

STUDIES IN THE ANALYTICAL CHEMISTRY OF DRUGS.
THE DETERMINATION OF TOTAL ALKALOIDS IN NUX VOMICA
PREPARATIONS.*¹

BY SAMUEL PALKIN AND H. R. WATKINS.

The process for the determination of total alkaloids in nux vomica preparations, such as described in the U. S. Pharmacopœia, IX, is tedious, due to the extreme slowness with which the last portions of alkaloid are extracted by the solvent and the need for purification of the alkaloidal solutions.

The U. S. P. IX assay for nux vomica preparations, unlike that of the U. S. P. VIII, provides for the determination of total alkaloids. Aside from the two well-known alkaloids, strychnine and brucine, present in nux vomica, a third alkaloid strychnicine, which possesses strongly amphoteric properties, has been isolated by Boorsma² from the leaves of *Strychnos nux vomica* grown in Java.

The brevity and simplicity of the directions for this quantitative method are in no way indicative of the time and effort usually necessary to carry it out. Nevertheless, in the hands of a patient and skilled analyst, surprisingly accurate results are possible.

This method requires three series of extractions based on the well-known principles of extraction with immiscible solvents: (1) extraction with chloroform of the alkaloids from the original preparation made alkaline with ammonia; (2) removal of the alkaloids from the chloroformic solution with dilute acid and (3) reextraction from this aqueous medium (after rendering alkaline) with chloroform.

Not less than six extractions, often eight or ten and sometimes as many as fifteen are required in each of the three steps to effect complete removal of the alkaloids. The process is usually rendered more tedious by emulsions.

The various steps involved in this assay have been studied in order to ascertain the cause for this abnormal behavior and if possible to revise and simplify the procedure so that a determination of the total alkaloids in nux vomica can be made with reasonable effort.

The behavior of brucine and strychnine as such on extraction with chloroform is fairly in accord with the solubilities recorded in the literature and the law governing distribution in immiscible solvents.

TABLE I.—EXTRACTION OF STRYCHNINE AND BRUCINE WITH CHLOROFORM.

Soln. A—20 cc. contained 250 mg. Strychnine in slightly acid solution.
Soln. B—20 cc. contained 250 mg. Brucine in slightly acid solution.
Soln. C—20 cc. contained 100 mg. Strychnine and 150 mg. Brucine in slightly acid solution.

2 cc. 8.5% NH₄OH used and extractions made with equal volumes of chloroform.

Alkaloid solution used.	Wt. strychnine taken.	Wt. brucine taken.	Wt. alkaloid recovered in 1st extraction.	Test for alkaloid in 3d extraction.
20 cc. A	0.2500 Gm.	...	0.2398 Gm.	Negative
20 cc. B	...	0.2500 Gm.	0.2395 Gm.	Negative
20 cc. C	0.1000 Gm.	0.1500 Gm.	0.2418 Gm.	Minute trace

* Presented before the Washington meeting of the American Chemical Society.

¹ Contribution from the Drug Control Laboratory, Bureau of Chemistry, U. S. Department of Agriculture.

² Henry, "Plant Alkaloids," p. 194.

Though these alkaloids are somewhat soluble in the presence of excess of ammonia, complete removal from the aqueous medium by chloroform is nevertheless possible in two extractions even where the quantity of ammonia used is considerably in excess of the amount theoretically required to liberate these alkaloids, as is shown in Table I.

A possible explanation for the stubborn retention of alkaloid exhibited by preparations of nux vomica is that some other, as yet unidentified, alkaloid either possesses a markedly different solubility from the other two or it exhibits strongly amphoteric properties. The latter explanation will appear more obvious from experiments shown in Table II(a) and (b), and graphically in Fig. 1.

