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ANALYTICAL METHOD DEVELOPMENT FOR SITAGLIPTIN USING REVERSE PHASE HPLC: A COMPREHENSIVE STUDY

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Abstract

The investigators in this work hope to one day be able to measure Sitagliptin in pharmaceutical formulations using a Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method. An essential medication for managing type 2 diabetes, sitagliptin acts as a selective inhibitor of the enzyme dipeptidyl peptidase-4 (DPP-4), which controls blood glucose levels. The need of precise quantification in providing consistent therapeutic effects and safety is highlighted by the research. Hypersil ODS C18 column and methanol/triethylamine mobile phase were optimized for RP-HPLC using high sensitivity UV detection at 237 nm. The method is suitable for routine pharmaceutical analyses since it is linear, exact, accurate, and resilient. The method's suitability for establishing a consistent Sitagliptin quality control is confirmed by its low limits of detection (LOD: 0.6773 µg/mL and LOQ: 2.1881/µg/mL), indicating a high level of sensitivity.

Keywords: Sitagliptin, RP-HPLC, DPP-4 Inhibitor, Pharmaceutical Formulations, Method Validation.

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1.INTRODUCTION

Indeed, one of the most often recommended medications for type 2 diabetes is sitagliptin. An key enzyme in glucose metabolism, dipeptidyl peptidase 4 (DPP-4) is inhibited, causing it to act. The incretin hormones, which regulate insulin secretion and blood glucose levels, are broken down by DPP-4. These hormones include glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptide (GIP). Sitagliptin reduces blood glucose levels by boosting the release of insulin and elevating these hormones by blocking DPP-4. As a result, sitagliptin has become an essential medication for the management of blood glucose in individuals with diabetes.
It is critical to have accurate and reliable quantification of Sitagliptin in pharmaceuticals because of its importance in diabetes treatment. To avoid potentially harmful side effects from overdosing or insufficient therapeutic results from underdosing, proper dosage is essential. In order to ensure that pharmaceutical goods containing Sitagliptin are of high quality and safe for patients, this work seeks to build a Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method for accurately measuring Sitagliptin concentrations.

1.1. Importance of Accurate Quantification in Pharmaceutical Products

Several factors make accurate pharmacological dosage determination of Sitagliptin essential. A change in the drug's composition could lead to unsuccessful therapy or unwanted side effects; the precise dosage determines the drug's therapeutic efficacy. The pharmaceutical preparations containing sitagliptin should be of excellent quality and homogeneity in order to ensure that each dosage unit or tablet contains the necessary amount of active ingredient. Treatment efficacy and patient safety might be compromised by improper formulation or contamination. To ensure that each batch of pharmaceuticals meets the specified standard, it is crucial to have reliable quality control methods of analysis for everyday quality control in the production process.

1.2. Reverse Phase HPLC Method for Sitagliptin Determination

The exceptional sensitivity, selectivity, and adaptability of Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) to complex pharmaceutical matrices have earned it the status of a gold standard analytical technology. Non-volatile, hydrophobic substances like Sitagliptin can

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be analyzed using RP-HPLC since it depends on the individual analytes' affinities in the hydrophobic stationary phase. This method is a solid choice for pharmaceutical quality control because of how exact and reliable it is. This study aims to develop and optimize a Sitagliptin RP-HPLC system that may be used as a routine analytical tool in pharmaceutical labs; this system will be fast, accurate, and dependable.

1.3. Research Objectives

The research aimed to design and validate an accurate RP-HPLC method for quantifying Sitagliptin in pharmaceutical preparations. The method's performance was evaluated in terms of sensitivity, linearity, precision, and accuracy.

- The goal is to develop a reliable RP-HPLC assay that can accurately and reliably measure the concentration of Sitagliptin in pharmaceutical formulations.
- In order to ensure that the RP-HPLC method is suitable for routine usage, it must be tested for linearity, precision, accuracy, and durability.
- Identifying the Sitagliptin Limit of Detection (LOD) and Limit of Quantification (LOQ) will allow us to gauge the method's sensitivity.

2. DRUG PROFILE OF SITAGLIPTIN

Molecular structure, action mechanism, solubility property, and adverse effects are all detailed in this section of the Sitagliptin pharmacological profile. With a generally positive safety profile, sitagliptin is a selective DPP-4 inhibitor used to improve blood sugar levels in type 2 diabetic patients.

