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NEW BIOANALYTICAL METHOD FOR THE ESTIMATION OF ORITAVANCIN IN RABBIT PLASMA BY USING LCMS/MS

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Abstract
An easy, quick, precise, active and reproducible LC-MS/MS technique was developed for the bio analytical method of Oritavancin with D_6-Oritavancin as internal standard. Separation was carried on X-Bridge phenyl column (250 mm x 4.6mm, 5µm) using a isocratic elution with a buffer containing 0.1% Tri fluoro acetic acid and Acetonitrile in the ratio of 40:60 as mobile phase with 1mL/min flow rate at ambient temperature . Analysis was carried out within 3 minutes over a good linear concentration range from 26 ng/mL to 204 ng/mL (r^2 = 0.9996) for Oritavancin. Precision and recovery study results are within the acceptable limit. This method has been successfully applied, exploring Oritavancin with its internal standard (D_6-Oritavancin) was extracted from rat plasma using liquid -liquid extraction. This strategy was applied for Freeze thaw, auto sampler, bench top and long-term stability studies, we found that the drugs were stable throughout the stability studies according to USFDA guidelines.
Key words: LC-MS/MS, Oritavancin, Rabbit plasma.

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.

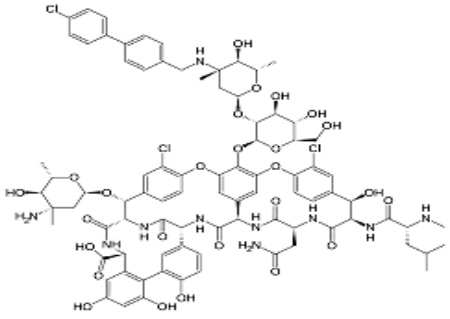

Fig. 1: Structure of Oritavancin

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 API of Oritavancin as reference standards were procured from Torrent Pharma, Ahmadabad.

Instrument and Conditions

For the development of a Bio-analytical assay, an HPLC device (Waters Alliance e2695 model) was connected to a mass spectrometer QTRAP 5500 triple quadrupole instrument (SCIEX). Chromatographic separation was achieved using an X-Bridge Phenyl (250 x 4.6 mm, 5 µm) column on an isocratic model at room temperature. The mobile step was a 40:60v/v mixture of 0.1% TFA and acetonitrile with a flow rate of 1.0 mL/min. The injection volume was 10 µL, and the total run time was 3 minutes. The analysis was carried out on a QTRAP 5500 triple quadrupole mass spectrometer with a positive ion electrospray ionisation interface. MRM mode was used to track the following mass ion pairs: m/z 1794.35 for Oritavancin, m/z 1800.51 for D_6-Oritavancin (Internal standard). Following are the working parameters of mass spectrometry after optimization: Ion spray voltage 5500V; temperature source 550°C; drying gas temperature 120-250°C; collision gas -Nitrogen; pressure 55psi; drying gas flow stream-5mL/min; Delustering potential 40V; Entrance potential 45V; Exit potential 15V; Capillary voltage 5500V and Dwell time 1sec.

Preparation of Standard stock solution:

Weighing 12 mg of Oritavancin into a 10 ml volumetric flask, adding approximately 7 ml of diluents, and sonicating for 15 minutes to dissolve. Then using diluents, get it up to mark. Take 0.1 mL of this solution and dilute it with diluents to make 10 mL. This is the parent stock of Oritavancin.

Take 1.70 mL of Oritavancin parent stock solution solution into another 10 ml volumetric flask, dilute it with diluents to make 10 mL. This is the stock of Oritavancin.

Like this prepare D_6-Oritavancin stock solution also.

Preparation of a standard solution for plasma sample

Aliquots of 200 µL of rabbit plasma specimens were spiked with 100 µL of internal normal (IS) and 100 µL of standard stock working solution. Following that, 1600 µL of acetonitrile and diluents were vortex mixed for 15 minutes, the samples were centrifuged at 5000 rpm for 15 minutes, and the supernatant handled solution was separated, collected, and filtered through a 0.45 µ nylon syringe filter into a vial before being injected into the HPLC system.

Validation of Bio analytical Method

Selectivity, Matrix Effect and Recovery

Oritavancin and its IS selectivity was tested by examining rabbit plasma specimens from 6 heaps of different rabbits for obstruction from unknown specimens at retention time. The peak zone proportion in the post extracted plasma sample from 6 separate medication free plasma samples and slick recovery samples was compared to calculate the effect matrix for Oritavancin. Trails were conducted in triplicate with six different lots of plasma at MQC levels, with a reasonable accuracy (percent CV) of 0.66%. The extraction efficiencies of Oritavancin was determined by looking at six repeats at each concentration of QC, and the degree of recovery was determined by comparing highlights of separate guidelines to non-extricated peak areas of standard.

