WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 9, Issue 4, 293-304. Review Article ISSN 2277– 7105

STABILITY INDICATING ASSAY METHOD DEVELOPMENT AND VALIDATION FOR SELEXIPAG IN PHARMACEUTICAL DOSAGE FORM

M. S. Aashka Joshi[1]* and Dr. C. N. Patel[2]

[1]Hemachandracharaya North Gujarat University, Patan, Gujarat.

[2]Shri Sarvajanik Pharmacy College, Mehsana, Gujarat.

Article Received on
30 Jan. 2020,

Revised on 20 Feb. 2020,
Accepted on 10 March 2020,

DOI: 10.20959/wjpr20204-17031

***Corresponding Author**
M. S. Aashka Joshi
Hemachandracharaya North Gujarat University, Patan, Gujarat.

ABSTRACT

A new simple, specific, accurate and stability-indicating reversed phase `high performance liquid chromatographic (HPLC) method was developed for the determination of Selexipag using a Hypersil BDS C18 column (150 mm×5.6 mm, 5.0 µm), a mobile phase consisting of Buffer(ph-5.0): Methanol 60:40, at a flow rate of 1.0 mL/min and ultraviolet detection at 296 nm. The retention times of Selexipag was found to be 5.390 min. Linearity was established for Selexipag in the range of 20-60 µg/mL with correlation coefficients >0.999. The percentage recovery of Selexipag was found to be in the range of 100.99-101.06%. Stress testing was carried out to demonstrate specificity of the method. The developed method could separate the potential degradation products from the Selexipag. This proposed method was suitable for analysis the content of Selexipag in Pharmaceutical dosage form. The method is validated as per ICH guidelines.

KEYWORDS: Selexipag, RP-HPLC Estimation, Analytical Method Validation, Stability.

1. INTRODUCTION

Selexipag (brand name Uptravi) is a drug developed by Actelion for the treatment of pulmonary arterial hypertension (PAH). Selexipag and its active metabolite, ACT-333679 (or MRE-269, the free carboxylic acid), are agonists of the prostacyclin receptor, which leads to vasodilation in the pulmonary circulation.[1]

Selexipag is an oral, selective, IP receptor agonist, and is structurally and pharmacologically distinct from prostacyclin and its analogues. Selexipag is hydrolyzed by carboxylesterase 1 to

yield its active metabolite, which is approximately 37-fold more potent than selexipag. Selexipag and the active metabolite are high-affinity IP receptor agonists with a high selectivity for the IP receptor versus other prostanoid receptors (EP1–EP4, DP, FP, and TP). Stimulation of the IP receptor by selexipag and the active metabolite leads to vasodilatory as well as anti-proliferative and anti-fibrotic effects.

The recommended starting dose is 200 mcg given twice daily. The dose is increased in increments of 200 mcg given twice daily, usually at weekly intervals, until adverse pharmacological effects that cannot be tolerated or medically managed are experienced, or until a maximum dose of 1,600 mcg twice daily is reached. The highest tolerated dose reached during dose titration should be maintained. If the therapy over time is less tolerated at a given dose, symptomatic treatment or a dose reduction to the next lower dose should be considered.[2]

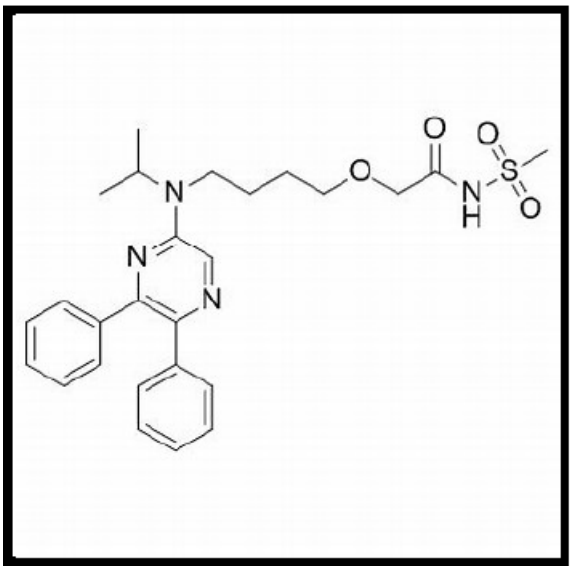

Figure 1: Structure of Selexipag.

2. MATERIAL AND METHOD: Chemicals

- Drug samples of Nebivolol Hydrochloride, Telmisartan, Selexipag, Riociguat were purchased from Zydus Pharmaceuticals Ltd., Changodar, Ahmedabad.

