assay method. Upon acidifying, adding potassium iodide and titrating with tenth normal sodium thiosulphate solution, the calculated amount of iodine was found to be liberated. Because of the extreme accuracy of the Thompson and Oakdale Method, the difference in results is thought to be due to some error in the U. S. P. assay method as yet unexplained.

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A COMPARATIVE STUDY OF THREE ASSAY PROCEDURES FOR CAMPHORATED TINCTURE OF OPIUM, U. S. P.*

BY A. R. BLISS, JR., E. L. DAVY, W. H. BLOME, N. T. CHAMBERLIN, R. I. GRANTHAM, R. W. MORRISON.

INTRODUCTION.

Camphorated Tincture of Opium, commonly called "*Paregoric*" (meaning "*soothing*") and originally known as "*Elixir Paregoricum*," is found in the U. S. P. X, but no assay procedure is provided in the Tenth Revision or in any previous revision. The small amount of Opium in this tincture and the presence of the other ingredients make an assay procedure for this preparation more or less involved.

Eaton (1) proposed an assay method for Camphorated Tincture of Opium which, in the hands of some workers, has given satisfactory results. This method was included in this study as "Method III."

Kippenberger (2), Warthle (3), and Puckner (4) each suggested methods of assay for this preparation which appeared to yield more or less satisfactory results as carried out by these investigators. Buchbinder (5) proposed a method in 1917 which was based on the work of the four investigators whose names have been mentioned above. This method was included also in this collaborative study as "Method I." St. John (6) elaborated a method which is adapted for small samples of this tincture. This method was studied too as "Method II."

Caines (7) suggested a colorimetric method for the determination of small amounts of morphine. Warren and McClosky (8) in commenting on this method state that, as applied to Camphorated Tincture of Opium, the morphine is obtained in comparative purity by suitable treatment, and the color produced with sulphuric acid and a saturated solution of potassium iodate is compared with a known standard under similar treatment.

The American Drug Manufacturers' Association, Subcommittee on Alkaloids and Drug Standards (9), studied the Buchbinder Method and an unpublished method devised by one of its members. In 1929 this sub-committee felt that the differences in the results obtained by four workers warranted further study. In 1930 this sub-committee reported that no further work is required on this product at this time. In view of the fact that the maximum and the minimum findings of this group were 0.055 and 0.027, one fails to understand the recommendation made by this subcommittee.

The three methods which follow were applied to portions of a very carefully prepared Camphorated Tincture of Opium, U. S. P. X.

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* Scientific Section, A. PH. A., Miami meeting, 1931.

ASSAY OF CAMPHORATED TINCTURE OF OPIUM.
(PAREGORIC.)

METHOD I.

Reagents.—Alkaline Salt Solution. Dissolve 25 Gm. of sodium hydroxide in 1000 cc. of water, saturate the solution with sodium chloride and filter.

Barium Chloride Solution.—Prepare a saturated solution of barium chloride in water.

(Adapted for large samples of tincture.)

Evaporate 200 cc. of the sample to a volume of 50 or 60 cc.; transfer the residue to a separator, rinsing the vessel in which the evaporation was made with several small portions of water. Add the rinsings to the separator. Shake out three times with chloroform, using 20 cc. each time. Collect the chloroform in another separator and wash it with 5 cc. of water. Reject the chloroform and add the wash water to the main aqueous solution. Withdraw the latter into a beaker and rinse the separator with several small portions of water, adding the rinsings to the beaker. Heat the beaker on the water-bath until the chloroform is expelled. Cool. Add 20 cc. of 10 per cent sodium hydroxide solution and rotate the beaker so as to mix the contents. Transfer the solution to a 200-cc. graduated flask containing 1 Gm. of powdered sodium chloride for every 3 cc. of the solution. Add 15 cc. of water, stopper the flask and shake gently until the salt is dissolved. Rinse the beaker with several portions of alkaline salt solution, adding the rinsings to the graduated flask. Add enough alkaline salt solution to make the volume of the contents of the flask about 175 cc. and rotate the flask gently so as to mix the contents without causing excessive frothing. Add 15 cc. of the barium chloride solution, reduce the froth by the addition of a little alcohol, and make up to volume with alkaline salt solution. Stopper the flask and shake thoroughly. Filter through a large, dry, fluted paper, rejecting the first 20 cc. of the filtrate. If the filtrate is not clear, refilter.

