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DEVELOPMENT AND VALIDATION OF ORITAVANCIN IN PHARMACEUTICAL FORMULATION BY USING RP-HPLC

[1,*] Tanniru Rajesh, [2] P. Venkateswara Rao
[1, 2]Department of Pharmacy, Vikas College of Pharmacy, Vissannapeta, AP.
rajeshtanniru5@gmail.com

Abstract

The present paper describes a simple, accurate and precise reversed phase High Performance Liquid Chromatography (HPLC) method for rapid and simultaneous quantification of Oritavancin. The chromatographic separation was achieved on Waters X-Bridge C_{18} (150x4.6mm, 3.5µ) column. Mobile phase contained a mixture of 0.1% Tri fluoro acetic acid and Acetonitrile in the ratio of 35:65 v/v, flow rate 1.0ml/min and UV detection at 370nm. The proposed method shows a good linearity in the concentration range of 50-300µg/ml of Oritavancin under optimized conditions. Precision and recovery study results are in between 98-102%. In the entire robustness conditions %RSD is below 2.0%. Degradation has minimum effect in stress condition and solutions are stable up to 24hrs. This method is validated for different parameters like Precision, linearity, accuracy, limit of detection (LOD), limit of quantification (LOQ), ruggedness, robustness and forced degradation study were determined according to the ICH Q2B guidelines. All the parameters of validation were found to be within the acceptance range of ICH guidelines.

Key words: RP-HPLC, Oritavancin.

INTRODUCTION

Oritavancin[1], sold under the brand name Orbactiv among others, is a semisynthetic glycopeptide antibiotic[2] medication for the treatment of serious Gram-positive bacterial infections. Its chemical structure as a lipoglycopeptide [3] is similar to vancomycin [4]. Oritavancin shares certain properties with other members of the glycopeptide class of antibiotics, which includes vancomycin, the current standard of care for serious Gram-positive infections in the United States and Europe. It possesses potent and rapid bactericidal activity in vitro against a broad spectrum [5] of both resistant and susceptible Gram-positive bacteria, including Staphylococcus aureus [6-8], MRSA, enterococci [9,10], and streptococci.[11,12] Oritavancin was more active than either metronidazole [13,14] or vancomycin against strains of Clostridium difficile tested. Oritavancin has potential use as a therapy for exposure to Bacillus anthracis,[15,16] the Gram-positive bacterium that causes anthrax, having demonstrated efficacy in a mouse model both before and after exposure to the bacterium. oritavancin demonstrates in vitro activity against both the planktonic [17] and biofilmstates of staphylococci associated with Prosthetic joint infection (PJI), albeit with increased minimum biofilm bactericidal concentration (MBBC) compared to Minimum inhibitory concentrations (MIC) values. More ever oritavancin has demonstrated activity against in vitro to vancomycin-susceptible enterococci (VSE) and vancomycin-resistant enterococci (VRE) in both planktonic and biofilm states.

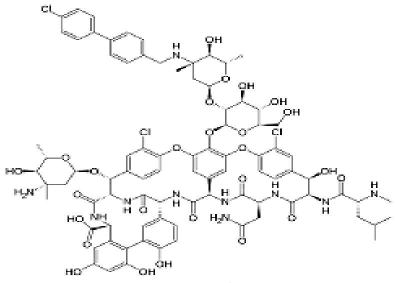

Fig. 1: Structure of Oritavancin

MATERIALS AND METHODS

Chemicals

Acetonitrile, Ortho Phosphoric acid (OPA) and water (HPLC grade), Tri fluoro acetic acid were purchased from Merck (India) Ltd. Worli, Mumbai, India. All APIs of Oritavancin as reference standards were procured from Torrent Pharma, Ahmadabad.

Equipment

Waters alliance -2695 chromatographic system consisting of quaternary pump, PDA detector-2996 and chromatographic software Empower-2.0 was used.

Chromatographic conditions

Chromatographic separation was carried out in isocratic mode at room temperature using Waters X-Bridge C_{18} (150x4.6mm, 3.5µ) column. The mixture of 0.1% TFA: Acetonitrile 35:65 v/v at a flow rate of 1.0ml/min was used as a mobile phase. The injection volume was 10µl and eluent was monitored at 370nm using PDA detector. The run time was 10min.