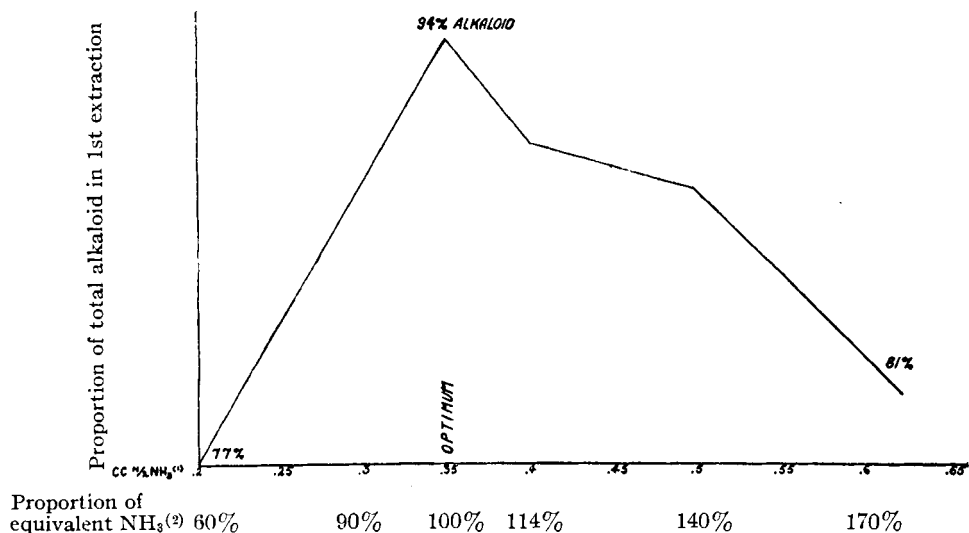


Fig. 1. Effect of NH₃ variation on extraction of total alkaloids (Table II-B)

(1) These quantities of NH₃ were added after the free acid in alkaloidal solution had been neutralized to Methyl Red. (2) The indicated percentages are calculated from the theoretical quantity of NH₃ necessary to completely liberate the alkaloids.

TABLE II(a)—VARIATION IN AMMONIA.

Constants: Fluid extract filtrate—5 cc. Soln. A.¹

Solvent—5 cc. CHCl₃.

Period of Shaking—2 minutes.

Variant = NH₄OH.

No.	N NH ₄ OH.	Alkaloids, 1st extraction.	Test for alkaloids in 4th extraction.	Test for alkaloids in 5th extraction.
1	0.45 cc. (not alkaline)	35.3 (56%)	...	Heavy ppt.
2	(0.45 + 0.2 cc.) 0.65 cc.	54.9 (87%)	Trace	Negative
3	(0.45 + 0.45 cc.) 0.9 cc.	52.0 (82%)	Ppt.	Light ppt.

¹ 5 cc. Soln. A—required 0.45 cc. N NH₄OH (methyl red indicator) to neutralize all free acid.

Total alkaloid present in 5 cc. Soln. A = 63 mgm.

1 cc. N NH₄OH = 364 mg. total alkaloid.

In these experiments a stock alkaloidal solution (designated Soln. A) was used which was prepared from a U. S. P. fluid extract of nux vomica in a manner similar to that described for the preliminary removal of impurities under "Method of Assay" in this paper. Twenty-five-cc. aliquots of the filtrate were employed for each ex-

traction. In this way, conditions absolutely analogous to those met with in actual practice were maintained, while studying the effect of the ammonia-variable.

TABLE II(b)—VARIATION IN NH_4OH —CONTINUED.

Constants: 5 cc. Soln. A¹ = alkaloid solution;

Solvent—5 cc. CHCl_3 ;

Period of shaking—2 minutes.

Variants = NH_4OH .

No.	$N/2 \text{ NH}_4\text{OH}$.	Quantity alkaloids in 1st extraction, mg.	Test for alkaloids tested in		
			3rd extraction.	4th extraction.	5th extraction.
1	(.9 + .2 cc.) 1.1 cc.	48.7 (77%)	heaviest	heavy	slight
2	(.9 + .35 cc.) ² 1.25	58.9 (94%)	slight test	negative	negative
3	(.9 + .4 cc.) ² 1.3	56.4 (89%)	heavier than 2	very slight test	negative
4	(.9 + .5 cc.) ² 1.4	55.3 (87.5%)	heavier than 3	heavier than 3	faintest trace
5	(.9 + .6 cc.) ² 1.5	51.3 (81%)	heavier than 4	heavier than 4	trace

¹ 5 cc. Soln. A required .9 cc. $N/2 \text{ NH}_4\text{OH}$ (methyl red indicator) to neutralize.

Total alkaloids in 5 cc. Soln. A—63 mg.

² 0.1 cc. $N/2 \text{ NH}_3 = 18.2$ mg. alkaloid; 0.35 cc. $N/2 \text{ NH}_3 = 63.7$ mg. alkaloid; 0.4 cc. $N/2 \text{ NH}_3 = 72.8$ mg. alkaloid.

As deduced from the above tables, the optimum alkalinity for exhaustion of the alkaloids is approximately that point where an amount of ammonia equivalent to the total alkaloid present has been added beyond the point neutral to methyl red.

Practical application of the information gained from these tables regarding ammonia, could not be made as the solubility variation due to this reagent is altogether too sensitive to be within the range of actual control by exact neutralization of the original sample.

In view of the possible complications caused by the presence of alcohol in the extractions, a series of experiments was run to determine the effects of varying the proportions of alcohol and chloroform in the extracting medium for nux vomica alkaloids.

In these experiments (Tables III(a) and (b)) an alkaloid solution was prepared similar to that employed in the experiments of Tables II(a) and (b). All other conditions such as alkalinity, period of shaking, volume of solvent, etc., were maintained fairly constant while the composition of the solvent was varied.

