RESEARCH PAPER

Application of RP-HPLC Technique for Quantitative Determination and Validation of Selexipag drug in bulk and tablet formulation

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ABSTRACT
A Simple, rapid and precise RP-HPLC method was validated for the quantitative determination of Selexipag in bulk and tablet dosage form. The HPLC method was developed by using C18 column; (4.6mm x 250mm;5µm) at 300nm, flow rate of 1.2ml/min, column oven temperature of 30ºC using Mobile phase as Acetonitrile and 0.1 % Isopropyl Alcohol (90:10v/v). The Retention time was found to be 4.1 min. Linearity was established for Selexipag in the range of 5-25µg/ml, r^2=0.999. The LOD and LOQ were obtained at 0.460 and 1.400µg/ml. The percentage recovery of Selexipag was found to be 101 %, and % RSD was found within limits (<2.0). The analytical method was validated according to ICH guidelines (ICH, Q2[R1]). The method can be suggested for routine analysis of Selexipag substance in pharmaceutical dosage form.
Keywords: Selexipag, Acetonitrile, Methanol, Validation.
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INTRODUCTION

Selexipag chemically known as *N-(methanesulfonyl)-2-{4-[(propan-2-yl) (pyrazin-2-yl)amino]butoxy}acetamide*, is a selective non-prostanoid agonist of the prostacyclin (IP) receptor. It is primarily used in the management of pulmonary arterial hypertensions to reduce blood pressure in the arteries connecting the heart and lungs.

Selexipag exhibits poor in aqueous media, particularly at low pH conditions, however it is freely soluble in methanol, acetonitrile, ethanol and DMSO. The molecular of selexipag is $C_{26}H_{32}N_4O_4S$, with a molecular weight of 496.63g/mol.

Pharmacokinetically, Selexipag demonstrate an onset of action within 1-3 hours and with peak effects observes around 3-4 hours post administration. The drug has a relatively short elimination half-life ranging from 0.8 to 2.5 hours. It is predominantly excreted via urine and feces.

Several analytical methods have been reported for the estimation and stability assessment of selexipag. Notably, Santhosh SS and Devi NK et al. developed and validated a stability-indicating RP-HPLC method for selexipag in bulk and pharmaceutical dosage forms. Similarly, Saloni Barode and J. Bhangale Charushila et al. reported a validation RP-HPLC method incorporating forced degradation studes for bulk drug and formulations. Furthermore, Yasmin M. Youssef, Marianne A. Mahrouse, and Eman A. Mostafa et al. established a Stability-indicating RP-HPLC method for quantitative determination of Selexipag in both bulk and dosage forms.

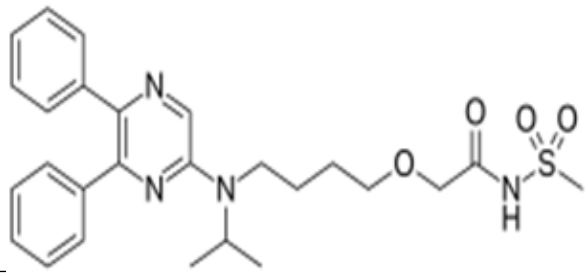

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Figure 1: Chemical structure of Selexipag

METHODS AND MATERIALS

Material

The Instruments were Shimadzu LC-20AD HPLC, Shimadzu UV-1800ENG240V UV Visible Spectroscopy, Shimadzu ATY224 Electronic balance, Shimadzu SONICA2200MH digital ultra sonicator, Hot air oven (VISION LAB EQUIPMENT), UV Cabinet (MONOQUARTZ).

Chemicals

The Chemicals used in this work included Selexipag, Methanol (HPLC grade Merck), Acetonitrile (HPLC grade Merck), Isopropyl Alcohol and Water for HPLC (Merck).

Chromatographic Conditions

High-Performance Liquid Chromatography (HPLC) analysis was performed using a Photodiode Array (PDA) detector. Separation was achieved on a C18 column packed with 5 µm particle size, having dimensions of 4.6 mm internal diameter × 250 mm length.

