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Research Article

HPLC - METHOD DEVELOPMENT AND VALIDATION OF TREPROSTINIL FOR ESTIMATION IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT
Treprostinil was estimated using RP-HPLC in a simple, precise approach. Stationary phase Agilent C18 (250mm*4.6mm5m), mobile phase 0.01N Kh2po4: Acetonitrile in the ratio 60:40, flow rate 1.0ml/min, detection wave length 224nm, column temperature 30oC, and diluent mobile phase. Optimised conditions were set. System appropriateness characteristics were studied by injecting the standard six times and scoring considerably below acceptability. R2 was 0.999 for linearity study between 25% and 150%.Precision was 0.3 for repeatability and moderate precision.LOD and LOQ are 0.11µg/ml and 0.33µg/ml. The aforesaid approach assayed commercial formulation and found 99.45%. All Treprostinil degradation investigations showed purity thresholds greater than purity angle and within acceptable limits. Full length procedure was not conducted; if done, it can be utilised for regular Treprostinil analysis.

Key Words: Treprostinil, Method development, Validation, RP-HPLC.

INTRODUCTION[1-10]
Treprostinil is a synthetic analog of prostacyclin (PGI2) that is primarily used in the treatment of pulmonary arterial hypertension (PAH). PAH is a progressive, life-threatening disorder characterized by elevated pulmonary arterial pressure, which leads to right ventricular heart failure. Treprostinil acts by dilating blood vessels in the lungs and inhibiting platelet aggregation, which helps reduce pulmonary vascular resistance and alleviate the strain on the heart. It mimics the effects of endogenous prostacyclin, enhancing blood flow and preventing the overgrowth of vascular smooth muscle cells in the pulmonary arteries.
Treprostinil is available in several formulations: subcutaneous (SC), intravenous (IV), inhalation, and oral. The versatility of these delivery methods allows clinicians to tailor the treatment to individual patient needs, ensuring long-term control of PAH symptoms. The development of various forms of treprostinil has improved patient compliance and quality of life, as it offers more convenient administration options compared to older prostacyclin analogs, like epoprostenol, which required continuous IV infusion and were associated with complex management issues.
The drug is particularly beneficial for patients with WHO Group 1 PAH, including idiopathic, familial, and connective tissue disease-associated PAH. Its long half-life relative to other prostacyclin analogs, such as epoprostenol, reduces the risk of rebound pulmonary hypertension if treatment is interrupted. Treprostinil's mechanism includes direct vasodilation of pulmonary and systemic arterial vascular beds, inhibition of platelet aggregation, and antiproliferative actions on smooth muscle cells.
Treprostinil has demonstrated efficacy in several pivotal clinical trials, including the pivotal studies of its SC, IV, inhalation, and oral formulations. These trials showed improvements in exercise capacity, symptom severity,

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and hemodynamic parameters in patients with PAH. The drug's side effects are generally related to its vasodilatory properties, such as headache, flushing, jaw pain, and hypotension, but they are typically manageable.

With its FDA approval in multiple formulations for the treatment of PAH, treprostinil remains a cornerstone therapy in the management of this challenging condition. Continuous research on treprostinil aims to further expand its use and improve delivery systems to enhance patient adherence and outcomes.

ANALYTICAL BACKGROUND[11]

Treprostinil is a stable analogue of prostacyclin , a prostaglandin that acts as an anti-thrombotic agent and a potent vasodilator. It is chemically known as 2-{[(1R,2R,3aS,9aS) -2-hydroxy-1-[(3S)-3-hydroxyoctyl]-1H,2H,3H,3aH,4H,9H,9aH-cyclopenta[b]naphthalen-5-yl]oxy}acetic acid

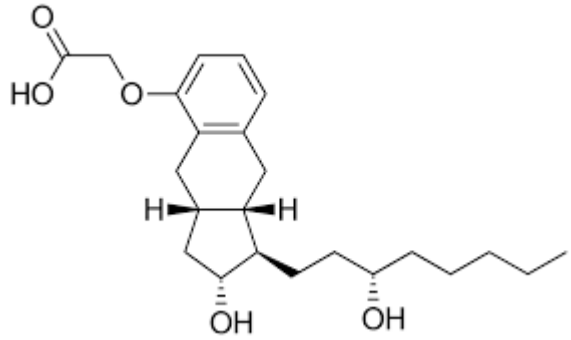

Figure 1 structure of Treprostinil

High Performance Liquid Chromatography (HPLC) plays a crucial role in the validation of Treprostinil, In the review of literature, more economical methods were observed[12-14], hence a simple, cost-effective stability-indicating simultaneous estimation of Treprostinil by RP-HPLC in pharmaceutical dosage form must be developed and validated as per the guidelines of ICH (Q2 specification).

