Some 25 lots of synthetic riboflavin have been tested. The solutions were made up in the same manner as the standard solution of riboflavin and compared with the standard riboflavin.

4.75%	of the samples assayed	950,000 μg./Gm.
4.75%	of the samples assayed	960,000 μg./Gm.
9.5%	of the samples assayed	970,000 μg./Gm.
28.6%	of the samples assayed	980,000 μg./Gm.
19.5%	of the samples assayed	990,000 μg./Gm.
33.0%	of the samples assayed	1,000,000–1,020,000 ug./Gm.

Of this series 81% showed 980,000 μg. or better per Gm. of material.

SUMMARY

1. The method of Hodson and Norris for the fluorophotometric determination of riboflavin and a modification of it have been applied to both simple preparations and complex mixtures, natural and artificial.

2. Fair agreement between biological and fluorophotometric determination of riboflavin exists.

The authors appreciate the help of Mr. Frank Howland, Development and Control Laboratory, in carrying out the determinations of riboflavin.

REFERENCE

(1) Hodson and Norris, *J. Biol. Chem.*, 131 (1939), 621.

Book Review

Traffic in Opium and Other Dangerous Drugs for the Year Ended December 31, 1940. Bureau of Narcotics, U. S. Treasury Department. 105 pages. 1941. Washington, D. C.: Government Printing Office. Price, paper, 25 cents.

This publication is the annual report of the Commissioner of Narcotics of the United States. It contains detailed information regarding raw materials, manufactured drugs, new legislation, administration of the Uniform Narcotic Drug Act, control of international trade and coöperative efforts of the nations of the world to control illicit traffic. The number of known medical drug addicts is estimated not to exceed 1 in 3000 population, which represents a reduction of at least 66 per cent in the past two decades. The report should make interesting reading for pharmacists and others interested in the problems of drug addiction.—A. G. D.

Photoelectric Determination of Nicotinic Acid*

*By Wm. S. Jones**

Nicotinic acid, nicotinamide or compounds containing the nicotinic acid nucleus play an important nutritional role as the black tongue and pellagra-preventing vitamin. Recognition of this makes it highly desirable that a rapid method be available for the assaying of materials and products containing this factor.

Many investigators have studied the cyanogen-bromide method of determining nicotinic acid. Harris and Raymond (1) and Kodicek (2) considered *p*-amino acetophenone a more satisfactory amine for this purpose. Kodicek studied further the various factors affecting the reactions and developed a satisfactory method for the extraction and determination of nicotinic acid. The sodium hydroxide hydrolysis to which the sample is subjected gives rise to highly colored substances that interfere badly with the subsequent measurement. Melnick and Field (3) overcame this difficulty by preferential adsorption of the colored substances on Darco G-60 also known as Darco, Coleman and Bell. In our hands this procedure for removal of color gave rise to a loss of nicotinic acid and we did not pursue it further. Other investigators, Arnold, Dehreffley and Lipsuirs (4), have followed the practice of extracting the colored cyanogen-amine compound of nicotinic acid with ethyl acetate and measuring the color photoelectrically. However, even under these conditions the blanks show more color than is desirable. Furthermore, the extra operations incident to the ethyl acetate extraction are time consuming. Moreover, we find that the intensity of the color in the ethyl acetate is subdued and is less, *i. e.*, $^1/_2$ to $^1/_3$, than that in aqueous alcoholic solutions and this is a disadvantage.

Giri and Naganna (5) describe a method in which the foodstuff is hydrolyzed with

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* From the Development Division of the Chemical and Pharmaceutical Laboratories of E. R. Squibb and Sons, Brooklyn, N. Y.

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sodium hydroxide, the nicotinic acid adsorbed on medicinal charcoal, then eluted with an alcoholic-sodium hydroxide solution and estimated by the cyanogen-*p*-amino-acetophenone reaction.

By means of nitrous acid, nicotinamide can be converted to the acid. Inasmuch as the factor needs to be in the form of nicotinic acid for measurement by this cyanogen-bromide method, this reaction serves as a means of making it possible to assay materials in which the amide is present.

There is also the possibility that enzyme hydrolysis might be used in place of the alkali or acid hydrolysis and the nitrous acid conversion of the amide as a means of preparing the material for assay. Thus, Thakur and Norris (6) have shown that an amidase, isolated from aspergillus flavus, splits off ammonia quantitatively, from asparagine at a pH 6.3–8.5. Also, in studying the "amide-splitting" ability of yeasts, Gorr, Gunther and Toagner (7) have shown that *Torula Utilis*, fresh or dried, splits off ammonia from certain amides.

