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A NEW ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR TREPROSTINIL BY USING RP-HPLC IN BULK FORM

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ABSTRACT
The method developed and validation was carried out by using isocratic mode with simple mobile phase preparation and separation was achieved on Phenomenex Luna C18 column (250 X 4.6mm, 5µm) using the mobile phase consisting of Methanol: 0.1% Ortho phosphoric acid in the ratio of (20:80 V/V). The mobile phase was pumped at a flow rate of 1.0mL/min and detection was done by UV detector at 223nm. The retention time was 3.06 min for Treprostinil. The linearity ranges for Treprostinil was 10-60µg/ml with correlation coefficient 0.9995. The LOD and LOQ values of Treprostinil were 0.691µg/ml and 2.093µg/ml obtained. This method can be applicable for routine analysis which shows good reproducible, precise and accurate of Treprostinil in pharmaceutical dosage forms.

KEYWORDS
Treprostinil, Methanol, O-phosphoric acid and RP-HPLC.

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INTRODUCTON
Treprostinil 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. This medication belongs to a class of drugs known as vasodilators and the Mechanism of action is direct vasodilation of pulmonary and systemic arterial vascular beds and inhibition of platelet aggregation. Its chemical structure has shown in Figure No.1.

DRUG PROFILE

Drug : Treprostinil
Drug category : pulmonary arterial hypertension
IUPAC Name : 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.
Molecular Formula : $C_{23} H_{34} O_5$
Molecular Weight : 390.5g/mol

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[5-11].

MATERIAL AND METHODS

Instrument

Shimadzu UFLC equipped with pump model LC 20 AD with PDA detector and C18 Phenomenex Luna (250X4.6mm; 5μ). Vibra Digital balance was used for weighing purpose. Sonication of the solutions was carried out using an Ultrasonic Cleaning Bath (Spinco tech, India).

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. Analytical grade ortho phosphoric acid 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.

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: 0.1% OPA in the ratio of (20:80 v/v) and the flow rate was 1ml/min it gave all system suitability parameters Satisfactory Results so this mobile phase was chosen for analysis of Treprostinil.

Preparation of standard stock solution

Weighed accurately 10mg of pure drug and transferred into 10mlvolumetric flask and add 10ml of mobile phase to obtain the concentration is 1000μg/ml. this stock solution sonicates in ultra-sonic 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 further method development.

METHODOLOGY

Chromatographic conditions are mobile phase methanol: 0.1% ortho phosphoric acid (20:80 V/V) and mobile phase pH was 4.0 it has been maintained through the analysis with 1.0ml/min flow rate and the separation was achieved on column was C18 Phenomenex Luna (250X4.6mm; 5μ) and temperature was ambient, with injection volume 20µl, and detected at 223nm 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.

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 No.1 and chromatogram showed in Figure No.3.

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 No.2.

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 Figure No.4 and the results were shown in Table No.3.

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 No.4.

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 No.5.

Detection of limits (LOD and 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 No.6.

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 No.7.

Table No.1: Results for Specificity

S.No	Peak Name	Observation (Peak Area)
1	Blank	Nil
2	Standard	287619

Table No.2: Results for System Suitability

S.No	Characteristic	Treprostinil
1	Capacity factor	2.13
2	Tailing factor	1.49
3	Theoretical plates	5193

Table No.3: Results for linearity

S.No	Concentration (µg/ml)	Peak Area
1	10	63012
2	20	137880
3	30	212749
4	40	287619
5	50	362490
6	60	447362

Table No.4: Results for Accuracy

S.No	Spiked Concentration (µg/ml)	Peak area	Amount added (µg/ml)	Amount Found (µg/ml)	Recovery	% Mean Recovery
1	20	145858	20.338	20.2849	99.74	99.34
		144888		20.1500	99.08	
		145092		20.1784	99.22	
2	40	294525	40.676	40.9604	100.70	100.03
		290212		40.3606	99.22	
		292981		40.7457	100.17	
3	60	442876	61.014	61.5920	100.95	100.78
		443127		61.6269	101.00	
		440399		61.2476	100.38	

Table No.5: Results for precision

S.No	Treprostinil	
	Intraday precision Area	Interday precision Area
1	289992	291987
2	291129	290324
3	292737	289589
4	289758	290985
5	288358	288896
6	291949	293984
Mean	290653.8333	290960.8
StdDev	1597.136	1829.981
%RSD	0.55	0.63

Table No.6: Results for LOD and LOQ

S.No	Method	Range (µg/ml)	Linear regression	R^2	LOD (µg/ml)	LOQ (µg/ml)
1	RP-HPLC	10-60	y = 7629.x – 15193	0.9995	0.691	2.093

