



Stability Indicating Method Development and Validation of Selexipag in Bulk and Pharmaceutical Dosage form by using RP-HPLC

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Research Article

Volume 5 Issue 2

Received Date: March 18, 2021

Published Date: May 27, 2021

DOI: 10.23880/oajpr-16000239

Abstract

A simple, efficient, precise, accurate & stable method has been developed and validated for determination of Selexipag in pharmaceutical tablet dosage form. An efficient high performance liquid chromatographic method was developed for Selexipag. The HPLC method was developed by using Symmetry C18 column; (150×4.6×5μ) column at 281nm, flow rate of 0.6ml/min., Injection volume of 20μl, column oven temperature of 25°C using equal volume of Methanol and Water used as mobile phase (50:50v/v). The retention times were found to be 2.682mins. The % purity was found to be 99.9 w/w. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The correlation coefficient (r²) was found to be 0.999%, recovery was 100.0%, %RSD for precision was found to be 0.2.

The HPLC method was found to be accurate, precise, economical and reproducible. The method can be suggested for routine analysis and method can be recommended for determination of substance related, relative substance of Selexipag in pharmaceutical dosage form.

Keywords: Selexipag; High performance liquid chromatography (HPLC); Analytical method; Validation

Introduction

High Performance Liquid Chromatography

HPLC is a form of liquid chromatography used to separate compounds that are dissolved in solution. HPLC instruments consist of a reservoir of mobile phase, a pump, an injector, a separation column, and a detector.

Compounds are separated by injecting a sample mixture onto the column. The different component in the mixture pass through the column at differentiates due to differences in their partition behavior between the mobile phase and the stationary phase [1,2].

High performance liquid chromatography (HPLC) is basically a highly improved form of column liquid

chromatography. Instead of a solvent being allowed to drip through a column under gravity, it is forced through under high pressures of up to 400 atmospheres. That makes it much faster.

All chromatographic separations, including HPLC operate under the same basic principle; separation of a sample into its constituent parts because of the difference in the relative affinities of different molecules for the mobile phase and the stationary phase used in the separation.

Types of HPLC

In general there are five techniques of HPLC. They are as follows:

1. Based on Polarity

Normal Phase HPLC: NP-HPLC uses polar stationary phase and non-polar mobile phase.

Reverse Phase HPLC: The stationary phase is nonpolar (hydrophobic), while the mobile phase is a polar.

2. Based on principle of separation

- Adsorption chromatography
- Ion exchange chromatography
- Ion pair chromatography
- Size exclusion chromatography
- Chiral chromatography

3. Based on Elution technique

- Isocratic
- Gradient

4. Based on scale of preparation

- Analytical HPLC
- Preparative HPLC

5. Based on type of analysis

- Qualitative
- Quantitative.



Figure 1: Showing HPLC Instrument.

Reversed Phase Chromatography

In 1960s, chromatographers started customizing the polar nature of the silanol group by chemically responding silicon with organic silanes. The object was to make silica much less polar or non-polar so that polar solvents will be used to divide water-soluble polar substances. Because the ionic nature of the chemically customized silica is now turned around i.e., it is non-polar or the nature of the stage is

returned, the chromatographic split up carried out with such silica is described as reversed-phase chromatography. For numerous purposes, reversed phase fluid chromatography (RPLC) is considered to be the tool of choice for the study of pharmaceutical substances, such as its compatibility with aqueous and natural solutions along with various detection systems and its high accuracy and repeatability.

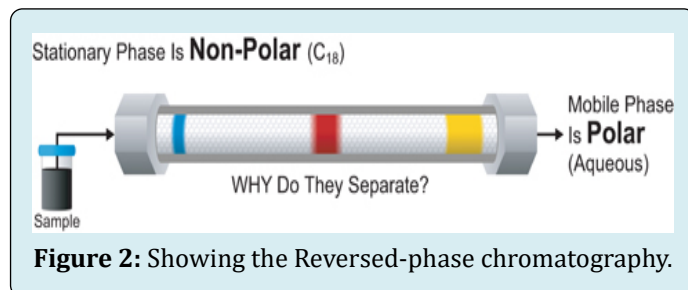


Figure 2: Showing the Reversed-phase chromatography.

Whether in the pharmaceutical or bio-analytical field, sensitive as well as precise RPLC analysis requires the use of stationary phases which offer proportional and reliable peaks. As a result, fixed stage suppliers are continually improving and offering new RPLC goods as well as high options for various forms of reversed fixed phase stages. The criteria for continuity as well as the globalization of the pharmaceutical industry demand that the strategies can definitely be moved from website to website, using either the same brand names or their matches. The fixed phase in the Turned around Stage chromatographic columns is a hydrophobic assistance that is consisted mainly of permeable particles of silica gel in various forms (spherical or irregular) at different diameters (1.7, 3, 6, 7, 10 μm etc.) at various pore dimensions (such as 50, 100, 120, 300). The surface area of these particles is covered with various chemical entities, such as numerous hydrocarbons (C1, C6, C4, C8, C18, etc). In many techniques utilized currently to separate medical materials, C18 columns are utilized, which often are called ODS (octadecylsilane) or RP-18. A polar solvent is made use of as mobile phase. The criteria that govern the retention backwards Stage systems are the following:

- A. The chemical nature of the fixed stage surface area.
- B. The sort of solvents that compose the mobile stage
- C. pH as well as ionic strength of the mobile phase

Normal Phase Chromatography

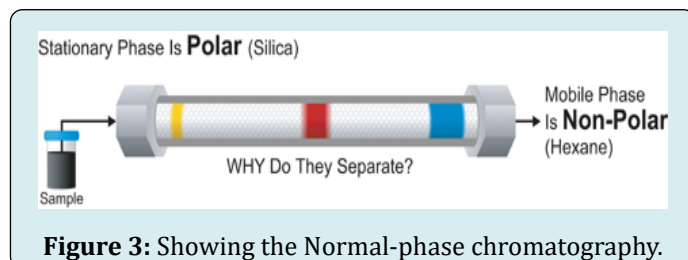


Figure 3: Showing the Normal-phase chromatography.

The mobile phase is the polar adsorbent in normal stage chromatography. A mixture of non-aqueous solvents is usually the mobile step. In standard stage separations, the silica structure is filled with the silanol team at the end.

These OH teams are statistically dispersed over the entire of the surface area. The silanol groups represent the energetic websites (extremely polar) in the stationary phase. This forms a weak bond with lots of particles in the vicinity when any one of the following interactions exist. Dipole-induced dipole, dipole-dipole, hydrogen bonding, complex bonding. These circumstances occur when the particle has one or numerous atoms with single set electrons or a dual bond.

The adsorption toughness as well as for this reason

'K' value (elution series) rise in the complying with order. Saturated hydrocarbon < olefins < aromatic < natural < halogen substances < sulphides < ethers < esters < aldehydes and also ketones < amines < sulphones < amides < carboxylic acids. The stamina of communications depends not only on the practical groups in the sample particle yet also on steric aspects. If a molecule has numerous useful groups, then one of the most polar one determines the reaction buildings.

Chemically customized silica, such as aminopropyl, cyanopropyl as well as diol stages are the stationary stages alternate to silica gel in typical phase chromatography. The aminopropyl and also cyanopropyl stages offer opportunities for particular communications in between the analyte as well as the stationary phase and therefore supply extra alternatives for the optimizations of splittings up.

