

METHOD DEVELOPMENT AND VALIDATION OF SELEXIPAG IN ITS BULK AND DOSAGE FORM BY RP-HPLC

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ABSTRACT

The present study described a new, simple, accurate and precise development for estimation of selexipag by RP-HPLC method. The chromatographic method was standardized using INERTSIL ODS 4.6×250mm, with a mobile phase ratio (70:30 v/v) ACN: 0.1 % OPA buffer pH 3 (pH was adjusted with NaOH) at a flow rate of 1ml/min using UV detection at 270 nm. The retention time was found to be 2.16min. The % purity of selexipag was found to be 100.43% respectively. The system suitability parameters for selexipag such as theoretical plates and tailing factor were found to be 2832.72, 1.17. The linearity study of selexipag was found in concentration range of 100 µg-500 µg and correlation coefficient (r^2) was found to be 0.999, % recovery was found to be 100.19%, %RSD for repeatability was 0.2, % RSD for intermediate precision was 0.3 respectively. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The described HPLC method was successfully employed for the analysis of selexipag.

KEY WORDS

Selexipag, Acetonitrile, Ortho phosphoric acid buffer, HPLC, Stress degradation.

1. Introduction^[1]

The Project entitled Method development and validation of selexipag in its bulk and dosage forms has not been reported by using RP-HPLC method. Hence, there is a need of new analytical method development for the estimation of selexipag. Aim of work is to develop a new, simple, fast, rapid, accurate, efficient and reproducible RP-HPLC method by optimizing the chromatographic conditions for the analysis of selexipag and to perform stress degradation studies. The developed method will be validated according to ICH guidelines Q2 (R1).

Selexipag is used for the treatment of pulmonary arterial hypertension (PAH) to delay disease progression and reduce risk of hospitalization. PAH is a relatively rare disease with usually a poor prognosis requiring more treatment options to prolong long-term outcomes. Selexipag and its active metabolite, ACT-333679 (MRE-269), act as agonists of the prostacyclin

receptor to increase vasodilation in the pulmonary circulation and decrease elevated pressure in the blood vessels supplying blood to the lungs.

Mechanism of Selexipag is a selective prostacyclin (IP, also called PGI₂) receptor agonist. The key features of pulmonary arterial hypertension include a decrease in prostacyclin and prostacyclin synthase (enzyme that helps produce prostacyclin) in the lung. Prostacyclin is a potent vasodilator with anti-proliferative, anti-inflammatory, and anti-thrombotic effects; therefore, there is strong rationale for treatment with IP receptor agonists.

Selexipag is administered orally, maximum concentration of drug and its active metabolites were observed with the bioavailability of 57% and 29% in rats and monkeys.

2. Instruments and Chemicals

Pure sample of Selexipag was gifted by Manus Aktteva Biopharma LLP. The HPLC is used of WATERS with an

ELICO UV detector, SHIMADZU digital balance and DANWER sonicator was used. All the solvents were of HPLC grade purchased from Merck.

3. Experimental Method ^[2]:

Optimized chromatographic conditions

Column	:	Inertsil Ods 4.6×250 mm
Mobile phase ratio	:	ACN: OPA (70: 30 % v/v)
Detection wavelength	:	270 nm
Flow rate	:	1.0ml/min
Injection volume	:	10µl
Column temperature	:	Ambient
Auto sampler temperature:	:	Ambient
Run time	:	8 min

3.1 Procedure

3.1.1 Preparation of OPA buffer:

1ml of OPA was taken into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water and pH was adjusted to 3 with NaOH. The resulting solution was sonicated and filtered.

3.1.2 Preparation of mobile phase:

Mix a mixture of above buffer 300 ml (30%) and 700 ml of ACN (HPLC grade-70%) and degassed in ultrasonic water bath for 5 minutes. Filter through 0.22 µ filter under vacuum filtration.

3.1.3 Preparation of the Selexipag standard and sample preparation:

Weighed 10mg of selixipag and transferred into 10ml volumetric flask and make up the solution with diluent up to the mark and then take 3ml of above solution in 10ml volumetric flask and make up with diluents up to the mark.

4. Method Validation ^[3, 4, 5]

The developed analytical method was subjected to validation with respect to various parameters such as specificity, Linearity, Range, Accuracy, Precision, Repeatability, Intermediate Precision, Detection Limit, Quantification Limit, Robustness were validated as per ICH guidelines using RP-HPLC.

