

Kinetic Determination of Ribavirin in Drug Formulations

A. M. El-Brashy, Z. A. Sheribah, M. K. Sharaf El-Din, R. M. El-Gamal

Department of Analytical Chemistry, Faculty of Pharmacy, University of Mansoura, Mansoura, Egypt

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 $\mu\text{g/ml}$ with detection limits of 0.028 $\mu\text{g/ml}$ in the first method and 0.229 $\mu\text{g/ml}$ 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-, adeno-, pox- and retro viruses. In vitro inhibitory concentration range is 3-10 $\mu\text{g/ml}$ for influenza, parainfluenza and respiratory syncytical (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) spec-

trophotometry (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-

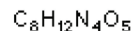
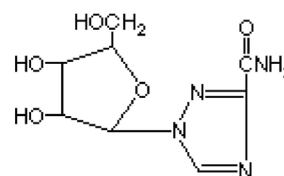


Figure 1. Structure of Ribavirin.

Corresponding author: A. M. El-Brashy, Department of Analytical Chemistry, Faculty of Pharmacy, University of Mansoura, Mansoura, 35516, Egypt. Tel: +20105057988; Fax: +2050-2247496; E-mail: amelbrash@yahoo.com.

Copyright: © 2007 A. M. El-Brashy *et al.* This is an open-access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/2.5/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

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 7.59×10^{-2} , 7.59×10^{-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 7.59×10^{-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 7.59×10^{-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 manganese 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 7.59×10^{-2} M KMnO_4 was adequate for the maximum absorbance. Higher concentra-

tions of KMnO_4 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 KMnO_4 concentration. It was found that 1 ml of $7.59 \times 10^{-3} \text{ M}$ KMnO_4 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 KMnO_4 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 $\mu\text{g/ml}$ keeping KMnO_4 and NaOH concentrations constant.

The reaction rate was found to obey the following equation:

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

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

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

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

$$\text{Log rate} = 0.2049 + 0.825 \text{ log } C \quad r=0.9986$$

(in the first method)

Hence $K' = 1.603 \text{ S}^{-1}$ and the reaction is first order ($n=0.825$).

$$\text{Log rate} = 0.3442 + 0.896 \text{ log } C \quad r=0.9988$$

(in the second method)

Hence $K' = 2.209 \text{ S}^{-1}$ 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 KMnO_4 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.

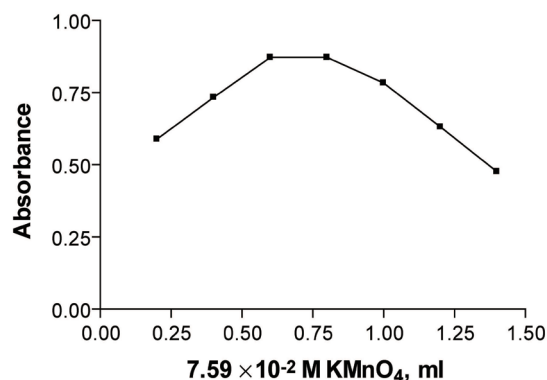


Figure 2. Effect of volume of potassium permanganate on the reaction product of $6.143 \times 10^{-3} \text{ M}$ ribavirin measured at room temperature after 20 min. (Method A).

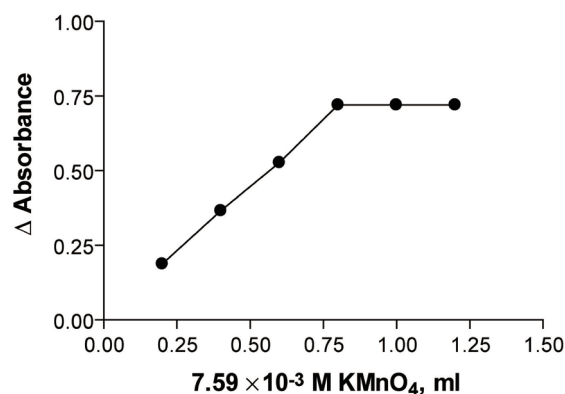


Figure 3. Effect of volume of sodium hydroxide on the reaction product of $6.143 \times 10^{-5} \text{ M}$ ribavirin measured at room temperature after 20 min. (Method A).