Proprietors	Normal Phase	Reversed Phase
Polarity of Stationary Phase	High	Low
Polarity of mobile Phase	Low to medium	Low to high
Sample elution order	Leader polar First	Most polar First
Retention will increase by	Increasing surface of stationary phase Increasing of n-alkyl chain length of stationary phase. Decreasing polarity of mobile phase Increasing polarity of sample	Increasing surface of stationary phase. Increasing polarity of mobile phase Decreasing polarity of sample molecules

Table1: Showing the comparison of NP-HPLC and RP-HPLC adsorption Chromatography.

The fixed phase is an adsorbent (like silica gel or any other packaging based on silica) and the separation is based on duplicated steps of adsorption-desorption.

Instrumentation of HPLC

The specific components HPLC and their working roles are reported below [3].

- Mobile phase and reservoir
- Solvent degassing system
- Pump
- Injector
- Column
- Detector
- Data system

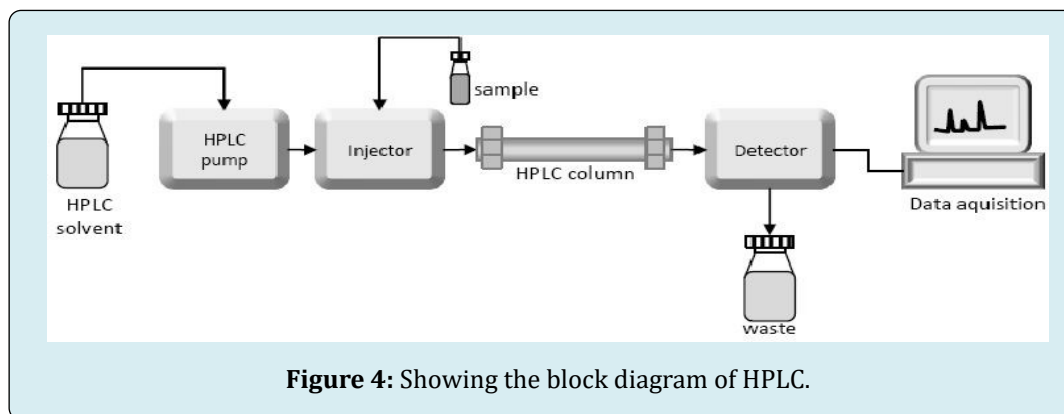


Figure 4: Showing the block diagram of HPLC.

Principle involved in HPLC

HPLC principle is based on both Adsorption as well as Partition, depending upon the nature of stationary phase. If stationary phase is solid then it is adsorption and if stationary phase is liquid then it is partition. When a mixture of components is introduced into a HPLC Column, they travel according to their relative affinities towards the stationary phase. The component which has more affinity towards the adsorbent, travels slower. The component which has less affinity towards the stationary phase travels faster. Since no two components have the same affinity towards the stationary phase, the components are separated.

Materials And Methods

Determination of absorption maxima by UV/Visible Spectroscopy

Precisely 50 mg of Selexipag was soaked up right into a 100 ml volumetric flask, and additionally 2 ml of methanol was added as well as sonicated for 2 mins to liquify in addition to watered down approximately the mark with Milli-Q water and also blended well. 1 ml of the above service was absorbed a 10 ml volumetric flask along with the volume was adjusted to 10 ml with water as well as combined well. (Focus of Selexipag is about 100µg/ ml). The resulting treatment was signed in the range of 200 nm to 400 nm [4-9]. From range 281 nm was selected as discovery wavelength. At this wavelength Selexipag showed great absorbance and also spectrum is displayed in Figure 14.

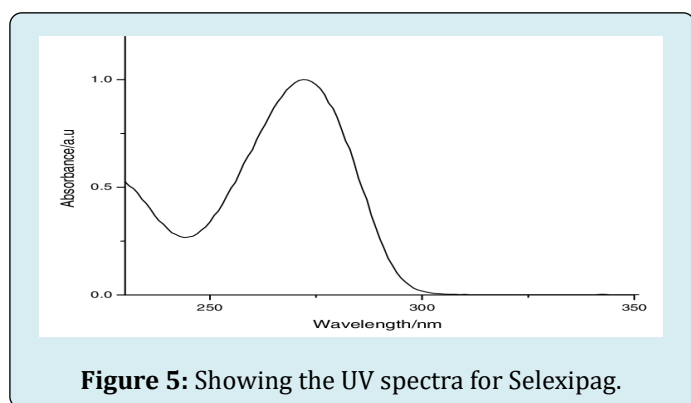


Figure 5: Showing the UV spectra for Selexipag.

Preparation of Standard Solution

Properly 50 mg of Selexipag is taken right into a 100 ml volumetric flask, as well as 2 ml of methanol was added and sonicated for 2 minutes to dissolve and watered down to the mark with Milli-Q water and mixed well. 1 ml of the above solution was added a 10 ml volumetric flask and the volume was adjusted to 10 ml with water as well as mixed well. (Concentration of Selexipag has to do with 5-30 µg/ml).

The resulting option was scanned in the range of 281nm. The mean value represents the point present on the calibration curve in the below figure

$y = 0.0374x + 0.0217$ was the linear regression equation. The correlation coefficient was greater than 0.999 [10,11].

Preparation Of Calibration Curve

Working solution was prepared from stock solution and a concentration range 5, 10 15, 20, 25, 30 µg/ml, respectively. These solutions were scanned from the range 301nm and calibration curve was obtained between concentrations of 5-30 µg/ml.

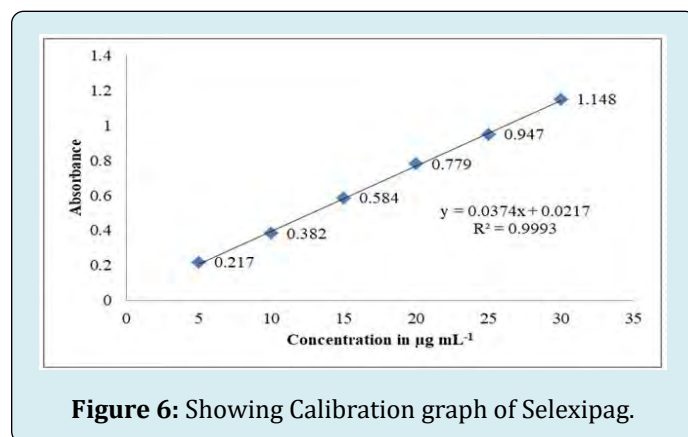


Figure 6: Showing Calibration graph of Selexipag.

Concentration	Absorbance
5	0.217
10	0.382
15	0.584
20	0.779
25	0.947
30	1.148

Table 2: Showing the Absorbance obtained in the calibration curve.

Trials

Trial - 1

Chromatographic Conditions

Stationary phase	: Seas C18 column (100 mm x 4.6 mm i.d, 5µm).
Mobile phase	: Methanol: Water (70:30 v/v).
Flow rate	: 0.6 ml/ minute.
Detector wavelength	: 281 nm.
Column temperature	: Ambient.
Injection quantity	: 20 µl

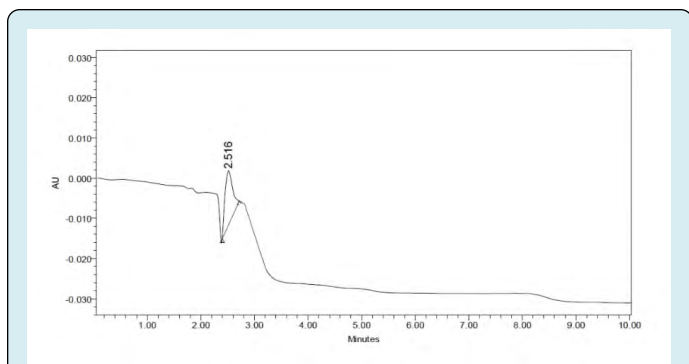


Figure 7: Chromatogram of Trial - 1.

