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Research Article

Derivative Spectrophotometric Methods for the Analysis and Stability Studies of Colistin Sulphate

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Simple spectrophotometric methods were developed for the quantitative determination of colistin sulphate in bulk and dosage forms. The methods were based on the measurement of first and second derivative spectra of colistin sulphate at 298 nm and 318 nm, respectively. Beer's law was obeyed in the concentration range 800–4000 IU/mL with good correlation coefficient (not less than 0.998) for both methods. The developed first derivative spectrophotometric method was then selected to study the degradation behavior of colistin sulphate in alkaline media at different temperatures as the second derivative method failed to give reproducible results for the stability study. The pH-rate profile indicates a first-order dependence of the degradation rate on $[OH^-]$ at pH ranging between 8 and 11. The obtained results for the photochemical study reflected photostability of colistin sulphate.

1. Introduction

Colistin (polymyxin E, Figure 1) is a polymyxin antibiotic produced by certain strains of *Bacillus polymyxa* var. *colistinus*. Colistin is a mixture of cyclic polypeptides colistins A and B. It is effective against most Gram-negative bacilli and is used as a polypeptide antibiotic. It remains one of the last-resort antibiotics for multidrug-resistant *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Acinetobacter* [1].

The knowledge of a drug chemical stability is of great importance to select the suitable storage conditions, estimation of drug shelf life, and anticipation of the drug interaction [2].

Literature survey revealed several methods for the determination of colistin sulphate in animals' body fluid and cow milk. These methods include mainly chromatographic methods [3–6]. None of these methods has been applied for the analysis and stability studies of colistin sulphate in dosage forms.

Derivative spectrophotometry has been widely applied in the analysis of different pharmaceutical dosage forms. It solves the problem of analysis associated with drug combination, stability studies of drug and degradation products, drug impurities, and interference of excipients in drugs.

Therefore, the aim of the present work is to develop simple and accurate derivative spectrophotometric methods for the determination of colistin sulphate in bulk and dosage forms and to investigate the effects of pH, temperature, and alkali on its stability.

Experiments

Instrumentation. UV spectrophotometric studies were carried out on Shimadzu UV-1800ENG240V (Kyoto, Japan). The operating conditions were as follows:

(i) Wavelength range: 250–450 nm.

(ii) Scan speed: medium, 0.2 nm/s.

(iii) Minimum ordinate readings recorded for the absorbance measurement (−0.005–−0.02).

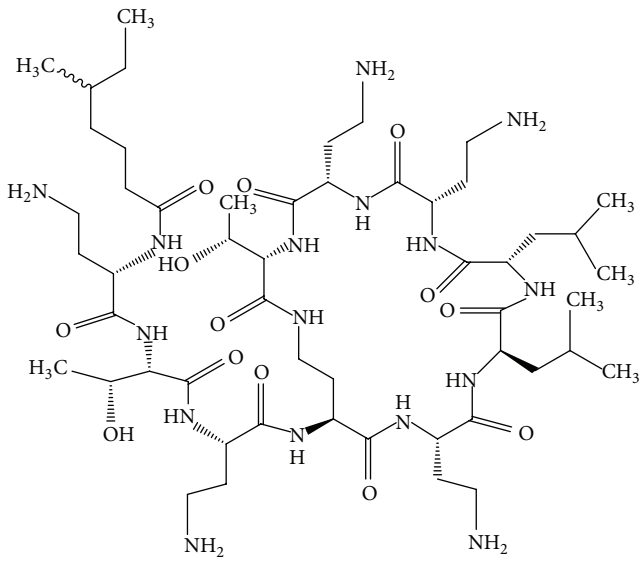

Figure 1: Chemical structure of colistin (used as sulphate).

2. Materials and Methods

All the materials and reagents used were of analytical grade. Colistin reference standard was obtained from the Central lab, Riyadh, Kingdom of Saudi Arabia. Colistin sample (Colidad powder; each 1 gram colistin (as sulphate) contains 800,000 IU) was obtained from Dar Aldawa Pharmaceuticals, Jordan. Disodium orthophosphate and sodium hydroxide were obtained from British Drug House (BDH), Poole, England; Mcllvaine universal buffer (pH range 2.2–8) [7] and phosphate buffer (pH range 9–11) [8] were prepared.

