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Differential Spectrophotometric Method for Determination of Cefquinome Sulphate

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Authors' contributions

This work was carried out in collaboration between all authors. Author SS performed experimental work, collected the literature survey and drafted the manuscript, Author EG took part in the discussions and versioned the paper. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Differential first derivative (ΔD_1) method is developed for the quantitative analysis of cefquinome sulphate (CS) in bulk and dosage form. The method is based on measuring the ΔD_1 of CS in alkaline solutions against its neutral aqueous solutions as blanks. The proposed method is sensitive and highly specific, since the interference of the excipients or impurities is nullified. Beer's law was obeyed over the concentration range 4-20 µg/mL with a limit of detection and limit of quantification 1.5 µg/mL and 4.8 µg/mL respectively.

Keywords: Differential; first derivative; CS; alkaline solution; spectrophotometry.

1. INTRODUCTION

CS [1] is a fourth generation cephalosporines (Fig. 1). It has a broad spectrum activity against Gram-positive and Gram-negative bacteria. It is intended for the treatment of bovine respiratory disease (BRD) [2].

Various methods have been reported for the determination of CS in dosage form and biological fluids. These methods include mainly HPLC [3-7]. Recently we developed a spectrophotometric and HPLC methods for determination of CS in bulk and dosage form [8].

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Difference spectrophotometry is an analytical technique which has been used to improve the selectivity and accuracy of the measurement. The main feature of difference spectrophotometric assay is that the measured value is the difference absorbance of two equimolar solution of the analyte in different chemical forms [9]. It not only eliminates matrix interference due to excipients, but also it resolves spectral overlap of other accompanying drugs [10]. The accuracy and selectivity of conventional UV absorption methods is also increased by conversion of normal zero-order or differential UV spectra into higher order [11,12].

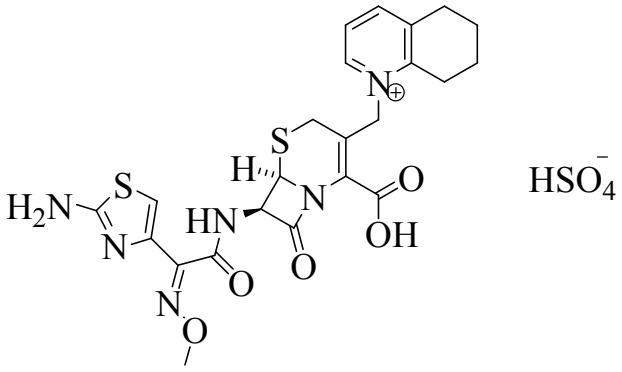

Fig. 1. Chemical structure of cefquinome sulphate

Therefore, the application of difference spectrophotometry is expected to have the totality of advantages of both derivative spectrophotometry (first, second, etc) combined with delta spectrophotometry.

Thus, the aim of the present work is to develop a simple and rapid differential spectrophotometric method for the assay of CS. The selectivity of the method permits the determination of CS in the presence of its degradation product (alkaline hydrolysis).

2. EXPERIMENTAL DETAILS

2.1 Apparatus

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

- Wavelength range, 400-240 nm.
- Scan speed, Medium.
- Ordinate maximum and minimum settings was +0.03 and – 0.02.

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 obtained from Intervet International. Sodium hydroxide (BDH), Pool, England.

2.3 Procedure

2.3.1 Preparation of standard stock solution

A stock solution of standard cefquinome sulphate (0.1%, w/v) was prepared in distilled water. One mL of this solution was further diluted to 25 mL with distilled water (40 µg/mL, solution A).

2.3.2 Procedure for suspension

1 mL of the suspension (shaken well) was transferred to 25 mL volumetric flask. About 10 mL of distilled water was added and the solution was shaken for about 10 minutes to ensure dissolution. The volume was then completed to mark and filtered. One mL of the filtrate was diluted to 25 mL with distilled water (solution B, 40 µg/mL).

2.4 Calibration Graph

Different accurately measured volumes (1-5 mL) of solution A were transferred into five stoppered glass tubes. 1 mL of 1M sodium hydroxide was added to each tube. The tubes were then heated in a boiling water bath for 30 minutes. The reaction was then quenched by cooling and the volumes were completed to 10 mL using distilled water. ΔD_1 spectrum was then recorded by measuring the absorbance of the CS alkaline solutions against the corresponding aqueous CS solutions as blanks. Regression analysis data was obtained from the ΔD_1-concentration graph.

For determination of suspension content, 3 mL of solution B was treated as under calibration graph.

A graph was also constructed from each of solution A and B to compare the slopes of these graphs as another means for suspension content determination.

3. RESULTS AND DISCUSSION

CS belongs to the ß-lactam group of antibiotics. Its antibacterial activity is dependent on the presence of the ß-lactam functionality which is not stable in aqueous conditions. This instability of those drugs leads them to undergo chemical degradation in alkaline medium [13,14].

