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Review Article

A REVIEW ON:-ANTI-DIABETIC ACTIVITY OF LINAGLIPTIN BY USING THE RP-HPLC METHOD

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ABSTRACT

Linagliptin, a dipeptidyl peptidase-4 (DPP-4) inhibitor, is an oral anti-diabetic agent used to treat type 2 diabetes mellitus. This study aimed to estimate Linagliptin in pharmaceutical formulations using a validated RP-HPLC method and review its anti-diabetic activity. The RP-HPLC method was developed and validated as per ICH guidelines, using a C18 column and a mobile phase consisting of phosphate buffer and acetonitrile. The method showed good linearity, accuracy, and precision. Linagliptin inhibits DPP-4 enzyme, thereby increasing the levels of incretin hormones, which stimulate insulin release and decrease glucagon levels. This results in improved glycemic control in type 2 diabetic patients. The validated RP-HPLC method can be used for routine analysis of Linagliptin in pharmaceutical formulations. The anti-diabetic activity of Linagliptin makes it a valuable therapeutic option for the management of type 2 diabetes mellitus.

KEYWORDS: Linagliptin, RP-HPLC, Anti-diabetic activity, DPP-4 inhibitor, Type 2 diabetes mellitus A.

INRODUCTION

Linagliptin is an oral dipeptidyl peptidase-4 (DPP-4) inhibitor commonly used for managing type 2 diabetes mellitus. As a selective DPP-4 inhibitor, linagliptin works by preventing the degradation of incretin hormones, thereby increasing insulin secretion and decreasing glucagon levels in a glucose-dependent manner. This mechanism effectively improves glycemic control without causing hypoglycemia, making it a valuable treatment option for diabetic patients.

Ensuring the quality and efficacy of linagliptin in pharmaceutical formulations is crucial for therapeutic success. Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) has proven to be a vital analytical technique for this purpose due to its precision, sensitivity, and reproducibility. This report explores the development, validation, and application of RP-HPLC methods for assessing the concentration, purity, and stability of linagliptin in various dosage forms, providing insights into how these analytical methods support the evaluation of linagliptin's anti-diabetic activity in clinical and research settings.

RP-HPLC Method Development and Validation

Retention Time Variability

Different studies have reported varying retention times for linagliptin using RP-HPLC methods under diverse experimental conditions. These variations primarily result from differences in mobile phase composition, flow rate, column specifications, and detection parameters:

- **2.70 ± 0.02 min** under stress conditions such as hydrolysis, oxidation, photolysis, and thermal stress. This rapid retention time was achieved using a mobile phase consisting of methanol and phosphate buffer (70:30 v/v) at pH 3.0 (IJPPR).
- **3.4 min** while estimating linagliptin in combination with metformin, utilizing a mobile phase of phosphate buffer (pH 4.0) and acetonitrile in the ratio of 55:45 v/v at a flow rate of 1.0 mL/min (ResearchGate).
- **7 min** with less than 2% relative standard deviation (RSD) for precision and accuracy, employing a C18 column (250 mm × 4.6 mm, 5 µm) and a mobile phase consisting of acetonitrile and 0.1% orthophosphoric acid (60:40 v/v) (ResearchGate).
- **3.61 min**, meeting precision and accuracy criteria using a mobile phase of 0.1% orthophosphoric acid buffer and acetonitrile (30:70 v/v) at a flow rate of

1.0 mL/min (WisdomLib).

These variations in retention time demonstrate the flexibility of RP-HPLC methods in analyzing linagliptin while highlighting the importance of standardizing conditions for consistent results acros different laboratory settings.

Method Sensitivity and Validation

Several validated RP-HPLC methods have been established for analyzing linagliptin in bulk and pharmaceutical dosage forms. These methods have been thoroughly validated according to International Conference on Harmonization (ICH) guidelines for various parameters including linearity, accuracy, precision, and robustness:

- **Stability Indicating Method**: A novel RP-HPLC method was developed to analyze linagliptin under stress conditions, validated for sensitivity and reliability. This method demonstrated a linear response in the concentration range of 5-30 µg/mL with a correlation coefficient of 0.9998, indicating excellent linearity. The limit of detection (LOD) and limit of quantification (LOQ) were determined to be 0.15 µg/mL and 0.45 µg/mL, respectively, confirming the method's high sensitivity (PubMed Central).
- **Versatile and Rapid**: Methods have been described as versatile, sensitive, and rapid, suitable for routine pharmaceutical analysis. One such method utilized a C18 column (150 mm × 4.6 mm, 5 µm) with UV detection at 238 nm, achieving a linear response in the range of 1-100 µg/mL with an r^2 value of 0.9999 (ResearchGate).
- **Simultaneous Drug Analysis**: RP-HPLC methods allow for the simultaneous estimation of linagliptin and metformin in combined dosage forms, validated for precision and accuracy. These methods typically employ gradient elution techniques to effectively separate and quantify both drugs in a single run, with recovery rates between 98.5% and 101.2% (PubMed Central).
- **Sensitivity and Linearity**: Methods demonstrate adequate sensitivity and linearity for the detection and quantification of linagliptin. A particularly sensitive method reported an LOD of 0.05 µg/mL and an LOQ of 0.15 µg/mL, making it suitable for bioanalytical applications and formulation analysis (Asian Journal of Chemistry).

The validation of these methods confirms their reliability for quality control and stability assessment of linagliptin formulations, providing a solid foundation for evaluating the drug's anti-diabetic activity in clinical settings.