By means of a pipette remove 100 cc. of the filtrate (corresponding to half the volume of the sample taken) and introduce the liquid into a separator (No. 1). Add concentrated hydrochloric acid in portions—toward the end not over $^1/_2$ cc. at a time—until the solution is acid to litmus; then add stronger ammonia water in portions—toward the end not over 4 drops at a time—until the mixture is alkaline; finally add 1 cc. in excess. (It is important that the quantities of acid and ammonia be added with the precision indicated.) Immediately shake out 6 times with chloroform containing from 5 to 7 per cent of alcohol, using 30, 20, 20, 15, 15 and 15 cc. Filter each successive chloroform fraction into a separator (No. 2) through a piece of cotton wetted with chloroform and wedged into the neck of a small funnel. Discard the liquid in separator No. 1.

Add 15 cc. of alkaline salt solution to separator No. 2, shake thoroughly and withdraw the chloroform layer into a separator (No. 3). To separator No. 3 add 5 cc. of alkaline salt solution. Shake well, withdraw the chloroform layer into a beaker and add the alkaline salt layer to separator No. 2. Return the chloroform to separator No. 3 and shake with a fresh portion of 5 cc. of alkaline salt solution. Reject the chloroform layer, and keep the alkaline salt layer (in separator No. 3) for later use. Shake out the alkaline salt solution in separator No. 2 twice, using 25 cc. of chloroform each time. Collect the chloroform in separator No. 3. Shake the separator No. 3, reject the chloroform layer and add the alkaline salt layer to the main alkaline salt solution in separator No. 2.

To separator No. 2 add concentrated hydrochloric acid very carefully, adding no more than 2 or 3 drops beyond the neutral point; then add 1 cc. in excess. Add 3 cc. of water and 4 cc. of alcohol; next add concentrated ammonia carefully, adding no more than 1 or 2 drops beyond the neutral point; then add 5 drops in excess. Immediately thereafter shake out 6 times with chloroform containing from 5 to 7 per cent of alcohol, using 30, 10, 10, 5, 5 and 5 cc. Filter each successive shake-out through cotton wetted with chloroform into a beaker.

Evaporate the chloroform on the water-bath to dryness. Add 10 cc. of neutral alcohol to the residue and heat until solution has taken place. Add 1 drop of methyl red T. S. Add 0.02 *N* sulphuric acid until the solution is acid with an excess of from 2 to 5 cc. At this stage

look out for any undissolved specks. Heat again if necessary. Evaporate most of the alcohol, and cool the residue. Titrate the excess acid with 0.02 *N* sodium hydroxide which has been ascertained to be sufficiently free from carbonates to give a sharp end-point with methyl red T. S. If more than 150 mg. of morphine are indicated, repeat the analysis with a smaller quantity of the sample.

Each cc. of 0.02 *N* H_2SO_4 = 0.00607 Gm. $C_{17}H_{19}O_3N + H_2O$.

NOTE: Both in the initial and final extraction of morphine with the organic solvent, test several drops of the sixth shake-out—after evaporation—with Marquis' reagent. If necessary, repeat extraction until a negative test for morphine is obtained. In that event, however, increase the quantities and volumes of all subsequent reagents so as to maintain the proportions prescribed.

METHOD II.

(Adapted for small samples of tincture.)

To 50 (or 100) cc. of the sample add 2 cc. of approximately *N* sulphuric acid and evaporate the mixture on the steam-bath to a volume of about 10 cc. Transfer the residue to a separator (which has been tested in the centrifuge and found to show no leak when centrifuged half full of chloroform for 5 minutes) or to an infant's 8-ounce milk bottle. Wash the evaporating dish twice with approximately 0.5 *N* sulphuric acid, using 10 cc. each time and adding the washings to the separator. Add about 9 Gm. of sodium chloride and carefully neutralize the solution by adding concentrated ammonia by drops, finally adding 5 drops in excess. Add 30 cc. of a mixture consisting of 85 volumes of chloroform and 15 volumes of alcohol. Shake the mixture and centrifuge it until a clear separation is obtained. Separate the immiscible solvent (by means of a pipette in case of the milk bottle) and run it into a separator (No. 2). Repeat the extraction of the alkaline solution with successive portions of 30, 20 and 20 cc. of the solvent mixture, collecting the extracts in separator No. 2. Test a few drops of the 4th extraction for alkaloids.[1]

