

*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.

of lemon peel color or other color. The U. S. P. "Tinctura Limonis" does *not* conform to these standards.

The sale of "Terpeneless" extract of lemon containing not less than 0.2% of citral by weight, is permitted by the regulations providing it is properly labeled so that in the mind of the purchaser, there is no confusion with a pure oil extract. This extract is made by dissolving citral in diluted alcohol, by shaking oil of lemon with diluted alcohol, or by dissolving terpeneless oil of lemon in diluted alcohol. Such an extract has the advantage of permanency. Terpeneless extract will not be considered in this paper.

Two methods for the estimation of the amount of oil in lemon extract are recognized by chemists, *i. e.*, the precipitation and the polarimetric. The accuracy of each under varying conditions, and especially in the hands of inexperienced analysts, has often been questioned, hence the investigations embodied in this paper. Results obtained by these methods express volume of oil in volume of extract.

#### PRECIPITATION METHOD.

The Precipitation Method involves the measurement of the volume of oil that separates from a definite volume of extract (20 cc.) upon dilution with water. The extract is measured into a Babcock milk bottle, diluted with water and centrifuged. The per cent of oil in the extract is read directly from the number of units occupied by the separated oil in the neck of the bottle, plus the factor 0.4 to correct for unseparated oil.

The object of the investigations in connection with this method centered upon two phases: First, (*a*) whether the entire length of the column of separated oil should be measured, or (*b*) the actual volume only, and secondly, the accuracy of the correction factor 0.4%.

A number of extracts of various strengths were manufactured in the laboratory and assayed for oil according to this method, with the results tabulated below. Five per cent extracts made from various oils are listed first, then extracts of various strengths made from oil No. 1-A.

TABLE I.  
5% EXTRACTS.

Oil.	Actual reading of separated oil in duplicate detns.		Per cent of oil after addition of correction factor.	
No. 1-A	4.5 units	4.6 units	4.9%	5.0%
No. 1-B	4.6 units	4.5 units	5.0%	4.9%
No. 2	4.4 units	4.6 units	4.8%	5.0%
No. 4	4.6 units	4.6 units	5.0%	5.0%
No. 6	4.7 units	4.8 units	5.1%	5.2%

TABLE II.  
EXTRACTS OF VARIOUS STRENGTHS MANUFACTURED FROM OIL NO. 1-A.

Per cent of oil used.	Actual reading of separated oil in duplicate determinations.		Per cent of oil after addition of correction factor.	
3%	2.7 units	2.7 units	3.1%	3.1%
4%	3.6 units	3.7 units	4.0%	4.1%
5%-A	4.5 units	4.6 units	4.9%	5.0%
5%-B	4.6 units	4.5 units	5.0%	4.9%
6%	5.7 units	5.6 units	6.1%	6.0%
8%	7.7 units	7.6 units	8.1%	8.0%
10%	9.8 units	9.8 units	10.2%	10.2%

The column of oil in the above assays was measured as under (a) above, *i. e.*, the height of the entire column was measured. When measured as under (b) consistently low results were obtained. Attention is also called to the necessity of using the average of two or more determinations in expressing results.

Following is the method recommended for the assay of lemon extracts by precipitation. The method is that of the A. O. A. C. (1924) with the suggested additions italicized to distinguish from the original method.

Pipette 20 cc. of the extract into a Babcock milk bottle; add 1 cc. of dilute hydrochloric acid (1-1), then 25-28 cc. of water previously warmed to 60° C.; mix; let stand in water at 60° C. for 5 minutes; centrifugalize for 5 minutes; *stand in water at 60° C. for 3 minutes and fill with warm water to bring the column of oil into the graduated neck of the bottle*; again centrifugalize for 2 minutes; place the *bottle* in water at 60° C. for a few minutes; *read the per cent of oil directly from the total length of the column of separated oil*. If the oil is present in amounts over 2%, add 0.4% to the percentage of oil noted to correct for the solubility of the oil. If less than 2% and more than 1% is present, add 0.3% for this correction.

Results obtained by following out the above instructions are reasonably accurate. It was noted that the results were the same whether the entire length of the separated oil column was used with the factor 0.4% or the actual volume of oil was considered and the correction factor 0.5% used. The former method has been recommended to avoid changing the correction factor, a figure familiar to all who have used this method any great number of times.

The Polarimetric Method is dependent upon the degree of rotation of the plane of polarized light by the oil in the extract. The A. O. A. C. method follows:

Without diluting polarize the extract at 20° C. in a 200-mm. tube. Divide the reading in degrees Ventske by 3.2; in the absence of other optically active substances (sugar), the result will be the percentage of oil by volume.

Alcohol is optically inactive, so theoretically the rotation of an extract should be proportional to the amount of oil present. The rotation of an alcoholic solution of lemon oil is actually about 92% of that calculated from the rotation of the oil. In other words, lemon oil loses about 8% in rotating power in a five per cent alcoholic solution. That this loss is fairly constant has been demonstrated by other investigators and is indicated by the table given later in this paper.

