DRUGS, COSMETICS, FORENSIC SCIENCES

Application of Inorganic Oxidants to the Spectrophotometric Determination of Ribavirin in Bulk and Capsules

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Eight spectrophotometric methods for determination of ribavirin have been developed and validated. These methods were based on the oxidation of the drug by different inorganic oxidants: ceric ammonium sulfate, potassium permanganate, ammonium molybdate, ammonium metavanidate, chromium trioxide, potassium dichromate, potassium iodate, and potassium periodate. The oxidation reactions were performed in perchloric acid medium for ceric ammonium sulfate and in sulfuric acid medium for the other reagents. With ceric ammonium sulfate and potassium permanganate, the concentration of ribavirin in its samples was determined by measuring the decrease in the absorption intensity of the colored reagents at 315 and 525 nm, respectively. With the other reagents, the concentration of ribavirin was determined by measuring the intensity of the developed colored reaction products at the wavelengths of maximum absorbance: 675, 780, 595, 595, 475, and 475 nm for reactions with ammonium molybdate, ammonium metavanidate, chromium trioxide, potassium dichromate, potassium iodate, and potassium periodate, respectively. Different variables affecting the reaction conditions were carefully studied and optimized. Under the optimum conditions, linear relationships with good correlation coefficients (0.9984–0.9998) were found between the absorbance readings and the concentrations of ribavirin in the range of 4–1400 μg/mL. The molar absorptivities were correlated with the oxidation potential of the oxidants used. The precision of the methods were satisfactory; the values of relative standard deviation did not exceed 1.64%. The proposed methods were successfully applied to the analysis of ribavirin in pure drug material and capsules with good accuracy and precision; the recovery values were 99.2–101.2 ± 0.48–1.30%. The results obtained using the proposed spectrophotometric methods were comparable with those obtained with the official method stated in the *United States Pharmacopeia*.

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Ribavirin, 1-β-D-ribofuranosyl-1H-1,2,4-triazole-3-carboxamide (Figure 1), is a synthetic guanosine analog that can inhibit the replication of a wide range of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) viruses, including influenza A and B, parainfluenza, respiratory syncytial virus, paramyxo viruses, hepatitis C virus (HCV), and human immunodeficiency virus Type 1 (HIV-1; 1). Ribavirin, in combination with interferon alfa-2b, has been approved for the treatment of chronic hepatitis C infection in patients with compensated liver disease (2–5). It also decreases the mortality in Lassa fever and other viral hemorrhagic fevers (1, 6).

Analytical methods that have been reported for determination of ribavirin include high-performance liquid chromatography (7–10), gas chromatography (11), capillary electrophoresis (12), radioimmunoassay (13), fluorometry (14), and spectrophotometry (15, 16). Spectrophotometry, being simple and available in most quality control laboratories, is considered the most recommended technique for determination of the drug in its bulk and dosage forms. Unfortunately, the few spectrophotometric methods reported for determination of ribavirin possess drawbacks, such as low sensitivity (15), complexity, and/or requirement of a critical derivatizing reagent (16). Therefore, our laboratory aimed to develop new spectrophotometric methods that overcome these drawbacks.

Oxidation-reduction reactions have been used as the basis for the development of simple and sensitive spectrophotometric methods for the determination of many pharmaceutical compounds (17–36). Reactions were considered in the present study. This approach was promoted by our previous report (14) that described the susceptibility of ribavirin for oxidation. In oxidimetric reactions, the most commonly used oxidizing agents are ceric ammonium sulfate (17–20), potassium permanganate (21–25), ammonium molybdate (26, 27), ammonium metavanidate (28–30), chromium trioxide, potassium dichromate (31–32), potassium iodate (33, 34), and potassium periodate (35, 36). Of these reagents, only potassium

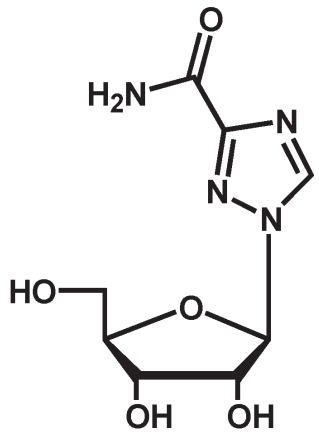

Figure 1. Structure of ribavirin.

periodate has been used for oxidimetric determination of ribavirin, but the analysis involved multiple indirect and difficult procedures (16). None of these reagents has been previously used for the direct, simple spectrophotmetric analysis of ribavirin. For these reasons, the present study was dedicated to investigate the application of these reagents in the direct spectrophotometric analysis of ribavirin in bulk drug and capsules.

Experimental

Apparatus

UV-1601 PC (Shimadzu, Kyoto, Japan) and Lambda-3 B (Perkin-Elmer Corp., Norwalk, CT) ultraviolet (UV)-visible spectrophotometers with matched 1 cm quartz cells were used for all measurements. An MLW type thermostatically controlled water bath (Memmert GmbH, Co. Schwabach, Germany) was used.

