Liquid Chromatographic Method Development and Validation for the Quantitation of Treprostinil in Bulk and Dosage Form

Tummala Maniratnam [1], Mahesh.M[2*]

[1]Department of Pharmaceutical Analysis, JNTUA-Oil Technological and Pharmaceutical Research Institute, Jawaharlal Nehru Technological University Anantapur (JNTUA)-Ananthapuramu-515001, Andhra Pradesh, India.

[2*]Assistant Professor, Department of Pharmaceutical Analysis, JNTUA-Oil Technological and Pharmaceutical Research Institute, Jawaharlal Nehru Technological University Anantapur (JNTUA)-Ananthapuramu-515001, Andhra Pradesh, India

*Corresponding Author:

Mahesh. M

Assistant Professor

Dept of Pharmaceutical Analysis,

JNTUA-OTPRI, Anantapur

Andhra Pradesh, India-515001

Abstract:

The present work mainly focuses on developing and validating a new robust method for the quantitation of Treprostinil in bulk and dosage form. Treprostinil is mainly used in treatment of pulmonary arterial hypertension and mainly works by relaxing and widening the blood vessels (arteries) in the lungs and other parts of the body so that blood can flow more easily. The main strategy of the present study is to develop a novel, precise, simple, accurate, and sensitive, stability indicating liquid chromatographic method for the estimation of the Treprostinil in bulk and pharmaceutical dosage form. The separation was achieved by using isocratic elution of the mobile phase containing a mixture of Methanol, Acetonitrile and water in the ratio of 35:35:30% v/v with a flow rate 0.9 ml/min. Chromatographic separation was achieved on a Phenomenex C_{18} (250mm×4.6mm, 5µm) and chromatographic retention time was stable at 3.064 min. The developed method was validated for linearity, precision (repeatability, intermediate precision), accuracy specificity, robustness, ruggedness, the limit of detection, and limit of quantification as per ICH Guidelines. The proposed method is simple, accurate and reproducible and can be employed for quantitation of Treprostinil.

Keywords: Treprostinil, Methanol, Acetonitrile, Validation

1. Introduction:

Treprostinil is chemically known as 2-[(1R,2R,3as,9as)-2-hydroxy-1-[(3s)-3-hydroxyoctyl]-2,3,3a,4,9,9a-hexahydro-1H-cyclopenta(9)napthalen-5-yl)oxy]acetic acid[1] which is used to treat a type of high blood pressure in the lungs (pulmonary arterial hypertension) and helps to improve symptoms such as shortness of breath and tiredness. It works by relaxing and widening the blood vessels (arteries) in the lungs and other parts of the body so that blood can flow more easily[2]. This medication belongs to a class of drugs known as vasodilators and the Mechanism of action is direct vasodilatation of pulmonary and systemic arterial vascular beds and inhibition of platelet aggregation[3]. Its chemical structure is shown in Figure No 1.

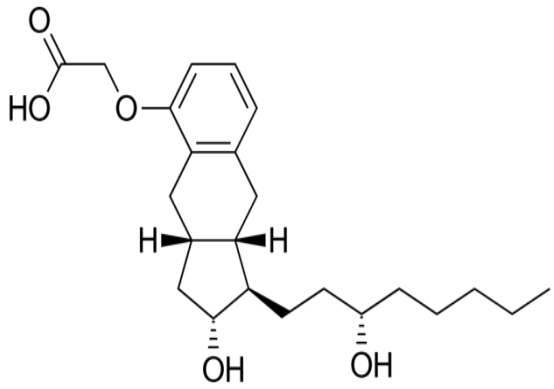

Figure 1: Chemical structure of Treprostinil

The Present work mainly focuses on developing and validating a reproducible, less cost, simple and rapid liquid chromatographic method for determination of Treprostinil in marketed formulations. Instead of routine analysis, the use of a rapid and uncomplicated is matter of highly importance. By conducting a literature review and concluded by collecting different articles related drug and its category and there is one bioanalytical[4] work has been done, there is no analytical work has carried[5-12].

2. Experimental

2.1 Instrument

The HPLC system was a Shimadzu LC-20 AD with a PDA detector, the ultra sonicator was a model 2200 MH Soltech (SPINCOTECH Pvt., ltd), and the digital balance was a model NO-HT 220 (VIBRA Shinko Denshi co. ltd), and the ultra filtration was a model 2200 MH Soltech (SPINCOTECH Pvt., ltd), and the ultra filtration (millipore Pvt.,ltd). The injection volume was 20µL and the chromatographic separation was done on a Phenomenex Luna C_{18} (250mm×4.6mm, 5m). The column was equilibrated for at least 30 minutes with the mobile phase running through the apparatus before injecting the drug solution. The data was collected, saved, and analyzed using Shimadzu LC-Solution chromatographic software.

