THE ASSAY OF NUX VOMICA BY A METHOXY-DIFFERENCE METHOD.*

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INTRODUCTION.

The value of Nux Vomica and its preparations as therapeutic agents is well known; they have been and still are recognized in many pharmacopœias including the United States Pharmacopœia.[4] The primary active constituent is Strychnine and efforts have been made to evaluate the drug in terms of this alkaloid. The proposed assay methods, however, have been found to be unreliable due to the interference of another alkaloid, Brucine, which is present in this drug and which has properties quite similar to Strychnine. The United States Pharmacopœia X, recognizes the fallibilities of these proposed methods by stating an assay requirement of not less than 2.5% *total* alkaloids in the place of a Strychnine requirement.

A study of the proposed methods shows that they may be classified as follows: (I) *Gravimetric Methods;* (II) Gravimetric Methods Involving Precipitation; (III) Volumetric Methods with or without Precipitation; (IV) Oxidation Methods Involving I, II, III or Colorimetry.

I. Gravimetric Methods.—Dunstan and Short,[5] Lyons,[6,7] and Farr and Wright[8] have devised methods involving the extraction of the alkaloids and weighing the alkaloidal residues.

II. Gravimetric Methods Involving Precipitation.—Of these methods, that involving the precipitation of the alkaloids as Ferrocyanides and effecting a separation by means of a difference in solubilities of the alkaloidal salts thus formed, is the oldest procedure. Such a method was first proposed by Dunstan and Short,[9] and was modified in turn by Lyons,[10] Holst and Beckurts,[11] Gordin and Prescott,[12] Bird,[13] and Linton.[14] All of these methods are time consuming and have been found to give inaccurate results since an accurate separation of the alkaloids cannot be effected.

In 1893, Nagelvoort[15] proposed a method involving the precipitation of the

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[4] United States Pharmacopœia, Tenth Edition, pages 148, 246 and 400.

[5] W. R. Dunstan and F. W. Short, "Yearbook of Pharm." (1883), 475–477.

[6] A. B. Lyons, "Manual of Pharm. Assaying," Haynes and Co., Detroit (1886), page 20.

[7] A. B. Lyons, *Pharm. Rev.*, 20 (1902), 250; *J. pharm. chim.* (6), 16, 139; "Yearbook of Pharm." (1903), 61.

[8] E. H. Farr and R. Wright, *Ibid.* (1906), 226; PROC. A. PH. A., 55, 933.

[9] W. R. Dunstan and F. W. Short, "Yearbook of Pharm." (1883), 469–475.

[10] A. B. Lyons, *Drugg. Circ.*, 30 (1886), 137.

[11] Holst and Beckurts, PROC. A. PH. A., 35 (1887), 524; *Drugg. Circ.*, 31 (1887), 104.

[12] H. M. Gordin and A. B. Prescott, *Am. J. Pharm.*, 71 (1899), 14.

[13] F. C. J. Bird, *Pharm. J.*, 65 (Ser. 4, Vol. XI) (1900), 574.

[14] W. H. Linton, *Ibid.*, 75 (1905), 864.

[15] J. B. Nagelvoort, PROC. A. PH. A., 41 (1893), 165.

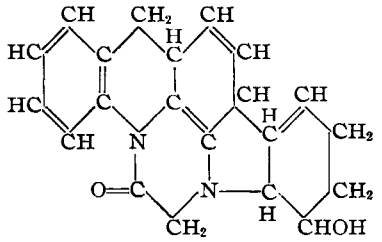
alkaloids as the picrates and in 1928, Stuber and Kejatschkina[1] developed a procedure precipitating them as the silicotungstates.

III. Volumetric Methods with or without Precipitation.—Dragondorff in his early work "Die Chemische Wertbestimmung" outlined a procedure using Potassium Mercuric Iodide as the precipitating agent and as a volumetric solution. Methods along this same line of attack were proposed or modified by Schweissinger,[2] Lyons,[3] Gordin and Prescott,[4] and Dulfilho.[5]