Materials and Reagent Solutions

Ribavirin (T3A, Assuit, Egypt), ammonium molybdate (El-Nasr Pharmaceutical Chemical Co., Abo-Zaabal, Egypt), and ammonium metavanidate (Veb Laborchemie Apolda, Germany) were 10% (w/v) in 20% (v/v) sulfuric acid. Ceric ammonium sulfate (Sigma-Aldrich Co. Ltd., Gillingham-Dorst, Germany) was 0.15% (w/v) in 0.25 M perchloric acid. Chromium trioxide (Mallinckrodt Chemical Works Ltd., Montreal, Canada) was 3% (w/v) in distilled water. Potassium dichromate (Cambrian Chemical, Croydon, UK) was 5% (w/v) in distilled water. Potassium iodate (Koch-light Laboratories Ltd., Colnbrook Bucks, UK) was 2% (w/v) in 20% (v/v) sulfuric acid. Potassium periodate (Winlab Co., London, UK) was 0.5% (w/v) in 20% (v/v) sulfuric acid. Potassium permanganate (El-Nasr Pharmaceutical Chemical Co.) was 0.06% (w/v) in distilled water. Ribavirin capsules (T3A) were labeled to contain 200 mg ribavirin/capsule. All solvents, acids, and other chemicals used throughout this study were of analytical grade. Double-distilled water was obtained through a Nanopure II water purification system (Barnstead/Thermolyne, Dubuque, IA) and used throughout the work.

Preparation of Standard Solutions

An accurately weighed amount (1 g) of ribavirin was transferred into a 100 mL volumetric flask and dissolved in about 40 mL distilled water. The resulting solution was diluted to the mark with water to provide a stock standard solution containing 10 mg/mL. Different volumes of this stock solution were then further diluted with water to

Table 1. Experimental conditions for the spectrophotometric determination of ribavirin by different inorganic oxidizing reagents

Oxidizing reagent	Concentration of reagent, % (w/v)	Acid volume, mL[a]	Reaction time, min[b]	λ_{max}, nm[c]
Ceric ammonium sulfate	0.15	3	15	315
Potassium permanganate	0.06	2	15	525
Ammonium molybdate	10	4	25	675
Ammonium metavanidate	10	5	5	780
Chromium trioxide	4	4	15	595
Potassium dichromate	5	4	15	595
Potassium iodate	3	4	5	475
Potassium periodate	0.05	2	5	475

[a] Acids were 0.25 M perchloric acid, 20% (v/v) sulfuric acid, and concentrated sulfuric acid for ceric ammonium sulfate, potassium permanganate, and the other reagents, respectively.

[b] Reactions were performed at 80°C in water bath for ceric ammonium sulfate and at room temperature (25 ± 5°C) for the other reagents.

[c] Measurements were performed after diluting the reaction mixtures with acetone for ammonium molybdate and with water for the other reagents.

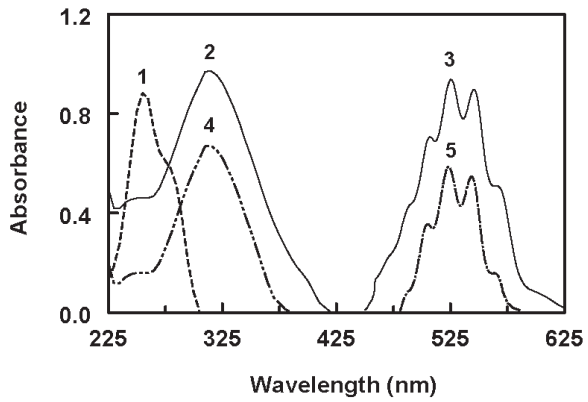

Figure 2. Absorption spectra of 15 μg/mL ribavirin (1), 0.15% (w/v) of ceric ammonium sulfate (2), 0.06% (w/v) of potassium permanganate (3), and the reaction mixtures of ribavirin with ceric ammonium sulfate and potassium permanganate (4 and 5, respectively). The concentrations of ribavirin were 5 and 30 μg/mL for reaction with ceric ammonium sulfate and potassium permanganate, respectively.

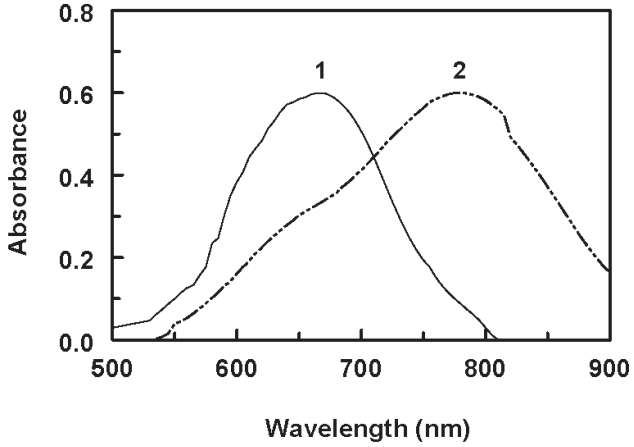

Figure 3. Absorption spectra of the reaction mixtures of ribavirin with 10% (w/v) of ammonium molybdate (1) and 10% (w/v) of ammonium metavanidate (2). The concentrations of ribavirin were 30 and 500 μg/mL for reaction with ammonium molybdate and ammonium metavanidate, respectively.

obtain the working standard solutions of concentrations suitable for analysis by the different oxidizing reagents.

Preparation of Capsule Samples for Analysis

The contents of 20 capsules were collected and weighed. An accurately weighed quantity of the capsule contents equivalent to 100 mg of the active ingredient was transferred into a 100 mL volumetric flask and dissolved in ca 40 mL distilled water. The contents of the flask were swirled, sonicated for 5 min, and diluted to the mark with water. The mixtures were mixed well and filtered, and the first portion of the filtrate was rejected. The prepared solution was diluted quantitatively with the distilled water to obtain a suitable concentration for analysis by each particular oxidant.