Table No.7: Results for Robustness

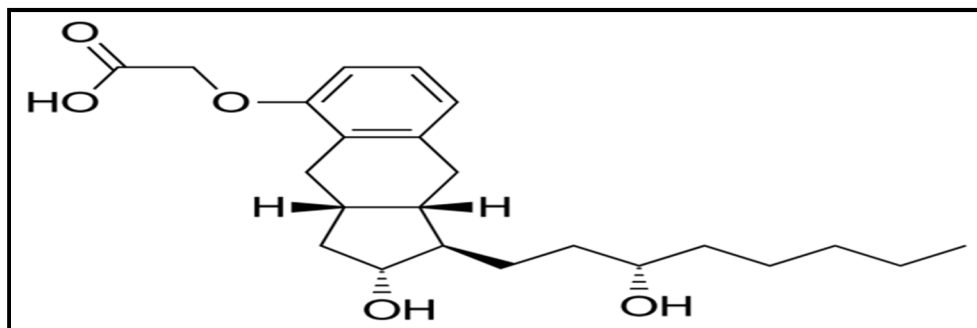| S.No	Robustness condition	% RSD	Tailing factor	Theoretical plates
1	Temp (30°c)	1.50	1.35	4897
2	Temp (35°c)	0.60	1.13	5210
3	Temp (40°c)	1.13	1.77	3563
4	Flow(0.9ml)	1.56	1.45	4117
5	Flow(1ml)	0.90	1.29	4980
6	Flow(1.1ml)	1.46	1.56	4872

Figure No.1: Chemical structure of Treprostinil
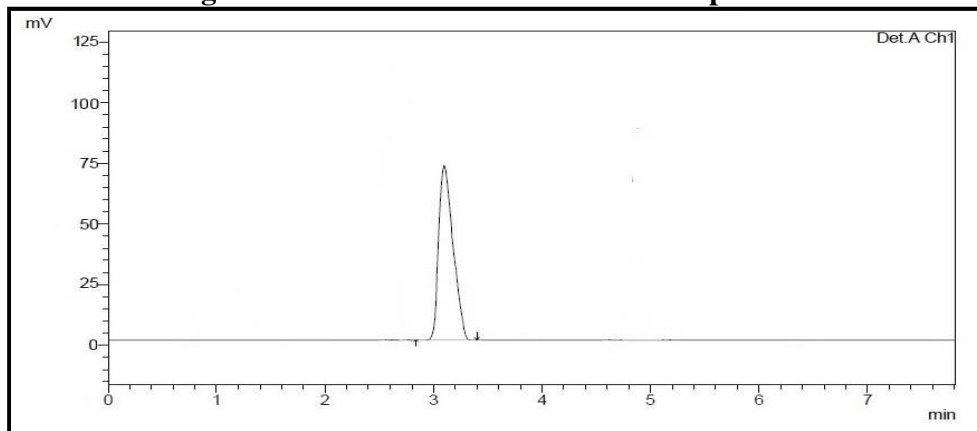

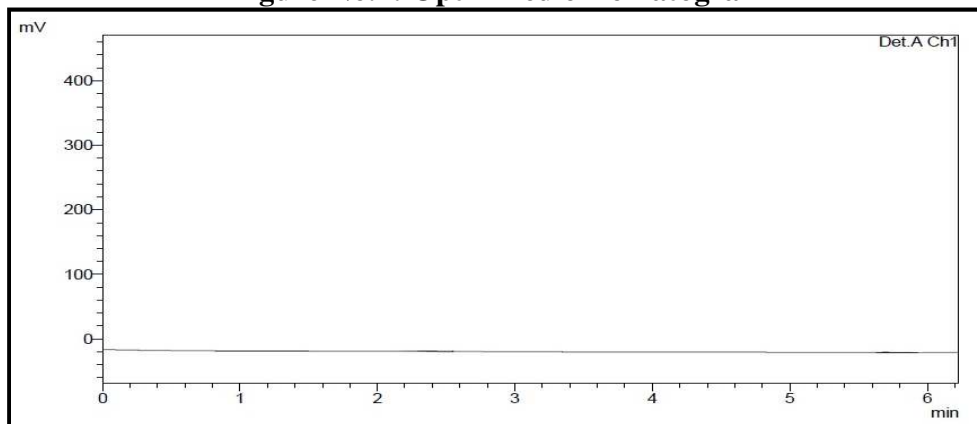**Figure No.2: Optimized chromatogram**
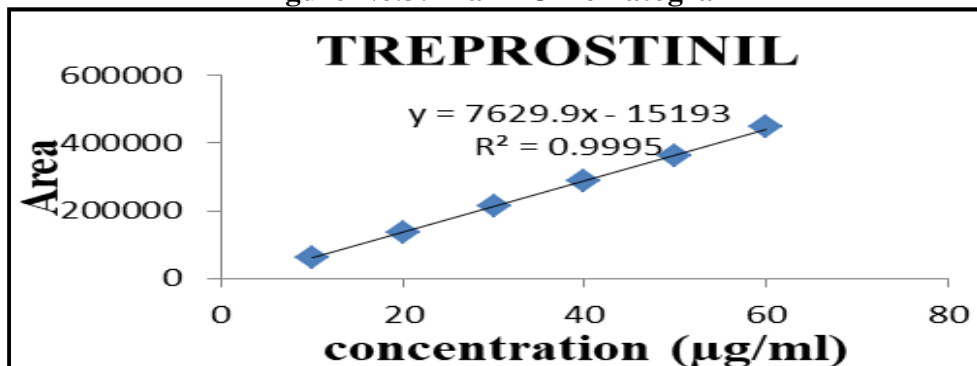

Figure No.3: Blank Chromatogram

Figure No.4: Calibration curve of Treprostinil

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 can be applied for the routine quality control analysis of analyte in dosage form.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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