THE ASSAY OF SPIRIT OF ETHYL NITRITE, U. S. P. X–U. S. P. XI.*

BY RAY S. KELLEY.[1]

The first official assay of Spirit of Ethyl Nitrite is found in the sixth revision of the United States Pharmacopœia. By that method 10 Gm. of sample are treated with a fixed amount of potassa and the alcohol is allowed to evaporate spontaneously. The mixture, after the addition of a slight excess of diluted sulfuric acid, must discharge the color of 0.335 Gm. of potassium permanganate. Any sample meeting this specification was assumed to contain "at least 4 per cent of real Ethyl Nitrite."

The nitrometer method of assay was introduced into the seventh revision of the Pharmacopœia. In this revision the Spirit of Ethyl Nitrite was required to yield "eleven times its own volume of NO." Five cubic centimeters of sample were used which must yield 55 cc. of NO equal to about 4 per cent of Ethyl Nitrite.

The eighth, ninth and tenth revisions of the Pharmacopœia continued to recognize the nitrometer method of assay and Spirit of Ethyl Nitrite was required to contain an average of 4 per cent Ethyl Nitrite.

The eleventh revision of the Pharmacopœia discontinues the use of the nitrometer and introduces a titration method for the assay of Spirit of Ethyl Nitrite. Both the nitrometer method and the titration method of assay depend upon the reaction between Ethyl Nitrite, potassium iodide and a mineral acid with the liberation of nitric oxide and iodine. The former method measures the volume of nitric oxide evolved from a definite weight of sample and from this volume of gas the per cent of Ethyl Nitrite is calculated. The present titration method determines the per cent of Ethyl Nitrite by direct titration of the iodine liberated from a definite amount of sample.

For the purpose of this investigation each sample of Spirit of Ethyl Nitrite was assayed by three different methods.

Method I.—Twenty-five cubic centimeters of sample were introduced into a tared 100-cc. volumetric flask, containing 20–30 cc. of alcohol, and the weight of the sample determined. The volume was made up to 100 cc. with alcohol; 10 cc. of this solution were introduced into a Bradley nitrometer, previously filled with a saturated solution of sodium chloride. Ten cubic centimeters of a 20 per cent solution of potassium iodide were then added followed by 5 cc. of sulfuric acid, T.S. After the gas ceased to be evolved, in about one hour, the volume of gas was determined at room temperature. Each cc. of NO is the equivalent of 0.00307 Gm. Ethyl Nitrite, C_2H_5ONO.

Calculation: $\frac{0.00307 \times (\text{cc. NO}) \times 100}{{}^1/_{10} \times (\text{weight of sample})}$ = per cent C_2H_5ONO. Correction was then made for any variation of temperature or pressure from the standard.

Method II.—(U. S. P. XI method.) The specific gravity of the sample was determined at 25° C. by means of a pycnometer. The apparatus to be used in the assay consisted of a 300-cc. Erlenmeyer flask fitted with a 2-hole rubber stopper. An aeration tube, tapered to an internal diameter of about 1 mm. at the lower end and leading to the bottom of the flask, was passed through one hole in the stopper. Through the other hole was passed a glass tube of about 6-mm. internal diameter and extending about 1 cm. above and below the stopper. The aeration tube was connected with a cylinder of air-free carbon dioxide.

Ten grams of potassium iodide and 40 cc. of boiling distilled water were placed in the flask and a stream of carbon dioxide, about five bubbles per second, was passed through the solution

---

* Scientific Section, A. Ph. A., Dallas meeting, 1936.

[1] Assistant Professor of Chemistry in the Massachusetts College of Pharmacy.

until it had cooled to room temperature. Then 10 cc. of hydrochloric acid (1 in 2) were added to the contents of the flask and the stream of carbon dioxide was continued for at least three minutes, and any color produced by liberated iodine was discharged by the careful addition of tenth-normal sodium thiosulfate through the outlet tube. The flow of carbon dioxide was decreased to about two bubbles per second and 10 cc. of sample were added from a pipette. This was accomplished by passing the pipette through the outlet tube until the tip was just above the surface of the potassium iodide solution. The adhering nitrite solution was removed by touching the tip of the pipette to the outlet tube and then rinsing the outlet tube with a fine jet of aldehyde free alcohol. The liberated iodine was at once titrated with tenth-normal sodium thiosulfate by introducing the tip of the burette through the outlet tube. Each cc. of tenth-normal sodium thiosulfate is the equivalent of 0.007505 Gm. of Ethyl Nitrite. Calculation:

$$\frac{0.007505 \times (\text{cc. } 0.1\ N\ Na_2S_2O_3) \times 100}{10 \times (\text{specific gravity of sample})} = \%\ C_2H_5ONO.$$