Glasswares

- Volumetric flask (5, 10, 25, 50, 100, 500 mL)
- Graduated pipette (1, 2, 5, 10 mL)
- Measuring cylinder (10, 100 mL)

- Glass Beaker (100, 250, 500 mL)
- Plastic beaker (500 mL)
- Syringe (1 mL)

Instruments

- Gradient high pressure liquid chromatographies (Shimadzu LC-2010C HT) with variable wavelength programmable UV/Vis detector (Shimadzu, Kyoto Japan), manual injector of 20 µl loop
- UV-1800 double beam UV-Visible spectrophotometer(Shimadz, Kyoto Japan) attached with computer operated software UV probe 2.0
- UV cabinet with dual wavelength UV lamp, Labtronic, Ahmedabad
- Sartorius CP224S analytical balance (Gottingen, Germany)
- Ultra sonic cleaner (Frontline FS 4, Mumbai, India)
- HPLC an Agilent's HPLC 1260 with photodiode array detector, software used was EZ Chrome
- Hypersil BDS C18 column (150 mm×5.6 mm, 5.0 µm)
- Analytical weight balance: Mettler Toledo, Schwerzenbach, Switzerland;
- Water bath : Metalab scientific industries Ltd;
- Oven : Lab line, India;
- pH meter : Lab line, India; 0.45 micron nylon filters.

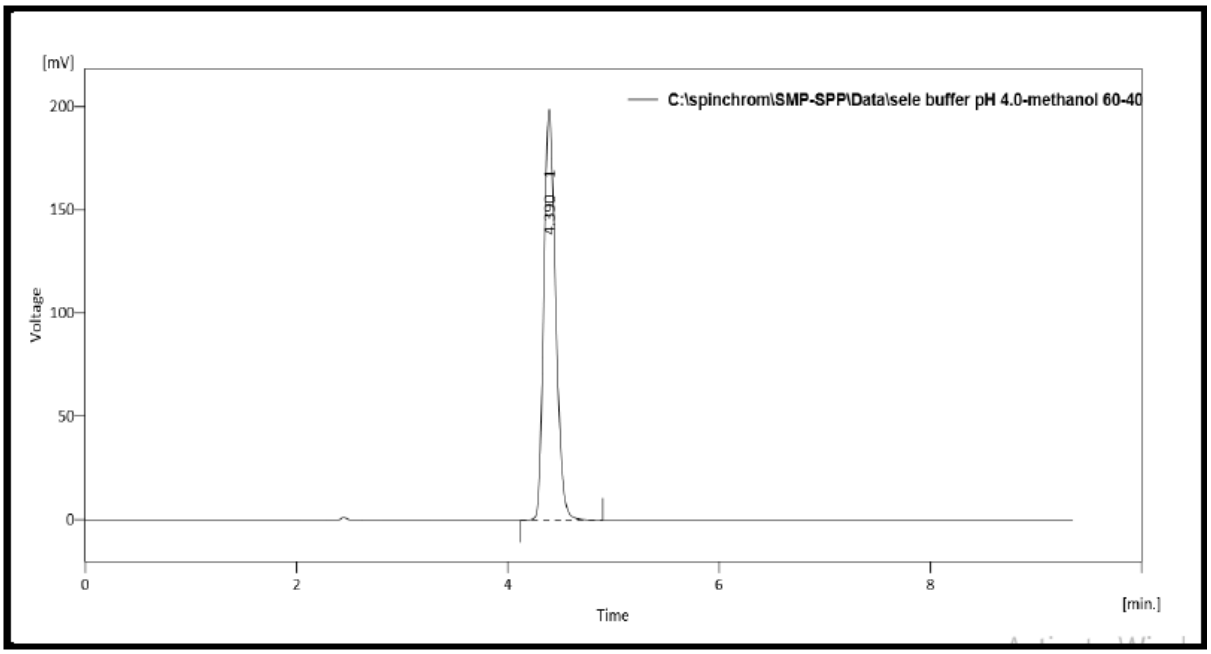

Figure 2: Chromatogram of Selexipag.

Table 1: System suitability parameters of Selexipag by RP- HPLC method.

Method Parameters	Optimized value
Column	Hypersil BDS C18 column (150 mm×5.6 mm, 5.0µm)
Analytical Wavelength	296 nm
Mobile phase	Buffer(ph-5.0): Methanol (60:40)
Pump mode	Isocratic
Flow rate	1.0 mL/min
Volume of Injection	20 µL
Run Time	10 in.

Method validation

- The developed method as described above was validated for various parameters like system suitability, robustness, specificity, linearity, precision, accuracy, LOQ and LOD.

System suitability

Table 2: System suitability parameters of SELEXIPAG by RP- HPLC method.

