Kinetic Determination of Ribavirin in Drug Formulations

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ABSTRACT

Two simple and sensitive kinetic methods were developed for the determination of ribavirin in bulk and in its pharmaceutical preparations using alkaline potassium permanganate as an oxidizing agent. The methods are based upon a kinetic investigation of the oxidation reaction of the drug at room temperature for fixed times of 20 and 30 minutes. In the first method, the absorbance of the colored manganate ion was measured at 610 nm, while in second method the reduction in the absorbance of permanganate was measured at 525 nm. The absorbance concentration plots were linear over the range of 3-15 μg/ml with detection limits of 0.028 μg/ml in the first method and 0.229 μg/ml for the second method. The proposed methods were applied successfully for the determination of the drug in its pharmaceutical formulations, the percentage recoveries were 100.15 ± 1.34, 100.06 ± 0.86 in the first method, and 99.60 ± 0.54, 100.43 ± 0.82 in the second method. The results obtained were compared statistically with those obtained by the official method and showed no significant differences regarding accuracy and precision.

Keywords: spectrophotometry; ribavirin; potassium permanganate; dosage forms

INTRODUCTION

Ribavirin (1-beta-D-ribofuranosyl-1H-1, 2, 4 thiazole-3-carboxamine) (Fig. 1) is a purine nucleoside analog with a modified base and D-ribose sugar (1). It inhibits the replication of a wide range of RNA and DNA viruses, including orthomyxo-, paramyxo-, arena-, bunya-, herpes-, adenovirus, pox- and retro viruses. In vitro inhibitory concentration range is 3-10 μg/ml for influenza, parainfluenza and respiratory syncytial (RSV) viruses (2). Similar concentrations may reversibly inhibit macromolecular synthesis and proliferation of uninfected cells and suppress lymphocytes responses in vitro. The reported methods for the determination of the drug include fluorimetry (3) spectrophotometry (4-6) and high performance liquid chromatography (HPLC) (7-10).

The catalytic kinetic spectrophotometric method is one of the most attractive approaches for the ultratrace determination of certain chemicals and has many advantages:

- Selectivity due to the measurement of the evolution of the absorbance with time of reaction instead of the measure of concrete absorbance value;
- Possibility of no interference of the colored and of turbidity background of the sample;
- Possibility of no interference of other active com-
pounds present in the commercial products, if they are resisting the chemical reaction conditions established for the proposed kinetic method (11).

The aim of the present work was to study the reaction between ribavirin and potassium permanganate in alkaline medium kinetically by two different methods in an attempt to evaluate the drug in its dosage forms. The proposed spectrophotometric methods were simple and did not need sophisticated instruments or special skills, sensitive, rapid and readily adaptable to both the bulk drug and dosage forms.

EXPERIMENTAL

Apparatus
UV - 1601, Shimadzu recording spectrophotometer (P/N 206 - 67001) equipped with kinetic accessory provided with temperature controlled cell (TCC - 240A) thermo-electrical temperature. Recording range, 0-1; wave-length, 610 and 525 nm; factor 1; number of cell, 1; reaction times, 20 and 30 min and cycle time, 0.1 min.

Materials and Reagents
• Ribavirin was kindly obtained from T3A (Cairo, Egypt). The purity of the drug was determined and confirmed by applying the official method (12).
• Pharmaceutical preparations containing the drug were purchased from different commercial sources in the local markets. Ribavirin 200 capsules: labeled to contain 200 mg ribavirin/capsule (lot No. 020303; T3A, Cairo, Egypt); Viracure capsules: labeled to contain 200 mg ribavirin/capsule (lot No. B31120; October Pharma Co, Cairo, Egypt).
• Reagents: All the reagents used were of analytical grade and water was always double distilled. Aqueous solutions of $7.59 \times 10^{-2}$, $7.59 \times 10^{-3}$ M potassium permanganate (Merck, Germany) and 2 M NaOH (BDH, UK) were prepared.
• Stock solutions.
The stock solution of the studied drug was prepared by dissolving 100 mg of ribavirin in 100 ml distilled water and solicited for few minutes. Working standard solutions were prepared by dilution of the stock solution with the same solvent. The solutions were stable for one week if kept in the refrigerator.

