JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF ANTI-DIABETIC DRUGS

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ABSTARCT

Analytical method development and validation play a crucial role in the pharmaceutical industry, particularly for quality control and regulatory compliance of antidiabetic drugs. With the rising global prevalence of diabetes mellitus, the need for effective, safe, and reliable antidiabetic medications is increasing. Accurate quantification and characterization of these drugs are essential throughout their development, production, and post-marketing stages. This study focuses on the development and validation of an analytical method for the estimation of a selected antidiabetic drug using techniques such as High-Performance Liquid Chromatography (HPLC), UV-visible spectrophotometry, or other suitable methods. Method development involves optimizing various parameters, including mobile phase composition, flow rate, detection wavelength, and column type to ensure the method's selectivity, sensitivity, and reproducibility.

The developed method is then validated according to ICH (International Council for Harmonization) guidelines, evaluating key parameters such as specificity, linearity, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ), and robustness. Validation ensures the method's suitability for routine analysis in quality control laboratories and guarantees consistent drug performance and patient safety. The results demonstrate that the proposed method is simple, rapid, cost-effective, and suitable for the reliable quantification of the antidiabetic drug in bulk and pharmaceutical dosage forms. This work supports the regulatory submission and manufacturing process, contributing to the assurance of pharmaceutical quality.

Key words – Detection wavelength, Regulatory compliance, pharmaceutical analysis, Flow rate.

INTRODUCTION

ANALYTICAL CHEMISTRY

Analytical Chemistry is defined as “The science and the art of determining the composition of materials in terms of the elements or compounds contained.” This branch of chemistry, which deals with both theoretical, practical science and is practiced in a large number of laboratories in many diverse ways. Methods of analysis are routinely developed, improved, validated, collaboratively studied and applied. In analytical chemistry, it is of prime importance to gain information about the qualitative and quantitative composition of substances and chemical species to find out what substance is composed of and exactly how much. In quantitative analysis, the question is how much is present. The research work in this thesis is based on this criterion. Pharmaceutical analysis deals not only with medicaments (drugs and their formulations) but also with their precursors, i.e., with the raw material on which the degree of purity and quality of medicament depends. The quality of the drug is determined after establishing its authenticity by testing its purity and the quality of the pure substance in the drug and its formulations. [1,2]

Importance of Drug Analysis

Medicines are a key part of the healthcare system. Numerous medicines are introduced into the world-market and also, and that is increasing every year. Due to the rapid growth of the pharmaceutical industry during the last several years, several pharmaceutical formulations enter as a part of the health care system, and thus, there has been rapid progress in the field of pharmaceutical analysis. [3,4]

Relevance of Analytical Methods

Analytical methods, which are a measure of the quality of the drugs, play a very comprehensive role in drug development and follow-up activities. It assures that a drug product meets the established standard, is stable and will continue to meet the purported quality throughout its shelf life. These methods should be selective and sensitive to monitor the known and unknown impurities and have to be written in a format such that they can be reproduced over a period of time and from laboratory to laboratory, i.e., these methods should be validated.[5]

CHROMATOGRAPHY

"Chromatography is defined as a procedure by which solutes are separated by a dynamic differential migration process in a system consisting of two phases, one of which moves continuously in a given direction and in which the individual substances exhibit mobilities by reasons of difference in adsorption, partition, solubility, vapor pressure, molecular size or ionic charge density." [6].

High Performance Liquid Chromatography (HPLC)

High Performance Liquid Chromatography (HPLC) was developed in the late 1960s and early 1970s. Today it is widely applied for separation and purification in a variety of areas including pharmaceuticals, biotechnology, environmental, polymer and food industries. High Performance Liquid Chromatography (HPLC) was known as high-pressure liquid chromatography. It is a form of column chromatography in which the stationary phase consists of small particles (3-50 μm) packing contained in a column with a small bore (2- 5mm), one end of which is attached to a source of pressurised liquid eluent (Mobile phase). The technique offers major speed improvements, resolving power, detection, quantification, convenience and applicability to new sample types. Modern HPLC techniques became available in 1969, but from the 1990s, HPLC became the most popular instrument for drug analysis, which is presently used in pharmaceutical research and development.[6]

The following are some important types of HPLC:

A. Based on modes of chromatography
1. Normal phase mode
2. Reverse phase mode
B. Based on the principle of separation
1. Adsorption chromatography
2. Ion exchange chromatography
3. Ion pair chromatography
4. Gel permeation chromatography
5. Affinity chromatography