Dilution Integrity and Carry over

Spiking matrix above the ULOQC with analyte concentration and diluting this test with a blank matrix should demonstrate dilution integrity. The analyte retained by the chromatographic device during the injection of a sample that occurs in subsequent blank or unknown samples is referred to as carry over.

Precision and Accuracy

Replication analysis of quality control specimens (n=6) was used to assess it at the lower quantification limit (LLOQ), low quality control (LQC), medium quality control (MQC), and high quality control (HQC) levels. Except for LLOQ, where CV should be less than 20%, the amount of CV should be less than 15%.

Stability

The area response of the analyte in the stability samples was compared to the region response of the specimen prepared from fresh stock solution to determine stock solution stability. Six replicates of each dose were used in plasma stability experiments at different concentration levels of LQC and HQC. According to the USFDA's guidelines, analyte was considered steady if the change was less than 15%. The stability of spiked rabbit plasma samples stored at room temperature for 24 hours was tested (bench top stability). The auto sampler stability of spiked rabbit plasma deposited in a 2-8°C auto sampler was tested for 24 hours. The auto sampler's stability was determined by comparing extract plasma samples injected immediately with samples re-injected at 2-8°C for 24 hours after storage in the auto sampler. The freeze-thaw durability was determined by comparing freshly spiked quality control samples with durability samples frozen at -30°C and thawed three times. The freeze-thaw stability test used six aliquots in each concentration spectrum in the LQC and HQC. For long-term stability testing, the concentration obtained after 24 hours was compared to the initial concentration.

Results

Bio-analytical Method development

In this step, the ESI has the most intense reaction over the chemical ionisation by atmospheric pressure (APCI) mode. The MRM mode has been used to quantify the ions of Oritavancin, and its internal standard. When compared to ion-negative mode, Oritavancin and its internal standard has a strong positive ion response mode. The details of the mass spectrum are shown in the figure 2 and 3.

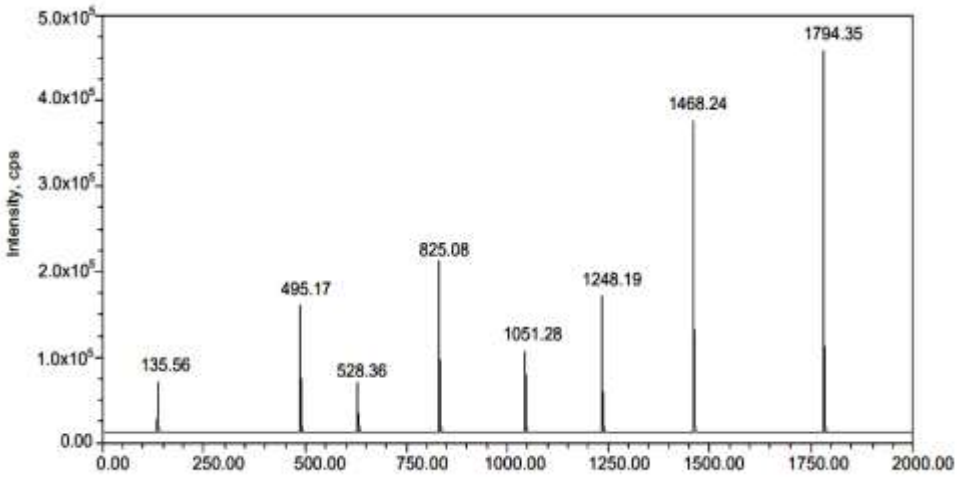

Fig. 2: Mass spectra of Oritavancin

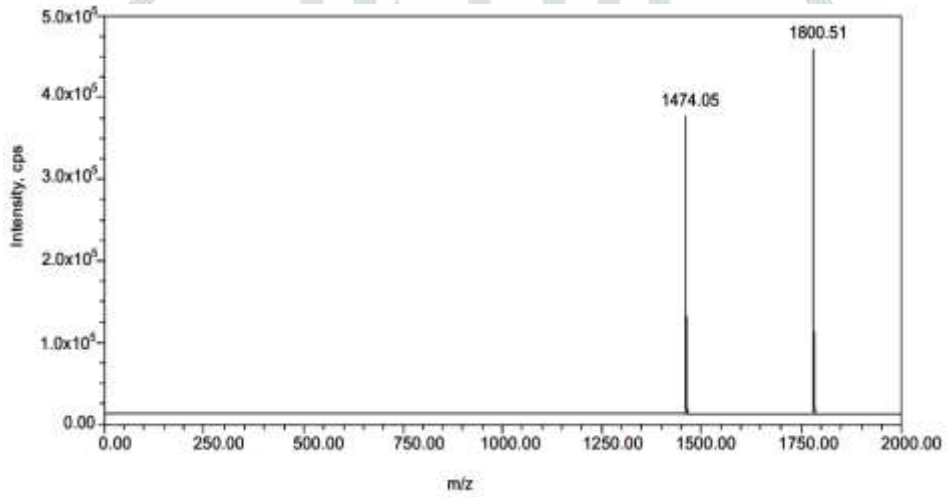

Fig. 3: Mass spectra of D_6-Oritavancin

Specificity

The specificity of the method to research Oritavancin is proved. The chromatograms of blank and standard as shown in figure 4, 5. The chromatograms of blank rabbit plasma and standard having no interference peaks were observed.

