IJPSR (2024), Volume 15, Issue 8 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 09 August 2023; received in revised form, 03 July 2024; accepted, 06 July 2024; published 01 August 2024

VALIDATION AND FORCED DEGRADATION BY RP-HPLC OF SELEXIPAG DRUG IN BULK AND DOSAGE FORM

Saloni Borade [*1] and J. Bhangale Charushila [2]

Department of Quality Assurance Techniques, PRES's [1], Pravara Rural Education Society's [2], Womens College of Pharmacy, Chincholi, Nashik - 422102, Maharashtra, India.

Keywords:

Selexipag, HPLC, ICH, Column, Dosage form, Validation

Correspondence to Author:
Ms. Saloni Borade

Research Scholar, Department of Quality Assurance Techniques, PRES's, Womens College of Pharmacy, Chincholi, Nashik - 422102, Maharashtra, India.

E-mail: saloniborade321@gmail.com

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 Grace C18 (250mm x 4.6ID, Particle size: 5 micron) a mobile phase consisting of Buffer (pH-7.4): Methanol 60:40, at a flow rate of 1.0 mL/min and ultraviolet detection at 294 nm. The retention times of Selexipag was found to be 4.296 min. Linearity was established for Selexipag in the range of 20-100 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 of the content of Selexipag in Pharmaceutical dosage form. The method is validated as per ICH guidelines.

INTRODUCTION: Chemical stability of pharmaceutical molecules is a matter of great concern as it affects the safety and efficacy of the drug product. The FDA and ICH guidelines state the requirement of stability testing data to understand how the quality of a drug substance and drug product changes with time under the influence of various environmental factors. Knowledge of the stability of molecule helps in selecting proper formulation and package as well as providing proper storage conditions and shelf life, which is essential for regulatory documentation [1-5].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.15(8).2517-22

This article can be accessed online on www.ijpsr.com

DOI link: https://doi.org/10.13040/IJPSR.0975-8232.15(8).2517-22

Forced degradation is a process that involves degradation of drug products and drug substances at conditions more severe than accelerated conditions and thus generates degradation products that can be studied to determine the stability of the molecule. The ICH guideline states that stress testing is intended to identify the likely degradation products which further helps in determination of the intrinsic stability of the molecule and establishing degradation pathways, and to validate the stability indicating procedures used [6-10].

The plethora subscribed in this research is directed towards the study of forced degradation of Selexipag and its formulation under thermal conditions. The various methods are reported for the estimation of Selexipag from bulk and formulation but very few analytical methods were directed towards the details of forced degradation of Selexipag bulk and formulations.

Hence in this research an attempt will be made to study forced degradation of Selexipag from bulk and formulations.

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.

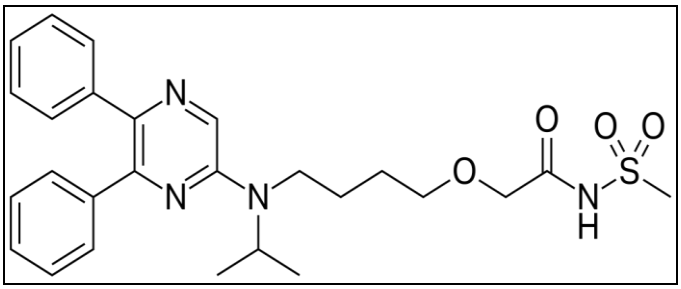

FIG. 1: STRUCTURE OF SELEXIPAG

MATERIAL AND METHOD: Selexipag was collected as a drug sample for research purposes. The solvents were procured from local chemical suppliers of Nashik and are of HPLC grade.

Preparation of Standard Stock Solution [11]: Accurately weigh and transfer 0.01gm (10mg) of pure Selexipag working standard separately into 10ml clean and dry volumetric flask, dissolved in the mobile phase and dilute to volume with the same solvent mixture to furnish stock solutions containing 1000µg/ml of Selexipag.1ml of above solution transferred in 10ml volumetric flask and the volume was made with diluents. The concentration of Selexipag is 100µg/ml.

Selection of Mobile Phase [12]: Selexipag was injected into the HPLC system and run in different solvent systems. Mixture of different solvents were tried in order to determine optimum chromatographic conditions for effective separation.

