

COLORIMETRIC DETERMINATION OF CEFQUINOME SULPHATE IN BULK AND DOSAGE FORM USING AMMONIUM MOLYBDATE

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ABSTRACT

This study was designed to develop two colorimetric methods for the determination of Cefquinome Sulphate (CS) in bulk and dosage forms using two different concentrations of molybdenum solution. The developed methods were based on the oxidation of CS with 2% ammonium molybdate, in the presence of sulphuric acid, producing a green colored product with λ_{\max} at 409 nm (Method I) and the oxidation of CS with 10% ammonium molybdate in acidic media to produce a blue colored product with λ_{\max} at 673 nm (Method II). The factors affecting the color development and stability were optimized and incorporated in the procedure. Beer's law was obeyed over the concentration range 16-80 $\mu\text{g mL}^{-1}$ (Method I) and 40-80 $\mu\text{g mL}^{-1}$ (Method II) with a correlation coefficient not less than 0.999. The limit of detection and limit of quantification were 5.7 and 18.9 $\mu\text{g mL}^{-1}$ for Method I, 4.25 $\mu\text{g mL}^{-1}$ and 14.2 $\mu\text{g mL}^{-1}$ for Method II respectively. The average recovery for the dosage form (suspension 2.5%) was 100.30% ± 0.59 ; n = 3, which reflected no interference by the suspension excipients. The results obtained by the developed methods for the suspension dosage form were statistically compared with those of a developed HPLC method and evaluated at 95% confidence limits. The developed methods were proved to be accurate and simple. The methods involved in the study covered, Colorimetric spectrophotometry, High Performance Liquid Chromatography. The molar ratio method is recommended to be conducted in order to determine the reaction stoichiometry.

Keywords: Determination, Spectrophotometry, Cefquinome Sulphate, Suspension, Ammonium Molybdate

1. INTRODUCTION

Cefquinome Sulphate (CS) is a quinolinium salt (Fig. 1) (Sweetman, 2009). It is a fourth generation cephalosporine and has a broad spectrum activity against Gram-positive and Gram-negative bacteria. It is used for the treatment of bovine respiratory disease (FDA, 2006).

In literature, methods used for analysis of CS include chromatographic methods either in animals' biological fluids (Uney *et al.*, 2010; Xie *et al.*, 2013; Sukren and Knappstein, 2003; Parlevliat *et al.*, 2009) or in dosage form (Janaki and Appala, 2012). Recently, we developed stability-indicating spectrophotometric and HPLC methods for the

determination of CS in bulk and dosage form (Shantier and Gadkariem, 2013).

Literature review revealed no previous colorimetric methods had been adopted for the assay of CS in its dosage forms.

It is well known that reduction of acidified molybdenum produces a colored solution which was utilized in analytical chemistry. Salts of Mo (VI) had been used as oxidizing agents for determination of various drugs: Tetracycline (Saleh, 1986), chloramphenicol (Morelli, 1987), cephalosporines (Prodromas, 1988a) and levodopa (Prodromas, 1988b). Spectrophotometry continues to be very popular, because of its simplicity and the general low operational cost compared to the recently developed sophisticated instrumental methods.

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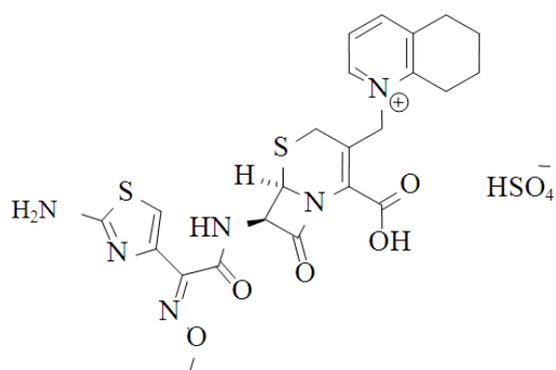


Fig. 1. Chemical structure of cefquinome sulphate

Therefore, the aim of the present work is to develop simple colorimetric methods for the determination of CS in bulk and dosage form that can be useful for routine quality control of the drug.

2. MATERIALS AND METHODS

2.1. Instrumentation

UV spectrophotometric studies were carried out on Shimadzu UV-1800ENG240V, (Koyoto, Japan).

2.2. Materials

All materials and reagents used were of analytical grade.