Method III.—The apparatus and method is similar to Method II with the following change. Twenty-five cubic centimeters of sample were introduced into a tared 100-cc. volumetric flask containing 20–30 cc. of alcohol and the weight of sample determined. The volume was made up to 100 cc. with alcohol and 10 cc. of this dilution were introduced into the Erlenmeyer flask and the liberated iodine at once titrated with tenth-normal sodium thiosulfate. Each cc. of tenth-normal sodium thiosulfate is the equivalent of 0.007505 Gm. of Ethyl Nitrite. Calculation:

$$\frac{0.007505 \times (\text{cc. } 0.1\ N\ Na_2S_2O_3) \times 100}{{}^1/_{10} \times (\text{weight of sample})} = \%\ C_2H_5ONO.$$

The following table shows the results obtained in the assay of Spirit of Ethyl Nitrite by the three methods described above:

TABLE OF RESULTS.

Sample.	Specific Gravity at 25° C.	Per Cent of Ethyl Nitrite Found. Method I.	Method II.	Method III.
1	0.8119	3.87	4.22	4.31
2	0.8124	4.03	4.10	4.22
3	0.8120	4.64	4.41	4.64
4	0.8120	4.25	4.21	4.60
5	0.8112	3.80	3.89	4.20
6	0.8123	3.79	3.69	3.98
7	0.8119	3.89	3.95	4.13
8	0.8117	4.12	4.34	4.68
9	0.8124	5.22	4.97	5.38
10	0.8128	4.48	4.36	4.75
11	0.8118	4.26	4.15	4.57
12	0.8124	4.05	4.07	4.39

CONCLUSIONS.

Method I.—This method is essentially that of the tenth revision of the United States Pharmacopœia. The actual time spent in carrying out the assay is somewhat less than that for the titration method. The results on duplicate assays check within 0.2 cc. of nitric oxide gas evolved.

Method II.—Gas was evolved through the sample when measured at 25° C. Iodine was liberated through the outlet tube when the sample came in contact with the acid potassium iodide solution in the flask. A variation of from 0.1 to 0.7 cc. of tenth-normal sodium thiosulfate was noted in the titration of duplicate samples. These three factors were especially noticeable in samples containing more than 4 per cent of Ethyl Nitrite.

Method III.—This modification seems to overcome the difficulties noted under Method II. The calculations are based on the actual weight of sample used rather than on a weight calculated from the specific gravity. A variation of 0.1 cc. of tenth-normal sodium thiosulfate was the maximum noted in duplicate assays.

THE PRESERVATIVE CAPACITY OF SODIUM FORMALDEHYDE SULFOXYLATE IN CERTAIN MEDICINAL PREPARATIONS.*

BY JOHN C. KRANTZ, JR., C. JELLEFF CARR AND RUTH MUSSER.[1]

Recently sodium formaldehyde sulfoxylate has been employed in the treatment of bichloride of mercury poisoning in experimental animals and man by Rosenthal (1 and 2). This work has conferred therapeutic prominence upon this compound. In working with this substance in a study of its effect in rat sarcoma (3) the authors became interested in its properties as a reducing agent in the preservation of medicinal preparations. The stability of sodium formaldehyde sulfoxylate in acid and alkaline solutions has been studied by Jurist and Christiansen (4).

MATERIALS AND METHODS.

The sodium formaldehyde sulfoxylate was obtained through the courtesy of Joseph Rosin, Merck and Co. Inc. of Rahway, N. J. The solutions studied under laboratory conditions in flint glass bottles were:

1. Epinephrine (in normal salt solution), 1–10,000
2. Physostigmine Salicylate, 0.2 per cent
3. Physostigmine Hydrochloride, 0.2 per cent
4. Morphine Sulfate, 1 per cent
5. Glycerite of Phenol
6. Solution of Resorcinol, 1 per cent
7. Sodium Bicarbonate-Sodium Salicylate, 6 per cent each.

To each of these solutions was added sodium formaldehyde sulfoxylate in concentrations of 1–500, 1–1000 and 1–5000, respectively. Observations for changes in color were made at 15-day intervals over a period of four months.

RESULTS.

Over the four-month period all solutions containing sodium formaldehyde sulfoxylate as a preservative in each concentration employed prevented color change. On the other hand the control solutions in each instance showed signs of color change from within one to four weeks. The characteristic color changes produced by oxidation were not found in any of the preserved solutions.

DISCUSSION.

The preservation of epinephrine solution has been the source of a considerable amount of investigation. Quite recently ascorbic acid has been employed for this purpose (5). In view of the fact that the pharmaceutical elegance of the preparation was preserved it was decided to test its medicinal potency. Under ether anesthesia, intravenous injections were made into dogs and the carotid blood pressure was measured. The results are shown in Table I. A study of

---

* Scientific Section, A. PH. A., Dallas meeting, 1936.

[1] Department of Pharmacology, School of Medicine, University of Maryland.