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Research Article

A NEW STABILITY INDICATING METHOD DEVELOPMENT AND VALIDATION FOR TREPROSTINIL IN PHARMACEUTICAL DOSAGE FORM AND BULK BY USING CHROMATOGRAPHIC AND SPECTROSCOPY TECHNIQUE.

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ABSTRACT:
Simple, novel and selective reverse phase-high performance liquid chromatography (RP-HPLC) and ultraviolet (UV) spectroscopic methods have been developed and optimized for the determination of treprostinil in bulk and dosage form. In the Uv-Visible Spectroscopy The mobile phase consisted of Methanol in the ratio of 100v/v and the wavelength to maximum absorbance at 288.0 nm. Chromatogram was run through HPLC Column ZORBAX Eclipse, XDB-C18, 80Å, 5 µm, 4.6 x 150 mm 0.1% OPA and Methanol taken in the ratio 60:40 was pumped through column at a flow rate of 1.2ml/min. Temperature was maintained at 25°C. Optimized wavelength selected was 288.0nm. Retention time of treprostinil was found to be 2.232min. %RSD of the treprostinil were and found to be 0.1%. %RSD of Method precision of treprostinil was found to be 0.5%. %Recovery was obtained as 99.79% for treprostinil. LOD, LOQ values obtained from regression equation of treprostinil were 0.12, 0.38, Regression equation of treprostinil is y = 13118x + 975.5, Analytical calibration curves were linear within a concentration range from 2.5 to 15 µg/ml and coefficient of correlation 0.999. %RSD was found to be less than 2 respectively, in UV spectroscopic method, The developed methods were found to be simple, accurate, reproducible, and precise. The treprostinil can be analyzed in dual techniques, i.e., chromatographic and UV spectroscopic methods for its routine analysis.

Key Words: Treprostinil, RP-HPLC, Uv-Visible Spectroscopy, Forced Degradation.

INTRODUCTION

Treprostinil—a stable, long-acting prostacyclin analogue—can be continuously infused subcutaneously.[1] In patients with pulmonary arterial hypertension, a rare condition with a dismal prognosis, subcutaneous Treprostinil has been demonstrated in a large multicentre randomized controlled study to improve exercise capacity, clinical state, functional class, pulmonary hemodynamic, and quality of life.[2,3] Face flush, headache, jaw discomfort, cramping in the abdomen, diarrhoea, and other side effects are common with prostacyclin and can be controlled with symptom-directed dose modifications.[4] In 7% to 10% of patients, infusion site pain may prevent further treatment. Patients with pulmonary arterial hypertension who get subcutaneous Treprostinil had comparable long-term survival rates to those who receive intravenous epoprostenol.[5]

Treprostinil chemically Fig-1 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, Treprostinil, a stable tricyclic counterpart of prostacyclin, inhibits platelet aggregation and encourages the dilatation of the pulmonary and systemic artery vascular beds.[6] Treprostinil was licensed by the FDA in 2002 for the treatment of pulmonary arterial hypertension (PAH) and pulmonary hypertension linked to interstitial lung disease. It helps patients with these conditions by reducing their symptoms.[7]

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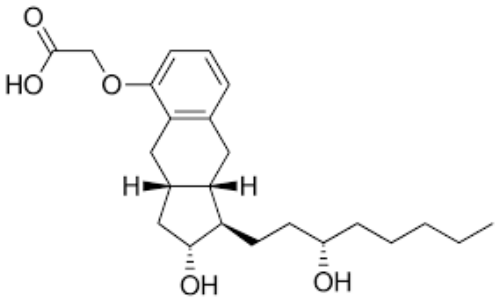


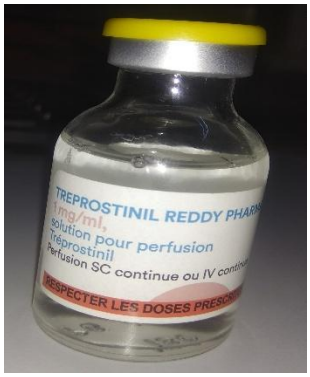
Fig 1: Chemical Structure of Treprostinil