C. Based on the elution technique

1. Isocratic separation
2. Gradient separation

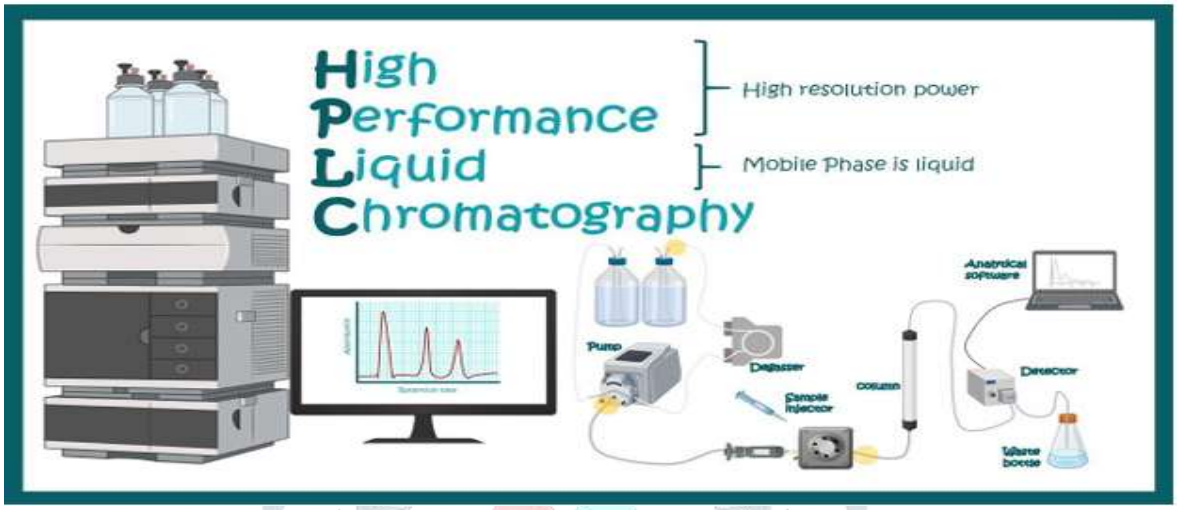
D. Based on the scale of operation
1. Analytical HPLC
2. Preparative HPLC

E. Based on the type of analysis
1. Qualitative analysis
2. Quantitative analysis

Reversed-phase high-performance liquid chromatography (RP-HPLC) involves the separation of molecules based on hydrophobicity. The separation depends on the hydrophobic binding of the solute molecule from the mobile phase to the immobilized hydrophobic ligands attached to the stationary phase, i.e., the sorbent. The solute mixture is initially applied to the sorbent in the presence of aqueous buffers, and the solutes are eluted by the addition of organic solvent to the mobile phase. Elution can proceed either by isocratic conditions where the concentration of organic solvent is constant, or by gradient elution, whereby the amount of organic solvent is increased over a period of time.

INSTRUMENTATION:

High-performance liquid chromatography is now one of the most powerful tools in analytical chemistry. It can separate, identify, and quantify the compounds that are present in any sample that can be dissolved in a liquid.



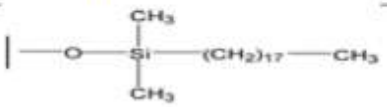
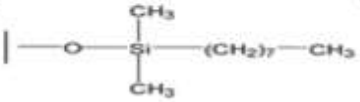
HPLC columns

The column is usually made up of heavy glass or stainless-steel tubing to withstand high pressure. The columns are usually 10-30 cm long and 4-10 mm inside diameter, containing a stationary phase at a particle diameter of 25 µm or less. Columns with an internal diameter of 5 mm give good results because of a compromise between efficiency, sample capacity, and the amount of packing and solvent required.

C18 and C8 HPLC Columns

- Classic reversed-phases for all general-purpose applications
- Excellent peak shape and efficiency compared to competitive columns
- Classic reversed-phase retention and selectivity
- C18 is generally more retentive than the C8

Table 1: Characteristics of C18 and C8 Column

Properties	C_{18}	C_8
Bonded Phase	Octadecylsilane, end capped	Octylsilane, end capped
Silica	Spherical, high purity (Fe <20; Na <7; Ca <7; Ti<1; Al <1; Mg <1ppm)	Spherical, high purity (Fe <20; Na <7; Ca <7; Ti<1; Al<1; Mg <1ppm)
Particle Size	5µm	5µm
Pore Size	180(Å)	180(Å)
Surface Area	200m^2/g	200m^2/g
%C	~12%	~7.5%
Coverage	~3µmoles/m^2	~3.4µmoles/m^2
Structure		

Column packing:

The packing used in modern HPLC consists of small, rigid particles having a narrow particle size distribution. There are three main types of columns packing in HPLC.

Porous, polymeric beds:

Porous, polymeric beds based on styrene divinyl benzene copolymers used doe ion exchange and size exclusion chromatography. For analytical purposes, these have now been replaced by silica-based ones, which are more efficient and more stable.

Porous layer beds:

Consisting of a thin shell (1-3 µm) of silica or modified silica on a spherical inert core (e.g., Glass). After the development of totally porous micro-particulate packings, they have not been used in HPLC.

