Analytical Method Development and Validation of Selexipag by different quantitative Analytical Techniques

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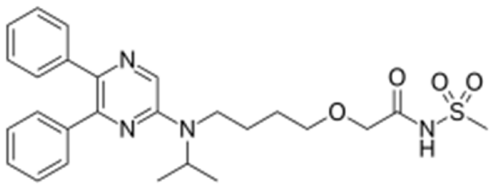
Abstract: Selexipag, a selective prostacyclin IP receptor agonist, is used for the treatment of pulmonary arterial hypertension (PAH). Analytical methods such as reversed-phase high-performance liquid chromatography (RP-HPLC), ultraviolet-visible (UV) spectroscopy, and their combination (RP-HPLC-UV) are critical for the accurate quantification and quality control of selexipag in bulk and pharmaceutical formulations. This review comprehensively discusses the development, optimization, and validation of RP-HPLC, UV, and RP-HPLC-UV methods for selexipag, focusing on their principles, applications, and compliance with International Conference on Harmonization (ICH) guidelines. Recent advancements, challenges, and future perspectives in these analytical techniques are also highlighted.

Keywords: Selexipag, RP-HPLC, UV Spectroscopy, Stability, Validation and ICH Guidelines.

Introduction: Selexipag N-(methanesulfonyl)-2-{4-[(propan-2-yl) (pyrazin-2-yl) amino] butoxy} acetamide is an orally active drug approved for the treatment of PAH. Its selective action on the prostacyclin receptor necessitates precise analytical methods to ensure its quality, stability, and efficacy in pharmaceutical formulations. RP-HPLC, UV spectroscopy, and RP-HPLC-UV are widely employed due to their sensitivity, specificity, and robustness. This review aims to consolidate the literature on these methods for selexipag, emphasizing method development, validation parameters, and their practical applications.



Chemical Structure of Selexipag

Analytical Techniques

1. LC-MS/MS method: [5-8]

A simple and sensitive LC-MS/MS method has been developed for the identification of process related impurities in Selexipag API. The method was developed using a Zorbax C18 15 × 0.46 cm, 5µ column with a gradient program at 35 ^{0}C and flow rate of 1.0 mL/min.

Selexipag was extracted and compared its % assay after protein precipitation technique from various biological materials such as rat plasma, rabbit plasma, human plasma and urine. Ambrisentan was selected as internal standard. Selected analytical column Waters, X-Bridge C18 3.5µ (50 x 4.6 mm), mobile phase consists of Hexane sulfonic acid and Acetonitrile (80:20 v/v) at a flow rate of 1.0 mL /min in isocratic mode and Selexipag was determined by the +ve mode of electrospray ionization by using Mass detector. The method was developed to assess the lower limit of detection (LLOD)(0.5 ng/mL), lower limit of quantification (LLOQ) (5 ng/mL) concentrations and Linearity range of 1 ng/mL to 20 ng/mL concentration with regression correlation coefficient 0.999 were observed for Selexipag in Rat plasma.

The validated liquid-liquid extraction method was applied for estimation of Selexipag in human plasma with Selexipag-D6 as an internal standard (ISTD) by using LC-MS/MS. The chromatographic separation was achieved with Acetonitrile: 10mM Ammonium formate (pH-4.0) (80: 20, v/v) using the CORTECS C18 COLUMN (100 x 4.6 mm, 2.7 µ). The total analysis time was 10 min and flow rate was set to 0.5 ml/min. Detection was done by turboionspray (API) positive mode with unit resolution.

2. Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC): [9-13]

RP-HPLC is the most prevalent chromatographic technique in pharmaceutical analysis due to its ability to separate analytes based on their hydrophobicity. It employs a non-polar stationary phase (e.g., C18 column) and a polar mobile phase (e.g., acetonitrile-IsopropylAlcohol mixtures). The separation of selexipag is achieved by optimizing parameters such as mobile phase composition, pH, flow rate, and column type. RP-HPLC is ideal for quantifying selexipag and its impurities in complex matrices.