Add 15 cc. of alkaline salt solution to separator No. 2. Extract the morphine from the chloroform-alcohol solvent by shaking, using 3 successive portions of 15, 10 and 10 cc. of the alkaline solution, respectively, and collecting the extracts in separator No. 3. Wash the combined alkaline salt solution with 10 cc. of chloroform and discard the chloroform. Exactly neutralize the alkaline salt solution by adding hydrochloric acid drop by drop, finally adding 1 cc. in excess. Cool the solution under the faucet and shake it with 10 cc. of chloroform. Remove the chloroform to another separator (No. 4) and shake it with 5 cc. of saturated salt solution to which 3 drops of hydrochloric acid have been added. Discard the chloroform in the fourth separator and add the acid salt solution to the solution in the third separator.

Add stronger ammonia water to the third separator till the solution is just alkaline, and then add 8 drops in excess. Cool the solution under the faucet and extract the alkaloid immediately with successive portions of chloroform containing from 5 to 7 per cent of alcohol by volume. Filter each successive chloroform fraction into a beaker through a piece of cotton wetted with chloroform and wedged into the neck of a small funnel. Discard the liquid in separator No. 3.

Evaporate the chloroform on the water-bath to dryness. Add 10 cc. of neutral alcohol to the residue and heat to dissolve the alkaloids. Add 1 drop of methyl red T. S.; add 0.02 *N* sulphuric acid until the solution is acid with an excess of from 2 to 5 cc. At this stage look out for any undissolved specks. Heat again if necessary. Evaporate most of the alcohol, and cool the residue. Titrate the excess acid with 0.02 *N* sodium hydroxide which has been ascertained to be sufficiently free from carbonates to give a sharp end-point with methyl red T. S.

Each cc. of 0.02 *N* H_2SO_4 = 0.00607 Gm. $C_{17}H_{19}O_3N + H_2O$.

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[1] NOTE: Both in the initial and final extraction of morphine with organic solvent, test the residue remaining on evaporation of several drops of the 4th shake-out—after evaporation—with Marquis' reagent. If necessary repeat the extractions until a negative test is obtained. In that event, however, increase the quantities and volumes of all subsequent reagents so as to maintain the proportions prescribed.

METHOD III.

Evaporate 100 cc. of camphorated tincture of opium to 15 cc. and transfer the residue to a separator, washing the container with small portions of water and adding the washings to the separator. Shake the mixture with two portions of 15 cc. each of water-washed ether and reject the ether. Add 50 cc. of lime water to the separator and agitate thoroughly. Filter, washing the filter well with lime-water and adding the washings to the filtrate. Shake the filtrate three times with 15 cc. each of chloroform and then with 15 cc. of water-washed ether.[1] Collect the ether-chloroform in another separator and wash it with 10 cc. of lime-water. Return the lime-water to the original solution and reject the immiscible solvent. Add 20 cc. of alcohol and 30 cc. of chloroform to the solution and enough of a 1 per cent solution of ammonium chloride to free the morphine. Shake the mixture thoroughly for several minutes and draw off the chloroform layer. Test the aqueous liquid for free ammonia with wet litmus paper held in the mouth of the separator. Shake out the aqueous layer with 4 successive additional portions of 30 cc. each of a mixture of 5 cc. of alcohol and 20 cc. of chloroform. Combine the alcohol-chloroform fractions and wash the solution with 10 cc. of water, rejecting the washings. Pass the chloroform through a filter wetted with chloroform into a beaker and wash the filter with fresh chloroform.

Evaporate the chloroform on the water-bath to dryness. Add 10 cc. of neutral alcohol to the residue and heat to dissolve the alkaloids. Add 1 drop of methyl red T. S. and sufficient 0.02 *N* sulphuric acid until the solution becomes acid with an excess of from 2 to 5 cc. Heat again if necessary. Evaporate most of the alcohol, and cool the residue. Titrate the excess acid with 0.02 *N* sodium hydroxide.

Each cc. of 0.02 *N* H_2SO_4 = 0.00571 Gm. of $C_{17}H_{19}O_3N$.

COLLABORATIVE WORK.

The collaborators' reports are given in the table below.

TABLE I.—ASSAYS OF CAMPHORATED TINCTURE OF OPIUM, U. S. P.

