more than 0.30 per cent of the alkaloids of belladonna leaves." I conducted a series of experiments to determine the cause of the low results obtained by the other analyst.

The following method for the assay of Belladonna Plaster official in U. S. P. X, page 129, was employed in making the analyses:

Introduce 10 Gm. of Belladonna Plaster into a 100-cc. flask. (If the plaster is spread on fabric, cut the portion to be assayed into strips, weigh accurately and introduce it into the flask.) Now add 50 cc. of chloroform, and shake the mixture until the plaster is dissolved. Pour the chloroform solution into a 250-cc. beaker, and wash the cloth upon which the plaster was spread with two successive portions of 25 cc. each of chloroform, adding the washings to the chloroform solution in the beaker. Then wash this cloth with 80 cc. of alcohol containing 1 cc. of ammonia T. S., and pour the washings into the chloroform solution in the beaker. Stir the mixture gently, and allow it to stand until the rubber has separated into a compact mass. Dry the cloth upon which the plaster was spread, weigh it, and subtract its weight from the first weight of the plaster. Pour the chloroform-alcohol solution into a 250-cc. separator, rinse the beaker and rubber with 10 cc. of alcohol, and add the rinsing to the separator. Completely extract the alkaloids from the chloroform-alcohol solution by shaking out repeatedly with weak sulphuric acid. Collect the acid washings in a separator, and add ammonia T. S. until the solution is decidedly alkaline to litmus paper, and completely extract the alkaloids by shaking out repeatedly with chloroform. Filter the chloroform solution through a pledget of purified cotton, evaporate it to dryness and dissolve the alkaloids from the residue in exactly 5 cc. of tenth-normal sulphuric acid and titrate the excess of acid with fiftieth-normal potassium hydroxide.

The U. S. P. on page 453, "Proximate Assays," "General Directions for Alkaloidal Assays," under the heading "Shaking Out with Acid," states that

"After extracting with the several portions of acid, test a few drops of the last portion used, for the presence of alkaloid. Extraction must be continued until not more than a very faint cloudiness results upon the addition of a drop of mercuric potassium iodide T. S. or, in the case of hydrastis or colchicum, upon the addition of a drop of iodine T. S."

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\* Presented at meeting of New York Pharmaceutical Association, June 1928.

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In the assay of belladonna plaster the sample is dissolved in 100 cc. of chloroform, the cloth washed with 80 cc. of alcohol containing 1 cc. of ammonia T. S., after which the rubber is washed with 10 cc. of alcohol making a total volume of 191 cc. of which 80 cc. or about 42 per cent is alcohol. The next step in the assay is to shake out the alkaloids from this mixture with acid, the exact wording being "completely extract the alkaloids from the chloroform-alcohol solution by shaking out repeatedly with weak sulphuric acid."

"Completely extract" means that "extraction must be continued until not more than a very faint cloudiness results upon the addition of a drop of mercuric potassium iodide T. S."

Allen (Commercial Organic Analysis, Vol. VI, Fourth Edition, 1912, page 191) says that in testing for alkaloids with Mayer's Reagent (mercuric potassium iodide T. S.) "the solution to be tested must be not more than slightly alcoholic" and "the precipitates yielded by alkaloids with Mayer's solution are more or less soluble in alcohol." Allen in the same volume on page 189 under precipitation of alkaloids with Wagner's Reagent (Iodine T. S.) says: "In alcoholic solutions the precipitates are sometimes not formed or are deposited very slowly."

Each time the chloroform-alcohol solution is shaken "with weak sulphuric acid" some alcohol is extracted and in an effort to determine to what degree the presence of alcohol interferes with test for alkaloids in the acid shake-out the following work was carried out:

About 2 cc. of the acid shake-out were placed on a watch glass, and a few drops of Mayer's Reagent added. There was neither precipitate nor turbidity indicating the absence of alkaloids. Another 2-cc. portion of this same acid shake-out was evaporated on a steam-bath until the alcohol was expelled, cooled and Mayer's Reagent added. The result was a heavy precipitate.

Another 2-cc. portion of this acid shake-out was tested with diluted Wagner's Reagent made by diluting 1 volume of Wagner's Reagent with 4 volumes of water. The reagent yielded a positive reaction.