4.1. Specificity

The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The specificity was performed by injecting blank.

4.2. Linearity

Preparation of stock solution

10 mg of selexipag working standard was accurately weighed and was transferred into a 10ml clean dry volumetric flask and makeup the volume with diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Five different levels of selexipag were prepared by taking stock solution samples of 1ml, 2ml, 3ml, 4ml, and 5ml in 10ml volumetric flask and diluted with mobile phase and each level was injected into the system, peak area and correlation coefficient was measured. The linearity range of 100µg/ml-500µg/ml of selexipag were measured.

4.3. Accuracy

Preparation of standard stock solution

Weigh 10mg of selexipag and transferred into 10ml volumetric flask and make up the solution with diluent up to the mark and then take 3ml of above solution in 10ml volumetric flask and make up with diluents up to the mark.

Preparation of sample solutions

For preparation of (50%, 100%, 150%) solutions 5mg, 10mg, 15mg of selexipag working standard was taken respectively into a 10-ml clean dry volumetric flask and makeup the volume with diluents. For second dilution take 3ml from above solution in 10ml flask and make up with diluent and then filter it with 0.45µ filter paper and transfer into HPLC vial. Later injected into the chromatographic system where the individual % recovery and mean % recovery values are calculated.

4.4 Precision/Repeatability

Preparation of stock solution for precision and intermediate precision:

Weigh 10mg of selixipag and transferred into 10ml volumetric flask and make up the solution with Diluent up to the mark and then take 3ml of above solution in 10ml volumetric flask and make up with diluents up to the mark.

Procedure: The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Intermediate Precision/Ruggedness

To evaluate the intermediate precision (also known as ruggedness) of the method, precision was performed on different days by using different make column of same dimensions.

4.5. Limit of detection (LOD) & Limit of quantification (LOQ)

LOD's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) at levels approximating the LOD according to the formula. The standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

4.6. Robustness

As part of the robustness, deliberate change in the flow rate was varied at 0.9ml/min to 1.1 ml/min. Standard solution 300 ppm of selixipag prepared and analysed using the varied flow rates along with method flow rate. The organic composition in the mobile phase was varied from $\pm 10\%$ standard solution 300 $\mu\text{g/ml}$ of Selexipag were prepared and analysed using the varied mobile phase composition along with the actual mobile phase composition in the method.

4.7. System suitability

Weigh 10mg of selixipag and transferred into 10ml volumetric flask and make up the solution with diluent unto the mark and then take 3ml of above solution in 10ml volumetric flask and make up with diluents up to the mark.

5. DEGRADATION STUDIES^[6]:

The International Conference on Harmonization (ICH) guideline entitled stability testing of new drug substances and products requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance.

Preparation of stock:

Weigh 10mg of selixipag and transferred into 10ml volumetric flask and make up the solution with diluent up to the mark and then take 3ml of above solution in 10ml volumetric flask and make up with diluents up to the mark.

5.1 Hydrolytic degradation under acidic condition:

Pipette 3 ml of above solution into a 10ml volumetric flask and 3 ml of 0.1N HCl was added. Then, the volumetric flask was kept at 60°C for 6 hours and then neutralized with 0.1 N NaOH and make up to 10ml with diluent. Filter the solution with 0.22 microns syringe filters and place in vials.

5.2 Hydrolytic degradation under alkaline condition:

Pipette 3ml of above solution into a 10ml volumetric flask and add 3ml of 0.1N NaOH. Then, it was kept at 60°C for 6 hours and then neutralized with 0.1N HCl and make up to 10ml with diluent. Filter the solution with 0.22 microns syringe filters and place in vials.

5.3 Thermal induced degradation:

Selexipag sample was taken in petridish and kept in Hot air oven at 110° C for 24 hours. Then the sample was taken and diluted with diluents and injected into HPLC and analyzed.

5.4 Oxidative degradation:

Pipette 3ml above stock solution into a 10ml volumetric flask and 1ml of 3% w/v of hydrogen peroxide and the volume was made up to the mark with diluents and kept at room temperature for 15 min. Filter the solution with 0.45 microns syringe filters and place in vials.

