ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR ESTIMATION OF SELEXIPAG IN BULK AND DOSAGE FORM

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ABSTRACT

The present study aimed to develop and validate a simple, rapid, accurate, precise, and cost-effective Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method for the quantitative estimation of Selexipag in bulk drug and pharmaceutical dosage forms. Selexipag is a selective prostacyclin (IP) receptor agonist used in the treatment of pulmonary arterial hypertension. Chromatographic separation was achieved using a C18 column under optimized chromatographic conditions with a suitable mobile phase composition and UV detection at 298 nm. The developed method provided a sharp and symmetrical peak for Selexipag with a retention time of approximately 4.33 min, indicating efficient separation and suitability for routine analysis.

The method was validated in accordance with ICH Q2(R1) guidelines for specificity, linearity, accuracy, precision, robustness, and system suitability. The calibration curve exhibited excellent linearity over the selected concentration range with a correlation coefficient (r^2) of 0.9998. Accuracy studies demonstrated satisfactory recovery values ranging from 99.37% to 100.32%, while precision studies showed low %RSD values, confirming the reproducibility of the method. Robustness evaluation indicated that minor deliberate variations in analytical conditions did not significantly affect the method performance. System suitability parameters were found to be within acceptable limits. The developed RP-HPLC method was found to be reliable, and suitable for routine quality control analysis of Selexipag in pharmaceutical formulations.

Keywords: Selexipag, Pulmonary Arterial Hypertension, RP-HPLC, Method Development, Method Validation, Pharmaceutical Analysis, Quality Control, ICH Guidelines.

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1.] INTRODUCTION

Selexipag(2-{4-[(5,6-diphenylpyrazin-2-yl)(propan-2-yl)amino]butoxy}-N-methanesulfonylacetamide; $C_{26}H_{32}N_4O_4S$;MW 496.63 g/mol;pKa:3.5). is an orally active, non-prostanoid agonist that is selective for the prostacyclin (IP) receptor and is indicated for the treatment of pulmonary arterial hypertension (PAH). Unlike prostacyclin (PGI2) or its synthetic analogues, selexipag is chemically distinct and displays high selectivity for the IP receptor over other prostanoid receptors. Following oral administration, it is rapidly hydrolysed by carboxylesterase 1 in the intestine and liver to its active metabolite, ACT-333679, which is approximately 37-fold more potent than the parent drug. Both selexipag and its metabolite are extensively (~99%) bound to plasma proteins, are metabolised principally by CYP2C8 and, to a lesser extent, CYP3A4, and exhibit terminal half-lives of 0.8–2.5 h and 6.2–13.5 h respectively. Co-administration with strong CYP2C8 inhibitors such as gemfibrozil is contraindicated, as it can raise plasma concentrations of selexipag and its active metabolite roughly two-fold and eleven-fold, respectively. [1,2,3]

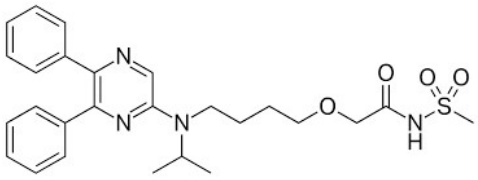


Fig.1 Structure of Selexipag

2] MATERIALS AND METHODS:

2.1 Chemical and reagents:

Working standard selexipag was procured from Vidisha Analytical, and the marketed formulation (selexipag tablets, 400 mcg) was sourced from MSN Laboratories. HPLC-grade acetonitrile and methanol, ammonium formate and formic acid (Merck Life Science), water (Rankem), and 0.45 µm nylon membrane and 0.45 µm PVDF syringe filters (Mdi) were used throughout the study.

2.2 Instrumentation:

The instruments used in the study included an Agilent 1260 Infinity II HPLC system, a Jasco UV-550 UV-Visible spectrophotometer, an Aczet CY224C digital analytical balance, a Thermo Scientific Orion Star A211 digital pH meter, and a Bio-Technic 13.5 L ultrasonic cleaner. These instruments were employed for chromatographic analysis, spectrophotometric measurements, weighing, pH determination, and sonication of samples and solvents.