Porous silica particles:

These packings have been widely used for analytical HPLC in recent years. Particles of diameter >20 µm are usually dry packed. While particles of diameter

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HPLC pumps:

The pump is one of the most important components of HPLC, since its performance directly affects retention time, reproducibility and detector sensitivity. Three main types of pumps are used in HPLC to propel the liquid mobile phase through the system.

1. *Displacement pump*: It produces a flow that tends to be independent of viscosity and back pressure, and also the output is pulse-free. But it possesses limited capacity (250 ml).

2. *Reciprocating pump*: It has a small internal volume (35 to 400 µl), high output pressure (up to 10,000 psi) and constant flow rates. But it produces a pulsed flow.

3. *Pneumatic or constant pressure pump:* They are pulse-free; suffer from limited capacity as well as a dependence of flow rate on solvent viscosity and column back pressure. They are limited to pressure less than 2000 psi.

HPLC injectors

Insertion of the sample into the pressurized column must be as a narrow plug so that the peak broadening attributable to this step is negligible. The injection system itself should have no dead (void) volume. There are three important ways of introducing the sample into the injection port.

In Loop injection - A fixed amount of volume is introduced by making use of a fixed volume loop injector.

In Valve injection, A variable volume is introduced by making use of an Injection valve. 3. On column injection- a variable volume is introduced using a Syringe through a septum.

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HPLC detectors

Selective detectors can be used advantageously to simplify the chromatography in many instances; two solutes need not be separated if the detector responds only to one of them. Detectors can be broadly classified into bulk property and solute property detectors.

Bulk property detectors

continuously monitor some property of the mobile phase, such as refractive index, conductance, or dielectric constant, which changes as solute is added to the mobile phase. Bulk property detectors have a finite signal in the absence of a solute, and this results in two serious limitations of these detectors.

1. The addition of a low concentration of solute will add only a small increment to what may already be a large background signal; as a \
2. Result, these detectors generally have poor limits of detection and are, in general, not suitable for trace analysis.

2. As they also respond to the mobile phase, the signal changes with changes in mobile phase conditions, and these detectors are largely incompatible with gradient elution techniques.

Solute property detectors respond to some specific property of certain compounds, such as the ultraviolet absorbance detector. These detectors generally have much lower limits of detection, and apply only to those compounds showing that specific property. Certain merits are relevant to all detection techniques, i.e., sensitivity, noise, detection limit, linearity, response time, cell volume and quantitation, etc. To choose any detector, these factors need to be taken into account. The different types of detectors used in HPLC are given as follows;

Applied certain techniques for measurement, i.e., Potential pulse techniques, scanning techniques, and multiple electrode techniques, all have been employed.

a) Conductance Detectors

• Differ from electrochemical detectors by non-Faradaic electrochemistry, i.e., no electron transfer reaction takes place

• Post-column method of chromatography, removing the background conductance from the mobile phase, makes the use of this detector wide.

b) Other Detectors

• Post-column Reaction Detector (derivatization and separation reaction product use to detect) • Hyphenated Techniques (LC-MS, GC-MS, FT-IR).

Modes of HPLC

1. *Normal phase chromatography*

• Mechanism: Retention by interaction of the stationary phase's polar surface with polar parts of the sample molecules.

• Stationary phase: SiO2, Al2O3, -NH2, -CN, -NO2

• Mobile phase: Heptane, hexane, cyclohexane, CHCL3

• Application: Separation of non-ionic, non-polar to medium polar substances.

2. *Reverse phase chromatography*

• Mechanism: Retention by interaction of the stationary phase's non-polar hydrocarbon chain with non-polar parts of the sample molecules.

• Stationary phase: n-octadecyl (PR-18), n-octyl (RP-8), ethyl (RP-2), phenyl.

• Mobile phase: Methanol or acetonitrile /water or buffer (sometimes with additives of THF or dioxane).

• Application: Separation of non-ionic and ion-forming non-polar to medium polar substances (carboxylic acid-hydrocarbon). If forming substances (as carboxylic acid) are to be separated, a pH control by buffers is necessary.

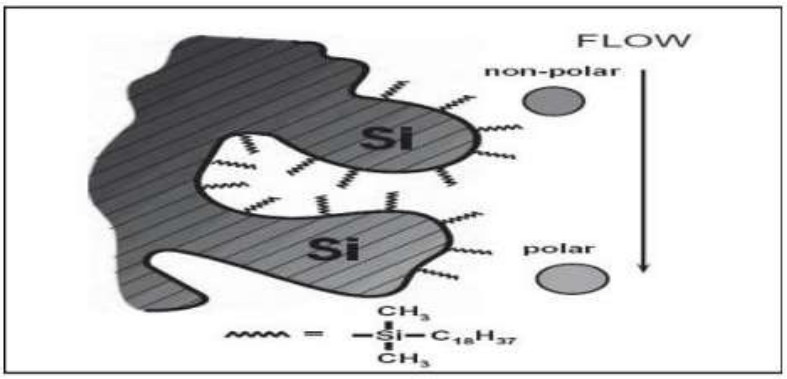

Fig 1.4: Separation modes of reverse phase chromatography

3. *Ion-exchange chromatography Mechanism:*

Retention by reversible ionic bonds to charged groups on the stationary phase. Application: Separation of substances which can form ions:

- Inorganic ions
- Organic acids, Organic bases

4. *Size Exclusion Chromatography*

This is a separation mode based on the analyte's molecular size. In this mode large molecule is excluded from the pores and migrates quickly, whereas a small molecule can penetrate the pores and migrate more slowly down the column.

