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# Stability Indicating Development and Validation for Simultaneous Estimation of Selexipag by RP-HPLC method

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## ABSTRACT

*A simple, precise, accurate method was developed for the estimation of Selexipag by RP-HPLC technique. Chromatographic conditions used are stationary phase DISCOVERY 250mm x 4.6 mm, 5µ Mobile phase 0.1% Perchloric acid: Acetonitrile in the ratio of 55:45 and flow rate was maintained at 1ml/min, detection wave length was 322nm, column temperature was set to 30°C and diluent was mobile phase conditions were finalized as optimized method. System suitability parameters were studied by injecting the standard six times and results were well under the acceptance criteria. Linearity study was carried out between25% to150 % levels, $R^2$ value was found to be as 0.999. Precision was found to be 0.5for repeatability and 0.6 for intermediate precision. LOD and LOQ are 0.48µg/ml and 1.45µg/ml respectively. By using above method assay of marketed formulation was carried out 98.92% was present. Degradation studies of Selexipag were done, in all conditions purity threshold was more than purity angle and within the acceptable range.*

**Keywords:** HPLC Selexipag, Method development. ICH Guidelines.

## 1. INTRODUCTION

Selexipag was affirmed by the United States FDA on December 22, 2015 for the treatment of pneumonic blood vessel hypertension (PAH) to defer ailment movement and decrease danger of hospitalization. PAH is a generally uncommon ailment with more often than not a poor visualization requiring greater treatment choices to draw out long haul results. Advertised by Actelion Pharmaceuticals under brand name Uptravi, selexipag and its dynamic metabolite, ACT-333679 (MRE-269), go about as agonists of the prostacyclin receptor to expand vasodilationin the pneumonic dissemination and diminishing raised weight in the veins providing blood to the lungs.

## 2. MATERIALS AND METHODS

### 2.1 Apparatus and Chromatographic Parameters

HPLC instrument used was of WATERS HPLC 2965 SYSTEM with Auto Injector and PDA Detector. Software used is Empower 2. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz was be used for measuring absorbance for Selexipag solutions.

### 2.2 Drug Samples

The Selexipag drug used for estimation for this study was procured from tablets.The brand name Uptravi, was used which is marketed by Actelion Pharmaceuticals The label claim was Selexipag 200 mcg in each tablet.

### 2.3 Reagent and solutions

HPLC grade Acetonitrile and Methanol, hplc grade/Merck glacial acetic acid, HPLC grade water and Selexipag drug was used in the study. A mixture Ortho phosphoric acid Acetonitrile in the ratio of 45:55%v/v was used as a mobile phase, adjusted with Ortho phosphoric Acid and it is also used as a diluent for preparing the working solution of drug. The mobile phase was degassed in ultrasonic water bath for 10

minutes and filtered through 0.45µm filter under vacuum filtration

### 2.4 PREPARATION OF THE SELEXIPAG STANDARD & SAMPLE SOLUTION

*2.4.1 Standard Solution Preparation*

Accurately Weighed and transferred 20mg Selexipag working Standard into a 10 ml clean dry volumetric flask, add 7ml of diluent, sonicated for 30 minutes and make up to the final volume with diluents .From the above stock solution, 1 ml was pipeted out in to a 10ml Volumetric flask and then make up to the final volume with diluent.

*2.4.2 Sample Solution*

Preparation: Tablets were weighed and calculated the average weight of each Tablet then the weight equivalent to 1 Tablet was transferred into a 100 ml volumetric flask, 50ml of diluent added and sonicated for 30 min, further the volume made up with diluent and filtered. From the filtered solution 1ml was pipette out into a 10 ml volumetric flask and made up to 10ml with diluent.

## 3. RESULTS AND DISCUSSION

### 3.1 METHOD DEVELOPMENT

Three trials were performed for the method development and the best peak with least fronting factor was found to be the third peak with RT=4.181 min (Fig 1).