Parameters	SELEXIPAG
Retention time (Minutes)	5.4
Theoretical plates (TP)	7062
Tailing factor (Tf)	1.4

Linearity and range

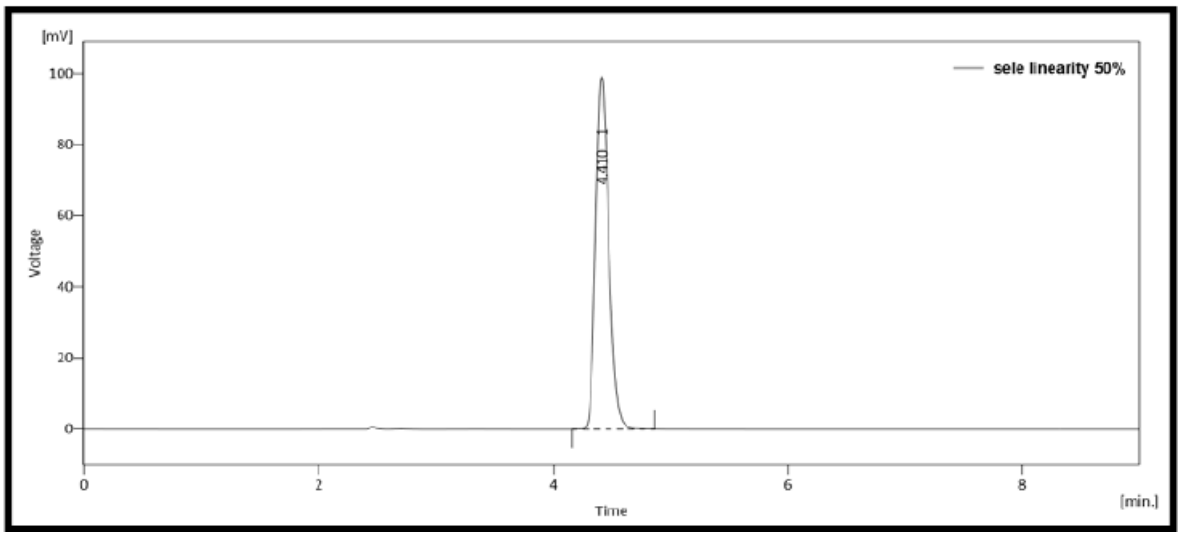

Figure 3: Linearity of Selexipag(50%).

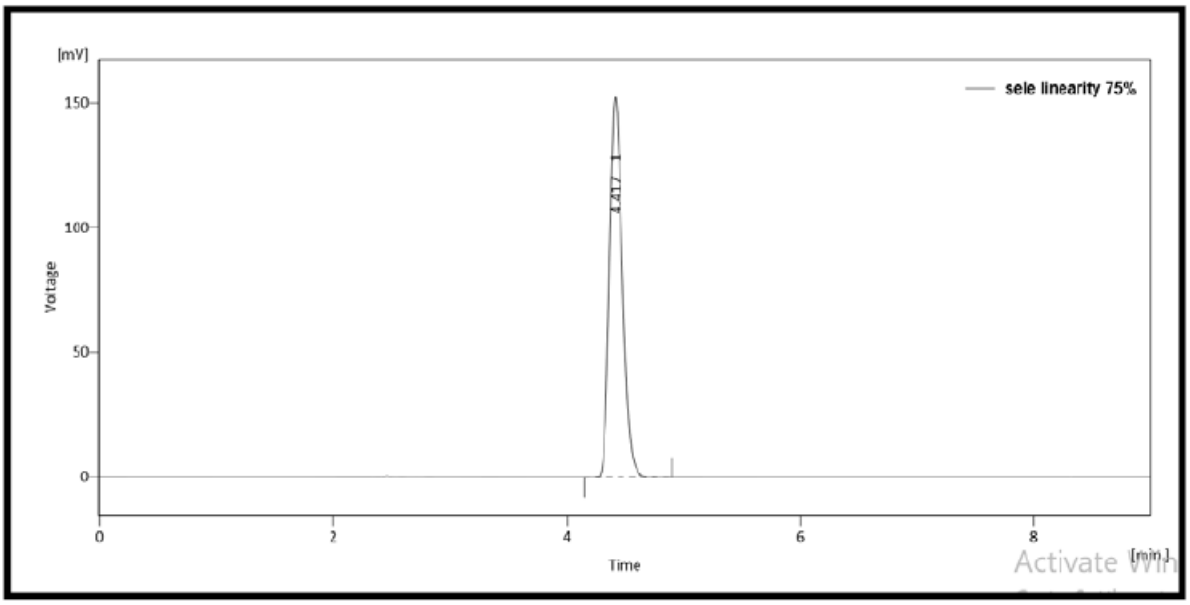

Figure 4: Linearity of Selexipag(75%).