5.5 Photo degradation:

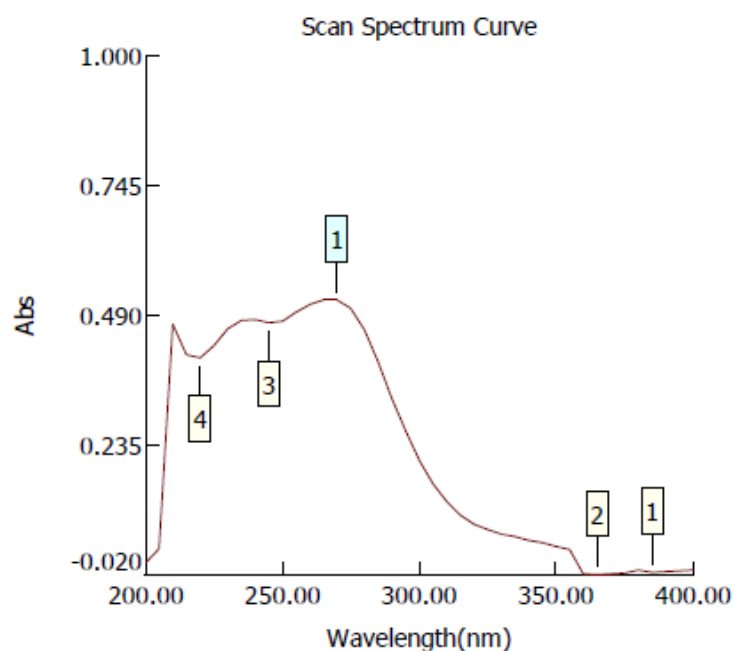
Pipette 3 ml above stock solution into a 10ml volumetric flask and expose to sunlight for 24hrs and the volume was made up to the mark with diluent. Filter the solution with 0.45 microns syringe filters and place in vials.

6. Results and Discussions

6.1 Method Development

The detection wavelength was selected by dissolving the drug in mobile phase to get a concentration of 10 $\mu\text{g/ml}$ for standard solution. The resulting solution was scanned in U.V range from 200-400nm. The spectrum of Selexipag was obtained and the absorption point of Selexipag showed maxima at 270 nm.

Fig.No.1. Figure showing spectrum of Selexipag



6.2 Validation parameters:

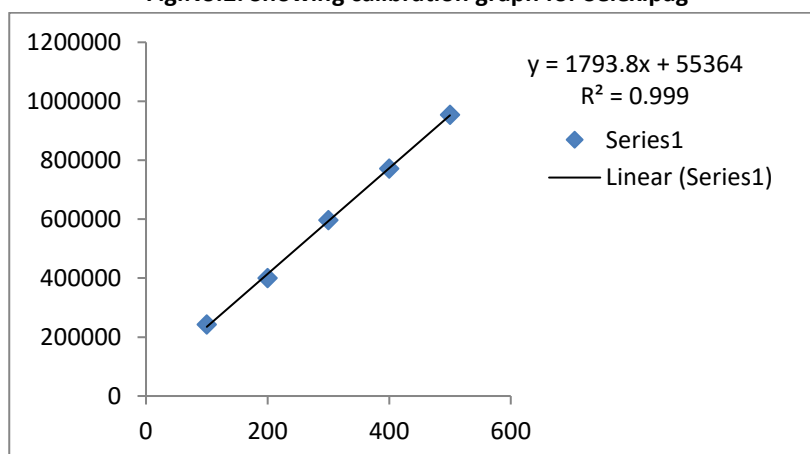
a) Linearity

The linearity study was performed for concentration range of 100µg/ml-500µg/ml selexipag and the correlation coefficient was found to be 0.999 (NLT 0.999)

Table.No.1: Linearity Results for Selexipag

S. No	Concentration (µg/ml)	Area
1	100	243038
2	200	400877
3	300	597435
4	400	771671
5	500	954583
Correlation coefficient		0.999

Fig.No.2. Showing calibration graph for Selexipag



b) Accuracy

The accuracy study was performed for 50%, 100% and 150 % for Selexipag. Each level was injected in triplicate into chromatographic system. The area of each level was used for calculation of % recovery.

Table.No.2. Showing accuracy results for Selexipag

% Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	299500	5	5.02	100.44%	100.19%
100%	595655	10	9.99	99.88%	
150%	896932	15	15.04	100.26%	

c) Precision

The Method precision study was performed for the %RSD of Selexipag was found to be 0.24 (NMT 2).