Selection of wavelength

The absorption spectra of solution of Oritavancin was scanned over the range of 200-400nm by using PDA detector and the spectra was recorded. The spectrum was shown in fig.3.

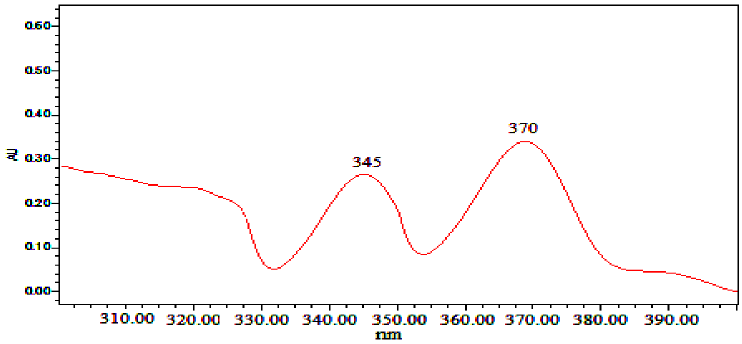

Fig. 2: PDA Spectrum of Oritavancin

Preparation of Standard solution:

Weigh accurately 200mg of Oritavancin working standards were taken into a 100ml volumetric flask, add 70ml of diluent and sonicated for 10min to dissolve the components, makeup to the mark with mobile phase. Further diluted 5ml of above solution to 50ml volumetric flask and diluted to volume with diluent.

Preparation of Sample solution

Weigh 200mg of Oritavancin equivalent weight of sample and transferred into 100ml volumetric flask and add 70ml of diluent and sonicated for 30min to dissolve the components and centrifuge for 30min then diluted up to the mark with diluent. Further dilute 5ml of above solution to 50ml diluent and it was filtered through 0.45µ nylon syringe filter.

Validation Procedure

The analytical method was validated as per ICH Q2 (R_1) guidelines for the parameters like system Precision, specificity, accuracy, precision, linearity, robustness, limit of detection (LOD), limit of quantification (LOQ) and forced degradation.

System Suitability

System suitability parameters were measured to verify the system performance. The parameters including USP plate count, USP tailing and % RSD are calculated and found to be within the limits.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of other components, which may be expected to be present in the sample and standard solution. It was checked by examining the chromatograms of blank samples and samples spiked with Oritavancin.

Accuracy

Accuracy is the closeness of the test results obtained by the method to the true value. It was assessed by the recovery studies at three different concentration levels. In each level, a minimum of three injections were given and amount of the drug present, percentage recovery and related standard deviation were calculated.

Precision

Precision of an analytical method is the degree of agreement among individual test results. It was studied by analysis of multiple sampling of homogeneous sample. The precision of the present method was assessed in terms of repeatability, intra-day and inter day variations. It was checked by analyzing the samples at different time intervals of the same day as well as on different days.

Linearity and range

Linearity of an analytical method is its ability to obtain results directly proportional to the concentration of the analyte in the sample within a definite range. The six series of standard solutions were selected for assessing linearity range. The calibration curve was plotted using peak area versus concentration of the standard solution and the regression equations were calculated. The least squares method was used to calculate the slope, intercept and correlation coefficient.

LOD and LOQ

LOD is the lowest amount of analyte in a sample that can be detected while LOQ is the lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy. LOD and LOQ were separately determined based on the calibration curves. The LOD and LOQ for Oritavancin were determined by injecting progressively low concentrations of standard solutions using the developed RP-HPLC method. The LOD and LOQ for calculated as 3.3s/n and 10s/n respectively as per ICH guidelines where s/n indicates signal-to-noise ratio.

Stress Degradation

Stress degradation should be no interference between the peaks obtained for the chromatogram of forced degradation preparations. Stress degradation studies were performed as per ICH guidelines Q_1A (R_2). The degradation peaks should be well separated from each other and the resolution between the peaks should be at least 1.0 and the peak purity of the principle peaks shall pass. Forced degradation studies were performed by different types of stress conditions to obtain the degradation of about 20%.