Observation: Fronting and Tailing of the peaks was reported.

Trial - 2

Chromatographic Conditions

Stationary phase : C18 column (100 mm x 4.6 mm i.d, 5 μ m).
 Mobile phase : Phosphate buffer pH 4.6: Methanol (50:50 v/v)
 Flow rate : 0.7 ml/ minute.
 Detector wavelength : 281 nm.
 Column temperature : Ambient.
 Injection quantity : 20 μ l

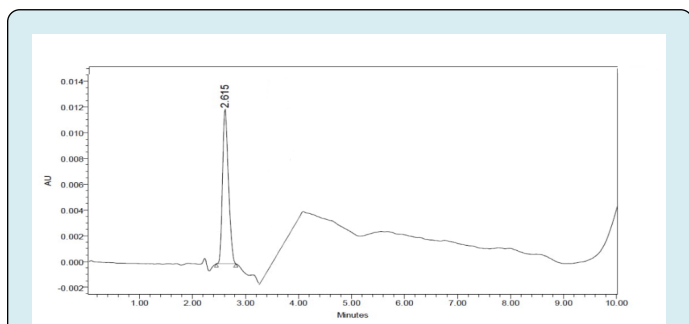


Figure 8: Chromatogram of Trial - 2.

Observation: Fronting and Tailing of the peaks was reported, so another trial was performed.

Trial - 3

Chromatographic Conditions

Stationary phase : C18 column (100 mm x 4.6 mm i.d, 5 μ m).
 Mobile phase : Methanol: Water (40:60 v/v)
 Flow rate : 0.7 ml/ minute.
 Detector wavelength : 281 nm.
 Column temperature : Ambient.
 Injection quantity : 20 μ l

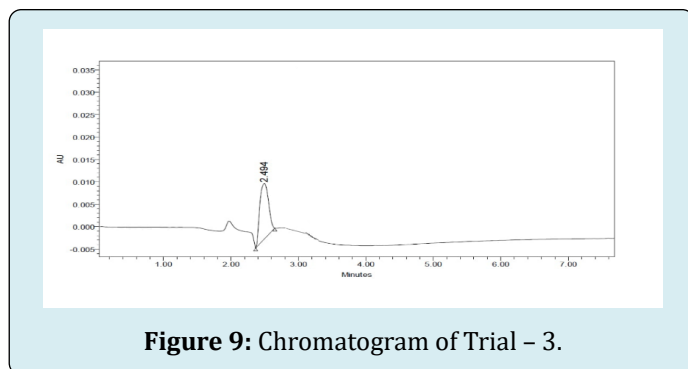


Figure 9: Chromatogram of Trial - 3.

Observation: Fronting and Tailing of the peaks was reported, so another trial was performed.

Trial - 4 (Optimized Method)

Chromatographic Conditions

Stationary phase : C18 column (100 mm x 4.6 mm i.d, 5 μ m).
 Mobile phase : Methanol: Water (50:50 v/v)
 Flow rate : 1.0 ml/ minute.
 Detector wavelength : 281 nm.
 Column temperature : Ambient.
 Injection quantity : 20 μ l

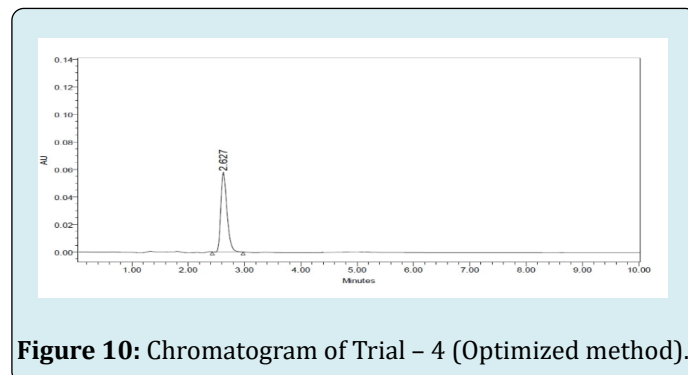


Figure 10: Chromatogram of Trial - 4 (Optimized method).

Observation: Sharp peak is observed. Retention is found to be 2.627.

Preparative Measures For Assay Method Development

Preparation of Solutions

Preparation of mobile phase: Mix a blend of HPLC Grade Water 500 ml (50%), 500 ml of Methanol HPLC (50%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45 microfilter under vacuum cleaner filtration.

Diluent Preparation: Use the mobile phase as diluents.

Preparation of the Selexipag Standard Solution Preparation: Accurately weigh 10 mg of Selexipag functioning standard into a 10ml in a tidy dry volumetric

flask add accurately 7ml of Diluent and sonicate to dissolve it completely as well as make volume as much as the mark with the same solvent.

Stock solution: Add 0.3 ml of the above stock into a 10ml volumetric flask and fill up to the mark with diluent.

Sample Solution Preparation

Accurately weigh and transfer 50 mg of Selexipag sample into a 100ml clean fully dry volumetric flask include about 70ml of Diluent and sonicate to dissolve it totally and make quantity as much as the mark with the exact same solvent.

Stock solution

Pipette out 0.1 ml of Selexipag of the above supply remedy right into a 10 ml volumetric flask and dilute up to the mark with diluent.

Procedure

Infuse 20ml of the required sample into the chromatographic system and determine the area for Selexipag optimal and determine the % Assay by utilizing the formulae.

%ASSAY =

Sample area Wt. of standard Dilution of sample Purity Weight of tablet $\times 100$

Std. area Dil. of standard Wt. of sample 100 Label claim

Assay

Selexipag is discovered to be soluble in methanol and water. The compound was dissolved in water to get concentration of 100 μ g/ml and the resulting remedy was checked under UV area. For this reason, 281nm was found maximum absorbance and the discovery wavelength for the suggested approach. In HPLC technique, the method was optimized to get an appropriate elution of compounds. Originally, various mobile phase make-ups were attempted to separate the labelled components.

The system with mobile phase of Methanol: Water in the proportion of 50:50(v/v) as well as a circulation rate of 1.0 ml/ minutes was located to be durable. The optimal wavelength for discovery was 281 nm as well as a run time of 7 minutes at which better detection was obtained. The standard chromatograms were considered the recommended approach and different system suitable specification.

The verified HPLC approach was utilized for the decision of Selexipag in solid dose form. In the assay experiment seven examples were evaluated independently and also analyzed. The mean assay results expressed as a percent of the label

claim. The outcomes indicate that the quantity of each drug in the tablet computers is within the requirements of 90-110% of the label claim.

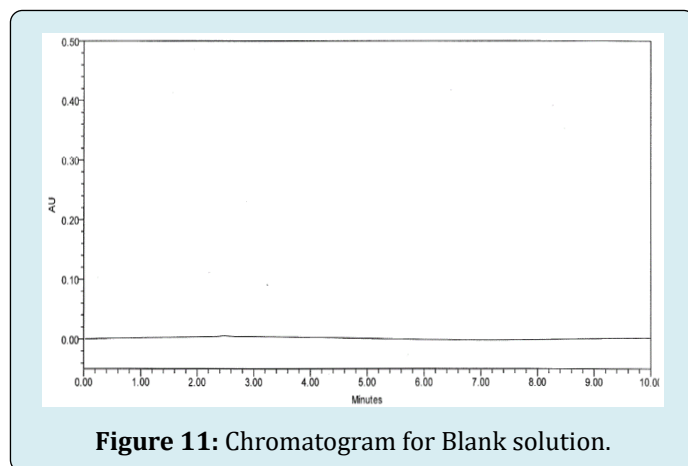


Figure 11: Chromatogram for Blank solution.

