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Research Article

Stability Indicating HPLC Method Development and Validation for The Estimation of Epoprostenol Sodium in Pharmaceutical Dosage Form

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ABSTRACT

A robust and precise HPLC (Reverse Phase High-Performance Liquid Chromatography) method was developed and validated for the estimation of Epoprostenol Sodium in Injectable dosage forms. The chromatographic separation was achieved on a C18 column using a mobile phase comprising Acetonitrile: Ammonium Acetate Buffer (60:40v/v) in an optimized ratio under isocratic conditions. The flow rate was set to 1.0 mL/min with detection at 217 nm. The method demonstrated excellent linearity for both Epoprostenol Sodium over their respective concentration ranges with correlation coefficients exceeding 0.98. The precision, accuracy, and recovery results were within acceptable limits as per ICH guidelines. The method was also evaluated for specificity, robustness, and system suitability, confirming its reliability for routine quality control analysis of Epoprostenol Sodium in combined dosage forms.

INTRODUCTION

The major actions of epoprostenol are vasodilatation of the pulmonary and systemic vascular beds (widening of narrowed blood vessels in the lung and other parts of the body), and inhibition of platelet clumping (aggregation). Improved survival and exercise capacity has been demonstrated in a 3-month study of intravenous epoprostenol given to patients with idiopathic

pulmonary arterial hypertension. An additional trial with intravenous epoprostenol included administration to patients with PAH associated with the scleroderma spectrum of connective tissue disease. This resulted in reduced symptoms and improved exercise capacity in patients.

Drug Name: Epoprostenol Sodium

Class: Prostaglandins

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Molecular Formula: C₂₀H₃₂O₅

Dosage Form: Injection (e.g., 1.5 mg/vial)

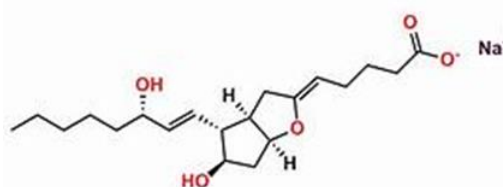
Mechanism of Action: Prostaglandins are present in most body tissues and fluids and mediate many biological functions. Epoprostenol (PGI₂) is a member of the family of prostaglandins that is derived from arachidonic acid. The major pharmacological actions of epoprostenol is ultimately inhibition of platelet aggregation. Prostacycline (PGI₂) from endothelial cells activate G protein-coupled receptors on platelets and endothelial cells. This activation causes adenylate cyclase to produce cyclic AMP which inhibits further platelet activation and activates protein kinase A. Cyclic AMP also prevents coagulation by preventing an increase in intracellular calcium from thromboxane A₂ binding. PKA then continues the cascade by phosphorylating and inhibiting myosin light-chain kinase which leads to smooth muscle relaxation and vasodilation. Notably, PGI₂ and TXA₂ work as physiological antagonists.

Bioavailability: ~74%

Half-life: ~ 6 Minutes

Excretion: Through the kidney.

Structure of Epoprostenol Sodium



MATERIAL AND METHOD

Chemicals And Reagents

Reagent	Purpose	Source
Epoprostenol Sodium (API)	Active Pharmaceutical Ingredient for analysis	Certified Supplier
Orthophosphoric Acid (1% v/v)	Non-toxic solvent for mobile phase preparation	Merck
Acetonitrile (HPLC Grade)	Organic solvent for mobile phase	Sigma-Aldrich
Methanol	Organic solvent for mobile phase	Sigma-Aldrich
Ammonium acetate Buffer	Organic solvent for mobile phase	Sigma-Aldrich
Water (HPLC Grade)	Mobile phase component	Milli-Q System
C18 Column (150 × 4.6 mm, 5 μm)	Stationary phase for chromatographic separation	Phenomenex
HPLC System with UV Detector	Quantitative analysis of APIs	Agilent Technologies
pH Meter	Measurement and adjustment of pH	Thermo Fisher Scientific
Analytical Balance	Accurate weighing of reagents and samples	Sartorius
Ultrasonicator	Dissolution of sample in diluent	Labman Instruments

Glassware (Volumetric Flasks, Pipettes)	Preparation of mobile phase and standard solutions	Borosil
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Instruments

Instrument	Model	Purpose
High-Performance Liquid Chromatograph (HPLC) with UV detector	Quantitative analysis of APIs	Agilent Technologies
C18 Column (250 × 4.6 mm, 5 μm)	Stationary phase for chromatographic separation	Phenomenex
pH Meter	Measurement and adjustment of pH	Thermo Fisher Scientific
Analytical Balance	Accurate weighing of reagents and samples	Sartorius
Ultrasonicator	Dissolution of sample in diluent	Labman Instruments
Glassware (Volumetric Flasks, Pipettes)	Preparation of mobile phase and standard solutions	Borosil

Identification of Drugs

The melting point of Epoprostenol Sodium were determined using the open capillary method, a standard technique for this purpose [30]. A small sample of Epoprostenol Sodium are placed in an

open capillary tube and heated gradually until it melts. The temperature at which the drug starts to melt is recorded as its melting point.