Preparation of CS fresh solutions was found necessary as these solutions were found to undergo slight decrease in concentration during the day; however CS does not undergo detectable hydrolysis unless heated with alkali.

As shown in Figs. 2a and 2b, the first derivative spectra of CS exhibited different absorption bands in aqueous and alkaline media. The UV spectrum of CS in aqueous media showed a maximum at 286 nm while in alkaline media (1M NaOH) showed a maximum at 311 nm. This indicates that CS undergoes chemical degradation which was found to be [OH-] dependant suggesting that OH- is playing the role of the nucleophile in the degradation process (intramolecular hydrolysis). Fig. 3 is a suggested alkaline-CS hydrolysis product which is probably giving the shift to wavelength 311 nm.

This bathochromic shift from 286 nm to 311 nm forms the basis of differential spectrophotometric method for determination of CS. Thus, CS can be quantitatively determined by measuring ΔD_1 at 289 nm (Fig. 4).

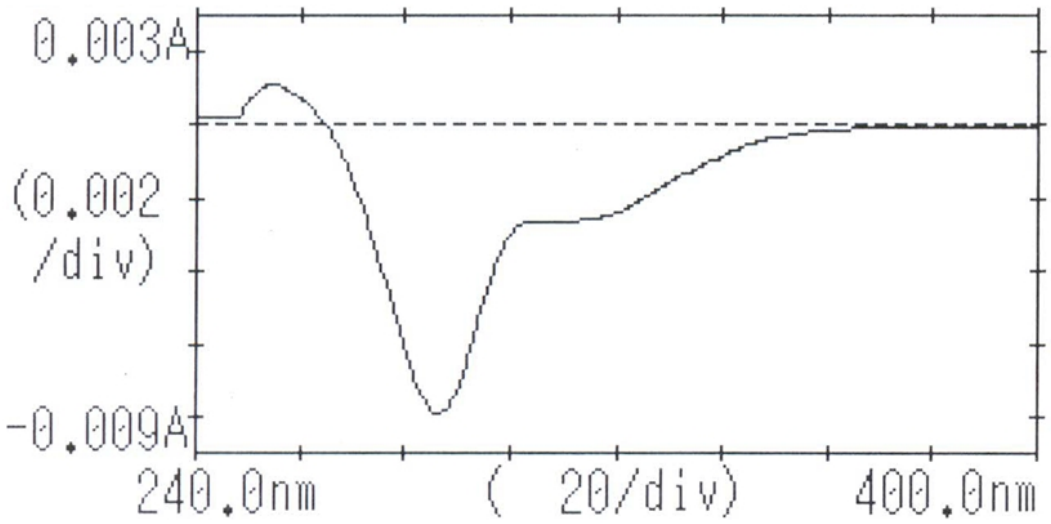

Fig. 2a. First derivative spectrum of CS solution (12µg/ml, 286nm)

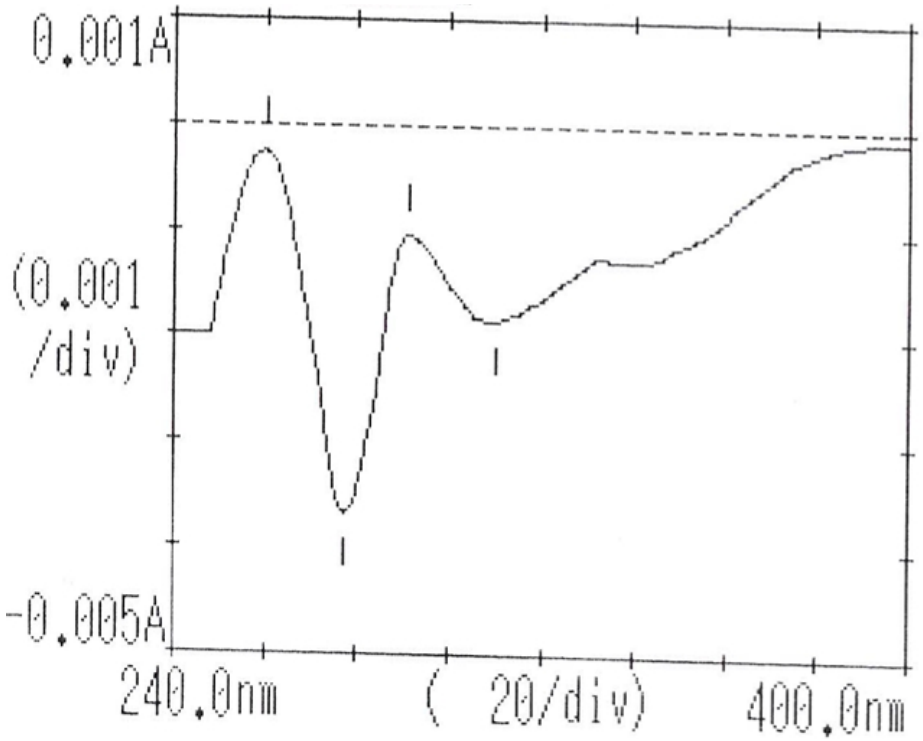

Fig. 2b. First derivative spectrum of CS alkaline solution (heating time 30 min)