2.1. Preparation of Standard Stock Solution. One gram of colistin sulphate standard was accurately weighed and transferred into 100 mL volumetric flask. The volume was completed to mark with distilled water (solution A; 8000 IU/mL).

2.2. Preparation of Sample Stock Solution. An amount of the powdered sample equivalent to 800,000 IU was accurately weighed and transferred into 100 mL volumetric flask. The volume was then completed to mark with distilled water (solution B; 8000 IU/mL).

3. Method Validation

3.1. Linearity. Serial dilutions were made from solution A by transferring accurately measured volumes (1–5 mL) to set of 10 mL volumetric flasks. The volumes were completed to mark with distilled water. The first and second derivative spectra were recorded over the range 250 nm–450 nm. The calibration curve was constructed by plotting the obtained absorbance values against drug concentrations in IU/mL.

3 mL of solution B was treated as under linearity. The content of the powdered sample can be determined from the regression data or by the direct comparison of sample/standard concentrations.

3.2. Precision. Within-day and between-day data were determined for three concentrations within the linearity range. The precision was then estimated in terms of relative standard deviation % (RSD%).

3.3. Accuracy. The accuracy of the developed method was determined by means of recovery study. Two mL of each of the solutions A and B was transferred into two separate stoppered glass tubes. In a third stoppered glass tube, 2 mL of solution A and 2 mL of solution B were mixed. Solutions in the three tubes were treated as under linearity and the percent recovery was calculated using the reported formula [9]:

$$\frac{A_{\text{mix}} - A_{\text{sam}}}{A_{\text{std}}} \times 100, \tag{1}$$

where A_{mix} is the absorbance of the mixture solution, A_{sam} is the absorbance of the sample solution, and A_{std} is the absorbance of the standard solution.

3.4. Limits of Detection and Quantification. LOD and LOQ were determined using the following formulae [10]:

$$\text{LOD} = \frac{3.3\delta}{S}, \qquad \text{LOQ} = \frac{10\delta}{S}, \tag{2}$$

where δ is the standard deviation of the intercept of the regression line; S is the slope of the calibration line.

4. Stability Studies

4.1. Effect of pH on the Stability of Colistin Solution. Aliquots of solution A (3 mL) were transferred into a set of volumetric flasks (10 mL). One mL of the phosphate buffers was added to each flask to obtain a range of pH values (2.2, 3, 4, 5, 6, 7, 8, 9, 10, and 11). The volumes were completed to mark with distilled water. The kinetics of drug degradation was then monitored at 5-minute intervals using the developed first derivative spectrophotometric method. The rate constant for each reaction mixture was calculated from the plot of % remaining drug versus time interval [11].

4.2. Effect of Alkali and Acid on the Stability of Colistin Solution. Three mL of solution A was transferred into 10 mL volumetric flask. 1 mL of 1 M NaOH was added, and the volume was completed to mark with distilled water. The drug degradation was monitored at 5-minute intervals using the developed first derivative spectrophotometric method (250–450 nm).

The same procedure was repeated adding 1 mL of 1 M HCl instead of NaOH to study the effect of acid on stability of colistin sulphate.

4.3. Effect of Temperature on the Stability of Colistin Solution. Aliquots from solution A (3 mL) were transferred into four stoppered glass tubes. 1 mL of Mcllvaine universal buffer

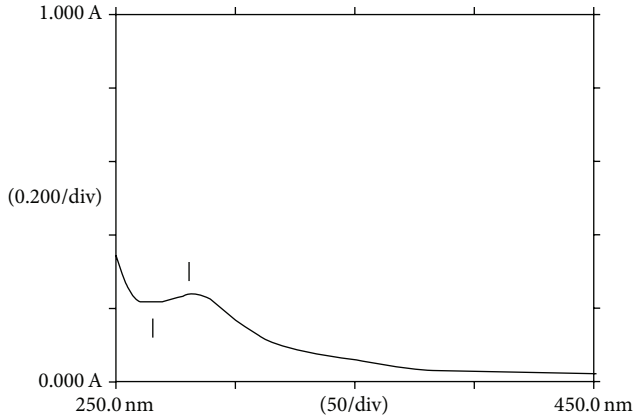

Figure 2: UV spectrum of colistin solution (280 nm, 1600 IU/mL).