IV. Oxidation Methods.—These methods depend upon the fact that Brucine is more liable to oxidation than Strychnine and by subjecting the residue of mixed alkaloids to oxidizing agents, the former is destroyed or converted in such a manner that the latter may be estimated gravimetrically, volumetrically or colorimetrically. These methods involve the use of nitric acid of varying concentrations or potassium chlorate. Gerock[6] as early as 1889 proposed such a method but it received little consideration until Keller's publication[7] was issued in 1893. Keller's method has been modified or studied by Stoeder,[8] Dowzard,[9] Gordin,[10] Smith,[11] Howard,[12] Reynolds and Sutcliffe,[13] Webster and Pursel,[14] Dott,[15] Heck[16] and Dufhilo.[17] The Nitric Acid-Oxidation Method was official in U. S. P. VIII (page 299) but apparently gave unsatisfactory results and was omitted from the following editions and is now receiving careful consideration by Sub-Committee No. 6 of the Revision Committee of U. S. P. XI.

PURPOSE OF THE INVESTIGATION.

Strychnine (mol. wt. 334.30) has the following formula:



[1] E. Stuber and B. Kejatschkina, *Arch. Pharm.*, 266 (1928), 33; *C. A.*, 22 (1928), 1213.

[2] O. Schweissinger, *Arch. Pharm.* (3), 23 (1885), 529; *Amer. Drugg.*, 14 (1885), 230.

[3] See references 6 and 7, page 298.

[4] H. M. Gordin and A. B. Prescott, *J. Am. Chem. Soc.*, 20 (1898), 706; *Am. J. Pharm.*, 71 (1899), 14. [5] M. E. Dulfilho, *Bull. soc. pharm., Bordeaux*, 66 (1928), 133; YEARBOOK A. PH. A., 17 (1928), 115.

[6] I. E. Gerock, *Arch. Pharm.* (Feb. 1889), 158; PROC. A. PH. A., 37 (1889), 702.

[7] C. Keller, *Z. Oest. Apoth. Ver.* (1893), 587; *Apoth. Ztg.*, 8 (1893), 542; *Z. angew. Chem.*, 35 (1893), 391.

[8] W. Stoeder, *Nederl. Tydsch. Pharm.*, 11, 1899; *Chem. Centbl.* (1889), 506.

[9] E. Dowzard, *Chem. News*, 86 (1902), 292. [10] H. M. Gordin, PROC. A. PH. A., 50 (1902), 336.

[11] F. J. Smith, *Am. J. Pharm.*, 75 (1903), 253. [12] D. L. Howard, *Analyst*, 30 (1905), 261.

[13] W. C. Reynolds and R. Sutcliffe, *J. Soc. Chem. Ind.*, 25 (1906), 512; "Yearbook of Pharm." (1906), 16; *Pharm. J.*, 76 (1906), 555; *Analyst*, 31 (1906), 303.

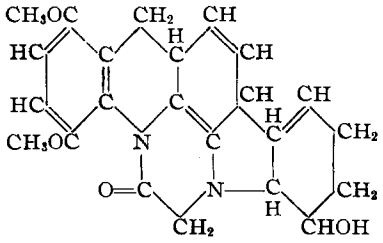
[14] M. H. Webster and R. C. Pursel, *Am. J. Pharm.*, 79 (1907), 1.

[15] D. B. Dott, "Yearbook of Pharm." (1914), 331; YEARBOOK A. PH. A., 3 (1914), 559.

[16] Heck, *Pharm. Ztg.*, 69 (1924), 240; YEARBOOK A. PH. A., 13 (1924), 451.

[17] E. Dufhilo, *Bull. soc. pharm., Bordeaux*, 65 (1927), 7; YEARBOOK A. PH. A., 16 (1927), 564.

and Brucine (394.346):



It will be observed that Brucine differs from Strychnine in chemical composition by two Methoxy groups, (CH_3O) which have been substituted for two hydrogens in the Strychnine molecule. A study of the literature revealed that no method has been proposed for the determination of Brucine in Nux Vomica by ascertaining the Methoxy value of the alkaloidal residues obtained upon the extraction of the crude drug or preparations of the drug and subsequently the amount of Strychnine by difference. The purpose of this investigation, therefore, was to study the possibilities of such a procedure.