Table.No.3. Showing% RSD results for Selexipag

Peak Results Name: selexipag						
	Name	RT	Area	Height	USP Plate Count	USP Tailing
1	selexipag	2.156	590409	53325	2904.1	1.22
2	selexipag	2.157	590348	51637	2978.1	1.2
3	selexipag	2.158	592414	52354	2927.12	1.23
4	selexipag	2.158	592149	49900	3107.57	1.21
5	selexipag	2.159	590131	50133	3052.70	1.29
6	selexipag	2.160	590771	46621	3131.72	1.22
Mean			591037.0			
Std. Dev.			989.3			
% RSD			0.2			

d) Intermediate precision

The intermediate precision was performed for %RSD of Selexipag was found to be 0.3 (NMT2).

Table.No.4. Showing results for Intermediate precision of Selexipag

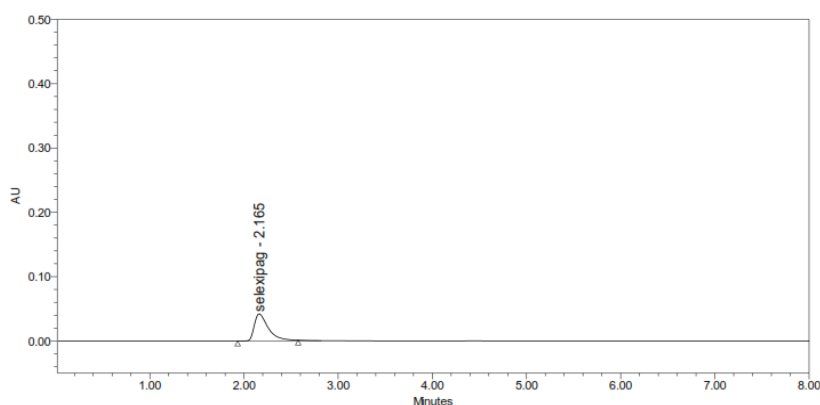
Peak Results Name: selexipag						
	Name	RT	Area	Height	USP Plate Count	USP Tailing
1	selexipag	2.156	592055	43500	3131.25	1.18
2	selexipag	2.160	592055	44034	3148.85	1.10
3	selexipag	2.162	592435	46598	3105.12	1.17
4	selexipag	2.163	590956	44918	3121.24	1.19
5	selexipag	2.163	590927	42166	3107.27	1.18
6	selexipag	2.165	596014	42081	3108.80	1.19
Mean			592407.0			
Std. Dev.			1873.8			
% RSD			0.3			

e) LOD

The LOD was performed for Selexipag was found to be 2.165 and 0.04 respectively.

Table.No.5. Showing results for Limit of Detection

Peak Name	RT	Area	Height	% Area	USP Tailing	USP Plate Count
selexipag	2.165	2177.6	197.0	100.00	1.39	3108.80

Fig.No.3. Chromatogram showing limit of detection


f) LOQ

The LOQ was performed for Selexipag was found to be 2.165 and 0.05 respectively.

Table.No.6. Showing results for Limit of Quantitation

Peak Name	RT	Area	Height	% Area	USP Tailing	USP Plate Count
selexipag	2.165	7317.6	662.0	100.00	1.19	3108.80

g) Robustness

The results are summarized on evaluation at different flow rates. It can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ± 0.2 ml/min. The method is robust only in less flow condition.

Table.No.7. Showing Robustness results for Selexipag

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.9	3132.20	1.21
2	1	2892.94	1.13
3	1.1	3167.78	1.21

h) System suitability

These tests are performed to verify resolution and reproducibility of the system and are adequate for the analysis.

