THE STANDARDIZATION AND STABILIZATION OF NUX VOMICA, GELSEMIUM AND VERATRUM AND THE HYDROGEN-ION CONCENTRATION FACTOR.—PAPER IV.

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I. INTRODUCTION.

In three previous articles (1, 2, 3), the standardization and stabilization of aconite preparations were reported. This report included the study of a bio-assay method, the deterioration of preparations of aconite, the unreliability of the chemical assay method, the prevention of deterioration, the cause of deterioration or hydrolysis of the aconitine alkaloid into the less potent alkaloids benzyl aconine and aconine, and finally the hydrogen-ion concentration, which was shown to control the deterioration and stabilization. Inasmuch as it was found that this factor controls the value of aconite preparations, the writers have studied it in relation to other drug preparations with the idea that perhaps each fluidextract and tincture has a hydrogen-ion concentration or p_H value which controls its deterioration and stabilization.

The drugs or fluidextracts of drugs chosen were fluidextracts of Nux Vomica U. S. P. IX, Gelsemium U. S. P. IX and of Veratrum U. S. P. IX. The chemical properties and chemical assay methods of the alkaloids of Nux Vomica, strychnine and brucine, are well known and are considered very stable in fluidextracts and tinctures. On the other hand, the tinctures and fluidextracts of Gelsemium and Veratrum are not so stable, the chemical assay methods being considered less reliable, therefore requiring a physiological assay.

II. DATA OF FLUIDEXTRACT OF NUX VOMICA U. S. P. IX.

Eleven samples of Fluidextract of Nux Vomica U. S. P. IX were selected, one for each year beginning 1917 to 1927 inclusive, each sample tested chemically and physiologically (White Mice, Lethal Dose Method). The following table represents the year made, chemical assay, bio-assay and per cent activity:

TABLE I.—FLUIDEXTRACT OF NUX VOMICA U. S. P. IX SAMPLES.

Sample.	Year made.	Chemical assay per cent total alkaloids.	Bio-assay M. L. D. M.	Bio-assay per cent.
1	1917	2.55	0.00015	100
2	1918	2.53	0.00015	100
3	1919	2.45	0.00015	100
4	1920	2.52	0.00015	100
5	1921	2.31	0.00015	100
6	1922	2.52	0.00015	100
7	1923	2.59	0.00015	100
8	1924	2.61	0.00015	100
9	1925	2.77	0.00015	100
10	1926	2.63	0.00015	100
11	1927	2.487	0.00015	100

Table I shows no loss in activity, either chemically or physiologically, over a period of ten years.

A fluidextract of Nux Vomica U. S. P. IX, was prepared, March 7, 1927, divided into eight parts, seven of these samples received various amounts of hydrochloric acid, the hydrogen-ion concentration determined in each sample, tested chemically for strychnine and brucine alkaloids and physiologically assayed. The following Table II represents the results of six months' aging.

TABLE II.—FLUIDEXTRACT OF NUX VOMICA U. S. P. IX.

Hydrogen-Ion Concentration Factor.

Sample no.	HCl Gm. in 100 cc.	p_H.	Bio-assay M. L. D. M.	Bio-assay per cent.	Chemical assay per cent total alkaloids.	Chemical assay strychnine.	Chemical assay brucine.
1	0.360 Gm.	0.75	0.00015	100	2.469	1.062	1.407
2	0.288 Gm.	1.92	0.00015	100	.....	.....	.....
3	0.144 Gm.	3.98	0.00015	100	.....	.....	.....
4	0.072 Gm.	4.20	0.00015	100	.....	.....	.....
5	0.036 Gm.	4.60	0.00015	100	.....	.....	.....
6	0.018 Gm.	4.83	0.00015	100	.....	.....	.....
7	0.009 Gm.	5.03	0.00015	100	.....	.....	.....
8	none	5.30	0.00015	100	2.484	1.052	1.432

The above table shows that the hydrogen-ion concentration does not control the deterioration or stabilization of Fluidextract of Nux Vomica U. S. P. IX., no acid being required to control its therapeutic value. Charts I and II represent the curve of aging and hydrogen-ion concentration.

III. DATA ON FLUIDEXTRACT OF GELSEMIUM U. S. P. IX.

The alkaloids of gelsemium have been extensively studied over a period of years by Sayre and his co-workers, (4, 5, 6, 7, 8, 9, 10). The alkaloids of gelsemium according to these investigators, are gelsemine, gelseminine and sempervirine, the principal alkaloid being gelseminine. The chemical assay is not considered reliable. Pittenger has described a physiological assay method (11), which is more accurate than the chemical method.

The following table represents data on samples of fluidextract of Gelsemium U. S. P. IX, 1917 to 1927 inclusive, each sample tested by the chemical and physiological method (White Mice, Lethal Dose Method).