General procedures
Construction of the calibration graph for the first method. An aliquot solutions of ribavirin containing 30-150 μg was transferred into a 10 ml volumetric flask, 2.5 ml of 2 M NaOH was added followed by 0.7 ml of $7.59 \times 10^{-2}$ M KMnO$_4$, the mixture was shaken well and completed to the volume with distilled water. The absorbance was scanned during 20 min. at room temperature at 610 nm against a similar blank prepared simultaneously.

Construction of the calibration graph for the second method. An aliquot solution of ribavirin containing 30-150 μg was transferred into a 10 ml volumetric flask, 3ml of 2 M NaOH was added followed by 1 ml of $7.59 \times 10^{-3}$ M KMnO$_4$, the mixture was shaken well and completed to the volume with distilled water. The reduction in absorbance was scanned during 30 min. at room temperature at 610 nm against a similar blank prepared simultaneously.

Procedures for determination of ribavirin in its dosage forms. An accurately weighed quantity of the mixed contents of 10 capsules equivalent to 50 mg of the drug was transferred into a 100 ml volumetric flask. About 70 ml distilled water were added and the mixture was sonicated for 15 min, filtered and then diluted to volume with distilled water. An aliquot of the filtrate was transferred into a 10 ml volumetric flask and either above procedure was adopted. The nominal content of the capsules were calculated by referring to the prepared calibration graphs or the corresponding regression equations.

RESULTS AND DISCUSSION

The reaction between ribavirin and KMnO$_4$ in alkaline medium yields a green color due to the production of manganate ions, which absorb at 610 nm. As the intensity of the color increases with time, this was used as a useful method for the determination of ribavirin in bulk as well as in dosage forms (first method).

At the same time owing to the consumption of KMnO$_4$ in the reaction the absorbance of KMnO$_4$ peaking at 525 nm decreases with time. This was also used as a useful method for the determination of ribavirin (second method).

The various experimental parameters affecting the development and stability of the reaction product in either method were optimized by changing each variable in turn while keeping all others constant.

Effect of KMnO$_4$
In the first method, the reaction rate and maximum absorbance increased with increasing KMnO$_4$ concentration. It was found that 0.6 ml of $7.59 \times 10^{-3}$ M KMnO$_4$ was adequate for the maximum absorbance. Higher concentra-
tions of KMnO₄ yielded lower absorbance values probably due to decomposition of the product (Fig. 2).

While in the second method, the reaction rate and maximum absorbance reduction increased with increasing KMnO₄ concentration. It was found that 1 ml of $7.59 \times 10^{-3}$ M KMnO₄ was adequate for the maximum absorbance reduction (Fig. 3).

**Effect of NaOH**

It was found that increasing the volume of 2 M NaOH would increase the absorbance of the reaction product up to 2.5 ml. (In the first method) (Fig. 4).

In the second method increasing the volume of 2 M NaOH would increase the reduction in the absorbance of KMnO₄ up to 3 ml (Fig. 5).

The rate of the reaction was found to be dependent on ribavirin concentration. The rate was followed at room temperature with various concentrations in the range of 3-15 μg/ml keeping KMnO₄ and NaOH concentrations constant.

The reaction rate was found to obey the following equation:

$$\text{Rate} = K' \cdot [\text{drug}]^n$$  
(Eq. 1)

where $K'$ is the pseudo - order rate constant and $n$ is the order of the reaction.

The rate of the reaction in either method may be estimated by the variable time method measurement as $\Delta A/\Delta t$, where $A$ is the absorbance and $t$ is the time in seconds. Taking logarithms of rate and concentrations (Table 1), Eq. 1 is transformed into

$$\log (\text{rate}) = \log \Delta A/\Delta t = \log K' + n \log [\text{drug}]$$  
(Eq. 2)

Log (rate) versus log [drug] gave the regression equation:

Log rate $= 0.2049 + 0.825 \log C$  
$r=0.9986$  
(in the first method)

Hence $K' = 1.603$ S⁻¹ and the reaction is first order ($n=0.825$).

Log rate $= 0.3442 + 0.896 \log C$  
$r=0.9988$  
(in the second method)

Hence $K' = 2.209$ S⁻¹ and the reaction is first order ($n=0.896$).