Table.No.8. Showing system suitability results for Selexipag

S. No	Change in organic composition in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	10 % less	3039.17	1.09
2	*Actual	2892.94	1.13
3	10 % more	3879.34	1.22

6.3 Degradation studies

Table.No.9. Showing Results of Degradation studies

Sample Name	Selexipag				
	Area	% Degraded	Purity Angle	Purity Threshold	Peak purity
Standard	595204	100			
Acid	572932	96.26	3.74	54.60	Passes
Base	575047	96.61	3.39	90.00	Passes
Peroxide	575327	96.66	3.34	90.00	Passes
Thermal	570258	95.81	4.19	90.00	Passes
Photo	576473	96.85	3.15	42.53	Passes

Fig.No.4. Chromatograph of Acid

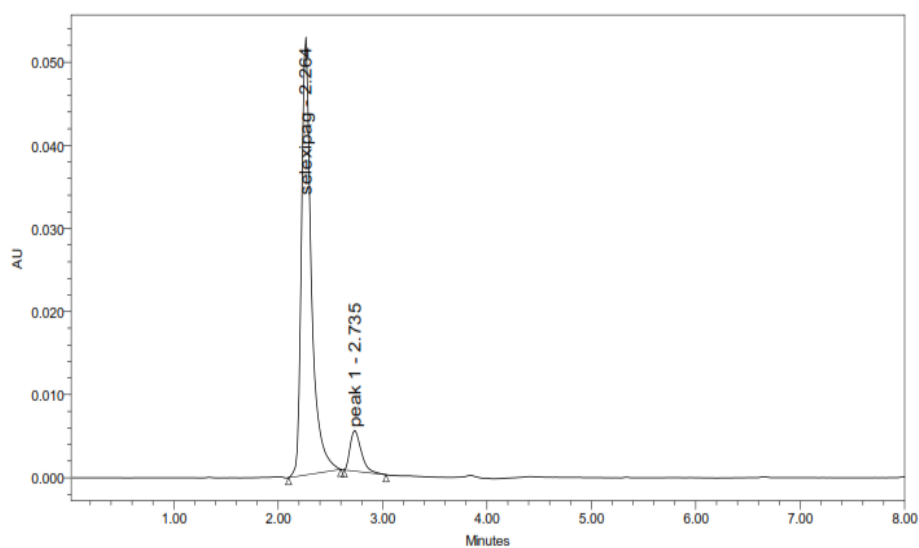


Fig.No.5. Chromatograph of Base

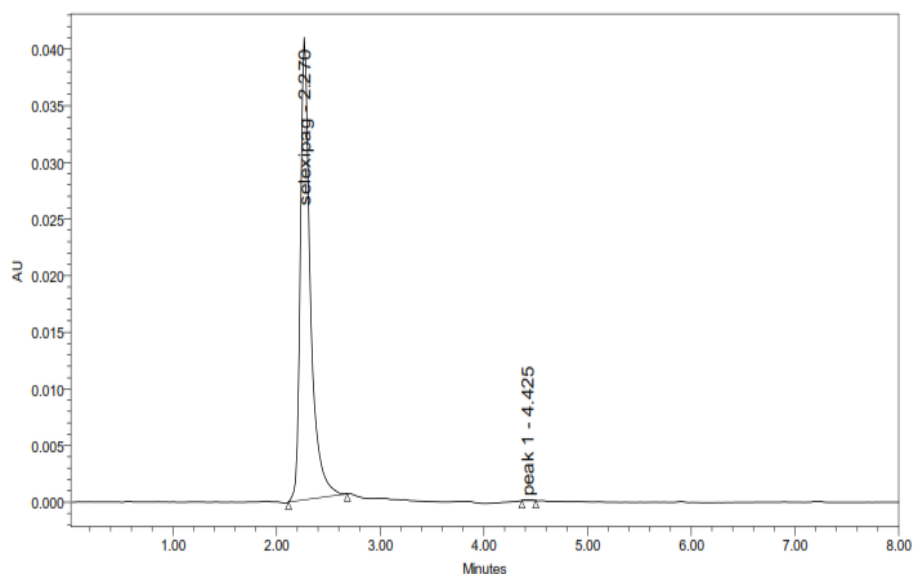


Fig.No.6. Chromatograph of thermal

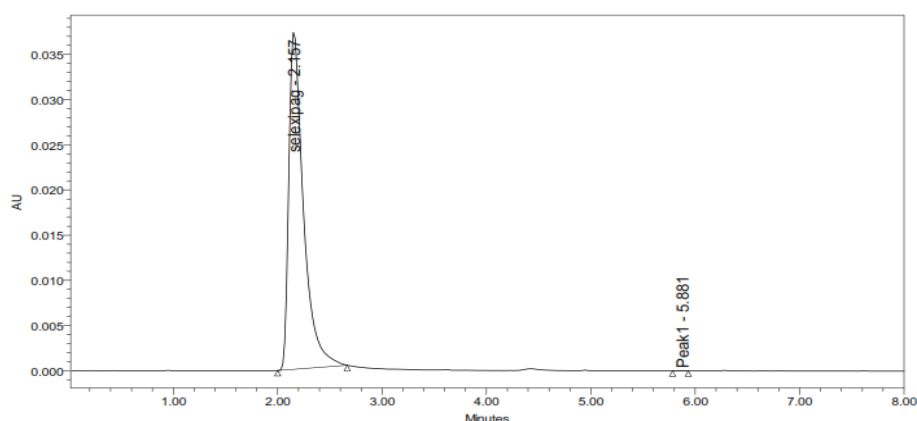


Fig.No.7. Chromatograph of peroxide

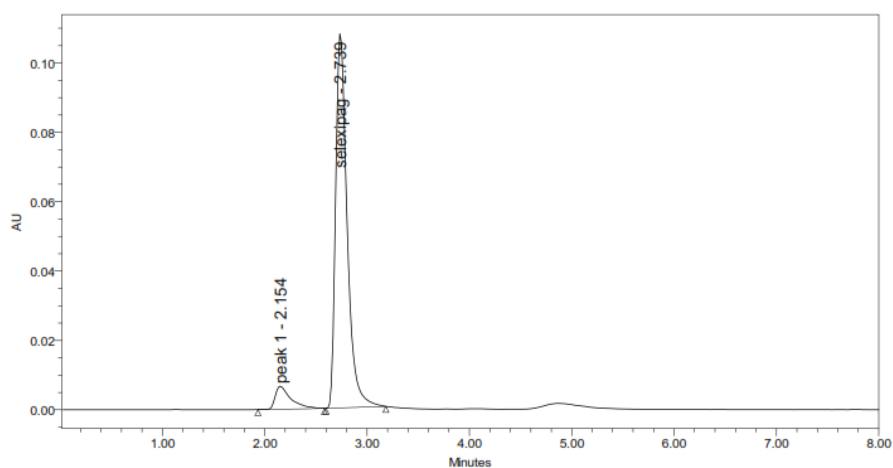
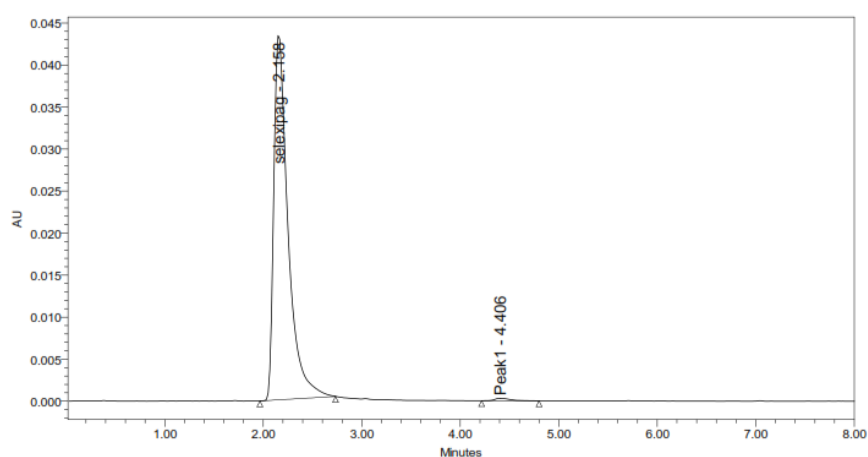


Fig.No.8. Chromatograph of photo



Conclusion:

A new method was established for simultaneous estimation of selexipag by RP-HPLC method.

The analytical method was validated according to ICH guidelines ICH, Q2 (R1). The degradation studies of selexipag in various conditions such as alkaline, acidic,

oxidation, photo and thermal were observed and quantitatively analysed by HPLC.

The results obtained from specificity, linearity range, LOD, LOQ, precision, accuracy, robustness, ruggedness and system suitability lie well within the acceptance criteria. Since all the results were within the limit, the

developed and validated analytical method is suitable for anticipated use and all parameters are subjected as per the ICH guidelines. Hence the suggested RP-HPLC method can be used for routine analysis of selexipag in API and Pharmaceutical dosage form.

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