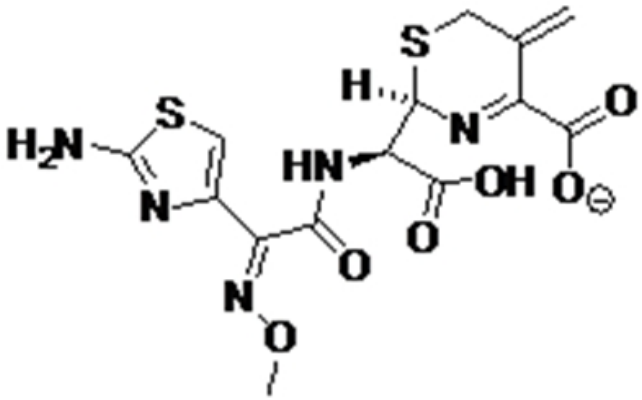

Fig. 3. Suggested structure of the degardation product

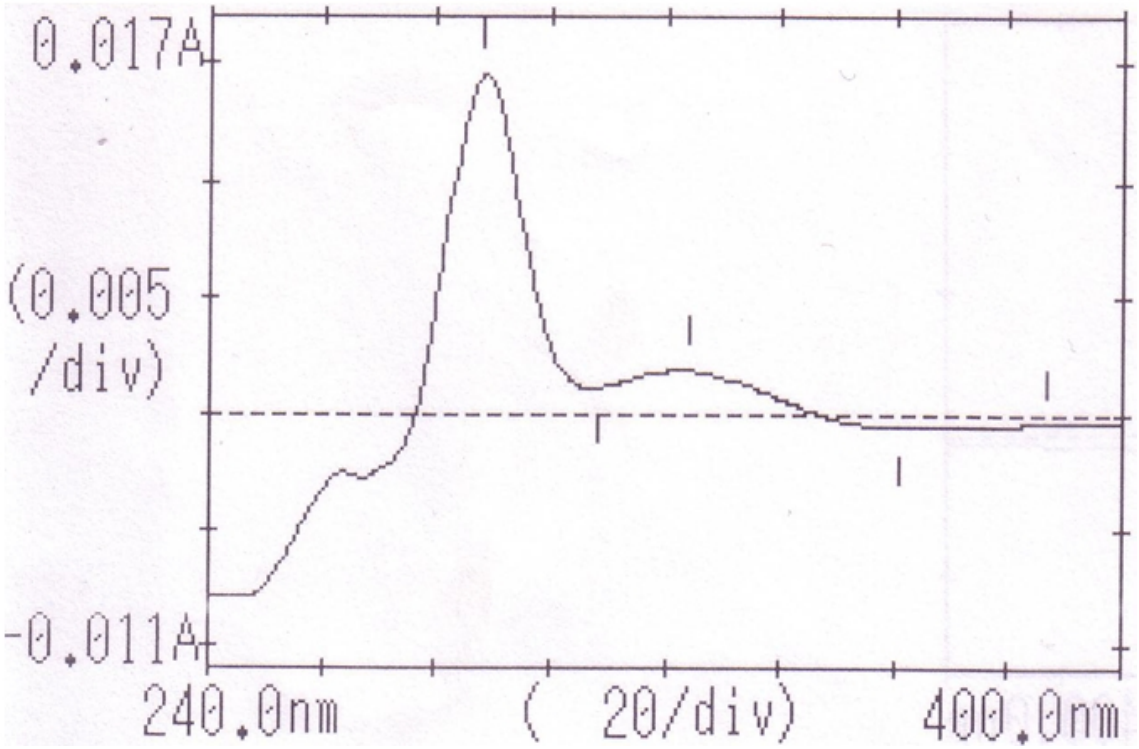

Fig. 4. First derivative differential spectrum of CS (12µg/ml)

Calibration graph of ΔD_1 versus the corresponding concentration of the drug showed linear relationship obeying Beer's law at concentration range 4-20 µg/mL (Fig. 5).