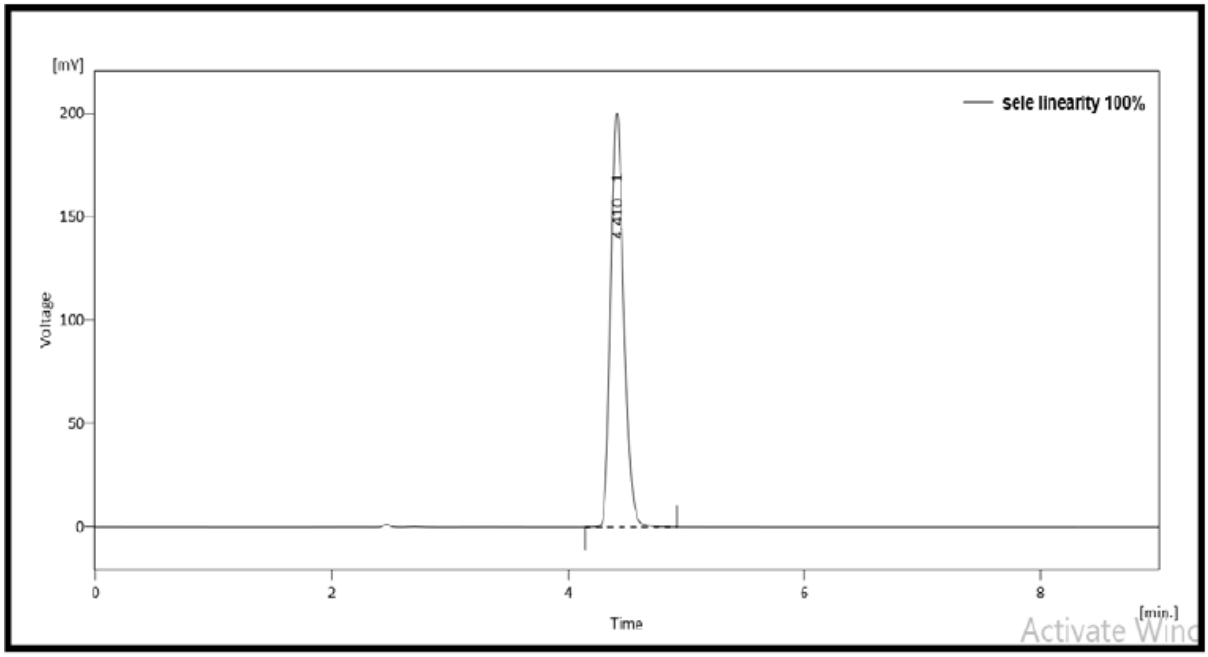

Figure 5: Linearity of Selexipag(100%).

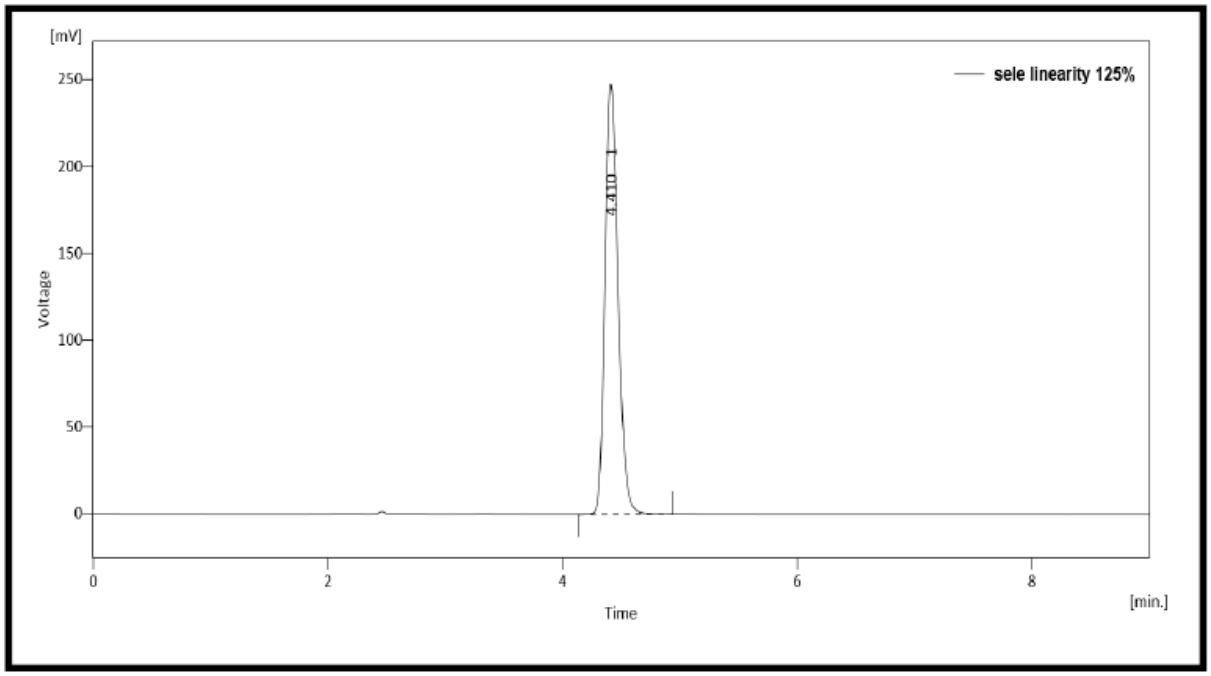

Figure 6: Linearity of Selexipag(125%).

