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Spectrophotometric and HPLC methods for the determination of Cefquinome Sulphate in Bullk and Dosage forms

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ABSTRACT

Simple, sensitive and rapid spectrophotometric and HPLC methods were developed for the determination of cefquinome sulphate in bulk and dosage forms. The methods were based on the measuring of the first and second derivative at 286nm and 300nm respectively. Regression analysis of Beer's plot showed good correlation (r=0.9997) in a concentration range of 4-12µg/ml. The recovery percentage was 100.1± 0.575% (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.

1. Introduction

Cefquinome sulphate (figure 1): 1-[[(6*R*,7*R*)-7-[[(2*Z*)-(2-Amino-4-thiazolyl)(methoxyimino)acetyl] amino] -2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0-oct-2-en-3-yl]methyl]-5,6,7,8-tetrahydroquinolinium inner salt, is a fourth generation cephalosporin. It acts by inhibition of bacterial cell wall synthesis. Efficacy against major mastitis and Bovine Pneumonia pathogens (bovine respiratory disease BRD) has been clearly demonstrated [1]. In literature, different methods for the determination of cefquinome in biological fluids are reported. They include HPLC methods with different systems [2-4] and other chromatographic methods [5, 6]. The present work describes new spectrophotometric and HPLC methods for the determination of cefquinome sulphate in bulk and suspension formulation.

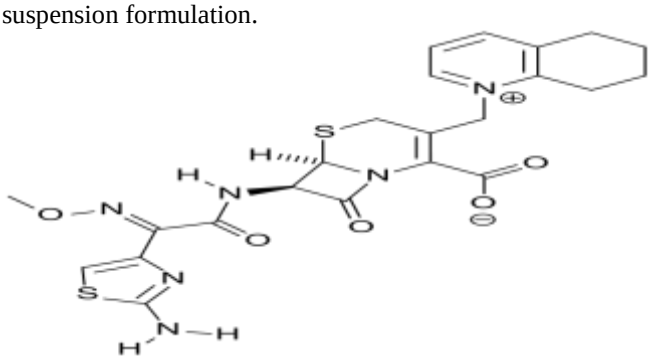

Figure 1: Chemical structure of cefquinome

2. Experimental

Apparatus

UV spectrophotometric studies were carried out on Shimadzu UV-1800ENG240V, (Koyoto, Japan). HPLC analysis was carried in Shimadzu liquid chromatograph, wavelength 268nm.

Chromatographic conditions

Shimadzu liquid chromatograph was used. The column used was Shimpack VP-ODS (250 x 4.6mm). The detector was SPD-20A prominence UV/VIS. The mobile phase consisted of ammonium acetate buffer: acetonitrile (80:20 v/v) at a flow rate 1 ml min^{-1}. Ultraviolet setting was at 268nm and 20µl volumes were injected onto the column at room temperature.

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 GmbH. Acetonitrile, Lobachemie, India. Ammonium acetate, E. Merck, Germany.

Preparation of solutions

Standard stock solution

A stock solution of cefquinome sulphate (0.1%, w/v) was prepared in distilled water. One ml of this solution was diluted to 50 mL with distilled water (20µg/mL, solution A).

Sample solution

1 ml of the suspension (shaken well) was transferred to 25ml volumetric flask. About 15ml 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 then filtered. One ml of the filtrate was diluted to 50ml with distilled water (solution B, 20µg/ml).

Procedure

Calibration graph

Different accurately measured volumes (2-6 ml) of solution A were transferred into five volumetric flasks (10ml). The volumes were completed to mark with distilled water. First and second order derivative spectra were recorded over the range 240-400nm. Regression analysis data was obtained from the absorbance-concentration graph.

HPLC chromatogram was also recorded by injecting 20µl volumes of the five solutions above.

Mobile phase polarity index calculation

Polarity indexes (P'abc) for the systems used were calculated using the following formula [7]:

$P'abc = \theta a + \theta b$

Where θ is the polarity index of the solvent, a is the fraction of aqueous phase, b is the fraction of acetonitrile.

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Precision

Within-day and between-day data were determined for four concentrations within the linearity range. The relative standard deviation (RSD) values were calculated.

