more than expected. To explain such deviations, several blank determinations were performed to ascertain if any solvent-titrant reaction occurred. No discernible reaction was observed, which suggests that the precision might be improved by further purification of these materials.

It has been found in these laboratories that the hydroxyl and phenolic protons of salicylic acid can be titrated separately in this solvent. Work on the differentiating ability of the solvent is currently in progress. This preliminary study shows 3-methyl-2-oxazolidone to be a promising solvent in pharmaceutical assays by titration.

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[x] To whom inquiries should be directed.

PHARMACEUTICAL TECHNOLOGY

Automated Analysis of Riboflavin in Multivitamin Preparations

OMER PELLETIER [x] and RENÉ BRASSARD

Abstract □ An automated procedure was developed for the determination of riboflavin by following the steps of the USP manual procedure. The automated procedure is applicable to different types of multivitamin products and yielded results equivalent to the manual procedure.

Keyphrases □ Riboflavin—automated analysis, multivitamin preparations, compared to official method □ Multivitamin preparations—automated analysis of riboflavin, compared to official method □ Automated analysis—riboflavin in multivitamin preparations

Methods recognized officially by the Association of Official Analytical Chemists (1) or USP (2) for the determination of riboflavin in pharmaceutical preparations are described exclusively as manual techniques. These determinations consist of measuring the fluorescence of riboflavin in an acetic acid medium after destroying interfering materials by permanganate oxidation and decolorizing excess permanganate with hydrogen peroxide. Other interfering materials are measured separately after reducing riboflavin with sodium hydrosulfite. Automated procedures (3–7) previously proposed differed from the official methods (1, 2) either by omitting some steps or by using different chemical principles. An automated system was developed that maintains the specificity and validity of the manual procedures by performing exactly the same steps.

EXPERIMENTAL

Equipment—The analytical system[1] consisted of the following modules: liquid sampler II, a proportioning pump (model II), a fluorometer II, and a recorder.

Reagents—*Hydrochloric Acid, 0.1* N—Dilute 8.5 ml of concentrated hydrochloric acid (analytical reagent) to 1 liter with distilled water.

Acetic Acid and Sodium Acetate Solution (pH 2.4)—Dissolve 10.25 g sodium acetate (analytical reagent) in 300 ml distilled water, add 600 ml acetic acid, and dilute to 1 liter with distilled water.

Potassium Permanganate, 0.2% (w/v)—Dissolve 0.4 g of potassium permanganate (analytical reagent) in 200 ml distilled water. Prepare fresh daily.

Hydrogen Peroxide, 0.15% (w/v)—Dilute 1 ml of 30% H_2O_2 (analytical reagent) to 200 ml with distilled water. Prepare fresh daily.

Sodium Hydrosulfite, 5% (w/v)—Dissolve 5 g of anhydrous sodium hydrosulfite (analytical reagent) in distilled water and dilute to 100 ml. Prepare daily just before use and keep in an amber bottle under toluene.

Ascorbic Acid, 4% (w/v)—Dissolve 4 g ascorbic acid[2] in 100 ml distilled water.

Standards—*Standard Riboflavin Stock Solution (10 μg/ml)*—

[1] Technicon.

[2] Nutritional Biochemicals Corp.