**Evaluation of the kinetic methods**

The quantitation of the drug under the optimized experimental conditions outlined above would result in a pseudo - first order with respect to the drug concentration where KMnO₄ concentration was at least 74 times of the initial concentration of the drug in the first method or 12 times of the initial concentration of the drug in the second method.
However the rate will be directly proportional to drug concentration in a pseudo-first rate equation as follows:
\[ \text{Rate} = K' \text{ [drug]} \] 
(Eq. 3)
where \( K' \) is the pseudo order rate constant.

Several experiments were then carried out to obtain drug concentration from the rate data according to (Eq. 3). Initial rate, rate constant, fixed concentration and fixed time methods (14, 15), were tried and the most suitable analytical method was selected taking into account the applicability, the sensitivity, the intercept and the correlation coefficient (r).

**Rate-constant method**

Graphs of log absorbance versus time for ribavirin in the range of \( 1.229 \times 10^{-5} - 6.143 \times 10^{-5} \) M were plotted and all appeared to be rectilinear. Pseudo-first order rate constant (\( K' \)) corresponding to different drug concentrations (C) were calculated from the slope multiplied by -2.303 and are presented in Table 2.

Regression of (C) versus \( K' \) gave equations:
\[ K' = -6.028 \times 10^{-4} + 4.187 \text{C} \] 
\( r=0.897 \)  
(in the first method)
\[ K' = -6.877 \times 10^{-4} + 2.683 \text{C} \]  
\( r=0.585 \)  
(in the second method)

**Fixed-concentration method**

Reaction rates were recorded for different concentrations of the drug in the range of \( 2.457 \times 10^{-5} - 4.914 \times 10^{-5} \) M in the first method and \( 1.229 \times 10^{-5} - 6.143 \times 10^{-5} \) in the second method. Preselected values of the absorbance (0.3) in the first method and (1.1) in the second method were fixed and the time was measured in seconds. The reciprocal of times (1/t) versus the initial concentrations of drug (Table 3) were plotted and the following equations of the calibration graphs were obtained:
\[ 1/t = -8.363 \times 10^{-3} + 386.350 \text{ C} \] 
\( r=0.9845 \)  
(in the first method)
\[ 1/t = -5.989 \times 10^{-4} + 94.028 \text{ C} \]  
\( r=0.9814 \)  
(in the second method)

**Fixed-time method**

Reaction rates were determined for different concentrations of the drug. At a preselected fixed time, which was accurately determined, the absorbance was measured.

| At 610 nm | At 525 nm |
|-----------|-----------|
| \( \text{Log } \Delta A/\Delta t \) | \( \text{Log [Ribavirin]} \) (M) | \( \text{Log } \Delta A/\Delta t \) | \( \text{Log [Ribavirin]} \) (M) |
| -3.844 | -4.910 | -4.059 | -4.910 |
| -3.608 | -4.609 | -3.859 | -4.689 |
| -3.445 | -4.433 | -3.567 | -4.388 |
| -3.335 | -4.309 | -3.529 | -4.309 |
| -3.285 | -4.212 | -3.436 | -4.212 |

| Table 2. Values of \( K' \) calculated from slopes of log A versus time graphs at 610 nm and 525 nm |
|-----------|-----------|
| At 610 nm | At 525 nm |
| \( K' \) (S\(^{-1}\)) | [Ribavirin] (M) | \( K' \) (S\(^{-1}\)) | [Ribavirin] (M) |
| -5.773 \times 10^{-4} | 1.229 \times 10^{-5} | -5.601 \times 10^{-4} | 1.229 \times 10^{-5} |
| -4.368 \times 10^{-4} | 2.457 \times 10^{-5} | -7.188 \times 10^{-4} | 2.048 \times 10^{-5} |
| -4.806 \times 10^{-4} | 3.686 \times 10^{-5} | -6.453 \times 10^{-4} | 4.095 \times 10^{-5} |
| -4.184 \times 10^{-4} | 4.914 \times 10^{-5} | -5.354 \times 10^{-4} | 4.914 \times 10^{-5} |
| -3.293 \times 10^{-4} | 6.143 \times 10^{-5} | -5.846 \times 10^{-4} | 6.143 \times 10^{-5} |

