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Research Article

A new HPLC method for selexipag analysis in pharmaceutical formulation and bulk form

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ABSTRACT

Selexipag is a new non-prostanoid prostacyclin receptor agonist used to treat pulmonary arterial hypertension. Selexipag is a long-acting IP receptor agonist with a shorter half-life than all other licensed drugs targeting the prostacyclin pathway, mostly administered intravenously or by subcutaneous infusion or inhalation. In this study, a new high performance liquid chromatography (HPLC) method was developed to analyze Selexipag in bulk and pharmaceutical formulations. The method used a column with Supelco Ascentis® Express (Sigma Aldrich, USA) model phenyl hexyl functional group (100×4.6 mm, ID, 2.7µm). Chromatographic separation was in isocratic elution mode, and the mobile phase mixture was acetonitrile containing 0.1% formic acid: water containing 0.1% formic acid (60:40, v/v) ratio. The method was linear in the concentration range of 15.7-117.6 µg/mL, and the LOD and LOQ were obtained as 2.4 and 3.1 µg/mL, respectively. Various method parameters have been tested according to the ICH Q2(R1) manual, and it is a method with high accuracy and precision. Therefore, the developed method is suitable for selexipag's bulk and pharmaceutical formulation analysis.

Keywords: Bulk, HPLC, Pharmaceutical formulation, Selexipag

1. INTRODUCTION

Pulmonary arterial hypertension (PAH) is a medical condition characterised by the presence of chronic and infrequent cardiovascular complications that can have severe consequences, including mortality. Selexipag (SLP) is a chemical compound with the chemical name 2-{4-[(5,6-Diphenylpyrazin-2-yl) (isopropyl)amino]butoxy}-N-(methylsulfonyl) acetamide The compound in question is a pharmacological agent that can be administered orally and exhibits selectivity towards the prostacyclin receptor, acting as an agonist. The term "orphan prodrug" refers to a pharmaceutical compound that is designed to undergo a specific metabolic transformation within the body in order to produce an active drug. In the context of the given statement, it is being used to describe a compound that falls under this category. Furthermore, the compound is identified as a platelet aggregation inhibitor, which refers to its ability to prevent the clumping together of platelets in the blood, thereby inhibiting the formation of blood clots. Additionally, the In order to mitigate the advancement of disease and decrease the likelihood of hospitalisation, the Food and Drug Administration granted approval for the use of SLP in 2015 as a therapeutic intervention for pulmonary arterial hypertension (PAH) in patients classified as functional class II or III. The active metabolite of SLP, known as ACT-333679, is a prodrug that

exhibits a significantly higher selectivity for the IP receptor, as indicated by a 130-fold increase in selectivity compared to other receptors [1]. SLP is distinguished by its minimal adverse effects in comparison to Prostaglandin I2 (PGI2) analogues, primarily due to its heightened selectivity. The suggested initial dosage is 200 µg administered twice daily, with subsequent increments of 200 µg twice daily on a weekly basis until the maximum tolerated dosage of up to 1600 µg twice daily is achieved. The determination of the maintenance dose is based on the level of tolerability [2].

SLP with the molecular formula $C_{26}H_{32}N_4O_4S$ and a molecular weight of 496.63 g/mol is a pyrazine derivative bearing two additional phenyl substituents at the fifth and sixth positions. Its molecular structure was given in Figure 1. It is a monocarboxylic acid amide, an ether, a member of the pyrazines, an aromatic amine, a tertiary amine compound, and an *N*-(methylsulfonyl)acetamide. It is functionally related to an ACT-333679. SLP is a light yellow crystalline powder, almost insoluble in water. Solid SLP is very stable and has no hygroscopic and photosensitivity properties [3].

It has more advantages over other analytical techniques of high performance liquid chromatography (HPLC) analysis in pharmaceutical formulation and finished product analysis especially quality control laboratories. It is an automated system with fast, high accuracy, and precision results. Adequate chromatographic separation can be eliminated in some problems such as matrix interferences, allowing technical and biological analysis. In addition, thanks to the developing column technology, lower detection limits, faster analysis, and better chromatographic separation and peak shape can be obtained. However, the biggest advantage of drug analysis is its ease of automation in analysis and data processing. This advantage indicates that the HPLC method will retain its place long [4].