Analyst.	Method I. Gm. per 100 cc.	Method II. Gm. per 100 cc.	Method III. Gm. per 100 cc.
E. D. Davy	0.0429	0.0460	0.0400
	0.0431	0.0453	0.0387
	0.0481	0.0456	0.0376
	Aver. 0.0447	Aver. 0.0456	Aver. 0.0388
N. T. Chamberlin	0.0454	0.0408	0.0445
	0.0446	0.0415	0.0433
	0.0432		0.0405
	Aver. 0.0444	Aver. 0.0412	Aver. 0.0426
R. I. Grantham	0.0404	0.0468	0.0341
	0.0432	0.0485	0.0308
	Aver. 0.0418	Aver. 0.0477	Aver. 0.0325
R. W. Morrison	0.0410	0.0447	0.0508
	0.0424	0.0440	0.0446
	0.0424	0.0447	0.0482
	Aver. 0.0419	Aver. 0.0445	Aver. 0.0478

[1] NOTE: Be sure to remove all uncombined alkaloids at this point; use more solvent if necessary.

A. R. Bliss	0.0433	0.0458	0.0362
	0.0412	0.0476	0.0382
	0.0428	0.0462	0.0366
	Aver. 0.0424	Aver. 0.0465	Aver. 0.0370
W. H. Blome	0.0436	0.0446	0.0450
	0.0436	0.0446	0.0450
	0.0433	0.0439	0.0444
	Aver. 0.0435	Aver. 0.0444	Aver. 0.0448

COLLABORATORS' COMMENTS.

E. D. Davy.—"I presume you also want preference if there be any, and my preference as to workability, time consumed, etc., is Method II.

"In Methods I and III the number of shakings made was double the number stated in the method and a faint test for morphine could still be gotten with Marquis' reagent. The test, however, when extraction was stopped, was so slight as to make further extractions unwarranted.

"Number 3 of Method I (see Table I) is considerably higher than the other two and can be accounted for only by the ammonia which may not have been entirely dissipated by heating, upon evaporation of the chloroform before titration."

N. T. Chamberlin.—"I am sorry to report that because of the breaking of our centrifuge the second method is somewhat curtailed. The second check of the second method was centrifugalized twice only and then shaken out. The first check was shaken out entirely. The third check was lost.

"I would like to point out the possibility of getting fair results by this method (Method II) without the use of the centrifuge."

R. I. Grantham.—"Method I is quite tedious, and the results obtained are lower than the results obtained by Method II. Method II is more expeditious and the results are higher than either of the other two methods, and appears to be satisfactory. Method III does not appear to be satisfactory because the results are quite low.

"It is noticed that in Method I and in Method II the factor for calculating the morphine is given with one molecule of water. I think the factor should be given for anhydrous morphine."

R. W. Morrison.—"Ten shakings were required before negative tests were obtained for morphine in Methods I and III.

"Some interesting gravimetric checks were obtained as follows:

Method I.	Gravimetric.	Volumetric.
A	0.0442	0.0410
B	0.0438	0.0424
C	0.0434	0.0424
	Average 0.0438	0.0419
Method II.		
A	0.0498	0.0447
B	0.0482	0.0440
C	0.0495	0.0447
	Average 0.0492	0.0445
Method III.		
A	0.0501	0.0508
B	0.0455	0.0446
C	0.0497	0.0482
	Average 0.0484	0.0478

"Several assays run according to Method II, but without the use of the centrifuge, gave results with inappreciable variations when compared with those obtained with the centrifuge."

L. F. Kebler and Co-workers (10).—"The directions should be followed to the letter and the determination should be carried out as rapidly as is consistent with careful manipulation. On no consideration should the morphine be allowed to remain in the alkaline salt solution for any considerable time, over $^1/_4$ to $^1/_2$ hour, as loss of morphine due to oxidation occurs.

"It has been found to be imperative to keep the proper ratio of NH_4OH to ammonium salts in the solution to be extracted, hence the specific directions in regard to the addition of the acid and ammonia.

"It is also imperative in the final shake-out with chloroform to shake immediately after making alkaline, for while freshly precipitated morphine is readily extracted by the solvent used, if allowed to stand it becomes crystalline and its extraction becomes very difficult.

"The procedure for estimating the morphine is somewhat involved on account of the small amount and the other ingredients contained therein. Considerable practice is also required to obtain accurate results. Experienced workers, however, obtain fairly concordant results."

