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Method Development for Simultaneous Estimation of Vancomycin and Ceftriaxone in Powder Form for Injection by RP-HPLC Method

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ABSTRACT

A simple, sensitive, rapid, robust and reproducible method for the simultaneous determination of ceftriaxone and vancomycin in formulation was developed using reverse phase high performance liquid chromatographic method. The analysis was performed on C8 (350×4.6 mm, 5 µm) column with a mobile phase consisting of 0.01 M of potassium di hydrogen ortho phosphate and 0.01M of disodium hydrogen phosphate buffer (pH 7.8), methanol in the ratio of 80:20 (v/v) with a flow rate of 1ml/min. The analyte was examined with UV detector at 213 nm. In the developed method vancomycin elutes at 3.8 min and ceftriaxone at 3.3 min. The proposed method can be readily utilized for determination of ceftriaxone and vancomycin.

KEYWORDS: Ceftriaxone; Vancomycin; RP-HPLC; Pharmaceutical formulation.

1.INTRODUCTION

Ceftriaxone is (6R,7R)- 3 [(acetyl - oxy) methyl] -7-[[(2Z)-(2- amino-4 thiazolyl) (methoxyamino)-acetyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (Figure 1).

Ceftriaxone is a third generation cephalosporin beta-lactam antibiotics used in the treatment of bacterial infections caused by vulnerable, generally gram positive organism. Bactericidal activity of ceftriaxone is due to inhibition of the cell wall synthesis and is facilitated by ceftriaxone binding to penicillin-binding proteins. It hinders the mucopeptide synthesis in the bacterial cell wall. The beta lactam moiety of the ceftriaxone binds to carboxypeptidase, endopeptidase, and transpeptidase in the bacterial cytoplasmic membrane, which are responsible for cell wall synthesis and cell division. Ceftriaxone results in the formation of defective cell walls and cell death by binding to these enzymes [1].

The bactericidal action of vancomycin is due to inhibition of cell-wall biosynthesis. Specifically, vancomycin avoids integration of N-acetylmuramic acid (NAM) and N-acetyl glucosamine (NAG)- peptide subunits from geting incorporated into the peptidoglycan matrix; which is the major structural component of Gram-positive cell walls. The huge hydrophilic molecule is capable to form hydrogen bond interactions with the terminal D-alanyl-D-alanine moieties of the NAM/NAG-peptides. Usually this is a five-point interaction.

The synergistic action of ceftriaxone-vancomycin has strong bactericidal activity against S.aureus and methicillin resistant strains. The combination works

well against enterococci, prevents development of resistance, and has been used successfully against a wide range of bacterial infections including bacterial meningitis.

However, there is only one method for the simultaneous estimation of ceftriaxone and vancomycin by HPLC with high linearity values and difficult to detect in lower concentration. The stability studies of the combined drugs are not reported in the determination of drugs. The aim of the present study was to develop a simple, sensitive, accurate, versatile, and fast stability indicating HPLC method for the simultaneous estimation of ceftriaxone and vancomycin in pharmaceutical injection dosage form.

2. MATERIALS AND METHOD

Chemicals and reagents

Pure sample of ceftriaxone and vancomycin was received from Vishnu Chemicals Ltd,Hyderabad. Formulation of ceftriaxone and vancomycin namely VANCOPLUSE was obtained from local pharmacy product was labeled to contain 1 mg of ceftriaxone and 0.5 mg of vancomycin. HPLC grade water, HPLC grade methanol were procured from Merck Pvt Ltd., Mumbai, India. All other chemicals used are analytical reagent grade (AR grade) like potassium dihydrogen orthophosphate and orthophosphoric acid and sodium dihydrogen phosphate.

Instrumentation

A HPLC equipped with UV detector was used for the present research work. The separation was achieved using C-8 column. The mobile phase was a mixture of phosphate Buffer of (pH-7.8), and methanol (70:30) v/v. The contents of mobile phase was filtered before use through 0.45 µ membrane filter and its was degassed with a helium sparge for 15 min at flow rate of 1.0 ml/min. Here the column temperature was maintained at 20 ± 10°C. The injection volume of samples was 10 µl and the analyte was monitored at wavelength of 213 nm. The chromatographic conditions are shown in Table 1.