After several permutation and combination, it was found that mixture of Methanol: Water (pH7.4), gives satisfactory results as compared to other mobile phases. Finally, the optimal composition of the mobile phase contains about 60volume of methanol and 40 volume of water (buffer pH 7.4), as it gave high resolution of Selexipag with minimal tailing.

TABLE 1: OPTIMIZED CHROMATOGRAPHIC CONDITION

Parameters	Values
Column	Grace C18
Wavelength	294 nm
Flow rate	1.0 ml/min
Injection Volume	20µl
Temperature	Ambient
Run time	7.78min

Validation of the Developed RP-HPLC Method [13-16]: The developed method was validated as per ICH guidelines for its system suitability, linearity, accuracy, precision, robustness, limit of detection, limit of quantification by using following procedures.

Specificity: Specificity is the ability to measure accurately and specifically the analyte of interest in the other components that may be expected to be present in the sample matrix.

Linearity: Linearity of an analytical method is its ability to elicit test results that are directly or by a well-defined mathematical transformation proportional in the concentration of analyte in sample within a given range.

Preparation of Standard Stock Solution: Standard solutions of Selexipag prepared at different concentrations level i.e., 10, 20, 30, 40, and 50ppm was used for this purpose. The peak areas of the chromatograms were plotted against the concentrations of Aceclofenac and Pregabalin to obtain the calibration curves. These five concentrations of the standard were subjected to regression analysis to calculate calibration equation and correlation coefficients.

Accuracy: The accuracy of an analytical method is the closeness of the test results obtained by that method to the true value. It is the measure of the exactness of the analytical method developed. Accuracy may often be expressed as percent recovery by the assay of a known amount of analyte added

Precision: Precision of an analytical method is the degree of agreement among Individual test results when the procedure is applied repeatedly to multiple sampling of a homogenous sample.

Limit of Detection and Limit of Quantification: LOD and LOQ were calculated from the average

slope and standard deviation from the calibration curve as per ICH guidelines.

$$LOD = 3.3 \times SD / S$$

$$LOQ = 10 \times SD / S$$

Forced Degradation Study:

Acid/ Alkali Hydrolysis: For acid/ alkali hydrolysis, 2ml of 0.1N HCL and 0.1N NaOH was added to the solutions. These solutions were kept aside for 1hr at 60ºC. Resultant solutions were injected in to system after neutralization and chromatogram were recorded to access stability

Oxidation Degradation: For oxidation degradation 3ml of 2% hydrogen peroxide (H2O2) was added and kept aside for 24 hr at 60ºC and injected in system and chromatogram were recorded.

Photo Degradation: For photo degradation solutions were exposed to near UV light for 24hr and resultant solutions were injected in chromatographic system and compared with standard drug solution.

Thermal Degradation: Selexipag was transferred into petri plate separately and kept in a hot air oven at 70ºC for 12hrs. from the above stressed sample, 10 mg was weighed accurately and transferred to 10 ml volumetric flask separately and volume was made up to the mark with the mobile phase to get the concentration 1000µg/ml of both drug solution. 5 ml of the above solution transferred in 10 ml volumetric flask and the volume was made with diluents. The concentration of Selexipag was 100µg/ml.

Stress Stability Testing of Selexipag: Thermal stability of SLG (1000µg/ml) was checked by exposing drug to different temperature conditions as per the guidelines of long-term stability testing. The different conditions of temperature are as follows

1. Thermal degradation studies at -20°C for 90 days.
2. Thermal degradation studies at -20°C for 180 days.
3. Thermal degradation studies at 25°C for 90 days.
4. Thermal degradation studies at 25°C for 180 days.
5. Thermal degradation studies at 40°C for 90 days.
6. Thermal degradation studies at 40°C for 180 days.

RESULTS AND DISCUSSION:

Determination of λmax of Selexipag (SLG): The standard solution of Selexipag was scanned at different Concentrations in the range of 200-400nm and the λmax was found to be 294 nm against reagent blank.

HPLC Method Development: The described method has been validated which includes parameters like system suitability, linearity, accuracy, precision, robustness, LOD (limit of detection) and LOQ (limit of quantification).

Optimized Chromatographic Conditions: Following are the optimized chromatographic conditions for RP-HPLC method.