3. Ultraviolet-Visible (UV) Spectroscopy: [14-18]

UV spectroscopy measures the absorption of light in the UV-visible range (200–800 nm) by selexipag, typically at its maximum absorbance wavelength (λ_max). It is simple, cost-effective, and suitable for routine analysis in bulk and formulations. However, its specificity is limited in the presence of interfering substances.

Method Development for Selexipag:

RP-HPLC Method Development: Several studies have reported RP-HPLC methods for selexipag. A notable example utilized an Inertsil ODS column (4.6 × 250 mm, 5 µm) with a mobile phase of acetonitrile (ACN) and 0.1% orthophosphoric acid (OPA) buffer (pH 3, adjusted with NaOH) in a 70:30 (v/v) ratio at a flow rate of 1 mL/min. The retention time was approximately 2.16 min, with detection at 270 nm. Optimization involved adjusting the mobile phase ratio, pH, and flow rate to achieve high resolution and minimal tailing. The method was linear over 100–500 µg/mL, with a correlation coefficient (r^2) of 0.999.

Another study developed a stability-indicating RP-HPLC method using an X-bridge phenyl column with an isocratic mobile phase of ACN and 0.1% formic acid. This method successfully separated selexipag from its five impurities, demonstrating robustness under stress conditions (acid, alkali, peroxide, thermal, hydrolysis, and UV).

Method Validation

Validation of analytical methods for selexipag adheres to ICH Q2(R1) guidelines, evaluating parameters such as specificity, linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), robustness, and stability.

Specificity: RP-HPLC and RP-HPLC-UV methods demonstrate specificity by resolving selexipag from its impurities and degradation products. For instance, stress testing under acidic, basic, oxidative, thermal, and photolytic conditions confirmed the stability-indicating nature of RP-HPLC methods, with no interference at the retention time of selexipag. UV methods, however, require careful selection of wavelengths to avoid interference.

Linearity: Linearity ranges for selexipag vary by method. RP-HPLC methods typically show linearity from 100–500 µg/mL ($r^2 = 0.999$), while UV methods cover 2–12 µg/mL. RP-HPLC-UV methods often achieve linearity over broader ranges (e.g., 5–75 µg/mL), suitable for both bulk and formulation analysis.

Accuracy and Precision: Accuracy is assessed via recovery studies, with RP-HPLC methods reporting recoveries of 98–102%. Precision, expressed as % relative standard deviation (%RSD), is typically <2%, with RP-HPLC-UV methods achieving %RSD values of 0.2–0.3 for repeatability and intermediate precision. UV methods also show high precision but are less robust for complex matrices.

LOD and LOQ: RP-HPLC-UV methods report LOD and LOQ values as low as 0.137 µg/mL and 0.574 µg/mL, respectively, indicating high sensitivity. UV methods, while sensitive, have higher LOD/LOQ due to limited specificity.

Robustness: Robustness is ensured by testing small variations in parameters (e.g., pH, flow rate, mobile phase composition). RP-HPLC methods using QbD approaches, such as Box-Behnken design, exhibit minimal impact on resolution and retention time, confirming their reliability.

Stability-Indicating Capability: Stability-indicating RP-HPLC methods are critical for selexipag, given its susceptibility to degradation. Forced degradation studies under ICH conditions (acid, base, peroxide, thermal, UV) demonstrate that RP-HPLC-UV methods can quantify selexipag in the presence of degradation products, making them suitable for stability studies.