Method Development

Taking in attention the instability of ceftriaxone and vancomycinin the strong alkaline and strong acidic conditions and pH value of the mobile phase should be limited within the range of 3-7. As mild acidic pH favors the retention and separation of two drugs on C-8 column. After some trials phosphate buffer with pH 7.8 was finally selected. The method development started with the methanol and phosphate buffer. In this mobile phase, there solution of the two drugs was not acceptable, so to change the selectivity the organic phase was changed from methanol to acetonitrile. Since both ceftriaxone and vancomycin in the mobile phase have no significant UV maximum but has end absorption, so to ensure the sensitivity of the method, the wavelength of 213 nm was employed for the detection. After a number of preliminary experiments, a Phenominax C-8 column and binary mixture of phosphate buffer pH 7.8 and methanol (80:20% v/v) was optimized as the mobile phase which produced good resolution, symmetric peak shape,and reasonable retention time for both the drugs. The retention times of ceftriaxone and vancomycin for six repetitions were found to be 3.3± 0.02 min and 3.8 ± 0.006 respectively.

Table 1: Optimized chromatographic conditions

Parameter	Optimized condition
Chromatograph	HPLC with UV-detector
Column	C8 Column
Mobile Phase	pH-7.8 phosphate buffer in the ratio and methanol of 80:20 (v/v)
Flow rate	1.00 ml/min
Detection	213 nm
Injection volume	10 µl.
Temperature column	Room temperature

Preparation of mobile phase

The content of the mobile phase was prepared from filtered and degassed mixture of 3.5 g Potassium dihydrogen orthophosphate and 14.5 g of disodium hydrogen phosphate is taken in 1000 ml flak and made up to mark with water and pH is adjusted to (pH 7.8) with ortho phosphoric acid and methanol in the ratio of 80:20 v/v. beneficiary profile.

Preparation of standard solutions

About 25 mg of pure sample of ceftriaxone and 12.5mg vancomycin was accurately weighed and dissolved in HPLC grade water in a 100 ml standard flask separately to get a standard stock solution concentration.

Preparation of working standard solutions

5 ml of the above solution was taken in 50 ml volumetric flask and there after made up to 50 ml with

diluents o get working standard solution.

Preparation of sample solution

Powder contains equivalent to 62 mg of the vancoplus formulation was mixed with 30 ml of diluents in 100 ml volumetric flask.The mixture was allowed to stand for 15 mins with intermittent sonication to ensure complete solubility of the drug and then filtered .Take 5 ml of above solution in 50 ml volumetric flask and made up to 50 ml with diluents to get sample solution.

Assay procedure

The column was equilibrated for minimum 30 min, with the mobile phase flowing through the system with a flow rate of 1ml/ min. Detector was set at a wavelength of 213 nm. Five sets of the drug solutions were prepared having 10-50 µg/ml for vancomycin and 20- 100 µg/ml ceftriaxone in mobile phase mixture. The retention time of vancomycin and ceftriaxone in bulk drug in five replicate samples were found to be 3.8 min and 3.3 min and the retention time of vancomycin and ceftriaxone in its pharmaceutical formulation were found to be 3.8 min and 3.3 min it is done by taking 40 µg/ml of VANCOPLUSE which contains (40 µg/ml of ceftriaxone and 20 µg/ml vancomycin) respectively and the peak areas of the drug concentration were calculated.

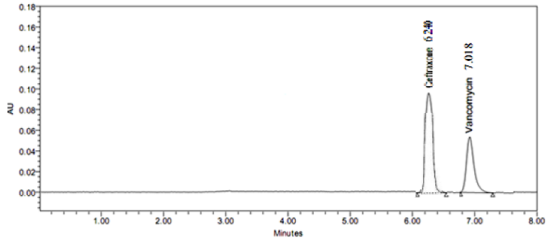
Results and Discussion

The method was developed to find the suitable column, mobile phase, flow rate and detector wavelength for the assay of vancomycin and ceftriaxone.