RP HPLC method development

RP HPLC method development is a critical step in analytical chemistry that involves the optimization of various parameters to achieve the desired separation and quantification of analytes in complex mixtures. RP HPLC stands for Reverse Phase High-Performance Liquid Chromatography, which is a widely used chromatographic technique for the separation of polar and non-polar compounds based on their hydrophobicity.

The development of an effective RP HPLC method involves several key steps, including the selection of an appropriate column, mobile phase, and detection method. The column is the heart of the RP HPLC system and is selected based on the properties of the analytes and the separation mechanism. The mobile phase is typically composed of a polar solvent, such as water, and an organic solvent, such as acetonitrile or methanol. The ratio of the two solvents can be adjusted to optimize the separation of analytes based on their hydrophobicity.

The detection method is also a crucial parameter in RP HPLC method development, as it determines the sensitivity and selectivity of the analysis. Common detection methods include UV-Vis spectroscopy, mass spectrometry, and fluorescence spectroscopy. The choice of detection method depends on the nature of the analytes and the level of sensitivity required for the analysis.

To optimize the RP HPLC method, a systematic approach is typically employed, such as Design of Experiments (DoE) or Quality by Design (QbD). These approaches involve the variation of multiple parameters simultaneously to identify the most significant factors that affect the separation and quantification of analytes. This allows for the determination of the optimal conditions for the RP HPLC method, such as the flow rate, column temperature, and gradient elution profile.

Recent advances in RP HPLC method development have also focused on the use of artificial intelligence, such as machine learning and deep learning, to optimize the method development process. These approaches can expedite the development of an effective RP HPLC method by reducing the time and resources required for method optimization.

A. Background and importance of RP HPLC method development

High-performance liquid chromatography (HPLC) is a widely used analytical technique that allows for the separation, identification, and quantification of complex mixtures of compounds. Reversed-phase HPLC (RP HPLC) is one of the most popular and versatile modes of HPLC used in pharmaceutical, food, and environmental industries, among others. The development of an effective RP HPLC method is a critical step in the analytical process, as it determines the accuracy, sensitivity, and selectivity of the analysis.

The background and importance of RP HPLC method development lies in its ability to provide accurate and precise quantification of analytes in complex mixtures. The specificity of RP HPLC makes it an essential tool in the development of new drugs and formulations, as well as in the analysis of environmental contaminants and food and beverage additives. RP HPLC is particularly useful in pharmaceutical analysis, where it is employed for drug discovery, quality control, and stability testing.

RP HPLC method development involves the optimization of various parameters, such as mobile phase composition, column type, and detection wavelength, among others. The development process requires a deep understanding of the chemical and physical properties of the analytes and the separation mechanism of the stationary and mobile phases. A well-developed RP HPLC method should provide high resolution, good reproducibility, and low detection limits.

The importance of RP HPLC method development is evident in the widespread use of this technique in a broad range of industries. In pharmaceutical analysis, RP HPLC is used to determine the purity and potency of drug substances and products, as well as to evaluate the stability and degradation of these compounds under various conditions. In food and beverage analysis, RP HPLC is employed to identify and quantify additives, contaminants, and natural compounds. In environmental analysis, RP HPLC is used to detect and measure pollutants, such as pesticides, herbicides, and toxic metals.

Table 1.1: Common challenges in RP HPLC method development [39-45]

Challenge	**Potential Solutions**
Selectivity	Use of alternative column chemistries, mobile phase modifiers, and/or additives
Resolution	Optimization of column dimensions, particle size, and/or mobile phase composition
Sensitivity	Optimization of detection parameters, including wavelength and/or flow cell volume
Reproducibility	Implementation of rigorous quality control procedures, including use of reference standards and standard operating procedures
Matrix effects	Sample preparation techniques, such as solid-phase extraction or protein precipitation, to remove interfering substances

Table1.2: Advantages and disadvantages of RP HPLC [46-48]

Advantage	**Disadvantage**
High resolution	Requires specialized equipment and expertise
Wide range of applications	Limited selectivity for some compounds
Compatible with many detection techniques	Can be time-consuming
High reproducibility	Requires careful optimization of mobile phase conditions
Robust and reliable	Limited capacity for large molecules

Table 1.3: Comparison of RP HPLC with other separation techniques [49-54]

Technique	Advantages	Disadvantages
Gas chromatography	High sensitivity and resolution for volatile and semivolatile compounds	Limited applicability to nonvolatile and thermally unstable compounds
Size exclusion chromatography	Separation based on size and shape of molecules	Limited resolution for small molecules
Ion exchange chromatography	Selective for charged molecules	Limited capacity for large molecules
Affinity chromatography	High selectivity for specific molecules	Limited capacity and specificity for some compounds
Capillary electrophoresis	High resolution and speed for charged molecules	Limited capacity and applicability to neutral and nonpolar molecules

These tables provide a useful summary of the key challenges, advantages, and disadvantages of RP HPLC method development, as well as a comparison of RP HPLC with other separation techniques.