2.3 chromatographic condition:

- **Detector:** U.V. Detector
- **Column**: AgilentC18
- **Column Dimension:** 250mmX 4.6mm, 5µm
- **Injection Volume:** 10µl
- **Wavelength:** 298nm
- **Mobile phase:** Phosphate Buffer (pH 4.0) : Methanol (35:65, v/v)
- **Flow Rate:** 1.2 ml/min
- **Run time:** 9 Minutes

2.4 Preparation of ammonium formate buffer pH 4.0:

Weigh and transfer 1.26 mg of ammonium formate into 1000 ml volumetric flask add water mixed well to dissolve. Adjust pH 4.0 0.05 with formic acid, mixed well.

2.5 Preparation of Mobile phase: Prepare mixture of buffer solution pH 4.0 and methanol, in the ratio of 35:65 v/v respectively, mix well. Filter through 0.45 nylon membrane disc filter. Sonicated for 15 min to degas the mobile phase.

2.6 Preparation of Diluent:

Prepare mixture of buffer solution pH 4.0 and methanol in the ratio of 20:80 v/v respectively, mix well.

2.6 Preparation of standard solution:

Weighed and transferred accurately about 50 mg of selexipag working standard into 100 ml clean and dry volumetric flask. Added about 80 mL of diluent, sonicate to about 30 minutes to dissolve and dilute up to the mark with diluent and mix. Further dilute above stock 2.0 mL of this stock solution to 100 mL with diluent and mix well. Filter the sample solution through 0.45 membrane PVDF filter. Discard first 4.0 mL of filtrate and then collected the sample. (Concentration of selexipag standard solution: 10 ppm)

2.7 Preparation of Sample Solution:

Weighed and transferred 10 selexipag tablets in to 100 mL clean and dry volumetric flask. Added about 80 mL of diluent, sonicate for 60 minutes with intermittent shaking, at control room temperature and make volume up to mark with diluent and mix. Further diluted above stock solution 5.0 mL of this sample stock solution to 20 mL volumetric flask make up with Diluent and mixed well. Filter the sample solution through 0.45 membrane PVDF filter. Discard first 4.0 mL of filtrate and then collected the sample. (Concentration of Sample Solution: 10 ppm).

3] METHOD VALIDATION:

The optimised method was validated as per ICH Q2(R1) guidelines for the following parameters.

3.1 System suitability:

System suitability studies showed excellent chromatographic efficiency with a %RSD of 0.18%, a tailing factor of 1.18, and 14,362 theoretical plates, all of which complied with the predefined acceptance criteria.

3.2 Specificity:

Specificity studies confirmed the absence of interference from blank and placebo solutions at the retention time of Selexipag. The retention times of the standard and sample solutions were found to be 4.33 min and 4.34 min, respectively, indicating accurate identification of the analyte. Peak purity analysis further confirmed the specificity of the method, as the purity angles for both standard (1.65) and sample (1.54) were lower than their corresponding purity thresholds (2.89 and 2.71).

3.3 Linearity:

Linearity studies demonstrated an excellent correlation between concentration and peak area over the selected range, with a correlation coefficient (r^2) of 0.9998 and a %Y-intercept of 0.14%, confirming proportional detector response.

3.4 Accuracy:

Accuracy studies showed mean recovery values of 99.37%, 100.32%, and 99.80% at 50%, 100%, and 150% concentration levels, respectively, indicating excellent accuracy of the method.

3.5 Precision:

Precision studies revealed a mean assay value of 98.29% with a %RSD of 0.39%, demonstrating good repeatability. Intermediate precision studies showed a pooled mean assay value of 98.44% and an absolute mean difference of 0.525%, confirming the ruggedness and reproducibility of the method.