To obtain a factor that would express the relation of rotation of an extract to the per cent of oil present, and at the same time correct for the 8% loss in rotation, a number of 5% extracts of representative oils were made up and their average rotation divided by 5. The factor thus obtained and adopted by the A. O. A. C. was 3.2 for the reading in Ventske degrees or 66.6 for the reading in angular minutes. In case the extract is polarized in a Ventske instrument, the reading divided by 3.2 expresses the per cent of oil; in an instrument reading in angular degrees, the reading of the extract in minutes divided by 66.6 expresses the per cent of oil. Investigators in the past have differed as to the correct factor for this work.

Three phases of this method were investigated by the writer as follows: (a) The accuracy of the method itself and the factor 3.2, (b) the effect of temperature upon the rotation of the extract and resultant calculated per cent of oil, and (c) the effect of aging.

The five per cent extracts under work upon the Precipitation Method with additional five per cent extracts, were used for this work. The results are tabulated below:

TABLE III.

A.	B.	C.	D.	E.	F.	G.
Oil.	Extract.	Rotation of oil in 100-mm. tube at 20° C.	Calculated rotation of extract.	Rotation of extract in 100-mm. tube at 20° C.	Loss in Rotation from that calculated.	Per cent of Oil calculated, using factor 66.6.
No. 1	A	59.75°	5.97°	5.53°	7.3%	4.98%
No. 1	B	59.75°	5.97°	5.50°	7.8%	4.95%
No. 1	C	59.75°	5.97°	5.50°	7.8%	4.95%
No. 1	D	59.75°	5.97°	5.50°	7.8%	4.95%
No. 2	A	59.50°	5.95°	5.48°	7.9%	4.93%
No. 2	C	59.50°	5.95°	5.43°	8.7%	4.89%
No. 2	D	59.50°	5.95°	5.44°	8.5%	4.90%
No. 4	A	60.07°	6.00°	5.51°	8.1%	4.96%
No. 4	C	60.07°	6.00°	5.53°	7.8%	4.97%
No. 4	D	60.07°	6.00°	5.56°	7.4%	5.00%
No. 6	A	59.54°	5.95°	5.46°	8.3%	4.92%
No. 6	C	59.54°	5.95°	5.51°	7.4%	4.95%
No. 6	D	59.54°	5.95°	5.50°	7.5%	4.95%
No. 8	A	62.78°	6.27°	5.73°	8.6%	5.15%
No. 8	C	62.78°	6.27°	5.69°	9.2%	5.12%
No. 8	D	62.78°	6.27°	5.71°	8.9%	5.13%
No. 10	C	59.42°	5.94°	5.47°	7.9%	4.92%
No. 10	D	59.42°	5.94°	5.48°	7.7%	4.93%
No. 12	C	60.87°	6.08°	5.59°	8.0%	5.03%
No. 12	D	60.87°	6.08°	5.60°	7.9%	5.04%

A study of this table indicated that the average loss in rotation of the oil in the alcohol was about 8.5% (Column F). Also, that the factor 66.6 for angular minutes or 3.2 for Ventzke degrees gave authentic results.

One of the sources of error in this method is the varying rotation of various samples of lemon oil. The U. S. P. allows a variation of 7°, *i. e.*, the oil must have a rotation between 57° and 64°. Considering the loss in rotation of lemon oil in alcoholic solution as 8.5%, an extract manufactured from an oil with a rotation of 57° would contain 4.69% of oil, while an extract from an oil with a rotation 64° would contain 5.27% of oil when calculated according to this method. The majority of lemon oils have a rotation between 59° and 62°, which would give a variation in calculated per cent of oil in a 5% extract of 0.26%.

The effect of temperature upon the rotation of a 5% extract was studied. Apparatus capable of maintaining the temperature within 0.1° C. was used for this work.

TABLE IV.

Temperature.	Rotation of extract.	Per cent of oil calculated from rotation of extract using factor 66.6.
15° C.	5.64°	5.07%
16° C.	5.61°	5.04%
18° C.	5.54°	4.98%
20° C.	5.49°	4.95%
22° C.	5.48°	4.93%
24° C.	5.46°	4.91%
26° C.	5.44°	4.89%

Previous work upon a commercial sample of lemon extract using less efficient methods of temperature control gave the results tabulated below.

TABLE V.

Temperature.	Extract E.	Extract F.
17.0° C.	....	4.39%
17.8° C.	5.08%	....
18 ° C.	....	4.40%
20 ° C.	5.06%	4.37%
22 ° C.	5.01%	4.35%

Considering Table IV. There was a variation of 0.18% between the extract at 15° C. and at 26° C. and when the ordinary range of room temperature is considered (19–25°) the difference or variation is only 0.07%, a negligible error for ordinary purposes of analysis.