| Table 3. Values of reciprocal of time taken at fixed absorbance for different rates of variable concentrations of ribavirin at constant concentrations of NaOH and KMnO\(_4\) at room temperature |
|-----------|-----------|
| At 610 nm | At 525 nm |
| \( 1/t \) (S\(^{-1}\)) | [Ribavirin] (M) | \( 1/t \) (S\(^{-1}\)) | [Ribavirin] (M) |
| 1.618 \times 10^{-3} | 2.457 \times 10^{-5} | 8.547 \times 10^{-4} | 1.229 \times 10^{-4} |
| 4.902 \times 10^{-3} | 3.686 \times 10^{-5} | 1.267 \times 10^{-3} | 2.048 \times 10^{-5} |
| 11.111 \times 10^{-3} | 4.914 \times 10^{-5} | 2.688 \times 10^{-3} | 4.095 \times 10^{-5} |
| 11.111 \times 10^{-3} | 4.914 \times 10^{-5} | 3.968 \times 10^{-3} | 4.914 \times 10^{-5} |
| 5.556 \times 10^{-1} | 6.143 \times 10^{-5} |
Calibration graphs of absorbance versus initial concentrations of ribavirin were established at fixed times of 5, 10, 15, 20 min. in the first method and 5, 10, 15, 20, 25, 30 min. in the second method with the regression equations assembled in (Table 4).

It is clear that the slope increased with time and the most acceptable values of the correlation coefficient (r) and the intercept were chosen as the most suitable time interval for measurement.

Calibration graphs

After optimizing the reaction conditions, the fixed time was applied to the determination of the drug in pure form over the concentration range 3-15 μg/ml. Analysis of the data gave the following regression equations:

\[
A = 0.0101 + 0.0575 C \quad r=0.9999 \text{ (in the first method)} \\
A = 0.0163 + 0.0469 C \quad r=0.9999 \text{ (in the second method)}
\]

The calibration graphs were shown in (Figs. 6, 7), the % recoveries of the drug compared with that obtained by the official method (12), were given in (Table 5).

Statistical analysis (16) of the results obtained by the proposed and reference method (13) using student’s t test and variance ratio revealed no significant difference between the performance of the methods regarding accuracy and precision.

The proposed methods were successfully applied for determination of the studied drug in its dosage forms, as shown in (Table 6), compared with the result obtained by the reference method.

Table 4. Regression equation for ribavirin at different fixed time over the range of 1.229 × 10⁻⁵ to 6.143 × 10⁻⁶

| Time (min) | Regression equation | (r)² | Time (min) | Regression equation | (r)² |
|------------|---------------------|------|------------|---------------------|------|
| 5          | A = -0.0335 + 0.0438 C | 0.9967 | 5          | A = -0.0221 + 0.0222 C | 0.8943 |
| 10         | A = -0.0114 + 0.0516 C | 0.9992 | 10         | A = -0.0207 + 0.0327 C | 0.9951 |
| 15         | A = 1.559 ×10⁻³ +0.0557 C | 0.9999 | 15         | A = -0.0166 + 0.0391 C | 0.9979 |
| 20         | A = 0.0101 + 0.575 C | 0.9999 | 20         | A = -7.810 ×10⁻³+0.0431 C | 0.9994 |
|            |                     |      | 25         | A = 3.382 ×10⁻⁵+0.0456 C | 0.9999 |
|            |                     |      | 30         | A = 0.0163 + 0.0469 C | 0.9999 |

*Correlation coefficient.