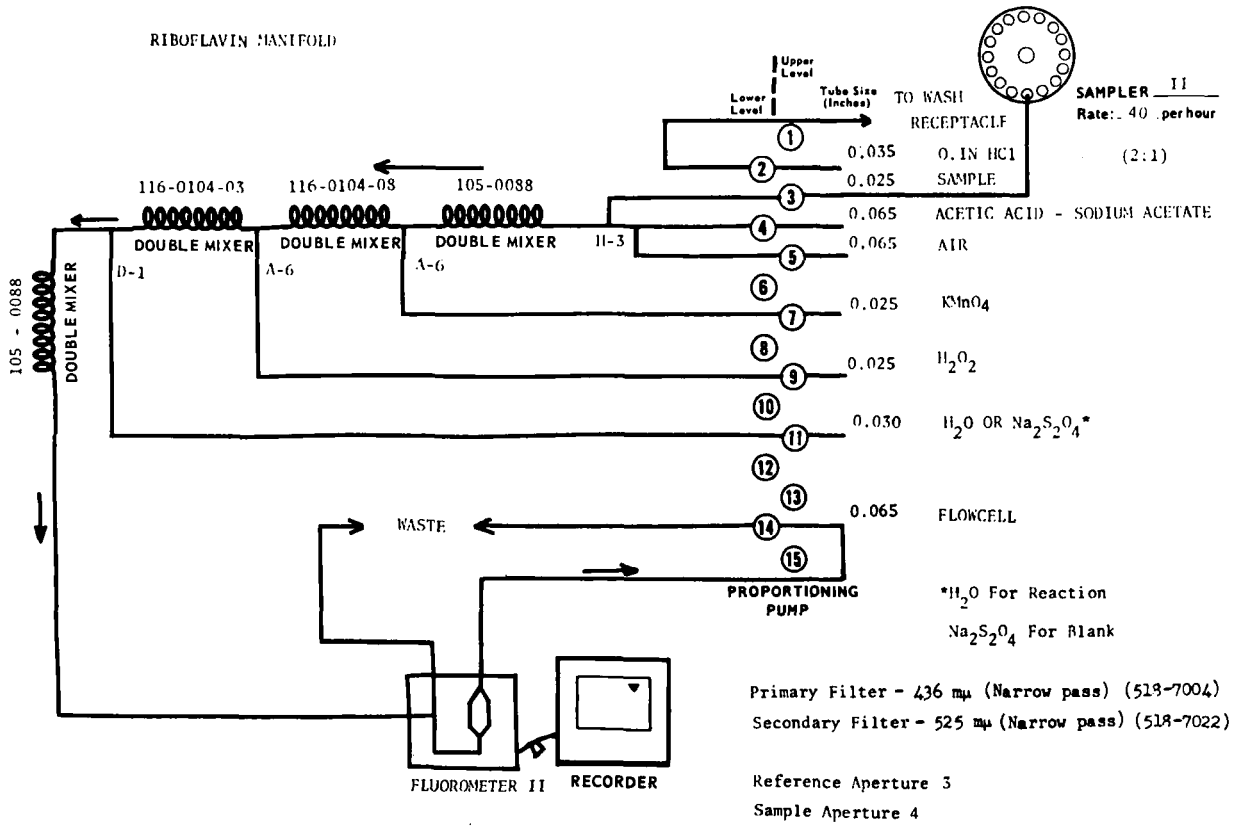

Figure 1—*Diagram of flowthrough system for determining riboflavin.*

Prepare as described for the USP procedure (2).

Intermediate Riboflavin Solution (1 μg/ml)—Dilute 10 ml standard riboflavin stock solution to 100 ml with 0.1 *N* HCl.

Working Riboflavin Solutions (0.05, 0.10, 0.15, 0.20, and 0.25 μg/ml)—Dilute 5, 10, 15, 20, and 25 ml of intermediate riboflavin solution (1 μg/ml) to 100 ml with 0.1 *N* HCl.

Controls—*Stock Riboflavin Control Solution (9 μg/ml)*—Prepare in the same way as described for the standard riboflavin stock solution (2) by weighing 45 mg and diluting to 9 μg/ml.

Working Control Solution (0.18 μg/ml)—Dilute 20 ml stock riboflavin control solution to 1 liter with 0.1 *N* HCl. Transfer volumes of approximately 40 ml into 50-ml brown[3] bottles. Store in a freezer and prepare monthly. Thaw one control bottle daily and mix vigorously before use.

Sample Preparation—Sample preparation and all the other steps of the analytical procedure must be performed in subdued light to prevent destruction of riboflavin by light.