General Assay Procedure

One mL of the standard or sample solution was transferred into a 10 mL volumetric flask. One milliliter of the oxidizing analytical reagent was added. Different volumes (Table 1) of acid were added; the acid was perchloric in case of ceric ammonium sulfate and sulfuric for the other reagents. The contents of the flasks were mixed, and the reactions were allowed to proceed for different periods of time (Table 1) at room temperature (25 ± 5°C), except in the case of ceric ammonium sulfate solution, whereas the reaction mixture was heated in a water bath adjusted to 80°C and then cooled to room temperature. After completion of the reactions, the solutions were diluted to volume with water, except in the case of ammonium molybdate, for which the solution was diluted with acetone. The absorbances of the resulting solutions were measured at their respective wavelength of maximum absorbance (λ_{max}; Table 1) against reagent blanks treated similarly. In the case of ceric ammonium sulfate and potassium permanganate, the positions of sample and blank cuvets were exchanged in order to directly obtain the difference in the absorbance values.

Results and Discussion

Reactions Involved and Spectral Characteristics

(**a**) *Reactions with ceric ammonium sulfate and potassium permanganate.*—Ceric ammonium sulfate, Ce(IV), is a strong oxidizing agent having a yellow color of λ_{max} 315 nm. The reduction of Ce(IV) to the colorless Ce(III) proceeds cleanly in acidic solution, and its potential is different according to the acid used. This reaction has been used for the spectrophotometric determination of many compounds either by direct measurement of the decrease in its yellow color (17–19) or indirect measurement of excess Ce(IV) with oxidizable color-producing reagents (20).

Potassium permanganate is a strong oxidant, as well, with an intense violet color of λ_{max} 525 nm. The oxidation of organic compounds with potassium permanganate was found to be pH-dependent. During the course of the reaction, the valance of manganese changes and the intermediate ions have been suggested to be participating oxidants. The species that are considered as potential oxidants depend on the nature of the substrate and the pH of the medium. In strong acidic medium (pH ≤ 1), potassium permanganate ($KMnO_4$) produces the Mn^{2+}, for a net transfer of 5 electrons (21). In neutral or basic media, manganese dioxide (MnO_2) is formed with corresponding net transfer of 3 electrons (22). In strongly alkaline solution (2 M NaOH), green manganate ion (MnO_4^{2-}) is produced (23–25).

Both ceric ammonium sulfate and potassium permanganate were tested for their oxidizing effect on ribavirin, and it was found that ribavirin reacted with both reagents in acidic solutions. This was evident from the decrease in the yellow color of ceric ammonium sulfate at

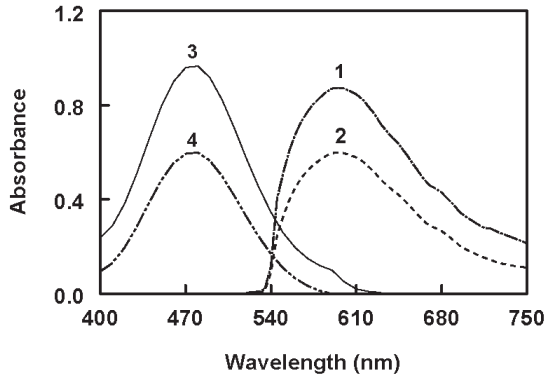

Figure 4. Absorption spectra for the reaction mixtures of ribavirin with 5% (w/v) of chromium trioxide (1), 3% (w/v) of potassium dichromate (2), 3% (w/v) of potassium iodate (3), and 0.06% (w/v) of potassium periodate (4). The concentrations of ribavirin were 100, 1500, 500, and 450 μg/mL for reaction with chromium trioxide, potassium dichromate, potassium iodate, and potassium periodate, respectively.

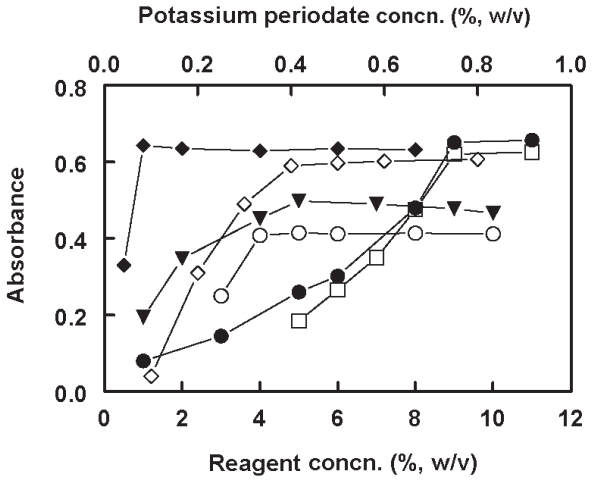

Figure 5. Effect of reagent concentrations on the absorption intensity of the reaction mixtures of ribavirin with ammonium molybdate (●), ammonium metavanidate (□), chromium trioxide (○), potassium dichromate (▼), potassium iodate (◆), and potassium periodate (◇, top scale). The concentrations of ribavirin were 20, 500, 700, 900, 400, and 450 μg/mL for reaction with ammonium molybdate, ammonium metavanidate, chromium trioxide, potassium dichromate, potassium iodate, and potassium periodate, respectively.

315 nm, and the violet color of potassium permanganate at 525 nm (Figure 2). This decrease in color was used for measurement of the concentration of ribavirin. It is worth noting that ribavirin has no absorption capabilities in regions of measurement of both reagents (315 and 525 nm for ceric ammonium sulfate and potassium permanganate solutions, respectively; Figure 2).

