A NOVEL VALIDATED RP-HPLC METHOD FOR IMPURITY PROFILING OF OMADACYCLINE IN BULK AND PHARMACEUTICAL DOSAGE FORM

Swetha Reddy[1], Adikay Sreedevi*, Bandi Swarupa[2] , Guttha Hema Latha[3]

ABSTRACT

An accurate, precise and robust analytical method was developed for impurity profiling of Omadacycline (Omd) bulk and tablet dosage form. Waters Alliance-E 2695 and Symmetry C_{18} (150x4.6mm 3.5m) column was used to separate Omadacycline and its Impurity-1(Imp-1) and Impurity-2(Imp-2). Acetonitrile, Ammonium formate (pH 2.5 adjusted with Tri Fluoro Acetic Acid (TFA) and Methanol were employed as Mobile phase (60:30:10), at flow rate of 1 ml/min, and 242nm wavelength. The peaks of Omadacycline and its impurities (1 and 2) were found at 3.64, 5.48, and 6.92 min, respectively. Omadacycline, Imp-1, and Imp-2 each had correlation coefficients of 0.9994, 0.9993, and 0.9996. Omadacycline and its impurities exhibited accuracy readings that ranged from 98.9- 100.1%, 100.1 - 100.4%, and 99.5 -100.1%, respectively. The highest degradation during the stability study was found with Peroxide (14.3%), and Thermal (0.7%) caused less degradation. The suggested approach is accurate, precise, robust, and linear.as a result, it may be utilised in quality control (QC) analysis during production to avoid the existence of impurities in pharmaceutical dosage form.

Keywords: Omadacycline, Impurity Profiling, Method validation, Stability studies.

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INTRODUCTION:

Impurity profiling has grown popular since it assists in the identification, quantification, and structure elucidation of the undesired elements. Since the presence of impurities may affect the safety and efficacy of a drug. The development of analytical methods for impurity profiling will aid in minimising production loss while monitoring safety, efficacy, and medication stability (4). Omadacycline is a tetracycline class antibiotic which is semi synthetic aminomethylcycline antibacterial. (11) .Omadacycline is used to treat Community acquired Bacterial infections, Skin infections and Bacterial Pneumonia (7).

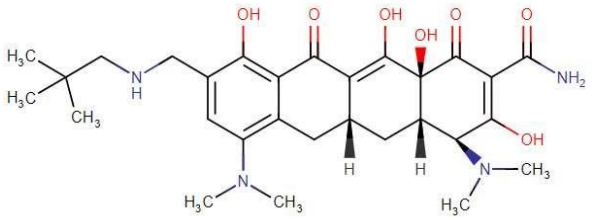


Figure. 1 Structure of Omadacycline

Only two bioanalytical procedures have been established for estimating Omadacycline in biological matrices using Liquid chromatography-Mass spectrometry (LC-MS) (6, 7). As per literature review there is no method for Omadacycline impurity profiling, hence the current study was planned to develop and validate a simple, accurate, and stability-indicating Reverse Phase High Performance Liquid Chromatography (RP-HPLC) method for the assessment of impurities in Omadacycline bulk and pharmaceutical dosage form . The present study was validated as per International Conference on Harmonization (ICH) Q1B (R2) guidelines.

MATERIALS and METHODS:

Chemicals, reagents and equipment's

HPLC grade Methanol and Acetonitrile were obtained from (Rankem, Mumbai, India.), Ammonium formate and Triflouro acetic acid are of analytical grade, used for mobile phase preparation. Omadacycline, imp-1 and imp-2 are purchased from (Zydus Cadila). The separation of impurities was achieved by using Waters Alliance-e 2695 symmetry C_{18} (150x4.6mm 3.5µm) column. The complete development and validation were conducted using HPLC Alliance e 2695-Empower software 2.0 version (Waters, USA), Alliance 2998 (Waters, USA) PDA detector.

Preparation of solutions

Mobile phase preparation

Acetonitrile, Ammonium formate (pH-2.5 adjusted with TFA), and Methanol were combined in the following proportions: 60:30:10 and used as mobile phase. To get rid of any contaminants that might interfere with the final chromatogram, it was filtered through a membrane filter with a 0.45 mesh size. Mobile phase is also used as diluent.

System suitability solution preparations

Standard stock solution preparation

15 mg of the Omadacycline working standard was accurately weighed and deposited into 10 ml volumetric flask which is clean and dry. A little amount of diluent then added, and was thoroughly dissolved using a solicitor before the volume was brought up to the required level.