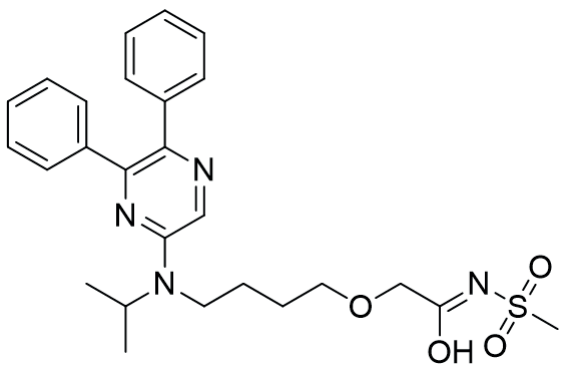

Figure 1. Structure of SLP

There are few studies on SLP analysis in the literature. These are HPLC analysis for SPL formulation and bulk analysis [5], stability indicating analysis with HPLC [6] and LC-MS/MS [7], and biological analysis with LC-MS/MS [3, 8-10], spectrophotometric method for determination of SLP in bulk and tablet formulation [9, 11]. Previous HPLC methods have disadvantages such as high flow, long column preference and more solvent and time consumption due to flour[5, 6]. It would also be better for them to make further improvements in method optimization and review system suitability parameters according to ICH (Q2) R1 [5]. This study proposes a fast, high-accuracy, and precision HPLC method for the analysis of SLP in bulk and pharmaceutical formulations.

2. MATERIALS AND METHODS

2.1 Chemical and reagents

Analytical grade chemicals, formic acid, acetic acid, hydrochloric acid, sodium hydroxide, and HPLC grade solvents, water, acetonitrile, and methanol were purchased from Sigma-Aldrich (USA). SLP hydrochloride standard with 99.9% (*w/w*) purity was obtained from TRC Company (Canada).

2.2 Instruments

The HPLC device used in the study is Shimadzu (Japan) brand LC-Nexera-i 2040C model and is a 3D compact system. Apart from this, RK 100 H model ultrasonic bath from Bandelin (Germany), XSE 105 Dual Range model analytical balance and SevenMulti model pH meter from Mettler Toledo (Switzerland), Rotina 380 R centrifuge device from Hettich (Germany), 20 in the preparation of solutions. They are Research model pipettors from Eppendorf (Germany) that can operate in the range of -100 µL and 100-1000 µL.

Table 1. The properties of used stationary phase

Properties	Value
Particle size	2.7
Surface area (m^2/g)	135
Carbon load (%)	7.1
Pore volume/Diameter	90 Å
pH range	2.0-9.0
USP Code	L43

2.3 Stationary Phase

The stationary phase used and its properties are given in Table 1. The method used a column Supelco Ascentis® Express (Sigma-Aldrich, USA) model phenyl hexyl functional group (100×4.6 mm, ID, 2.7µm).

2.4 Experimental Parameters

During the analysis using HPLC, the flow rate of the mobile phase introduced into the system was set at 0.5 mL/min. Additionally, the temperature of the column furnace was maintained at 30.05 °C. The temperature of the autosampler thermostat was set at 15±0.1 °C in order to ensure the stability of both the sample and standard solutions. Additionally, the injection volume was determined to be 1 µL.

The wavelength at which the maximum absorbance of SLP was observed was determined to be 204 nm. Consequently, the photodiode array detector in the high-performance liquid chromatography (HPLC) system was adjusted to this specific wavelength. Furthermore, the spectra were observed in the detector within the wavelength range of 190 to 380 nm. The data sampling frequency was set at 1.5625 Hz, and a time constant of 0.640 seconds was applied.

2.5 Preparation of Solutions

1 mg of SLP was weighed and added to a 5 mL acetonitrile flask. Then the volume was completed with acetonitrile, and the stock solution concentration was calculated as 200 µg/mL. Working solutions were obtained by diluting this stock solution with acetonitrile.

In the recovery studies, while the solutions were prepared, they were kept in an ultrasonic bath for 30 min and then filtered with a PTFE (22/25 mm, 0.22 µm pore size, Isolab, Germany) type syringe.

In the experimental procedure, isocratic elution chromatography was employed as a technique for the separation of compounds. The mobile phase utilised in this study consisted of a mixture of acetonitrile and water, with the ratio of 60:40 (v/v). To enhance the chromatographic separation, both acetonitrile and water were supplemented with 0.1% formic acid. In this study, we employed non-sterile Sartorius cellulose acetate membrane filters sourced from Germany. These filters possessed a diameter of 47 mm and a pore size of 0.22 m. Following the dissolution of the solutions in an ultrasonic bath for a duration of 15 minutes, the aforementioned filters were utilised to filter the resultant solutions.