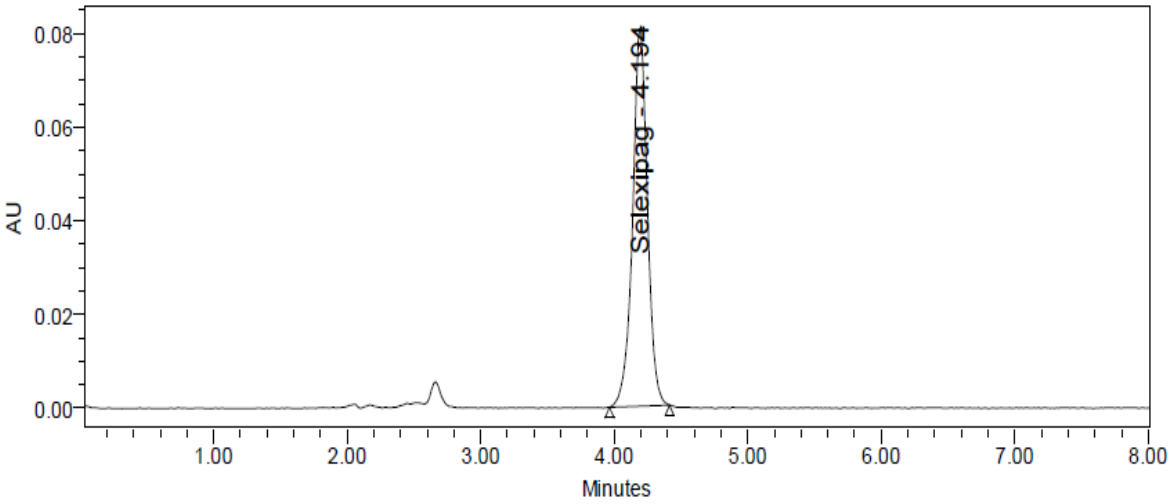



**Figure 1:** Chromatogram peak of Selexipag.

### 3.2 METHOD VALIDATION

*3.2.1 Precision*

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

*3.2.2 Acceptance Criteria*

The % RSD for the area of five standard injections results (Table 1) should not be more than 2%.

**Table 1:** % RSD data

| S. No | Peak area | Average peak area | Standard deviation | %RSD |
|---|---|---|---|---|
| 1 | 621510 | | | |
| 2 | 623303 | | | |
| 3 | 63307 | 626419 | 4104.2 | 0.7 |
| 4 | 627673 | | | |
| 5 | 625527 | | | |

### 3.3 Accuracy

Three Concentrations of 50%, 100%, 150% are Injected in a triplicate manner Amount added for selexipag and the individual recovery and mean recovery values.

### 3.4 Acceptance Criteria

The % Recovery for each level (Table 2) should be between 98.0 to 10.0%.

**Table 2:** Recovery data

| %concentration at specification level | Area | Amount spiked | % Recovery | Mean recovery |
|---|---|---|---|---|
| 50% | 317714 | 100 | 98.64% | |
| 100% | 635780 | 200 | 100.37% | 99.70% |
| 150% | 944258 | 300 | 100.87% | |

### 3.5 Recovery Studies

To determine the accuracy and precision of the proposed method recovery studies were carried out. A fixed amount of sample was taken and standard drug was added at 50%, 100% and 150% levels. The results were analyzed and the results were within the limits. The % recovery, Mean recovery and %Relative standard deviation value for selexipag drug was found to be 98.64% 100.37%, 100.87% and 0.7 respectively.