2.2 Reagents and chemicals

Treprostinil pure drug was procured as a gift sample from Emmennar Pharma (P) LTD Hyderabad. HPLC grade Methanol and Acetonitrile by Merck., India. HPLC grade water by Merck, Mumbai India, has been used for analysis. Distilled water was prepared using Milli-Q system in laboratory and was used throughout the study.

2.3 Preparation of Solutions:

Selection of Mobile Phase: During optimization Different Composition and proportions of mobile phases are tried such as Methanol: Water and Acetonitrile: water. In these mobile phase system suitability tests was not satisfactory. Mobile phase containing Methanol, Acetonitrile and water in the ratio of 35:35:30% v/v and the flow rate was 0.9 ml/min and had given all system suitability parameters within the limits. Hence this mobile phase was chosen for analysis of Treprostinil.

Preparation of Standard Stock Solution:

Weigh accurately 10 mg of pure drug and transfer into a 10 ml volumetric flask and add 10 ml of mobile phase to obtain the concentration is 1000 µg/ml and kept for sonication in water bath for removal of air bubbles. From this solution withdraw 1ml and add 10ml of mobile phase in volumetric flask to get concentration is 100µg/ml. From this solution a series of aliquots were prepared for carrying out further method development.

2.4 Chromatographic conditions:

Liquid chromatographic analysis was performed by using mobile phase containing Methanol, Acetonitrile and water in the ratio of 35:35:30% v/v and the flow rate was 0.9 ml/min and the separation was achieved on column was C_{18} Phenomenex Luna(250X4.6mm;5µ) and temperature was ambient, with injection volume 20µl, and detected at 223 nm wave length. According to the ICH Q2 (R1) guidelines, all validation parameters are performed by applying these chromatographic conditions. Chromatogram meets acceptance criteria like tailing factor, theoretical plates and capacity factor and injection reproducibility. Optimized chromatogram showed in Figure no. 2

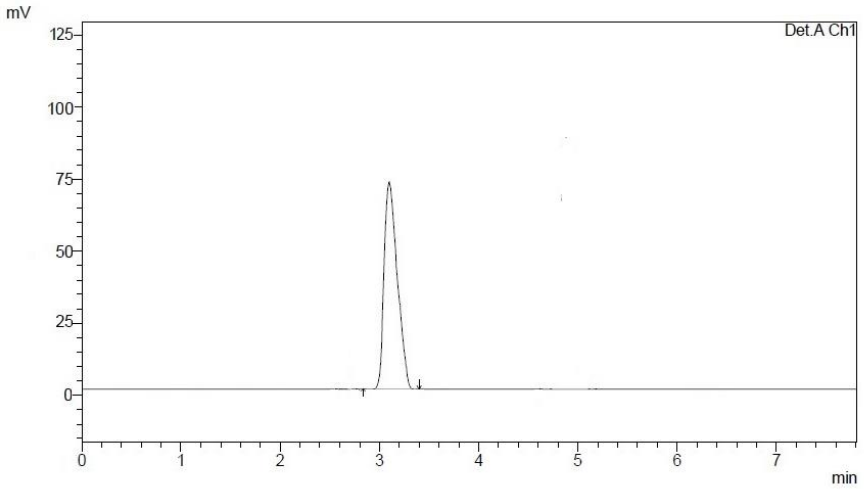

Figure 2: Optimized Chromatogram of Treprostinil

2.5 Method Validation:

The analytical method was validated as per ICH Q2 (R1) guidelines for the parameters like system suitability, specificity, accuracy, precision, linearity, robustness, LOD, and LOQ.

System Suitability

System suitability parameters are performed by injecting the prepared standard solution in six times and measured parameters like theoretical plates, retention time, tailing factor and %RSD.

Accuracy

Accuracy was performed for this method by conducting the recovery studies were carried out by adding three different concentration levels 50%, 100%, and 150% respectively. Percentage of recovery, % RSD was calculated.

Specificity

The comparison of the data of the drug solution before spiking and the spiked drug solution revealed that there was no significant interference of blank with the recovery of Treprostinil, inferring that the method was specific.

Precision

The method shows repeatability and reproducibility for the estimated sample analysis which has been done by six replicates of fixed concentration from the standard stock solution. Performing intraday precision and inter-day precision at confidence intervals. The % RSD for the method were found to be less than 2%.

Linearity and Range

Linearity was conducted by preparing different standard solutions of Treprostinil at different concentration levels. The standard solutions were prepared in the concentration range of 10-60µg/ml of Treprostinil. Each concentration was injected into the HPLC system and recorded the areas obtained. Plot a graph between the area taken on Y-axis and concentration on X-axis.

LOD and LOQ

LOD was measured by diluting the standard solution of Treprostinil and determining the concentration was the response of sample peaks are three times the noise peak. LOQ was measured by diluting the standard solution of Treprostinil and determining the concentration was the response of sample peaks are ten times the noise peak.