Tablets and Capsules[4]—Grind a sample of at least five tablets to a fine powder and transfer quantitatively to a 1-liter flask with approximately 900 ml 0.1 *N* HCl. For soft core tablets or capsules, replace the grinding process by blending for about 2 min with 0.1 *N* HCl in the glass container of a blender[5]. Similarly, blend hard core capsules with 0.1 *N* HCl but use 2 ml of ethylene dichloride and 0.2 ml of acetic acid per capsule. Transfer the blended material quantitatively with 0.1 *N* HCl to a 1-liter volumetric flask and make to a volume of approximately 900 ml. Heat flask in a hot (nearly boiling) water bath with frequent shaking until a good dispersion is obtained and continue heating for an additional 15 min or autoclave at 15 lb of pressure for 15 min. Let the solution cool and dilute to 1 liter with 0.1 *N* HCl. Filter and dilute an aliquot with 0.1 *N* HCl to an expected concentration of about 0.2 μg riboflavin/ml.

Liquid Materials—Dilute an aliquot of the sample to an expected concentration of about 0.2 μg riboflavin/ml with 0.1 *N* HCl.

[3] Nalgene.

[4] Automated Technicon equipment (solid sampler) is also available to solubilize riboflavin from tablets and capsules.

[5] Waring.

Procedure—Start the automated system (Fig. 1) about 30 min before initiating the analysis. Set the reference and sample aperture of the fluorometer at the No. 3 and No. 4 positions, respectively. To avoid the formation of manganese dioxide precipitate, introduce the acetic acid and sodium acetate solution before potassium permanganate. Pump all reagents until an equilibrium is obtained and set the baseline between 2 and 5%.

Place the standards, samples, and controls into polystyrene cups of 2 ml after rinsing each cup with the solution to be analyzed to avoid contamination. Run the sequence as described in Table I.

Before determining blanks, run three cups of the 0.20-μg/ml standard. To obtain blanks, pump 5% sodium hydrosulfite in place of distilled water through tube No. 11 (Fig. 1) and run the sequence described in Table I. Rinse tube No. 11 by pumping dis-

Table I—Disposition of Standards[a] and Samples[b]

Baseline, 0.1 *N* HCl
Standard curve 1
0.05 μg/ml
0.10 μg/ml
0.15 μg/ml
0.20 μg/ml
0.25 μg/ml
Baseline, two cups 0.1 *N* HCl
Standard curve 2 (as curve 1)
Baseline, two cups 0.1 *N* HCl
Standard, 0.20 μg/ml
Control, 0.18 μg/ml
Ten samples
Baseline
Standard, 0.20 μg/ml
Control, 0.18 μg/ml
Ten samples
Baseline
Standard, 0.20 μg/ml[c]

[a] For blanks, run the standard of 0.20 μg/ml in triplicate before and after the sodium hydrosulfite treatment. [b] Every hour, run a cup of 4% ascorbic acid to remove traces of coating by potassium permanganate. [c] For additional samples, continue the same sequence starting with one standard and one control before every 10 samples.