TABLE III.—FLUIDEXTRACT OF GELSEMIUM U. S. P. IX SAMPLES.

Samples.	Year made.	Chemical assay.	Bio-assay M. L. D. M.	Bio-assay per cent.
1	1917	0.521	0.00020	100
2	1918	0.490	0.00025	80
3	1919	0.525	0.00035	57.1
4	1920	0.550	0.00025	80
5	1921	0.490	0.00040	50
6	1922	0.510	0.00030	$66^2/_3$
7	1923	0.520	0.00025	80
8	1924	0.506	0.00035	57.1
9	1925	0.540	0.00025	80
10	1926	0.572	0.00030	$66^2/_3$
11	1927	0.530	0.00025	80

The above data show that the chemical and physiological methods do not correlate.

A fluidextract of Gelsemium U. S. P. IX was prepared on March 7, 1927, this was divided into 8 parts, 7 parts received various amounts of acid (HCl), the hydrogen-ion concentration of each sample determined and assayed chemically and biologically. The following table represents the data of six months' aging.

The determination of the hydrogen-ion concentration in fluidextract of Gelsemium is very difficult. The method found to give the best results is to dilute the fluidextract 1:2, filter and then make the p_H reading or determination.

TABLE IV.—FLUIDEXTRACT OF GELSEMIUM U. S. P. IX.

Hydrogen-Ion Concentration Factor.

Sample no.	HCl Gm. in 100 cc.	p_H.	Bio-assay M. L. D. M.	Bio-assay per cent.	Chemical assay.
1	0.360 Gm.	less than 1	0.00025	100	0.568%
2	0.288 Gm.	less than 1	0.00025	100	.....
3	0.144 Gm.	1.12	0.00025	100	.....
4	0.072 Gm.	2.43	0.00025	100	.....
5	0.036 Gm.	2.87	0.00025	100	.....
6	0.018 Gm.	3.15	0.00025	100	.....
7	0.009 Gm.	3.25	0.00025	100	.....
8	None Gm.	3.45	0.00025	100	0.572%

The above data show that the hydrogen-ion concentration does not control the deterioration and stabilization of Gelsemium. No acid is required to prevent deterioration in 6 months' time, however the sample with no acid has a distinct acid reaction and a p_H value of 3.45.

IV. DATA ON FLUIDEXTRACT OF VERATRUM U. S. P. IX.

The chemistry of veratrum alkaloids has been studied by Eden, (12), the pharmacological action by Pilcher and Sollmann, (13). The chemical assay of veratrum alkaloids as concluded by several authorities is not an indication of its therapeutic value. Houghton and Hamilton, (14), in 1905 reported the assay of a veratrum preparation on frogs; later, Pilcher, (15), reported a bio-assay method on frogs, and still more recently Rowe, (16), described the use of mice as a bio-assay method.

The following data represent the assay by the chemical and bio-assay (White Mice, Lethal Dose Method) methods of Fluidextract of Veratrum U. S. P. IX, samples made in 1920 to 1927, inclusive.

TABLE V.—FLUIDEXTRACT OF VERATRUM U. S. P. IX SAMPLES.

Sample.	Year made.	Chemical assay.	Bio-assay M. L. D. M.	Bio-assay per cent.
1	1920	1.020	0.00080	31
2	1922	0.940	0.00035	71
3	1923	1.029	0.00040	62.5
4	1924	1.010	0.00040	62.5
5	1925	1.060	0.00035	71
6	1927	1.080	0.00065	38.4

The above data show that the chemical method is unreliable and does not correlate with the bio-assay method.

A fluidextract of Veratum U. S. P. IX was prepared on March 7, 1927. This preparation was divided into 8 parts, to 7 parts various amounts of HCl were added, the hydrogen-ion concentration of each sample determined. Each sample

was assayed chemically and biologically. The following data represent 6 months' aging.

TABLE VI.—FLUIDEXTRACT OF VERATRUM U. S. P. IX.

Hydrogen-Ion Concentration Factor.

Sample no.	HCl Gm. in 100 cc.	p_H	Bio-assay M. L. D. M.	Bio-assay per cent.	Chemical assay.
1	0.360 Gm.	0.97	0.0060	4	1.066%
2	0.288 Gm.	1.15	0.0045	5	.....
3	0.144 Gm.	1.63	0.00095	26	.....
4	0.072 Gm.	3.12	0.00050	50	.....
5	0.036 Gm.	4.02	0.00055	45	.....
6	0.018 Gm.	4.37	0.00050	50	.....
7	0.009 Gm.	4.55	0.00050	50	.....
8	none	5.07	0.00070	35.7	1.047%

The above data indicate that the hydrogen-ion concentration will control the deterioration and stabilization of veratrum. Further study is necessary to determine definitely the required hydrogen-ion concentration.