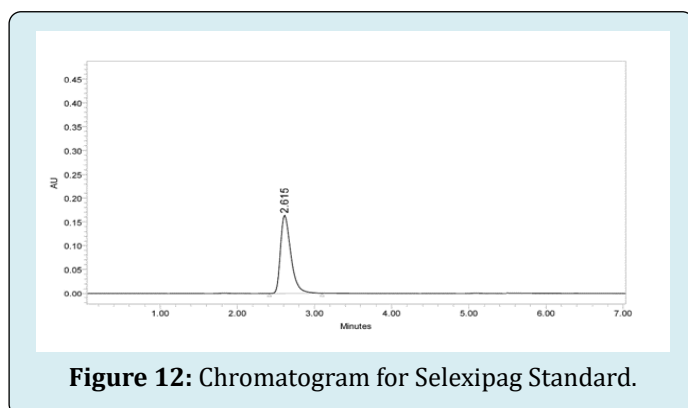


Figure 12: Chromatogram for Selexipag Standard.

S.no	Peak Name	RT	Area	Height
1	Selexipag	2.615	6008963	163883

Table 3: Showing the Area under the curve for the Assay.

Selexipag	Observations
Standard area	6008963
Sample area	6014491
Standard wt.	20mg
Sample wt.	1442mg
Avg wt.	1442mg
%purity	99.9

Table 4: Showing the observations for the Assay.

Retention time was reported to be 2.6 min and the percentage purity of the Selexipag was reported to be 99.9 % w/w.

System Suitability

To check the reproducibility of a chromatographic

system, system suitability tests are used. System suitability tests on freshly prepared standard solutions were performed to assess their effectiveness.

S. No.	Parameter	Selexipag	Acceptance criteria
1	% Relative standard deviation	0.1	Should not be more than 2.0%
2	Tailing factor for analyte peak in standard solution	1.4	Should not be more than 2.0
3	Plate count for analyte peak in standard solution	2684.8	Should be not less than 2000

Table 5: Showing the System suitability values for Selexipag.

Specificity

The specificity of the method was defined by the non-interference of the null, internal and mobile phases of the standard. The process demonstrated excellent specificity. Based on these chromatograms, we may tell that blank interference is not present.

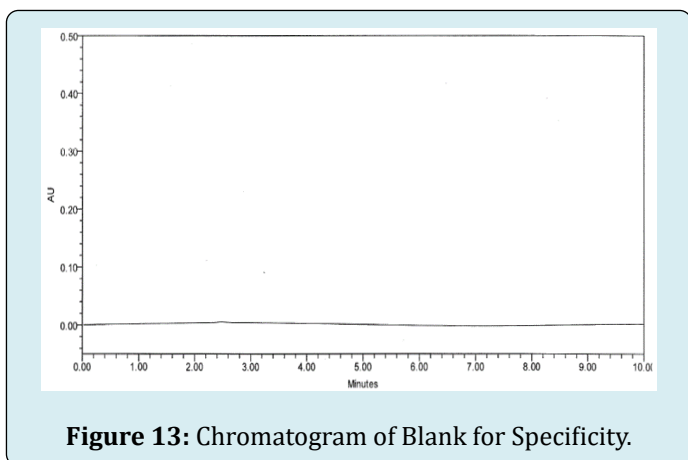


Figure 13: Chromatogram of Blank for Specificity.

The specificity analysis for the blank was carried out and the chromatograms indicate that there is no interference in the analyte's retention time.

Linearity

Linearity of an analytical method can be explained as its capability to show results that are directly proportional to the concentration of the analyte in the sample.

Linearity level	Volume of stock solution(ml)	Diluted to(ml)	Linearity conc. ($\mu\text{g/ml}$) of Selexipag
1	0.1	10	10
2	0.2	10	20
3	0.3	10	30
4	0.4	10	40
5	0.5	10	50

Table 6: Showing the observations for the Linearity.

Procedure

The linearity of the technique was demonstrated over the concentration range of 10-50 $\mu\text{g/ml}$. Aliquots of 0.1, 0.2, 0.3, 0.4, 0.5 $\mu\text{g/ml}$ was prepared from example remedy. Each level remedy was injected into chromatographic system as well as the peak area was determined. A chart of peak location versus focus (on X-axis concentration and also on Y-axis Peak location) was outlined and the relationship coefficient was calculated.

Acceptance Criteria

Regression coefficient need to not be less than 0.999.

Concentration $\mu\text{g/ml}$	Response
10	543883
20	1082795
30	1540742
40	2044051
50	2524015

Table 7: Showing the observations for the response of Selexipag at various concentrations.

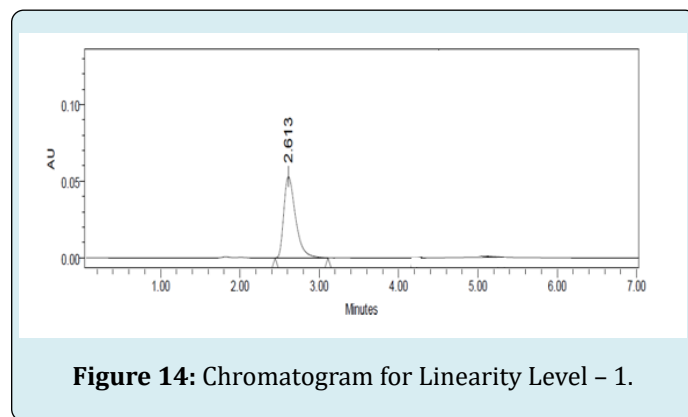


Figure 14: Chromatogram for Linearity Level - 1.

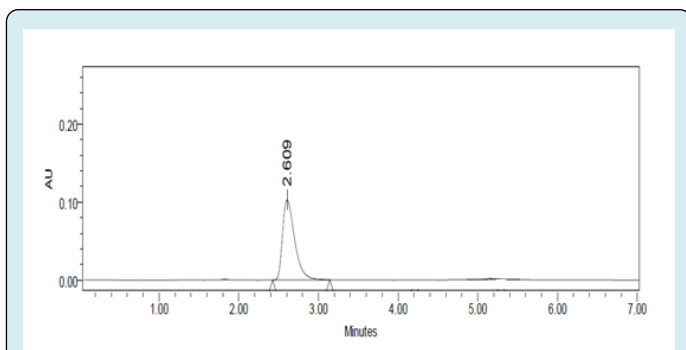


Figure 15: Chromatogram for Linearity Level – 2.

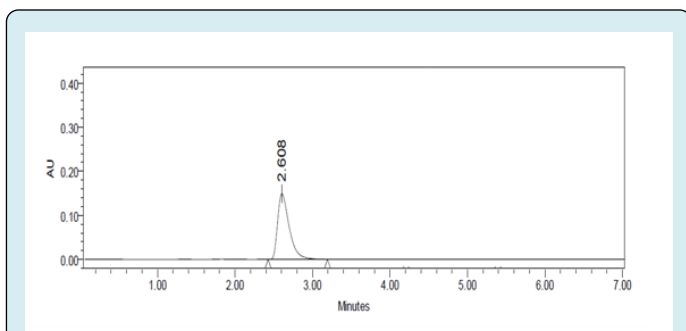


Figure 16: Chromatogram for Linearity Level – 3.

