

SCIENTIFIC SECTION

INFLUENCE OF THE ALKALOIDAL PURIFICATION PROCEDURE IN ASSAY OF ALKALOIDAL DRUGS.

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In assaying certain alkaloidal drugs and their preparations the initial immiscible solvent extract containing the alkaloids is often so light-colored as to appear free from substances likely to interfere with titration of the alkaloids. This is especially true when a fluid preparation has been dried on saw-dust prior to extraction with ammoniacal, immiscible solvent. Such a light-colored extract may tempt the operator to resort to what we will term the "short" procedure of merely evaporating the extract directly for the titration, in an effort to avoid the time-consuming "complete" alkaloidal purification procedure as typified by the U. S. P. method outlined on pages 452, 453 and 454 of the U. S. P. X.

While the results so obtained by this "short" procedure often check well with the results obtained by the "complete" purification procedure this practice fails to yield correct results often enough to condemn it for practical use as shown by the following table of results by the 2 methods:

Drug Product.	"Complete" Procedure. Per Cent.	"Short" Procedure. Per Cent.
Aconite Root 1	0.488	1.24
Aconite Root 2	0.551	0.632
Ignatia Bean 1	3.097	3.203
Ignatia Bean 2	2.907	3.006
Ignatia Bean 3	2.657	2.631
Ignatia Bean 4	3.08	3.29
Ipecac 1	1.58	1.58
Ipecac 2	2.04	2.06
Ipecac 3	2.34	2.32
Ipecac 4	2.50	2.43
Ipecac 5	2.462	2.496
Ipecac 6	2.11	2.23
Ipecac 7	2.15	2.25
Fldext. Ipecac 1	1.38	1.36
Nux Vomica 1	2.77	4.02
Nux Vomica 2	2.67	3.78
Nux Vomica 3	2.68	2.57
Nux Vomica 4	2.80	2.80
Nux Vomica 5	2.76	2.94
Nux Vomica 6	2.44	2.84
Tinct. Nux Vomica 1	0.0943	0.0996
Tinct. Nux Vomica 2	0.305	0.305
Tinct. Nux Vomica 3	0.1974	0.1970
S. E. Nux Vomica 1	12.2	12.7
S. E. Nux Vomica 2	14.37	18.2
S. E. Nux Vomica 3	12.5	12.24
P. E. Nux Vomica 1	16.85	17.29
P. E. Nux Vomica 2	20.5	21.6
F. E. Nux Vomica 1	1.918	1.85

F. E. Nux Vomica 2	1.97	1.893
F. E. Nux Vomica 3	1.80	1.80
F. E. Nux Vomica 4	2.32	2.28

A powdered extract of Nux Vomica which assayed 16.2% total alkaloids by the U. S. P. ("complete") procedure gave a result of 24.9% when the initial chloroformic extracts were evaporated directly and the alkaloidal residue then titrated. The alkaloidal residue contained magnesium which was evidently present as a chloroform-soluble magnesium "soap" in the powdered extract. This type of contamination of alkaloidal residues by "soaps" of the "earthy" elements was pointed out by Watkins and Palkin (*J. A. O. A. C.*, Vol. X, No. 1 (Feb. 15, 1927)). It is likely that the divergences often shown by incomplete or "short" alkaloidal purification procedures are caused by the inclusion of these titratable "earthy soaps" in the alkaloidal residue. Another possible contaminant of alkaloidal residues obtained by incomplete purification procedures is that represented by ammonium salts extracted along with the alkaloid as shown by the results reported by the writer in *JOUR. A. PH. A.*, 19 (1930), 26-27.

Thus it is evident that alkaloidal residues obtained by these incomplete alkaloidal purification procedures may be contaminated by "earthy soaps" and ammonium compounds. Both of these contaminants show titration value and consequently these "short" procedures sometimes give results which are too high. This is shown by the above comparative results by the 2 methods.

In an article in the *JOUR. A. PH. A.*, 18 (1929), 241, the writer has shown that the ammoniacal chloroformic extractives of various vegetable drugs have decided basic effect. This basic effect is often due to the presence of calcium and magnesium compounds and also sometimes to the presence of ammonium compounds. When sodium bicarbonate is used in place of ammonia in making the chloroformic extractions basic effect is also shown by the extractives but the basicity, while certain, is of much less magnitude than that yielded by ammonia, and is, in fact, relatively negligible.

Drug.	Gentian.	Podophyllum.	Capsicum.	Cannabis.	P.E. Jalap.
Amount of drug.....	0.66 Gm.	0.33 Gm.	(See note)	0.66 Gm.	0.33 Gm.
Basic effect using ammonia...	0.00735	0.00641	0.0238	0.0191	0.00641
Basic effect using sodium bicarbonate	0.000267	0.000869	0.00267	0.00367	0.00214

NOTES: No record was kept of the amount of capsicum used, the extracts having a light amber color. Basic effects are stated in the table in terms of Gms. of strychnine alkaloid for convenience in comparison.