An exhaustive literature study revealed that Treprostinil has been determined separately by UV spectroscopy and RP-HPLC [8,9,10]. However, no single study has reported that Treprostinil was found to be stable, indicating that a study of the selected superior method for separating the active analyte present in the pharmaceutical dosage is being conducted, making the current study unique and novel. As a result, we have worked hard to develop precise, accurate, sensitive, and cost-effective procedures and compare them based on analytical outcomes such as sensitivity, percentage recovery, and drug assay. The approach was validated in accordance with ICH Q2 (R1) requirements.[11]

MATERIALS AND METHODS

Chemicals and reagents

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



Instrumentation: The instrument used in the study were electronic balance (Make: Mettler Toledo, Model: XP56), sonicator (Make: Elma, Model: S300H), hot air oven (Make: Serve well Instruments, Model: H02436), digital meter (Make: Mettler Toledo) and UV-Visible chamber (Make: Mack Equipment, Model: MK-2). HPLC (Waters 2695 with PDA detector 2996) was monitored and integrated using Empower 3 software, A double-beam UV-1800 Shimadzu UV spectrophotometer along with a pair of matched quartz cells 10 mm is used.

Sample preparation for UV and RP-HPLC method development

Diluent: Based up on the solubility of the drugs, diluent was selected, Methanol and Water taken in the ratio of 50:50

Buffer preparation:

1ml of ortho phosphoric acid was diluted to 1000ml with HPLC grade water.

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 autosampler 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).

Determination of Treprostinil maximum absorbance (λmax)

A typical Treprostinil working solution in the range of 400-200 nm is scanned. The absorption maxima were found to be 288 nm, which was chosen as the analytical wavelength for future investigation. The spectrum was recorded as indicated in Figure 2.

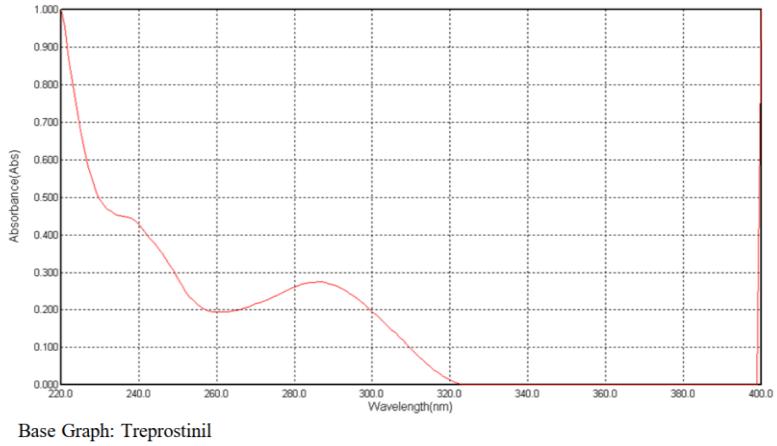


Fig 2. Spectra of Treprostinil

HPLC method development

To achieve optimized chromatographic conditions, the below parameters were modified in each trial. The trial runs are shown in Table 1.

Table: 1 Trial run for optimization of Treprostinil

Trails	**Mobile phase, Stationary phase**	**Flow rate (mL/min)**	**Retention time (min)**	**Comment**
Trail-1	Symmetry C18 (4.6 x 150mm, 5µm) Methanol: Water (50:50 v/v)	1.0 ml/min	8.417 min	Broad peak shape and more retention time
Trail-2	Symmetry C18 (4.6 x 150mm, 5µm) 0.1% Formic acid: Methanol (60:40 v/v)	1.0 ml/min	7.890 min	peak was eluted but peak long retention time
Trail-3	Discovery C18 (4.6 x 250mm, 5µm). 0.1% OPA : Methanol(60:40v/v)	1.0 ml/min	2.906 min	but plate count less and baseline noise was observed
Trail-4	HPLC Column ZORBAX Eclipse, XDB-C18, 5 µm, 4.6 x 150 mm 0.1% OPA: Methanol(60:40)	1.2 ml/min	2.232 min	all the suitability parameters were passed

The optimal chromatographic setting was chosen from the experiments based on the tailing factor falling within a predetermined range and the peak shape being crisp as assessed by theoretical plates, and retention time is 2.232 min which is much less. Therefore, separation of Treprostinil was performed on a HPLC Column ZORBAX Eclipse, XDB-C18, 5 µm, 4.6 x 150 mm. consisting of 0.1% OPA: Methanol (60:40) as a mobile phase; by using a membrane filter it was filtered and degassed. The flow rate was retained at 1.2 mL/min. The injection volume was kept at 10 µl at a column oven temperature of 30 °C, and analyte eluted at detected at 288 nm. The mode of separation was isocratic. The chromatogram of Treprostinil is shown in Fig no 3 respectively.