3.6 Robustness:

Robustness studies performed by varying flow rate (±0.1 mL/min) and wavelength (±2 nm) demonstrated no significant impact on chromatographic performance, and all results remained within acceptable limits. These findings confirm that the developed RP-HPLC method is specific, linear, accurate, precise, robust, and suitable for routine quality control analysis of Selexipag in bulk drug and pharmaceutical dosage forms.

4] RESULT AND DISCUSSION:

4.1 CHARACTERISATION OF DRUG SUBSTANCE:

4.1.1. Physical Characteristics:

1) Colour, odour and appearance

Table no 1 : Physical characteristics

o	Name	Colour, odour and appearance of drug
	selexipag	hite, odourless powder

selexipag was evaluated for parameters like color; odour & appearance are shown in result**.**

4.1.2 Selection of solvent:

Table 2: Drug Solubility Summary

Sr. No.	Name of Solvent	Observation	Conclusion
1	Water	Drug Particles seen after sonication	Drug was not found soluble in water.
2	Methanol	No Drug Particles seen after sonication	Drug was found soluble in methanol.
3	Acetonitrile	Drug Particles seen after sonication	Drug was not found soluble in Acetonitrile.
7	Water: Methanol (30:70 v/v)	No Drug Particles seen after sonication	Drug was found solubl e in water;Methanol.

Final Conclusion: Water: Methanol (30:70 v/v) will be used as a diluent for preparing stock solution.

4.2. INFRARED SPECTROSCOPIC METHOD

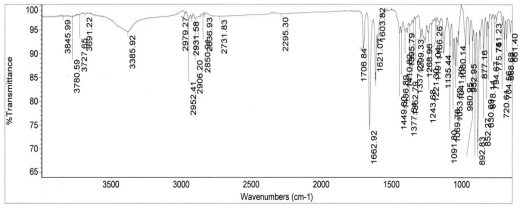


Fig. 2 FTIR spectrum of selexipag.

FTIR spectrum API was identified which shows characteristics absorption of various functional group of API as given above figure.

Table 3 IR peak Assignment value of selexipag

Standard IR Ranges (cm^{-1})	IR Ranges (cm^{-1})	Functional Group
3400-3300	33856.92	N-H Stretching
3000-2840	2979.27	C-H Stretching
1720-1706	1706.84	C=O Stretching
1690-1640	1662.92	C=N Stretching
1410-1380	1386.89	S=O Stretching
1250-1020	1243.68	C-N Stretching

4.3 METHODS:

4.3.1 UV SPECTROSCOPIC METHOD:

4.3.1.1 Selection of wavelength:

Blank spectra:

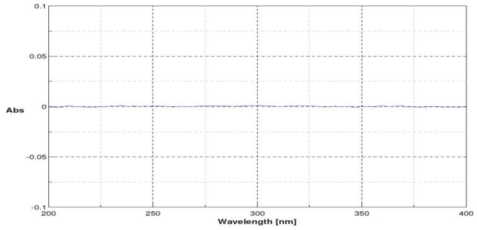


Fig. 3: UV spectrum of Blank.

4.1.1.2 Standard Solution spectra:

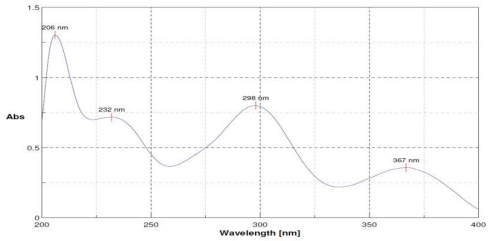


Fig. 4 UV spectrum of Selexipag.

Observation: The standard solution was scanned from 400 nm to 200 nm. Wavelength of maximum absorption was determined for drug. Selexipag showed maximum absorbance at 367, 298, 232 and 206 nm. 298 nm considered as an analytical wavelength for further determination as it's having highest absorption as compare to 367 and 232 nm. 206 nm cannot select as its below cut off wavelength region of solvents.

Table 4 : Determination of λ max of Selexipag

Sr. No.	Wavelength (nm)	Absorbance
1.	298	0.8069 A^0

Conclusion:

The lambda max of Selexipag 298 nm is selected for further analysis.

5] REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD DEVELOPMENT

Different trials taken were as follows:

TRIAL: 1

Chromatographic Conditions:

Column	Zorbax SB C-8, 150 x 4.6mm, 3.5µ
Mobile Phase	Water: Acetonitrile (90:10 v/v)
Flow Rate	1.5 mL/min
Injection Volume	50 µL
Wavelength	298 nm
Column Temp.	25°C
Auto sampler Temp.	25°C
Run time	8.0 min.
Needle wash	Water : Methanol (90:10 v/v)
Seal wash	Water : Methanol (10:90 v/v)

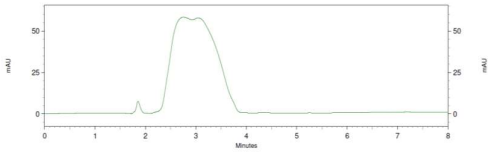


Fig. 5 Typical chromatogram for Trial- 1

Observation: Selexipag eluted at 3.0minutes with unacceptable chromatography (Asymmetry:0.60 and Theoretical plates 605) broad peak observed.

Conclusion: Selexipag shows unacceptable chromatography hence, Method rejected.

TRIAL: 2

Chromatographic Conditions:

Column	Zorbax SB C18, 150 x 4.6mm, 3.5µ
Mobile Phase	Water: Methanol (90:10 v/v)
Flow Rate	1.5 mL/min
Injection Volume	50 µL
Wavelength	298 nm
Column Temp.	25°C
Auto sampler Temp.	25°C
Run time	7.0 min.
Needle wash	Water : Methanol (90:10 v/v)
Seal wash	Water : Methanol (10:90 v/v)

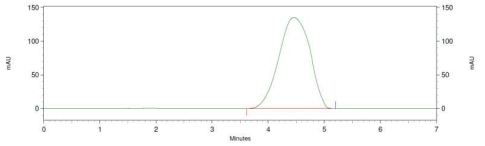


Fig. 6 Typical chromatogram for Trial- 2

Observation: Selexipag eluted at 4.4 minutes with unacceptable chromatography (Asymmetry:0.70 and Theoretical plates 920) broad peak observed.

Conclusion: Selexipag shows unacceptable chromatography hence, Method rejected.

TRIAL: 3

Chromatographic Conditions:

Column	Waters symmetry C18, 250mm x 4.6mm, 5µ.
Mobile Phase	Water: Methanol (50:50 v/v)
Flow Rate	1.2 mL/min
Injection Volume	50 µL

Wavelength	298 nm
Column Temp.	25°C
Auto sampler Temp.	25°C
Run time	8.0 min.
Needle wash	Water : Methanol (90:10 v/v)
Seal wash	Water : Methanol (10:90 v/v)

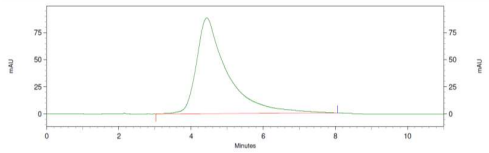


Fig. 7 Typical chromatogram for Trial- 3

Observation: Selexipag eluted at 4.3 minutes with unacceptable chromatography (Asymmetry:0.63 and Theoretical plates 1480) peak tailing observed.

Conclusion: Selexipag shows unacceptable chromatography hence, Method rejected.

TRIAL: 4

Chromatographic Conditions:

Column	Waters symmetry C18, 250mm x 4.6mm, 5µ.
Mobile Phase	water: Methanol (30:60v/v)
Flow Rate	1.0 mL/min
Injection Volume	10 µL
Wavelength	298 nm
Column Temp	25ºC
Sample Temp	25ºC
Run Time	5.0 minutes
Seal Wash	Water: Methanol(90:10) v/v
Needle Wash	Water: Methanol(10:90) v/v

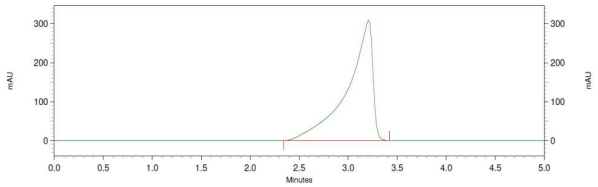


Fig. 8 Typical chromatogram for Trial-4

Observation: Selexipag eluted at 3.3 minutes with acceptable chromatography (Asymmetry: 2.5 and Theoretical plates 1865) fronting observed.

