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NEW DEVELOPMENT AND VALIDATION OF REVERSE PHASE-HPLC DETERMINATION FOR THE OMADACYCLINE IN API AND MARKETED PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, rapid, specific and accurate reverse phase high performance liquid chromatographic method has been developed for the validated of Omadacycline in bulk as well as in marketed pharmaceutical dosage form. This separation was performed on a Symmetry ODS C_{18} (4.6×250mm, 5µm) column with Methanol: Phosphate Buffer (35:65) V/V as mobile phase at a flow rate of 1.0 mL min−1 with UV detection at 235 nm; the constant column temperature was Ambient. The run time under these chromatographic conditions was less than 8 min. The retention time of Omadacycline was found to be 2.252. The calibration plot was linear over the concentration range of 6–14 µg mL−1 with limits of detection and quantification values of 1.2 and 3.6 ng mL−1 respectively. The mean % assay of marketed formulation was found to be 99.86%, and % recovery was observed in the range of 98-102%. Relative standard deviation for the precision study was found <2%.The developed method is simple, precise, specific, accurate and rapid, making it suitable for estimation of Omadacycline in bulk and marketed pharmaceutical dosage form dosage form.

Keywords: Omadacycline, RP-HPLC, Validation and ICH Guidelines.

INTRODUCTION

Omadacycline is an oral, tetracycline-like antibiotic. Chemically it is (4S,4aS,5aR,12aS)-4,7- bis (dimethylamino)-9-{[(2,2-dimethyl propyl) amino] methyl} -3,10,12,12a-tetra hydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide[1] (Figure.1). Omadacycline is indicated for the treatment of community acquired bacterial pneumonia and acute bacterial skin and skin structure infections caused by Omadacycline-susceptible organisms in adults[2-3]. A thorough literature survey revealed that there are a few analytical methods developed for the estimation Omadacycline by [LCMS][4] in pharmaceutical formulations. The aim of the work is to develop new simple, sensitive, accurate and economical analytical method for the estimation of Omadacycline in bulk and marketed pharmaceutical dosage form.

MATERIALS AND METHODS

Omadacycline (Pure) is gifted by Sura labs, India. The chromatography system is a WATERS Alliance 2695 separation module, Software: Empower 2, equipped with 996 PDA detector. Water, acetonitrile and Methanol for HPLC is procured from Lichrosolv (Merck).

Preparation of the solutions

Preparation of mobile phase

Accurately measured 350 ml (35%) of Methanol, 650 ml of Phosphate buffer (65%) were mixed and degassed in digital ultrasonicator for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration. The Mobile phase was used as the diluent.

Omadacycline standard solution

Accurately weigh and transfer 10 mg of Omadacycline working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol. Further pipette 0.1ml of the above Omadacycline stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Sample preparation

Weight 10 mg equivalent weight of Omadacycline sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 0.1ml of Omadacycline above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

RESULTS AND DISCUSSION

Method development

The method was performed with various C_{18} columns like, X- bridge column, Xterra, and C_{18} column. Symmetry ODS C_{18} (4.6 x 250mm, 5µm) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow. The solvent system consisting of phosphate buffer and methanol in a ratio 35:65 parts by volume results in good peak shape and short retention time. The analyte is monitored at 235 nm at a flow rate of 1.0 mL/min. The optimized chromatographic conditions are given in Table 1.

Method validation

System suitability

The suitability gave an excellent peak shape (tailing factor 1.20) and good plate count as 6589 at retention time 2.27 min. Both parameters are found to be within the acceptance criteria. Hence, it is evident that the preferred chromatographic system is suitable.

Specificity

Analytical method was tested for specificity to measure accurately quantitate Omadacycline in drug product. No interference from placebo is observed at the retention time of Omadacycline. Therefore it can be evident that no interference due to placebo and standard for the quantification of Omadacycline in formulations. Hence, the method is specific and selective.

Linearity

The proposed method is linear over the concentration range 6-14 µg/mL and the correlation coefficient is highly impressive as its value is 0.999. The regression analysis indicates that the method has excellent linearity over the wide concentration range. The plot of Concentration (x) versus the Average Peak Area (y) data of Omadacycline is a straight line. The linearity plot is given in Fig.3. The results of the linearity is shown in Table 2.

Precision

The % RSD for intra-day precision of the sample is found as 0.17. The inter-day precision is achieved by performing the method in between the days and the % RSD is found as 0.38. In all the cases studied, the % RSD has been found by the proposed method is within 2.0 % that it indicates a consistency in its precision and can be seen in the results of precision studies in Table 3.

Accuracy

Recovery studies are performed on three different levels at 50, 100, and 150 in three replicates in each level in the present study. Standard drug is spiked to the pre-analyzed sample and injected into an HPLC system to determine the amount recovered by the proposed method. The % recovery values are observed to be in the range of 100.42 % - 101.20 % and the mean % recovery of the drug is found to be 100.72 and the % RSD is observed to be less than 1.0 at every spiking level. The results obtained for recovery at 50%, 100%, 150% are within the limits (98-102%). Hence, method is accurate. Results for the accuracy study are shown in Table 4.