(**b**) *Reactions with ammonium molybdate, ammonium metavanidate, chromium trioxide, and potassium dichromate.*—The reactions of ribavirin with these reagents were studied, and it was found that they proceeded by oxidation of ribavirin and, consequently, the reduction of ammonium molybdate, Mo(VI), and metavanidate, VO_3^{-3}, to the corresponding Mo(V) and VO_3^{-4}, respectively. These ions were blue, with λ_{max} values at 675 and 780 nm, respectively (Figure 3). Ribavirin was also found to be oxidizable by both chromium trioxide and potassium dichromate, yielding green Cr(III) ions with λ_{max} 595 nm (Figure 4).

(**c**) *Reactions with potassium iodate and potassium periodate.*—Potassium iodate (33, 34) and periodate (35, 36) are strong oxidizing agents that have been used in the oxidation-based spectrophotometric determination of many pharmaceutical compounds. Potassium periodate has been used as selective reagent for cleavage of 1,2-diols and related compounds (37). The resulting dialdehydes of these compounds were determined by proper signal-producing

Table 2. Effect of acid type on the absorption intensity of the reaction products of the oxidation of ribavirin with different oxidants

	Absorbance[a]				
Oxidant	Sulfuric acid	Hydrochloric acid	Nitric acid	Perchloric acid	Acetic acid
Ceric ammonium sulfate (9)[b]	0.377	0.300	0.310	0.564	0.186
Potassium permanganate (45)	0.533	0.462	0.476	0.266	0.203
Ammonium molybdate (40)	0.750	0.423	0.467	0.241	0.124
Ammonium metavanidate (300)	0.404	0.228	0.237	0.177	0.104
Chromium trioxide (700)	0.388	0.321	0.340	0.217	0.145
Potassium dichromate (700)	0.367	0.333	0.345	0.228	0.122
Potassium iodate (400)	0.624	0.512	0.531	0.322	0.214
Potassium periodate (400)	0.676	0.544	0.562	0.363	0.240

[a] Values are the mean of 3 determinations.

[b] Figures in parentheses are the ribavirin concentrations, in μg/mL, used in the study.

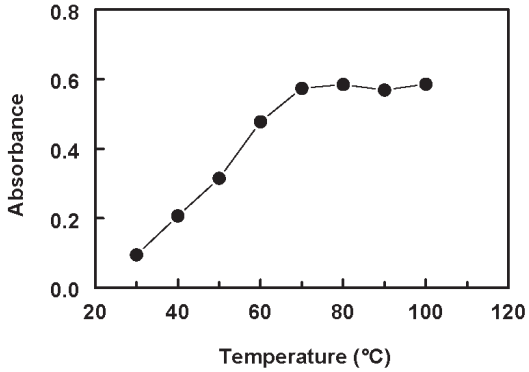

Figure 6. Effect of temperature on the absorption intensity of the oxidation product of ribavirin (10 μg/mL) with ceric ammonium sulfate (0.15%, w/v).

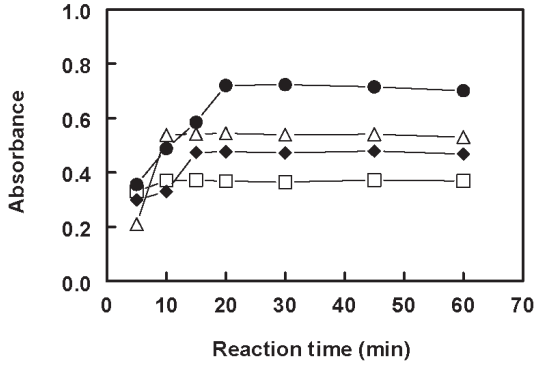

Figure 7. Effect of reaction time on the absorption intensity of the reaction mixtures of ribavirin with ceric ammonium sulfate (◆), ammonium molybdate (●), chromium trioxide (□), and potassium dichromate (△). The concentrations of ribavirin were 8, 35, 600, and 1000 μg/mL for reaction with ceric ammonium sulfate, ammonium molybdate, chromium trioxide, and potassium dichromate, respectively.

chemical reactions. In the present study, ribavirin was subjected to the oxidation by both reagents, and it was found to produce a red-colored chromogen with λ_{max} 475 nm (Figure 4). Structure elucidation for the chromogen is currently under investigation.

Optimization of Reaction Variables

(**a**) *Effect of oxidant concentration.*—According to the above-mentioned reactions, ceric ammonium sulfate and potassium permanganate solutions should be added in excess to react with ribavirin. By measuring the excess reagent, the consumed reagent would correspond to the amount of the drug. The highest concentration of either reagent that gave the highest absorption value within the practical sensitivity range of absorption values (approximately 0.9) was found to be 0.15 and 0.06% (w/v) for ceric ammonium sulfate and potassium permanganate, respectively (Figure 2).

For the other oxidizing reagents, the effect of concentration on the reactions was studied by performing the reactions using 1 mL of different concentrations in the ranges cited in Figure 5. It was observed that the reactions increased by increasing the concentration until maximum absorbance was obtained. Further increase in the concentration of the reagents had no effect on the reactions. The optimum concentration selected for further experiments was the concentration at which maximum absorption was obtained in the plateau region, if any, of the concentration-absorption curve. The optimum concentrations selected for further experiments are given in Table 1.