B. Scope and limitations

The scope of the RP HPLC method is extensive, and it has become one of the most commonly used analytical techniques in various industries, including pharmaceutical, food and beverage, and environmental analysis. RP HPLC is used to separate, identify, and quantify analytes in complex mixtures, making it a valuable tool in modern analytical chemistry. RP HPLC is highly sensitive and selective, allowing for the detection and quantification of trace amounts of analytes.

However, like any analytical technique, RP HPLC has limitations. The limitations of the RP HPLC method include its inability to separate compounds that have similar structures and physicochemical properties. This can lead to co-elution of analytes, resulting in decreased resolution and sensitivity. RP HPLC also has limitations in terms of sample compatibility with the mobile phase and the stationary phase, which may result in poor peak shape and decreased accuracy of the analysis. In addition, RP HPLC may require lengthy analysis times and may not be suitable for high-throughput analyses.

Furthermore, the development of an effective RP HPLC method can be challenging and time-consuming, as it requires optimization of various parameters, such as the column type, mobile phase composition, and detection method. The complexity of the method development process may also require highly skilled personnel and specialized equipment, which can increase the cost and time required for the analysis. [55-57]

RP HPLC method development

A. Principles of RP HPLC method development

RP HPLC method development is based on the principles of liquid chromatography, which involves the separation of components in a mixture based on their physicochemical properties, such as size, shape, charge, and hydrophobicity. RP HPLC is a type of liquid chromatography that uses a stationary phase with a hydrophobic surface and a mobile phase with a polar solvent and an organic solvent. The separation is based on the differential affinity of analytes for the stationary and mobile phases.

The principles of RP HPLC method development include the selection of an appropriate stationary phase, mobile phase, and detection method, as well as the optimization of various parameters, such as the column type, column temperature, flow rate, and gradient elution profile.

The stationary phase in RP HPLC is typically a silica-based material that has been modified with a hydrophobic functional group, such as octadecylsilane (C18) or octyl silane (C8). The selection of the stationary phase is based on the properties of the analytes and the separation mechanism. For example, C18 columns are commonly used for the separation of non-polar and moderately polar compounds, while C8 columns are more suitable for the separation of highly polar compounds. The mobile phase in RP HPLC typically consists of a polar solvent, such as water, and an organic solvent, such as methanol or acetonitrile. The ratio of the two solvents can be adjusted to optimize the separation of analytes based on their hydrophobicity. A gradient elution profile can also be employed to improve separation, where the ratio of the two solvents is varied over time.

The detection method in RP HPLC is also important, as it determines the sensitivity and selectivity of the analysis. Common detection methods include UV-Vis spectroscopy, mass spectrometry, and fluorescence spectroscopy. The choice of detection method depends on the nature of the analytes and the level of sensitivity required for the analysis.

The optimization of various parameters in RP HPLC method development is typically achieved through a systematic approach, such as Design of Experiments (DoE) or Quality by Design (QbD). These approaches involve the variation of multiple parameters simultaneously to identify the most significant factors that affect the separation and quantification of analytes. This allows for the determination of the optimal conditions for the RP HPLC method.

In summary, the principles of RP HPLC method development involve the selection of an appropriate stationary phase, mobile phase, and detection method, as well as the optimization of various parameters to achieve the desired separation and quantification of analytes in complex mixtures. [58-65]

B. Key factors affecting RP HPLC method development

There are several key factors that can affect the development of a successful RP HPLC method.

These factors can influence the separation and quantification of analytes in a complex mixture and can include the following:

- Stationary phase: The choice of the stationary phase is crucial in RP HPLC method development. The surface chemistry of the stationary phase determines its selectivity, capacity, and retention behavior towards analytes. The most commonly used stationary phases in RP HPLC are C18, C8, and phenyl-based phases. The selection of the appropriate stationary phase depends on the nature of the analytes and the separation mechanism.

- Mobile phase: The mobile phase is also a critical factor in RP HPLC method development. The mobile phase should be optimized to provide the necessary selectivity, resolution, and sensitivity required for the separation of analytes. The composition of the mobile phase, such as the ratio of water to organic solvent and the pH, can impact the retention behavior of analytes.