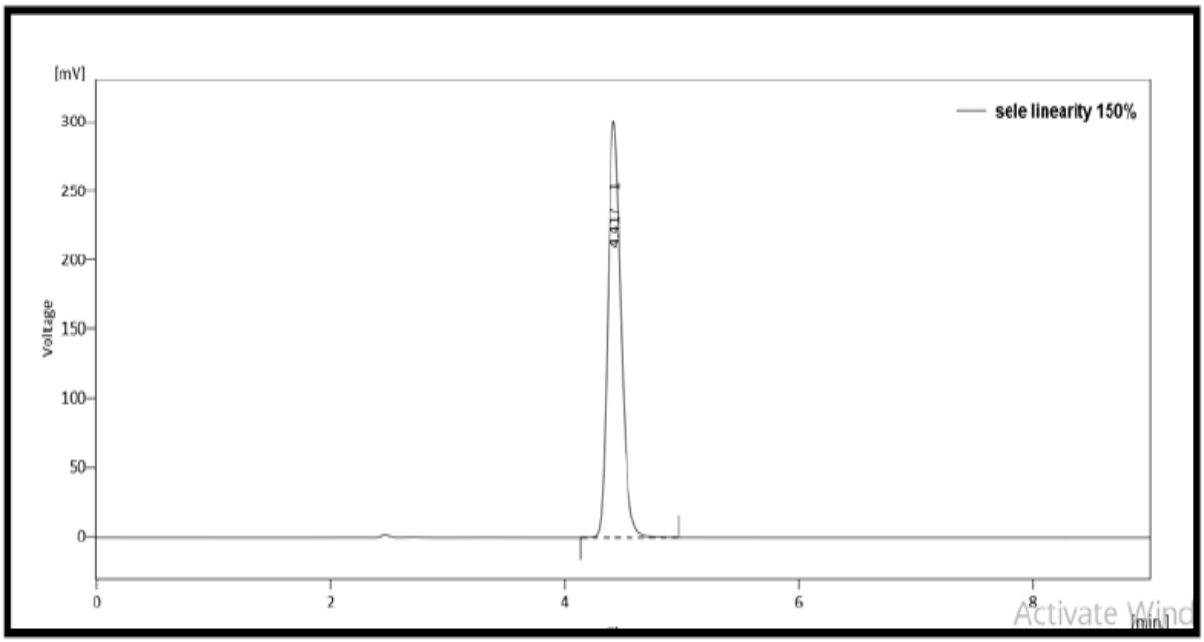

Figure 7: Linearity of Selexipag(150%).

- **Analysis data**

Result Table (Uncal - sele linearity 50%)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	4.410	770.565	99.262	100.000
	Total	770.565	99.262	100.000

Result Table (Uncal - sele linearity 75%)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	4.417	1186.479	152.552	100.000
	Total	1186.479	152.552	100.000

Result Table (Uncal - sele linearity 100%)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	4.410	1559.379	200.742	100.000
	Total	1559.379	200.742	100.000

Result Table (Uncal - sele linearity 125%)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	4.410	1924.313	247.686	100.000
	Total	1924.313	247.686	100.000

Result Table (Uncal - sele linearity 150%)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	4.417	2346.120	301.491	100.000
	Total	2346.120	301.491	100.000

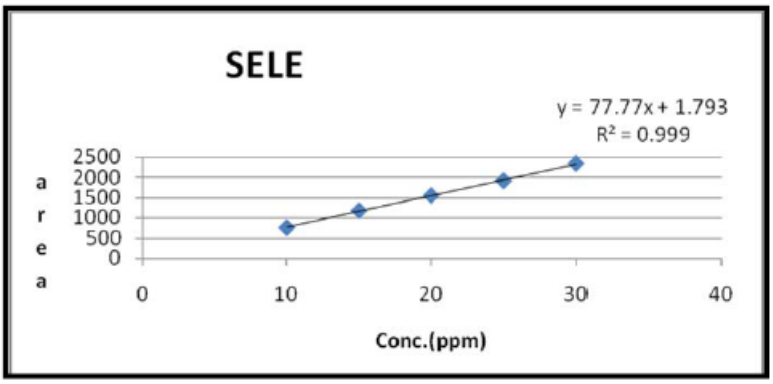

Figure 8: Calibration curve of Selexipag.

Accuracy

Table 3: Percentage recovery of Selexipag.

Drug	Level	Amount taken (µg/mL)	Amount added (µg/mL)	Amount recovered (µg/mL) (n=3)	Mean % Recovery (n=3)
SELEXIPAG	80%	10	8	8.079825842	100.997823
	100%	10	10	10.10478124	101.0478124
	120%	10	12	12.12787391	101.0656159

Precision

Method precision (Repeatability)

Table 5: Method precision data for Selexipag.