Impurity Stock Solution Preparation:

5mg of Omadacycline Impurity-1 and 5 mg Omadacycline Impurity-2 were accurately weighed, transferred to a 10 ml volumetric flask, 7 ml of diluent added, and it was sonicated for up to 30 minutes for complete dissolution. The volume is then made up to mark with same diluent. 2 ml of the above-mentioned solution was pipetted into a 10 ml volumetric flask, then diluent was added to make the volume appropriate.

Spiked Standard solution preparation:

Transferred 1ml standard solution and 1ml impurity Stock solution in 10 ml volumetric flask and diluent is added up to the mark. Then filtered through 0.45µ filter paper.

Sample stock solution preparation:

The 77mg of Omadacycline sample was weighed and transferred into 10 ml volumetric flask. Diluent was added, and sample sonicated for 30 minutes to dissolve. Then it was centrifuged for 30 minutes for complete dissolution of the sample. The volume was then brought up to the volume using the same diluent. The 0.45 micron Injection filter (Stock solution) is then used to filter it.

Preparation of Spiked sample solution:
1ml of sample solution and 1ml of impurity Stock solution was taken in 10 ml volumetric flask and diluent was added up to the mark. Then filtered through 0.45µ filter paper.

Method validation
System suitability
The system suitability was studied by injecting single injection of blank (diluent) and six times of standard solution. USP plate count, USP tailing and Percent Relative Standard Deviation (%RSD) of Omadacycline and its impurities and peak area from six replicate injections of standard solution were determined.

Specificity
To observe specificity of developed method, the HPLC system was injected with blank, placebo, standard, and sample solutions, the corresponding chromatograms were recorded.

Accuracy
Accuracy was conducted by spiking the impurities with working concentrations of 50%, 100%, and 150% of Omadacycline and recovery percentage was computed.

Precision
The System precision, method precision and intermediate precision were carried out according to ICH guideline Q2B (R1).

Linearity
The six solutions were made from stock solutions of spiking sample at various concentrations, i.e. 25, 50, 75, 100, 125, and 150 µg/ml. The peak area was measured after injecting each level into the chromatographic apparatus. Peak area (Y-axis) vs concentration (X-axis) was displayed on a graph and the correlation coefficient was computed.

Limit of Detection and Limit of Quantification (LOD and LOQ)
The solutions are injected into HPLC for LOD of Omadacycline 3% solution i.e., 0.03ml of standard solution taken and made up to 10ml with diluent and for LOQ 10% solution i.e., 0.1 ml of standard spiked solution taken and made up to 10ml.

Robustness
In order to test the method's robustness, a planned adjustment in Flow rate and Mobile Phase composition is made. Flow minus (0.9 ml/min), Flow plus (1.1 ml/min), Organic minus (54:37:9), and Organic plus (66:23:11) v/v are among the modifications implemented.

Forced degradation studies
Forced degradation studies were carried out by exposing the sample to relevant stress conditions as per ICH (Q1A and Q1B) guidelines like Hydrolysis, Acid degradation, Alkali degradation, Oxidation, Reduction, Thermal and Photolytic degradation. These stressed samples were analysed by HPLC.

RESULTS:
Wavelength detection with a PDA detector

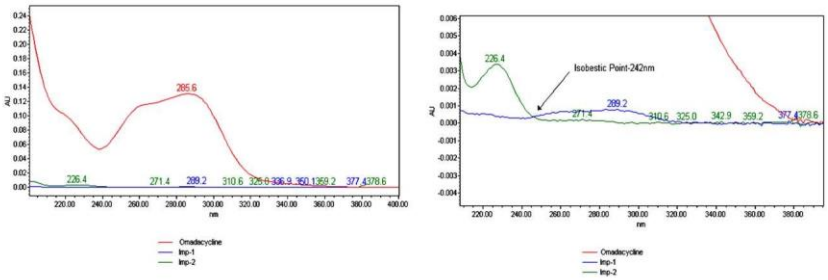


Figure 2 PDA spectrum of Omadacycline and its impurities

The Isobestic point is visible on the absorption curve at 242nm.As a result, the detector wavelength for the HPLC chromatographic process was chosen to be 242 nm.