- Column dimensions: The dimensions of the column, such as the length and diameter, can also impact the separation and quantification of analytes. Longer columns can provide better resolution, but at the cost of longer analysis times, while wider columns can provide higher sample throughput but may result in lower resolution.

- Flow rate: The flow rate of the mobile phase through the column can impact the separation and resolution of analytes. Too high a flow rate can lead to band broadening and decreased resolution, while too low a flow rate can result in long analysis times.

- Gradient elution: The use of a gradient elution profile can improve separation and resolution in RP HPLC method development. The gradient can be adjusted to optimize the separation of analytes based on their hydrophobicity.

- Detection method: The choice of detection method can impact the sensitivity and selectivity of the analysis. Common detection methods include UV-Vis spectroscopy, mass spectrometry, and fluorescence spectroscopy. The choice of detection method depends on the nature of the analytes and the level of sensitivity required for the analysis.

- Sample preparation: Proper sample preparation is crucial for the success of RP HPLC method development. Sample preparation can include steps such as extraction, purification, and derivatization to improve the separation and quantification of analytes. [66,67]

The key factors that can affect RP HPLC method development include the stationary phase, mobile phase, column dimensions, flow rate, gradient elution, detection method, and sample preparation. Proper optimization of these factors can lead to the successful separation and quantification of analytes in complex mixtures.

C. Optimization strategies for RP HPLC method development

Optimization of RP HPLC method development is crucial to achieve the desired separation and quantification of analytes in a complex mixture. Here are some optimization strategies that can be applied during RP HPLC method development:

- Stationary phase screening: The choice of stationary phase is a critical factor in RP HPLC method development. By screening different stationary phases, the most suitable phase can be identified based on selectivity, retention behavior, and capacity towards the analytes of interest.

- Mobile phase optimization: The mobile phase composition can also be optimized to achieve the desired separation and resolution. By varying the ratio of organic solvent to water, pH, and buffer concentration, the retention behavior of analytes can be controlled.

- Column dimensions optimization: Column dimensions can impact the separation and analysis time. By optimizing the column length and diameter, the desired resolution and analysis time can be achieved.

- Flow rate optimization: Flow rate optimization can improve the separation efficiency and minimize analysis time. A suitable flow rate should be chosen to prevent band broadening while maintaining the separation of analytes.

- Gradient elution optimization: Gradient elution can be used to improve separation and resolution in RP HPLC method development. The gradient can be optimized by varying the slope, duration, and endpoint conditions to achieve the desired separation and resolution.

- Temperature optimization: Temperature can impact the separation and retention of analytes. By varying the column temperature, the retention time and selectivity can be optimized.

- Sample preparation optimization: Proper sample preparation is crucial for the success of RP HPLC method development. Sample preparation optimization can include extraction, purification, and derivatization to improve the separation and quantification of analytes.

Overall, optimization strategies for RP HPLC methods development should be based on the specific nature of the analytes, the intended separation mechanism, and the desired resolution and analysis time. Proper optimization can lead to the successful separation and quantification of analytes in complex mixtures. [68,69]

Table 1.4: New stationary phases for RP HPLC [70,71]

Type of Stationary Phase	**Properties**	**Applications**
Core-shell	High efficiency, reduced backpressure, improved selectivity	Pharmaceuticals, peptides, proteins, natural products
Porous organic polymers	High surface area, tunable pore size and chemistry, stability in a wide pH range	Environmental analysis, natural products, synthetic polymers
Zwitterionic	Unique selectivity, stability at extreme pH, reduced nonspecific adsorption	Proteins, peptides, natural products
HILIC	Retention of polar and hydrophilic compounds, complementary to RP HPLC	Glycoproteins, carbohydrates, nucleotides

Table 1.5: New detection techniques for RP HPLC [72,73]

Type of Detection	Properties	Applications
Mass spectrometry	High sensitivity, selectivity, and accuracy, ability to identify unknown compounds	Pharmaceuticals, metabolomics, environmental analysis
Fluorescence	High sensitivity, selectivity, and speed, nondestructive	Pharmaceuticals, food and beverage, environmental analysis
Electrochemical	High sensitivity, selectivity, and speed, low cost and simple instrumentation	Pharmaceuticals, food and beverage, environmental analysis

Table 1.6: New applications and techniques for RP HPLC [74]

Type of Application	Properties	Examples
Metabolomics	Comprehensive analysis of metabolites, identification of biomarkers	Disease diagnosis, drug discovery
Proteomics	Identification and quantification of proteins and peptides, characterization of post-translational modifications	Biomarker discovery, drug development
Glycomics	Analysis of glycoproteins, glycolipids, and glycans, characterization of carbohydrate structures	Disease diagnosis, vaccine development
Microfluidics	Integration of multiple analytical steps into a single device, high throughput and automation	Point-of-care diagnostics, drug screening
Lab-on-a-chip	Miniaturization of analytical systems, reduced sample and reagent consumption	Environmental monitoring, food safety testing

These tables provide a useful summary of the key advances and trends in RP HPLC method development, and can help researchers and practitioners stay up-to-date with the latest developments in the field.