(**b**) *Effect of acid type and concentration.*—The oxidation reactions of ribavirin by different inorganic oxidants were performed in acid medium. In order to determine the most appropriate acid, different acids (sulfuric, hydrochloric, nitric, perchloric, and acetic) were tested. As shown in Table 2, sulfuric acid gave the highest readings with all oxidants except with ceric ammonium sulfate, for which perchloric acid gave

Table 3. Effect of diluting solvent on the absorption intensity of the reaction products of the oxidation of ribavirin with different oxidants

	Absorbance[a]					
Oxidant	Water	Acetonitrile	Methanol	Ethanol	Acetone	Isopropanol
Ceric ammonium sulfate (9)[b]	0.562	0.536	0.501	0.452	0.478	0.410
Potassium permanganate (45)	0.532	0.488	0.498	0.504	0.512	0.507
Ammonium molybdate (40)	0.014	0.025	0.231	0.630	0.816	0.780
Ammonium metavanidate (300)	0.314	0.284	0.277	0.255	0.257	0.245
Chromium trioxide (700)	0.387	0.314	0.307	0.303	0.298	0.305
Potassium dichromate (700)	0.362	0.323	0.314	0.306	0.312	0.304
Potassium iodate (400)	0.624	0.572	0.577	0.582	0.532	0.512
Potassium periodate (400)	0.576	0.513	0.532	0.524	0.507	0.517

[a] Values are the mean of 3 determinations.

[b] Figures in parentheses are the ribavirin concentrations, in μg/mL, used in the study.

Table 4. Quantitative parameters and statistical data for the spectrophotometric determination of ribavirin with various oxidants

	Parameter						
Oxidant	Range, μg/mL	Intercept (a)	Slope (b)	Correlation coefficient (r)	$\varepsilon \times 10^3$, L/mol/cm	LOD, μg/mL	LOQ, μg/mL
Ceric ammonium sulfate	4–12	–0.0514	0.0689	0.9984	14.89	0.39	1.30
Potassium permanganate	5–60	0.0105	0.0127	0.9985	3.25	1.39	4.61
Ammonium molybdate	5–35	0.0167	0.0194	0.9993	4.82	0.77	2.57
Ammonium metavanidate	100–700	0.0211	0.0013	0.9998	0.32	10.00	40.00
Chromium trioxide	100–1400	0.0426	0.0005	0.9993	0.13	25.50	76.60
Potassium dichromate	100–1300	0.0310	0.0005	0.9986	0.13	22.30	67.00
Potassium iodate	100–500	0.0121	0.0016	0.9995	0.39	6.92	23.06
Potassium periodate	100–550	–0.0901	0.0016	0.9993	0.31	9.77	32.59

the highest readings. This was attributed to the fact that the potential of Ce(IV) in perchloric acid (E^0 = 1.7 V) is higher than that in sulfuric acid (E^0 = 1.4 V; 38). Therefore, sulfuric acid was selected for further testing with all reagents except ceric ammonium sulfate, for which perchloric acid was selected.

Preliminary experiments indicated that oxidation of ribavirin with the oxidants required a high concentration of the acid. In order to determine the most appropriate concentration, the reactions were performed using varying volumes (0.5–6 mL) of concentrated acids. It was found that all of the reactions were dependent on the concentration of sulfuric acid. The absorption intensity increased as the concentration of the acid increased. After attaining the maximum readings, different behaviors were attained. The optimum amount of sulfuric acid at which the maximum readings were obtained was 2 mL (for potassium permanganate and potassium periodate), 4 mL (for ammonium molybdate, chromium trioxide, potassium dichromate, and potassium iodate), or 5 mL (for ammonium metavanidate). The optimum amount of perchloric acid used for reaction with ceric ammonium sulfate was 3 mL.

(**c**) *Effect of temperature and reaction time*.—The reactions between ribavirin and the oxidants (except ceric ammonium sulfate) were performed in relatively high volumes of concentrated acid, thus heat was generated. The generated heat was found to be sufficient for completion of the reaction. The reaction of ribavirin with ceric ammonium sulfate, which was performed in perchloric acid, required heating for its completion. The effect of heating temperature on the oxidation of ribavirin by ceric ammonium sulfate was studied by performing the reaction in a thermostatically controlled water bath at varying temperatures (25–100°C). It was found that the reaction increased with increasing temperature until it became optimum in the range of 70–100°C (Figure 6). Therefore, further experiments were performed at 80°C.

The effect of heating time on the reactions was studied by carrying out the reactions for different periods of time at 80°C for ceric ammonium sulfate and at room temperature for all other reagents. The reactions of ribavirin with ammonium metavanidate, potassium iodate, and potassium periodate were instantaneous; however, for more precise readings, the reaction mixtures were allowed to stand for 5 min. For the other reagents, the reactions were dependent on the time, and the maximum absorption intensities were attained after different periods of time in the range of 5–20 min (Figure 7). After attaining the maximum readings, longer reaction times up to 60 min had no effect. The optimum reaction times selected for subsequent experiments are given in Table 1.

(**d**) *Effect of diluting solvent*.—The effect of diluting solvent on the absorption intensity of the oxidation reaction products of ribavirin with the different reagents was studied. It was found that water was the optimum diluting solvent because it gave maximum readings in all cases except with

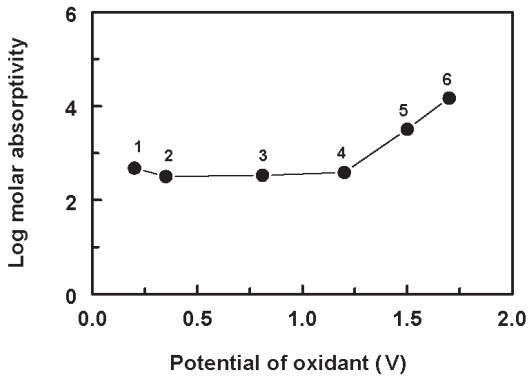

Figure 8. Correlation between the log of the molar absorptivity value and the corresponding potential of the oxidant: ammonium molybdate (1), ammonium metavanidate (2), potassium periodate (3), potassium iodate (4), ceric ammonium sulfate (5), and potassium permanganate (6).