Melting Point of Drugs

Sr. No.	APIs	Melting Point	
		Reported	Measured
1	Epoprostenol Sodium	182.2°C	182-183°C

Identification by FTIR

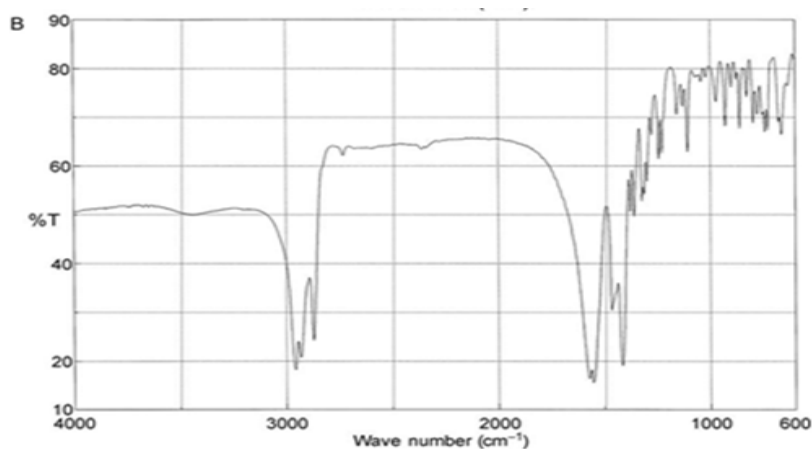


Fig 1: IR Spectra of Standard Epoprostenol Sodium

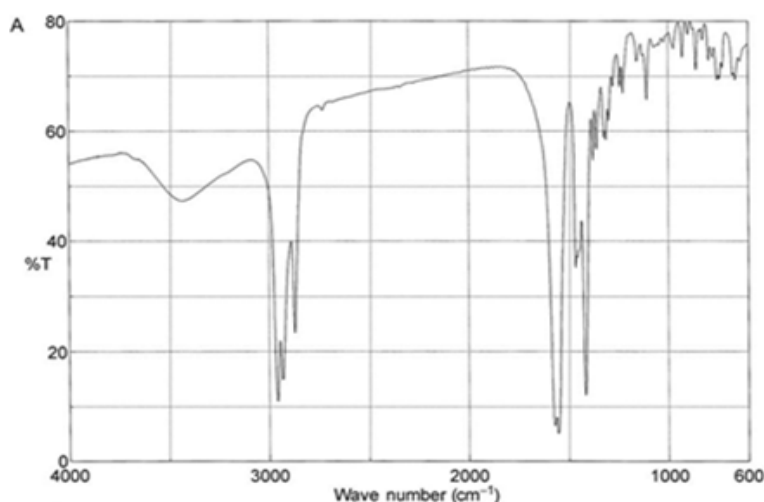


Fig 2: IR Spectra of Sample Epoprostenol Sodium

Table 1: IR Spectra Interpretation for Epoprostenol Sodium

Wavenumber (cm ⁻¹)	Vibration
3452–3440	–OH stretching
1650–1640	–OH bending
1450–1408	O–C–O stretching
1080–1005	Si–O–Si and Si–O–Al asymmet

Stability

For stability studies, the solubility of Epoprostenol Sodium were practically determined by adding 100 mg of Epoprostenol Sodium to 100 mL volumetric flasks, then adding an appropriate quantity of solvent (e.g., water or ethanol) at room temperature and shaking for a few minutes [31]. The solubility was then classified based on the amount of solvent required to dissolve the solute.

Table 3: Solubility Table

Description Terms	Relative Quantities of solvent for 1 Parts of solute
Very soluble	Less than 1 part
Freely soluble	From 1 to 10 parts
Soluble	From 10 to 30 parts
Sparingly soluble	From 30 to 100 parts
Slightly soluble	From 300 to 1000 parts
Very slightly soluble	From 1000 to 10000 parts
Practically Insoluble	More than 10000 parts

Reversed Phase High Pressure Liquid Chromatography (RP - HPLC)

Reversed Phase High-Performance Liquid Chromatography (RP - HPLC) is a more precise and sensitive analytical technique for identifying and quantifying Epoprostenol Sodium in Injection formulations.