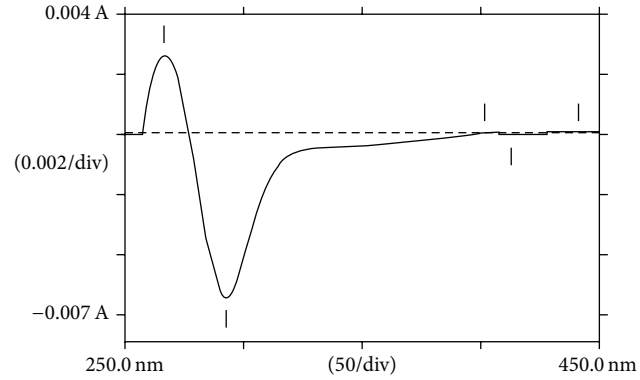

Figure 3: First derivative spectrum of colistin solution (298 nm, 1600 IU/mL).

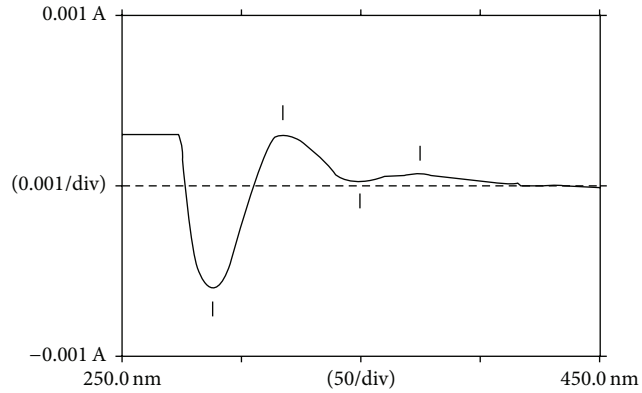

Figure 4: Second derivative spectrum of colistin solution (318 nm, 2400 IU/mL).

(pH 9) was added to each tube. The volume of the first tube was completed to 10 mL with distilled water and the first derivative spectra were recorded at room temperature (25°C) with time intervals ranging within 5–20 minutes. The other three tubes were heated at 40°C at the same time interval. The reaction was then quenched by cooling and the volumes were completed to 10 mL with distilled water. The kinetics of the degradation was monitored using the developed method (250–450 nm).

4.4. Effect of Light on the Stability of Colistin Solution. The effect of sunlight in the day time was studied by placing colistin solution in stoppered glass tube (3 mL of solution A completed to 10 mL with distilled water; 2400 IU/mL) and exposed to sunlight. The photodegradation was then monitored by the developed method at time intervals ranging within 1–6 hrs.

5. Results and Discussion

Direct spectrophotometric methods (zero-order) are widely used in pharmaceutical analysis although they lack selectivity and did not prove in most cases to be a useful tool in stability-indicating procedure. However, the development of derivative spectroscopy presented the analyst with a tool suited for the analysis of different pharmaceutical dosage forms as it has proved to be an accurate stability-indicating method. Therefore, our main objective was to develop an accurate and precise method for the analysis of colistin sulphate which can also be applied for the stability studies under stress conditions as per ICH requirements [12].

The original UV spectrum of colistin solution showed a broad peak at 280 nm (Figure 2). First and second derivative spectra showed sharper and better resolved peaks at 298 nm and 318 nm, respectively (Figures 3 and 4).

5.1. Linearity. A calibration curve was prepared using the developed method at concentration of 800–4000 IU/mL for colistin with correlation coefficient value not less than 0.998. The regression analysis data was calculated at 95% confidence level for the developed methods using the following formulae [10]:

$$\begin{aligned} &\left(b \pm ts_b\right), \\ &\left(a \pm ts_a\right), \end{aligned} \tag{3}$$

where b is the slope, a the intercept, s_b standard deviation of slope, and s_a standard deviation of intercept and the t-value is at 95% confidence level for $(n - 2)$. The results obtained reflected the consistency of the prepared calibration graphs (Table 1).

The developed derivative spectrophotometric methods were applied for the drug uniformity testing in colistin powder where good assay results were obtained (98.8 ± 1.8%, $n = 3$).