Name	Area of SELEXIPAG
Method Precision-1	1545.415
Method Precision-2	1555.684
Method Precision-3	1536.066
Method Precision-4	1552.966
Method Precision-5	1560.72
Method Precision-6	1568.532
Mean	1553.063833
SD	11.38373022
% RSD	0.73298534

Intermediate precision (Reproducibility)

Table 6: Intermediate Precision data of Selexipag.

Drug	Conc µg/mL	Intra-day measured mean area ± % RSD (n=3)	Inter-day measured mean area ± % RSD (n=3)
SELEXIPAG	50%	0.80710433	0.512074
	100%	0.404159041	0.450207
	150%	0.373659757	0.474621

Table 7: Assay results for Selexipag in formulation.

Formulation	Label claim (mg)	Amount found (mg)	% Assay ± S.D
SELEXIPAG	50	49.27208972	98.5417945 ±**0.466**

Limit of Detection and Quantification

Table 8: Limit of Detection and Quantification.

Selexipag	LOD	LOQ
	0.10219175 µg/ml	0.309671971 µg/ml

Forced degradation of Selexipag API

Table 9: Results of forced degradation study for Selexipag API.

Area of std 1539.291				
Condition	**Area**	**% Deg Std**	**Area**	**% Deg Sample**
Acid	1341.793	12.83	1351.065	12.23
Base	1388.333	9.81	1355.336	12.02
Thermal	1315.358	15.61	1326.248	13.84
Oxidation	1301.3	15.46	1288.948	16.26
Photolytic	1353.082	12.10	1366.62	11.22

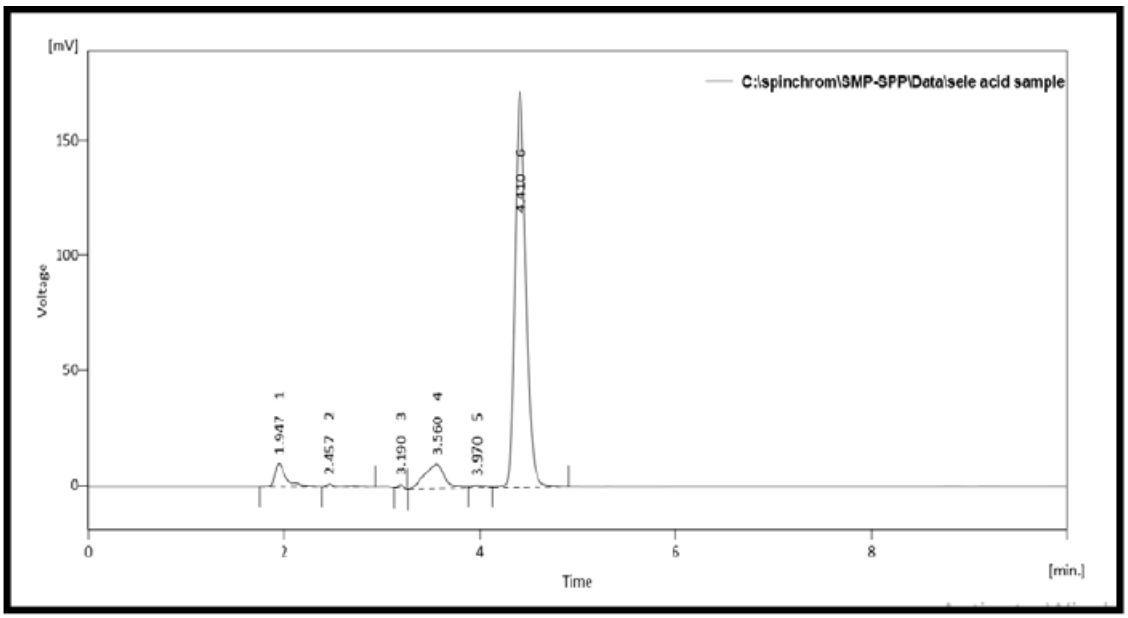

Figure 9: Chromatograms of SELEXIPAG in HCl at room temperature.