RP - HPLC Method

- **Stationary Phase:** C18 reversed-phase column (e.g., 150 mm × 4.6 mm, 5 μm particle size).
- **Mobile Phase:** Acetonitrile: Ammonium acetate Buffer (60:40v/v).
- **Detection:** Set the UV detector to 217 nm to simultaneously detect Epoprostenol Sodium.
- **Flow Rate:** 1.0 ml/min

- **Injection Volume:** 20 μ L
- **Column Temperature:** Ambient or specific to ensure reproducibility.
- **Run Time:** Approximately 10 minutes.

Optimization of Chromatographic Conditions:

Conduct preliminary trials with different ratios of the mobile phase components and pH levels to optimize the resolution and symmetry of the peak for Epoprostenol Sodium.

Procedure:

Preparation of Standard Solution:

Accurately weigh and transfer an appropriate amount of Epoprostenol Sodium into separate volumetric flasks. Dissolve the drugs in a suitable solvent (such as methanol or acetonitrile) to make standard stock solutions of known concentration. Further dilute the stock solutions with the mobile phase to prepare working standard solutions of different concentrations (e.g., 10, 20, 30, 40, and 50 μ g/mL for both drugs).

Solution Preparation for Validation and Analysis

Preparation of Standard Stock Solution:

Accurately weighed quantity of Epoprostenol Sodium 10 mg was transferred into 100 mL volumetric flask, dissolved in methanol and diluted up to mark with methanol. This will give a stock solution having strength of 100 μ g/mL. Withdraw 0.4 ml from Stock Solution and make up to 10 ml with to get 4 μ g/mL.

Preparation of Working Standard Solutions:

Dilute the stock solutions with the mobile phase to prepare standard solutions at concentrations of: 10

μ g/mL, 20 μ g/mL, 30 μ g/mL, 40 μ g/mL, and 50 μ g/mL for Epoprostenol Sodium.

Forced Degradation Studies ^[10-12]

Acidic degradation

Prepare a stock solution of Epoprostenol Sodium at a concentration of 1 mg/mL in a small volume of methanol. Dilute the solution with distilled water. Transfer 10 mL of the prepared stock solution into a volumetric flask and add 10 mL of 0.1 M HCl. Incubate the acidic mixture at room temperature for 1–2 hours. Periodically sample aliquots to monitor the degradation process. After the specified reaction time, neutralize the acidic mixture with an equivalent volume of 0.1 M NaOH to halt further degradation. Filter the neutralized solution to remove any insoluble impurities. Inject an aliquot of the solution into the RP-HPLC system using the optimized chromatographic conditions. Observe the chromatogram to identify degradation products and determine the extent of degradation. Significant degradation of both Epoprostenol Sodium is expected within 1 hour under acidic conditions, as indicated by additional peaks corresponding to degradation products.

Alkaline degradation

Prepare a stock solution of Epoprostenol Sodium at a concentration of 1 mg/mL in a small volume of methanol. Dilute the solution with distilled water. Transfer 10 mL of the stock solution into a volumetric flask and add 10 mL of 0.1 M NaOH. Incubate the alkaline mixture at room temperature for 1–2 hours. Periodically sample aliquots to monitor the degradation process. After the specified reaction time, neutralize the alkaline mixture with an equivalent volume of 0.1 M HCl. Filter the neutralized solution to remove any insoluble impurities. Inject an aliquot of the

solution into the RP-HPLC system. Observe the chromatogram to identify degradation products and determine the extent of degradation. Significant degradation is expected within 1 hour under alkaline conditions, with the appearance of degradation peaks in the chromatogram.

Oxidative Degradation:

Prepare a stock solution of Dapagliflozin propanediol monohydrate and 10 mL of 3% hydrogen peroxide (H₂O₂). Transfer 10 mL of the stock solution into a volumetric flask and add 10 mL of 0.1 M NaOH. Incubate the mixture at room temperature for 1–2 hours, with periodic sampling to monitor degradation. Filter the solution to remove any insoluble impurities. Inject an aliquot of the solution into the RP-HPLC system. Examine the chromatogram for peaks corresponding to degradation products. Moderate to significant degradation is expected under oxidative conditions, with the formation of additional peaks in the chromatogram.

Thermal Degradation:

Place a weighed amount of Epoprostenol Sodium (solid state) in a clean and dry glass container. Expose the sample to 60°C in a hot air oven for 1–2 hours. After the specified time, dissolve the thermally stressed sample in methanol and dilute it with the mobile phase to the desired concentration. Filter the solution to remove any impurities and inject an aliquot into the RP-HPLC system. Minimal to moderate degradation is expected under thermal stress, with possible formation of new peaks in the chromatogram.