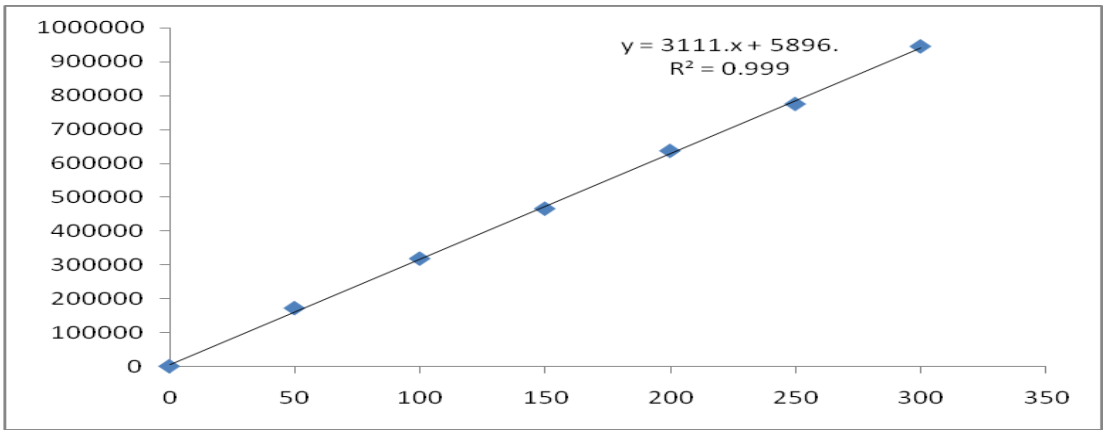
### 3.6 Linearity and Calibration Curve

To demonstrate the linearity of assay method, inject 6 standard solutions with concentrations of about 50ppm to 300ppm of Selexipag. Plot a graph to concentration versus peak area. Slope obtained was 3111 Y-Intercept was 5896 and Correlation Co-efficient was found to be 0.999.

**Table 3:** Different concentration values of selexipag

| linearity Level(%) | Concentration (ppm) | Area |
|---|---|---|
| 0 | 0 | 0 |
| 25 | 50 | 171476 |
| 50 | 100 | 317714 |
| 75 | 150 | 465091 |
| 100 | 200 | 635780 |
| 125 | 250 | 774253 |
| 150 | 300 | 944258 |

**Linearity graph**




### 3.7 Limit of Detection and Limit of Quantification

**Limit of Detection** (LOD) is the lowest concentration of an analyte in a sample that can be detected but not quantified. LOD is expressed as a concentration at a specified signal to noise ratio. The LOD will not only depend on the procedure of analysis but also on the type of instrument. In chromatography, detection limit is the injected amount that results in a peak with a height at least twice or thrice as high as baseline noise level. The LOD for Selexipag was found to be 4.147 at 0.20µg/ml solution

**Limit of Quantification** (LOQ) is defined as lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy and reliability by a given method under stated experimental conditions. LOQ is expressed as a concentration at a specified signal to noise ratio. In chromatography, limit of quantification is the injected amount that results in a peak with a height, ten times as high as base line noise level. The LOQ for selexipag was found to be 1.224 at 0.60/ml solution.

### 3.8 Robustness

Robustness is determined by making deliberate changes in the chromatographic conditions like change in flow rate, mobile phase composition and temperature and evaluated for the impact on the method. It was observed from the chromatograms that the results were within the limits. This indicates that the method developed is robust.

A simple, rapid and precise method has been developed and validated for the drug selexipag The estimation was carried out with A mixture Ortho phosphoric acid Acetonitrile in the ratio of 45:55%v/v was used as a mobile phase, adjusted with Ortho phosphoric Acid Precision of the methods were studied by making repeated injections of the samples and system precision values were determined (Table 3, 4). The retention time was 4.181 min. The calibration curve was linear over the concentration range of 50- 90 µ g mL $^{-1}$. The LOD and LOQ values were found to be 0.20µg/ml and 0.60µg/ml. The high percentage of recovery and low percentage coefficient of variance confirm the suitability of the method. Hence it was concluded that the RP-HPLC method developed was very much suitable for routine analysis.

**Table 4:** System precision values of selexipag

| S. No | Parameter | Acceptance criteria | Observed value |
|---|---|---|---|
| 1 | Accuracy | 95-105% | 99..70% |
| 2 | Precision | RSD within 2% | 0.7% |
| 3 | Linearity | 0.99 | $R^2$=0.999 |
| 4 | LOD | S/N=3 | 0.20µg/ml |
| 5 | LOQ | S/N=10 | 0.60µg/ml |

## 4. ACKNOWLEDGEMENTS

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## 5. CONCLUSION

The proposed study describes new and simple RP-HPLC method for the estimation of Selexipag. The method validated was found to be simple, accurate and precise. Therefore the proposed study method can be used for quantification of selexipag in bulk and pharmaceutical dosage form.

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