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A Study of the Assay of Strychnine in Tincture of Nux Vomica*

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Various assay methods for the determination of strychnine have resulted from investigations conducted with a view to improving the official processes. Such factors as the presence of fixed oil, resins and coloring matter tend to cause considerable variation in results when assaying the drug. Controversy has centered around the nitric acid method for destroying brucine. The elimination of emulsions during the extraction of the alkaloids is worthy of consideration. It has been suggested that certain agents might be used which would quanti-

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tatively precipitate one alkaloid leaving the other in solution. Our work has been carried on in an effort to devise an assay method which would be more efficient than previously offered processes.

In addition to the official methods, numerous other processes have been suggested. Some of the better known are as follows: Kolthoff and Lingane (1) employed ferrocyanide in acid medium to produce slightly soluble crystalline hydroferrocyanides with strychnine and brucine. The former crystallizes much more rapidly and is less soluble than the latter. The same investigators have used potassium dichromate in both volumetric and gravimetric determinations (2). Another method has been devised whereby the brucine content of the alkaloidal residue is determined through its methoxyl groups, and strychnine is obtained by difference (3). A dilute alcoholic solution of picrolonic acid precipitates strychnine picrolonate from dilute aqueous, alcoholic or ethereal solutions of either the free base or one of its salts (4). According to Elgazin (5), strychnine may be separated from brucine on a commercial scale by dissolving 1 part of mixed salts in 10 parts of 10 per cent NH_4Cl and treating with 10 per cent NH_4OH at 80° to 85° C. until precipitation is complete.

Numerous processes for special types of work have also been devised, such as the recently suggested electrodialysis method (6).

EXPERIMENTAL

During this study, an apparatus was devised to obviate the familiar hand process of shaking out the alkaloidal solutions, one or two at a time. An attachment has been constructed which fits the ordinary box type mechanical shaker and which holds nine separatory funnels. The speed of the shaker has been adjusted so that uniform backward and forward motion is produced. Troublesome emulsions were not encountered as readily with its use. Tests with other drugs show that the method is apparently efficient in all cases.

There is a great divergence of opinion regarding the estimation of strychnine and brucine by the various assay methods employed. In this investigation, we have compared some of the many processes to learn their relative efficiencies in determining the amount of strychnine in the tincture.

The first study was made of the B. P. 1932 method. This process uses, in addition to nitric acid, an equal volume of 5 per cent sodium nitrite solution to destroy the brucine.

The method of the U. S. P. VIII for the determination of the strychnine content of Nux Vomica employed concentrated nitric acid to destroy the brucine. The remaining liquid was then analyzed for its strychnine. In this process, the tincture was evaporated to dryness and the assay continued as though it were a powdered extract.

The fixed oil has already been indicated as a factor which causes difficulty in the assay of tincture of Nux Vomica. Accordingly, several other methods were compared in which the oil is removed, either from the tincture in manufacture or from the sample during the assay process.

The results obtained by the U. S. P. XI Interim Revision method indicated that the alkaloidal content of the preparations was only slightly affected by this method. Removal of the fixed oil gave somewhat lower results than were given by the original U. S. P. XI method. This tends to confirm the statements of Sabalitschka and Jungermann (7).

A series of assays by the U. S. P. XI process was performed on samples of the tincture from which the fixed oil had been removed by shaking with petroleum ether.

Previous experiments suggested that the oil might be more completely removed by chilling the tincture at a lower temperature than directed by the U. S. P. XI. Samples of freshly made tincture were cooled to −7° C., filtered and analyzed.

The Beal and Hamilton method (8) uses lead acetate solution as a clarifying agent to remove contaminating extractive materials.

The method of Palkin and Watkins (9) was also examined. The contaminating extractive material was precipitated in a flocculent form by evaporating the acidified tincture and redissolving the residue in water.

The use of potassium dichromate for precipitating the strychnine as advocated by Kolthoff and Lingane (2) was not satisfactory when applied to mixtures of known solutions of strychnine and brucine sulfate.

All the experiments described thus far are summarized in Table I.

It was suggested that potassium iodide T.S. might be a suitable precipitating agent for strychnine (unpublished data communicated to us by other workers). Accordingly, solutions containing 20 cc. of a mixture of equal volumes of brucine sulfate and strychnine sulfate were treated with varying amounts (5 to 20 cc.) of potassium iodide T.S. to determine its efficiency in precipitating the strychnine.