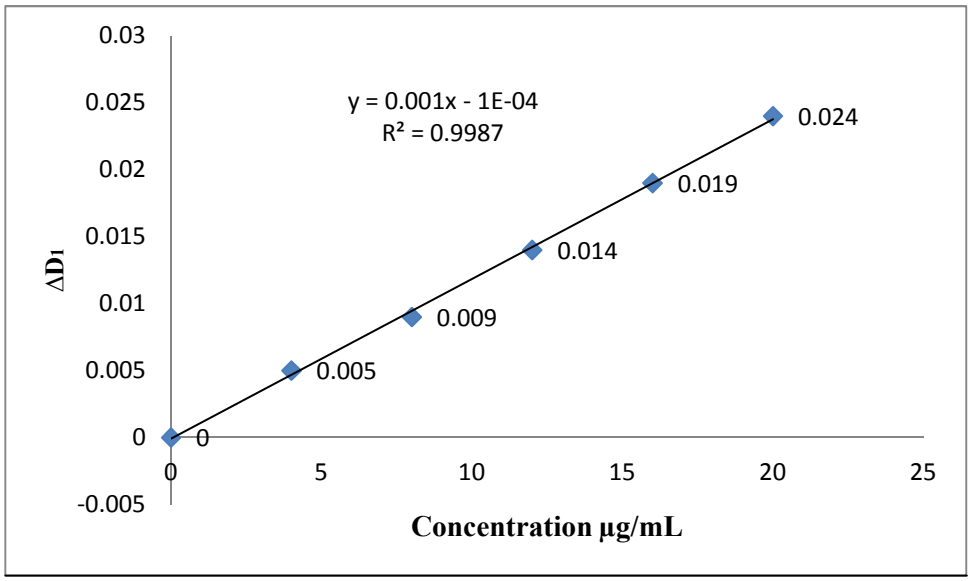

Fig. 5. Calibration graph for ΔD_1 vs CS concentration

The results obtained for linearity data of the proposed method are summarized in Table 1. The low values of the standard errors of the slope and correlation coefficient values (not less than 0.9987) reflected the consistency of the prepared calibration graphs.

The limit of detection and limit of quantification were determined from calibration curves of the proposed method using an adopted equation which is a reduced form of the formula in reference [15]:

3 SB / b
10 SB/ b

Where SB = Sy/x (calculated from the regression analysis data), b is slope.

Table 1. Spectral data of the proposed method

λ_{max} (nm)	Linearity range µg/mL	Limit of quantification µg/mL	Limit of detection µg/mL	Intercept ± standard error* (n=6)	Slope ± standard error* (n=6)	Correlation coefficient
289	4-20	4.8	1.5	-0.00008 ± 0. 0.0019	0.00117 ± 0. 0.00013	0.9987

**Slope and intercept were evaluated at 95% confidence level [15]*

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 cefquinome to sample solution in the ratio of 1:1. The results showed good recovery (100.00% ± 0% (RSD), n=3). The %± SD data for CS assay showed good results (103.00 ± 1.72). The slopes of the sample and standard graphs can also be used to determine the suspension content using the following formula [16]:

$$\frac{\text{Mean slope of sample graph}}{\text{Mean slope of authentic graph}} \times 100$$

This sample calibration graph can also be considered a useful indication of absence of excipients interference (superimposed parallel graphs) as it uses a range of low to high concentrations of sample solution (4-20 µg/mL).

The reproducibility and repeatability of the derivative method were obtained by the follow-up of within-day and between-day data for four concentrations within the linearity range. The results obtained are shown in Table 2.Good reproducibility and precision was reflected by the low SD values.

Table 2. Reproducibility and precision data as evaluated by SD

Concentration (µg/ml)	Within-day (n=3) SD	Between days (n=3) SD
8	7×10^{-4}	0
12	7×10^{-4}	0
16	1.4×10^{-3}	0
20	1.4×10^{-3}	7×10^{-4}

The validity of the developed method was assessed by comparing the statistical results obtained with those of a developed HPLC method [8]. Data of Table 3 show the obtained assay results and the calculated *t*- and *F*-values as compared to the corresponding tabulated values at 95% confidence level.

Table 3. Validation results of the Proposed Method compared to the Developed HPLC method

	Content % of CS ± SD	*t cal, t(tab)	F* cal, F (tab)
Proposed method	103.00±1.72	2.04, (2.78)	1,18 (19)
Developed HPLC	100.00±1.87	-	

** = t and F calculated and tabulated*

As the calculated *t*-value at 95% confidence limit was less than tabulated one, the result of developed UV method can be considered as accurate and precise as the liquid chromatography method.

4. CONCLUSION

The stability-indicating property, coupled with the selectivity and simplicity of application, of the derivative spectrophotometry (first, second…etc) and ΔD_1 make these methods more preferable to use for drug analysis than the costly HPLC methods, especially in developing countries.

In conclusion, difference spectrophotometry provides a selective method for the determination of cefquinome sulphate in bulk and dosage form. The simplicity, rapidity and sensitivity of the proposed method together with the wide availability of spectrophotometers

would encourage the use of the proposed method for the routine analysis and the quality control of CS.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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