III. Recent Advances in RP HPLC Method Development

A. Modern approaches for RP HPLC method development

In recent years, there have been several advances in RP HPLC method development that have improved the efficiency, sensitivity, and selectivity of the technique. Here are some modern approaches for RP HPLC method development:

• UHPLC: Ultra-high performance liquid chromatography (UHPLC) is a modern approach that uses smaller particle sizes and higher pressures than traditional HPLC. This results in higher resolution, shorter analysis times, and higher sensitivity.

• Monolithic columns: Monolithic columns are a modern alternative to traditional particle-based columns. These columns have a highly porous structure that allows for faster analysis times and higher throughput.

• 2D-LC: Two-dimensional liquid chromatography (2D-LC) is a modern approach that combines two different separation mechanisms, such as RP and ion-exchange chromatography. This can lead to improved separation and sensitivity.

• Mixed-mode chromatography: Mixed-mode chromatography uses a combination of RP and ion-exchange or size exclusion mechanisms. This approach can lead to improved selectivity and sensitivity.

• Chiral chromatography: Chiral chromatography is a modern approach that separates enantiomers, or mirror-image isomers, of a molecule. This technique can be used in the pharmaceutical industry to separate active pharmaceutical ingredients (APIs) and improve drug efficacy and safety.

• Multidimensional chromatography: Multidimensional chromatography is a modern approach that combines multiple separation mechanisms, such as RP, size exclusion, and ion-exchange chromatography. This approach can lead to improved separation and sensitivity for complex mixtures.

• Automation and computer modelling: Modern RP HPLC method development also involves automation and computer modelling to optimize and simulate separation conditions. This approach can save time and resources while also improving the efficiency and accuracy of RP HPLC method development.

Overall, these modern approaches to RP HPLC method development offer improved efficiency, sensitivity, selectivity, and accuracy, making them valuable tools for the analysis of complex mixtures in various industries. [75,76]

B. Emerging trends in RP HPLC method development

In addition to the modern approaches for RP HPLC method development mentioned previously, there are also emerging trends in the field that are worth discussing. Here are some examples:

• Green RP HPLC: With growing concerns over environmental sustainability, there is a trend towards developing RP HPLC methods that are more environmentally friendly. This includes the use of green solvents, such as water or ethanol, and reducing the use of toxic solvents.

• Microscale and nanoscale RP HPLC: Microscale and nanoscale RP HPLC methods are gaining popularity due to their ability to analyses small sample volumes with high sensitivity. These methods are particularly useful in proteomics and metabolomics research.

• Online and hyphenated techniques: Online and hyphenated techniques combine RP HPLC with other analytical techniques, such as mass spectrometry or infrared spectroscopy, to provide more comprehensive analysis of complex mixtures.

• High-throughput RP HPLC: High-throughput RP HPLC methods are becoming increasingly important for screening large numbers of samples in a short amount of time. This is particularly useful in the pharmaceutical industry for drug discovery and development.

• Quality-by-design (QbD) approach: QbD is a systematic approach to method development that focuses on identifying and controlling sources of variability to ensure consistent and high-quality results. This approach is becoming more popular in RP HPLC method development to ensure robust and reliable methods. [43,44]

Overall, these emerging trends in RP HPLC method development are driven by the need for more efficient, sensitive, and environmentally friendly methods that can handle complex mixtures. Researchers and practitioners in the field should be aware of these trends and adapt their methods accordingly to stay at the forefront of analytical chemistry.

C. Novel technologies for RP HPLC method development

In recent years, novel technologies have been developed to enhance the efficiency and effectiveness of RP HPLC method development. Here are some examples:

• Advanced stationary phases: The development of new stationary phases with improved selectivity and efficiency has been a major focus of research in RP HPLC method development. For example, core-shell particles have been developed with higher surface area and better packing efficiency than traditional fully porous particles.

• Monolithic columns: Monolithic columns are a single piece of stationary phase with a continuous network of pores. They offer faster separation times and lower backpressure than traditional particle-packed columns.

• Ultra-high-pressure liquid chromatography (UHPLC): UHPLC uses columns packed with sub-2µm particles and operates at higher pressures than traditional HPLC, resulting in faster separations and improved resolution.

• Two-dimensional liquid chromatography (2D-LC): 2D-LC involves the separation of a sample using two different modes of chromatography. This technique can improve the separation of complex mixtures and has applications in proteomics and metabolomics research.

• Intelligent software for method development: Intelligent software can aid in method development by automatically selecting optimal parameters based on experimental data, reducing the time and effort required for optimization. [45,46]

Overall, these novel technologies have the potential to significantly improve the efficiency, selectivity, and sensitivity of RP HPLC method development. Researchers and practitioners should keep abreast of these developments and consider their potential applications in their work.