Conclusion: Selexipag shows acceptable chromatography but reduction of retention time required.

TRIAL: 5

Chromatographic Conditions:

Column	Waters symmetry C18, 250mm x 4.6mm, 5µ.
Mobile Phase	Buffer pH 4.0: Methanol (40:60v/v)
Flow Rate	1.0 mL/min
Injection Volume	10 µL
Wavelength	298 nm
Column Temp	25ºC
Sample Temp	20ºC
Run Time	9.5 minutes
Seal Wash	Water: Methanol(90:10) v/v
Needle Wash	Water: Methanol(10:90) v/v

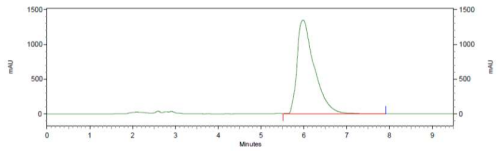


Fig. 9 Typical chromatogram for Trial- 5

Observation: Selexipag eluted at 6.1 minutes with unacceptable chromatography (Asymmetry: 1.0 and Theoretical plates 2351) peak tailing observed. Retention time need to reduce.

Conclusion: Selexipag shows unacceptable chromatography hence, Method rejected.

TRIAL: 6

Chromatographic Conditions:

Column	Waters symmetry C18, 250mm x 4.6mm, 5µ.
Mobile Phase	Buffer pH 4.0: Methanol (35:65v/v)
Flow Rate	1.2 mL/min
Injection Volume	10 µL
Wavelength	298 nm
Column Temp	35ºC
Sample Temp	20ºC
Run Time	9.0 minutes
Seal Wash	Water: Methanol(90:10) v/v
Needle Wash	Water: Methanol(10:90) v/v

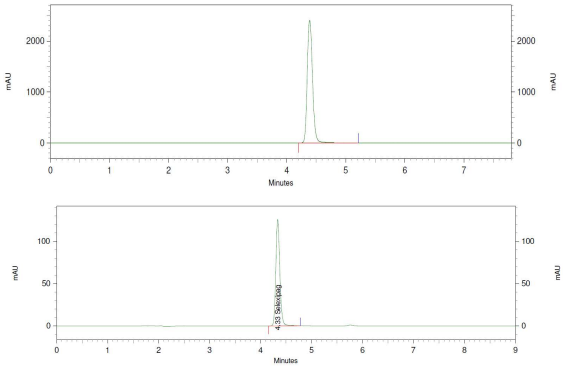


Fig. 10 Typical chromatogram for Trial- 6

Observation: Selexipag eluted at 4.33 minutes with acceptable chromatography (Asymmetry: 1.18 and Theoretical plates 14362)
Conclusion: Method can be used for further analysis and will be subjected for validation.

6] METHOD VALIDATION

System Suitability, Specificity, Linearity, Accuracy, Precision, System Precision, Method Precision, Intermediate Precision, Robustness Thise parameters were considered for the analytical method validation of title ingredients.
6.1 SYSTEM SUITABILITY: System suitability test is a pharmacopoeial requirement and is used to verify, whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be done.

Table 5 system suitability test of Selexipag

Tailing Factor	**1.18**
Theoretical plates	**14362**
Injection No.	**Area**
1	11521052
2	11562531
3	11532486
4	11545638
5	11572429
Mean	**11546827**
%RSD	**0.18**

The tests were performed by collecting data from Single injection of blank (Diluent) and five replicate injections of Standard solution were injected into the chromatograph. The data obtained is summarized in Table.