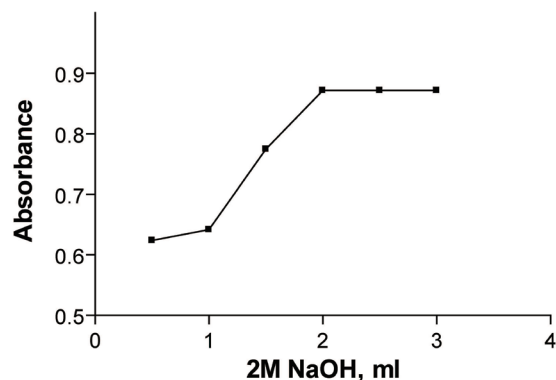


Figure 4. Effect of volume of potassium permanganate on the reaction product of $6.143 \times 10^{-3} \text{ M}$ ribavirin measured at room temperature after 30 min. (Method B).

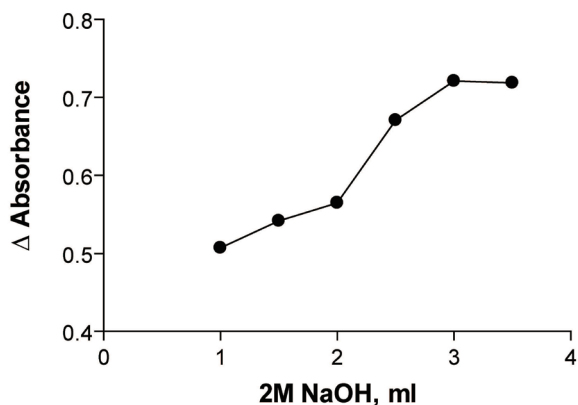


Figure 5. Effect of volume of sodium hydroxide on the reaction product of 6.143×10^{-5} M ribavirin measured at room temperature after 30 min. (Method B).

However the rate will be directly proportional to drug concentration in a pseudo - first rate equation as follows:

$$\text{Rate} = K' [\text{drug}] \quad (\text{Eq. 3})$$

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 1.229×10^{-5} - 6.143×10^{-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:

$$K' = -6.028 \times 10^{-4} + 4.187 C \quad r=0.897$$

(in the first method)

$$K' = -6.877 \times 10^{-4} + 2.683 C \quad r=0.585$$

(in the second method)

Fixed-concentration method

Reaction rates were recorded for different concentrations of the drug in the range of 2.457×10^{-5} - 4.914×10^{-5} M in the first method and 1.229×10^{-5} - 6.143×10^{-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:

$$1/t = -8.363 \times 10^{-3} + 386.350 C \quad r=0.9845$$

(in the first method)

$$1/t = -5.989 \times 10^{-4} + 94.028 C \quad r=0.9814$$

(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.

Table 1. Logarithms of rate for different concentrations of ribavirin at room temperature and at 610 nm, 525 nm

At 610 nm		At 525 nm	
Log $\Delta A/\Delta t$	Log [Ribavirin] (M)	Log $\Delta A/\Delta t$	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)
-5.773×10^{-4}	1.229×10^{-5}	-5.601×10^{-4}	1.229×10^{-5}
-4.368×10^{-4}	2.457×10^{-5}	-7.188×10^{-4}	2.048×10^{-5}
-4.806×10^{-4}	3.686×10^{-5}	-6.453×10^{-4}	4.095×10^{-5}
-4.184×10^{-4}	4.914×10^{-5}	-5.354×10^{-4}	4.914×10^{-5}
-3.293×10^{-4}	6.143×10^{-5}	-5.846×10^{-4}	6.143×10^{-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)
1.618×10^{-3}	2.457×10^{-5}	8.547×10^{-4}	1.229×10^{-5}
4.902×10^{-3}	3.686×10^{-5}	1.267×10^{-3}	2.048×10^{-5}
11.111×10^{-3}	4.914×10^{-5}	2.688×10^{-3}	4.095×10^{-5}
		3.968×10^{-3}	4.914×10^{-5}
		5.556×10^{-3}	6.143×10^{-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 $\mu\text{g/ml}$. 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^{-5}

At 610 nm			At 525 nm		
Time (min)	Regression equation	(r) ^a	Time (min)	Regression equation	(r) ^a
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	$A = 1.559 \times 10^{-3} + 0.0557 C$	0.9999	15	$A = -0.0166 + 0.0391 C$	0.9979
20	$A = 0.0101 + 0.575 C$	0.9999	20	$A = -7.810 \times 10^{-3} + 0.0431 C$	0.9994
			25	$A = 3.382 \times 10^{-3} + 0.0456 C$	0.9999
			30	$A = 0.0163 + 0.0469 C$	0.9999

^aCorrelation coefficient.