Drug sample (Cobactan[®], 2.5%) was kindly provided by Intervet Schering-Plough, European Union. The reference standard was provided by Intervet International GmbH.

CS standard stock solution was freshly prepared to obtain 800 $\mu\text{g mL}^{-1}$ (solution A) and 500 $\mu\text{g mL}^{-1}$ (solution B). Sulphuric acid H_2SO_4 (Sd fine Chem.Limited, Mumbai): 3 M and upto 11 M were prepared in the usual way from the concentrated acid (98%). Ammonium molybdate $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$; Sd fine Chem. Limited, Mumbai): Solutions were freshly prepared by dissolving 0.5 g in 25 mL distilled water to obtain 2% m/v (Mo-1) and dissolving 2.5 g in 25 mL of 50% v/v H_2SO_4 to obtain 10% m/v (Mo-2).

2.3. Procedure Calibration Graph

2.3.1. Method I

Different accurately measured volumes (100-500 μL) of solution A were transferred into five stoppered glass tubes. 1 mL of Mo-1 solution was added to each tube, followed by 3 mL of 9 M H_2SO_4 . The tubes were then

heated in a boiling water bath for 30 min. After cooling, the volumes were completed to 5 mL with distilled water. The blank was prepared in the same manner using distilled water instead of CS solution. The absorbance was measured at 409 nm and calibration graph was constructed by plotting the absorbance values against the drug concentrations series.

2.3.2. Method II

Different accurately measured volumes (400-800 μL) of solution B were transferred into five stoppered glass tubes. About 1 mL of Mo-2 solution was added to each tube, followed by 3 mL of 7 M H_2SO_4 . The tubes were then heated in a boiling water bath for 15 min. After cooling, the volumes were completed to 5 mL with distilled water. The blank was prepared in the same manner using distilled water instead of CS solution. The absorbance was measured at 673 nm and calibration graph was constructed by plotting the absorbance values against the drug concentrations series.

2.4. Procedure for the Assay of CS Suspension

CS sample solutions were freshly prepared to obtain 800 $\mu\text{g mL}^{-1}$ (solution C) and 500 $\mu\text{g mL}^{-1}$ (solution D) using water as diluents.

About 300 μL of solution C and 600 μL of solution D were treated as under calibration graph. The concentration of both sample solutions (C and D) were obtained from the regression analysis data of the standard absorbance/concentration plot.

3. RESULTS AND DISCUSSION

The cephalosporins are among the widely used antibiotics for the treatment of bacterial infections in humans and animals. CS, one of these β -lactam cephalosporines, is the target of the present work. It has a free amino group and expected to undergo oxidation-reduction reaction.

It is well known that a colored solution is obtained as a result of the reduction of acidified molybdenum (Mo (VI)) solution. It has several oxidation states, the most stable being +4 and +6. The highest oxidation state is common in the molybdenum (VI) trioxide (MoO_3). When MoO_3 is dissolved in neutral or alkali solution the simple MoO_4^{2-} anion is produced which gives upon reduction the hydroxide $\text{MoO}(\text{OH})_2$ (green colour). As the pH is reduced the first species to be formed is the heptamolybdate (Pope and Muller, 1997).

The developed methods involved the reaction of CS with Mo-1 solution, in acidic media, to produce a green product with a wavelength maximum at 409 nm and with Mo-2 solution to produce a blue colored product at λ_{\max} 673 nm (Method I and II) respectively (**Fig. 2**).

It is proposed that the reaction between CS and molybdenum could be through a charge-transfer reaction (metal/ligand) leading to the observed lambda shift into the visible region (409, 673 nm) depending on the molybdenum state.

3.1. Optimization of Different Experimental Parameters

The optimum conditions were established based on the maximum color intensity and the stability of the reaction product.

3.2. Effect of Sulphuric Acid Concentration

The concentration and volume of sulphuric acid is one of the important parameters affecting the oxidation-reduction reaction. Different concentrations of the acid upto 11 M were investigated.

For method I, the maximum color intensity with good linearity and stability was obtained using 9 M H_2SO_4 . At lower concentration, the color was not obtained which indicates that the reaction product was not formed; while at higher concentrations (11 M), although it gives more intense color, the color stability and the linearity were decreased, (**Table 1 and Fig. 3**). Other acids such as acetic acid and HCl were also studied. It was found that either no color reaction was produced with acetic acid or a light color was obtained with hydrochloric acid which was unstable.