2.6 Method validation

The validity of the developed method has been tested as specified in the ICH guideline and has been shown to meet the analytical criteria. Linearity tests confirmed method validity, the lower limit of detection, specificity, precision, limit of detection, system suitability, and accuracy.

The prepared SLP stock solution was diluted with acetonitrile and kept at -20 °C for freeze-thaw cycles and different times and then analyzed for the stability of the mobile phase and solution, and the solution was stable.

3. RESULTS AND DISCUSSION

This study aimed to develop a method to distinguish SLP from other compounds in drugs used in the treatment of pulmonary arterial hypertension. The HPLC system can separate and detect each compound by the difference in the velocity of each compound in the column. In this way, it is possible to distinguish SLP from other compounds. For this reason, the HPLC method was seen as the most suitable method for this analysis.

First, studies were carried out for stationary phase selection. In the analyzes performed on acetonitrile and methanol, it was decided that more relevant results were obtained for our analysis of acetonitrile. Then, experiments were done with different ratios

Table 2. Calculated system suitability parameters

SST parameters	Calculated value	Accepted value (USP)
Retention time	4.5	-
Number of theoretical plate	12056	N>2000
Tailing factor	1.1	2≤T
Resolution	1.4	Rs>1.5
Peak asymmetry	1.1	0.95≤As≤2
Repeatability of the peak area	0.5	%BSS<1.5 General separation
		%BSS<5 Biological sample
		%BSS 5-15 Trace element analyzes

of the organic phase. In order to measure the effect of different temperatures, experiments were done with different mobile phase ratios at 30 °C, 35 °C, and 40 °C and it was determined that 40 °C was the most suitable. In addition, it was determined that the most relevant results were obtained with acetonitrile/water (60:40, *v/v*) as the mobile phase. System suitability parameters for the developed method are given in the Table 2. Previous HPLC methods have disadvantages such as high flow, long column preference and more solvent and time consumption due to flour. It would also be better for them to make further improvements in method optimization and review system suitability parameters according to ICH Q2 R1. Each parameter appears to comply with the ICH (Q2) R1 guideline. In this respect, the method outperforms the method of Damireddy et al. [5]. In addition, it is a faster, cheaper and greener method due to shorter columns and a lower flow rate. Calculations were made to determine method validity considering the result obtained from high-performance liquid chromatography and the prepared analyte concentration. A calibration chart was created by looking at the peak area corresponding to the analyte concentration. For the method, analyses were made considering all method validity parameters. The results of the precision and linearity studies for the method are given in Table 3. Also linearity of SLP was shown Figure 2. This method has the lowest linearity compared to its counterparts. In addition, lower LOD and LOQ were obtained. Accuracy studies for the HPLC method developed and optimized for the analysis of SLP were performed after precision and linearity studies. One of the samples collected from the market was selected for recovery, its solution was prepared, and

Table 3. Precision and linearity data for SLP

Parameter	Calculated value
Linearity (µg/mL)	15.7-117.6
Slope (n=7)	2955
Intercept (n=7)	7572
Regression coefficient (n=7)	0.9917
Standard deviation of slope	135
Standard deviation of the slope	10235
LOD (µg/mL)	2.4
LOQ (µg/mL)	3.1
ANOVA	F (2.13)=0.90
	P=0.35821(P>0.05)

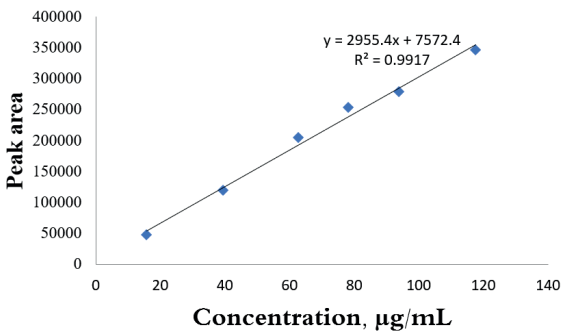

Figure 2. Linearity of SLP

SLP was added. The recovery studies were carried out with pharmaceutical formulation of SLP was Uptravi®. These analyzes were performed at three different concentrations and nine different analyzes. The obtained results were given in Table 4. Also recovery chromatogram for 25 µg/mL was given in Figure 3.