In our effort to set up a simple method for determining nicotinic acid, we have resorted to the procedure of hydrolyzing the sample with 20% sodium hydroxide solution, neutralizing to a pH of 4–5 with 60% sulfuric acid solution (keeping the volume small) and treating with acetone to precipitate the proteins, salts and practically all colored substances, the nicotinic acid remaining in solution. The acetone solution of the nicotinic acid is filtered off and evaporated. Any residual foreign coloring matter can be further reduced by treating the acetone solution before evaporation with Darco G-60 (0.2 Gm. per 100 cc.). The subsequent aqueous solutions then yield satisfactory, low-colored blanks. Using a method of this type, described in detail below, a variety of material has been assayed and the findings are shown in attached table.

METHOD

(*A*) *Reagents.*—1. Cyanogen bromide solution: Cold 10% potassium cyanide solution is added dropwise to cold saturated bromine water until the latter is just decolorized.

2. Amino reagent: Five Gm. of *p*-amino acetophenone and 10.2 cc. of concentrated hydrochloric acid (sp. gr. 1.19) are made up to 50 cc. with distilled water.

3. Twenty per cent sodium hydroxide solution (243.8 Gm. sodium hydroxide per liter).

4. Sixty per cent sulfuric acid solution (899 Gm. sulfuric acid per liter).

5. Standard nicotinic acid solution 20 μg. per cc.—prepared daily by diluting an alcoholic solution, containing 1000 μg. nicotinic acid per cc., with water.

6. Phosphate buffer solution: To 16.5 Gm. of 85% potassium hydroxide, 19.1 cc. 85% phosphoric acid and 82 cc. of *N* sodium hydroxide solution is added sufficient distilled water to make 250 cc.

7. Absolute alcohol.

8. Acetone.

(*B*) *Hydrolysis, Neutralization and Acetone Treatment.*—To the sample (this should be of the order of 0.1 to 0.5 Gm. depending upon the potency of the material being assayed) in a 125-cc. Erlenmeyer flask are added 3 cc. of 20% sodium hydroxide solution and 5 cc. of distilled water. The mixture is heated on the steam bath for one hour with occasional shaking—a glass funnel is placed in the mouth of the Erlenmeyer flask to prevent excessive loss of water by evaporation. The mixture is cooled, neutralized and adjusted to a pH of 4–5 by means of 1.0 cc. of 60% sulfuric acid. The volume is kept small and at this point certain protein material and humins (colored substances) precipitate out. After 80 cc. of acetone have been added, the contents of the flask are heated at the boiling point with agitation for a minute and then cooled to room temperature. At this point the bulk of the sodium sulfate, proteins and colored substances exist as a solid phase which is removed by suction filtration through a filter aid on paper on a Büchner funnel. The residue on the filter is washed with several 10-cc. portions of acetone. The combined acetone filtrate and washings is freed of its acetone by evaporation on the steam bath. The small residual aqueous phase is taken up in 20 cc. of distilled water[1] and transferred to a 250-cc. volumetric flask. Then 5 cc. of the phosphate buffer solution are added and the volume made up to the mark with distilled water. The pH varies from 6–6.5.

(*C*) *Opening Pyridine Ring.*—Four 25-cc. amber graduated cylinders are employed. To cylinders 3 and 4 are added, respectively, 1 cc. and 2 cc. of the standard nicotinic acid solution. To each of the four cylinders are added 10 cc.[2] of the "sample solution." To cylinders 1, 2 and 3 are added, respectively, 4 cc., 2 cc. and 1 cc. of distilled water. The cylinders are stoppered and heated in a hot water bath at 70–80° C. for 10 minutes. To each of cyl-

[1] If any precipitate or oil (fatty acids from the saponification of fats) is present, the aqueous phase is filtered through paper, washing with several 20-cc. portions of distilled water.

[2] This 10 cc. should contain 20 μg. or less of nicotinic acid. If that is not the case the "sample solution" should be diluted further.

inders 2, 3 and 4 are added 2 cc. of the cyanogen-bromide solution allowing exactly 10 seconds between the additions to the several cylinders. Each is vigorously shaken and returned to the hot water bath and heated for another 5 minutes. At the end of the 5-minute period the cylinders are removed in order at 10-second intervals and transferred to an ice water bath and chilled quickly.