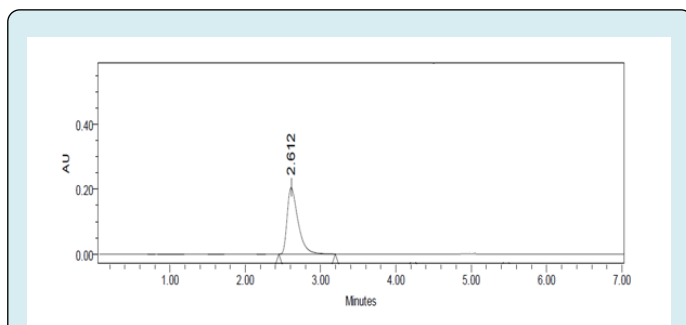


Figure 17: Chromatogram for Linearity Level – 4.

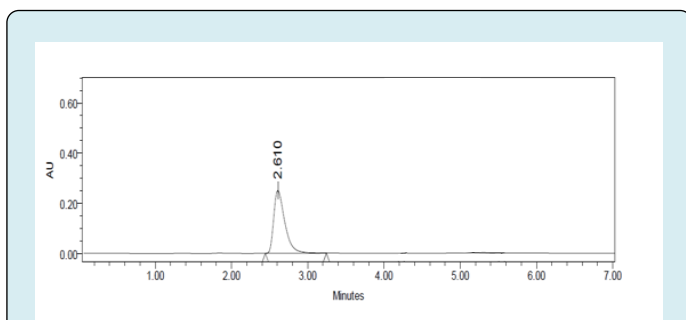


Figure 18: Chromatogram for Linearity Level – 5.

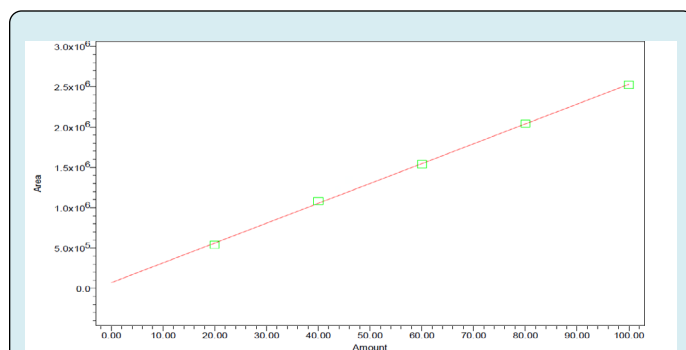


Figure 19: Linearity plot of Selexipag.

Acceptance Criteria

Regression co-efficient need to not be less than 0.999. The regression coefficient was found to be 0.999 which is within the limits.

Accuracy

The accuracy of an analytical method is the degree of agreement of test results generated by the method to the true value.

Determination

Accuracy is executed in 3 various levels of Selexipag. Spiked the amount of Selexipag at 50%, 100%, and 150% level into the placebo. Analyzed examples in three for each level. From the results, % recuperation was determined.

Preparation of Standard stock solution

Accurately weigh and transfer 10 mg of Selexipag working standard into a 10ml tidy completely dry volumetric flask add concerning 7 ml of Diluent and sonicate to dissolve it totally and make volume as much as the mark with the same solvent.

Stock Solution

Further pipette 0.3 ml of Selexipag of the above supply service right into a 10ml volumetric flask and thin down up to the mark with diluents.

For preparation of 50% solution (Relative to target Assay concentration)

Preparation of Standard stock solution: Accurately weigh and transfer 0.5ml of Selexipag working standard into a 10ml

tidy completely dry volumetric flask add concerning 7ml of Diluent and sonicate to dissolve it totally and make volume as much as the mark with the same solvent.

For preparation of 100% solution (Relative to target Assay concentration)

Preparation of Standard stock solution:

Accurately weigh and transfer 1ml of Selexipag working standard into a 10ml tidy completely dry volumetric flask add concerning 7 ml of Diluent and sonicate to dissolve it totally and make volume as much as the mark with the same solvent.

For preparation of 150% solution (Relative to target Assay concentration)

Preparation of Standard stock solution:

Accurately weigh and transfer 1.5ml of Selexipag working standard into a 10ml tidy completely dry volumetric flask add concerning 7ml of Diluent and sonicate to dissolve it totally and make volume as much as the mark with the same solvent.

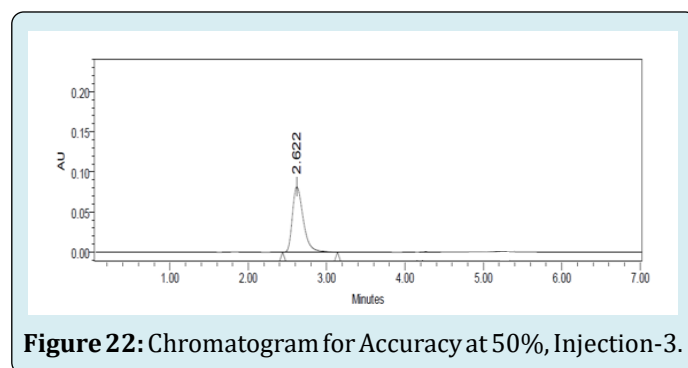
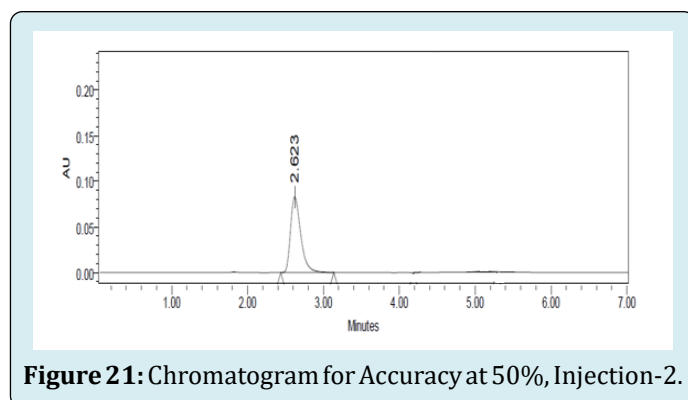
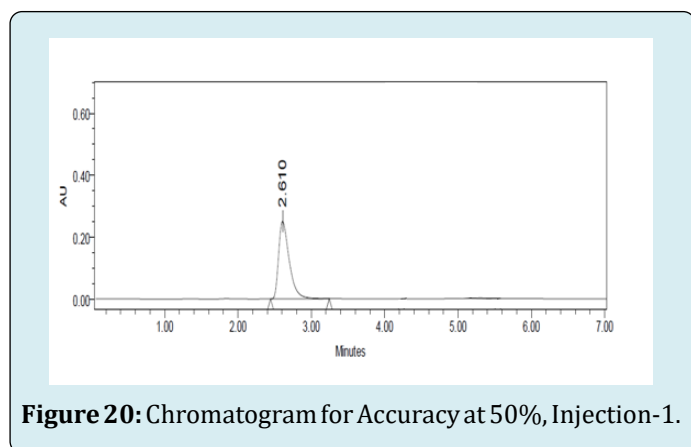
Procedure

Infuse the common solution, Accuracy-50%, Accuracy -100% and Accuracy-150% solutions. Compute the Quantity found as well as Quantity added for Selexipag and also compute the recuperation and imply healing worth and recovery research information for Selexipag are tabulated.