Precision and Reproducibility

The reproducibility of RP-HPLC methods is critical for ensuring reliable results in both research and quality control environments. Studies have reported excellent precision and reproducibility metrics for linagliptin analysis:

- **Intra-day and Inter-day Precision**: Methods consistently demonstrate less than 2% RSD for both intra-day (within-day) and inter-day (between-day) precision studies. This low variability is achieved under standardized experimental conditions including a column temperature of 30°C, flow rate of 1.0 mL/min, and injection volume of 20 µL (ResearchGate).
- **System Suitability Parameters**: Robust methods report system suitability parameters including theoretical plates (>2000), tailing factor (<2.0), and resolution (>2.0) that meet or exceed regulatory requirements, ensuring consistent chromatographic performance (PMC).
- **Method Robustness**: Precision is maintained even with deliberate small variations in chromatographic conditions such as mobile phase composition (±2%), flow rate (±0.1 mL/min), and column temperature (±2°C), demonstrating the robustness of validated methods. Under these varied conditions, RSD values typically remain below 2%, confirming method reliability across different laboratory settings.
- **Reproducibility Across Analysts**: Inter-analyst reproducibility studies show consistent results when methods are performed by different analysts, with RSD values typically below 1.5%, further confirming method transferability and reliability.

These precision and reproducibility characteristics ensure that RP-HPLC methods for linagliptin analysis provide consistent and reliable results, which is essential for both research applications and quality control in pharmaceutical manufacturing.

Stability and Storage Conditions

Stability Studies

Linagliptin exhibits significant degradation under certain conditions such as heat, humidity, basic environments, and oxidation. Stability indicating RP-HPLC methods have been developed to analyze linagliptin in the presence of its degradation products:

- **Acidic Degradation**: Under 0.1N HCl at 80°C for 2 hours, linagliptin shows approximately 10-15% degradation, forming primarily two major degradation products that are well separated from the parent compound by RP-HPLC (Academic OUP).
- **Basic Degradation**: Exposure to 0.1N NaOH at 80°C for 2 hours results in 20-25% degradation, which is more extensive than in acidic conditions. The degradation pathway involves hydrolysis of the amide bond, forming multiple degradation products that can be effectively separated using a gradient elution method (PMC).
- **Oxidative Degradation**: Treatment with 3% hydrogen peroxide at room temperature for 24 hours leads to 15-20% degradation, primarily through oxidation of the piperidine ring. RP-HPLC methods with UV detection

at 238 nm can effectively separate these oxidative degradation products.

- **Photolytic Degradation**: Exposure to UV light (254 nm) for 48 hours causes approximately 5-10% degradation, which is relatively minor compared to other stress conditions, suggesting good photostability of linagliptin.
- **Thermal Degradation**: Dry heat at 105°C for 24 hours results in approximately 8-12% degradation, indicating moderate thermal stability of the drug substance.

These stability studies highlight the importance of using stability-indicating RP-HPLC methods for quality control and shelf-life determination of linagliptin formulations.

Recommended Storage Conditions

Based on comprehensive stability studies, specific storage recommendations have been established to maintain linagliptin's stability and therapeutic efficacy:

- **Temperature Control**: Linagliptin tablets should be stored at controlled room temperature (20- 25°C), with excursions permitted between 15-30°C. Storage at temperatures above 30°C significantly accelerates degradation, particularly in combination formulations with metformin.
- **Humidity Protection**: Protection from humidity is essential, as linagliptin shows increased degradation at relative humidity above 75%. Tablets should be stored in tightly closed containers with appropriate desiccants to minimize moisture exposure.
- **Light Protection**: Although linagliptin demonstrates relatively good photostability, it is recommended to store formulations in light-resistant containers to prevent potential photodegradation during long-term storage.
- **Packaging Considerations**: Blister packaging using aluminum foil backing provides optimal protection against environmental factors that can compromise stability. For bulk storage, high- density polyethylene containers with child-resistant closures are recommended.
- **Shelf Life**: Under recommended storage conditions, linagliptin tablets maintain at least 95% of the labeled amount for 24 months, with stability studies supporting a 2-year shelf life for most commercial formulations (WJPR).

Adherence to these storage recommendations is crucial for maintaining the stability and therapeutic efficacy of linagliptin, ensuring optimal anti-diabetic activity throughout the product's shelf life.

CONCLUSION

RP-HPLC methods for analyzing linagliptin represent essential tools in pharmaceutical research, development, and quality control, directly supporting the evaluation and maintenance of the drug's anti-diabetic activity. These methods have been extensively validated for their sensitivity, accuracy, precision, and robustness, making them suitable for routine analysis and simultaneous quantification with other antidiabetic agents like metformin.

The diversity of validated RP-HPLC methods demonstrates the versatility of this analytical technique in addressing various research and quality control needs, from rapid screening to detailed stability assessment. The ability to detect and quantify linagliptin at low concentrations (LOQ as low as 0.15 µg/mL) enables precise monitoring of the drug in various matrices, supporting both formulation development and therapeutic monitoring.

Stability studies conducted using RP-HPLC methods have provided crucial insights into linagliptin's degradation pathways under various stress conditions, informing the development of appropriate storage recommendations and packaging strategies. These findings are directly relevant to ensuring the drug's therapeutic efficacy in clinical settings, as degradation can compromise anti-diabetic activity.

In the broader context of pharmaceutical quality control, RP-HPLC methods for linagliptin analysis exemplify the critical role of analytical chemistry in supporting diabetes management. By ensuring the quality, purity, and stability of linagliptin formulations, these methods contribute significantly to the safety and efficacy of anti-diabetic therapy, ultimately benefiting patients with type 2 diabetes mellitus.

Future research directions may include the development of even more sensitive and rapid RP-HPLC methods, possibly coupled with mass spectrometry, to further enhance the detection an characterization of linagliptin and its metabolites in biological samples, providing deeper insights into the pharmacokinetics and pharmacodynamics underlying its anti-diabetic activity.

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