(*D*) *Production of Color.*—After standing 5 minutes in the ice water bath, 0.5 cc. of the *p*-amino-acetophenone solution is added to each cylinder. The volume is now 14.5 cc. The cylinders are placed in the dark.

(*E*) *Measurement of Color.*—After standing 5 minutes in the dark the volume is made up to 25 cc. with absolute alcohol (11.2 cc. required) and the color is measured photoelectrically within the next 4 minutes in the Pfaltz and Bauer Fluorophotometer using a G. E. Mercury Lamp, Type H3, 85 Watts as the source of light. Filter glass (Jena), a combination GG-3 and BG-12 (Corning combination is 038 and 511) giving a peak at 4500 A., is placed between the solution and the light source. The iris diaphragm, which controls the amount of light admitted to the solution, is practically closed.

To set the instrument place 12.5 cc. of aqueous alcohol (14.5 cc. distilled water and absolute alcohol *q. s.* 25 cc.) in the 4-cm. cuvette; set the transmission switch at highest intensity and the variable resistance at 10, and the TrH reading on the galvanometer is then set at zero extinction by means of a shunt resistance.

Now place 12.5 cc. of aqueous alcoholic solution from each of the four cylinders individually in order into the 4-cm. glass cuvette and read the extinction directly from the galvanometer. The extinction for the blank (cylinder 1) is subtracted from the readings for the solutions in cylinders 2, 3 and 4, thereby giving values known as *A*, *B* and *C*. The nicotinic acid is calculated as follows:

$$\frac{A}{(B + C) - 2A} \times \frac{60}{1} \times \frac{25}{1} \times \frac{1}{\text{amt. of sample}}$$

(Amt. = cc., grams, capsules, tablets taken.)

In the attached table the materials tested and the potencies found are shown together with the values which, from other work, have been considered the nicotinic acid content of the substance in question. These latter values are in some instances based on bio- and microbiological assays, and, in others, on known amounts of nicotinic acid (or amide) used in making the mixtures.

SUMMARY

1. Cyano-*p*-amino-acetophenone reaction has been applied satisfactorily to the determination of nicotinic acid in pharmaceutical products.

2. By treating the alkali hydrolyzates of materials containing nicotinic acid with acetone the salts, proteins and colored substances, which interfere with photoelectric measurement of the cyano-*p*-amino-acetophenone color compound of nicotinic acid, are removed to a point where they no longer have any significant effect.

Table of Potencies

	Sample Taken or Potency Expected	Found
Nicotinic acid	0.500 mg.	0.499 mg.
Nicotinamide	0.500 mg.	0.495 mg.
Nicotinic acid tablet	100 mg./tab.	111.7 mg./tab.
	50 mg./tab.	40 mg./tab.
Nicotinamide capsule	50 mg./cap.	56.8 mg./cap.
Yeast powder, type A	0.3–0.66 mg./Gm.	0.50 mg./Gm.
		0.53 mg./Gm.
		0.86 mg./Gm.
		0.68 mg./Gm.
		0.65 mg./Gm.
Powder containing B complex, type B	0.5 mg./Gm.	0.80 mg./Gm.
		0.70 mg./Gm.
		0.54 mg./Gm.
		0.64 mg./Gm.
		0.67 mg./Gm.
Powder containing B complex, type C	2.0–2.5 mg./Gm.	2.63 mg./Gm.
		2.38 mg./Gm.
		2.31 mg./Gm.
		2.29 mg./Gm.
Syrup containing B complex	2.2–2.5 mg./cc.	2.46 mg./cc.
		2.79 mg./cc.
		3.29 mg./cc.
Yeast concentrate, liquid	1.25–2.75 mg./cc.	1.56 mg./cc.
Mixture of B factors in capsules	5.0 mg./cap.	5.6 mg./cap.
		5.04 mg./cap.
		5.43 mg./cap.
		5.2 mg./cap.
Paste in capsules, type III	10 mg./cap.	10.8 mg./cap.
Paste in capsules, type IV	10 mg./cap.	9.85 mg./cap.
		11.83 mg./cap.
Paste in capsules, type V	20 mg./cap.	20.6 mg./cap.
Paste in capsules, type VII	5.0 mg./cap.	5.27 mg./cap.
		5.20 mg./cap.
		4.85 mg./cap.
Paste in capsules, type IX	5.0 mg./cap.	5.5 mg./cap.
		5.1 mg./cap.
		5.3 mg./cap
Paste in capsules, type XI	5.0 mg./cap.	4.95 mg./cap

The items listed as Paste in capsules are in all instances multivitamin mixtures with suitable diluents and vehicles in soft elastic capsules.