As early as 1885, S. Zeisel[1] proposed a method of differentiating essential oils by estimating the proportion of Methoxy or equivalent alkyl radicals by treating with hydriodic acid. The substances are heated with concentrated HI and the liberated alkyl halide is absorbed in an alcoholic solution of silver nitrate according to the following reaction:

$$RI + AgNO_3 = AgI + RNO_3$$

From the weight of the silver iodide produced the amount of the alkyl radical may be calculated as follows:

$$\text{Wt. of AgI} \times 0.132 = \text{wt. of } CH_3O$$

Zeisel's original method was found to give inaccurate results chiefly because the acid might contain sulphur and phosphorous compounds and there is danger of the acid distilling over into the silver nitrate solution. Accordingly the method has been modified by Benedict and Grüssner,[2] Herzig,[3] Gregor,[4] Ehmann,[5] Hewett and Moore,[6] Perkin,[7] Kaufler[8] and Kropheschek.[9]

Instead of weighing the silver iodide formed, Gregor[4] suggested the absorption of the alkyl halide in a solution of known silver content and determining the excess silver in the solution after the silver halide was filtered off and washed. A 0.1 *N* alcoholic solution of silver nitrate is prepared by dissolving approximately 17 Gm. of the pure salt in thirty cubic centimeters of distilled water and then diluting to

[1] S. Zeisel, *Monatsh.*, 6 (1885), 989.
[2] Benedict and Grüssner, *Chem. Ztg.*, 13 (1889), 872.
[3] J. Herzig, *Monatsh.*, 9 (1888), 544.
[4] G. Gregor, *J. Soc. Chem. Ind.*, 17 (1898), 609.
[5] L. Ehmann, *Chem. Ztg.*, 14 (1890), 1767.
[6] J. T. Hewett and T. S. Moore, "Proc. Chem. Soc.," 8, 101; *Analyst*, 27 (1902), 126.
[7] W. H. Perkin, *J. Chem. Soc.*, (T) 83 (1903), 1367.
[8] F. Kaufler, *Monatsh. f. Chem.*, 22 (1901), 1105; *Analyst*, 27 (1902), 126.
[9] W. Kropheschek, *Monatsh. f. Chem.*, 25 (1904), 583.

a liter with absolute aldehyde-free alcohol. The solution is finally standardized against 0.1 *N* potassium thiocyanate after acidifying slightly with nitric acid.

EXPERIMENTAL WORK.[1]

After conducting a large number of preliminary experiments the following method was devised which seems to have definite possibilities:

A. Extraction of the Drug.—The drug in No. 20 powder was extracted by the following methods:

1. As directed in the U. S. P. X, page 247.

2. Procedure as described in *Bulletin 23*, page 81 of Sub-Committee No. 6 of the Committee of Revision of the Pharmacopœia: "Introduce 15 Gm. of Nux Vomica in No. 20 powder, into a 250-cc. Erlenmeyer flask. Add 150 cc. of a mixture of 2 volumes of ether and 1 volume of chloroform, agitate and allow to stand 2 minutes, then add 10 cc. of Stronger Ammonia Water and agitate thoroughly. Stopper the flask securely and shake frequently, but gently, during one hour and let stand 12 hours or over night in a cool place. At the expiration of this period, shake the flask gently for 15 minutes, and then let stand to allow the mixture to separate. Decant 100 cc. of the liquid (representing 10 Gm. of Nux Vomica), into a 100-cc. measuring cylinder and transfer it to separatory funnel, rinsing out the cylinder with a little chloroform and adding the rinsings to the funnel. Add 40 cc. of approximately normal sulphuric acid to the separatory funnel and shake the mixture gently for 5 minutes, then allow the liquids to separate and draw off the acid layer into another separatory funnel. Repeat the shaking out with successive portions of 25 and 15 cc. of acid of the same strength. To make sure that all of the alkaloid is extracted, it is well to shake a fourth time with 5 cc. of acid and test for complete extraction of the alkaloid by shaking 0.5 cc. of this acid solution with Mayer's Reagent. If it still shows alkaloids, shake again with 5-cc. portions of acid until all of the alkaloids are removed. To the combined acid solutions in the separator, add a small piece of red litmus paper and 50 cc. of chloroform, and then follow with sufficient ammonia water to make the aqueous layer alkaline, after gentle shaking, then add 2 or 3 cc. more of the ammonia. Shake thoroughly but gently for 10 minutes and allow the liquids to separate. Draw off the chloroform into an Erlenmeyer flask of 200–250 cc. capacity, and repeat shaking out with 30- and then with 15-cc. portions of chloroform until all of the alkaloid is extracted. Test for completeness of extraction by evaporating 0.5 cc. of the chloroform solution to dryness. Warm the residue with 2 drops of diluted hydrochloric acid and 1 cc. of water, transfer to a test-tube and add a few drops of Mayer's Reagent. Not more than a slight turbidity should be produced."