2.1. Molecular Structure

One key enzyme in glucose metabolism is dipeptidyl peptide-4 (DPP-4), and sitagliptin is a specific and powerful inhibitor of this enzyme. The chemical formula is C16H15F6N5O and the molecular weight is 407.314 g/mol; it is a triazolopyridine derivative. The binding capacity and effectiveness in inhibiting the DPP-4 enzyme and improving the insulin secretion mechanism are both enhanced by the structure's many functional entities. Patients with diabetes may benefit from

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its long-term use because of its unique composition, which allows it to dodge rapid metabolic breakdown.

2.2. Mechanism of Action

One mechanism by which sitagliptin exerts its effects is by inhibiting Dipeptidyl Peptidase-4 (DPP-4), an enzyme that is responsible for the degradation of incretin hormones like GLP-1 and GIP. Important for controlling blood sugar levels, incretin hormones are secreted naturally all across the GI system in response to eating. Pancreatic beta cells secrete more insulin and alpha cells secrete less glucagon when sitagliptin inhibits DPP-4, which in turn increases GLP-1 and GIP levels. Both effects work together to improve glycemic control in type 2 diabetes by lowering blood glucose levels, particularly after eating. One benefit of this method is that it has no hypoglycemia risk, unlike other anti-diabetic medications, because its action is glucose-dependent. This means that insulin production is increased in response to an increase in blood glucose levels.

2.3. Solubility

For the most part, sitagliptin is water- or alcohol-soluble. To dissolve it, you need a polar organic solvent like methanol or N,N-dimethyl formamide (DMF). But its poor solubility in water might reduce its absorption when taken orally. The formulation of Sitagliptin makes it more acceptable to boost absorption and guarantee that this drug can be effective in its therapeutic use, which is a frequent difficulty for most oral drugs due to their low solubility. Tablet formulations that include excipients to increase the rate of dissolving are primarily responsible for addressing the low water solubility.

2.4. Adverse Effects

Compared to other oral anti-diabetic medications, sitagliptin is thought to have a superior adverse effects profile, and the drug's side effects are usually acceptable. However, some people may experience negative effects because it is a medicine. Common adverse effects include stuffy nose, headaches, and infections in the upper respiratory tract. These are just minor side effects that, in most cases, go away after a few days of use. Pancreatitis, or inflammation of the pancreas, is another rare but serious side effect of Sitagliptin that has been documented in a small number of

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post-marketing monitoring studies. Patients with a history of pancreatitis are less likely to be prescribed Sitagliptin due to concerns about the potential danger, even though the exact cause-and-effect relationship between the two has not been completely determined. A low blood sugar, or hypoglycemia, is not a typical side effect of sitagliptin when used alone but may occur when combined with insulin, sulfonylureas, or other anti-diabetic medications. Less common but still possible are gastrointestinal problems including nausea and diarrhea. Sitagliptin has a reduced risk of weight gain, which is good news for many people who have type 2 diabetes.

3. REVIEW OF LITREATURE

In order to determine the Sitagliptin phosphate content in tablets, Tang et al. (2012) developed and validated a stability-indicating RP-HPLC method. The goal of this process was to remove any potential contaminants from the analytical data by isolating Sitagliptin and its breakdown products. We utilized a UV detector operating at 237 nm in conjunction with a methanol and water mobile phase and a Symmetry C18 column. The method is suitable for regular stability testing of Sitagliptin in pharmaceutical preparations because of its effectiveness, linearity, precision, and strength.

Ravanello et al. (2013) created an additional LC method for Sitagliptin phosphate tablets that indicates stability. At 267 nm, they found the product using an isocratic mobile phase consisting of acetonitrile and phosphate buffer. It was determined that the method was accurate, exact, linear, and selective. Based on the results of the experiment, the method might be used to test the stability of Sitagliptin in commercial pharmaceutical formulations since it could distinguish between Sitagliptin and its degradation products.

To estimate Sitagliptin phosphate in pharmaceutical dose forms, Monila et al. (2014) established visible spectrophotometric techniques. Sitagliptin was complexed with two dyes, Bromo Thymol Blue (BTB) and Bromo Cresol Green (BCG), to form colored complexes that could be measured at 412 nm and 419 nm, respectively, according to these procedures. These procedures have been proven to be accurate, sensitive, and reproducible; they provide a cheap, easy way to check the quality of your pharmaceutical analyses.

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Sitagliptin phosphate may be easily and accurately measured in pharmaceutical and bulk forms using a new spectrophotometric technique developed by Balasekaran and Indumathy (2012). The procedure yielded a yellow substance by means of a condensation reaction involving Sitagliptin phosphate, acetyl acetone, and formaldehyde. We tested the method for linearity, accuracy, and precision, and then we conducted the spectrophotometric measurement at 430 nm. Its low price and high reliability make it an excellent choice for regular use in pharmaceutical quality control analyses.