Table 5. Validity of the proposed method for the determination of the studied drug

| Proposed methods | Official method |
|------------------|----------------|
|                  | 1st method     | 2nd method     | 
| Amount taken     | Amount found   | Recovery %     | Amount taken | Amount found | Recovery % |
| (μg/ml)          | (μg/ml)        |                 | (μg/ml)       | (μg/ml)      |              |
| 3                | 2.955          | 98.49           | 3             | 3.000        | 100          |
| 6                | 5.998          | 99.97           | 5             | 4.983        | 99.66        |
| 9                | 9.129          | 101.43          | 10            | 10.079       | 100.79       |
| 12               | 11.929         | 99.41           | 12            | 11.977       | 99.81        |
| 15               | 14.990         | 99.93           | 15            | 15.004       | 100.03       |
| X¹               | 99.85          | 100.06          |               |              |              |
| SD               | 1.07           | 0.44            |               |              |              |
| t                | 0.03 (2.31)    | 1.008 (2.31)    |               |              |              |
| F                | 1.21 (6.39)    | 4.95 (6.39)     |               |              |              |

Each result is the average of three separate determinations. *The values between brackets are the tabulated student t-test and variance ratio test (at $P=0.05$) (16). X, mean; SD, Standard deviation.
Mechanism of the reaction

The stoichiometry of the reaction was studied adopting the limiting logarithmic method (17). The ratio of the reaction between ribavirin and KMnO₄ in alkaline medium was calculated by dividing the slope of KMnO₄ curve over the slope of the drug curve (Fig. 8a, 8b). It was found that the ratio was (1:1) KMnO₄ to drug. The proposed pathway of the reaction is given in Figure 9.

CONCLUSION

The proposed methods were simple, accurate, precise, sensitive, rapid and low cost. Furthermore, the proposed methods do not require elaboration of procedures, which are usually associated with chromatographic methods. The proposed methods could be applied successfully for determination of the studied drug in pure form as well as in dosage form.
KINETIC DETERMINATION OF RIBAVIRIN

Table 6. Application of the proposed methods to the determination of the studied drug in dosage forms

| Preparation                  | Amount taken (μg/ml) | Amount found (μg/ml) | Recovery % | Amount taken (μg/ml) | Amount found (μg/ml) | Recovery % | Amount taken (μg/ml) | Amount found (μg/ml) | Recovery % |
|------------------------------|----------------------|----------------------|------------|----------------------|----------------------|------------|----------------------|----------------------|------------|
| Ribavirin 200 capsules       | 3                    | 3.017                | 100.55     | 3                    | 3.008                | 100.27     | 5                    | 4.943                | 98.85      |
|                              | 9                    | 8.916                | 99.07      | 5                    | 5.019                | 100.39     | 10                   | 9.946                | 99.46      |
|                              | 12                   | 12.068               | 100.57     | 10                   | 10.178               | 101.78     | 15                   | 15.072               | 100.48     |
|                              |                      |                      |            | 12                   | 12.016               | 100.13     |                      |                      |            |
|                              |                      |                      |            | 15                   | 14.934               | 99.56      |                      |                      |            |
| Mean ± SD                    | 100.06 ± 0.86         |                      |            | 100.43 ± 0.82        |                      |            | 99.29 ± 0.81         |                      |            |
| Student’s t test             | 1.07 (2.78)*         |                      |            | 1.84 (2.45)*         |                      |            | 4.85 (6.94)*         |                      |            |
| F test                       | 1.13 (19)*           |                      |            | 1.03 (19.25)*        |                      |            |                      |                      |            |
| Viracure 200 capsules        | 3                    | 3.050                | 101.67     | 3                    | 2.997                | 99.09      | 5                    | 4.961                | 99.22      |
|                              | 6                    | 5.949                | 99.16      | 5                    | 5.005                | 100.09     | 10                   | 10.156               | 101.56     |
|                              | 9                    | 8.966                | 99.63      | 10                   | 9.929                | 99.29      | 15                   | 15.101               | 100.67     |
|                              |                      |                      |            | 12                   | 12.032               | 100.27     |                      |                      |            |
|                              |                      |                      |            | 15                   | 14.891               | 99.27      |                      |                      |            |
| Mean ± SD                    | 100.15 ± 1.34         |                      |            | 99.60 ± 0.54         |                      |            | 100.48 ± 1.18        |                      |            |
| Student’s t test             | -0.32 (2.78)*        |                      |            | -1.504 (2.45)*       |                      |            |                      |                      |            |
| F test                       | 1.28 (19)*           |                      |            | 4.85 (6.94)*         |                      |            |                      |                      |            |

Each result is the average of three separate determinations. *The values between brackets are the tabulated student t-test and variance ratio test (at P=0.05) (16).

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