For method II, the maximum color intensity with good linearity and stability was obtained using 7 M H_2SO_4 . The color intensity increased gradually with increasing sulphuric acid concentration upto 7 M sulphuric acid, then started to decrease when using higher acid concentrations (9 and 11 M). Therefore, 7 M sulphuric acid was found to be optimum to produce stable and intense color (**Table 2 and Fig. 4**).

The effect of different volumes of H_2SO_4 was also investigated. About 3 mL of the acid was found to be sufficient for color production and stability.

3.3. Effect of Ammonium Molybdate Concentration

Ammonium molybdate was used as color producing reagent. The adopted concentrations and volume, 1 mL of 2% solution (Method I) and 1 mL of 10% solution (Method II), were sufficient for maximum absorbance

values and stability. Below or above these concentrations and volume, the absorbance was found to decrease.

3.4. Effect of Heating Time

The heating time was one of the important parameters studied for proper color development. The results obtained showed that heating in boiling water bath for 45 and 30 min, for method I and II respectively, gave higher absorbance but the color stability was decreased. Below 30 min (method I) and 15 min (method II), the reaction products were not completely formed. Therefore, heating for 30 and 15 min. (method I and II respectively) was found to be the optimum condition to develop an intense and stable color.

Also, the order of addition of the reactants recommended in the general procedure was found important for the development of a color with maximum intensity and stability.

The formed products were found to remain stable for at least 3 h (Method I) and 24 h (Method II).

3.5. Analytical Curves, Recovery and Precision

The optimized conditions were utilized to construct the calibration graphs using authentic CS. Beer's law plots were obeyed for drug concentrations within a range of 16-80 $\mu g mL^{-1}$ (Method I) and 40-80 $\mu g mL^{-1}$ (Method II). Spectral data for the reaction of molybdate with CS are presented in **Table 3**.

The low values of the standard errors of the slope, the intercept and correlation coefficient values (not less than 0.999) reflected the consistency of the prepared calibration graphs.

The accuracy of the procedure and freedom of interference by the suspension excipients were confirmed by the results obtained for recovery testing of added amount of authentic CS to suspension solution in the ratio of 1:1. The results showed good recovery (100.30%±0.59, n = 3).

To examine the repeatability and reproducibility of the methods, replicate determinations (n = 3) were made for four different concentrations of the standard curve. The calculated RSD values were found to be within the accepted limits (less than 2%).

Table 1. Effect of sulphuric acid concentration (Method I)

Sulphuric acid conc	3M	5M	7M	9M	11M
Absorbance (60 $\mu g mL^{-1}$)	0	0	0.116	0.283	0.395
Correlation coefficient	-	-	-	0.999	0.987

Table 2. Effect of sulphuric acid concentration (Method II)

Sulphuric acid conc	3M	5M	7M	9M	11M
Absorbance (100 $\mu g mL^{-1}$)	0	0.117	0.503	0.21	0.058

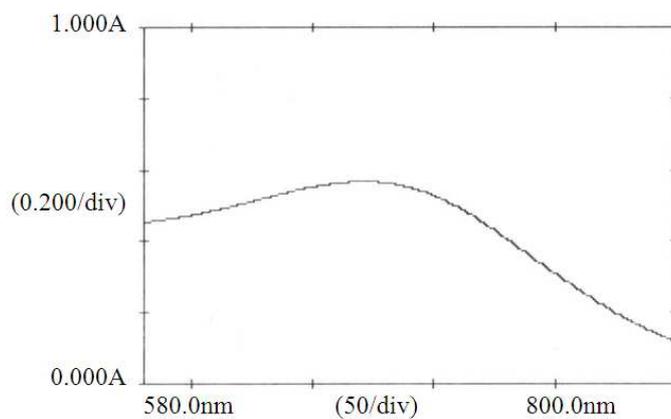


Fig. 2. UV/VIS spectrum of the colored product (673nm)