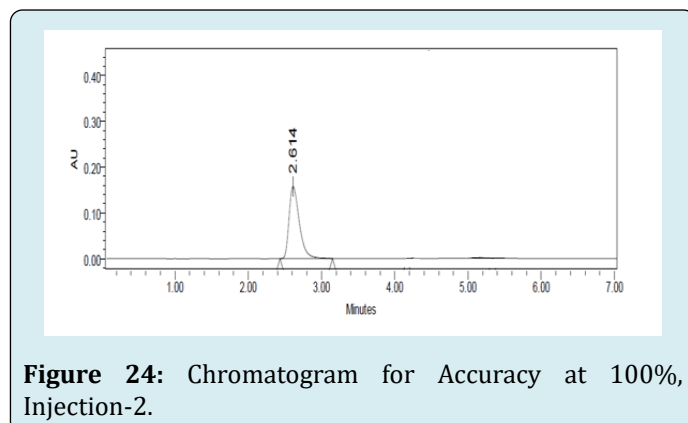
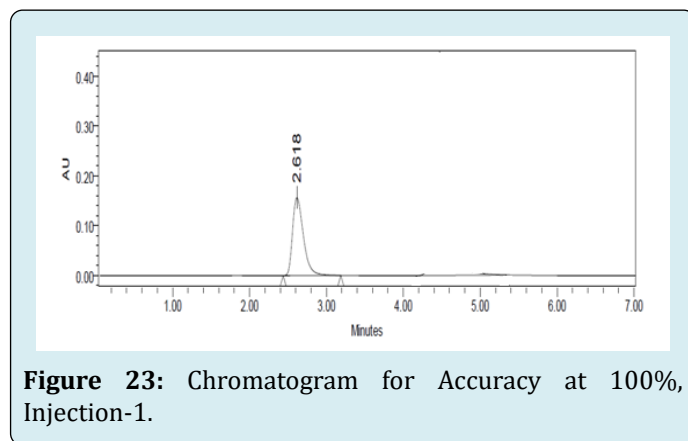
Acceptance Criteria

% Recuperation at each spike level will be not less than 98.0 and not greater than 102.0%.

Level 1 (50%)



Level 2 (100%)



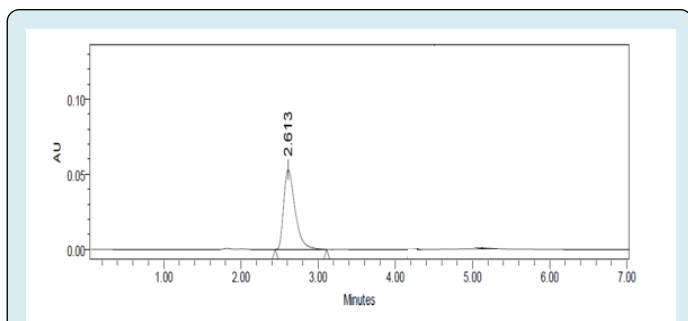


Figure 25: Chromatogram for Accuracy at 100%, Injection-3.

Level 3 (150%)

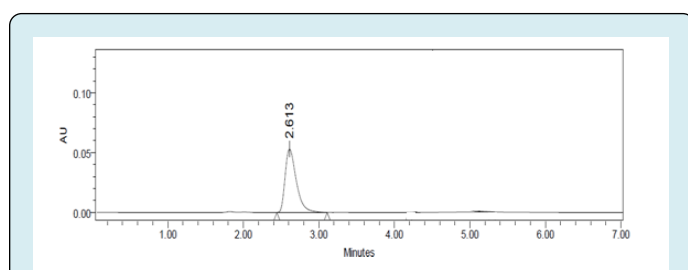


Figure 26: Chromatogram for Accuracy at 150%, Injection-1.

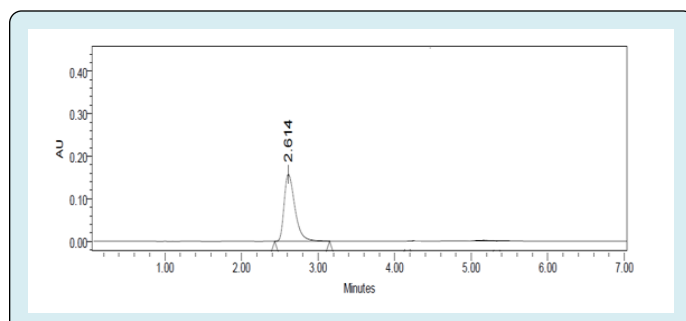


Figure 27: Chromatogram for Accuracy at 150%, Injection-2.

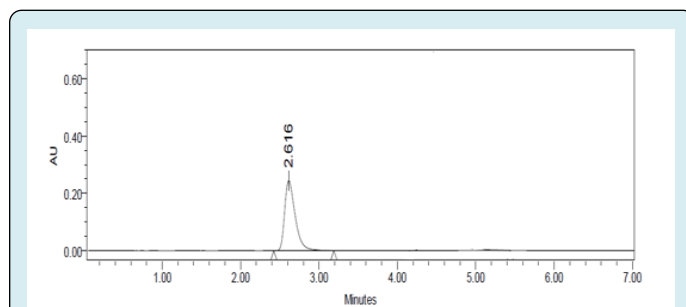


Figure 28: Chromatogram for Accuracy at 150%, Injection-3.

Injection sample	Conc.(μ g)	Area 1	Area 2	Area 3	Avg. area
Selexipag	50%	3202.879	3193.192	3213.687	3203.252
	100%	5994.595	6016.317	6008.074	6006.328
	150%	8744.326	8742.155	8736.633	8741.038

Table 8: Showing the Peak results for Accuracy of Selexipag.

Drug	Conc. of sample(mg)	Avg area	%recovery	Mean % Recovery
Selexipag	50%	3203.253	101.7%	100.0%
	100%	6006.329	100.9%	
	150%	8741.038	98.1%	

Table 9: Showing the Percentage recovery for Accuracy of Selexipag.

Acceptance criteria

The percentage recovery of Selexipag was reported to be 100% (NLT-98% & NMT-102%).

Precision

It is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings. Precision is measured by injecting a series of

standards or analyzing series of samples from multiple samplings from a homogeneous lot.

Preparation of Standard stock solution

Accurately weigh and transfer 10 mg of Selexipag working standard into a 10ml tidy completely dry volumetric flask add concerning 7 ml of Diluent and sonicate to dissolve it totally and make volume as much as the mark with the same solvent. Later transfer 3ml from above solution and

make up to 10ml with same diluent.

Procedure

The standard solution was infused for 5 times and determined the area for all five shots in HPLC. The % RSD for the location of five replicate shots was located to be within the defined restrictions.

Acceptance Criteria

The % RSD for area of five conventional shots results should not be greater than 2%.

S. No.	Retention Time	Area
1	2.616	1547.033
2	2.619	1545.256
3	2.619	1548.422
4	2.620	1549.235
5	2.621	1541.013
Mean	-	1546191.9
SD	-	3265.0
%RSD	-	0.2

Table 10: Showing the AUC for precision of Selexipag.

Acceptance Criteria

The % RSD for area of five conventional shots was found to be 0.2% (NMT 2%).

Intermediate Precision

It is the variation within the laboratory such as different days, i.e. Intraday and Inter day, different laboratories etc.

Preparation of Standard stock solution

Accurately weigh and transfer 10 mg of Selexipag working standard into a 10ml tidy completely dry volumetric flask add concerning 7 ml of Diluent and sonicate to dissolve it totally and make volume as much as the mark with the same solvent. Later transfer 3ml from above solution and make up to 10ml with same diluent.

Procedure

The standard solution was infused for 5 times and determined the area for all five shots in HPLC. The % RSD for the location of five replicate shots was located to be within the defined restrictions.

Acceptance Criteria

The % RSD for area of five conventional shots results should not be greater than 2%.

Intermediate Precision/ Ruggedness

Using different columns of the same scale, the intermediate precision analysis was performed on different days. Each normal injection in the chromatographic system has been injected. For the percent RSD measurement, the area of each standard injection was used.