Photolytic Degradation:

Spread a thin layer of Epoprostenol Sodium powder in a glass Petri dish. Expose the sample to

UV light (254 nm) or direct sunlight for 24 hours. Dissolve the photolytically stressed sample in methanol and dilute it with the mobile phase to the desired concentration. Filter the solution to remove any impurities and inject an aliquot into the RP-HPLC system. Significant degradation is expected under photolytic conditions, resulting in new peaks corresponding to degradation products.

2. Method Validation Procedure ^[13-15]

1. Specificity:

A blank solution (mobile phase) and a placebo solution (tablet excipients without active ingredients) were prepared. Both solutions were injected into the RP-HPLC system to confirm the absence of any interfering peaks at the retention times of Epoprostenol Sodium. Result: No peaks were observed at the retention times of the active ingredients, confirming the method's specificity.

2. LOD & LOQ:

Perform serial dilutions of the standard solutions and inject into the system. LOD & LOQ of the drug was calculated by using following equation as per ICH guideline.

3. Precision:

1. Repeatability:

A target concentration of 30 µg/mL was selected for the repeatability study. Six replicates of the 30 µg/mL solution were prepared in the solvent mixture. Each replicate solution was analysed under identical experimental conditions. The absorbance of all six replicate solutions were measured at the specified wavelength. The standard deviation (SD) and relative standard deviation (%RSD) for the measured absorbance were calculated. The %RSD was found to be ≤ 2%, confirming that the method is repeatable.



1. Intra-day:

A target concentration of 30 µg/mL was selected for the intra-day precision study. Three replicate solutions of the target concentration were prepared in the solvent mixture. Each replicate solution was analysed at three different time intervals (e.g., 0 hours, 2 hours, and 4 hours) under identical conditions on the same day. The absorbance of all solutions were measured at the specified wavelength. The standard deviation (SD) and relative standard deviation (%RSD) for the absorbances were calculated.

2. Inter-day:

A target concentration of 30 µg/mL was selected for the inter-day precision study. Three replicate solutions of the target concentration were prepared in the solvent mixture. Each replicate solution was analysed on three different days (e.g., Day 1, Day 2, and Day 3) under identical conditions. Fresh solutions were prepared for analysis on each day to maintain accuracy. The absorbance of all replicate solutions were measured at the specified wavelength. The standard deviation (SD) and relative standard deviation (%RSD) were calculated for the absorbance across the three days. The %RSD values were found to be ≤ 2%, confirming the method's precision over multiple days.

3. Accuracy (Recovery study):

A target concentration of 30 µg/mL was selected for the accuracy study. Three solutions of the target concentration were spiked with 50% (1.5 µg/mL), 100% (3.0 µg/mL), and 150% (4.0

µg/mL) of the standard drug, respectively. The spiked solutions were prepared in triplicate for each level to ensure robustness in measurements. The absorbance of the spiked solutions were measured at the specified wavelength (e.g., 217 nm).

4. Robustness:

The robustness of the method was tested by making small, deliberate variations in the analytical conditions. For each variation, the target concentration of 30 µg/mL was analysed in triplicate. The absorbance of all solutions were measured, and the % relative standard deviation (%RSD) was calculated.

RESULT AND DISCUSSION

1. Selection of Wavelength

To determine wavelength for measurement, standard spectra of Epoprostenol Sodium were scanned between 200-400 nm against diluents. Absorbance maxima of Epoprostenol Sodium have detected at 217. Chromatogram was taken at 217 nm, drug give good peak height and shape. So, 217 nm was selected for Simultaneous estimation of Epoprostenol Sodium in their formulation.

Selection of Mobile phase

Trail 1	
Column	C-18 (id 4.6 x 150 mm, 5 µm)
Mobile Phase	: Acetonitrile: Water(30:70v/v).
Detection	217 nm
Flow rate	1ml/min
Run Time	10 min
Observations	No peak detected