LOD and LOQ

The LOD and LOQ is determined from the linearity plot and it is found satisfactory since the lowest amount that can be detected by this method is 1.2 µg/mL and the minimum

concentration of the analyte that can be quantified is found as 3.6 µg/mL.

Robustness

The robustness was performed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Omadacycline. The method is robust only in less flow condition. The standard of Omadacycline was injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

CONCLUSION

The developed method ensures an accurate estimation of Omadacycline in pharmaceutical formulations and it confirms an excellent linearity, accuracy and the precision. Hence, this method was recommended for quantitative estimation of Omadacycline.

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Table 1: Method optimization

Parameter	Optimized
Column	Symmetry ODS C18 (4.6×250mm, 5µm)
Column temperature	25°C
Mobile phase	Methanol : Phosphate buffer pH 3.6(35:65,v/v)
Wavelength	235nm
Flow rate	1ml/min
Run time	8 min
Retention time	2.260 min
Inference	Good separation

Table 2: Linearity results of Omadacycline

Injection no.	Concentration µg/mL	Average Peak Area
1	0	0
2	6	1078475
3	8	1461129
4	10	1808358
5	12	2211573
6	14	2593778

Table 3: Precision results for Omadacycline

Type	Intra-day precision		Inter-day precision	
S. no	Area (µV*sec)	USP Plate Count	Area (µV*sec)	USP Plate Count
1	1658954	6785	1678541	6587
2	1658745	6854	1685985	6321
3	1659865	6852	1685745	6385
4	1653254	6784	1685987	6580
5	1654781	6895	1698526	6721
Mean	1657120		1686788	
SD	2913.5		6463.46	
%RSD	0.17		0.38	

Table 4: Accuracy results of Omadacycline

%Concentration (accuracy Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	109068.3	5	5.021	100.420	100.72%
100%	202187	10	10.054	100.540	
150%	297032.3	15	15.181	101.206	

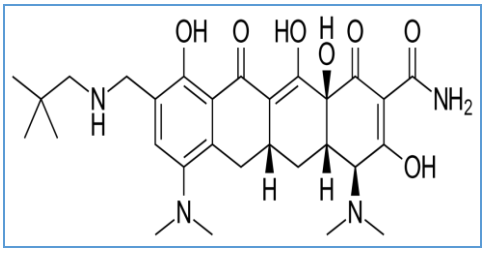

Fig. 1: Structure of Omadacycline

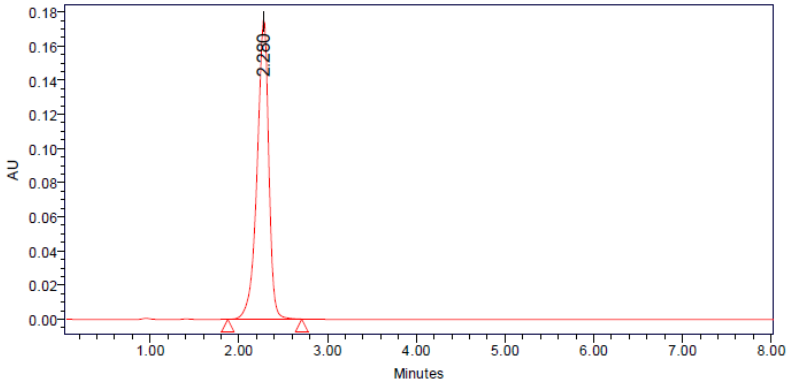

Fig. 2: Chromatogram of standard Omadacycline

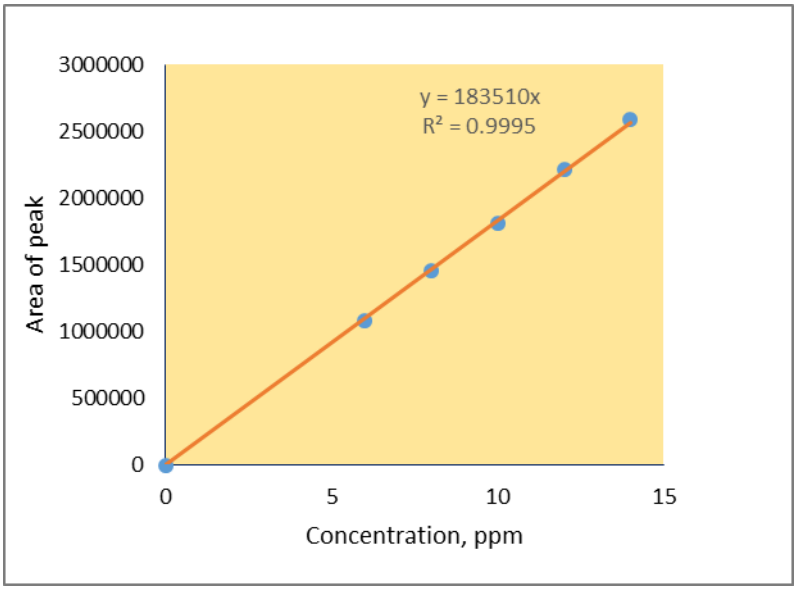

Fig. 3: Linearity plot of Omadacycline

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