Both strychnine and brucine are less soluble in alcohol than in chloroform. Nevertheless the mixture of chloroform and alcohol extracts the alkaloids as well, if not more rapidly, than chloroform alone. But the relative freedom from contaminating impurities when chloroform alone is used offsets the advantage in rapidity of extraction gained by the use of the mixed solvent except where obstinate emulsions are formed. Table III(b) shows similar results obtained when applying information gained in Tables II(a) and (b) as regards optimum alkalinity.

As indicated in the outline of the U. S. P. assay two series of steps, additional to the original exhaustion of the alkaloids from the nux vomica preparation, are necessary to render the alkaloids clean enough for determination by titration. It is in this process of purification from resinous and other natural contaminations that the greatest amount of time and labor is consumed. It was therefore thought possible that a preliminary treatment of the original nux vomica preparation for the removal of the major portion of these impurities might render the subsequently extracted alkaloid sufficiently clean for titration. Some experiments

TABLE III(a)—EXTRACTION WITH MIXED SOLVENTS.

Constants:
 Period of shaking = 2 minutes; $\text{NH}_3 = 2\frac{1}{2}$ cc. 10%; 10 cc. H_2O ; Vol. solvent equal to aqueous volume.

Variant—solvent (proportion of alcohol and CHCl_3 by volume).

Solvent.	Alkaloid residues from			Total in three shakings, Gm.
	1st extraction, Gm.	2nd extraction, Gm.	3rd extraction, Gm.	
(1) CHCl_3 , alone	0.2331	0.0209	0.0041	0.2581
(2) CHCl_3 , 4 parts; alcohol, 1 part	0.2466	0.0150	0.0003	0.2619
(3) CHCl_3 , 2 parts; alcohol, 1 part	0.2524	0.0069	0.0003	0.2596

Seven extractions were necessary to remove all alkaloids. Test of the eighth extraction with Mayer's reagent was negative.

TABLE III(b)—VARIATION IN SOLVENT.

Constants: 5 cc. Soln. A, 1.3 cc. $N/2$ NH_4OH (optimum alkalinity derived from data in Tables II(a) and (b) and period of shaking 2 minutes.

Variant: Solvent¹

Number.	1st extraction.		2nd extraction.		3rd extraction.		Test for alkaloid with Mayer's reagent.	
	Wt. of alkaloids in mg.	% alkaloids extracted.	Wt. of alkaloids in mg.	% alkaloids extracted.	Wt. of alkaloids in mg.	% alkaloids extracted.	4th extraction.	5th extraction.
1	59.37	95.7	2.17	3.5	0.72	1.1	Minute trace	Negative
2	59.37	95.7	1.81	2.9	1.09	1.7	Minute trace	Negative
3	58.64	94.6	1.81	2.9	0.36	0.6	Trace	Slight trace
4	58.64	94.6	1.45	2.3	0.72	1.1	Trace

¹ No. 1—5 cc. CHCl_3 ; No. 2—5 cc. CHCl_3 ; No. 3—5 cc. chloroform-alcohol 3:1; No. 4—5 cc. chloroform-alcohol.

using lead salts showed this means to be possible but not practicable. A very simple and direct means for the removal of these impurities was found by evaporating the acidified preparation to low volume and then rediluting with water. In most cases, the resinous impurities are precipitated very largely by this means and the alkaloids can be extracted from the clear filtrate in a condition suitable for titration. Incidentally, the tendency for emulsions is either eliminated or greatly reduced by this preliminary treatment.

The following short method (described in detail below) has been applied to a representative number of commercial samples of nux vomica, fluid extracts and tinctures, and results as compared with their U. S. P. assays are shown in Table IV.

METHOD OF ANALYSIS.

Fluid Extract.—Pipette 25 cc. of sample into a 50-cc. volumetric flask. Add about 3 cc. normal sulphuric acid and evaporate on the steam-bath (using air blast to hasten evaporation)¹ to a volume of about 10 cc. To the residue add about 30

¹ The evaporation can be carried out in a small beaker but great care will have to be exercised in transferring the whole to a volumetric flask to insure complete transfer of the alkaloidal solution.

TABLE IV—COMPARISONS OF ANALYSES OF FLUIDEXTRACTS OF NUX VOMICA BY THE TWO METHODS.

	Alkaloids in 10 cc.			Alkaloids in 10 cc.	
	U. S. P. method. Gm.	Revised method. Gm.		U. S. P. method. Gm.	Revised method. Gm.
1	0.2469	0.2459	8	0.1680	0.1654
	0.2426	0.2410		0.1694	0.1654
2	0.2534	0.2548	9	0.0407	0.0422
	0.2548	0.2534		0.2147	0.2126
3	0.2287	0.2291	10	0.2133	0.2164
	0.2258	0.2269		0.3021	0.3021
4	0.2099	0.2074	11	0.1944	0.1929
		0.2042		0.3378	0.3340
5	0.2796	0.2788	12		
	0.2796	0.2788			
6	0.2730	0.2715	13		
	0.2498	0.2433			
7	0.2484	0.2433	14		

COMPARISONS OF ANALYSES OF TINCTURES OF NUX VOMICA BY THE TWO METHODS.