Neither at room temperature, nor at 40–45° C. is potassium iodide T.S. efficient as a precipitating agent. The results were variable even when the

same relative amounts of reagent were used. In no case was more than 15 per cent of the strychnine recovered.

Acetate and citrate buffer solutions of varying p_H (4.63 to 9.03) were used in conjunction with potassium iodide T.S. to determine if p_H affected the action of the reagent as a precipitant for strychnine. Of the 112 samples treated with the different buffer solutions and various amounts of potassium iodide T.S., all failed to show any appreciable precipitate of strychnine.

Each 100 cc. of solution contained 0.1470 Gm. of strychnine sulfate (0.1150 Gm. of strychnine) and each 100 cc. of brucine sulfate solution contained 0.1350 Gm. of that salt (0.1150 Gm. of brucine). Distilled water was used as the solvent.

Solutions of strychnine sulfate and brucine sulfate were tested separately to ascertain the amount of $N/1$ sodium hydroxide completely precipitating the alkaloid concerned. When 10 cc. of the above strychnine solution was used, it was found that from 1 to 2 cc. of the $N/1$ sodium hydroxide completely precipitated the alkaloid. However, 10 cc. of the brucine sulfate solution were not completely precipitated by 10 cc. of the alkali. On this basis, 10 cc. each of the strychnine and brucine solutions were mixed and tested with the alkali to determine how much would be required to cause the precipitation of the strychnine from the mixture.

The samples were allowed to stand for a definite period of time so that complete precipitation might

Table I.—Summary of Results of Various Assay Methods[a]

No.	B. P. 1932, Gm.	U. S. P. VIII, Gm.	U. S. P. XI, Gm.	U. S. P. XI Interim Revision, Gm.	Removal of Oil at −7° C., Gm.	Removal of Oil with Pet. Ether, Gm.	Palkin and Watkins, Gm.	Beal and Hamilton, Gm.
1	0.1353	0.1358	0.1445	0.1340	0.1227	0.1254	0.1431	0.1304
2	0.1362	0.1212	0.1450	0.1373	0.1228	0.1254	0.1469	....
3	0.1361	0.1235	0.1418	0.1340	0.1219	0.1256	0.1431	0.1248
4	0.1360	0.1254	0.1418	0.1376	0.1431	0.1260	0.1430	0.1267
	Ave.	Ave.	Ave.	Ave.	Ave.	Ave.	Ave.	Ave.
	0.1359	0.1265	0.1433	0.1357	0.1251	0.1256	0.1440	0.1273

[a] Each of the above calculations is based on 100 cc. of tincture.

Table II.—Normal Sodium Hydroxide as the Precipitating Agent of Strychnine Solutions[a]

No.	$N/1$ NaOH C. F. 0.9875 Cc.	$N/10$ H_2SO_4 C. F. 1.0084 Cc.	$N/50$ NaOH C. F. 0.9750 Cc.	Strychnine Recovered Gm.	Per Cent Recovered
1	1.00	6.00	30.00	0.0063	54.0
2	1.00	6.00	28.85	0.0077	66.6
3	1.00	6.00	29.70	0.0087	75.2
4	1.00	6.00	29.70	0.0087	75.2
5	1.25	6.00	29.85	0.0077	66.6
6	1.25	6.00	29.75	0.0083	72.4
7	1.25	6.00	29.50	0.0099	86.5
8	1.25	6.00	29.75	0.0083	72.4
9	1.50	6.00	29.90	0.0074	63.9
10	1.50	6.00	29.40	0.0106	92.1
11	1.50	6.00	29.40	0.0106	92.1
12	1.50	6.00	29.50	0.0099	86.5
13	1.75	6.00	29.40	0.0106	92.1
14	1.75	6.00	29.30	0.0115	100.0
15	1.75	6.00	29.40	0.0106	92.1
16	1.75	6.00	29.35	0.0107	92.3

[a] Each sample consisted of 10 cc. of strychnine sulfate solution containing 0.0147 Gm. of strychnine sulfate (0.0115 Gm. strychnine). All analyses were made at room temperature. The solutions were allowed to stand four hours before filtering