Optimized chromatographic conditions
Use suitable High-Performance Liquid Chromatographic equipped with PDA detector.
Column : Symmetry C18 (150X4.6mm, 3.5µ)
Mobile phase : Acetonitrile and Ammonium formate pH-2.5/TFA and Methanol (60:30:10)
Wavelengt : 242 nm
Flow rate : 1ml/min
Injection volume : 10µl
Run time : 10min

Method validation
The created method was validated in accordance with ICH guideline Q2B (R1).

System suitability

To develop a robust analytical method, in present study the system suitability parameters like retention time, resolution, theoretical plates, tailing factor, %RSD were Detected.

Table No 1 System suitability parameters

parameters	Results observed		
	Omadacycline	Imp-1	Imp-2
API Concentration (µg/ml)	15	5	5
Retention time (min)	3.641	5.487	6.922
Theoretical plates	9378	5889	6455
Resolution		5.36	4.61
Tailing factor	0.84	0.94	1.01
%RSD	0.56	0.48	0.39

Specificity

There was no interference found in the chromatographic separation of Omadacycline, Imp-1 and Imp-2 with blank and placebo.

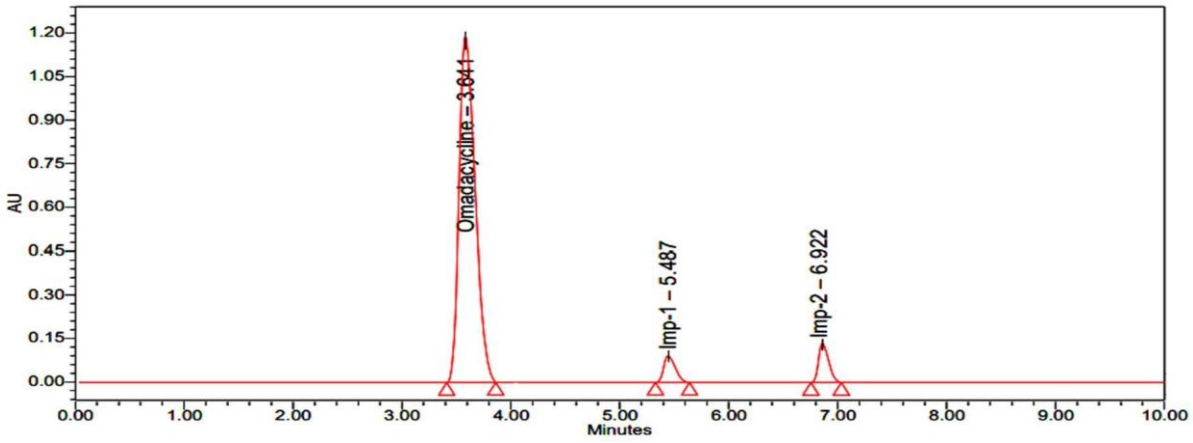


Figure.3 Chromatogram for Omadacycline and its impurities (standard)

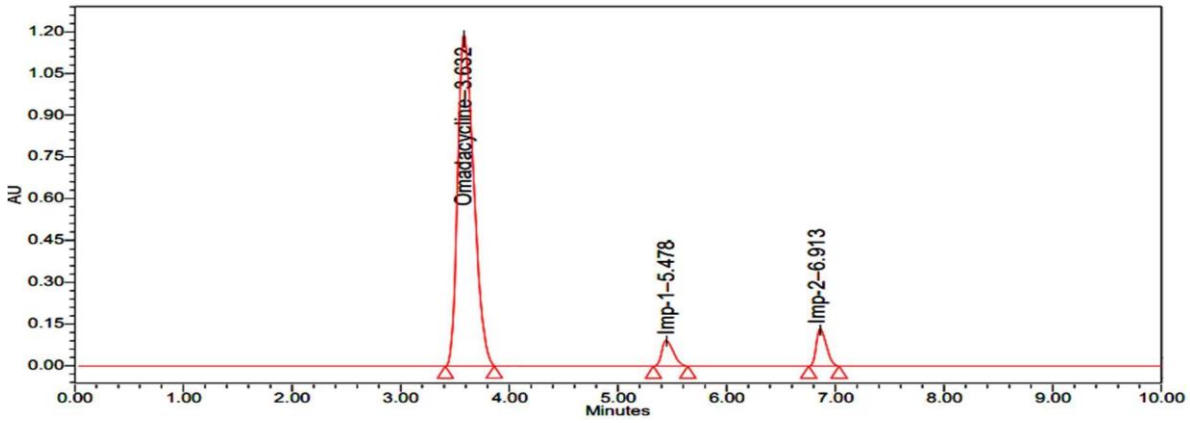


Figure. 4 Chromatogram for Omadacycline and its impurities (Sample)