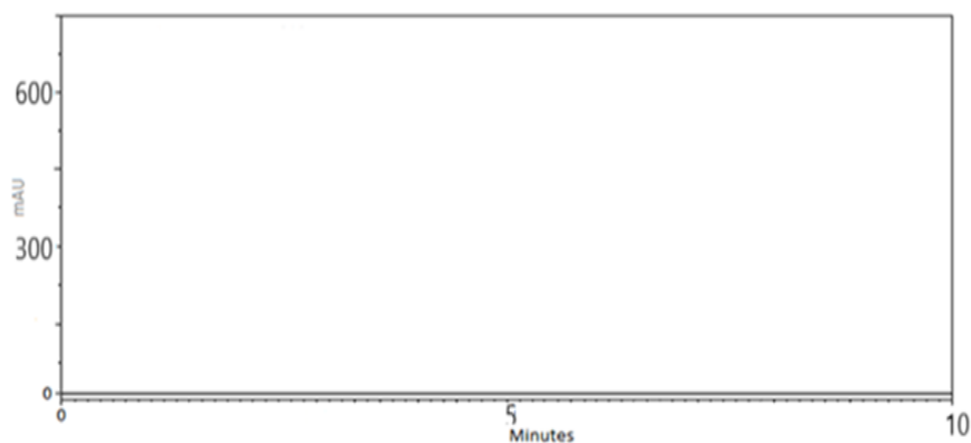


Fig 3: Chromatogram of Epoprostenol Sodium Acetonitrile: Water (30:70v/v)

Trail 2	
Column	C-18 (id 4.6 x 150 mm, 5 μ m)
Mobile Phase	Acetonitrile: Water(50:50v/v)

Detection	217 nm
Flow rate	1 ml/min
Run Time	10 min
Observations	Broad peak detected.

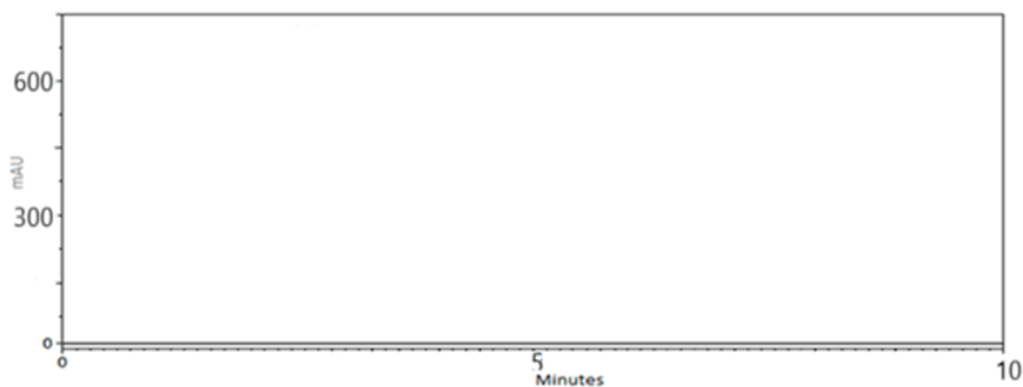


Fig 4: Chromatogram of Epoprostenol Sodium Acetonitrile: Water (50:50v/v)

Trail 3	
Column	C-18 (id 4.6 x 150 mm, 5 μ m)
Mobile Phase	Acetonitrile: Water(80:20v/v).
Detection	217 nm

Flow rate	1 ml/min
Run Time	10 min
Observations	Peak detected but broad peaks observe.

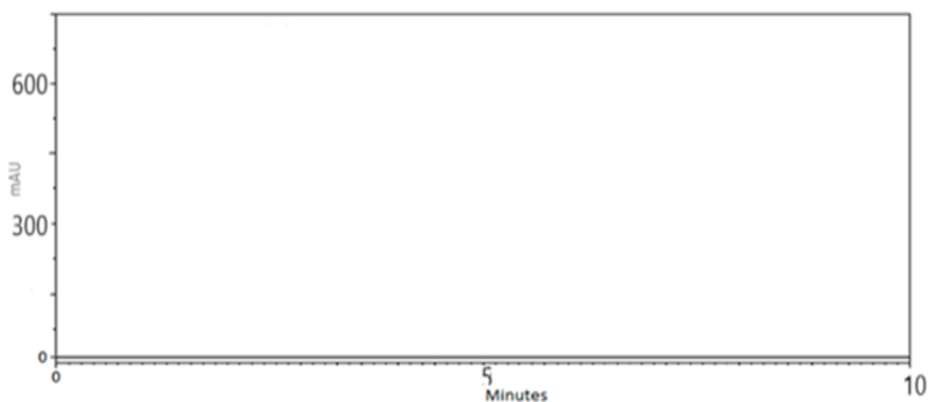


Fig 5: Chromatogram of Epoprostenol Sodium Acetonitrile: Water(80:20 v/v)

Trail 4	
Column	C-18 (id 4.6 x 250 mm, 5 μ m)
Mobile Phase	Acetonitrile: Ammonium acetate Buffer (60:40v/v)
Detection	217 nm

Flow rate	1 ml/min
Run Time	10 min.
Observations	Good peak with Adequate solution was observed.