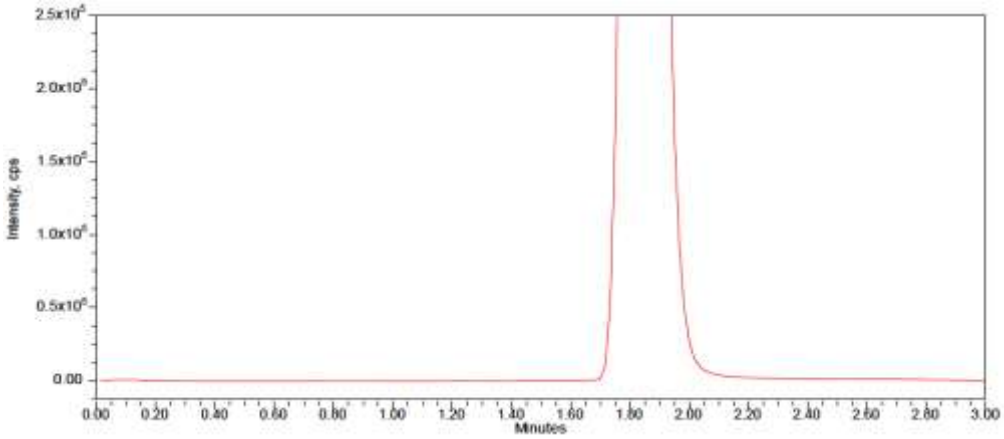

Fig. 4. Chromatogram of blank

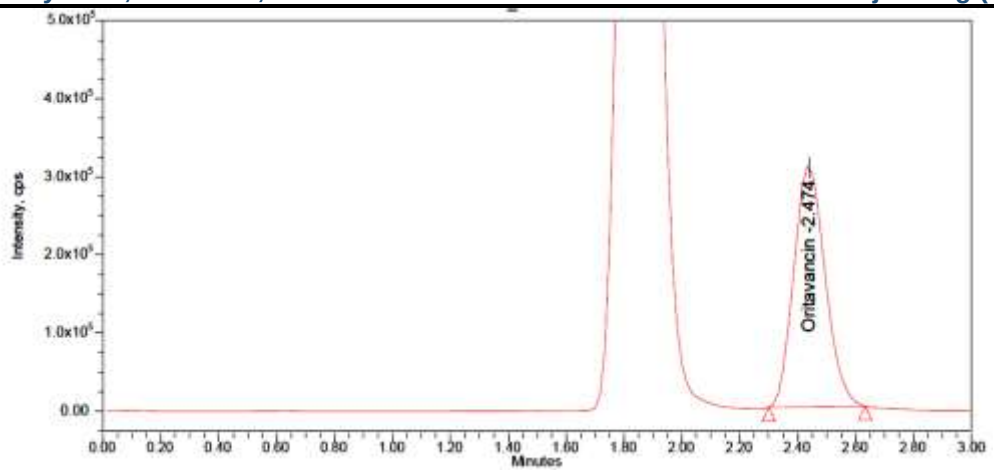

Fig. 5. Chromatogram of standard

Matrix effect

Percent RSD for within the signal, ion suppression/enhancement was observed as 1.0 percent for Oritavancin in LC-MS/MS, suggesting that under these circumstances the matrix effect on analyte ionization is within an acceptable range of ionization. In matrix effect LQC and HQC of Oritavancin were 101.34and 98.32. %CV of the drug at LQC and HQC level were 4.45, 0.18 respectively. It indicates that the matrix effect on the ionization of the analyte is within the suitable limit.

Linearity

The peak area ratio of calibration standards was proportional to the concentration. The concentration range of Oritavancin is 26 - 204 ng/ml. Linearity results of Oritavancin was shown in following table 1 and their calibration plots were shown in figure 6. The calibration curves were appeared linear and coefficient of correlation was found to be 0.999 for Oritavancin.