A spectrofluorimetric technique for the determination of Sitagliptin in both tablet form and spiking human serum was devised by Caglar et al. (2013). Aside from assessing Sitagliptin's inherent fluorescence, this study also used a method to measure its reaction with fluorescein, which yielded a derivative with enhanced fluorescence. The linear range of the first approach was 0.5-10.4 µg/mL, whereas the second method covered 0.4-1.8 µg/mL. Both approaches were shown to be very effective in detecting Sitagliptin in complicated pharmaceutical and biological matrices, and they were verified for linearity, precision, sensitivity, and accuracy.

4. METHODOLOGY

This study aimed to design and establish a dependable Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method of the quantification of Sitagliptin in pharmaceutical preparations.

4.1. RP-HPLC Method Development

The RP-HPLC technique involved the use of Hypersil ODS C18 column (250 mm x 4.6 mm, 5 uM) where the mobile phase consisted of methanol and 0.5% triethylamine in a 66:34 v/v mixture. Optimum flow rate was set at 1.0 mL/min with UV recording at 237 nm that is sensitive to the quantification of Sitagliptin. Optimization was made to these conditions to obtain good separation and peak resolution.

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4.2. Sample Preparation

Sitagliptin tablets were powdered and triturated then dissolved in methanol. The vacuum filter was used to filter the solution and proper dilution of the solution was made in methanol to be within the linear range of the calibration curve.

4.3. Instruments Used

- HPLC-UV detector: It is necessary to quantify Sitagliptin due to its absorbance of 237 nm.
- UV-Visible Spectrophotometer: This instrument is applicable in the determination of the λmax of Sitagliptin.
- pH Meter: The meter was used to titrate the pH of the mobile phase to 3.0 ± 0.1.
- Vacuum Filter: S ensured sample clarity by eliminating the particulates prior to analysis.

4.4. Method Validation

The technique was tested as linear, precise, accurate, and robust in accordance to ICH requirements. Calibration curves were plotted and reliability of the method was tested using study of repeatability and accuracy.

5. RESULT

This part includes the findings of the RP-HPLC method development of Sitagliptin quantification, determination of 1 / 1max, method optimization, linearity, precision, accuracy, and sensitivity. The results confirm the strength of the technique, sensitivity, and the applicability of the technique to normal pharmaceutical studies.

5.1. Determination of λmax

In the methanol absorption spectrum, the absorption spectra of Sitagliptin were acquired and the drug showed a typical peak at 237 nm which was chosen to be used in HPLC analysis in U.V. detection. The absorption spectrum of Sitagliptin in methanol in figure 1 has a typical peak at 237 nm which was chosen as the UV absorbing wavelength in the HPLC analysis.

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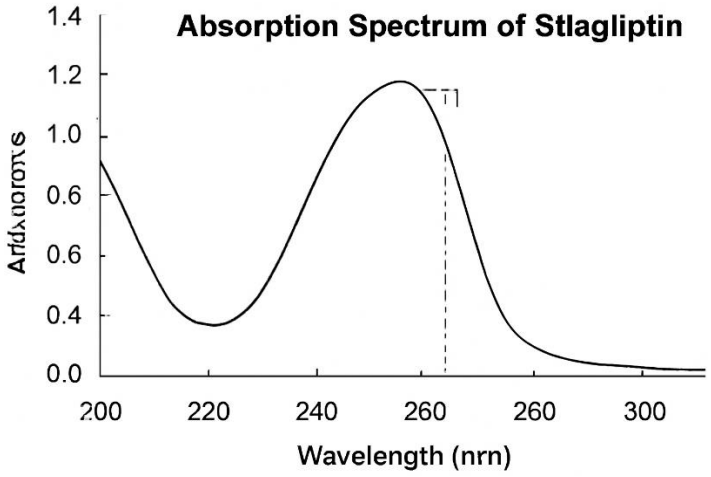

Figure 1: Absorption spectrum of Sitagliptin at 237 nm.

The highest point at 237 nm indicates the maximum absorption wavelength (maximum absorption) (6) of Sitagliptin meaning the wavelength which the drug absorbs UV light best. The selected wavelength (UV) was used in the HPLC analysis to detect the UV in order to achieve a high level of sensitivity and precise determination of Sitagliptin in pharmaceutical preparations. The steepness of the peak indicates that the technique is very selective to Sitagliptin and other compounds will not interfere with the technique.