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

Proposed methods						Official method		
1 st method			2 nd method					
Amount taken ($\mu\text{g/ml}$)	Amount found ($\mu\text{g/ml}$)	Recovery %	Amount taken ($\mu\text{g/ml}$)	Amount found ($\mu\text{g/ml}$)	Recovery %	Amount taken ($\mu\text{g/ml}$)	Amount found ($\mu\text{g/ml}$)	Recovery %
3	2.955	98.49	3	3.000	100	5	4.913	98.26
6	5.998	99.97	5	4.983	99.66	10	10.064	100.64
9	9.129	101.43	10	10.079	100.79	15	15.028	100.19
12	11.929	99.41	12	11.977	99.81	20	20.100	100.50
15	14.990	99.93	15	15.004	100.03	25	24.895	99.58
X ^a		99.85			100.06			99.83
SD		1.07			0.44			0.97
t		0.03 (2.31) ^a			1.008 (2.31) ^a			
F		1.21 (6.39) ^a			4.95 (6.39) ^a			

Each result is the average of three separate determinations. ^aThe 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 KMnO_4 in alkaline medium was calculated by dividing the slope of KMnO_4 curve over the slope of the drug curve (Fig. 8a, 8b). It was found that the ratio was (1:1) KMnO_4 to drug. The proposed pathway of the reaction is given in Figure 9.

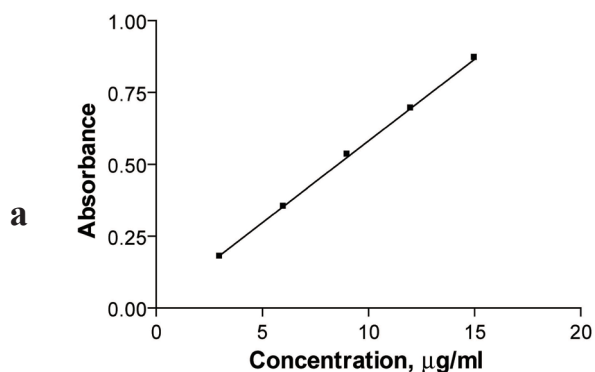


Figure 6. Kinetic spectrophotometric calibration curve for the reaction between ribavirin and alkaline potassium permanganate. (Method A).

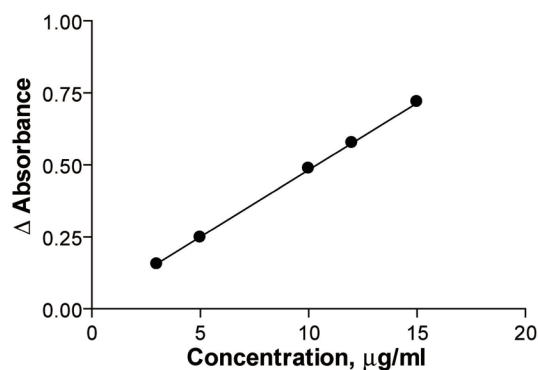


Figure 7. Kinetic spectrophotometric calibration curve for the reaction between ribavirin and alkaline potassium permanganate. (Method B).

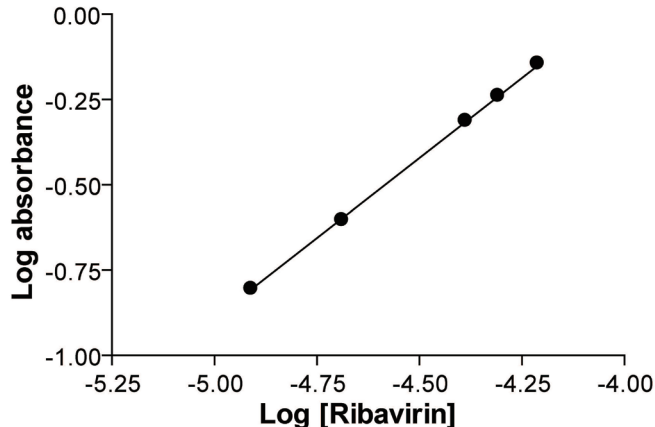
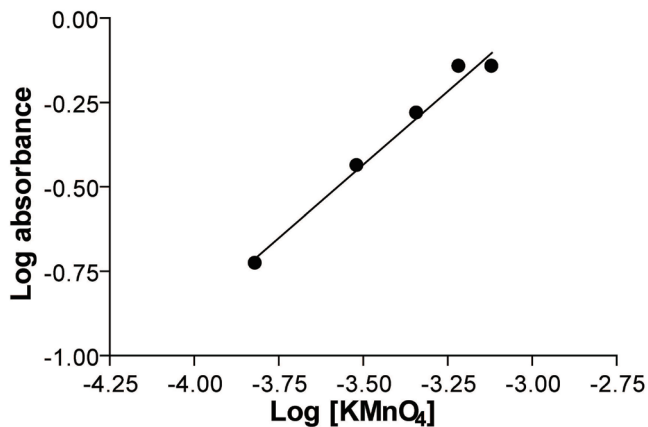


Figure 8. Stoichiometry of the reaction between ribavirin and alkaline potassium permanganate adopting limiting logarithmic method.