MATERIALS:

Treprostinil pure drug (API), Treprostinil formulation (Orenitram), Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from Rankem.

INSTRUMENTATION

The development and method validation were conducted using a WATERS HPLC, specifically the model 2695 SYSTEM, equipped with a Photo diode array detector. The system also included an automated sample injector and the Empower 2 software.

Table 1: Chromatographic Conditions:

Mobile phase	0.1%TFA: MeOH (70:30 v/v)
Flow rate	1.0 ml/min
Column	Kromasil C18 (4.6 x 150mm, 5µm)
wave length	224 nm
Column temperature	30°C
Injection volume	10µL
Run time	10.0 min
Buffer	0.1%TFA

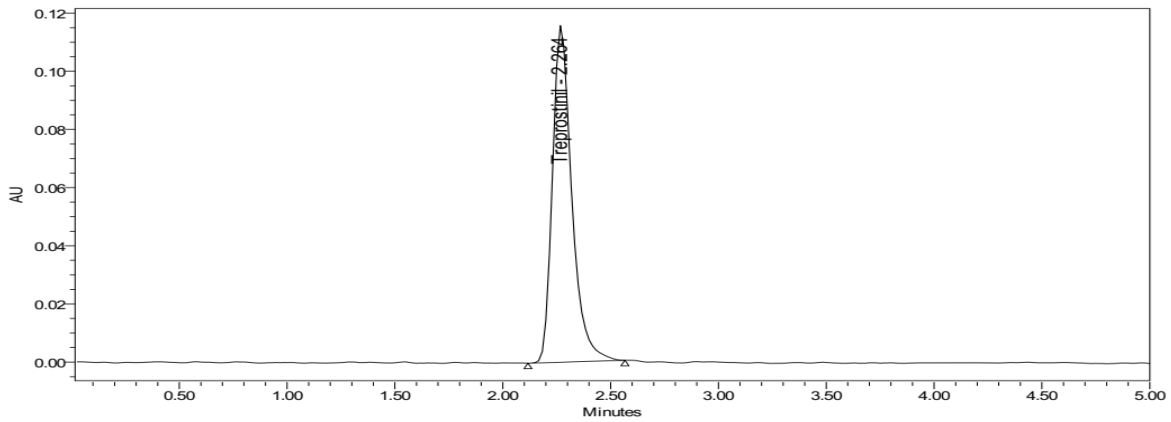

Figure 2: Optimized Chromatogram

Methods:

Preparation of Standard stock solutions: Accurately weighed 5mg of Treprostinil is transferred to 50ml volumetric flask. 3/4 th of diluents was added to the flask and sonicated for 10 minutes. Flask was made up with diluents and labeled as Standard stock solution. (100µg/ml of Treprostinil)

Preparation of Standard working solutions (100% solution): 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (10µg/ml of Treprostinil).

Preparation of Sample stock solutions: Pipette out 1ml of Treprostinil injection sample from autosampller vial into a 100 volumetric flask, 50ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters. (100µg/ml of Treprostinil)

Preparation of Sample working solutions (100% solution): 1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (10µg/ml of Treprostinil)

Validation:

System suitability parameters:

The system suitability parameters were determined by preparing standard solution of Treprostinil (6 ppm) and the solution were injected six times and the parameters like peak tailing, resolution and USP plate count were determined.

The % RSD for the area of six standard injections results should not be more than 2%.

Specificity (Selectivity): Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So, this method was said to be specific. Representative chromatogram is shown in Figure 3 and experimental data is given in Table 2

Table: 2 System suitability parameters for Treprostinil

S no	Treprostinil		
Inj	RT(min)	USP Plate Count	Tailing
1	2.281	2916	1.29
2	2.283	2950	1.30
3	2.284	3016	1.30
4	2.286	2918	1.30
5	2.286	3031	1.31
6	2.288	2959	1.33