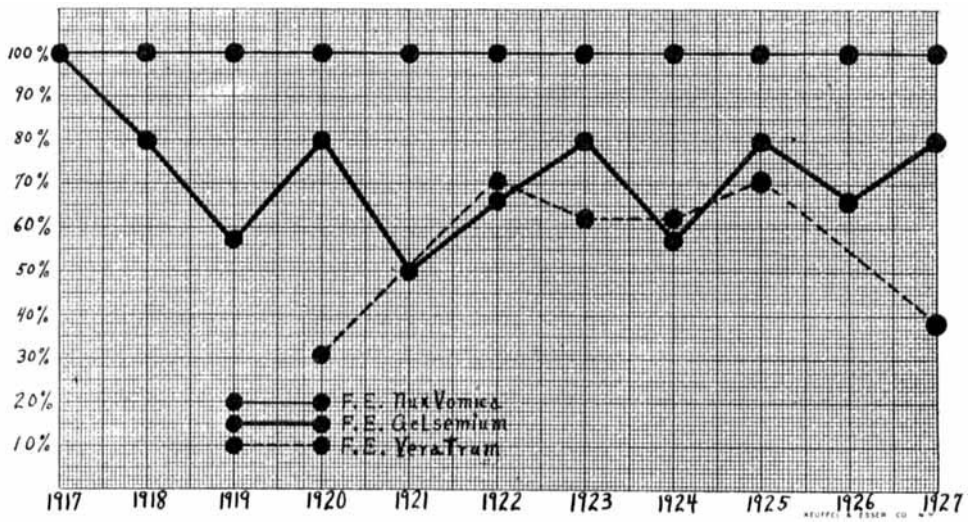

Chart I.—Aging test.

In Chart I the curve shows variation in the biological assay of samples prepared in the last seven years. In Chart II the hydrogen-ion concentration seems to show some control of the stability and deterioration of veratrum. The two curves represent the assay of the fluidextract of Veratum U. S. P. IX, with water dilution and 50% alcohol dilution. The water dilution shows a more regular constant curve.

CONCLUSIONS.

A discussion of the above data leads one to believe that not all fluidextracts require a definite hydrogen-ion concentration to control their deterioration and stabilization. Fluidextract of Nux Vomica U. S. P. IX is stable and requires no hydrogen-ion concentration factor, the chemical method and bio-assay method giving correlative results.

The fluidextract of Gelsemium U. S. P. IX requires no definite hydrogen-ion concentration to stabilize its alkaloids. However, the chemical method is not reliable and this preparation should be biologically assayed.

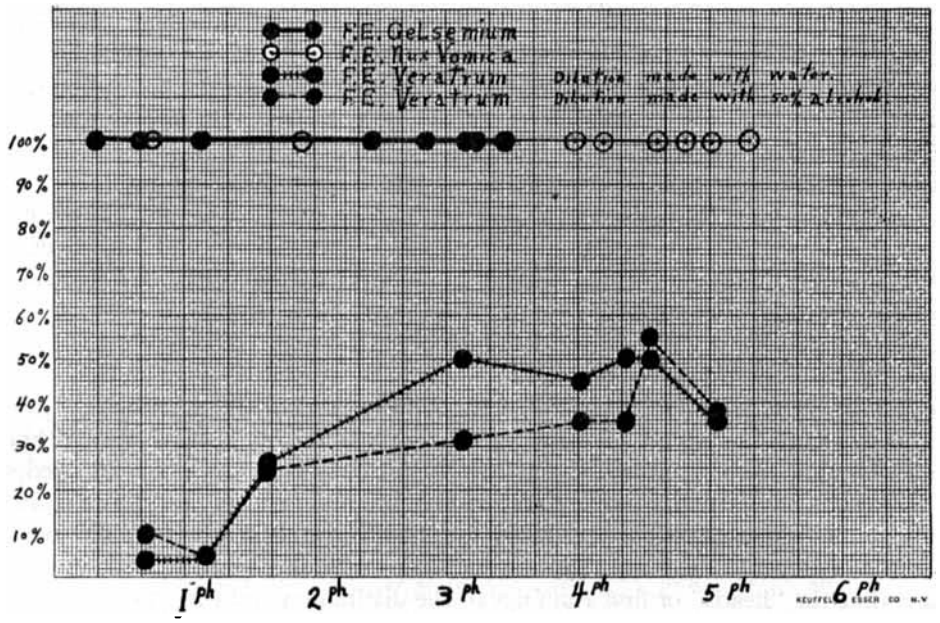

Chart II.

The fluidextract of Veratrum U. S. P. IX seems to require some definite hydrogen-ion concentration to control its deterioration and stabilization. The chemical method is unreliable and should be assayed biologically.

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