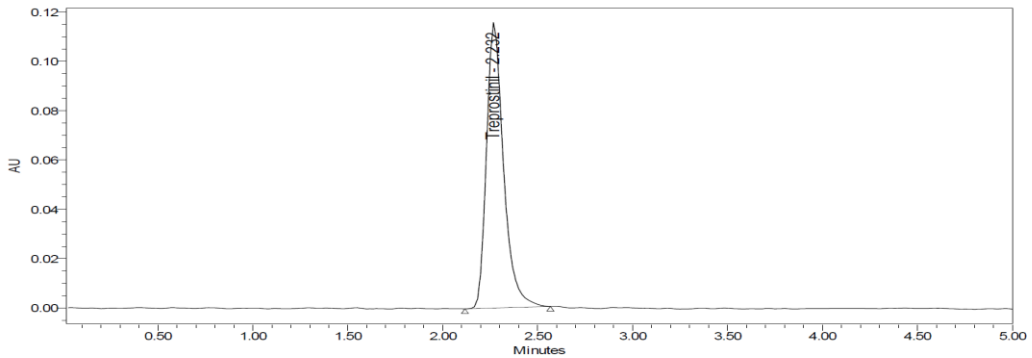


Fig 3 Optimized Chromatogram

Method Validation: The method was validated in accordance with ICH recommendations Q2R1. System appropriateness, specificity, linearity, accuracy, precision, LOD& LOQ, and robustness are among the validation parameters.

RESULTS AND DISCUSSION

System suitability parameters: The system suitability parameters were assessed by making standard solutions of Treprostinil (10µg/ml) and injecting them six times. Peak tailing, resolution, and USP plate count were all determined. For three medications in combination, the USP Plate count exceeded 2000 and the tailing factor was less than 2. All the system's appropriate parameters were passed and remained within the limitations. Table 2 shows the results.

Table: 2 System suitability parameters for Treprostinil

S no	Treprostinil		
Inj	RT (min)	USP Plate Count	Tailing
1	2.276	3033	1.29
2	2.278	3037	1.29
3	2.279	3026	1.30
4	2.280	3026	1.31
5	2.281	3016	1.31
6	2.281	3037	1.3

Specificity: In the optimised method, the interference is checked. Treprostinil, had retention time of 2.280 minutes. We did not find any interfering peaks in the chromatograms of blank and placebo samples during the retention periods of the drug in our approach. As a result, this procedure was stated to be. Figures 4 show the chromatograms for specificity.

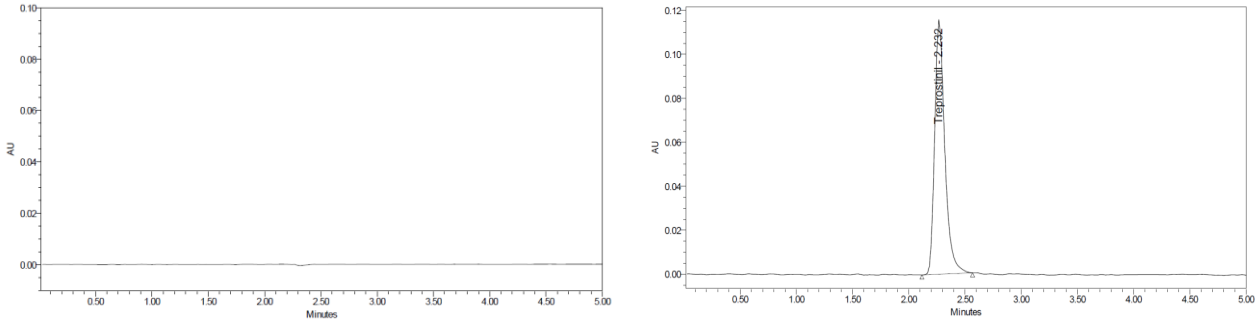


Figure No.4 Specificity Chromatograms

Linearity: Six linear concentrations of Treprostinil (2.5-15µg/ml) was injected in duplicate manner. Correlation coefficients obtained was 0.999 for all the three drugs. The results were shown in table 3 and fig 5.