Parameter	Selexipag
Precision (%RSD)	0.2
Intermediate precision (%RSD)	0.2

Table 11: Showing the Values obtained for precision & Intermediate precision of Selexipag.

The precision study was conducted and the percentage RSD of Selexipag was reported to be 0.2 and 0.2 (Intermediate precision).

Limit Of Detection

LOD is defined as the lowest amount of analyte in the sample that can be detected.

Preparation of Standard stock solution

Accurately weigh and transfer 10 mg of Selexipag working standard into a 10ml tidy completely dry volumetric flask add concerning 7ml of Diluent and sonicate to dissolve it totally and make volume as much as the mark with the same solvent. Later transfer 3ml from above solution and make up to 10ml with same diluent.

Preparation of 0.02µg/ml solution

Pipette 1ml of the above solution into a 10ml volumetric flask and thin down approximately the mark with diluent. Additional pipette out 1ml of the above supply option into a 10ml volumetric flask as well as dilute as much as the mark with diluent. Pipette 0.2 ml of service into a 10 ml of volumetric flask and water down as much as the mark with diluent.

Acceptance Criteria

S/N Proportion value will be 3 for LOD.

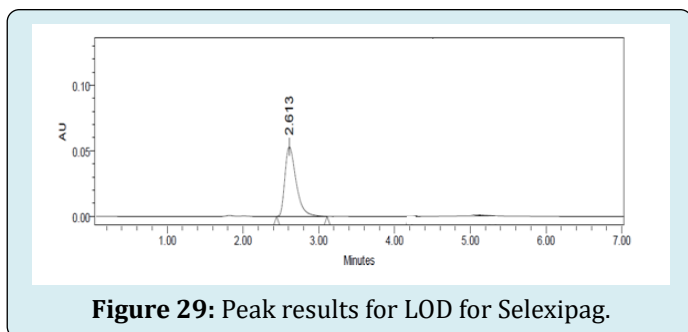


Figure 29: Peak results for LOD for Selexipag.

S.No.	Peak Name	RT	Area	Height
1	Selexipag	2.613	1295	127

Table 12: Showing the Peak results for LOD for Selexipag.

Limit Of Quantification

LOQ is defined as the lowest amount of analyte in the sample that can be quantitatively determined.

Preparation of Standard stock solution

Accurately weigh and transfer 10 mg of Selexipag working standard into a 10ml tidy completely dry volumetric flask add concerning 7ml of Diluent and sonicate to dissolve it totally and make volume as much as the mark with the same solvent. Later transfer 3ml from above solution and make up to 10ml with same diluent.

Preparation of 0.08µg/ml solution

Pipette 1ml of the above solution into a 10ml volumetric flask and thin down approximately the mark with diluent. Additional pipette out 1ml of the above supply option into a 10ml volumetric flask as well as dilute as much as the mark with diluent. Pipette 0.8 ml of service into a 10 ml of volumetric flask and water down as much as the mark with diluent.

Acceptance Criteria

S/N Proportion value will be 10 for LOQ.

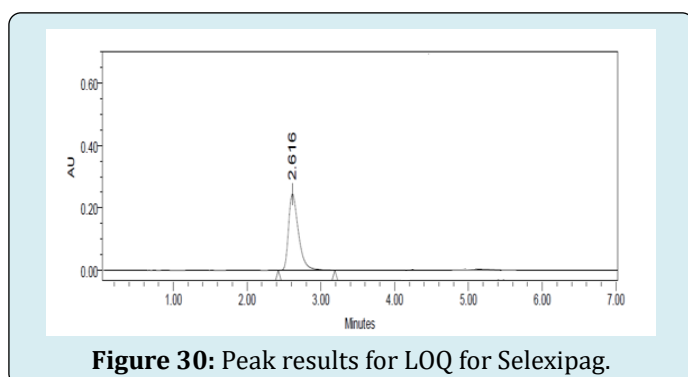


Figure 30: Peak results for LOQ for Selexipag.

S.No.	Peak Name	RT	Area	Height
1	Selexipag	2.616	4365	428

Table 13: Showing the Peak results for LOQ for Selexipag.

Validation parameter	Results
Retention time (Minutes)	2.627
Accuracy (% Recovery)	100%
Linearity and Range	10 µg/ml-50 µg/ml
Regression coefficient (r ²)	0.999
Limit of Detection By equation method	0.102 µg/mL
Limit of Quantification By equation method	0.309 µg/mL
Precision Repeatability	0.2%
Intermediate precision Intraday Precision	0.2%
Assay	99.9%
System suitability	Meets the System Suitability Criteria

Table 14: Consolidated results of all the Validation parameters for Selexipag.

Stability Studies

Acid Degradation

Degradation of drug sample is carried out by acid stress agents in which 1 ml of drug stock solution and 2 ml of 0.1 N HCl solution mixed in 10 ml of flask and kept for five hours at 70 °C temperature and refluxed in to 250 ml of round bottom flask kept the solution on bench top to attain the room temperature. Then the final volume was made up with diluent to achieve 10µg/ml for Selexipag concentrations and the chromatograms were recorded to access the stability of sample.

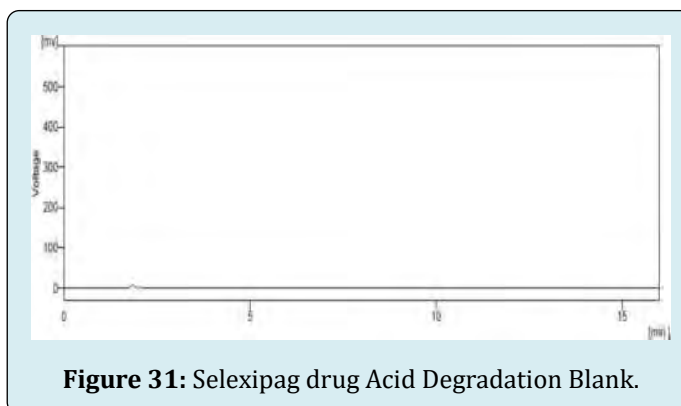


Figure 31: Selexipag drug Acid Degradation Blank.

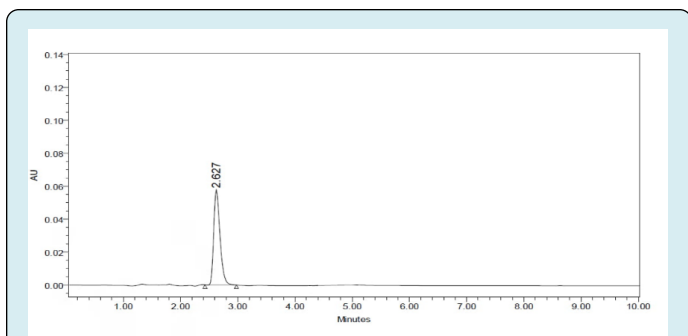


Figure 32: Selexipag drug Acid Degradation Standard.

Base Degradation

Degradation of drug sample is carried out by alkali stress agents in which 1 ml of drug stock solution and 2 ml of 0.1 N NaOH solution mixed in 10 ml of flask and kept for five hours at 70 °C temperature and refluxed in to 250 ml of round bottom flask. Kept the solution on bench top to attain the room temperature.

Then the final volume was made up with diluent to achieve 10µg/ml for Selexipag concentrations and the chromatograms were recorded to access the stability of sample.