Limit of detection (LOD) and limit of quantification (LOQ)

LOD and LOQ were determined from calibration curves of the proposed methods using the following formulas [8]:

3SB / b

10 SB/ b

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

Results and Discussion

CS is a drug used in veterinary medicine; therefore all the previous reported methods were directed towards the assay of the drug in biological fluids [2-3, 5-6]. In the point of view of the pharmaceutical analysis, the drug in its pharmaceutical formulations needs to be evaluated chemically (content %) to check its efficacy, safety and stability. Therefore, we deemed it useful to develop chemical methods (derivative spectrophotometric and HPLC methods) for its evaluation and which can be used for its routine analysis.

Derivative spectroscopy is a simple powerful technique. It is suitable for analysis of turbid solutions [9], and can be used successfully for the assay of pharmaceutical formulations.

The original UV spectrum (zero order) of cefquinome solution has a broad peak at 268nm. First and second order derivative showed better and sharper peaks at 286nm and 300nm respectively figure 2 and 3.

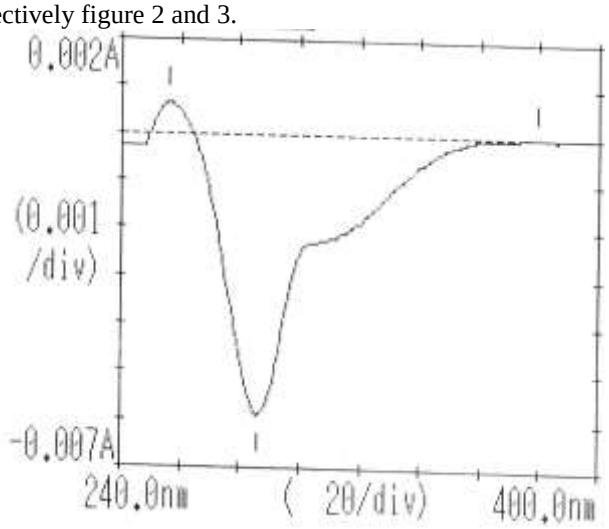

Figure 2: First derivative spectrum of cefquinome solution

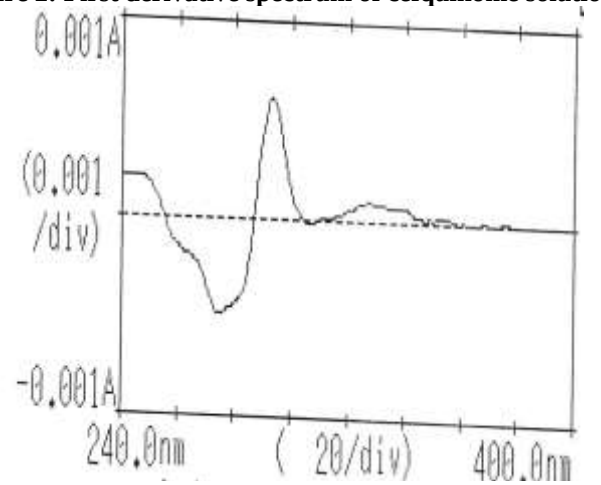

Figure 3: Second derivative spectrum of cefquinome solution

Selection of mobile phase

In order to obtain a column/mobile phase system that would be suitable for the quantification of cefquinome sulphate, different columns and mobile phases were investigated. A 50% v/v mixture of acetonitrile and orthophosphoric acid was first investigated at flow rate 1 ml min^{-1} using those columns. This system didn't elute CS even when the solvent pecentages was changed. A solvent percentage was consisting of 70:30 v/v potassium dihydrogen phosphates: acetonitrile at flow rate 1.5ml min^{-1} eluted the CS at ≈ 15 min with a remarkable tailing. The polarity index of the system was 8.88. When the same constituents were used with a flow rate 2.5 ml min^{-1} and composition of 80:20 v/v, the CS peak was eluted at 10 min again with remarkable tailing. Another system consists of 80:20v/v ammonium acetate buffer (0.02M): acetonitrile at flow rate of 1 ml min^{-1} resulted in a reasonable retention time for the elution of cefquinome peak (≈ 5 min) and good peak symmetry **(figure 4)**. The polarity index of this system was 9.32.