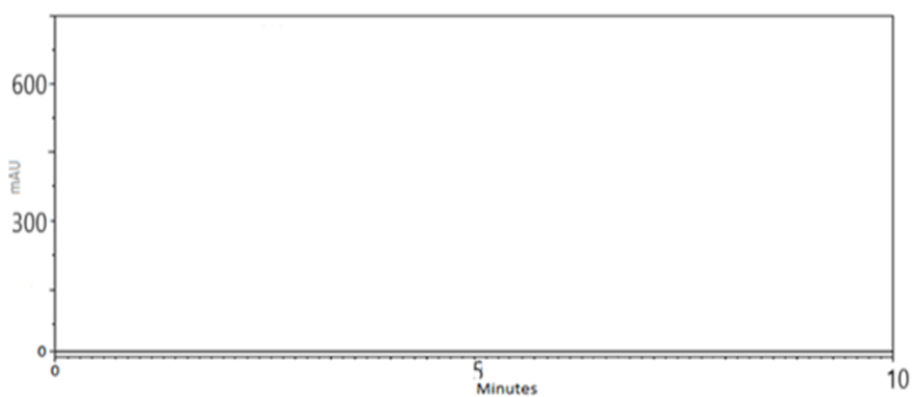


Fig 6: Chromatogram of Epoprostenol Sodium Acetonitrile: Ammonium acetate Buffer (60:40v/v)

Chromatographic conditions for optimized mobile phase trial:

Column	C-18 (id 4.6 x 250 mm, 5 μ m)
Mobile Phase	Acetonitrile: Ammonium acetate Buffer (60:40v/v).

Detection	217 nm
Flow rate	1 ml/min
Run Time	10 min.
Detector	UV Detector
Injection Volume	20 μ l
Column Temperature	40 $^{\circ}$ C
Mode	Isocratic

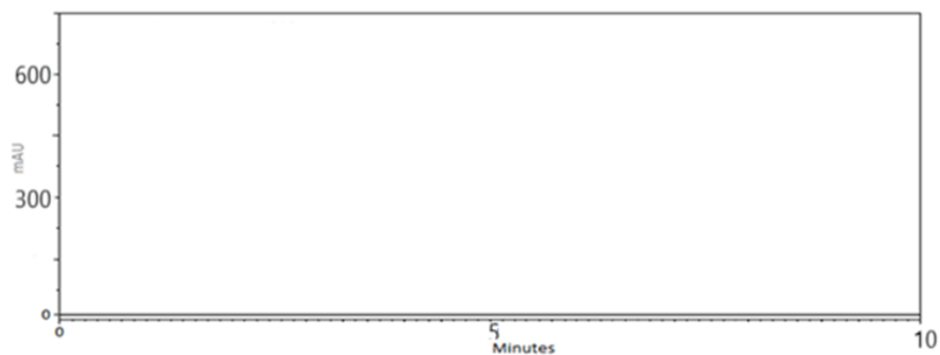


Fig 7: Optimized mobile phase trial for optimized chromatogram of Std. Epoprostenol Sodium:2.115 min

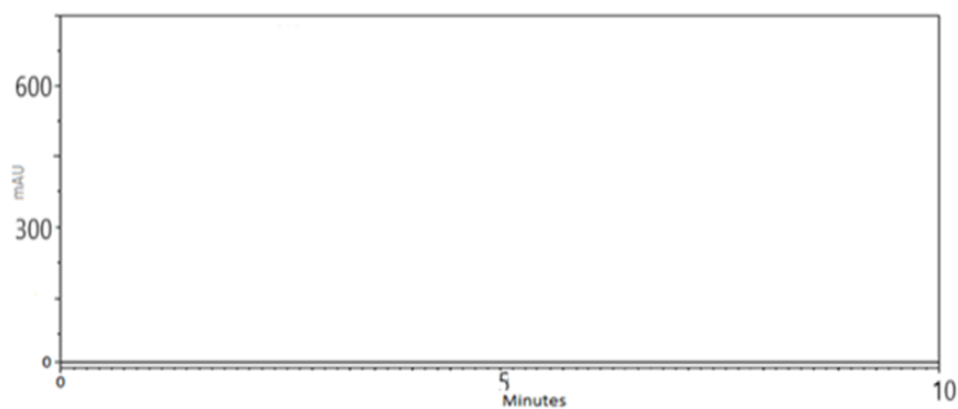


Fig 8: Chromatogram of blank Epoprostenol Sodium Acetonitrile: Ammonium acetate Buffer (60:40v/v)

Method Validation

Linearity:

For the purpose of linearity, accurately weighed amount of Epoprostenol Sodium (10 mg) was taken into the volumetric flask (10 ml) and volume of the flask was raised to 10 ml with methyl alcohol to

give stock solution containing 100 $\mu\text{g/ml}$ of Epoprostenol Sodium. Various aliquots from this stock solution were transferred to another 10 ml volumetric flask and volume was raised to the mark with mobile phase to give final solutions containing 4, 6, 8, 10 and 12 $\mu\text{g/ml}$ of Epoprostenol Sodium.