Parameters	Values
Column	Grace C18 (250mm x 4.6ID, Particle size: 5 micron)
Mobile Phase	Methanol: Phosphate Buffer (40:60)
pH	7.4
Wavelength	294 nm
Flow rate	1.0ml/min
Injection volume	20µl
Run time	7.78min
Retention time	4.296 min SLG

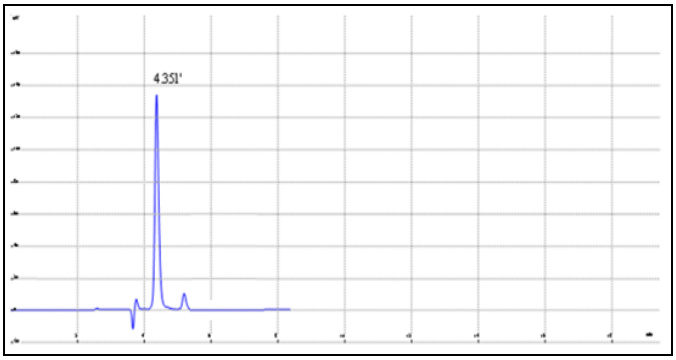

FIG. 2: CHROMATOGRAM OF SELEXIPAG

System Suitability: System suitability is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system.

System suitability and chromatographic parameters were validated such as resolution, theoretical plates, and tailing factor was calculated.

TABLE 2: SYSTEM SUITABILITY PARAMETERS FOR SLG

System suitability parameters	SLG
Retention time	4.327min
Theoretical plate no.	8433
Tailing factor	1.21
Resolution	1.04

Analysis of Injection Formulation of SLG: After analysis of SLG injection it was found that the amount of SLG found after calculation was within the limit of label claim as mentioned in **Table 10**.

TABLE 3: ANALYSIS DATA FOR INJECTION FORMULATION

Drug	Label claim (mg/inj)	Amount found (mg/inj)	Percent label claim
SLG	18	17.64	98

TABLE 4: SPECIFICITY DATA FOR PROPOSED HPLC METHOD

Drug Sample	Area	Amount added	Amount recovered	Percent recovery	SD	RSD
SLG	1752741	40	20	50	2186.79	0.10
	2234838	40	40	100		
	2603520	40	60	150		

Linearity: The calibration curves exhibited linear relationship of peak area to concentration in the range 20-100µg/ml for SLG. The regression coefficients (r^2) for SLG were 0.998, maintaining good correlation close to unity.

The graph of concentration vs Average area was plotted which is showing straight line passing through all points. So, as per ICH guidelines, the proposed HPLC method for the determination of SLG was found to be linear.

TABLE 5: LINEARITY DATA FOR SLG

Drug	Concentration (ppm)	Area
SLG	20	871908
	40	1341247
	60	1753841
	80	2235938
	100	2604620

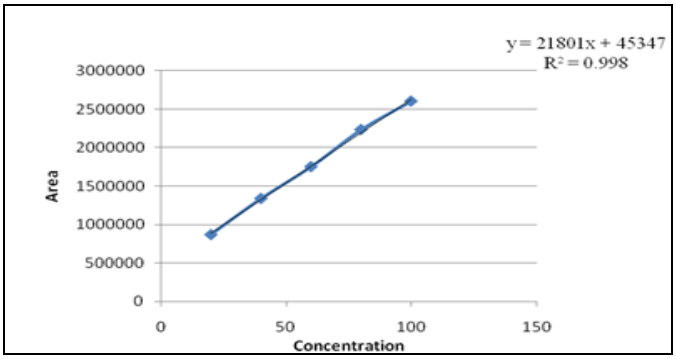

FIG. 3: LINEARITY GRAPH FOR SLG

Repeatability:

TABLE 6: REPEATABILITY DATA FOR SLG

Drug	Conc (ppm)	Area	Mean ±SD (n=3)	% RSD
SLG	20ppm	871908	1036.57	0.11
		873931		
		873312		

Accuracy:

TABLE 7: ACCURACY DATA FOR SLG

Conc. (%)	Sample amount (ppm)	Amount added (ppm)	Amount recovered (ppm)	% recovery	%mean recovery	SD	%RSD
50%	40	20	60.06	100.100	100.09	2360	0.10
	40	20	59.95	99.93			
	40	20	60.14	100.24			
100%	40	40	79.84	99.80	99.9		