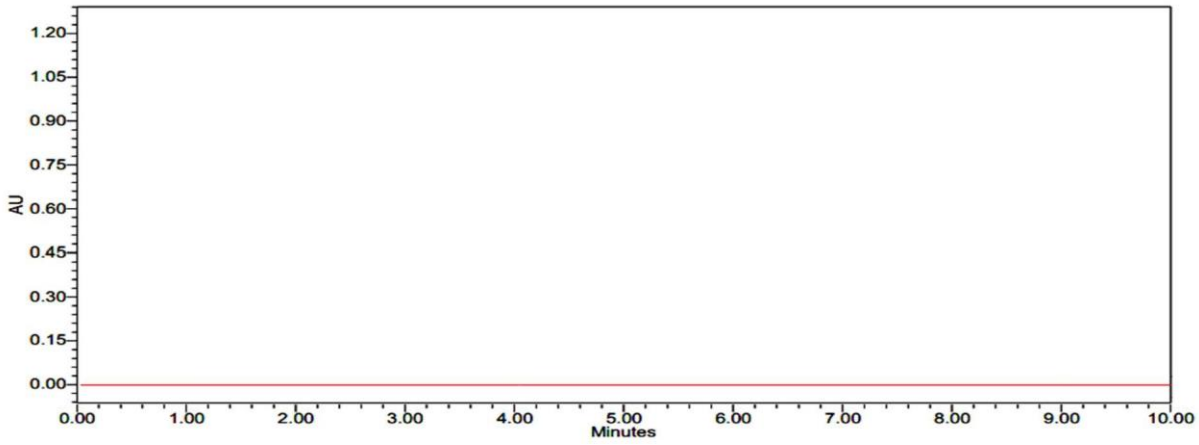


Figure.5 Chromatogram of blank

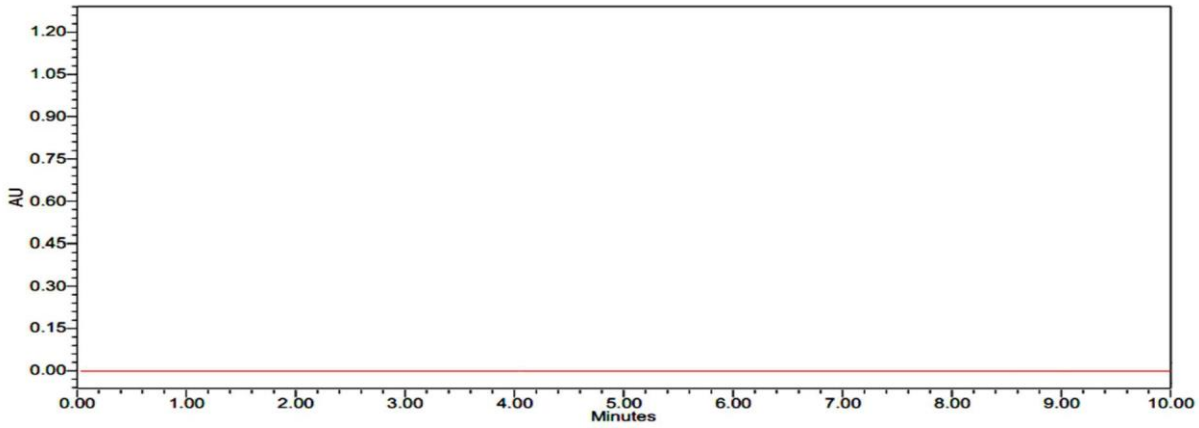


Figure.6 Chromatogram of Placebo

Accuracy

When spiked at 50% - 150% of the working concentration, the devised method was proven to be capable of accurately recovering the contents.

Table No 2 Accuracy study

Recovery sample name	% Recovery of Omadacycline		% Recovery of Imp-1		% Recovery of Imp-2	
	%Recovery	%RSD	% Recovery	%RSD	% Recovery	%RSD
50%-1	100.9	0.70	100.3	0.25	100.1	0.70
50%-2						
50%-3						
100%-1	100.4	0.65	100.1	0.20	100.0	0.21
100%-2						
100%-3						
150%-1	98.0	0.06	100.4	0.83	99.5	0.10
150%-2						
150%-3						
	%RSD – 0.47		%RSD – 0.42		%RSD – 0,33	