Table 5. Precision of the spectrophotometric analysis of ribavirin by oxidation with different oxidants

Oxidant	Absorbance of samples									Mean	RSD, %
	1	2	3	4	5	6	7	8	9		
Ceric ammonium sulfate (10)[a]	0.624	0.633	0.621	0.625	0.634	0.632	0.624	0.629	0.633	0.628	1.34
Potassium permanganate (30)	0.407	0.405	0.412	0.410	0.412	0.405	0.407	0.416	0.413	0.410	0.90
Ammonium molybdate (30)	0.588	0.604	0.597	0.597	0.599	0.602	0.609	0.604	0.607	0.600	1.05
Ammonium metavanidate (600)	0.797	0.788	0.789	0.785	0.774	0.792	0.788	0.775	0.787	0.786	0.99
Chromium trioxide (700)	0.342	0.337	0.340	0.342	0.329	0.343	0.343	0.350	0.341	0.340	1.64
Potassium dichromate (900)	0.499	0.495	0.487	0.491	0.490	0.499	0.495	0.488	0.508	0.494	1.34
Potassium iodate (350)	0.574	0.571	0.566	0.567	0.580	0.556	0.571	0.584	0.564	0.571	1.51
Potassium periodate (350)	0.378	0.389	0.375	0.378	0.381	0.369	0.380	0.377	0.375	0.378	1.43

[a] Figures in parentheses are the ribavirin concentrations, in μg/mL, used in the study.

Table 6. Analysis of ribavirin in the presence of common excipients by the proposed spectrophotometric methods using different oxidants

Oxidant	Recovery (% ± SD)[a]						
	Sucrose (100)[b]	Lactose (80)[b]	Glucose (90)[b]	Citric acid (5)[b]	Gum acacia (70)[b]	MCC[c] (6)[b]	Starch (10)[b]
Ceric ammonium sulfate	102.3 ± 0.83	101.1 ± 0.15	101.4 ± 0.32	100.2 ± 0.41	100.9 ± 0.12	99.8 ± 0.55	100.6 ± 1.20
Potassium permanganate	100.4 ± 0.92	101.2 ± 0.88	102.4 ± 0.47	99.6 ± 0.44	98.8 ± 1.02	100.7 ± 1.33	99.4 ± 0.25
Ammonium molybdate	100.9 ± 0.88	101.6 ± 0.67	102.3 ± 0.47	99.7 ± 0.89	99.4 ± 0.72	100.3 ± 0.34	100.6 ± 0.22
Ammonium metavanidate	101.1 ± 0.45	102.2 ± 0.35	101.8 ± 0.41	100.7 ± 0. 7	100.3 ± 0.22	99.2 ± 0.67	101.4 ± 1.03
Chromium trioxide	100.8 ± 0.71	102.1 ±1.30	102.9 ± 0.91	100.4 ± 0.24	98.8 ± 0.86	99.6 ± 0.64	100.4 ± 0.42
Potassium dichromate	103.1 ± 0.67	103.7 ± 0.71	104.5 ± 1.45	101.3 ± 0.63	99.3 ± 0.24	100.3 ± 0.15	101.0 ± 0.74
Potassium iodate	103.2 ± 1.20	102.6 ± 0.62	104.1 ± 0.76	98.5 ± 0.34	100.6 ± 0.67	101.1 ± 0.61	99.8 ± 0.97
Potassium periodate	101.1 ± 1.30	101.4 ± 0.77	102.5 ± 0.81	100.3 ± 0.14	99.3 ± 0.70	98.9 ± 0.61	98.7 ± 0.32

[a] Mean of 3 determinations.
[b] Figures in parentheses are the amount of powders, in mg, added to 200 mg ribavirin.
[c] MCC = Microcrystalline cellulose.

ammonium molybdate, for which acetone gave the highest reading (Table 3). Therefore, acetone was selected for further work with ammonium molybdate and water for all other reagents.

(**e**) *Effect of time on stability of readings.*—The effect of time on the stability of the readings after dilution was studied. It was found that the readings of the reaction mixtures were stable for at least 1 h after diluting the reaction mixtures. This gave the advantage of allowing measurement at any time within that period without changes in the values of the readings. This advantage is beneficial when the processing of a large number of samples is necessary.

Validation of the Proposed Methods

(**a**) *Linearity, detection, and quantitation limits.*—Under the above-mentioned optimum conditions, the calibration graphs correlating the absorption intensity with the corresponding concentration of ribavirin were constructed for all the reagents used. Regression analysis for the results was performed using the least-squares method. In all cases, Beer's law plots (n = 5) were linear with very small intercepts (–0.0901–0.0426) and good correlation coefficients (0.9984–0.9998) in the concentration ranges cited in Table 4. The limit of detection (LOD) and limit of quantitation (LOQ) values were determined (39) using the formula:

$$\text{LOD or LOQ} = \kappa SD_a/b$$

where κ = 3 for LOD and 10 for LOQ, SD_a is the standard deviation of the intercept, and b is the slope. The LOD and LOQ values were 0.39–25.5 and 1.30–76.6 μg/mL, respectively. Results in Table 4 indicated that the analytical performance (in terms of slopes, molar absorptivities, and detection limits) for both chromium trioxide and potassium dichromate were identical. This was attributed to the conversion of chromium trioxide in acidic medium to the dichromate anion. It was also observed that the reagents that have the lowest oxidation potential, E^0 (chromium trioxide and potassium dichromate), gave the lowest sensitivities (lowest ε values and highest LOD values). However, reagents that have the highest E^0 (ceric ammonium sulfate and potassium permanganate) gave the highest sensitivities (highest ε values and lowest LOD values). The correlation of the oxidation potential of the oxidants with the obtained ε values indicated that there was no significant difference between the ε values obtained with the reagents having potentials less than 1.2 V (Figure 8). Beyond this value, the relation was found to obey the linear relation equation:

$$\text{Log } \varepsilon = -4.2009 + 3.1545\ E^0 \ (r = 0.9992)$$

This excellent correlation proved the validity of the proposed equation, which was derived for calculating the ε value for a particular reagent based on its potential, for a perfect prediction of the ultimate sensitivity of the analytical method.