Table 1. Linearity results of Oritavancin

Linearity	Oritavancin conc. (ng/mL)	Oritavancin Area response ratio
1	26	0.296
2	51	0.585
3	77	0.899
4	102	1.165
5	128	1.437
6	153	1.719
7	204	2.284
Slope	0.0113	
Intercept	0.01091	
CC	0.99967	

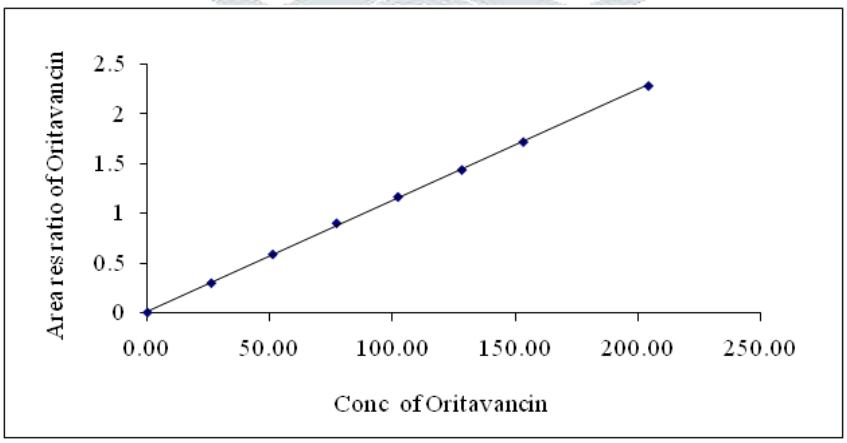

Fig. 6: Linearity plot for Oritavancin

Precision and Accuracy

By pooling all individual assay results of different internal control samples, the accuracy and precision were calculated. It was obvious, based on the data provided, that the strategy was precise and effective. The precision results of Oritavancin were shown in Table 2. Oritavancin accuracy results in quality control samples 99.31-112.37. Half of Oritavancin CV is < 5% of total internal control samples.

Table 2: Precision and Accuracy of Oritavancin

QC Name	LLQC	LQC	MQC	HQC
Conc.(ng/ml)	5.1 ng/ml	51 ng/ml	102 ng/ml	153 ng/ml
Mean	$0.3547x10^5$	$1.6238x10^5$	$3.1254x10^5$	$4.6239x10^5$
SD	0.00647	0.00854	0.00958	0.01054
%CV	0.13	0.08	0.06	0.17
Accuracy	112.37	104.63	99.71	99.31

Recovery

The recovery for Oritavancin at LQC, MQC and HQC levels the results demonstrated that the bio-analytical method had good extraction efficiency. This also showed that the recovery wasn't hooked into concentration. The recovery for Oritavancin (94.57% - 106.77%) at LQC, MQC and HQC levels and % CV ranged from 0.28-1.47 for Oritavancin. The results demonstrated that the bioanalytical method had good extraction efficiency.

Ruggedness

The percent recovery and percent CV of Oritavancin determined with two different analysts and on two different columns were within acceptable criteria in HQC, LQC, MQC and LLQC samples. The results proved method is ruggedness. The percent recoveries ranged from 98.85%-106.12% for Oritavancin. The %CV values ranged from 0.18-0.46 for Oritavancin. The results proved method is ruggedness.

Auto sampler carryover

Peak area response of Oritavancin, wasn't observed within the blank rabbit plasma samples after successive injections of LLQC and ULQC at the retention time of Oritavancin. In auto sampler carryover this method doesn't exhibit auto sampler carryover.

Stability

Oritavancin solution was prepared with diluents for solution stability analysis and placed in a refrigerator at 2-8°C. Fresh stock solutions were associated with stock solutions that were prepared 24 hours earlier. The plasma stability of the bench top and auto sampler was stable for twenty four hours, and 24 hours at 20°C in the auto sampler. It became apparent from future stability that Oritavancin was stable at a storage temperature of -30°C for up to 24 hours. The overall stability results of Oritavancin have been stated in the below table 3.

Table 3: Stability results of Oritavancin

Stability experiment spiked plasma		Spiked plasma conc. (n=6,ng/ml)	Conc. measured (n=6,ng/ml)	%CV
Bench top stability	LQC	51	51.23	0.85
	HQC	153	153.16	1.22
Auto sampler stability	LQC	51	51.41	0.46
	MQC	102	102.08	0.99
	HQC	153	153.13	1.10
Long term (Day28) stability	LQC	51	51.34	1.46
	HQC	153	153.50	0.77
Wet extract stability	LQC	51	51.02	0.51
	HQC	153	153.42	0.68
Dry extract stability	LQC	51	51.38	1.02
	HQC	153	153.26	1.44
Freeze thaw stability	LQC	51	51.54	1.01
	HQC	153	153.17	0.89
Short term stability	LQC	51	51.09	1.38
	HQC	153	153.21	0.65

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 Oritavancin was eluted within 3mins. The proposed LCMS/MS 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.