	40	40	79.96	99.95	
	40	40	79.96	99.95	
150%	40	60	100.04	100.04	100.03
	40	60	99.95	99.95	
	40	60	100.10	100.10	

Robustness:

Change in Flow Rate:

TABLE 8: DATA FOR ROBUSTNESS (AT DIFFERENT FLOW RATE)

Drug Sample	Flow rate(ml/min)	Area	Mean	SD	%RSD
SLG	0.9	1342872	1341293	1556.02	0.116
	1.0	1341247			
	1.1	1339761			

Limit of Detection and Quantization: The limits of detection (LOD) and quantification (LOQ) were determined separately, on the basis of the standard deviation of the y intercept and slope of the calibration plots.

TABLE 9: DATA FOR LOD AND LOQ

Sr. no.	Drug	LOD	LOQ
1	SLG	0.35	1.08

Forced Degradation Study:

Acid /Alkali Hydrolysis: For Acid/Alkali hydrolysis, 2ml of 0.1M Hydrochloric acid (HCL) / 2ml of 0.1N Sodium hydroxide (NaOH) were added to solutions. These solutions were kept aside for 1hr at 60°C. Resultant solutions were injected in to system after neutralization and chromatograms were recorded to access stability.

Oxidation Degradation: For oxidation degradation, 3ml of 2% Hydrogen Peroxide (H2O2) was added and kept aside for 24hrs at 60˚C and injected in system and chromatograms were recorded.

Photo Degradation: For photo degradation solutions were exposed near UV light for 24hrs and resultant solutions were injected in chromatographic system and compared with the standard drug solution.

Thermal Degradation: SLG transferred to petri plate separately and kept in a hot air oven at 70°C for 12hrs. From the above stressed sample, 10mg was weighed accurately and transferred to 10ml volumetric flask separately and volume was made up to the mark with the methanol to get the concentration of 1000µg/ml of both drug solution. 5ml of above solution transferred in 10 ml volumetric flask and volume was made with diluents.

TABLE 10: SUMMARY OF DEGRADATION DATA FOR SLG

Stress Condition	Retention Time	Area of Peak	Degradation (%)	API after degradation %
Std. Drug	4.296	2604620	-	-
Acidic (0.1N HCL)	4.123	2209541	84.83160691	15.16839309
Alkaline (0.1 N NaOH)	4.288	1656071	63.58205804	36.41794196
Oxidation (3% H2O2)	4.056	1971288	75.68428408	24.31571592
Photolytic (UV)	4.438	2543282	97.64503075	2.354969247
Thermal	4.438	2582716	99.1590328	0.840967204

Stress Stability Testing of SLG: Thermal stability of SLG (1000µg/ml) was checked by exposing drug to different temperature conditions as per the guidelines of long term stability testing. The different conditions of temperature are as follows

1. Thermal degradation studies at -20°C for 90 days.
2. Thermal degradation studies at -20°C for 180 days.
3. Thermal degradation studies at 25°C for 90 days.
4. Thermal degradation studies at 25°C for 180 days.
5. Thermal degradation studies at 40°C for 90 days
6. Thermal degradation studies at 40°C for 180 days.

TABLE 11: STRESS STABILITY TESTING OF SLG

Sr. no.	Thermal Degradation condition	% Recovery	Remark
1	Thermal degradation studies at -20°C for 90 days	98.75	No degradation
2	Thermal degradation studies at -20°C for 180 days	98.95	No degradation
3	Thermal degradation studies at 25°C for 90 days	99.15	No degradation
4	Thermal degradation studies at 25°C for 180 days	99.25	No degradation
5	Thermal degradation studies at 40°C for 90 days	99.04	No degradation
6	Thermal degradation studies at 40°C for 180 days	99.05	No degradation

CONCLUSION: Development and validation of RP-HPLC method was found to be linear, accurate, precise, specific and robust according to acceptance criteria and with high level of LOD and LOQ. The results show that the HPLC method presented here can be considered suitable for the analytical determination of SLG in bulk and tablet dosage form. The developed method was validated. The good % recovery in tablet forms suggests that the excipients present in the dosage forms have no interference in the determination. The %RSD was also less than 2% showing a high degree of precision of the proposed method. The method was successfully applied to the available marketed formulation without any interference due to the excipients and can have an application in the industry. The forced degradation of SLG has suggested that the method is stable at different conditions of temperature and humidity.