5.2. Accuracy and Precision. The accuracy of the procedure and freedom of interference by the powder excipients were confirmed by the results obtained for recovery testing of added amount of authentic colistin to sample solution in the ratio of 1 : 1. The results showed good recovery (99.7 ± 1.6%, $n = 3$), which indicates the accuracy of the developed method.

Table 1: Linearity data for the developed methods.

Parameter	Zero-order	1st derivative	2nd derivative
λ_{max}	280 nm	298 nm	318 nm
Slope $\pm ts_b$	0.00027 ± 0.000	0.000005 ± 0.000	0.0089 ± 0.0008
Inter. $\pm ts_a$	0.015 ± 0.089	−0.0006 ± 0.0017	−0.43 ± 2.7
LOD	335 IU/mL	343 IU/mL	239 IU/mL
LOQ	1015 IU/mL	1040 IU/mL	725 IU/mL
R	0.998	0.998	0.999

Table 2: Precision of the developed methods.

Concentration (IU/mL)	RSD%; $n = 3$	
	Within-day	Between-day
1600	1.1	2
2400	0.54	1.3
3200	0.77	0.8

Table 3: K_{obs}, $t_{1/2}$, and t_{90} values for the degradation of colistin sulphate at room temperature in different pH values.

pH	K_{obs} min^{-1}	$t_{1/2}$ min	t_{90}
2.2–7.4	0.0028	247.50	37.50
8	0.0050	138.60	21.00
9	0.0079	87.70	13.30
10	0.0115	60.30	9.13
11	0.0200	34.65	5.25

The precision of the developed method was confirmed by the calculated RSD values, which were found to be within the accepted limit (less than 2%) (Table 2).

5.3. Stability Studies

5.3.1. Effect of pH. The degradation of colistin at different pH values ranging within 2.2–11 was monitored by the developed first derivative spectrophotometric method. A plot of log K_{obs} (degradation rate constant) versus pH values gave a positive slope on the alkaline side. This suggested first order dependence of the degradation rate on [OH$^-$]. The obtained pH profile (Figure 5) resembles subtype BCD in the generalized pH polygon [11], where K_{obs} increases, and hence $t_{1/2}$ decreases, at high pH values (Table 3).

5.3.2. Effect of Alkali. The effect of different alkali concentrations with different time intervals on the degradation rate of colistin sulphate solution was studied using the developed first derivative method. 1 M NaOH with 5-minute intervals was found appropriate to give good correlation coefficient with measurable degradation rate. The first derivative spectrum of colistin solution treated with 1 M NaOH showed a decrease in colistin sulphate peak at 298 nm with the consequent appearance of major degradation peak at 338 nm (Figure 6).

The degradation rate constant was calculated by plotting log % remaining drug versus time interval (Figure 7).

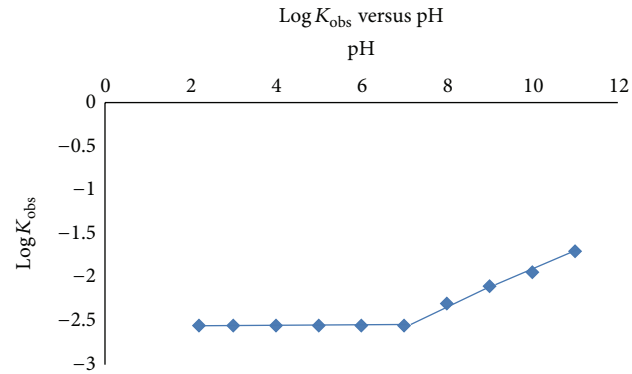

Figure 5: pH profile of colistin degradation at room temperature.

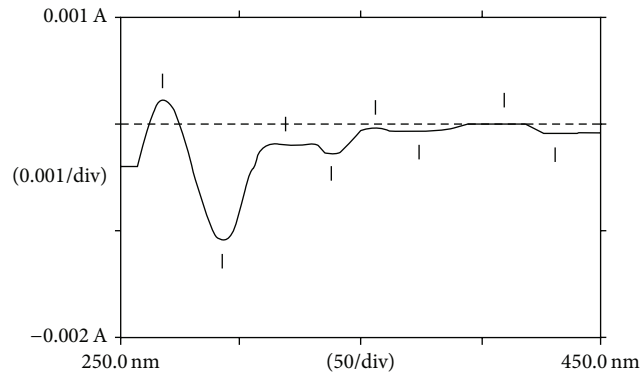

Figure 6: First derivative spectrum of colistin solution treated with 1 M NaOH for 15 minutes.