Conclusion:

The data demonstrates that the system suitability is within the acceptance criteria, thus the system is suitable.

Specificity: (Identification, Interference & Peak Purity)

ect Blank (Diluent), standard solution, cebo solution and sample solution. The data ained is summarized in Table

Table 6 Specificity (Identification and Interference)

Solution	**Specificity data**		
	Retention time (min)	**Purity Match**	
Blank solution	NA	NA	
Placebo solution	NA	NA	
Standard solution	4.33	Purity angle	Purity threshold
		1.65	2.89
Sample solution	4.34	1.54	2.71

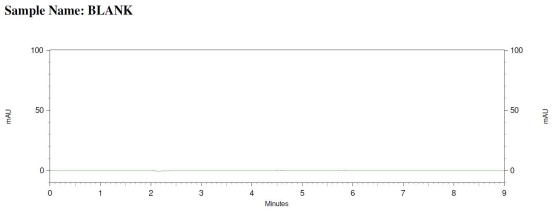


Fig .11 Chromatogram of Blank

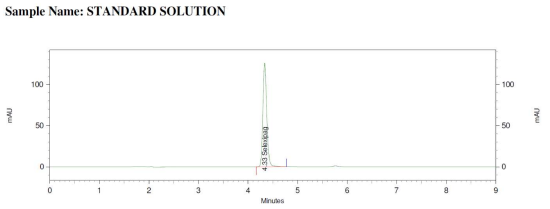


Fig 12 Chromatogram of Standard

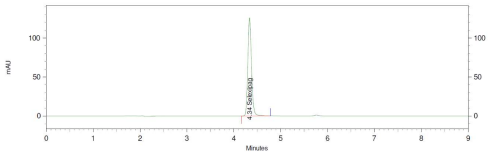
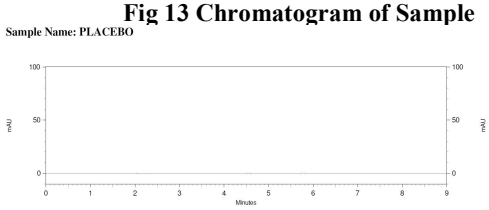


Fig 13 Chromatogram of Sample



.14 Chromatogram of Placebo

Conclusion:

- The data demonstrates that retention time in standard and sample is same for Selexipag peak.
- The data demonstrates that there is no interference in blank and placebo at the retention time of Selexipag peak. Peak Purity match in both chromatograms obtained from Standard and Sample solution.

6.3 Linearity:

Linearity was evaluated in the range of 50 % to 150 % of Selexipag for working concentration. The working concentration of Selexipag in solution is 10 µg/mL. The data summarized in Table.

Level	Conc (µg/mL)	Area	Mean
50%	5	5735214	5744176
		5742106	
		5755208	
75%	7.5	8658524	8656096
		8660237	
		8649527	
100%	10	11540873	11540846
		11551428	
		11530238	
125%	12.5	14565210	14506699
		14470128	
		14484759	
150%	15	17252106	17344250
		17235428	
		17245217	
Corr. Coeff			0.9998
Intercept			16113
Slope			11540846
% Y-intercept			0.14

Table 7 Linearity of Selexipag

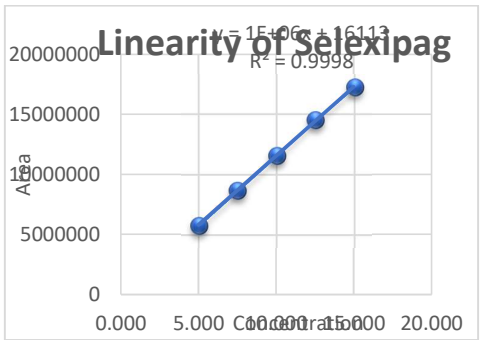


Fig .15 Linearity plot of Selexipag

Conclusion:

- The data shows that system suitability is fulfilled.
- The data shows that the response is found to be linear.
- Co-relation coefficient (r^2 was found 0.9998.