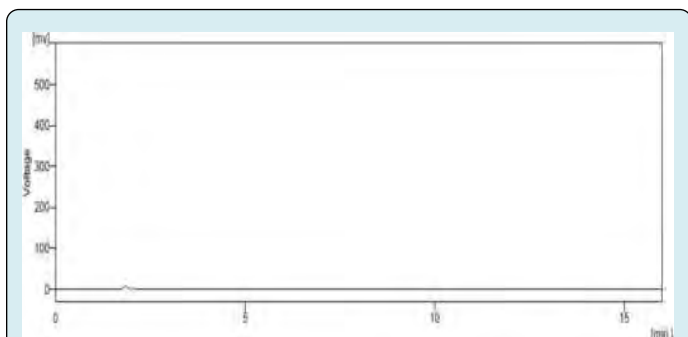


Figure 33: Selexipag drug Base Degradation Blank.

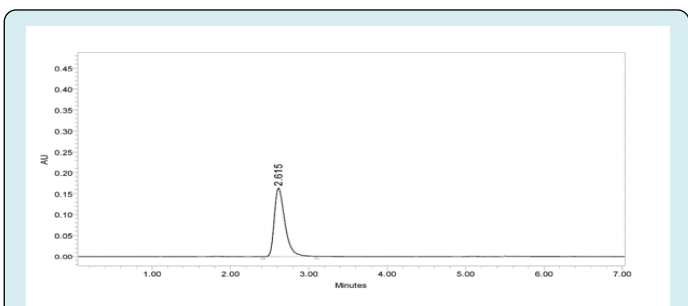


Figure 34: Selexipag drug Base Degradation Standard.

Oxidative Degradation

Degradation of drug sample is carried out by oxidative stress agents in which 1 ml of drug stock solution and 2 ml of 3% H₂O₂ solution mixed in 10 ml of flask and kept for 3.5 hours at 70 °C temperature and refluxed in to 250 ml of round bottom flask. Kept the solution on bench top to attain the room temperature.

Then the final volume was made up with diluent to achieve 10µg/ml for Selexipag concentrations and the chromatograms were recorded to access the stability of sample.



Figure 35: Selexipag drug Oxidative Degradation Blank.

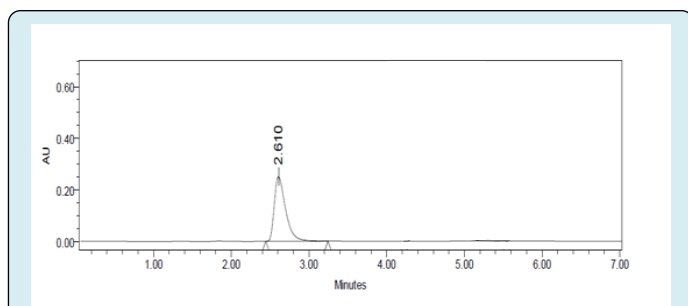


Figure 36: Selexipag drug Oxidative Degradation Standard.

Photo Degradation

Degradation of drug sample is carried out by photolytic stress agents in which 1ml of drug stock solution was transferred in to 10 ml of volumetric flask and kept for 48 hours at natural sun light. Then the final volume was made up with diluent to achieve 10µg/ml for Selexipag concentrations and the chromatograms were recorded to access the stability of sample



Figure 37: Selexipag drug Photo Degradation Blank.

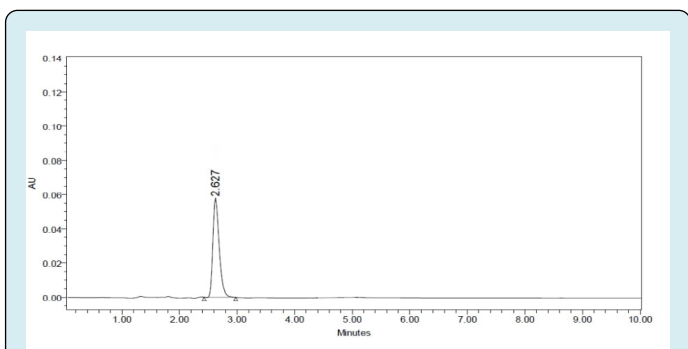


Figure 38: Selexipag drug Photo Degradation Standard.

Thermal Degradation

Degradation of drug sample is carried out by temperature stress agents in which 1 ml of drug stock solution was

incorporated in to 10 ml of volumetric flask and kept for 5 hours at 110 °C temperature in oven.

Then the final volume was made up with diluent to achieve 10µg/ml for Selexipag concentrations and the chromatograms were recorded to access the stability of sample.

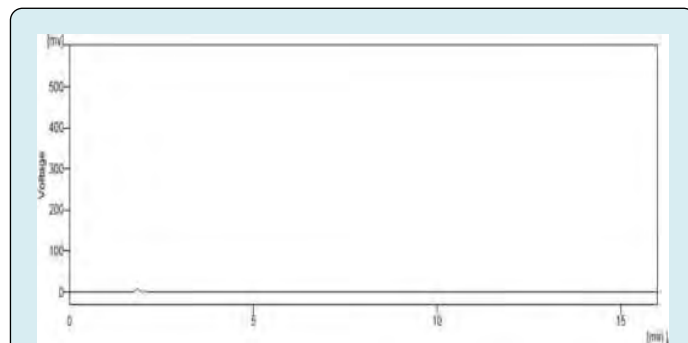


Figure 39: Selexipag drug Thermal Degradation Blank.

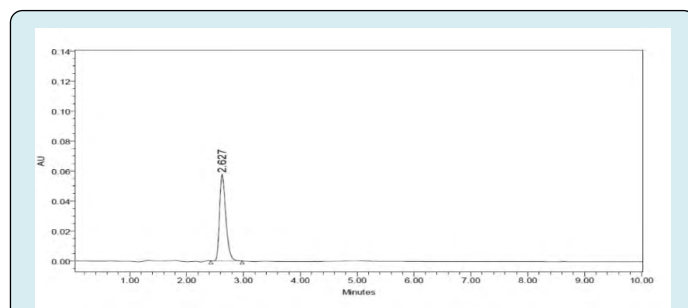


Figure 40: Selexipag drug Thermal Degradation Standard.

API Area of Standard 1539.291				
Condition	Area	% Degradation Standard	Area	% Degradation Sample
Acid	1341.793	3.654	1351.065	3.765
Base	1388.33	3.548	1355.336	3.832
Thermal	1315.35	2.434	1326.248	2.21
Oxidation	1301.3	2.81	1288.948	2.798
Photolytic	1353.082	1.078	1366.62	1.202

Table 15: Consolidated results of Stability studies for Selexipag.

Summary and Conclusion

The project summarizes a method development and validation for determination of Selexipag in pharmaceutical tablet dosage form.

An effective HPLC (high performance liquid chromatographic) technique was established for Selexipag. The HPLC method was using C18 column; (150 × 4.6 × 5µ)

column at 281nm, flow rate of 1.0 ml/min., Injection quantity of 20µl, column temperature was maintained ambient, equal quantity of Methanol as well as Water used as mobile phase (50:50 v/v). It was found that retention time were 2.627 mins. It was found that the percent purity was 99.9 w/w. According to ICH guidelines, the logical approach was validated (ICH, Q2 (R1)). The regression coefficient (r^2) was 0.999, recovery was 100.0%, and % RSD was found to be 0.2. The present stability indicating method for Selexipag in pharmaceutical

formulations is specific because the drug peak was well separated even in presence of degradation products. Overall, the data demonstrated that the excipients and the degradation products did not interfere with Selexipag peak, indicating the selective method.

The HPLC method was found to be reliable, specific, economical, stable and reproducible for routine analysis, the technique can be suggested, and the approach can be recommended for the resolution of the associated drug Selexipag compound and in pharmaceutical dosage form.

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