Table 3 Linearity table for Treprostinil

Treprostinil	
Conc (µg/mL)	Peak area
0	0
2.5	33754
5	67805
7.5	99400
10	132101
12.5	165890
15	196556

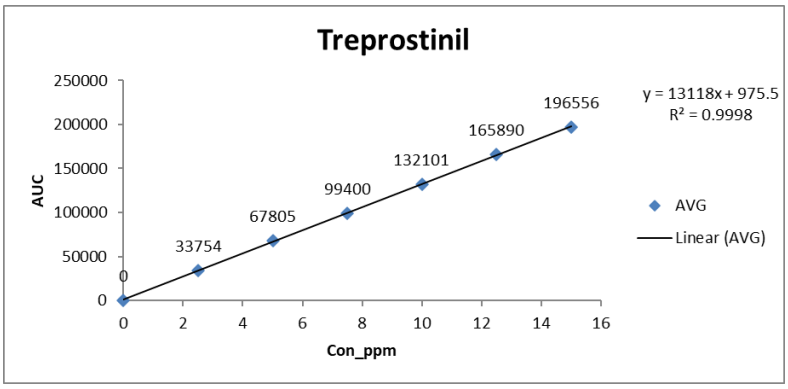


Fig No.5 Calibration curve of Treprostinil

Precision:

Repeatability: Multiple samples were taken from a sample stock solution, and six working sample solutions of the same concentrations (10µg/Treprostinil) were created. Each injection was given from each working sample solution, and the results are shown in table 3. The average area, standard deviation, and % RSD for the medication were computed and found to be 0.5% for Treprostinil. The system precision was passed for this procedure since the precision limit was less than "2 %." Table 4 shows the information results.

Table 4.Repetability table of Treprostinil

S. No	Area of Treprostinil
1.	131738
2.	131636
3.	131454
4.	132744
5.	132743
6.	132847
Mean	**132194**
S.D	**647.6**
%RSD	**0.5**

Intermediate Precision: Multiple samples were taken from a sample stock solution, and six working sample solutions of the same concentrations (15µg/ml of Treprostinil) was prepared. Each injection from each working sample solution was given on the following day of the sample preparation, and the obtained areas are listed in table 5. The average area, standard deviation, and % RSD for the medication was computed and found to be 0.6% for Treprostinil. Because the precision limit was less than "2%" the intermediate precision was used for this procedure. Table 5 shows the information results.

Table 5 Intra-Day precision table of Treprostinil

S. No	Area of Treprostinil
1.	131456
2.	131654
3.	130744
4.	130565
5.	129456
6.	130765
Mean	**130773**
S.D	**777.3**
%RSD	**0.6**

Accuracy: The conventional addition procedure was used to create three levels of accuracy samples. Triplicate injections were administered at each degree of accuracy, and the mean % recovery for Treprostinil was found to be 99.79 %. Tables 6 show the outcomes. Because satisfactory recover values were achieved, the accuracy for this approach was passed.

Table 6 Accuracy data of Treprostinil

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	5	4.978	99.56	99.79%
	5	4.985	99.70	
	5	4.994	99.88	
100%	10	9.924	99.24	
	10	9.933	99.33	
	10	9.933	99.33	
150%	15	15.283	101.89	
	15	14.986	99.91	
	15	14.886	99.24	

Robustness: Robustness conditions such as flow minus (1.0ml/min), flow plus (1.3ml/min), mobile phase minus (55B:45A v/v), mobile phase plus (65B:35A v/v), temperature minus (20°C), and temperature plus (30°C) were maintained, and samples (50µg/ml Treprostinil) was injected in triplicate. The % RSD was computed and determined to be within the acceptable range. Table 7 shows the data.