REFERENCES

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The Solubility of Ephedrine in Liquid Petrolatum*

By Joseph Rosin, G. K. Eger and Harry Mack

Ephedrine base is extensively used as a therapeutic agent. It is generally formulated as a solution in light liquid petrolatum, commonly called "spray" or "inhalation." Accurate knowledge of the solubility of ephedrine in this solvent is, therefore, very desirable. There is, however, very little information on this subject in the literature. Moreover, ephedrine base is available in the form of a hemihydrate and also as the anhydrous article. The U. S. P. specifications permit both the hemihydrate and the anhydrous, and some difference in the degree of solubility might be expected.

The laboratory of the AMERICAN PHARMACEUTICAL ASSOCIATION (1) has reported the solubility of ephedrine in light and heavy liquid petrolatum at 25° C. It is not, however, clear from their report what type of ephedrine they used, whether anhydrous or the hydrated, and if the latter, how much water it contained and whether or not it was dried before use in their determinations. As will be shown, the water content of the ephedrine has a profound effect on the degree of solubility.

In addition to the solubility at 25° C. we also determined the solubility at 20° C.—a temperature which is frequently met with in the stock rooms of manufacturers and retail pharmacists.

EXPERIMENTAL

The solubility determinations at 20° and 25° C. were made with different lots of ephedrine and of liquid petrolatum. The hydrated ephedrine used at 20° C. showed by titration 95.26% anhydrous ephedrine, and by toluene distillation it indicated 4.65% of water. The "anhydrous" ephedrine titrated 99.8%, and by toluene distillation showed 0.27% water. The light liquid petrolatum used in these determinations had a specific gravity of 0.845^{25}_{25}.

* Presented to the Scientific Section of the A. PH. A., Detroit meeting, 1941.

For the solubility determinations at 25° C. the hydrated ephedrine used titrated 95.12% of anhydrous ephedrine and the "anhydrous" alkaloid 99.37%. The light liquid petrolatum used in these tests had a specific gravity of 0.852, and the heavy, 0.868 at 25/25. The kinematic viscosity at 37.8° C. of the light oil was 0.183, and of the heavy, 0.438.

The solubilities at 20° C. were made as follows, except 24[e] which was obtained with the ephedrines and the oils used for solubilities at 25° C.

The liquid petrolatum was heated between 45° and 50° C. An excess of the ephedrine amounting to 50–100% of the assumed solubility was added, and the mixture shaken in a mechanical shaker for about 6 hours. After allowing to stand over night or longer, at room temperature, the mixture was maintained at 20° C. for 5 to 6 hours with frequent agitation. A portion of the mixture was filtered through a glass filter, and the ephedrine determined in an accurately measured volume of 10 or 15 cc. of the clear filtrate. The remainder of the mixture was again shaken at room temperature in a mechanical shaker for several hours and on the following day kept at 20° C. for 5 to 6 hours with frequent shaking, and the ephedrine again determined in a filtered portion. The results are shown in Table I.

Table I.—Solubility of Ephedrine in Light Liquid Petrolatum at 20° C.

Number of Hours of Contact	Hemihydrated Ephedrine Gm. of Anhydrous Ephedrine per 100 Cc. of Soln.	Anhydrous Ephedrine Gm. of Anhydrous Ephedrine per 100 Cc. of Soln.
48	0.87	2.26
96	0.81	2.24
24[e]		2.207
Av.	0.84	2.235

The solubility at 25° C. was determined in a similar manner, except that the petrolatum was not preheated. The ephedrine was added to the petrolatum at room temperature (about 25° C.), the mixture shaken at room temperature for 6 hours or longer, and before withdrawal of a portion for the determination, the mixture was maintained at 25° C. for 5 to 6 hours with frequent shaking. The solubility at 25° C. was also determined for light and heavy liquid petrolatum. The results are shown in Table II.

The extraction of the alkaloid from the oil solution was effected with a moderate excess of approximately half-normal sulfuric acid. The acid solution was alkalinized with sodium hydroxide and shaken with 6 to 8 portions of ether. The combined ether extracts were shaken with two 10-cc. portions of water to remove any sodium hydroxide, and the water washings were extracted with two 10-cc. portions of ether and the latter added to the main ether solution. The bulk of the ether was allowed to evaporate at practically room temperature, then a measured excess of twentieth-normal sulfuric acid added. The mixture was then heated gently to expel all the ether, cooled, and the excess acid titrated