Trial: 1

Column	: ODS-3V analytical column (350 x 4.6 mm, 5 µm particle size)
Flow rate	: 1ml/min
Detector	: PDA-detector
Wavelength	: 213nm
Injection volume	: 10µL
Column oven temperature	: Ambient
Run Time	: 8 minutes
Elution	: Isocratic
Mobile phase	: Phosphate buffer (7.8): Methanol (80:20)

chromatogram 1



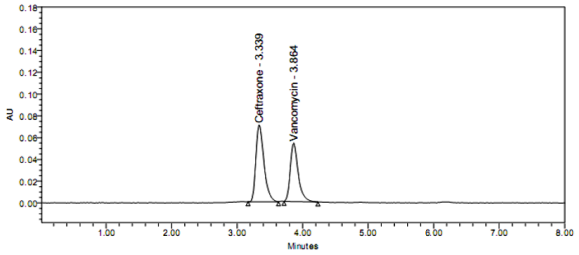
The Reasons for disqualifying this trial are

- The retention of Vancomycin and Ceftriaxone is more.
- The tailing factor of ceftriaxone is more.

Trial: 2(Final)

Column	: ODS-3V analytical column (350 x 4.6 mm, 5 µm particle size)
Flow rate	: 1ml/min
Detector	: PDA-detector
Wavelength	: 213nm
Injection volume	: 10µL
Column oven temperature	: Ambient
Run Time	: 8 minutes
Elution	: Isocratic
Mobile phase	: Phosphate buffer (7.8): Methanol (50:50)

CHROMATOGRAM 2

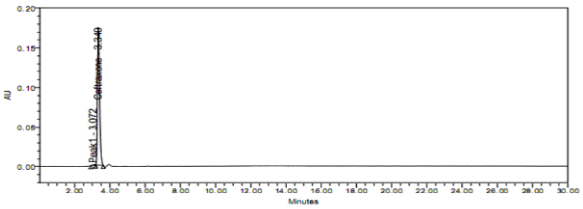


	Peak Name	RT	Area	% Area	RT Ratio	USP Plate Count	USP Resolution	USP Tailing
1	Ceftraxone	3.339	604948	57.23		3587.23		1.39
2	Vancomycin	3.864	452035	42.77	1.157	5153.73	2.35	1.40

Remarks

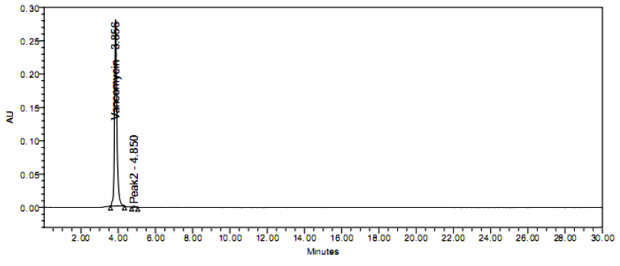
- The tailing factor of vancomycin and ceftriaxone was within the limit.
- The theoretical plates of vancomycin and ceftriaxone was more than 2000

Standard Chromatogram for ceftriaxone:



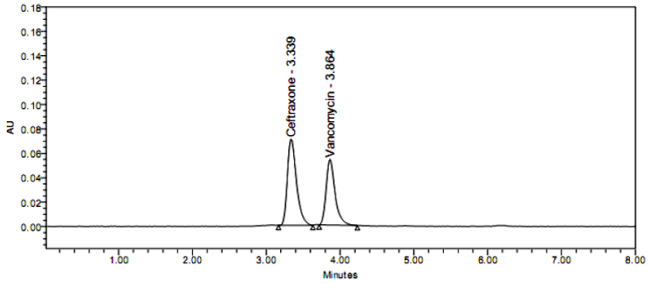
	Peak Name	RT	Area	% Area	RT Ratio
1	Peak1	3.072	6734	0.45	0.920
2	Ceftraxone	3.340	1489602	99.55	

Standard Chromatogram for vancomycin:



	Peak Name	RT	Area	% Area	RT Ratio
1	Vancomycin	3.856	2454595	99.36	
2	Peak2	4.850	15738	0.64	1.258

Standard Chromatogram for mixed ceftriaxone and vancomycin:



3. SUMMARY AND CONCLUSION

A RP-HPLC method was developed for the simultaneous estimation of Vancomycin and Ceftriaxone in powder form for injection utilizing empower separation module with PDA detector and c8 column ,injection volume 10µl and the mobile phase used was phosphate buffer of pH 7.8 and methonal in(80:20) at a flow rate 0.8 ml/min and detected at 213 nm.The peak retention time was 3.3 min for Ceftriaxone and 3.8 min for Vancomycin respectively.

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