5.2. Method Optimization

The optimum mobile phase (Methanol:0.5% Triethylamine, 66:34 v/v) gave a retention time (Rt) of 3.6 minutes which provided good peak resolution. The chromatogram presented below shows that Sitagliptin is separated significantly in the tablet matrix in comparison with other excipients. The figure shows the optimized chromatogram of Sitagliptin, which was obtained in the application of a mobile phase, made up of a mixture of methanol:0.5% triethylamine (66:34 v/v). A retention time (Rt) of 3.6 minutes is observed in the chromatogram, which indicates that Sitagliptin is effectively separated with other excipients in the tablet matrix.

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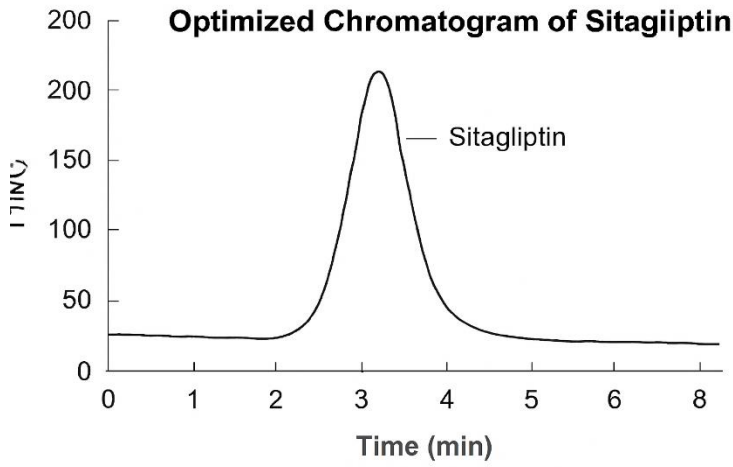

Figure 2: Optimized Chromatogram of Sitagliptin.

It can be seen that the chromatogram shows that the optimized mobile phase is capable of separating Sitagliptin and excipients well with a sharp and distinct peak at 3.6 minutes. This confirms the approach ability to give the straight answer thus creating the right quantification of Sitagliptin in the pharmaceutical formulations. The clear distinction underlines the applicability of the method in the regular quality control in pharmaceutical industry.

5.3. Linearity and Calibration

The figure represents the calibration curve of Sitagliptin that indicates the dependence of the peak area on the concentration (20-200 µg/mL). The high linear relationship with a correlation coefficient of 0.9999 assures the strength and quality of method to measure Sitagliptin.

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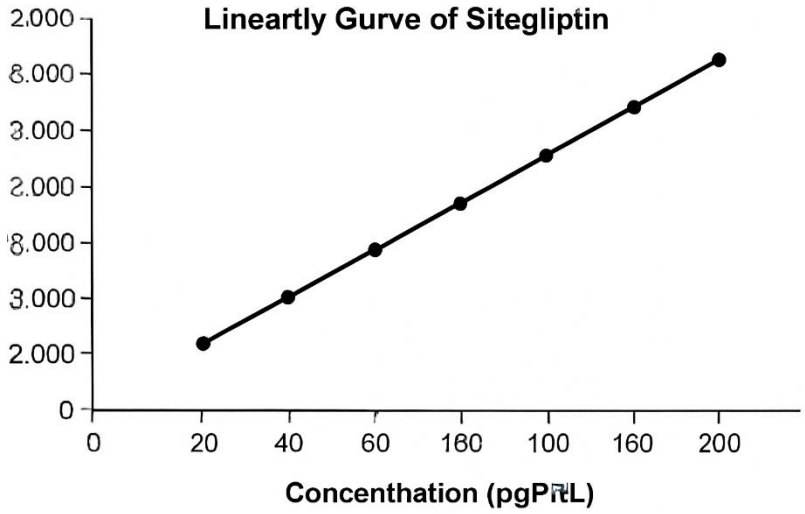

Figure 3: Linearity curve of Sitagliptin.

The linearity curve shows that the concentration of Sitagliptin is directly proportional to the area of the peak with high correlation coefficient (0.9999) indicating that the technique is effective and precise in measuring the concentration of Sitagliptin in pharmaceutical preparations.

Calibration was developed with a concentration of 20-200 ug/ ml. The data on the linearity (Table 1) showed a correlation of 0.9999 which validated the strength of the method in the determination of the correct quantification.

Table 1: Linearity data for Sitagliptin.

Conc. (µg/mL)	Peak Area
20	1.973
40	3.767
60	5.452
80	7.331

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120	10.843
160	14.365

The results demonstrate a steady rise in the peak area with the Sitagliptin concentration, which proves the existence of a strong linear correlation between them and validity of the approach to precise quantification.

5.4. Precision

Repeatability and intermediate precision tests were run to ensure the technique was accurate. The approach is reproducible across and within trials, as shown by the low values of the percent RSD in Tables 2 and 3.