The mobile phase consisted of a mixture of acetonitrile and isopropyl alcohol in the ratio of 90:10 (v/v). This composition was optimized after evaluating different solvent systems to achieve suitable chromatographic performance in accordance with standard analytical guidelines.

A standard stock solution of Selexipag was prepared by accurately weighing 5 mg of the drug and dissolving it in 10 mL of acetonitrile. Further dilutions were carried out using the mobile phase to obtain a working standard solution with a concentration of 10 µg/mL.

Prior to analysis, the sample was filtered through a 0.45 µm membrane filter to remove particulate matter and ensure system suitability.

Under the optimized chromatographic conditions, Selexipag was eluted at a retention time of approximately 4.1 minutes, with a total run time of 5.5 minutes.

RESULTS AND DISCUSSION

System Suitability:

The chromatographic system was filled with prepared standard solutions. All of the parameters retention time, theoretical plates, tailing factor, and HETP were found within limits based on the system suitability studies. Under optimized conditions of concentration 10µ/ml, is presented in the values of the system suitability parameters are listed in table 1, and figure 2 displays the system suitability representative chromatograms.

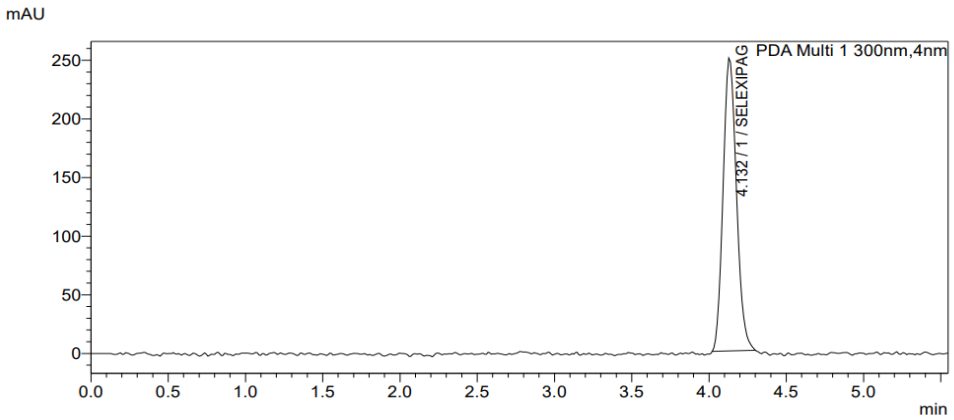

Figure-2: Chromatogram of System Suitability

Table-1 System Suitability Data

S.NO	PEAK AREA	RET TIME	PLATE COUNT	PEAK HEIGHT	TAILING
1	1497885	4.132	8669	249630	1.155
2	1499478	4.134	8789	255045	1.153
3	1495764	4.131	8695	251158	1.124
4	1498498	4.135	8629	248448	1.133
5	1491149	4.133	8688	247209	1.151
6	1496963	4.137	8650	250869	1.15
Average	1496622.83	4.13	8686.67	250393.17	1.26
STDEV	2969.09	0.00	55.72	2718.67	0.01
% RSD	0.20	0.05	0.64	1.09	1.01
Limits	_	_	>2000	_	<2.0
% RSD	<2.0	<2.0	<2.0	<2.0	<2.0

Linearity:
Linearity range was found to be 5-25µg/ml for Selexipag. The correlation coefficient was found to be 0.999. A calibration curve prepared by plotting peak area as a function of concentration of drug solution and the graph of same shown in figure-3.