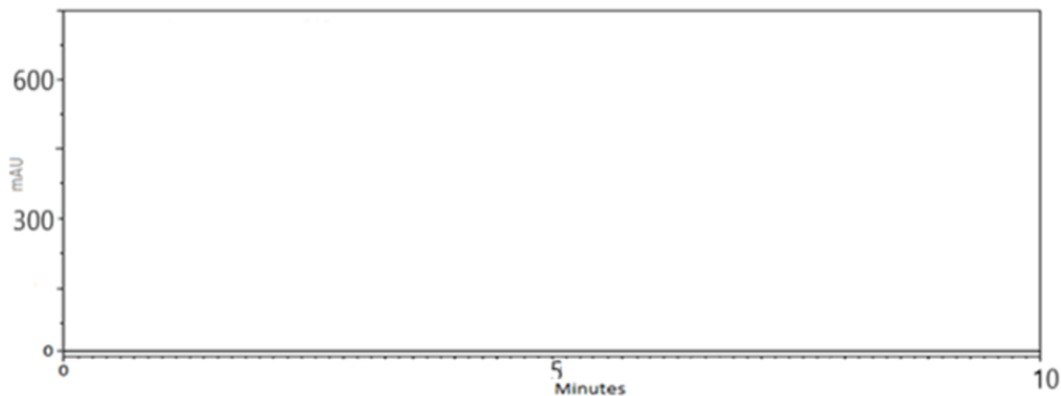


Fig 9: Overlain Linearity Spectra of Epoprostenol Sodium

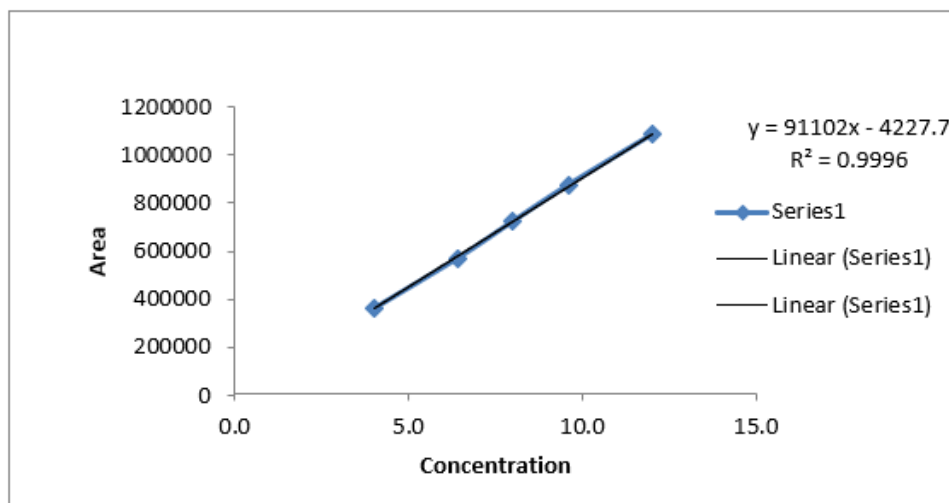


Fig 10: Calibration curve of Epoprostenol Sodium

Table 4: Linearity results for Epoprostenol Sodium

Regression Analysis	Epoprostenol Sodium
Concentration Range	4-12 µg/mL

Regression equation	$y = 91102x - 4227.7$
Correlation co-efficient	0.9996

Table 5: Linearity data for Epoprostenol Sodium

Conc. (µg/ml)	Epoprostenol Sodium		
	Mean Area	± SD (n=5)	% RSD
4	362791	362791 ± 149.01	0.04
6	571467	571467 ± 5693.60	1.00
8	725650	725650 ± 1086.59	0.15
10	877038	877038 ± 1749.10	0.20
12	1085987	1085987 ± 942.35	0.09

Precision

Repeatability



The data for repeatability for Epoprostenol Sodium is shown in table. The % R.S.D For Repeatability data was found to be 1.10 % for Epoprostenol Sodium.

Table 6: Repeatability data for Epoprostenol Sodium

Drugs	Conc. (µg/ml)	Mean Peak Area ± SD	%RSD
Epoprostenol Sodium	4	724860 ± 1041.54	1.10

Inter-day precision

The data for interday precision for Epoprostenol Sodium is shown in table. The % R.S.D for intraday precision was found to be 0.25-1.05 % for Epoprostenol Sodium.