Robustness

The robustness of an analytical procedure is a measure of its ability to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness study was performed by injecting standard solution into the HPLC system and altered chromatographic conditions such as flow rate (±0.2), organic content in the mobile phase (±10%). The separation factor, retention time and peak asymmetry were calculated by determining the effect of the modified parameters.

RESULTS AND DISCUSSION

Method Validation

In this method system suitability, linearity, precision, accuracy, LOD, LOQ, robustness, forced degradation are validated for the selected drug Oritavancin. The proposed method having standard solution and sample solution chromatograms are shown.

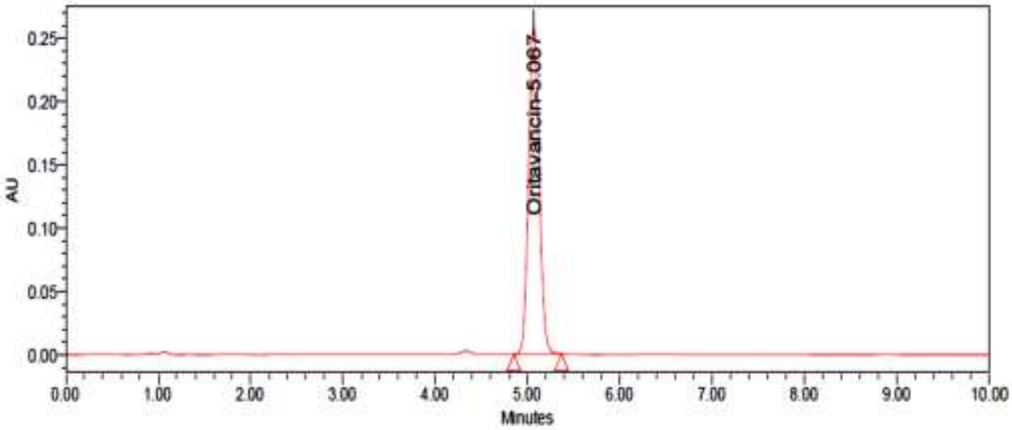

Fig. 3: Chromatogram of standard

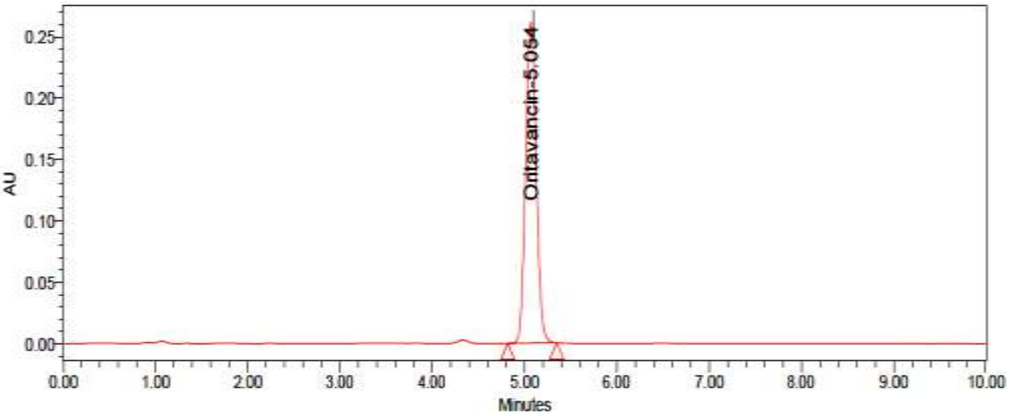

Fig. 4: Chromatogram of sample

System suitability

The HPLC system was stabilized for 60min to get a stable baseline. Six replicate injections of the standard solution containing 200µg/ml of Oritavancin were assessed to check the system suitability. The number of theoretical plate count for Oritavancin was 9113 respectively. Tailing factor for Oritavancin was 1.08 respectively. All the parameters were found to be within limit.

Linearity

Linearity of the method was evaluated by preparing a standard solution containing 200µg/ml of Oritavancin. Sequential dilutions were performed to the given solutions at 25, 50, 75, 100, 125 and 150% of the target concentrations. These were injected and the peak areas are used to plot calibration curves against the concentration. The correlation coefficient values of these analytes were 0.999. The results were shown in table 1.