Precision

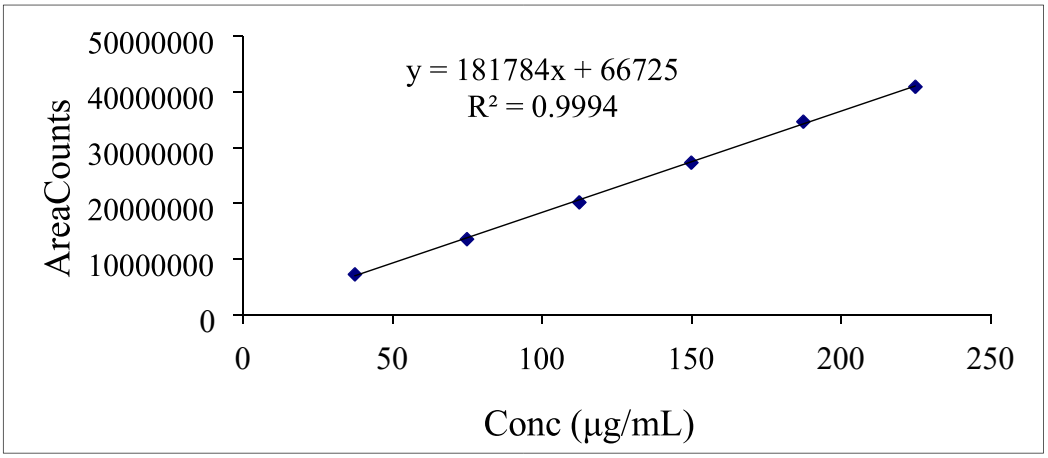
The system precision investigations revealed that all the parameters such as the peak areas, %RSD fall within acceptable boundaries.

Table No 3 Precision study

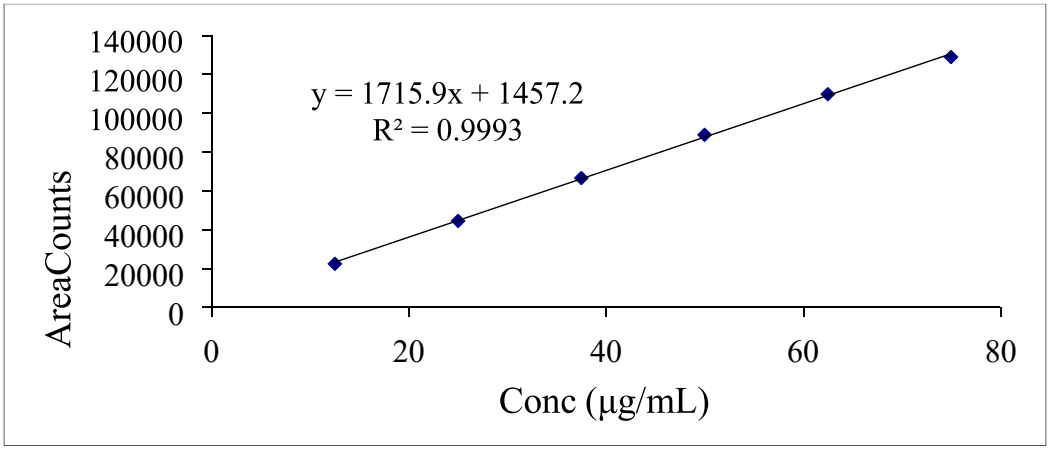
Sample	Omadacycline		Imp-1		Imp-2	
	Peak area MP	Peak area IP	Peak area MP	Peak area IP	Peak area MP	Peak area IP
1	27445962	27248622	88468	88359	58874	58259
2	27291457	27157862	88328	88329	58481	58751
3	27385692	27435476	88614	88574	58674	58863
4	27503384	27558742	88657	88486	58774	58673
5	27596120	27465716	88587	88234	58817	58533
6	27643271	27335412	87298	87527	58572	58842
Mean±SD	131343.772	152682.984	517.219	419.740	151.295	228.875
%RSD	0.48	0.54	0.59	0.42	0.26	0.39