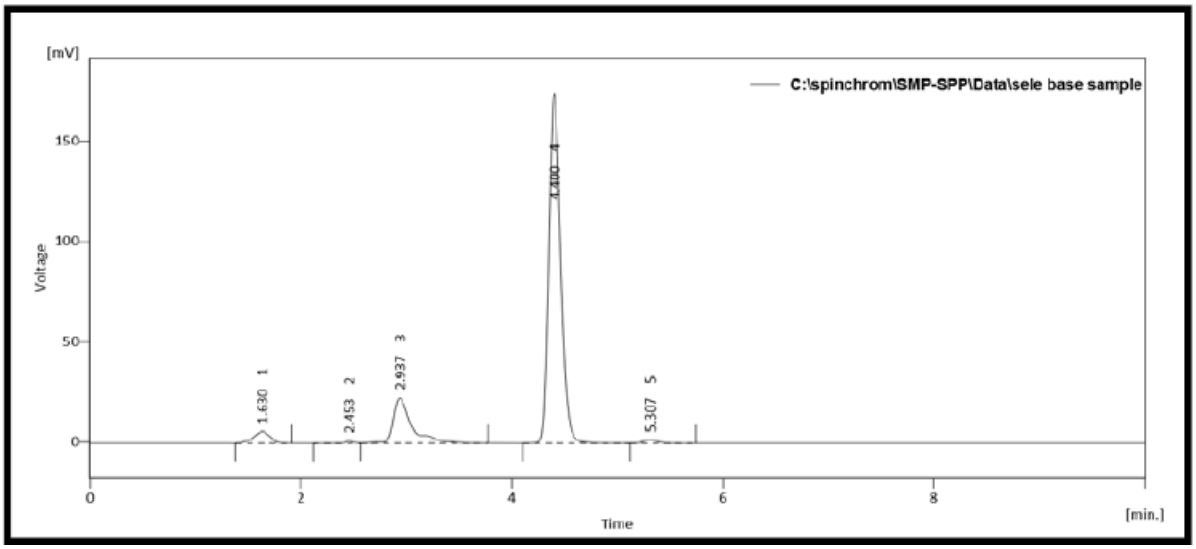

Figure 10: Chromatograms of SELEXIPAG in NaOH at room temperature.

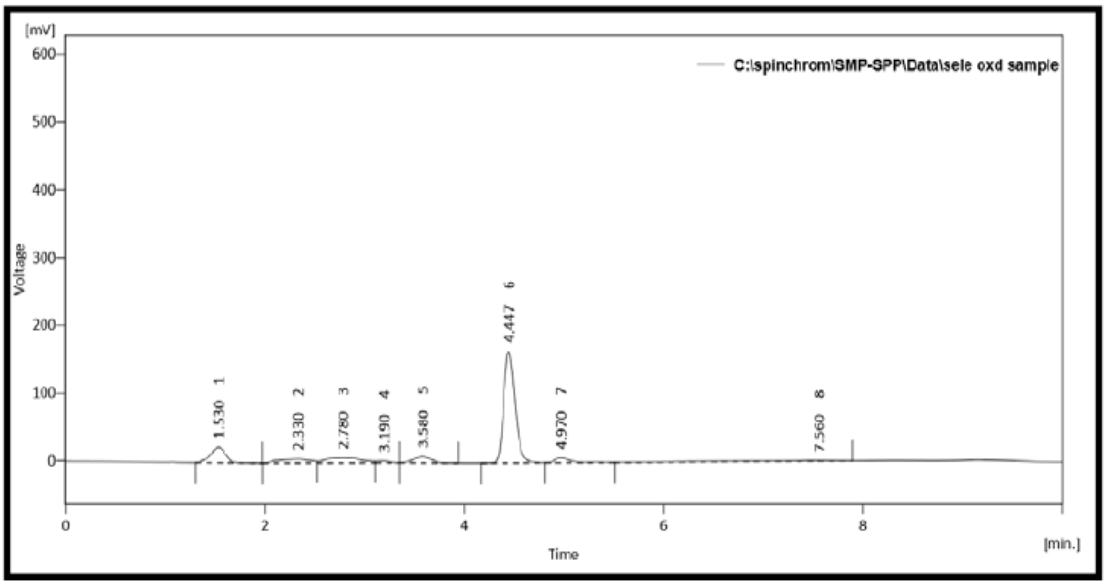

Figure 11: Chromatograms of SELEXIPAG in H_2O_2.