Table 7: Inter-day precision data for estimation of Epoprostenol Sodium

Mcg/ml	Epoprostenol Sodium		
	4	8	12
	365487	724634	1088445
	362312	723328	1093425
	369980	720945	1083341
MEAN	365926.3	722969	1088404

Table 9: Recovery data for Epoprostenol Sodium

	Epoprostenol Sodium					
	50%		100%		150%	
	Amount of drug recovered (mg)	%Recovery	Amount of drug recovered (mg)	%Recovery	Amount of drug recovered (mg)	%Recovery
	1.46	99.76	2.97	99.20	4.54	100.20
	1.40	97.70	2.89	99.01	4.56	100.22
	1.56	100.50	3.09	100.01	4.68	100.30
Mean	1.49	96.65	2.98	99.43	4.69	100.24
%RSD	0.02	1.30	0.04	1.75	0.05	0.68

LOD and LOQ:

0.23	0.72
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Table 10: LOD and LOQ Limit for Epoprostenol Sodium

Epoprostenol Sodium	
LOD(µg/ml)	LOQ(µg/ml)

Selectivity:

There is no interference in the mixture.

Robustness:

± SD	3852.832	1870.519	5042.127
RSD	1.052898	0.258727	0.463259

Intra -day precision

The data for intra-day precision for Epoprostenol Sodium is shown in table. The % R.S.D for intraday precision was found to be 0.43-1.16 % for Epoprostenol Sodium.

Table 8: Intra-day precision data for estimation of Epoprostenol Sodium accuracy

Mcg/ml	Epoprostenol Sodium		
	4	8	12
	369809	724351	1093652
	365544	729876	1085467
	361287	729801	1094357
MEAN	365546.7	728009.3	1091159
± SD	4261.001	3167.432	4941.716
RSD	1.165652	0.435219	0.452887

Accuracy

Accuracy of the method was confirmed by recovery study from synthetic mixture at three level standard additions. Percentage recovery for Epoprostenol Sodium was found to be 99.48-99.78%. The results are shown in table.

The method is found to be robust as the results were not significantly affected by slight variation in Mobile Phase Composition and flow rate of mobile phase.

Table 11: Robustness data for Epoprostenol Sodium

Parameter	Level of Change	Effect on assay volume	
		Epoprostenol Sodium	
		Assay \pm SD	RSD
Flow rate	0.9 mL/min	97.70 \pm 0.50	0.49
	1.1 mL/min	101.09 \pm 0.72	0.72
Mobile phase composition	50:50	97.47 \pm 0.53	0.53
	60:40	97.39 \pm 0.99	0.98
	30:70	99.51 \pm 0.67	0.67

Analysis of marketed product:

The proposed method was successfully applied to analysis of the commercially available tablet

formulation. The % drugs were found satisfactory, which is comparable with the corresponding label claim.

Table 12: Analysis of marketed formulations

Drug	Amount taken (μ g/mL)	Amount found (μ g/mL)	% Assy
Epoprostenol Sodium	3	2.93 \pm 0.04	99.80 \pm 1.20

Summary of Method Validation:

Table 13: Summary of validation parameter of RP-HPLC method

Optimized chromatographic Condition	
Stationary Phase	C-18 (id 4.6 x 150 mm, 5 μ m)
Mobile Phase	Acetonitrile: Ammonium Acetate Buffer (60:40v/v)
Detection wave Length	217 nm
Flow rate	1 ml/minute
Run time	10 minutes
Retention Time	2.115 min

Validation parameters			
Parameter	Limit	Result	Conclusion
		Epoprostenol Sodium	
Linearity and Range	R2 > 0.995	0.9996 (4-12 μ g/mL)	Method was linear
Repeatability	RSD < 2	1.10	Method was repeatable
LOD	-	0.23	-
LOQ	-	0.72	-
Intra-day Precision	RSD < 2	0.25.-1.05	Method was precise



Inter-Day Precision	RSD<2	0.43-1.16	Method was precise
% Recovery	98-102%	99.35 ±0.83– 100.01±0.03 %	Method was accurate
Robustness	RSD<2	0.41– 0.63	Method was robust
Assay%		99.80 ±1.20	-

CONCLUSION

In this study, a novel and eco-friendly HPLC method was successfully developed and validated for the simultaneous estimation of Epoprostenol Sodium in Injectable dosage form. The method demonstrated high sensitivity, accuracy, and precision, making it suitable for routine quality control applications. The stability-indicating nature of the method was confirmed by stress degradation studies, which ensured that the method could effectively differentiate between the drug substances and their degradation products under various stress conditions. The developed method was also environmentally sustainable, employing green chemistry principles such as the use of an aqueous mobile phase Acetonitrile: Ammonium Acetate Buffer (60:40v/v) % v/v.

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