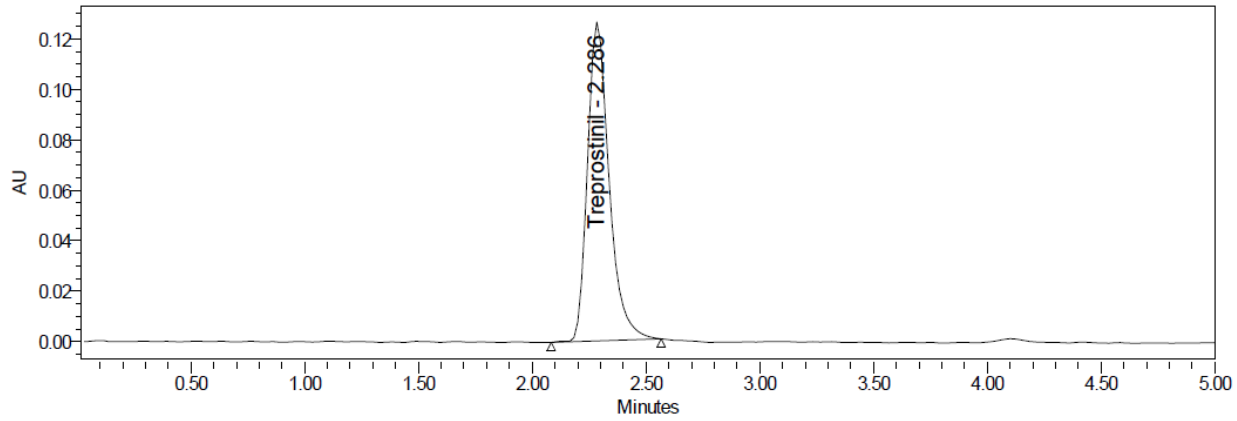

Figure 3: System Suitability Chromatogram of Treprostinil

Table 3: Specificity Data

Peak name	Rt	Area	USP plate count	Tailing
Treprostinil	2.264	773663	3659.4	1.2

Specificity:

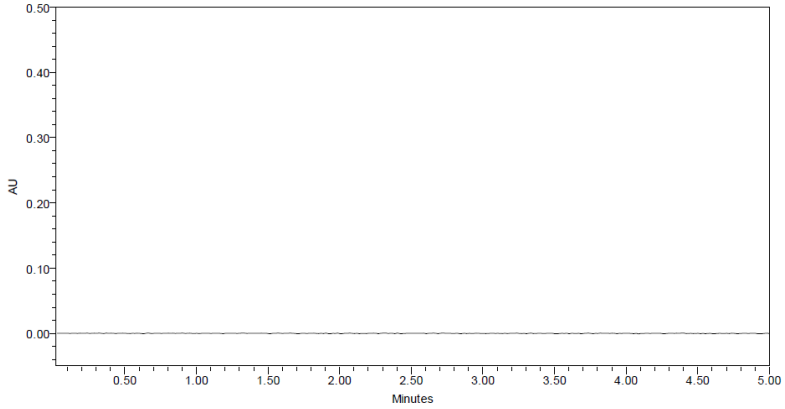

Figure 4 Chromatogram of blank.

The forced degradation conditions are mentioned in Table 4 and the results are mentioned in Table 5

Table 4: Forced degradation conditions for Treprostinil

Stress condition	Solvent	Temp(^{0}C)	Exposed time
Acid	2N HCL	60^{0}c	30 mins
Base	2N NAOH	60^{0}c	30 mins
Oxdation	20% H_2O_2	60^{0}c	30 mins
Thermal	Diluent	105^{0}c	6 hours
Photolytic	Diluent	-	-
Hydrolytic	Water	60^{0}c	

From the results, degradation peaks were observed when the samples were exposed to acid. According to the stress study, none of the degradant co-eluted with the active drug peaks formed.

Table 5: Degradation profile results

Degradation Condition	% Drug Un Degraded	% Drug Degraded
Acid	96.04	3.96
Base	96.50	3.50
Oxidation	96.51	3.49
Thermal	99.32	0.68
Photolytic	99.0	0.95
Hydrolytic	99.8	0.18