In terms of concentration (mg/ml), peak area with standard deviation (SD), and percentage relative standard deviation (percent RSD), the findings of the precision (repeatability) of the Sitagliptin quantification method are displayed in the table below.

Table 2: Precision (Repeatability) study results.

Conc. (µg/mL)	Area ± SD	%RSD
20	1.836 ± 0.01	0.54
40	3.684 ± 0.04	1.08
80	7.192 ± 0.07	0.97
120	10.943 ± 0.16	1.46
200	17.434 ± 0.28	1.60

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Because the percent RSD numbers are modest, we can say that the approach is highly consistent and repeatable. The method is applicable for routine analysis since it accurately quantifies Sitagliptin and the percentage relative standard deviation values fall within the range of 0.54 to 1.60.

The results of the different trials for Sitagliptin at an intermediate level of accuracy are shown in this table together with their concentration (µg/mL), peak area with standard deviation (± SD), and percentage relative standard deviation (% RSD).

Table 3: Precision (Intermediate Precision) study results.

Conc. (µg/mL)	Area ± SD	%RSD
20	1.820 ± 0.02	1.09
40	3.678 ± 0.04	1.08
80	7.256 ± 0.10	1.37
120	10.988 ± 0.16	1.45
200	17.492 ± 0.28	1.60

Results from the repeatability study and the intermediate precision study for the percentage of RSD are comparable, demonstrating that the approach maintains its accuracy and reproducibility when tested by different operators. The soundness of the methodology and its capacity to give consistent results in varied situations are demonstrated by the estimated logarithms of RSD, which range from 1.09 percent to 1.60.

5.5. Accuracy

Spiking standard solutions with 50, 100, and 150 percent of the correct concentration allowed for the determination of accuracy. The method's accuracy was demonstrated by the recovery values, which ranged from 100.97 to -101.3. Table 4 shows the area responses and percentages of recovery

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from a Sitagliptin recovery study conducted at different concentration levels (50, 100, and 150 percent).

Table 4: Results of Recovery.

Level (%)	Area Response SITA	Recovery (%)
50	5.617	101.86 ± 0.10
100	7.211	100.97 ± 1.5
150	9.125	101.09 ± 0.8

Proof of the method's accuracy and reliability are recovery percentages near 100, 100.97-101.86. Low standard deviation values indicate that the approach is suitable for quantitative analysis of pharmaceutical preparations since it produces consistent and trustworthy results across a range of concentrations.

5.6. LOD and LOQ

The verification of the method's sensitivity led to the determination of the limit of detection (LOD) as 0.6773 µg/mL and the limit of quantitation (LOQ) as 2.188 1µg/mL. Table 1 displays the Limit of Detection (LOD) and Limit of Quantification (LOQ) for Sitagliptin, which indicate the lowest values at which the method can be used with any degree of certainty.

Table 5: Result of LOD and LOQ.

Parameter	Sitagliptin
LOD (µg/mL)	0.6773
LOQ (µg/mL)	2.1881

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A LOD value of 0.6773 0g/ml and LOQ of 2.1881 0g/ml prove the high sensitivity of the method. The above values affirm that the technique can identify and quantify Sitagliptin accurately even at a low concentration and thus it is used in the accurate analysis of pharmaceutical products.

6.CONCLUSION AND RECOMMENDATIONS

In order to quantify sitagliptin in pharmaceutical preparations, this research was able to develop and demonstrate an effective Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method. Across a wide concentration range of 20200 µg/mL, the method demonstrated exceptional sensitivity, accuracy, and precision. A correlation coefficient of 0.9999, an optimum retention time of 3.6 minutes, and effective separation of Sitagliptin from other excipients proved that the approach was robust enough for routine usage. Validation results, such as small values of the percent reproducible s.d. and high recovery levels, show that the approach is suitable for quantifying Sitagliptin and quality controlling it in commercial preparations. Furthermore, the method's great sensitivity is supported by its low detection and quantification limits (LOD: 0.6773 ug/mL, LOQ: 2.1881/mL).

- Routine Quality Control Testing: The designed RP-HPLC technique can be implemented in routine to measure the concentrations of Sitagliptin in the pharmaceutical products to provide control over the product quality and safety of the patients throughout the production batches.
- Additional Method Optimization: Additional optimization of the mobile phase composition and column configurations can be considered in future applications to improve the efficiency and throughput of the method, which might decrease the time of analysis of large-scale testing.
- Application to Biological Samples: It is suggested that the method should be adapted to analyze Sitagliptin in biological samples including serum or plasma to add value to its application in pharmacokinetic research and in therapeutic drug monitoring.

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