	Alkaloids in 100 cc.			Alkaloids in 100 cc.	
	U. S. P. method.	Revised method.		U. S. P. method.	Revised method.
15	0.2644	0.2630	21	0.1674	0.1668
	0.2636	0.2659		0.2031	0.2056
16	0.2291	0.2288	22	0.2082	0.2096
		0.2316		0.2781	0.2824
17	0.2982	0.2979	23	0.1332	0.1301
	0.2996			0.2460	0.2457
18	0.2130	0.2088	24	0.2380	0.2357
	0.3694	0.3722			
19	0.2358	0.2329	25		
20			26		
21			27		

COMPARISONS OF ANALYSES OF FLUIDEXTRACTS AND POWDERED EXTRACTS OF NUX VOMICA BY THE TWO METHODS.

	Alkaloids in 10 cc.			Alkaloids in 10 cc.	
	U. S. P. method.	Mechanical extractor method.		U. S. P. method.	Mechanical extractor method.
28	0.2250	0.2280	33	0.0407	0.0436
29	0.2290	0.2290	34	0.2147	0.2160
30	0.2380	0.2417	35	(Powdered extracts)	
		0.2417		12.96%	12.74%
31	0.1914	0.1940	36	14.49%	14.89%
		0.1940		37	15.35%
32	0.2500	0.2518			

cc. of water while rotating the flask, cool to room temperature and dilute to volume. Allow to stand five minutes and filter through dry filter paper. The major portion of contaminating extractive matter will have precipitated in a flocculent form.¹ Pipette 20 cc. (equivalent to 10 cc. original sample) into a separatory funnel (this is done in duplicate), add 1 cc. of ammonium hydroxide and extract the alka-

¹ In some cases the extractive matter is not sufficiently precipitated by this process, and in that event the solution is re-*evaporated* to a smaller volume than 10 cc.—to about 2 or 3 cc. or a new portion taken, and then taken up in water as usual.

loids with equal volumes of chloroform¹ until extraction is complete testing the final extracted residue with Mayer's reagent. Eight or more extractions are usually necessary. Each portion of chloroform extract is successively washed in another separatory funnel with 10 to 15 cc. of water and drawn off into a beaker or preferably into a 200-cc. Erlenmeyer flask and the combined extracts evaporated on the steam-bath using air blast. By evaporating the first four or five extracts, while the remaining extractions are being made, the process can be materially hastened.

Take up the alkaloidal residue in a few cc. of neutral alcohol warming on the steam-bath to insure complete solution, add 10 cc. *N*/10 sulphuric acid, dilute with about 20 cc. water and titrate back the excess acid with *N*/50 alkali using methyl red as indicator. One cc. *N*/10 acid = 36.4 mg. total alkaloid.

Tinctures.—Use 125 cc. sample, 2 cc. *N* acid and evaporate in a beaker and transfer into a volumetric flask as described in footnote 1.

Powdered Extracts.—These preparations require special treatment for their analysis. While it is possible to prepare fluid extracts from known weights of these solid extracts and then treat these solutions (for assay) in the manner described for the assay of fluid extracts and tinctures, this form of nux vomica preparation lends itself much more readily to analysis by means of an automatic extractor which has been developed in this laboratory. This device is equally serviceable for the other products of nux vomica and its description and application will follow in a later publication.

SUMMARY.

The steps involved in the extraction, purification, and determination of the total alkaloids in nux vomica preparations have been studied experimentally with the view to a simplification of the method of assay.

The following conclusions have been reached: (a) the extreme slowness with which the last portions of alkaloid are extracted by the solvent is probably due to the presence of some alkaloid, other than strychnine or brucine; (b) the extraction can be facilitated by careful control of the ammonia used; and (c) the essential purification can be effected by a simple preliminary precipitation. The latter step incidentally reduces the tendency for emulsion.

A shorter method is described and tables showing comparative results so obtained are given.

THE ISOLATION AND PROPERTIES OF THE ALKALOIDS AND OIL OF LARKSPUR SEED (*DELPHINIUM CONSOLIDIDA*).

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The present investigation was undertaken for the purpose of determining the insecticidal constituents of larkspur seed (*Delphinium consolida*) and of proprietary preparations made therefrom.

¹ Troublesome emulsions are rarely encountered after this preliminary purification but when they do occur the use of alcohol with the chloroform will be found advantageous and if not used in greater proportion than volume of CHCl_3 this will not vitiate the results as shown in Table III(b).

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