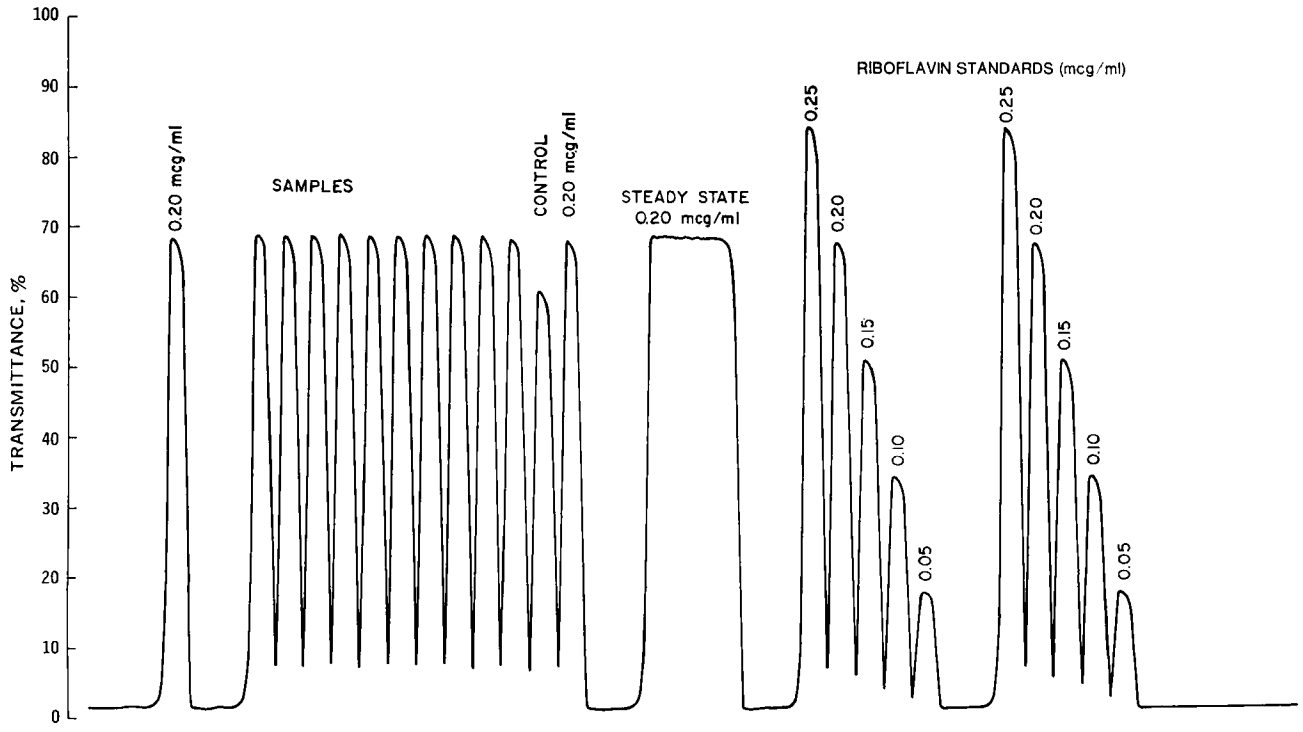

Figure 2—*Typical recordings from the automated analysis of riboflavin.*

tilled water for about 5 min before running three additional cups of the 0.20-μg/ml standard.

After completing the analysis, pump distilled water through all of the tubing except No. 14, which must be placed into water only after the potassium permanganate solution is pumped out.

Calculations—*Standard Curves*—Calculate the net fluorescence (% F) of each level of standard by subtracting from the peak the baseline averaged from before and after the standards. Calculate the slope, b, by:

$$b = \sum Y / \sum X \qquad \text{(Eq. 1)}$$

where X is the concentration, and Y is the net % F of the standard. Using the net % F of the 0.20-μg/ml standard, calculate its concentration and calculate its efficiency, E, by:

$$\text{concentration} = \frac{Y}{b} \qquad \text{(Eq. 2)}$$

and:

$$E = \frac{\text{concentration} \times 100}{0.2} \qquad \text{(Eq. 3)}$$

Follow the same procedure for the second standard curve and obtain the average slope, $\bar{b}$, and efficiency, $\bar{E}$, for the two standard curves.

Samples—Before and after every group of 10 samples, average the % F of baselines and subtract from the mean % F of the two peaks of 0.20 μg/ml standard to obtain the net % $\bar{F}_{1,2}$. Also subtract the average baseline value from the control and sample peaks to obtain the net % $F(Y)$.

Calculate the corrected slope, b^1, the percentage change of b^1 relative to b, and the concentration, X, by the following:

$$b^1 = \frac{\%\ \bar{F}_{1,2}}{0.01\bar{E} \times 0.2} \qquad \text{(Eq. 4)}$$

$$\%\ \text{change of}\ b^1 = \frac{b^1 \times 100}{\bar{b}} \qquad \text{(Eq. 5)}$$

$$X = \frac{Y}{b^1} \qquad \text{(Eq. 6)}$$

Blanks—Average the % F of the baseline before and % F of the baseline after the blanks of all of the samples, and subtract from the mean % F of three peaks of the 0.20-μg/ml standard which precede and follow these blanks. Subtract the average baseline (before and after 10 samples) from the blanks of the standards, control, and samples treated with sodium hydrosulfite. A value of about zero should be obtained with the standards, control, and most samples. Make calculations of the corrected slope, the percent change of the slope, and concentrations with Eqs. 4–6. Subtract the blank value from the corresponding sample value and multiply by the dilution factors to obtain the true riboflavin concentration.