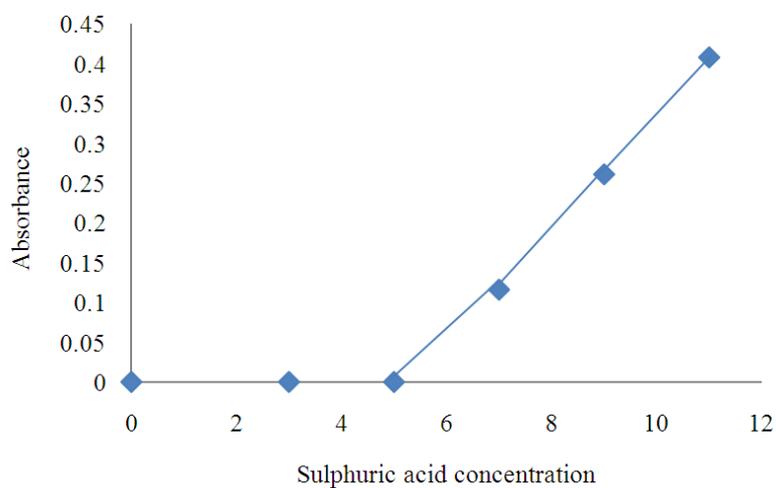


Fig. 3. Effect of sulphuric acid concentration on the color intensity

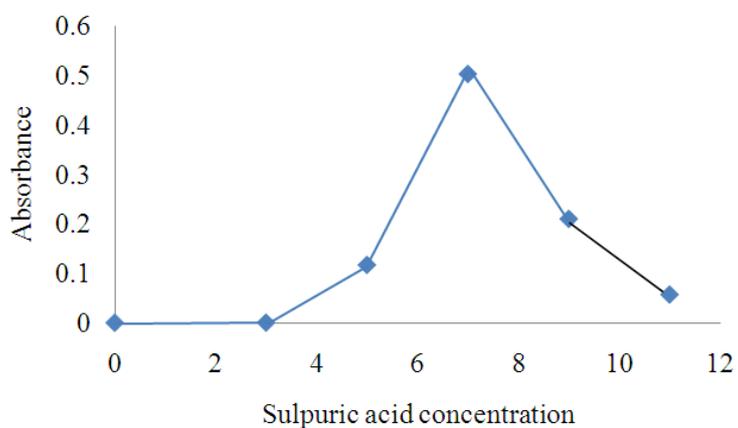


Fig. 4. Effect of sulphuric acid concentration on the color intensity

Table 3. Spectral data of the reaction of CS with ammonium molybdate

Parameter	Method 1	Method 2
Slope \pm SE*	0.004 \pm 0.00048	0.0033 \pm 0.00047
Intercept \pm SE*	-0.0014 \pm 0.026	-0.00058 \pm 0.029
Correlation coefficient	0.999	0.999
Range	16-80 $\mu\text{g mL}^{-1}$	40-80 $\mu\text{g mL}^{-1}$
LOD	5.7 $\mu\text{g mL}^{-1}$	4.25 $\mu\text{g mL}^{-1}$
LOQ	18.9 $\mu\text{g mL}^{-1}$	14.2 $\mu\text{g mL}^{-1}$
Molar absorptivity ($\text{L mol}^{-1} \text{cm}^{-1}$)	2185	1715

*Standard error calculated at 95% confidence limit

Table 4. Validation results of developed method compared to the developed HPLC method

	Content% of CS \pm RSD%	*t cal, t(tab)	*F cal, F(tab)
Method I	101.00 \pm 0.64	0.78, (2.78)	7 (19)
Method II	102.00 \pm 0.83	1.44, (2.78)	5 (19)
Liquid chromatographic method	100.00 \pm 1.87	-	-

* = t and F calculated and tabulated

The methods were applied for the drug uniformity testing in CS suspension (2.5%) where good assay results ($X \pm \text{RSD} (\%)$, n) were obtained.

The validity of the developed methods for the determination of cefquinome in bulk or dosage form was assessed by comparison of the statistical results obtained with those of the developed HPLC method (Shantier and Gadkariem, 2013). Data of **Table 4** show the obtained assay results and the calculated t-and F-values as compared to the corresponding tabulated values at 95% confidence level. As the calculated t-and F-values were less than tabulated ones, this indicates very good accuracy and precision of the developed methods.

4. CONCLUSION

The proposed methods are simple, sensitive, accurate, precise and inexpensive analytical techniques for the determination of CS in bulk and dosage form. Statistical comparison of the results with the developed HPLC method indicates no significant difference at 95% confidence limit. The proposed methods are the only colorimetric methods for the determination of CS in its dosage form. They have low operational cost compared to the chromatographic methods. Another advantage of the proposed methods is that there is no need for pretreatment of the sample (extraction step).

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