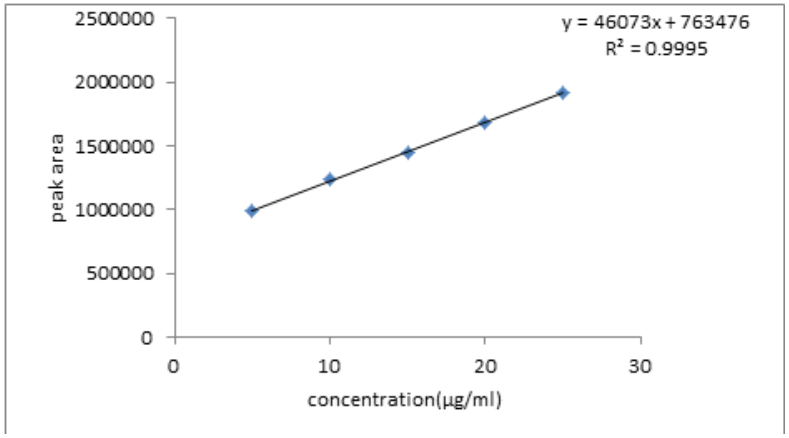

Figure-3: Calibration curve for Linearity

Precision:
The Chromatographic system was injected with a 100% concentration (15µg/ml), and %RSD was less than 2 which passes the test for precision. The values being exhibited in table 2 (a) & 2 (b) and the representative chromatogram in figure 2(a) and (b).

Table 2 (a): Inter-Day Precision for proposed HPLC method

S. No	Day 1	Day 2	Day 3
1	1596806	1588267	1508429
2	1571539	1590730	1502757
3	1586958	1591421	1500664
4	1563329	1586950	1516253
5	1591260	1589894	1495348
6	1585800	1590268	1512667
Average	1582615.33	1589588.33	1506019.67
STDEV	12647.17	1668.78	7847.62
%RSD	0.80	0.10	0.52
Limits	%RSD: <2.0	%RSD: <2.0	%RSD: <2.0

Table 2 (b): Intra Day Precision for proposed HPLC Method

S. No	9:00am	1:00pm	5:00pm
1	1543884	1567965	1508976
2	1568288	1559569	1524567
3	1549710	1556049	1502345
4	1581101	1509271	1502692
5	1572268	1579944	1538896
6	1588305	1593185	1501132
Average	1567053.60	1560997.17	1513101.33
STDEV	19453.61	28794.83	15369.86
%RSD	1.24	1.84	1.02
Limits	%RSD<2.0	%RSD<2.0	%RSD<2.0

Accuracy:
A series of 50%, 100% and 150% solutions were occupied by taking the tablet powder equivalent to Selexipag drug. The resulting chromatograms for the above were interpreted and percentage recovery seem to be within limits i.e., 98-102%. The values being showed in table 3 and the resulted chromatograms are presented in figure 4.

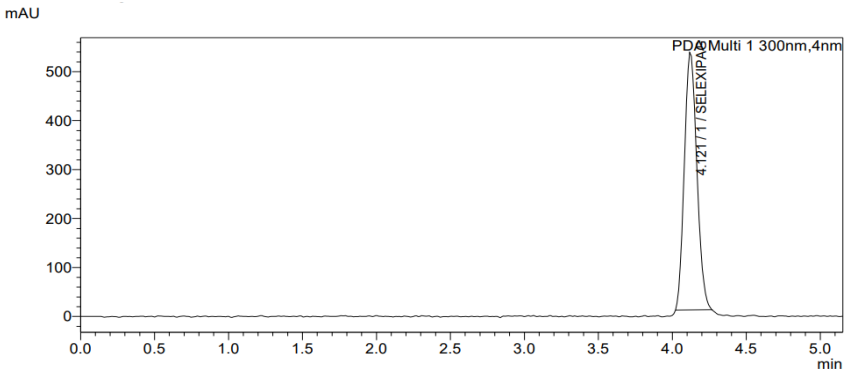

Figure-4: Chromatogram with 100% Accuracy

Table 3: Accuracy displaying the Recovery Percentage

%spiked	Sample ppm	Std ppm	Amount found	%recovery
50%	7.5µg/ml	15µg/ml	7.5742	100.9890
	7.5µg/ml	15µg/ml	7.3571	98.0951
	7.5µg/ml	15µg/ml	7.5742	100.9890
100%	15µg/ml	15µg/ml	14.9480	99.6533
	15µg/ml	15µg/ml	14.9708	99.8052
	15µg/ml	15µg/ml	15.1207	100.8047
150%	22.5µg/ml	15µg/ml	22.6643	100.7302
	22.5µg/ml	15µg/ml	22.5379	101.9649
	22.5µg/ml	15µg/ml	22.5379	100.1683