W. H. Blome.—"Method I is exceedingly long and for that reason expensive. While it seems to be a very good method, it is too long and too expensive for routine industrial use. The morphine is very difficultly soluble in the chloroform-alcohol mixture, the shaking out directed in the last paragraph of the first page requiring fourteen shakings before the solution was extracted of its contained morphine. The shaking directed in the second paragraph, page 2, required eight shakings. The results are very close to the theoretical morphine content of the tincture.

"We found Method II for the assay of Paregoric to be much simpler and to require much less time than Method I.

"Method III is apparently good but takes much more time than Method II. Emulsions form which are more difficult to handle than is the case with Method II. We had to shake out twelve times instead of four times as directed in the method.

"While Method I may give excellent results, we feel it is not practical for the reason that it takes altogether too much time, and is therefore too costly and would tie up containers and mechanical apparatus too long in a manufacturing pharmacist's laboratory. We feel that Method II is as accurate, but much easier to carry out and much less expensive."

A. R. Bliss, Jr.—"In so far as the reported results are concerned, both Methods I and II yield results which are close to the theoretical amount of morphine present. The average for the six reporting collaborators by Method I is 0.0431, and by Method II, 0.0450. Method II gave higher yields than Method I in the hands of five of the six reporting workers. However, Method II is preferred because it is less involved, less tedious, more rapid, and, in addition, it yields higher values. Our experiments indicate, also, that in the hands of a careful, experienced worker the use of the centrifuge may be dispensed with.

"Method III on the whole seems to be unsatisfactory. While the lowest yield with Method I was 0.0404 and the highest 0.0481 (see Davy's comments); and with Method II the lowest was 0.0408 and the highest 0.0485; with Method III the lowest was 0.0308 and the highest 0.0508, a range of 0.02 Gm. Three of the six workers obtained their lowest yields with Method III, and two their highest."

CONCLUSIONS.

1. Method I is too long, tedious and expensive.
2. Method II is less involved, more expeditious and is less expensive.
3. Method III is the least satisfactory of the three procedures studied; yields the least concordant results.

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INCOMPATIBILITIES OF SOME IMPORTANT NEWER CHEMICALS.*

BY CHARLES F. LANWERMEYER.

In his daily compounding of prescriptions, the pharmacist is often confronted with the problem of incompatibility. The physician knows what action he needs in the particular case and prescribes the individual drugs in combination; but it is up to the pharmacist to make an elegant, chemically and physically compatible mixture. The various textbooks on pharmacy devote some space to incompatibilities and there are also books like Ruddiman's "Incompatibilities" devoted exclusively to this subject.

In this day of multifarious new chemical production, the authors of these texts would have to publish annual editions in order to keep step with the manufacturers. It was in order to fill this gap that the following work was undertaken.

This work presents the Literature References in a condensed form as well as the results obtained by the author in making mixtures such as the dispensing or manufacturing pharmacist might be asked to compound. The following chemicals, which are listed either in the U. S. P. or in the "New and Nonofficial Remedies," were used in this study: Acriflavine (Base), Acriflavine Hydrochloride, Amidopyrine, Barbital, Sodium Barbital, Benzocaine, Butesin, Butyn, Ephedrine Hydrochloride, Ephedrine Sulphate, Neonal, Phenobarbital and Procaine Hydrochloride. References to these items in the literature were examined, and combinations with other drugs were made up and allowed to age in the laboratory.

AMIDOPYRINE.

(Also known as Pyramidon and Amidozone.)

The solubility of Amidopyrine stated in the U. S. P. is 1 Gm. in 18 cc. of water. Other texts claim 1 in 11 (E) (X), 1 in 20 (Y), 1 in 9 (B). The author found that the U. S. P. solubility is correct. According to *Comptes rend.*, 1927-185, p. 284, "the difference in water solubilities was accounted for because of this often being a mixture of A and B Pyramidon."

It is incompatible with acacia producing a colored solution (R). This is due to the oxydase present, and can be prevented by heating the acacia to 85° C. which destroys the oxydase (S). A blue-violet color is produced by many oxidizing agents like ferric chloride, silver nitrate, nitric acid, spirit of nitrous ether, lead dioxide,

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* Scientific Section, A. PH. A., Miami meeting, 1931.