Controls—Calculate the mean value of the controls and their standard deviations to evaluate the reproducibility on the day of analysis and the variation from day to day.

Table II—Sample Interactions[a] at Four Levels

Sample Levels, μg/ml	Range[b] of Interaction	Range[b] of Coefficient of Variation	
		Random	Continuous
0.05	1.00–1.02	0.5–0.7	0.4–0.7
0.10	1.00–1.01	0.5–1.0	0.4–0.5
0.15	1.00–1.01	0.4–0.9	0.3–0.4
0.20	1.00–1.00	0.4–0.8	0.2–0.8

[a] Interaction = ratio of 20 samples at one level randomly mixed with 20 samples from each of the other three levels over 20 samples of the same level in one continuous sequence. [b] Range obtained from assays on 3 different days.

RESULTS AND DISCUSSION

The system (Fig. 1) performs the manual procedure (2) automatically. The sampler regulates the sampling and wash times to 1 and 0.5 min, respectively. Reproducible volumes of standards or samples are introduced by the constant flow rate induced by the action of the proportioning pump on the flexible manifold tubing. Air bubbles divide each sample or wash in several segments to prevent interaction due to diffusion and to ensure proper mixing of the reagents as they are added to the continuous flow in the same sequence as in the manual procedure. The wash that separates each sample further eliminates residual interactions by removing traces left from the previous sample. The air bubbles are removed immediately before the reaction product is pumped

Table III—Comparison of Results by USP Manual Method and Automated Procedure with Various Vitamin Preparations

Sample	Composition	Riboflavin[a], mg/Tablet, Capsule, or ml		Coefficient of Variation	
		USP	Automated	USP	Automated
Tablets	Multivitamin	3.01	3.09	1.68	0.78
Capsules	Multivitamin	2.20	2.19	4.87	1.44
Tonic	Water-soluble vitamins plus minerals	0.12	0.12	5.69	1.57
Syrup	Water-soluble vitamins	0.16	0.16	4.17	1.30
Capsules	Multivitamin plus minerals	5.20	5.15	4.64	1.24
Tablets	Multivitamin plus minerals	5.62	5.63	3.00	1.37
Tablets	Multivitamin plus minerals	3.03	3.04	2.40	0.86
Tablets	Multivitamin	0.83	0.83	3.12	0.77

[a] Values represent the mean of eight assays on 8 different days.

through the flow cell, where the fluorescence is continuously measured and recorded on a chart. An example of such recording is shown in Fig. 2. Sample interactions were found to be effectively negligible (Table II) by analyzing levels of 0.05, 0.10, 0.15, and 0.20 μg riboflavin/ml arranged in groups of 20 containing one single concentration and four groups of 20 containing random mixtures of the four levels.

As shown in Table III, results obtained with the automated procedure compared favorably with those of the USP method in the analysis of eight different types of vitamin products. The mean values from eight assays on 8 different days were essentially the same by both procedures. The coefficient of variation with each sample was significantly smaller by the automated procedure.

In summary, results obtained in these laboratories indicate better reproducibility by the proposed automated procedure in comparison to the official USP manual procedure.

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[x] To whom inquiries should be directed.

NOTES

TLC Separation and Identification of Diastereomers of D-Ergonovine Maleate

DAVID L. SONDACK

Abstract □ The resolution of D-lysergic acid D-2-propanolamide from D-lysergic acid L-2-propanolamide (ergonovine) by TLC and column chromatography is described. The compound was identified by comparison with authentic samples using TLC, circular dichroism, and other physical characteristics.

Keyphrases □ Ergonovine maleate—TLC separation and identification of diastereomers □ Lysergic acid derivatives—TLC separation and identification of diastereomers of D-ergonovine maleate □ TLC—separation and identification of diastereomers of D-ergonovine maleate

During TLC examination of some samples of D-lysergic acid L-2-propanolamide maleate (ergonovine maleate), in preparation for gas–liquid partition chromatography (1), a hitherto unobserved minor spot became evident. Since it had not been observed before in this laboratory, even in aged samples, it