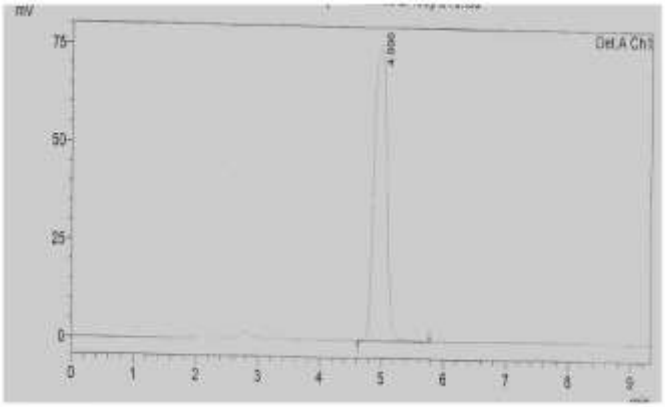

Figure 4: Typical chromatogram for standard cefquinome

It has been found that the polarity index of the mobile phase is a sufficient criteria for good resolution and peak symmetry. In addition, the ratio of solvents in the mixture composing the mobile phase is also important. This is confirmed by comparing results obtained for solvent mixtures of polarity 9.1 and the other solvent mixture with polarity index 9.32.

Linearity

A calibration curve was prepared using the developed methods at concentration of 4-12 µg/ml for CS. The obtained correlation coefficient values (r) for spectrophotometric and HPLC methods were 0.9997 and 0.9993 respectively. The regression analysis data was calculated at 95% confidence level for the developed methods using the following formulas [8]:

$(b \pm ts_b)$ and $(a \pm ts_a)$

Where b is the slope, a the intercept, s_b standard deviation of slope, s_a standard deviation of intercept, the t-value at 95% confidence level for (n – 2).

The results obtained reflected the consistency of the prepared calibration graphs.

Assay

The derivative spectrophotometry and HPLC methods were applied for the drug uniformity testing in Cobactan suspension. Good assay results (X ± RSD (%), n) were obtained

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.

Table 2 shows the %± SD data for cefquinome assay and the added recovery using the developed methods (UV spectrophotometry and HPLC).

Table 1: Linearity data of the proposed methods

Developed method	λ_{max}	Slope	Intercept
1st derivative	286nm	0.00135 ± 0.00016	-0.000014 ± 0.00133
2nd derivative	300nm	0.26 ± 0.0157	-0.0357 ± 0.042
HPLC method	268nm	48869 ± 5731	-165.7 ± 48594

Table 2

	Assay %			Mean ± SD % (n=3)	Recovery % ± SD
UV spectrophotometric method	100	101.1	100	100.4± 0.635	100.1± 0.575
HPLC method	100.3	101.7	98	100 ± 1.869	

Table 3

Concentration (µg/ml)	Within-day variation (RSD%, n=6)	Between-days variation (RSD%, n=6)
6	0.65	0.65
8	0.58	0.88
10	0.48	0.82
12	0.65	0.75

Table 4

Developed Method	LOD (µg/ml)	LOQ (µg/ml)
1st derivative spectrophotometry	0.7	2.34
2nd derivative spectrophotometry	0.36	1.2
HPLC method	0.7	2.33

Both results reflected the accuracy of the methods and the freedom of interference by the suspension excipients.

The validity of the derivative spectrophotometric method 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. As the calculated *t*-value (0.35) at 95% confidence limit was less than tabulated one (3.18), the result of developed UV method can be considered as accurate and precise as the liquid chromatography method.

Precision

Between-days and within-day precisions were determined for four different concentrations of the standard curve. The calculated RSD values were found to be within the accepted limits(less than 2%) table (3).

Limit of detection and quantification

The results for LOD and LOQ were summarized in table 4.The obtained low levels for LOD and LOQ indicate that the developed methods are sensitive and suitable for the determination of cefquinome sulphate.

Conclusion

The developed derivative spectrophotometric and HPLC methods proved to be simple, rapid, accurate and reproducible for the determination of CS in bulk and dosage forms. In addition, the major advantage of the developed methods is that the procedure does not require extraction step or great number of chemicals.

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