UV Spectroscopy Method Development: UV methods for selexipag often exploit its diazo coupling reaction with p-nitroaniline in an alkaline medium, forming a green-colored azo dye with maximum absorption at 510 nm. Beer's law was obeyed over 2–12 µg/mL, with a molar absorptivity of 3.33×10^4 L/mol/cm. Optimization focused on reaction conditions to enhance sensitivity and stability of the colored complex. UV methods are advantageous for their simplicity but lack the specificity required for impurity

Applications:

Pharmaceutical Analysis: RP-HPLC and RP-HPLC-UV methods are used for assaying selexipag in bulk, tablets, and other dosage forms. They ensure compliance with pharmacopoeial standards by quantifying the active pharmaceutical ingredient (API) and detecting impurities.

Stability Studies: Stability-indicating RP-HPLC methods monitor selexipag's degradation under various conditions, supporting shelf-life determination and formulation development.

Bioanalytical Applications: RP-HPLC coupled with mass spectrometry (LC-MS/MS) extends to bioanalysis, quantifying selexipag in biological matrices like plasma. A validated LC-MS/MS method using a Zorbax C18 column achieved a runtime of 5 minutes, with a linearity range of 0.001–100 µg/mL.

Quality Control: UV spectroscopy is employed for routine quality control due to its simplicity and cost-effectiveness, particularly in resource-limited settings. However, RP-HPLC-UV is preferred in regulated environments for its specificity and robustness.

Recent Advancements

Quality by Design (QbD); QbD approaches, such as Box-Behnken and Taguchi designs, optimize RP-HPLC methods for selexipag, reducing trial-and-error and enhancing method robustness. These methods use statistical tools to evaluate critical parameters like mobile phase composition and pH.

Green Analytical Chemistry:

Eco-friendly RP-HPLC methods minimize organic solvent use (e.g., replacing methanol with ethanol) and employ shorter runtimes to reduce environmental impact. Such methods align with sustainability goals in pharmaceutical analysis.

Hyphenated Techniques: RP-HPLC coupled with advanced detectors (e.g., photodiode array, mass spectrometry) improves sensitivity and specificity. LC-MS/MS methods for selexipag offer lower LODs and are ideal for trace analysis.

Miniaturization: Ultra-performance liquid chromatography (UPLC) reduces analysis time and solvent consumption compared to traditional RP-HPLC. UPLC methods for selexipag are emerging, offering higher throughput for quality control.

Challenges

- Interference in UV Spectroscopy: UV methods suffer from limited specificity in complex matrices, necessitating careful wavelength selection.
- Impurity Profiling: Resolving selexipag's impurities requires optimized RP-HPLC conditions, which can be time-consuming.
- Cost and Accessibility: Advanced detectors (e.g., PDA, MS) increase method sensitivity but are costly, limiting their use in smaller laboratories.
- Stability of Selexipag: Selexipag's susceptibility to degradation under stress conditions complicates method development, requiring robust stability-indicating methods.

Future Perspectives

- Automation: Automated RP-HPLC systems with real-time data processing will enhance efficiency and reduce human error in selexipag analysis.
- Novel Stationary Phases: Development of hybrid or chiral stationary phases could improve separation of selexipag and its enantiomers or impurities.
- Portable Analytical Devices: Miniaturized UV and HPLC systems could enable on-site quality control, particularly in resource-limited settings.
- Integration with Artificial Intelligence: AI-driven optimization of chromatographic conditions could streamline method development for selexipag.

Conclusion: RP-HPLC, UV, and RP-HPLC-UV methods are indispensable for the analysis of selexipag, offering high sensitivity, specificity, and compliance with ICH guidelines. RP-HPLC-UV stands out for its ability to separate and quantify selexipag in the presence of impurities and degradation products, making it ideal for stability studies and quality control. UV spectroscopy, while simpler, is best suited for routine analysis in controlled settings. Recent advancements, such as QbD, green chemistry, and hyphenated

techniques, have enhanced the efficiency and sustainability of these methods. Addressing challenges like cost, specificity, and stability will further strengthen their application in pharmaceutical analysis. This review underscores the importance of tailored analytical strategies to ensure the quality and safety of selexipag-based therapies.

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