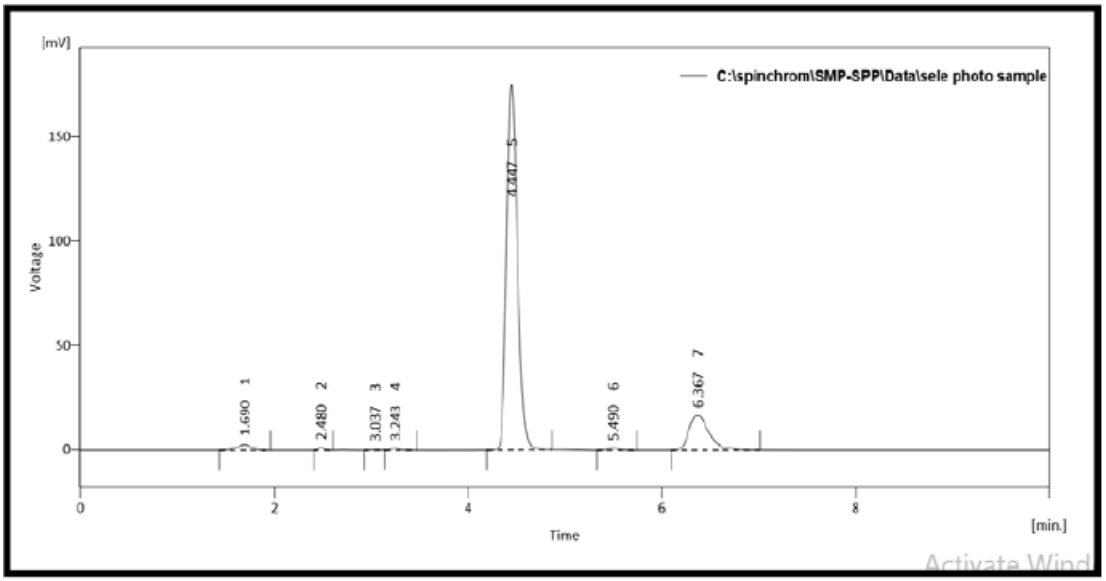

Figure 12: Chromatograms of SELEXIPAG in U.V.

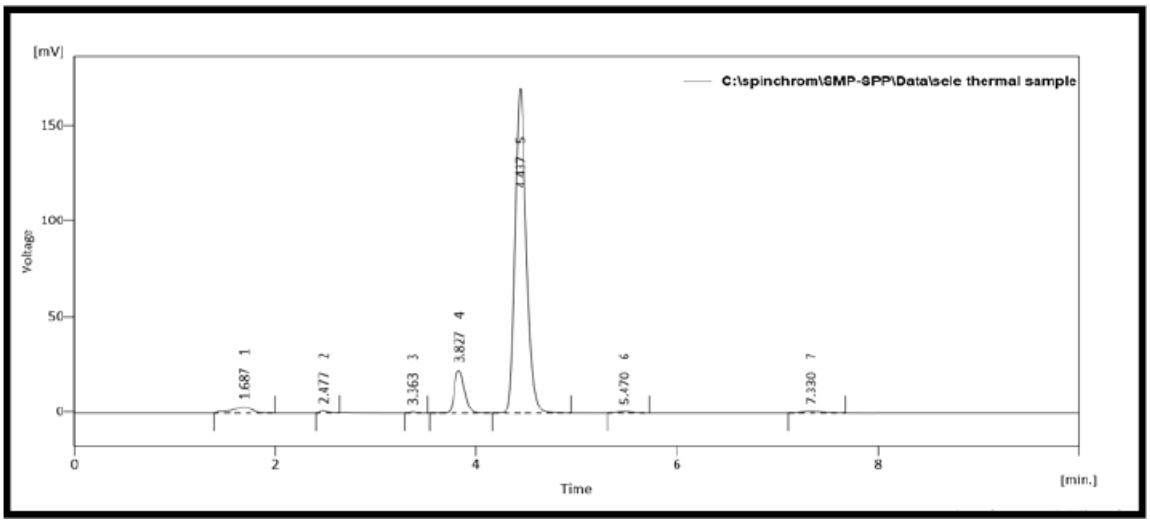

Figure 13: Chromatograms of SELEXIPAG thermal.

Summary of Validation Parameters

Table 10: Data indicating results of validation parameters.

Validation parameter		Results
Retention time (Minutes)		5.4
Accuracy (% Recovery)		100.99-101.06%
Linearity and Range		20 µg/mL-60 µg/Ml
Correlation coefficient (r2) (n=6)		0.999
Limit of Detection By equation method		0.1021 µg/mL
Limit of Quantification By equation method		0.3096 µg/mL
Precision		
Repeatability		0.73%
Intermediate precision	**Intraday Precision**	0.37-0.80%
	Inter-day Precision	0.45-0.51%
Assay		98.54%
System suitability		Meets the system suitability Criteria

3. CONCLUSION

Based on the results of the above studies, it is concluded that the method for determination of assay of Selexipag is precise, linear over the concentration range, stability indicating, and robust. The method is specific for the quantization of assay of Selexipag in pharmaceutical formulation. So the developed method can be easily applied for routine analysis of Selexipag in its Pharmaceutical dosage form.

The method was found to be simple accurate economical and rapid and it can be applied for routine analysis in laboratories and suitable for the quality control of bulk and pharmaceutical

formulations.

4. REFERENCES

1. Sitbon, O.; Morrell, N. (2012). "Pathways in pulmonary arterial hypertension: The future is here". European Respiratory Review, 21(126): 321–327. doi:10.1183/09059180.00004812. PMID 23204120.
2. Information des Bundesamtes für Sicherheit im Gesundheitswesen zu Uptravi (in German), Österreichisches Bundesamt für Sicherheit im Gesundheitswesen, 2017- 06-07 Chobanian AV, Bakris GL, Black HR, et al. The seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. JAMA, 2003; 289: 2560–72.