Robustness

In robustness, the method was determined by making slight changes in the method parameters and measuring the effect on the method by monitoring system suitability test results.

2.6 Forced Degradation Study:

The degradation samples were prepared by transferring intact tablets, samples were employed for acidic, alkaline and oxidant media and also for thermal and photolytic conditions. After the degradation treatments were completed, the stress content solutions were diluted with diluent to attain about 100 µg/ml concentration.

a) Acidic Degradation:

Acidic degradation study was performed by weigh 1mg drug and dissolved in 10ml of mobile phase and the sample is heated for one hour at 100° C and cooling to room temperature. The solution was further diluted with 0.1 N HCl to attain concentrations of 100 µg/ml.

b) Alkali Degradation:

Alkaline degradation was performed by weigh 1mg drug and dissolved in 10ml of mobile phase and the sample is heated for one hour at 100° C and cooling to room temperature. The solution was further diluted with 0.1 N NaoH to attain concentration of 100 µg/ml.

c) Oxidative Degradation Study:

Oxidative degradation study was performed by weigh 1mg drug and dissolved in 10ml of mobile phase and the sample is heated for one hour at 100° C and cooling to room temperature. The solution was further diluted with 10% v/v H_2O_2 to attain concentration 100µg/ml.

d) Thermal Degradation Study:

Thermal degradation study was performing by weigh 1mg drug and dissolved in 10ml of mobile phase and the sample is heated for one hour at 100° C and cooling to room temperature. This solution is used dilution to attain 100µg/ml.

3. Results and Discussion:

Specificity:

The comparison of the data of the drug solution before spiking and the spiked drug solution revealed that there was no significant interference with the recovery of Treprostinil and, inferring that the method was specific. The results were shown in table 2 and chromatogram showed in figure 3

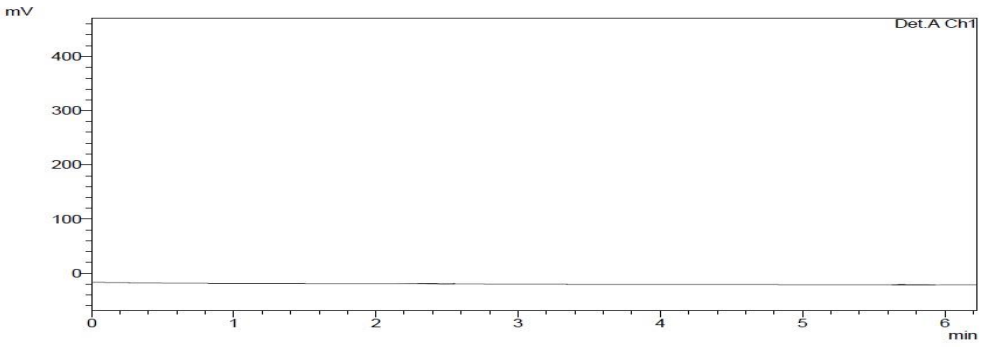

Figure 3: Blank Chromatogram

S.No	Peak Name	Peak area
1	Blank	Nil
2	Standard	397214

Table 2: Results of specificity

System Suitability:

System suitability parameters are performed by injecting prepared standard solution in six times and measured the parameters like theoretical plates, retention time, tailing factor and %RSD. All the results shown in table 3.

S.No.	Parameters	Results	Limits
1	Capacity factor	2.14	1 – 5

2	Tailing factor (T)	1.57	$T \leq 2$
3	Theoretical plates(N)	5420	N > 2000
4	%RSD	0.83%	RSD < 2%

Table 3: Results of system suitability

Linearity and Range:

The proposed linearity range of the study was carried out by plotting concentration against absorbance of the analyte it shows a good relationship between concentrations and the absorbance of the Treprostinil. The linearity graph is shown in fig. no 4 and the results were shown in table 4

Sl. No.	Concentration (µg/ml)	Peak Area response (mV)
1	10	59326
2	20	188520
3	30	282918
4	40	398122
5	50	511375
6	60	622791
Regression Equation		Y=11146x-46267
R2		0.999