Table 7 Robustness data of Treprostinil

S.no	Condition	%RSD of Treprostinil
1	Flow rate (-) 1.0ml/min	0.8
2	Flow rate (+) 1.3ml/min	0.4
3	Mobile phase (-) 55B:45A	0.9
4	Mobile phase (+) 65B:35A	0.7
5	Temperature (-) 20°C	0.4
6	Temperature (+) 30°C	0.6

Assay: Kilfat bearing the label claim Treprostinil 200mg. Assay was performed with the above formulation. Average % Assay for Treprostinil obtained was 98.42%. The chromatogram is depicted in Fig. 6.

$$\%\ \text{Assay} = \frac{AT}{AS} \times \frac{WS}{50} \times \frac{1}{10} \times \frac{100}{1} \times \frac{10}{1} \times \frac{p}{100} \times \frac{AvWt}{L.C} \times 100$$

AT	Average Peak area of in test solution
AS	Mean peak area of in standard solution
WS	Weight of working standard taken in mg
P	Potency of working standard in % on dried basis
L.C	Label Claim
Avwt	Average weight of drug

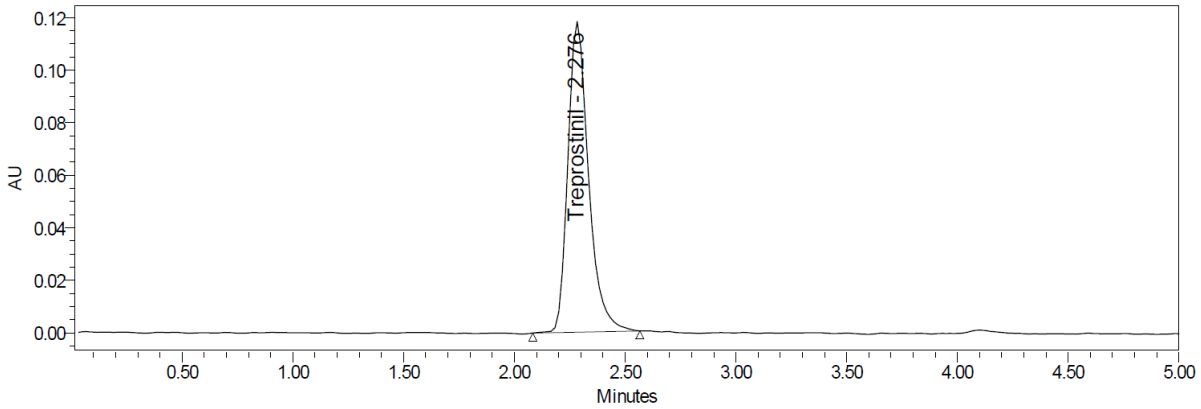


Fig No.6: Sample Chromatogram

Degradation: To conduct the forced degradation experiment, standard stock solutions of Treprostinil was exposed to various stress conditions, including 1 mL of 20% H2O2 (for oxidative degradation), 1 mL of 2N HCL (for acidic degradation), and 1 mL of 2N NAOH (for acidic degradation) (for basic degradation). The produced solutions were refluxed for 30 minutes at 60oC. To examine the descent, the standard solutions were also subjected to UV radiation and temperature conditions.13,14,15 The resulting solutions were diluted to yield 15µg/ml of Treprostinil for degradation studies. To examine sample stability, 10µl samples were fed into the system and chromatograms were obtained, there is no interaction of the degradation peak with that of the Treprostinil peak. Hence, the proposed HPLC method was stability indicating and specific. The peak purity index values of Treprostinil peak and degradation peaks were found to be within acceptable limits, Table 8 and fig no 7, 8.

Table No.8: Degradation Data

S.No.	Condition	Treprostinil %Degraded	Treprostinil %Undegraded	Purity angle	Purity Threshold
1	**Acid**	4.61	95.39	0.310	0.325
2	**Base**	3.25	96.75	0.394	0.450
3	**Oxidation**	3.31	96.69	0.293	0.331
4	**Dry heat**	1.14	98.86	0.253	0.303
5	**UV Light**	0.98	99.02	0.278	0.304
6	**Hydrolytic**	1.15	98.85	0.278	0.304