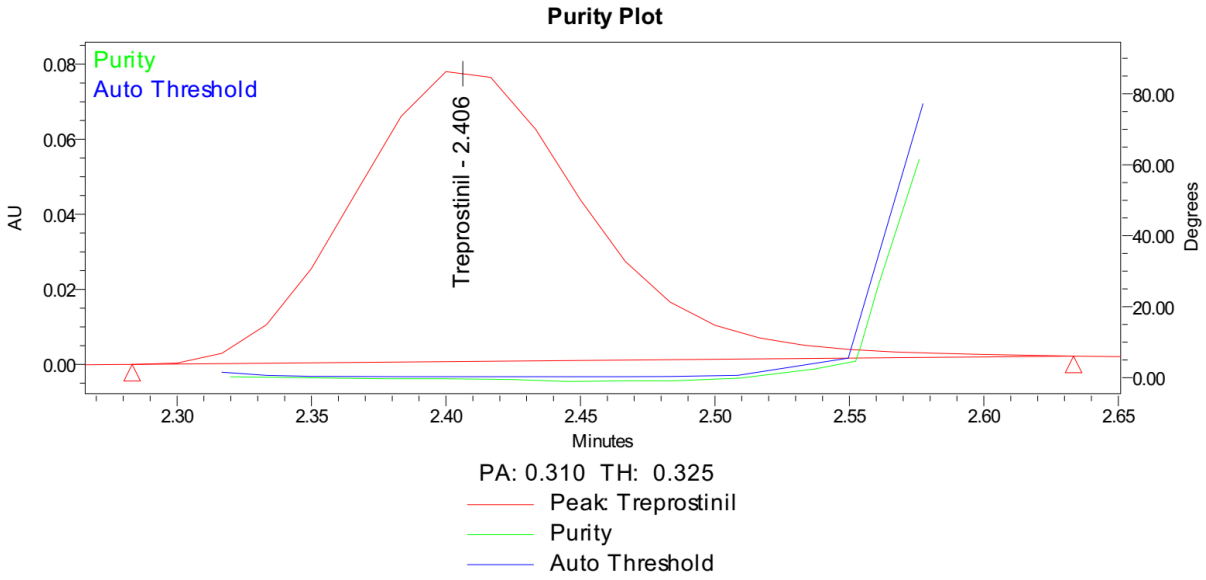

Figure 5: Purity Plot of Acid

Limit of detection (LOD) The detection limit is considered as very low level of concentration of an analyte in a sample that can be detected, but not necessarily quantitated.

Limit of quantitation (LOQ): The limit of quantitation is considered as the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy of the method.

The LOD values obtained for Treprostinil are listed in Table 6.

Table 6: Summary of limit of detection

Sample	Conc (µg/ml)
LOD	0.08
LOQ	0.26

Linearity: The linearity of the method was demonstrated for Treprostinil by analyzing the solutions ranging from 25% to 150% of the specification limit (Table 7). The correlation coefficient for Treprostinil was 0.999. This indicates good linearity

Linearity:

Calibration data is given in table 7 and regression data in table 8 and calibration curve in figure 6

Table 7: Calibration data of Treprostinil

Treprostinil	
Conc (µg/mL)	**Peak area**
0	0
2.5	196694
5	381623
7.5	577758
10	772163
12.5	963708
15	1147590

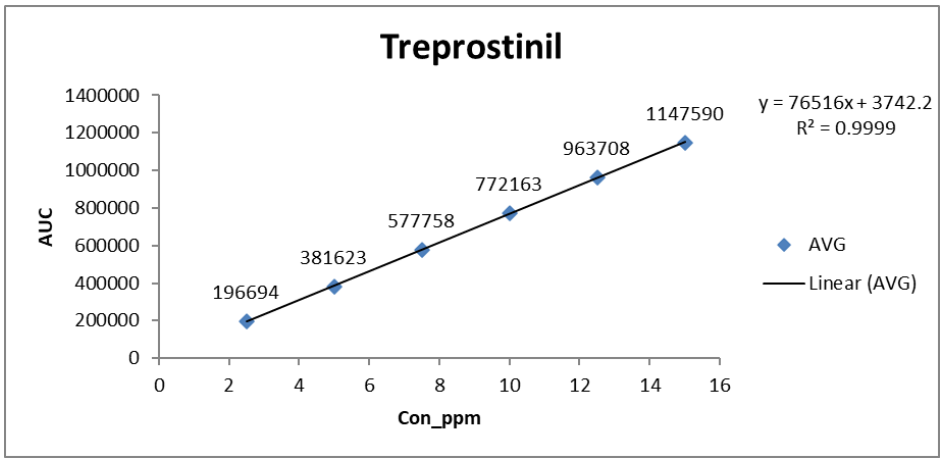

Figure 6: Calibration curve of Treprostinil

Table 8: regression data

Parameter	Treprostinil
Conc range (µg/mL)	2.5-15µg/ml
Regression Equation	y = 76516x + 3742.2
Co-relation	0.999