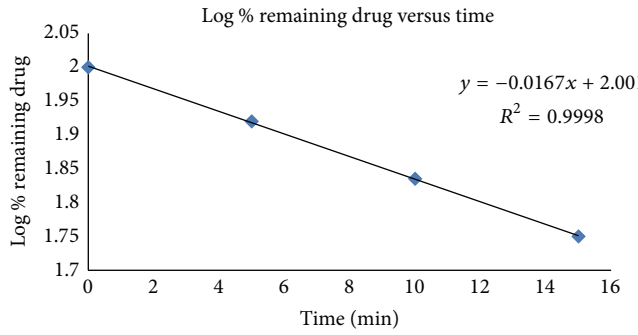

Figure 7: Effect of NaOH (1 M) on the degradation of colistin.

The degradation rate was found to increase and $t_{1/2}$ decreases with increased time and alkali concentration.

Higher concentrations of sodium hydroxide resulted in the immediate disappearance of the colistin peak and the appearance of the degradation products peaks.

Using hydrochloric acid, the concentration and first derivative spectrum of colistin sulphate were not affected with the different concentrations of acid even at high temperatures.

5.3.3. Effect of Temperature. The developed first derivative method was applied to monitor the time-course decomposition of colistin in phosphate buffer (pH 9) at temperatures

Table 4: K, $t_{1/2}$, and t_{90} values for colistin degradation at pH 9 at different temperatures (5, 25, and 40°C).

Temp.	K (min^{-1})	$t_{1/2}$ (0.693/K)	t_{90} (0.105/K)
5°C	0.0008	866.25	131.25
25°C	0.0079	87.70	13.30
40°C	0.035	19.80	3.00

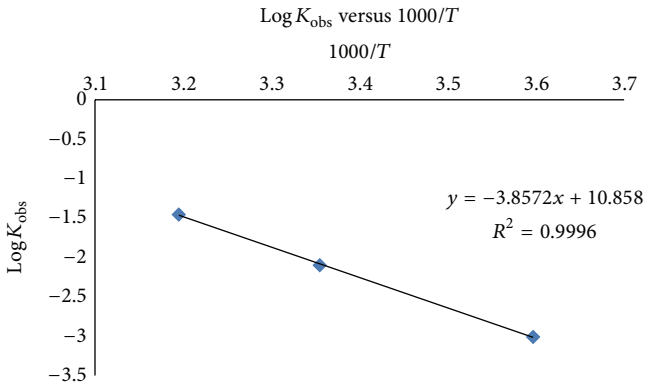

Figure 8: Arrhenius plot for degradation of colistin sulphate in phosphate buffer pH 9 and temperatures 5°, 25°, and 40°C.

of 5°C, 25°C, and 40°C. The linearity of the constructed plots at different temperatures (log % remaining drug versus time) reflected the dependence of the decomposition reaction on temperature. The activation energy (E_a) was calculated (18.5 Kcal mol^{-}) using Arrhenius plot (Figure 8), which was then utilized to calculate the shelf life ($t_{1/2}$) and t_{90} for colistin sulphate at different temperatures (Table 4).

5.3.4. Effect of Light (Photodegradation). The effect of light is often considered an important factor in drug stability. The photodegradation study on colistin was intended to obtain useful information about the sensitivity of the drug to light. Conditions of irradiation were controlled to study the effect of light and not energy (heat). The decomposition of irradiated colistin solution with sunlight for about 6 hours was monitored using first derivative spectrophotometry. The solution remained stable and there was no change neither in spectrum maximum at 298 nm nor in the concentration. This reflected the stability of colistin solution under these conditions.

6. Conclusion

In this work, simple, accurate, precise, and stability-indicating spectrophotometric methods were developed for the analysis of colistin sulphate in bulk and dosage forms. The degradation product peak was well separated using the developed first derivative spectrophotometric method.

Colistin sulphate was found to undergo hydrolysis which appeared to be [OH^{-}] dependent. It was stable along the acidic pH even at temperatures above 40°C. Colistin sulphate was found to be photostable under the study conditions.

The developed methods can be useful for the routine quality control analysis and stability study of colistin sulphate.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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