(**b**) *Precision.*—The precision of the proposed method was determined (39) by replicate analysis of 9 separate

Table 7. Robustness of the proposed methods for analysis of ribavirin by various oxidizing reagents

Method/conditions	Recovery, % ± SD[a]
Ceric ammonium sulfate	
No variation	100.2 ± 0.87
Acid volume, mL: 3, 5	99.8 ± 0.94, 99.4 ± 0.72
Reaction time, min: 15, 25	100.3 ± 0.34, 99.9 ± 0.67
Potassium permanganate	
No variation	99.6 ± 0.76
Acid volume, mL: 1.5, 2.5	100.2 ± 0.64, 99.4 ± 0.87
Reaction time, min: 15, 25	98.8 ± 0.94, 99.3 ± 0.74
Ammonium molybdate	
No variation	100.3 ± 0.87
Reagent concentration, % (w/v): 9, 11	99.9 ± 0.87, 99.6 ± 0.71
Acid volume, mL: 3, 5	100.2 ± 0.94, 100.1 ± 0.82
Reaction time, min: 25, 35	99.7 ± 0.67, 99.4 ± 0.80
Ammonium metavanidate	
No variation	99.4 ± 0.75
Reagent concentration, % (w/v): 9, 11	99.7 ± 0.76, 99.2 ± 0.81
Acid volume, mL: 4, 6	99.7 ± 0.68, 99.3 ± 0.77
Reaction time, min: 10, 20	99.9 ± 0.76, 99.8 ± 0.69
Chromium trioxide	
No variation	99.2 ± 0.72
Reagent concentration, % (w/v): 4, 6	98.9 ± 0.64, 99.1 ± 0.92
Acid volume, mL: 4, 6	98.6 ± 0.72, 98.8 ± 0.89
Reaction time, min: 10, 20	99.7 ± 0.96, 99.5 ± 0.87
Potassium dichromate	
No variation	99.1 ± 0.76
Reagent concentration, % (w/v): 9, 11	99.3 ± 0.82, 98.6 ± 0.73
Acid volume, mL: 3, 5	99.5 ± 0.95, 98.7 ± 0.83
Reaction time, min: 15, 25	99.1 ± 0.86, 98.8 ± 0.80
Potassium iodate	
No variation	99.3 ± 0.88
Reagent concentration, % (w/v): 2, 4	99.3 ± 0.93, 98.8 ± 0.87
Acid volume, mL: 3, 5	100.1 ± 0.96, 99.8 ± 0.77
Reaction time, min: 10, 20	99.8 ± 0.74, 99.6 ± 0.72
Potassium periodate	
No variation	100.3 ± 0.80
Reagent concentration, % (w/v): 9, 11	100.1 ± 0.67, 99.7 ± 0.77
Acid volume, mL: 3, 5	99.6 ± 0.63, 99.4 ± 0.71
Reaction time, min: 25, 35	99.9 ± 0.66, 100.1 ± 0.72

[a] Values are the mean of 3 determinations ± SD.

Table 8. Ruggedness of the proposed methods for the analysis of ribavirin by various oxidizing reagents

	Recovery, % ± RSD[a]				
	Laboratory-to-laboratory		Day-to-day		
Reagent	Laboratory 1[b]	Laboratory 2[b]	Day 1	Day 2	Day 3
Ceric ammonium sulfate	99.8 ± 0.56	99.3 ± 0.45	100.3 ± 0.78	99.8 ± 0.45	99.7 ± 1.35
Potassium permanganate	99.7 ± 0.89	99.8 ± 0.64	98.7 ± 1.29	99.3 ± 1.37	99.8 ± 0.47
Ammonium molybdate	99.9 ± 0.19	98.7 ± 0.81	98.6 ± 1.34	99.7 ± 0.87	99.2 ± 1.07
Ammonium metavanidate	99.7 ± 0.55	100.3 ± 1.31	100.1 ± 0.34	98.7 ± 0.56	99.3 ± 0.93
Chromium trioxide	100.3 ± 0.93	99.6 ± 0.53	99.9 ± 0.38	100.1 ± 0.36	99.5 ± 0.79
Potassium dichromate	99.7 ± 0.91	98.9 ± 0.31	99.8 ± 0.72	99.1 ± 0.45	98.5 ± 0.81
Potassium iodate	99.8 ± 0.57	99.9 ± 1.37	100.2 ± 0.77	99.7 ± 0.45	99.1 ± 0.56
Potassium periodate	99.9 ± 1.19	98.7 ± 0.81	98.6 ± 1.43	99.7 ± 0.87	99.2 ± 0.47

[a] Values are the mean of 3 determinations ± RSD.
[b] The spectrophotometers used in Laboratory 1 and Laboratory 2 were UV-1601 PC and Lambda-3 B, respectively.

solutions of the working standards at one concentration level. The relative standard deviation (RSD) values were less than 2% with all of the tested reagents, indicating the good reproducibility of the proposed methods (Table 5). This precision level is adequate for the routine analysis of the investigated drugs in quality control laboratories.