Table III.—Normal Sodium Hydroxide as the Precipitating Agent on Mixed Solutions of Strychnine and Brucine[a]

No.	$N/1$ NaOH C. F. 0.9875 Cc.	$N/10$ H_2SO_4 C. F. 1.0084 Cc.	$N/50$ NaOH C. F. 1.036 Cc.	Strychnine Recovered Gm.	Per Cent Recovered
1	4.25	6.00	27.60	0.0111	96.3
2	4.25	6.00	27.59	0.0112	96.9
3	4.25	6.00	27.61	0.0110	95.7
4	4.25	6.00	27.60	0.0111	96.3
5	4.50	6.00	27.60	0.0111	96.3
6	4.50	6.00	27.60	0.0111	96.3
7	4.50	6.00	27.61	0.0110	95.7
8	4.50	6.00	27.61	0.0110	95.7

[a] Each sample contained 20 cc. of a mixture of equal volumes of strychnine sulfate and brucine sulfate solutions. Each contained 0.0147 Gm. of strychnine sulfate (0.0115 Gm. strychnine) and 0.01350 Gm. brucine sulfate (0.0115 Gm. brucine). The solutions were allowed to stand 36 hours at 40–45° C. before filtering.

Our studies eventually led us to test the possibility of utilizing precipitating agents which would take advantage of the different dissociation constants of strychnine and brucine. The difference in these constants is large enough to make it seem reasonable to assume that these two alkaloids can be separated from each other by means of alkali.

Known amounts of strychnine sulfate and brucine sulfate were weighed into separate volumetric flasks.

take place. The precipitate was washed onto a quantitative filter paper. Part of the filtrate was used to repeatedly wash it and thus prevent any loss of alkaloid. After continuing the above for two or three operations, distilled water was used to wash the product until the filtrate gave a negative test for brucine. To the filter paper and precipitate placed in a suitable flask were added 6 cc. of $N/10$ sulfuric acid and 20 cc. of neutral distilled water. The contents of the flask were warmed on a steam bath until the alkaloid was completely dissolved. The solution was cooled to room temperature and titrated with $N/50$ sodium hydroxide. Tables II and III show the results obtained in these experiments.

Because of the difference in the p_H values of known solutions of brucine and strychnine sulfate, it was thought that certain indicators might render possible the differential titration of the two alkaloids. Bromcresol purple, chlorphenol red and para nitrophenol were the indicators tried. The results obtained were entirely unsatisfactory. The reason is evident from Fig. 1, as indicated below.

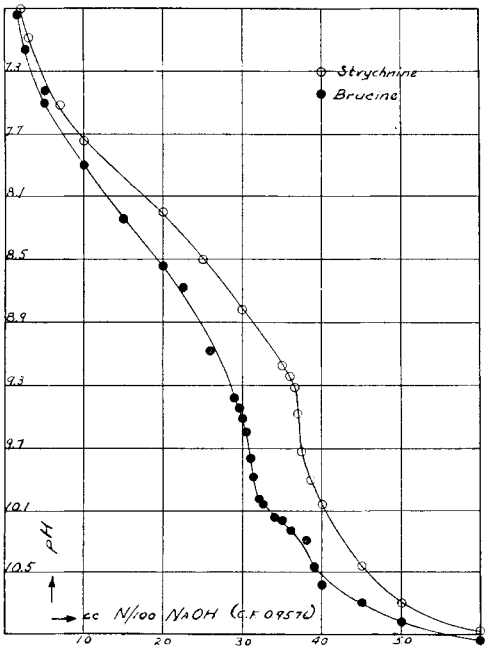

Fig. 1.—Approximate Titration Curves of Brucine and Strychnine Sulfates.

As the use of the indicators tried was not successful, the approximate titration curves of the known solutions of brucine and strychnine sulfates were determined. A Beckman p_H meter was used for the experiments. The results are shown in Fig. 1. These curves show a maximum deviation per unit of added alkali within approximately the same p_H range. This accounts for the fact that indicators cannot be used to determine separately these two alkaloids. The same statement would apply to buffer solutions.