3. Introduce 15 Gm. of Nux Vomica in No. 20 powder into a Palkins-Watkins Automatic Extractor, Type S,[2] pack firmly and moisten throughout with chloroform. Carefully add a mixture of 20 cc. of ether, 10 cc. of alcohol and 10 cc. of stronger ammonia water, stopper well and allow to macerate over night. Extract for 3–4 hours or until the extraction liquid is no longer colored. Transfer the chloroformic liquid to a separatory funnel, add 40 cc. of approximately normal sulphuric acid and proceed as directed in A-2.

The final combined chloroformic extracts obtained by A-1, A-2 or A-3 are received into a tared Erlenmeyer flask (125–150 cc.). Carefully evaporate almost to dryness on a steam-bath and dry the residue to constant weight at 105–110° C., recording all weights.

B. The Methoxy Determination (See Fig. 1).—To flask No. 2 containing the alkaloidal residue obtained and dried as in (A), is added 10 cc. Hydriodic Acid (Sp. Gr. 1.5 to 1.7) and 5 cc. of Acetic Acid (8%). The flask is then immersed in glycerin contained in a beaker (1000 cc.), which is placed upon an asbestos pad set upon a tripod. The glycerin bath is maintained at a temperature of 130–140° C. Carbon dioxide furnished from a tank is bubbled into a flask No. 1

[1] The writers wish to express their appreciation to Messrs. R. K. Miller, C. H. Bender, J. T. Mullins and J. E. Ball, Senior Students in the School of Pharmacy, Purdue University, for their assistance in conducting many of the preliminary experiments necessitated by this investigation.

[2] Palkins and Watkins, *Ind. Eng. Chem.*, 19 (1927), 535.

containing silver nitrate solution (10%) at the rate of 120–150 bubbles per minute. This flask is connected to flask No. 2 and the alkyl halide generated in this flask is carried by the stream of carbon dioxide into an empty container No. 3. Container No. 4 is provided with distilled water, flasks Nos. 5, 6 and 7 hold a 2% solution of Arsenious Acid and 2% of Potassium Carbonate; No. 8 receives distilled water. Containers Nos. 3–7 are immersed in a water-bath maintained at 40–50° C. Receiver No. 9 is an Erlenmeyer flask (125–150 cc.) containing 40 cc. of 0.1 *N* alcoholic Silver Nitrate solution and No. 10 is a flask of the same size provided with 10 cc. of the standard silver solution; in each case the solution is slightly acidified with nitric

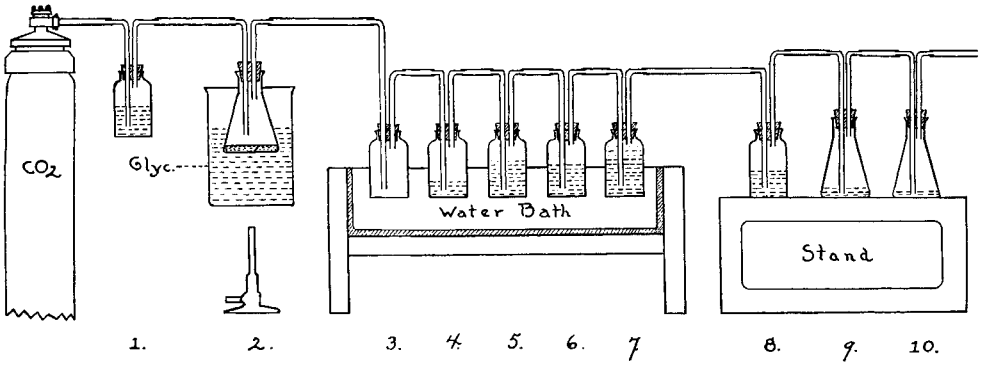


Figure. I.

acid. The reaction is allowed to continue for two hours after which time the flasks Nos. 9 and 10 are disconnected and the latter flask is connected to the apparatus and gas allowed to pass 15 minutes longer to be assured that the alkyl halide has been completely washed from the apparatus. The contents of flasks Nos. 9 and 10 are combined and filtered through a tared Gooch crucible provided with an asbestos mat and the residue after washing with acidulated water is dried to constant weight at 105–110° C. The weight of Brucine may be calculated as follows:

$$X \text{ (wt. of Brucine)} : (\text{wt. of Residue} \times 0.132) :: \underset{\text{Brucine}}{394.346} : \underset{\text{Methoxy Group}}{62.048}$$

(Mol. wt. Brucine; Mol. wt. of Methoxy Group)

or: wt. of Silver Iodide × 0.83970 = wt. of Anhydrous Brucine.