TABLE VI.

Extract.	Rotation April 6, 1927 at 30° C.	Rotation August 20, 1927 at 20° C.	Increase in rotation in 4½ months' period.	Per cent of oil calc. from rotation, April 6, 1927.	Per cent of oil calc. from rotation, August 20, 1927.	Increase in per cent over 4½ months' period.
1-C	5.50°	5.89°	7.0%	4.95%	5.30%	0.35%
1-D	5.50°	5.83°	6.0%	4.95%	5.24%	0.29%
2-C	5.43°	5.81°	7.0%	4.89%	5.22%	0.33%
2-D	5.44°	5.82°	7.0%	4.90%	5.23%	0.33%
4-C	5.53°	5.57°	0.7%	4.97%	5.01%	0.04%
4-D	5.56°	5.62°	1.0%	5.00%	5.05%	0.05%
6-C	5.51°	5.736°	3.9%	4.95%	5.15%	0.20%
6-D	5.50°	5.57°	0.2%	4.95%	5.02%	0.07%
8-C	5.69°	5.79°	1.8%	5.12%	5.21%	0.09%
8-D	5.71°	5.74°	0.5%	5.13%	5.16%	0.03%
10-C	5.47°	5.61°	2.5%	4.92%	5.04%	0.12%
10-D	5.48°	5.54°	1.1%	4.93%	4.98%	0.05%
12-C	5.59°	5.80°	3.7%	5.03%	5.22%	0.19%
12-D	5.60°	5.65°	0.9%	5.04%	5.08%	0.04%

Aging tends to increase the rotation of an extract. Work upon this phase has been only partially completed. A number of the 5% extracts indicated in Table III were allowed to stand in tightly-stoppered containers in a moderate light.

A perusal of this table indicates that there is a considerable increase in rotation in the case of some extracts and very little change in the case of others. At the same time, the results of this preliminary work indicate that the age of an extract affects analytical results to a varying degree. Error from this cause would appear to the casual observer to be beyond the control of the analyst or mathematician.

The following method is recommended for the estimation of oil in lemon extract by means of the polarimeter. The additions to the A. O. A. C. method are underlined:

Without diluting, polarize the *filtered* extract at *room temperature* (19° C.–24° C.) in a 200-mm. tube. Divide the readings in degrees Ventzke by 3.2 in the case of lemon extract and by 5.2 in the case of orange extract; in the absence of other optically active substances the result will be the percentage of oil by volume.

## SUMMARY.

Experimental results indicate that the precipitation method for estimation of per cent of oil in lemon extracts is accurate, providing the height of the column of oil is considered instead of the actual volume.

The polarimetric method is the less accurate of the two, due to a number of factors, *i. e.*, the variation in rotation of various lemon oils and the effect of the aging of an extract upon its rotation. In the past most of the experimental work has been performed upon extracts of recent manufacture. The present A. O. A. C. method is readily adaptable for such extracts. The fact that the rotation of extracts increases upon standing introduces a source of considerable error, and after scrutinizing the results in Table VI, one wonders if, after all, it is not advisable to increase the factor 3.2 (66.6) to make the method applicable to the older extracts.

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## THE ASSAY OF PHOSPHORIC ACID.\*

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Most methods of determining phosphoric acid gravimetrically are troublesome and time consuming, and since much manipulation is necessary it is correspondingly inexact. Perhaps the most common gravimetric analysis of phosphoric acid is carried out by precipitation as ammonium phosphomolybdate.

Baxter (*Am. Ch. Jr.*, vol. 28, p. 298) has made a thorough study of the composition of ammonium phosphomolybdate and found the precipitate quite useful and efficient when weighed directly as a means of determining phosphoric acid. Although Baxter's method has been found to give satisfactory results in my hands, and is perhaps the shortest gravimetric method developed, it is obviously quite desirable to have a reliable method of determining phosphoric acid by titration with volumetric solutions.

Of all the volumetric methods which have been developed for this assay, perhaps three have stood out as the most reliable and each of these methods experienced a vogue at different periods. Unfortunately, neither of these methods show any marked advantage over the others and seem to have superseded each other only as a matter of course in the trend of time.

First we had Pemberton's volumetric method of solution of the ammonium phosphomolybdate in standard potassium hydroxide and titration with standard acid. This was subject to the errors due to the loss of ammonia, and to the uncertainty of the end-point with phenolphthalein in the presence of ammonium salts.

Next came the method of direct neutralization with standard potassium hydroxide, which at one time was used in the U. S. P. A weighed quantity of the acid was diluted with water and sufficient pure sodium chloride was added to saturate the solution. Methyl orange was then used as the indicator, and titration with normal potassium hydroxide begun and continued until the red color changed

\* Scientific Section, A. P. H. A., St. Louis meeting, 1927.