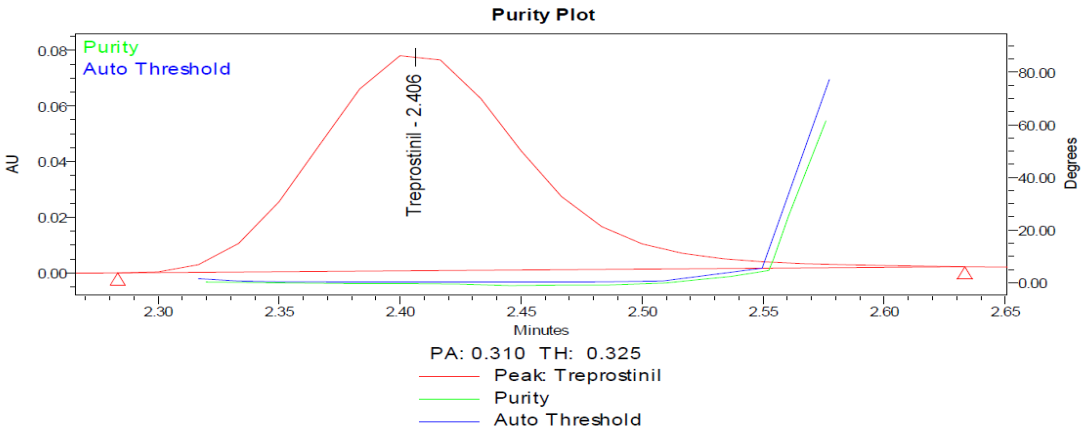


Fig No 7: Acid Purity plot

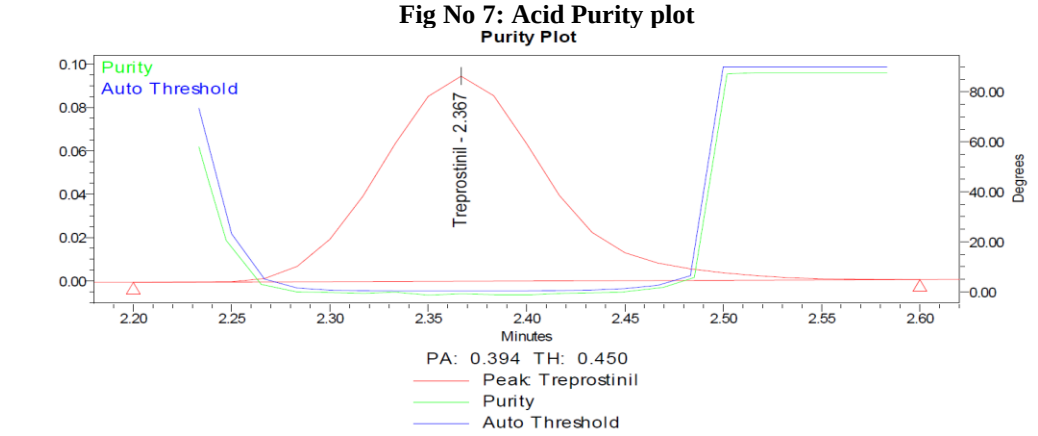


Fig No 8: Base Purity plot

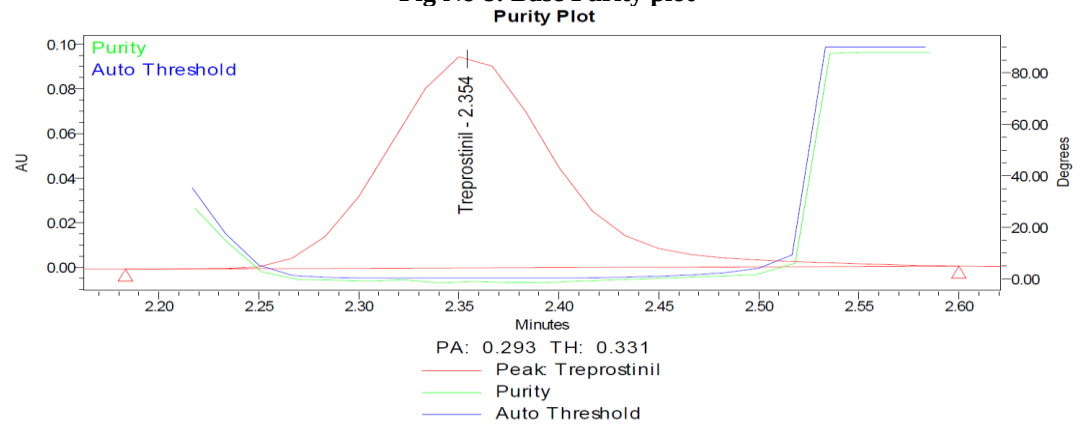


Fig No 9: Peroxide Purity plot

Conclusion:

A few RP-HPLC techniques have been created to measure Treprostinil. These techniques were used to determine Treprostinil using UV and rphplc, and to perform the Stability method using forced degradation experiments. The procedures that were projected differ from those that were published. The entire run time for the RP-HPLC technique was five minutes. It was discovered that the linearity range for the UV spectroscopic method was between 2.5 µg mL-1 and 15 µg mL-1 µg mL-1, and for the RP-HPLC method, it was between 2.5 µg mL-1 and 15 µg mL-1, For both methods, the regression coefficients (R2) were determined to be equivalent at 0.999. It was discovered that the developed methods were straightforward, precise, accurate, and reproducible. The RP-HPLC approach was comparatively more sensitive than the UV spectroscopic method, as demonstrated by the collected data from both methods.

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REFERENCES:

1. Skoro-Sajer N, Lang I, Naeije R. Treprostinil for pulmonary hypertension. Vascular Health and Risk Management. 2008 Jun 30;4(3):507-13.
2. Waxman A, Restrepo-Jaramillo R, Thenappan T, Ravichandran A, Engel P, Bajwa A, Allen R, Feldman J, Argula R, Smith P, Rollins K. Inhaled treprostinil in pulmonary hypertension due to interstitial lung disease. New England Journal of Medicine. 2021 Jan 28;384(4):325-34.
3. Oudiz RJ, Schilz RJ, Barst RJ, Galié N, Rich S, Rubin LJ, Simonneau G, Treprostinil Study Group. Treprostinil, a prostacyclin analogue, in pulmonary arterial hypertension associated with connective tissue disease. Chest. 2004 Aug 1;126(2):420-7.
4. Gomberg-Maitland M, Tapson VF, Benza RL, McLaughlin VV, Krichman A, Widlitz AC, Barst RJ. Transition from intravenous epoprostenol to intravenous treprostinil in pulmonary hypertension. American journal of respiratory and critical care medicine. 2005 Dec 15;172(12):1586-9.
5. Channick RN, Voswinckel R, Rubin LJ. Inhaled treprostinil: a therapeutic review. Drug design, development and therapy. 2012 Jan 24:19-28.
6. Feldman J, Habib N, Fann J, Radosevich JJ. Treprostinil in the treatment of pulmonary arterial hypertension. Future Cardiology. 2020 Nov 1;16(6):547-58.
7. Horn EM, Barst RJ. Treprostinil therapy for pulmonary artery hypertension. Expert Opinion on Investigational Drugs. 2002 Nov 1;11(11):1615-22.
8. Alluri NR, Bandlamudi MR, Kuppusamy S, Morais SR. Implementation of the QbD Approach to the Analytical Method Development and Validation for the Estimation of the Treprostinil Injection Dosage Form by RP-HPLC. ACS omega. 2025 Apr 28;10(17):17827-35.
9. Gallucci GM, Agbabiaka MO, Ding M, Gohh R, Ghonem NS. Quantification of treprostinil concentration in rat and human using a novel validated and rapid liquid chromatography-tandem mass spectrometry method: Experimental and clinical applications in ischemia–reperfusion injury. Clinica Chimica Acta. 2024 Jul 15;561:119837.
10. Plaunt AJ, Islam S, Macaluso T, Gauani H, Baker T, Chun D, Viramontes V, Chang C, Corboz MR, Chapman RW, Li Z. Development and characterization of treprostinil palmitil inhalation aerosol for the investigational treatment of pulmonary arterial hypertension. International Journal of Molecular Sciences. 2021 Jan 7;22(2):548.
11. Borman P, Elder D. Q2 (R1) validation of analytical procedures: text and methodology. ICH quality guidelines: an implementation guide. 2017 Sep 27:127-66.
12. Singh S, Junwal M, Modhe G, Tiwari H, Kurmi M, Parashar N, Sidduri P. Forced degradation studies to assess the stability of drugs and products. TrAC Trends in Analytical Chemistry. 2013 Sep 1;49:71-88.
13. Ngwa G. Forced degradation as an integral part of HPLC stability-indicating method development. Drug delivery technology. 2010 Jun;10(5):56-9.
14. Iram F, Iram H, Iqbal AZ, Husain A. Forced degradation studies. Journal of Analytical & Pharmaceutical Research. 2016;3(6):73.