The per cent of Brucine is estimated as follows:

$$\frac{\text{Wt. of Brucine found} \times 100}{\text{Wt. of sample}}$$

The per cent of Strychnine is estimated:

$$\frac{\text{Constant wt. of total alkaloids} - \text{wt. of Brucine found} \times 100}{\text{Wt. of sample}}$$

The filtrate and washing are carefully reserved and the excess of silver is titrated with 0.1 *N* Potassium Thiocyanate, using Ferric Ammonium Sulphate as an indicator. 1 cc. of 0.1 *N* $AgNO_3$ = 0.01972 Gm. Anhydrous Brucine.

SAMPLES USED IN THE INVESTIGATION.

Series O.

This sample consisted of a commercial product purchased from S. B. Penick and Company. Fineness labeled as No. 40.

Series I.[1]

Sample 1.—A commercial sample of Nux Vomica purchased from S. B. Penick and Company. Fineness labeled as No. 20.

[1] Bulletin 20, page 73, Sub-Committee No. 6 of Revision Committee of the U. S. P.

Sample 2.—Exhausted Nux Vomica No. 20 (0.028% total alkaloids)..... 177.445 Gm.
Strychnine Sulphate (856.75) in fine powder..... 4.000
Brucine Sulphate (1012.81) in fine powder..... 1.800

Sample 3.—Exhausted Nux Vomica (see Sample 2)..... 180.000 Gm.
Strychnine Sulphate..... 1.700
Brucine Sulphate..... 4.400

Series II.[1]

Sample 1.—Exhausted Nux Vomica No. 20 powder (0.056% Total Alkaloids..... 330.000 Gm.
Strychnine Sulphate..... 7.500
Brucine Sulphate..... 3.500

Sample 2.—A commercial sample purchased from S. B. Penick and Company. Labeled as No. 20 powder.

Sample 3.—Exhausted Nux Vomica (see Series II, Sample 1)..... 330.000 Gm.
Strychnine Sulphate..... 3.500
Brucine Sulphate..... 3.500

Series III.

Sample 1.—Exhausted Nux Vomica..... 120.000 Gm.
Strychnine Sulphate..... 1.200
Brucine Sulphate..... 1.200

TABLE OF RESULTS.