Table 1: Linearity study results

Analyte	Linearity range	Equation of calibration curve	Correlation coefficient
Oritavancin	50-300µg/ml	Y= 16537.97x +52345.29	0.9998

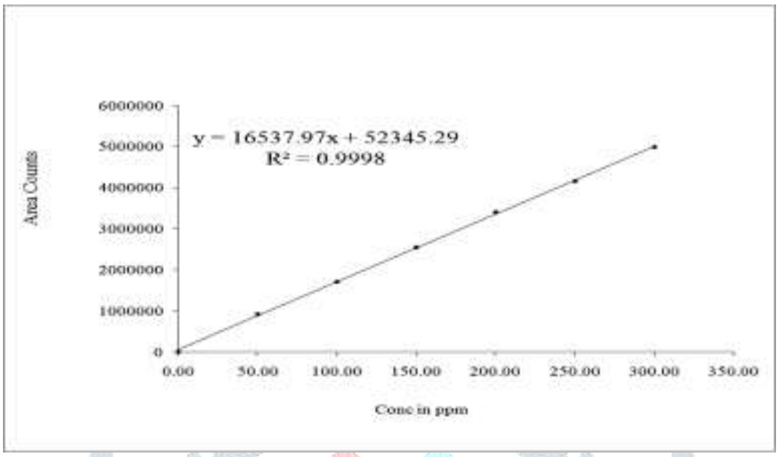

Fig. 5: Linearity plot for Oritavancin

LOD and LOQ

LOD and LOQ minimum concentration level at which the analyte can be reliably detected, quantified by using the standard formulas. LOD values for Oritavancin was 6µg/ml. LOQ values for Oritavancin were 20µg/ml.

Precision

Method precision was investigated by the analysis of six separately prepared samples of the same batch. From these six separate samples solution was injected and the peak areas obtained used to calculate mean and percentage RSD values. The present method was found to be precise as %RSD of the less than 2.0%. The results are given in table 2.

Table 2: Method Precision results

Analyte	Concentration (µg/ml)	% RSD
Oritavancin	200	0.27

Accuracy

Accuracy was determined by recovery studies which were carried out in three different concentration levels (50%, 100% and 150%). APIs with concentration 100, 200 and 300µg/ml of Oritavancin were prepared. As per the test method the test solution was injected to three preparations each spike level and the assay was performed. The percentage recovery values were found to be in the range of 98-102%. The results are given in table 3.

Table 3: Accuracy (recovery) study results

% of target concentration	Oritavancin (% Recovery)
50	101.0
100	100.3
150	100.6
Mean (% Recovery)	100.6

Ruggedness

Ruggedness of the method was studied and showed that chromatographic patterns did not significantly change when different HPLC system, analyst, column. The value of percentage of RSD was below 2% exhibits the ruggedness of the developed method.

Robustness

Robustness of the method was found to be %RSD should be less than 2%. Slightly variations were done in the optimized method parameters like flow rate (±20%), Organic content in mobile phase (±10%). The results are given in table 4.

Table 4: Robustness results

Drug Name	Flow Plus (1.2ml/min)	Flow Minus (0.8ml/min)	Organic Plus (71.5:28.5)	Organic Minus (58.5:41.5)
	%RSD			
Oritavancin	0.19	0.27	0.54	0.83

Forced Degradation

Forced degradation conditions such as acidic, basic, oxidation, reduction, photo and water stress were attempted as per ICH Q1A (R2). The effect of assay on their results are shown below table 5.

Table 5: Forced Degradation results

Degradation	Oritavancin (% Degradation)
Control	0
Acid	14.0
Alkali	15.1
Peroxide	13.3
Reduction	16.6
Photo	0.7
Hydrolysis	1.2

CONCLUSION

This method described the quantification of Oritavancin in bulk and pharmaceutical formulation as per ICH guidelines. The developed method was found to be accurate, precise, linear and reliable. The advantage lies in the simplicity of sample preparation and the less expensive reagents were used. In addition compound is eluted within 10mins. The proposed HPLC conditions ensure sufficient resolution and the precise quantification of the compounds. Statistical analysis of the experimental result indicates that the precision and reproducibility data are satisfactory. The developed chromatographic method can be effectively applied for routine analysis in drug research.