The weaker basicity yielded by sodium bicarbonate may be due partly to the absence of ammonium compounds in the extracts and partly to the breaking up of the organic compounds of calcium and magnesium by the sodium bicarbonate whereby they are rendered insoluble in chloroform. This action of sodium bicarbonate appeared to offer promise in the assay of alkaloidal drugs by the incomplete purification method but the following experiments show that this apparently useful action of sodium bicarbonate is offset by its lower efficiency in liberating alkaloids from plant tissues:

Drug.	Extraction Method.	Alkaloidal Purification Process.	Assay: Per Cent.
Ipecac No. 6	Ether-Ammonia	Extracts evaporated directly and titrated	2.23
	Ether-Sod. Bicarb.-Water	Extracts evaporated directly and titrated	1.97
	U. S. P.	U. S. P.	2.11
Ipecac No. 7	Ether-Ammonia	Extracts evaporated directly and titrated	2.25
	Ether-Sod. Bicarb.-Water	Extracts evaporated directly and titrated	1.90
	U. S. P.	U. S. P.	2.15
Nux Vomica No. 5	Ether-Chloroform-Ammonia	Extracts evaporated directly and titrated	2.94
	Ether-Chloroform-Sod. Bicarb.-Water	Extracts evaporated directly and titrated	2.34
	U. S. P.	U. S. P.	2.76

Thus it will be noted that whereas the use of sodium bicarbonate resulted in lower yields of alkaloids this decrease was not entirely due to elimination of titratable "earthy soaps" and ammonium compounds from the alkaloidal residues, but was partly due to incomplete liberation of the alkaloids since the U. S. P. purification process which practically entirely eliminates these interfering substances yielded higher results than incomplete or "short" procedures in which sodium bicarbonate was used.

Sodium carbonate, like sodium bicarbonate, also proved less effective than ammonia in liberating alkaloids from vegetable tissue and consequently is inadmissible for the purpose of obviating the necessity for the use of ammonia followed by "complete" purification procedure:

Drug.	Extraction Method.	Alkaloidal Purification Process.	Assay: Per Cent.
Ipecac No. 6	Ether-Ammonia	Extracts evaporated directly and titrated	2.23
	Ether-Sod. Carb.-Water	Extracts evaporated directly and titrated	1.56
	U. S. P.	U. S. P.	2.11
Ipecac No. 7	Ether-Ammonia	Extracts evaporated directly and titrated	2.25
	Ether-Sod. Carb.-Water	Extracts evaporated directly and titrated	1.43
	U. S. P.	U. S. P.	2.15
Ignatia No. 4	Chloroform-Ammonia	Extracts evaporated directly and titrated	3.29
	Chloroform-Sodium Carbonate-Water	Extracts evaporated directly and titrated	2.70
	Chloroform-Ammonia-Ether	Complete purification procedure	3.08
Nux Vomica No. 5	Chloroform-Sodium Carbonate-Water	Extracts evaporated directly and titrated	2.33
	U. S. P.	U. S. P.	2.76
Nux Vomica No. 6	Chloroform-Ammonia-Ether	Extracts evaporated directly and titrated	2.84
	Chloroform-Sodium Carbonate-Water	Extracts evaporated directly and titrated	2.36
	U. S. P.	U. S. P.	2.44

Alkaloidal residues whether obtained by evaporation of the initial immiscible solvent extracts ("short" procedure) or by the U. S. P. purification process ("complete" procedure) invariably show the presence of calcium, and often, magnesium upon analysis. Alkaloidal residues obtained by the U. S. P. procedure always show very much less of these "earthy" compounds than do the residues obtained by the "short" procedure.

Immiscible solvent solutions of alkaloidal residues from numerous single assays of Belladonna, Ipecac, Ignatia and Nux Vomica when evaporated to dryness, ignited, dissolved in a few drops of hydrochloric acid, made alkaline with ammonia, then acid with acetic acid and treated with ammonium oxalate yielded decided reactions for calcium ranging from turbidities to small precipitates. When the calcium oxalate was completely precipitated with ammonium oxalate and ammonia by allowing to stand over night, filtered off and sodium phosphate and ammonia was added to the filtrate distinct traces of crystalline precipitate were obtained in the case of Ipecac and Nux Vomica thus indicating the presence of magnesium. Blanks were conducted in all cases with negative results. To make certain of the identity of the calcium oxalate the precipitates from a large number of alkaloidal residues were consolidated and confirmatory tests made. To ascertain the possible influence of the "earthy" compound content of alkaloidal residues purified by the U. S. P. procedure upon analytical results a large series of immiscible solvent solutions of alkaloidal residues each representing single assays of Belladonna, Ipecac and Nux Vomica were individually evaporated, and the residues then ignited to constant weight. In every case, quite evident white stains remained unvolatilized but the quantity was "unweighable" (less than 0.5 mgm.), so that it is likely that the influence of these "earthy" compounds is negligible. A corresponding series of quantitative tests made on initial immiscible solvent extracts of alkaloidal drugs obtained by the "short" procedure showed as much as 0.0030 Gm. of residue upon ignition from single assays of Aconite Root; 0.0011 Gm. from Ipecac and 0.0023 Gm. from Nux Vomica. Ignatia showed less than 0.5 mgm. residue upon ignition in this test although obviously more than when the alkaloids were purified by the "complete" procedure.

SUMMARY.

Incomplete alkaloid purification in the assay of alkaloidal drugs may cause inaccurate results due to inclusion in the alkaloidal residue of titratable calcium, magnesium and ammonium compounds.

Sodium carbonate or bicarbonate cannot be used to replace ammonia in an effort to eliminate the above-mentioned interfering substances as their possible usefulness in this direction is offset by their low efficiency in liberating alkaloids from plant tissues.

The U. S. P. alkaloidal purification process ("complete" procedure) essentially eliminates these interfering substances but even residues so purified may still retain traces of calcium and magnesium, although the proportion is not significant for ordinary assay purposes.

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W. D. CHAPMAN, M.D., in *Illinois Medical Journal*.