Fig 6.4 Accuracy (Recovery):

Evaluated accuracy from 50% to 150% of Selexipag tablet, working concentration level. Each level prepared in triplicates.

Table 8 % Recovery for Selexipag

Level (%)	Selexipag Added Conc (µg/mL)	Selexipag Recovered conc	Area	% Recovery	Mean % Recovery
50	5.00	5.01	57850100	100.20	99.37
	5.00	4.92	57352188	99.34	
	5.00	4.86	56914225	98.58	
100	10.00	9.99	11520124	99.77	100.32
	10.00	10.04	11632428	100.74	
	10.00	10.02	11600227	100.46	
150	15.00	15.00	17320749	100.00	99.80

	15.00	14.07	17 28 42 40	99. 79	
	15.00	15.05	17 25 41 27	99. 62	

Conclusion:

The data shows that the Mean recovery for 50% to 150% is in the range of 98.0%-102.0% and individual recovery for 50% to 150% is in the range of 98.0% - 102.0%.

6.5 Precision:

6.5.1 Method Precision:

Single injection of blank (Diluent), Standard solution (five replicates) and sample solution (six preparations) was injected on the system.

Table 9 Method precision for Bulk(API)

Sample	Area	% Assay
Sample 1	11512534	99.90
Sample 2	11535201	100.02
Sample 3	11542569	100.30
Sample 4	11549568	99.86
Sample 5	11505231	99.76
Sample 6	11505698	99.66
Mean		**99.92**
STD DEV		**0.2244**
% RSD		**0.225**

Table 9 Method precision for Pharmaceutical dosage form(Tablet)

Sample	Area	% Assay
Sample 1	11352413	98.51
Sample 2	11328328	98.23
Sample 3	11254729	97.80
Sample 4	11325010	97.92
Sample 5	11400587	98.86
Sample 6	11362418	98.42
Mean		**98.29**
STD DEV		**0.3909**
% RSD		**0.398**

Conclusion:

The data shows that system suitability is fulfilled. The data shows that % RSD for % Assay is within the acceptance criteria and hence the method is precise.

6.6* Intermediate Precision*:

six independent sample preparations were prepared on different day and by different analyst and injected on the HPLC.

Table 10 Intermediate Precision

Sample	Area	% Assay
Sample 1	11420157	99.13
Sample 2	11406876	98.91
Sample 3	11282415	97.76
Sample 4	11432784	99.03
Sample 5	11275416	97.84
Sample 6	11433207	98.86
Mean		**98.59**
STD DEV		**0.6178**
% RSD		**0.627**

Table 11 Intermediate Precision pool Data

Parameter	Method Precision (Analyst-I)	Intermediate Precision (Analyst-II)
HPLC NO.	**AD/HPLC-02**	**AD/HPLC-04**
Column No.	**HPLC-014**	**HPLC-031**
Sample No.	**%Assay**	
1	98.51	99.13
2	98.23	98.91
3	97.80	97.76
4	97.92	99.03
5	98.86	97.84
6	98.42	98.86
Mean	**98.29**	**98.59**
Mean of Precision % Assay	**98.44**	
Absolute Mean difference % assay	**0.525**	

Conclusion:

- The data shows that system suitability is fulfilled.
- The data shows that % Assay is of six samples is not more than 2.0
- The data shows that % Assay is within the acceptance criteria and hence the method is rugged.

6.7 Robustness:

This parameter was studied by making small, deliberate changes in the chromatographic conditions and Assay parameters, observing the effect of these changes on the system suitability and results obtained by injecting the standard and sample solutions.

Table 12 Robustness for Selexipag

Change in	Condition	Area	% Assay	Absolute differe

parameter				nce of % Assay
Control	As per method	11352413	98.51	NA
Change in flow rate1.0 ml/min (±0.1 ml/min)	1.3 ml/min	11254268	97.66	0.85
	1.1 ml/min	11482134	99.63	-1.12
Change in wavelength (±2 nm)	300 nm	11191215	97.11	1.4
	296 nm	11525341	100.01	-1.5

Conclusion:

- System suitability criteria were fulfilled.
- The difference of % assay value in each modified condition is within acceptance criteria.