Table 4: Results of Linearity by RP-HPLC method

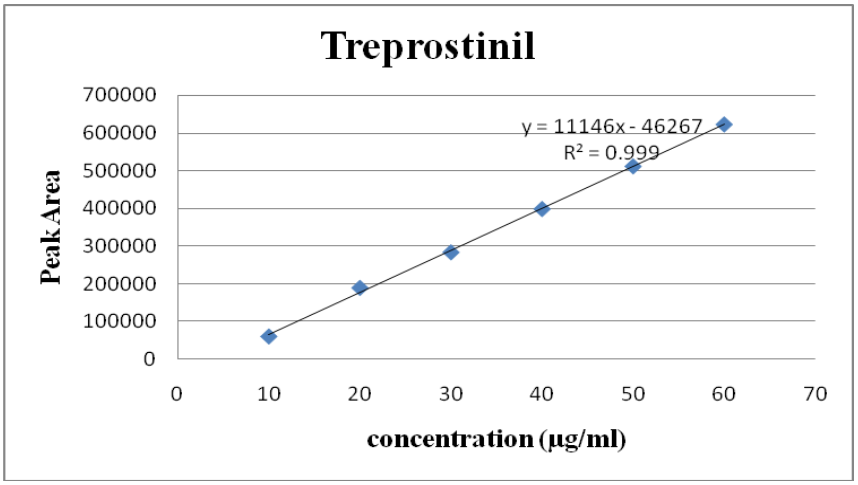

Figure 4: Calibration curve of Treprostinil

Accuracy:

The accuracy was performed for this method by conducting recovery studies of triplet standard addition method at different concentration levels of 50%, 100% and 150%. By adding known amount of Treprostinil to pre analysed samples and was subjected to the proposed method. Results of recovery studies are shown in table 5.

Spiked Concentration (µg/ml)	Peak area	Amount added (µg/ml)	Amount Found (µg/ml)	Recovery	% Mean Recovery
20	199858	20.226	20.0801	99.28	99.01
	198988		19.9927	98.85	
	199092		20.0031	98.90	
40	408525	40.452	40.0405	101.47	100.53
	403712		40.5616	100.27	
	401981		40.3877	99.84	
60	602876	60.678	60.5720	99.83	99.87
	601127		60.3963	99.54	
	605399		60.8255	100.24	

Table 5: Results of Accuracy

Precision:

The method shows repeatability and reproducibility by the estimated sample analysis which has been done by six replicates of fixed concentration from the formulation. The Interday and intraday also conducted at confidence interval and the results obtained. The %RSD was found below the 2% it indicates that as good precision for the method these results were tabulated in table 6.

S.No.	Intraday precision Area	Interday precision Area
1	389897	395643
2	395146	394761
3	392915	389965
4	389478	398746
5	388917	397158

6	391868	398577
Mean	391370.1667	395808.3333
Std Dev	2394.151	3267.592
%RSD	0.61	0.83

Table 6: Results of Precision

Detection of limits (LOD&LOQ):

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The limit of detection of analyte can be calculated LOD=3.3 * standard deviation (σ)/ s. The Limit of quantitation LOQ=10* standard deviation (σ)/ s. The results of LOD&LOQ were tabulated in table 7.

S.No	**Method**	**Range (µg/ml)**	**LRa**	**R2**	**LOD (µg/ml)**	**LOQ (µg/ml)**
1	RP-HPLC	10-60	11146x-46267	0.999	0.709	2.148

Table 7: Results of detection and quantification limits

Robustness:

It demonstrates the analytical method which will unaffected while small changes made in the analytical procedure, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The robustness data were shows in table 8.

Variation	**% RSD**	**Tailing factor**	**Theoretical plates**
Temp (30^0c)	1.50	1.47	4954
Temp (35^0c)	0.60	1.09	5257
Temp (40^0c)	1.13	1.77	3785
Flow(0.9ml)	0.99	1.38	4028

Flow(1ml)	0.27	1.11	5218
Flow(1.1ml)	1.07	1.68	4963

Table 8: Results of robustness

4. Forced Degradation Study:

The degradation samples were made by transferring intact tablets, and they were tested in acidic, alkaline, and oxidant media, as well as under thermal and photolytic conditions. After the degradation treatments were finished, the stress content solutions were diluted with diluent to a concentration of about 100µg/ml. The following conditions were described in detail. The procedure was specific. There was no other interfering peak around the Treprostinil retention time, and the base line showed no significant noise. Table 9 shows the percent degradations observed when the stress conditions were applied.

S.NO	Stress degradation condition	Normal peak area	Stress peak area	Peak purity	% Degradation
1	Alkali degradation	387619	381725	97.95	2.05
2	Acidic degradation	387619	379639	97.23	2.77
3	Oxidative degradation	387619	369836	93.82	6.18
4	Thermal degradation	387619	378947	96.98	3.02

Table 9: Result for Forced degradation studies

5. Conclusion

The chromatographic method for the determination of Treprostinil has been successfully developed and validated and the method adheres to regulatory requirements for linearity, accuracy, precision and recovery studies. This method indicated the suitability of the method to study the stability of Treprostinil, it can be applied for the routine quality control analysis of analyte in dosage form.

Acknowledgement

The authors are very thankful to Director, JNTUA-OTPRI, Ananthapuramu for providing the laboratory facilities, chemicals to carryout entire research work.

Conflict of Interest

The authors declare that they have no conflict of interest

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