Linearity

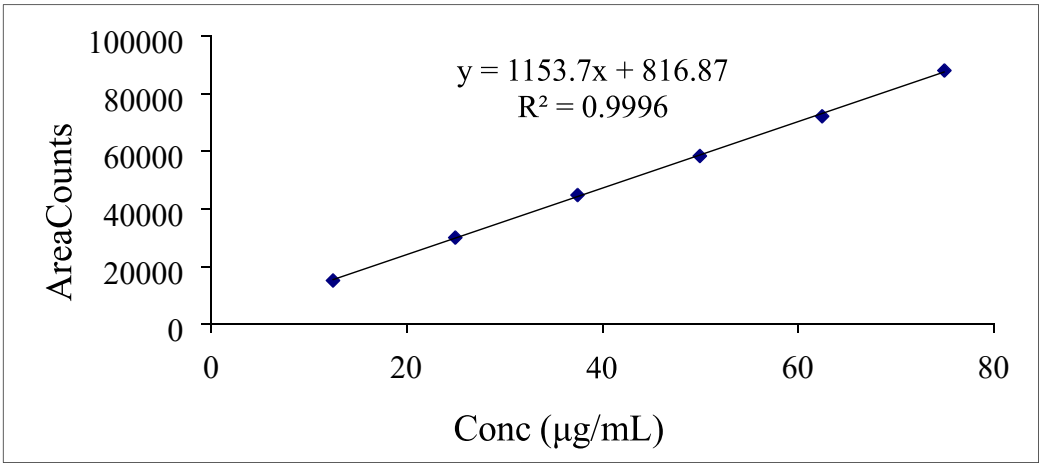
The area under the curve for Omadacycline, Imp-1 and Imp-2 was determined in the range of 25 - 150 µg/ml. The correlation coefficient of Omadacycline, Imp-1, and Imp-2 was found to be 0.9994, 0.9993, and 0.9996 respectively.



Calibration curve of Omd



Calibration curve of Imp-1



Calibration curve of Imp-2

LOD and LOQ

LOD for Omadacycline, impurity-1and imp-2 were found to be 4.5, 0.03, 0.03µg/mL and LOQ for Omadacycline, impurity-1and imp-2 were found to be 15, 0.1 and 0.1µg/ml respectively.

Robustness

During the robustness investigation we found that changes in the flowrate (0.9-1.1), ratio of organic phase (OP Minus-54:37:9; OP Plus-66:23:11) had no effect on the system characteristics including RT, and resolution

Table No 4 Robustness study

Parameter	Modification	Omadacycline		Imp-1		Imp-2	
		RT	%RSD	RT	%RSD	RT	%RSD
Flow rate	0.9ml/min	3.85	0.63	5.52	0.17	7.13	0.22
	1.1ml/min	3.51	0.42	5.267	0.12	6.86	0.29
Organic phase	54:37:9	3.986	0.62	5.703	0.10	7.25	0.21
	66:23:11	3.431	0.63	5.14	0.14	6.70	0.30

Study of forced degradation (FD)

We found no significant degradation throughout the FD research under Acidic (12.2%), Alkaline (12.7%), Peroxide (14.3%), Reduction (10.5) Thermal (0.7%), Photolytic (10.9%) and Hydrolysis (2.2) conditions. highest degradation was seen in the presence of peroxide stress. We found no interfering peaks during the FD analysis during the RT of the principal analyte peak and recognised impurities. Purity angle is found to be less than threshold angle in all forced degradation studies. This demonstrated the method's stability-indicating character.

Table NO.5 Forced Degradation Studies

Results: % Degradation results	Omadacycline				
	Area	% Assay	% Deg	Purity Angle	Purity Threshold
Control	16458918	100	0	14.462	20.522
Acid	14455097	87.8	12.2	14.427	20.524
Alkali	14366199	87.3	12.7	14.434	20.537
Peroxide	14106507	85.7	14.3	14.448	20.587
Reduction	14730246	89.5	10.5	14.485	20.575
Thermal	16358271	99.3	0.7	14.464	20.565
Photolytic	14673308	89.1	10.9	14.405	20.519
Hydrolysis	16103325	97.8	2.2	14.469	20.544

DISCUSSION:

Only two bioanalytical methods have been established so far to estimate the presence of Omadacycline in biological matrices. There is no known method for estimating Omadacycline on its own and in combination with impurities using

HPLC. Hence the present study was developed with a precise, accurate, and reliable method for impurity profiling of Omadacycline.

CONCLUSIONS:

The findings of the current study indicate that the current method is precise, accurate, linear, and robust because all of results were within acceptable bounds. The technique is used for stability analysis of the formulation product and Omadacycline estimation utilising HPLC. As a result, the suggested approach helps researchers by reducing the time and expense associated with development. The investigation doesn't involve any potentially harmful chemicals hence the method is safe to use. The developed method can be used for the routine testing of impurities in pharmaceutical products during manufacturing of batches.

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