DISCUSSION :

Method development was initiated to achieve a rapid, selective, and efficient RP-HPLC method for the determination of Selexipag. Several chromatographic parameters, including stationary phase, mobile phase composition, flow rate, and column temperature, were systematically optimized to obtain acceptable chromatographic performance. Initial experiments using Zorbax SB C8 and Zorbax SB C18 columns with aqueous-organic mobile phases (Trials 1 and 2) resulted in broad and asymmetrical peaks with low theoretical plate counts (605 and 920, respectively), indicating poor column efficiency and inadequate separation. In Trial 3, the use of a Waters Symmetry C18 column and a water:methanol (50:50 v/v) mobile phase improved retention behavior; however, peak tailing and insufficient theoretical plates (1480) were still observed.Further modification of the mobile phase composition in Trial 4 improved chromatographic behavior and reduced the retention time to 3.3 min. Nevertheless, significant peak fronting (asymmetry factor 2.5) and inadequate column efficiency (1865 theoretical plates) suggested that the method was not yet suitable for quantitative analysis. Introduction of a pH 4.0 buffer in Trial 5 enhanced peak symmetry and increased theoretical plates to 2351, confirming the beneficial effect of pH control on chromatographic performance. However, the retention time increased to 6.1 min and peak tailing persisted, making the method less desirable for routine analysis.

- To overcome these limitations, further optimization was carried out by adjusting the buffer-to-methanol ratio, flow rate, and column temperature. The optimized conditions employed in Trial 6, consisting of Buffer (pH 4.0):Methanol (35:65 v/v), a flow rate of 1.2 mL/min, and a column temperature of 35°C, produced a sharp, symmetrical, and well-resolved Selexipag peak with a retention time of 4.33 min, tailing factor of 1.18, and theoretical plate count of 14,362. The substantial improvement in peak symmetry and column efficiency demonstrated the suitability of the optimized chromatographic conditions. Therefore, Trial 6 was selected as the final method and subsequently validated according to ICH Q2(R1) guidelines for its intended analytical application.

CONCLUSION

A simple, accurate, precise and economical reversed-phase high-performance liquid chromatographic (RP-HPLC) method was developed and validated for the estimation of selexipag, a selective prostacyclin (IP) receptor agonist used in the treatment of pulmonary arterial hypertension, in bulk drug and tablet dosage form. Chromatographic separation was achieved on a Waters Symmetry C18 column (250 mm × 4.6 mm, 5µm) using a mobile phase comprising ammonium formate buffer (pH 4.0) and methanol in the ratio of 35:65v/v at a flow rate of 1.2 mL/min, with UV detection at 298 nm. Under these conditions selexipag eluted at a retention time of 4.33 min within a total run time of 9.0 min, with a tailing factor of 1.18 and 14,362 theoretical plates. The method was validated as per ICH Q2(R1) guidelines for system suitability, specificity, linearity, accuracy, precision and robustness. The drug response was linear over the range of 5–15 µg/mL (50–150% of the working concentration) with a correlation coefficient of 0.9998. Mean percentage recovery ranged from 99.37% to 100.32%, and the %RSD for method precision and intermediate precision was 0.225–0.627%, well within the ICH acceptance limit of ≤2.0%. The method remained unaffected by small, deliberate changes in flow rate and detection wavelength, confirming its robustness.The proposed RP-HPLC method is simple, accurate, precise, reproducible and suitable for the routine quality-control analysis of selexipag in bulk drug and pharmaceutical dosage form.

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CONFLICT OF INTEREST

The authors declare no conflict of interest. The selexipag working standard was provided by Vidisha Analytical Laboratory, Pune, and the tablet formulation was obtained from Alkem Laboratories Ltd. Neither organization had any role in study design, data collection, analysis, interpretation of results, manuscript preparation, or the decision to publish this work.

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