Another 2-cc. portion of this acid shake-out evaporated on the steam-bath until the alcohol expelled was tested with diluted Wagner's Reagent. The result was a heavy precipitate.

This acid shake-out was discarded and the chloroform-alcohol mixture shaken with another portion of diluted sulphuric acid which was tested for alkaloids before and after evaporation. Shaking out with fresh portions of diluted acid was continued until the acid shake-outs were free from alkaloids, the results being as follows:

#### *Second Shake-out*

Before Evaporation of Alcohol  
Mayer's Reagent—No reaction  
Wagner's Reagent—Distinct reaction  
After Evaporation of Alcohol  
Mayer's Reagent—Heavy reaction  
Wagner's Reagent—Heavy reaction

#### *Third Shake-out*

Before Evaporation of Alcohol  
Mayer's Reagent—No reaction  
Wagner's Reagent—Faint reaction  
After Evaporation of Alcohol  
Mayer's Reagent—Pronounced reaction  
Wagner's Reagent—Pronounced reaction

#### *Fourth Shake-out*

Before Evaporation of Alcohol  
Mayer's Reagent—No reaction  
Wagner's Reagent—No reaction  
After Evaporation of Alcohol  
Mayer's Reagent—Faint reaction  
Wagner's Reagent—Distinct reaction

#### *Fifth Acid Shake-out*

Before Evaporation of Alcohol  
Mayer's Reagent—No reaction  
Wagner's Reagent—No reaction  
After Evaporation of Alcohol  
Mayer's Reagent—Very faint reaction  
Wagner's Reagent—Distinct reaction

*Sixth Acid Shake-out*

Before Evaporation of Alcohol  
 Mayer's Reagent—No reaction  
 Wagner's Reagent—No reaction  
 After Evaporation of Alcohol  
 Mayer's Reagent—No reaction  
 Wagner's Reagent—Faint reaction

*Seventh Acid Shake-out*

Before Evaporation of Alcohol  
 Mayer's Reagent—No reaction  
 Wagner's Reagent—No reaction  
 After Evaporation of Alcohol  
 Mayer's Reagent—No reaction  
 Wagner's Reagent—No reaction

These results clearly indicate that if Mayer's Reagent is applied to the acid shake-outs from the chloroform-alcohol mixture in the assay of belladonna plaster without evaporating the alcohol the absence of alkaloids is indicated, even in the first separation, shaking out with acid is discontinued while a large quantity of alkaloid is still present and as a consequence low results are obtained.

The U. S. P. Sub-Committee on Proximate Assays should include in the "General Directions for Alkaloidal Assays" a statement that in testing the acid shake-outs in the assay of belladonna plaster the alcohol should be evaporated on a steam-bath and the material cooled before testing with Mayer's Reagent.

## SUMMARY.

1. In the assay of belladonna plaster low results will be obtained if the acid shake-outs are tested for freedom from alkaloids with Mayer's Reagent unless the alcohol is evaporated.

2. It is imperative to evaporate the alcohol from these acid shake-outs before testing for alkaloids with Mayer's Reagent due to the fact that in the presence of alcohol even the first shake-out fails to yield an alkaloidal reaction thus indicating the absence of alkaloids where a large amount is present.

3. The U. S. P. Sub-Committee on Proximate Assays should incorporate a statement under "General Directions for Alkaloidal Assays" calling attention to the necessity of evaporating the alcohol from the acid shake-outs in the assay of belladonna plaster before testing for alkaloids with Mayer's Reagent.

The author takes this opportunity of acknowledging his indebtedness to his assistant, Mr. Berl S. Alstodt, for the considerable aid rendered in making many of the tests and assays.

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 THE ESTIMATION OF OIL IN EXTRACT OF LEMON.

A DISCUSSION OF THE PRECIPITATION AND POLARIMETRIC METHODS: THE INFLUENCE OF TEMPERATURE AND AGING UPON THE LATTER.\*

BY C. V. NETZ.

The regulations of the U. S. Department of Agriculture define Lemon Extract as "The flavoring extract prepared from oil of lemon, or from lemon peel, or both and containing not less than 5% by volume of oil of lemon." At the present time all lemon extracts (except terpeneless extracts) are manufactured by dissolving the oil in alcohol of sufficient strength, with the optional addition of a small amount

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\* Scientific Section, A. Ph. A., St. Louis meeting, 1927.