	Weight of Total Alkaloids.			Per Cent Brucine.				Per Cent Strychnine.				
Series.	U. S. P. X ext. No. 1.	Extraction method No. 2.	Extraction method No. 3.	Calculated.	Grav. detn.	Vol. detn.	Av. grav. and vol.	Calculated.	Collabr. results sub-comm. 6	Grav. detn.	Vol. detn.	Av. grav. and vol.
O	0.1403*	....	....	...	1.21	1.36	1.29	...	...	1.60	1.45	1.52
	0.2130**	....	....	...	1.29	1.43	1.36	...	...	1.55	1.40	1.48
					Av. 1.25	1.39	1.32			1.57	1.43	1.50
	....	0.3041	....	...	1.00	1.11	1.06	...	...	2.04	1.93	1.98
	....	0.3086	....	...	..	1.14	1.14	...	...	..	1.95	1.95
					Av. 1.00	1.12	1.10			2.04	1.94	1.96
	....	....	0.4930	...	1.07	1.14	1.10	...	...	2.21	2.15	2.18
	....	....	0.4945	...	1.04	1.08	1.06	...	...	2.26	2.22	2.24
					Av. 1.06	1.11	1.08			2.23	2.19	2.21
I—1A	....	....	0.4080	...	0.85	0.88	0.87	...	1.274	1.87	1.84	1.86
1B	....	....	0.4022	...	0.83	0.81	0.82	...	...	1.86	1.87	1.86
1C	....	....	0.4142	...	0.94	0.98	0.96	...	...	1.82	1.79	1.81
					Av. 0.87	0.82	0.88			1.85	1.83	1.84
1D	0.1534*	....	....	...	1.02	1.15	1.08	...	...	2.05	1.92	1.98
1E	0.1502*	....	....	...	1.01	1.08	1.04	...	...	1.99	1.92	1.95
					Av. 1.02	1.11	1.06			2.02	1.92	1.96
2A	....	....	0.3962	0.779	0.94	0.98	0.96	1.718	1.701	1.70	1.66	1.68
2B	....	....	0.3768	...	0.69	0.79	0.74	...	...	1.82	1.73	1.77
					Av. 0.82	0.88	0.85			1.76	1.70	1.74
2C	0.1344*	....	....	...	0.90	0.98	0.94	...	...	1.79	1.80	1.79
2D	0.1340*	....	....	...	0.90	0.90	0.90	...	...	1.78	1.78	1.78
					Av. 0.90	0.94	0.92			1.78	1.79	1.78
3A	....	....	0.3942	1.855	1.91	1.87	1.89	0.727	0.619	0.71	0.76	0.74
II—1A	....	....	0.3809	0.827	0.70	0.75	0.73	1.744	1.670	1.84	1.78	1.81
2A	....	....	0.3869	...	1.14	1.04	1.09	...	1.240	1.62	1.54	1.58
3A	....	....	0.2500	0.837	0.68	0.77	0.73	0.839	0.801	0.98	0.90	0.94
III—1A	....	....	0.2388	0.763	..	0.75	..	0.766	...	..	0.84	..
	....	....	0.2390	...	..	0.75	0.72	...	...	0.86	0.84	0.85
					Av. 0.00	0.75	0.72	...	...	0.86	0.84	0.85

* 50 cc. aliquot from total volume of 150 cc.
** 100 cc. aliquot from total volume of 100 cc.

[1] Bulletin 23, page 79, Sub-Committee No. 6 of Revision Committee of the U. S. P.

It has been found that the following precautions are necessary and important:

1. Between each determination the tube leading into the silver nitrate solution in flask No. 9 should be cleaned by allowing to stand in concentrated Nitric Acid over night and rinsing with distilled water.

2. Careful regulation of the speed of the stream of carbon dioxide and the temperature of the baths is necessary. It has been found that the formation of a black residue often occurs if the temperature becomes too high and the speed of the gas is too great. The gases are, thereby, not allowed to cool sufficiently and the silver nitrate may be reduced.

3. Reagents used should be pure. This is especially true of the Hydriodic Acid used. It should not contain free iodine, sulphur or phosphorous compounds.

4. All connections should be tight.

5. Analytical technique should be employed at all times.

COMMENTS.

1. It has been found that, in general, the volumetric determination (for Brucine) gives higher results than the gravimetric method.

2. The values for Brucine are higher than the calculated amount in the known samples; this might indicate that the residues are not sufficiently pure, therefore contain impurities which tend to give high Methoxy values. The Strychnine values, however, are not so affected.

3. It will be observed that with commercial samples the extraction method No. 3 gives higher results than by other extraction methods used, indicating that the usual methods of extraction might not be sufficiently thorough or that this method extracts impurities which tend to give high results. Time did not permit a further study which might clarify the situations involved in 2 and 3 (see Series O, Series I, IA, IB, IC, Series II, IIA).

4. The values for Strychnine content on samples of known composition are quite in accord with the calculated values and compare well with those values obtained with the samples by the Oxidation method directed in *Bulletin 23 of Sub-Committee* No. 6 (see page 299).

SUMMARY.

1. Three extraction methods are studied. The use of the automatic extractor gives higher results than the usual methods of extraction.

2. A method is proposed whereby alkaloidal residues from Nux Vomica may be examined for Brucine content by ascertaining the Methoxy value of the residue of known weight and the amount of Strychnine by difference.

3. The values for Brucine are apparently affected by the character of the residue. This fact does not seem to affect the values for Strychnine content since the experimental results check well with calculated values.

4. The writers deem the method worthy of consideration in the commercial laboratory where routine analyses are conducted on Nux Vomica by a single worker or a group of workers since the development of technique is necessary.

5. The method proposed is not as rapid but apparently is as accurate as the oxidation method under consideration by Sub-Committee No. 6.

6. The fact that results may be checked gravimetrically as well as volumetrically is an advantage.