ACKNOWLEDGEMENT: Nil

CONFLICTS OF INTEREST: Nil

REFERENCES:

1. Kotwal TS, Patwardhan DM, Amrutkar SS and Wagh MP: development and validation of simple UV spectrophotometric method for the determination of Selexipag in API and its bulk dosage form, Indo American J of Pharma Research 2017; 7(05): 857
2. Joshi MSA and Patel CN: Stability indicating assay method development and validation for Selexipag in pharmaceutical dosage form, WJPR 9(4): 293-304.
3. Giri Prasad Gorumutchu and Venkata Nadh Ratnakaram: Diazo coupling for the determination of selexipag by visible spectrophotometry. IJGP 2018; 12(4): 822.
4. Snigdha Damireddy, Pravalika K, Praveen MG and SM Anusha: Method development and validation of selexipag in its bulk and dosage form by RP-HPLC. International Journal of Pharmacy and Biological Sciences 84-92.
5. Koya Prabhakara Rao, Namburi LA Amarababu, Kalyani Koganti, Babji Palakeeti and Koduri S. V. Srinivas: Related substances method development and validation of an LCMS/ MS method for quantification of selexipag and its related impurities in rat plasma and its application to pharmacokinetic studies. SN Applied Scienc 2021; 3: 321.
6. Sistla Mounica Prathyusha, Choppala Asha Deepti, Raghu Raj Naik and Mukthinuthalapati Mathrusri Annapurna: Development and validated of spectrophotometric methods for the determination of Selexipag (An anti-hypertensive agent), Research J Pharm and Tech 2020; 13(3): 1-5.
7. Iram F, Iram H, Iqbal A and Husain A: Forced Degradation Studies. J Anal Pharm Res 2016; 3(6): 00073.
8. Benedito Roberto de Alvarenga Junior and Renato Lajarim Carneiro, Chemometrics Approaches in Forced Degradation Studies of Pharmaceutical Drugs. Molecules 2019; 24: 3804.
9. Blessy M, Patel RD, Prajapati PN and Agrawal YK: Development of forced degradation and stability indicating studies of drugs. A review. JPA 2014; 4(3): 159-165.
10. Panchumarthy Ravisankar: Current Trends in Performance of Forced Degradation Studies and Stability Indicating Studies of Drugs. IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) 2017; 12(6): 17-36.
11. Sharma MK and Murugesan M: Forced Degradation Study an Essential Approach to Develop Stability Indicating Method. J Chromatogr Sep Tech 2017; 8: 349.
12. Bhaskar Rajveer, Ola Monika, Vinit Agnihotri, Chavan Arjun and Girase Harpalsing: Current trend in performance of forced degradation studies for drug substance and drug product's. Journal of Drug Delivery & Therapeutics 2020; 10(2): 149-155.
13. Pandey S, Pandey R and Shukla SS: Appliance of the ICH guidelines: forced degradation studies on abafungin and development of validated stability indicating method by 1st order derivative spectroscopy. Indian J of Pharmaceutical Education and Res 2021; 55(1): 249-55.
14. Farias FF, Martins VAP, Yano HM, Trujillo LM and Pinto E: Forced degradation studies to identify organic impurities in pharmaceuticals: a Brazilian perspective. Rev Ciênc Farm Básica Apl 2021; 42: 729.
15. Patel D, Patel M, Ahir K and Singh S: A review article on development of forced degradation and stability indicating studies for drug substance and drug product. J Pharm Sci Bioscientific Res 2019; 9(2): 165- 172.
16. Shreya M. Durgule, Pranoti M. Patil, Ankita P. Kore, Muskan T. Singh and Sandeep D. Kadam: An introduction to forced degradation studies for drug substance & drug products. Journal of Emerging Technologies and Innovative Research 2021; 8(9): 260-269.

How to cite this article:
Borade S and Charushila JB: Validation and forced degradation by RP-HPLC of selexipag drug in bulk and dosage form. Int J Pharm Sci & Res 2024; 15(8): 2517-22. doi: 10.13040/IJPSR.0975-8232.15(8).2517-22.