SUMMARY AND CONCLUSIONS

1. The special shaking apparatus devised for the studies decreased the time necessary for a series of assays. Other studies not described in this paper indicate that it may be universally used in place of the usual hand shaking-out process in alkaloidal analysis.

2. The B. P. method needs little comment other than emphasizing the necessity of carrying out the method strictly in detail. Complete destruction of brucine was obtained at ordinary temperatures with a small loss of strychnine. The error caused is taken into account by the correction factor 1.02.

3. The U. S. P. VIII method appears to yield only approximate results. It uses only nitric acid to destroy the brucine. The following possible reasons are suggested in explanation of the greater efficiency of the B. P. method over the U. S. P. VIII: (*a*) The tincture should not be evaporated to complete dryness; our experience shows that this is definitely a factor in the analysis. (*b*) The B. P. method for the destruction of brucine allows adequate time for the reaction to be complete, whereas the U. S. P. VIII procedure apparently does not.

4. The fixed oil which occurs in tincture of Nux Vomica seems to be a very troublesome factor in the U. S. P. XI method. In some of the samples, there appeared to be more oil present than in others. No explanation of this can be offered. Shaking out the combined chloroform solutions with dilute sulfuric acid as per the U. S. P. XI Interim Revision method is a decided improvement over the original U. S. P. XI process.

5. Cooling to $-7°$ C. slightly decreased both the oil and the alkaloids in the tincture.

6. The method of Beal and Hamilton (8) was difficult to perform. The removal of the fine precipitate of lead sulfide required much time and probably occluded some of the alkaloid, accounting for the decreased amount recovered.

7. The procedure of Palkin and Watkins (9) was very satisfactory and easily performed. The contaminating extractive ma-

terial was precipitated by evaporating the acidified tincture and redissolving the residue in water. The results obtained by this assay were slightly higher than by other methods, due probably to the shorter procedure.

8. The use of potassium dichromate as a precipitating agent as suggested by Kolthoff and Lingane (2) was satisfactory for solutions of either strychnine or brucine salts alone, but not for mixtures of the two. Because of this nothing was done to adapt it to the tincture of Nux Vomica.

9. Potassium iodide T.S., with or without the buffer solutions tried, is of no value as a quantitative precipitant for strychnine.

10. Quantitative precipitation of strychnine from solutions of its salts, and from mixtures of strychnine and brucine salts, by the use of normal sodium hydroxide was reasonably efficient. The discrepancies in percentage recovery of strychnine would probably become less with continued work in handling such small amounts of material.

It is suggested that the principle utilized may be employed to good advantage with other alkaloidal drugs in which there exists a difficult problem of separation of two similar components. Elgazin (5) and Baker and Jordan (10) have already suggested applications of this type.

11. A solution containing 0.1470 Gm. of strychnine sulfate in 100 cc. (0.1150 Gm. of strychnine) had a determined p_H of 5.79 using a Beckman p_H meter. A brucine sulfate solution containing 0.1350 Gm. in 100 cc. (0.1150 Gm. of brucine) had a p_H value of 6.1. The use of chlorphenol red, bromcresol purple and para nitrophenol as differential indicators proved unsuccessful.

12. The approximate titration curves of brucine and strychnine were determined. A study of the curves shows why the indicators were unsuccessful, and also why buffers cannot be used.

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Hydrogenated Oil as an Ointment Base. III. Potassium Iodide Ointment*

By George W. Fiero†

Potassium Iodide Ointment of the N. F. V was prepared with benzoinated lard as a base. Even though the ointment contained one per cent of sodium thiosulfate, it soon developed a yellow color due to oxidation of potassium iodide to form free iodine. This is due to rancidity of the lard. In rancidity peroxides are formed; one test for rancidity is to shake the molten fat with potassium iodide solution—the liberation of free iodine being an indication of rancidity. Obviously, a potassium iodide ointment must be prepared with a fat which will not readily rancidify. The product of the N. F. VI overcomes this through the use of lanolin and petrolatum. The former may be allergic to some skins; the latter is said to be nonabsorbent.

Hydrogenation of oils, by reducing those fatty glycerides most susceptible to oxidation, results in a product which is less susceptible to rancidity. These products, unlike petrolatum and wool fat, are true fats and by proper degree of hydrogenation may be obtained with almost any desired melting point. The hydrogenated oils used in these

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