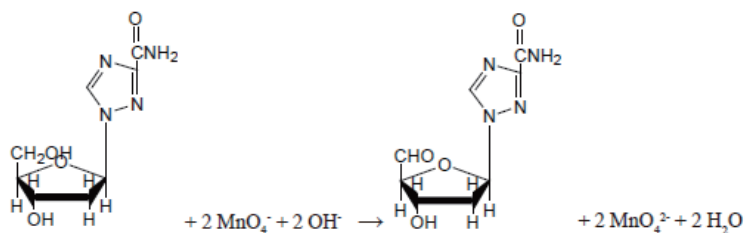


Figure 9. The proposed pathway of the reaction.

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.

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

Preparation	1 st method			2 nd method			Official method		
	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 (ribavirin, 200mg/capsule)	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) ^a			1.84 (2.45) ^a			
F test			1.13 (19) ^a			1.03 (19.25) ^a			
Viracure 200 capsules (ribavirin, 200mg/capsule)	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) ^a			-1.504 (2.45) ^a			
F test			1.28 (19) ^a			4.85 (6.94) ^a			

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

REFERENCES

- Budavari S. The Merck Index, An Encyclopaedia of Chemicals, Drugs and Biologicals. 12th ed. *NJ: Merck & Co.* 1996; 1409.
- Reynolds LEF. Martindale, (The Extra Pharmacopoeia) 31st ed. *London: The Pharmaceutical Press.* 1996; 626.
- Darwish IA, Khedr AS, Askal HF, Mahmoud RM. Simple fluorimetric method for determination of certain antiviral drugs via their oxidation with cerium (IV). *Farmaco.* 2005; 60 (6-7): 555.
- Darwish IA, Khedr AS, Askal HF, Mahmoud RM. Application of inorganic oxidants to the spectrophotometric determination of ribavirin in Bulk and Capsules. *J. AOAC Int.* 2006; 89 (2): 341.
- Sharaf El-Din MK, El-Brashy AM, Sheribah ZA, El-Gamal RM. Spectrophotometric determination of acyclovir and ribavirin in their dosage forms. *J. AOAC Int.* 2006; 89.
- Milovanovic GA, Caker MM, Vucic NB, Jokanovic M. Selective indicator reaction for kinetic determination of traces of manganese (II), ribavirin and tiazofurin. *Mikrochim. Acta.* 2000; 135 (3-4): 173.
- Liu Y, Yan CR, Lim C, Yeh LT, *et al.* Sensitive and specific LC -MS/MS method for the simultaneous measurements of viramidine and ribavirin in human plasma. *J. Chromatog. B.* 2006; 832 (1):17.
- Yeh L, Nguyen M, Lourenco D, Lin C. A sensitive and specific method for the determination of total ribavirin in monkey liver by high-performance liquid chromatography with tandem mass spectrometry. *J. Pharma. Biomed. Anal.* 2005; 38 (1): 34.
- D'Avolio A, Ibañez A, Sciandra M, Siccardi M, *et al.* Validation of liquid/liquid extraction method coupled with HPLC-UV for measurement of ribavirin plasma levels in HCV-positive patients. *J. Chromatog. B.* 2006; 835 (1-2): 127.
- Z. Shou W, Bu H, Addison T, Jiang X, *et al.* Development and validation of a liquid chromatography/tandem mass spectrometry (LC/MS/MS) method for the determination of ribavirin in human plasma and serum. *J. Pharma. Biomed. Anal.* 2002; 29 (1-2):83.
- Espinosa-Mansilla A, Acedo-Valeenzulea MI, Salinas F, Canda F. Kinetic determination of ansamicins in pharmaceutical formulations and human urine. Manual and semiautomatic (stopped-flow) procedures. *Anal. Chim. Acta.* 1998; 376 (3): 365.
- The United States Pharmacopoeia XXII. National Formulary XVII. Rockville, MD: USP Convention. 2000; 1644.
- Weisberger A, Friess SL, L Lewis ES. Techniques of Organic Chemistry, III, vol. 3. New York: Interscience. 1953.
- Yatsimirskii KB. Kinetic Methods of Analysis. *Oxford: Pergamon Press.* 1996.
- Laitinen HA, Harris WE, Chemical Analysis. 2nd ed. *New York: McGraw-Hill.* 1975.
- Miller JC, Miller JN. Statistics for Analytical Chemistry. New York: John Wiley & Sons. 1984; 1-94.
- Rose J. Advanced Physicochemical Experiments. *London: Pitman and Sons.* 1964; 67.