Table 4: LOD and LOQ

S.No	Parameter	Results
1	LOD	0.460µg/ml
2	LOQ	1.400µg/ml

Degradation Studies:

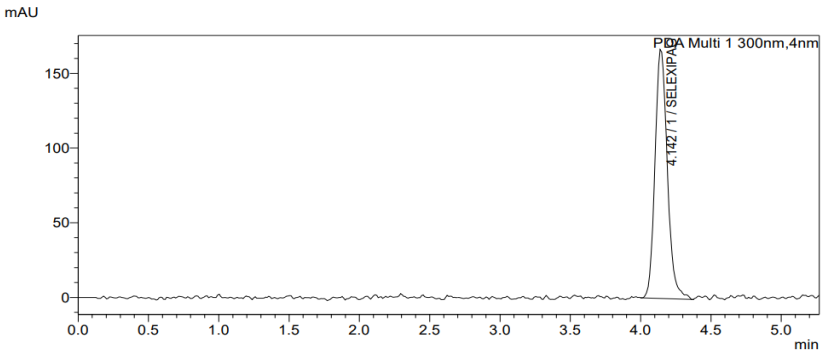

Figure 5 (a): Acid Degradation

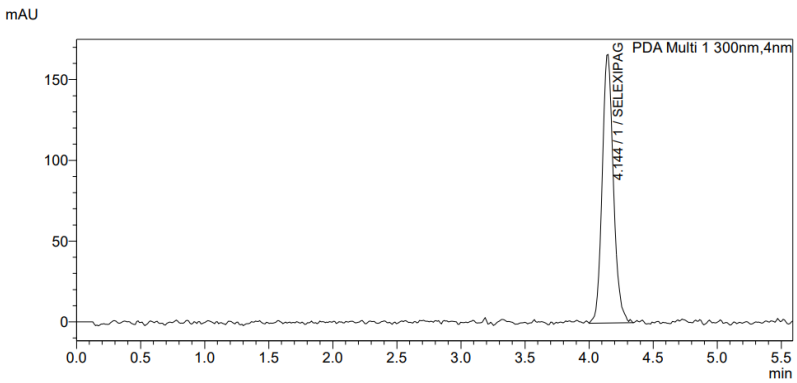

Figure 5 (b): Base Degradation

Table 5: Degradation studies

S. No	Condition	Peak Area	% Assay	% Degradation
1	Acid	1117599	93.624	6.376
2	Base	1122629	94.045	5.955
3	Temperature	1139071	95.422	4.578
4	Peroxide	1105670	92.624	7.376
5	UV	1103262	92.423	7.577
Average		-		
STDEV				
% RSD				
Limits	**Average Assay: 90; % Degradation:10%**			

CONCLUSION

Selexipag in both active pharmaceutical ingredient (API) and tablet dosage form was quantitatively estimated using a developed Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method. The method was successfully validated in accordance with ICH Q2(R1) guidelines for analytical method validation and ICH Q1A(R2) guidelines for stability studies.

The chromatographic system demonstrated satisfactory performance, with theoretical plate count exceeding 2000, indicating good column efficiency. The method exhibited excellent linearity over the concentration range of 5–25 µg/mL.

Validation parameters, including accuracy, precision, and robustness, were found to be within acceptable limits, as evidenced by percentage relative standard deviation (%RSD) values of less than 2%, complying with regulatory requirements. The sensitivity of the method was confirmed by determining the limit of detection (LOD) and limit of quantification (LOQ), which were found to be 0.460 µg/mL and 1.400 µg/mL, respectively.

Forced degradation studies were performed on the tablet dosage form to evaluate the stability-indicating capability of the method. Approximately 10% degradation of the drug was observed under stress conditions. Importantly, the method was able to effectively separate Selexipag from its degradation products, confirming its specificity and suitability as a stability-indicating method. The retention time of Selexipag was consistently observed at 4.1 minutes.

Based on the obtained results, the developed RP-HPLC method can be considered sensitive, accurate, precise, reproducible, cost-effective, and rapid. Therefore, it is suitable for routine analysis of Selexipag in both bulk drug and pharmaceutical dosage forms with high reliability and confidence.

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