REFERENCES

1. Yan, Qun, Melissa Karau J, and Robin Patel. In Vitro Activity of Oritavancin against Biofilms of Staphylococci Isolated from Prosthetic Joint Infection. Diagnostic Microbiology and Infectious Disease 92, no. 2. October 2018, 155–57.
2. Gould K. Antibiotics: from prehistory to the present day. The Journal of Antimicrobial Chemotherapy. March 2016, 71 (3): 572–5.
3. Zhanel, George G, Calic, Divna, Schweizer, Frank, Zelenitsky, Sheryl, Adam, Heather, Lagacé-Wiens, Philippe R.S, Rubinstein, Ethan, Gin, Alfred S, et al. New lipoglycopeptides: a comparative review of dalbavancin, oritavancin and telavancin. Drugs. 2010-05-07, 70 (7): 859–886.
4. Domenech O, Francius G, Tulkens PM, Van Bambeke F, Dufrêne Y, Mingeot-Leclercq MP. Interactions of oritavancin, a new lipoglycopeptide derived from vancomycin, with phospholipid bilayers: Effect on membrane permeability and nanoscale lipid membrane organization (PDF). Biochimica et Biophysica Acta (BBA) - Biomembranes. September 2009, 1788 (9): 1832–40.
5. Ory EM, Yow EM. The use and abuse of the broad spectrum antibiotics. JAMA. July 1963, 185 (4): 273–9.
6. Begier E, Seiden DJ, Patton M, Zito E, Severs J, Cooper D, Eiden J, Gruber WC, Jansen KU, Anderson AS, Gurtman A. SA4Ag, a 4-antigen Staphylococcus aureus vaccine, rapidly induces high levels of bacteria-killing antibodies. Vaccine. February 2017, 35 (8): 1132–1139.
7. Mead GC, Adams BW. Chlorine resistance of Staphylococcus aureus isolated from turkeys and turkey products. Letters in Applied Microbiology. 1986, 3 (6): 131–133.
8. Masalha M, Borovok I, Schreiber R, Aharonowitz Y, Cohen G. Analysis of transcription of the Staphylococcus aureus aerobic class Ib and anaerobic class III ribonucleotide reductase genes in response to oxygen. Journal of Bacteriology. December 2001, 183 (24): 7260–72.
9. Fisher K, Phillips C. The ecology, epidemiology and virulence of Enterococcus. Microbiology. June 2009, 155 (Pt 6): 1749–57.
10. Jin G, Jeng HW, Bradford H, Englande AJ. Comparison of E. coli, enterococci, and fecal coliform as indicators for brackish water quality assessment. Water Environment Research. 2004, 76 (3): 245–55.
11. ohnsborg O, Eldholm V, Bjørnstad ML, Havarstein LS. A predatory mechanism dramatically increases the efficiency of lateral gene transfer in Streptococcus pneumoniae and related commensal species. Mol. Microbiol. 2008, 69 (1): 245–53.
12. Whitworth JM. Lancefield group F and related streptococci (PDF). J. Med. Microbiol. November 1990, 33 (3): 135–51.
13. Kraft MY, Kochergin PM, Tsyganova AM, Shlikhunova VS. Synthesis of metronidazole from ethylenediamine. Pharmaceutical Chemistry Journal. 1989, 23 (10): 861–863.

14. Visapää JP, Tillonen JS, Kaihovaara PS, Salaspuro MP. Lack of disulfiram-like reaction with metronidazole and ethanol. The Annals of Pharmacotherapy. June 2002, 36 (6): 971–4.
15. Spencer R C. Bacillus anthracis. Journal of Clinical Pathology. 1 March 2003, 56 (3): 182–187.
16. Choudhury B, Leoff C, Saile E, Wilkins P, Quinn C P, Kannenberg E L, & Carlson R W. The Structure of the Major Cell Wall Polysaccharide of Bacillus anthracis is Species-specific. Journal of Biological Chemistry. 2006, 281(38), 27932–27941.
17. Vázquez-Domínguez E, Vaqué D, Gasol JM. Ocean warming enhances respiration and carbon demand of coastal microbial plankton. Global Change Biology. 2007, 13 (7): 1327–1334.