4. CONCLUSION

Table 4. Recovery studies for Uptravi® (n=3)

			Precision	Accuracy	
Added (µg/mL)	**Found (µg/mL)±CI***	**SD**	**RSD (%)**	**Recovery (%)**	**Error (%)**
20.00	20.01±0.11	0.21	1.05	100.1	+0.10
25.00	25.71±0.23	0.13	0.51	102.8	+2.80
30.00	30.30±0.41	0.22	0.73	101.0	+1.0

*95% confidence level

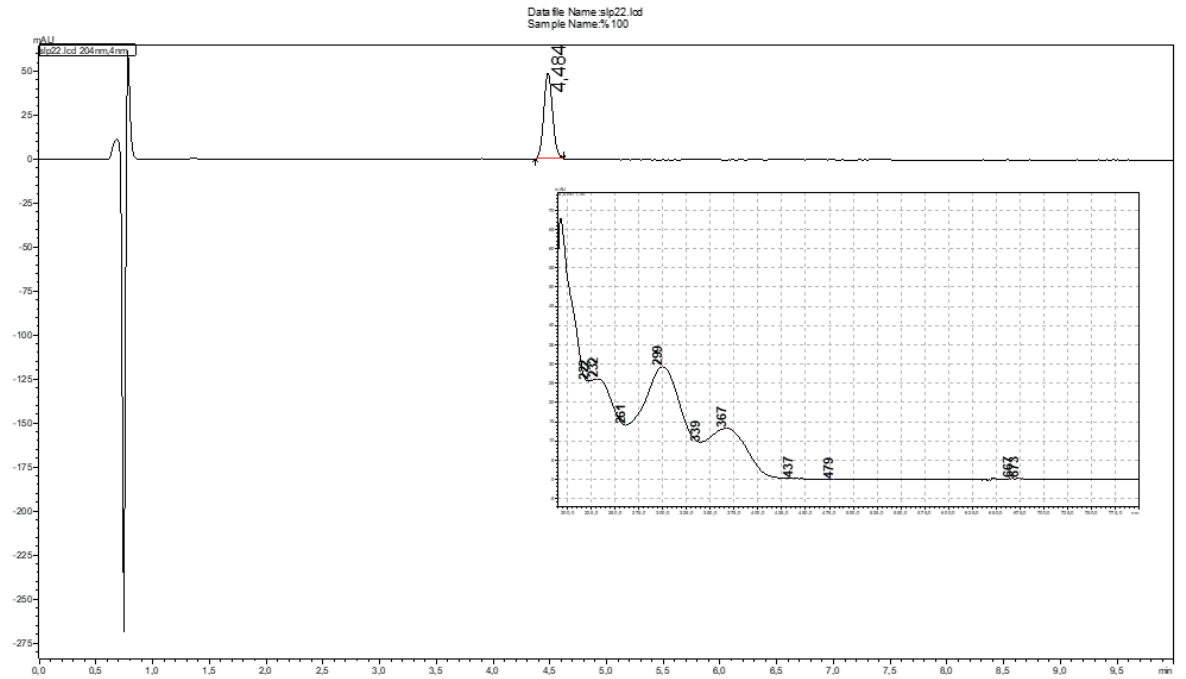

Figure 3. Chromatogram of 25 µg/mL SLP recovery solution

HPLC system is a chromatographic method that provides the opportunity to distinguish very well with the developing technology. It is the most widely used analytical instrument in analysis laboratories. HPLC separates compounds dissolved in a liquid sample and allows for qualitative and quantitative analysis of which components and how much of each component is present in the sample. In this study, a new HPLC method with high accuracy and reproducibility was developed to analyze SLP in bulk and pharmaceutical formulations. The developed method is a faster, less solvent-consuming, greener and reliable method when compared to similar studies in the literature. The current method is especially suitable for routine formulation and finished product analysis and will provide great convenience to analysts in quality control laboratories.

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Ethical approval

Not applicable, because this article does not contain any studies with human or animal subjects.

Author contribution

Concept: SÖ, NÖC; Design: SÖ, NÖC; Supervision: SL, NÖC; Materials: NÖC; Data Collection and/or Processing: SÖ, SL; Analysis and/or Interpretation: EGÖ; Literature Search: SÖ, SL; Writing: SÖ; Critical Reviews: SL, NÖC.

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Conflict of interest

The authors declared that there is no conflict of interest.

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