(**c**) *Accuracy and interference.*—To check the accuracy of the proposed methods, recovery studies at different drug concentrations were carried out using the standard addition method. The results obtained revealed the good accuracy of the proposed methods in terms of mean recoveries (99.1–100.3 ± 0.72–0.88%). Before proceeding with the analysis of ribavirin in its capsules, interference studies were performed to explore the effect of common additives that might be added during formulations. Samples were prepared by mixing a known amount (200 mg) of ribavirin with various amounts of the common additives, such as gum acacia, sucrose, glucose, lactose, starch, citric acid, and microcrystalline cellulose, and the analysis was then performed. The good recoveries for ribavirin obtained from these synthetic mixtures indicated the absence of interference effects from these additives (Table 6). Although the methods are not selective, because they are based on oxidation reactions, the good recoveries ensure their suitability for the analysis of ribavirin capsules without interference from the common reducing additives. This was attributed to the sensitivity of the methods, which necessitated dilution of the samples; consequently, the additives were present below concentrations at which they could interfere.

(**d**) *Robustness and ruggedness.*—Robustness was examined by evaluating the influence of the small variation of method variables, including concentration of oxidants, reaction time, volume of acid (perchloric for ceric ammonium sulfate and sulfuric for the other reagents), and temperature on the performance of the proposed methods. In these experiments, one parameter was changed while the others were kept unchanged, and the recovery was calculated each time. It was found that none of these variables significantly affected the method; the recoveries ranged from 98.6 to 100.3 ± 0.34 to 0.96% (Table 7). This provided an indication of reliability during routine application of the proposed methods in the analysis of ribavirin.

Ruggedness was tested by applying the proposed methods to the assay of ribavirin using the same operational conditions but with 2 different instruments in 2 different laboratories and with different elapsed times. Results were found to be reproducible, because RSD values did not exceed 2% (Table 8).

Application of the Proposed Method to Analysis of Ribavirin Capsules

It is evident from the aforementioned results that the proposed methods gave satisfactory results with the bulk drug. Thus, ribavirin-containing capsules were subjected to analysis for their contents of ribavirin by the proposed methods and the official method (7). The recoveries ranged from 98.3 to 101.5 ± 0.52 to 1.02% (Table 9). These results were compared with those obtained from the official method by statistical analysis with respect to the accuracy (*t*-test) and precision (*F*-test). No significant differences were found between the calculated and theoretical values of the *t*- and *F*-tests at 95% confidence level, proving similar accuracy and precision in the analysis of ribavirin in capsules. It is evident from these results that all of the proposed methods are applicable to the analysis of ribavirin in its bulk form and in capsules with comparable analytical performance. The critical recommendations of some of these methods might be based on their relative sensitivities (depending upon the amount of specimen available for analysis) and experimental conditions (reaction time, temperature, diluting solvent, etc.). For example, the methods

Table 9. Analysis of ribavirin capsules by the proposed and official methods

Method	Found, % ± SD[a]	*t*-value[b]	*F*-value[b]
Ceric ammonium sulfate	100.4 ± 0.52	1.41	2.84
Potassium permanganate	98.9 ± 0.77	1.59	1.09
Ammonium molybdate	98.3 ± 0.85	1.02	1.22
Ammonium metavanidate	99.1 ± 0.81	1.89	2.05
Chromium trioxide	99.2 ± 1.02	0.87	2.15
Potassium dichromate	101.5 ± 0.95	2.10	3.53
Potassium iodate	98.9 ± 0.57	1.24	1.83
Potassium periodate	100.8 ± 0.79	1.12	3.74
Official method[c]	100.2 ± 0.72		

[a] Values are the mean of 5 determinations.

[b] Theoretical values for *t* and *F* at 95% confidence limit and $n = 5$ were 2.78 and 6.39, respectively.

[c] Ref. 7, high-performance liquid chromatography.

involving ceric ammonium sulfate, ammonium molybdate, and potassium permanganate gave more sensitive assays than the other reagents. However, the assay involving ceric solution needed an extra piece of apparatus, a water bath. Ammonium molybdate gave a sensitive assay; however, it needed acetone, rather than water (with other reagents), as the diluting solvent.

Conclusions

The present study developed simple and accurate spectrophotometric methods for the analysis of ribavirin. The methods were based on the oxidation of ribavirin with inorganic oxidants, and subsequent monitoring of the reactions spectrophotometrically at the corresponding λ_{max}. The methods are reliable for the accurate determination of ribavirin in bulk form and capsules without interference from common additives. The proposed methods are superior to the previously reported spectrophotometric methods (15, 16) in terms of simplicity and sensitivity. The methods are superior to the previously reported nonspectrophotometric methods for determination of both types of samples in terms of simplicity because they do not require prederivatization of ribavirin prior to the analysis. The proposed methods involved measurement in the visible region, which leads to selectivity and avoids potential intereferences from UV-absorbing excipients that are encountered in methods involving measurements in UV region. The range of different sensitivities achieved with various reagents gives the opportunity for choosing from these methods based on the amount of specimen available for analysis. The wide linear dynamic range that has been achieved in the proposed methods allows ease in preparation of the samples for analysis, and all of the analytical reagents are inexpensive, have excellent shelf life, and are available in any analytical laboratory. Therefore, these methods can be recommended for the routine analysis of ribavirin in quality control laboratories but, being based on oxidation, their selectivity is not adequate for application to biological fluids.

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