Accuracy: The accuracy of the method was determined by using solutions containing spiked samples of Treprostinil at 50%, 100% and 150% of the working strength. All the solutions were prepared in triplicate and analysed. The percentage recovery results obtained for each impurity was listed in Table 9

Table 9 Accuracy table of Treprostinil

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% recovery
50%	5	5.030	100.61
	5	4.974	99.47
	5	4.974	99.48
100%	10	10.003	100.03
	10	9.907	99.07
	10	9.917	99.17
150%	15	14.932	99.54
	15	14.982	99.88
	15	14.921	99.47
Mean % recovery			**99.64**

System Precision: The system precision was performed by analyzing six replicate injections of standard solution at 100% of the specified limit with respect to the working strength of Treprostinil. Results of peak area are summarized in Table 10

Table 10 System precision table of Treprostinil

S. No	Area of Treprostinil
1.	776584
2.	772533
3.	767156
4.	773663
5.	766722
6.	771638
Mean	**771383**
S.D	**3827.0**
%RSD	**0.5**

Method Precision: The precision of the method was determined by analyzing a sample of Treprostinil). Data obtained is summarized in Table 11

Table 11 Repeatability table of Treprostinil

S. No	Area of Treprostinil
1.	771978
2.	772432
3.	771578
4.	773028
5.	769310
6.	768888
Mean	**771202**
S.D	**1704.4**
%RSD	**0.2**

Intermediate precision: It is differently from the repeatability, the precision obtained within a single laboratory over a longer period (generally at least several months) and considers more changes than repeatability. Data obtained is summarized in Table 12

Table 12 Intermediate precision table of Treprostinil

S. No	Area of Treprostinil
1.	844648
2.	846929
3.	843642
4.	845783
5.	846012
6.	844302
Mean	**845219**
S.D	**1226.8**
%RSD	**0.1**

Robustness: The chromatographic conditions were deliberately changed to evaluate the robustness of the existing method. To determine the robustness of method, system suitability solution is prepared as per methodology and injected into HPLC at different altered conditions to check the method's ability like flow rate (± 10%), column oven temperature (± 5°C) and Mobile phase (± 10%) from actual method conditions. No significant change is observed by changing flow, temperature, Mobile phase, and system suitability also complied as per methodology. The robustness results are summarized in Table 13.

Table 13 Robustness data for Treprostinil

Condition	%RSD of Treprostinil
Flow rate (-) 0.9ml/min	0.3
Flow rate (+) 1.1ml/min	0.7
Mobile phase (-) 65B:35A	0.4
Mobile phase (+) 75B:25A	0.4
Temperature (-) 27°C	0.1
Temperature (+) 33°C	0.2

Assay data: -

Orenitram Tablet bearing the label claims Treprostinil 300 mg. Assay was performed with the above formulation. Average % Assay for Treprostinil obtained was 99.78%. Assay data shown in table no 14.

Figure 7: Treprostinil Marketed Drug

Formula to calculate assay:

$$\% \text{ Assay} = \frac{AT}{AS} \times \frac{WS}{100} \times \frac{1}{10} \times \frac{10}{1} \times \frac{P}{5} \times \frac{FV}{100} \times \frac{100}{L.C}$$

AT	Average Peak area of Carboprost in test solution
AS	Mean peak area of Carboprost in standard solution
WS	Weight of Carboprost working standard taken in mg
P	Assay of Carboprost working standard in % on dried basis
L.C	Label Claim
FV	Filled volume(1ml of a vail)

Table 14: Assay Data of Treprostinil

S.no	Standard Area	Sample area	% Assay
1	776584	771978	99.9
2	772533	772432	99.9
3	767156	771578	99.8
4	773663	773028	100.0
5	766722	769310	99.5
6	771638	768888	99.5
Avg	**771383**	**771202**	**99.78**
Stdev	**3827.0**	**1704.4**	**0.22**
%RSD	**0.5**	**0.2**	**0.2**

CONCLUSION

The Treprostinil HPLC investigation found that this approach can properly measure the drug's concentration and purity. This technology is ideal for pharmacokinetic studies and standard quality control since it may be used repeatedly with exact peak resolutions and steady retention times. HPLC analysis is used to determine Treprostinil's efficacy and safety in medicinal applications, as well as to verify its chemical makeup.

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