IV. Applications of RP HPLC Method Development

RP HPLC is a widely used analytical technique with numerous applications in various industries. Here are some examples:

• Pharmaceutical industry: RP HPLC is an essential tool in drug discovery and development. It is used for drug purity analysis, impurity identification, and quantification of active pharmaceutical ingredients (APIs) in formulations.

• Food and beverage industry: RP HPLC is used for the analysis of food additives, preservatives, and contaminants in food and beverages. It can also be used for the determination of nutritional components, such as vitamins and amino acids.

• Environmental analysis: RP HPLC is used for the analysis of pollutants, such as pesticides, herbicides, and industrial chemicals, in environmental samples. It is also used for the determination of organic compounds in water and soil samples.

• Forensic science: RP HPLC is used for the analysis of drugs of abuse in biological samples, such as blood and urine. It can also be used for the analysis of toxic compounds in post-mortem samples.

• Biotechnology industry: RP HPLC is used for the analysis of proteins, peptides, and nucleic acids in biotechnology products, such as monoclonal antibodies and recombinant proteins. [77,78]

Overall, the versatility and sensitivity of RP HPLC make it a valuable tool in a wide range of applications. Its ability to separate and quantify complex mixtures of compounds with high precision and accuracy has made it a standard technique in many industries.

V. Challenges and Future Directions

A. Common challenges in RP HPLC method development

Despite its widespread use and effectiveness, RP HPLC method development is not without its challenges. Here are some common challenges that researchers and practitioners may encounter:

• Stationary phase selectivity: Selecting the most appropriate stationary phase for a particular sample can be challenging, especially when dealing with complex mixtures. The use of alternative selectivity columns or mixed-mode columns can help address this challenge.

• Optimization of parameters: Optimizing the parameters of an RP HPLC method, such as column temperature, flow rate, and mobile phase composition, can be time-consuming and require significant trial and error. Intelligent software and automation can help expedite this process.

• Matrix interference: Sample matrices can interfere with separation and detection, leading to reduced sensitivity and selectivity. Sample preparation techniques, such as solid-phase extraction, can help remove matrix interference.

• Column degradation: Columns can degrade over time due to sample matrix effects, column overload, and other factors. Periodic column maintenance and replacement can help address this challenge.

• In terms of future directions, the following areas are likely to see continued development and improvement in RP HPLC method development:

• Column technology: As discussed earlier, new column technologies, such as monolithic columns and core-shell particles, are likely to see increased use and development.

• Stationary phase design: Researchers are continuing to explore new stationary phase chemistries and designs to improve selectivity and efficiency.

• Automation and artificial intelligence: The use of automation and artificial intelligence in RP HPLC method development is likely to increase, with the potential for more efficient and effective optimization strategies.

• Miniaturization: Miniaturization of RP HPLC systems, such as microfluidic chips, could lead to improved speed, sensitivity, and portability. [79,80]

Overall, RP HPLC method development will continue to play an important role in modern analytical chemistry, with ongoing improvements and innovations addressing existing challenges and expanding its range of applications.

C. Emerging challenges in RP HPLC method development

• Analysis of large biomolecules: RP HPLC is commonly used for the analysis of small molecules, but its application to large biomolecules, such as proteins and peptides, can be challenging due to their size, complexity, and hydrophobicity. New column technologies and sample preparation techniques, such as size-exclusion chromatography and protein digestion, are being developed to address these challenges.

• Analysis of chiral compounds: Chiral compounds are molecules that exist in two or more mirror-image forms, and their separation is critical in many industries, including pharmaceuticals, agrochemicals, and flavors and fragrances. While RP HPLC can be used for chiral separations, it often requires the use of chiral stationary phases or derivatization techniques, which can be time-consuming and costly.

• Analysis of polar compounds: RP HPLC is not well-suited for the analysis of highly polar compounds, such as carbohydrates and organic acids, due to their poor retention on RP columns. Alternative modes, such as hydrophilic interaction chromatography (HILIC), are being developed to address this challenge.

• Analysis of trace impurities: RP HPLC is commonly used for the analysis of impurities in pharmaceuticals and other products, but its sensitivity for trace impurities can be limited. The use of high-resolution mass spectrometry (HRMS) and other advanced detection techniques can improve sensitivity and selectivity for trace impurities. [81,82]

In summary, the emerging challenges in RP HPLC method development reflect the growing demand for more efficient, sensitive, and selective analytical techniques in various industries.

Table 1.7: Recent advances in RP HPLC method development [83]

Technique	Description	Advantages
Ultra-high performance liquid chromatography (UHPLC)	Utilizes columns packed with smaller particles (typically < 2 µm) and higher pressures (up to 1000 bar) for faster separations and higher resolution	Improved speed, resolution, and sensitivity
Monolithic columns	Consist of a single piece of porous material, providing higher flow rates and faster separations	Improved speed and resolution, reduced backpressure
Stationary phase coatings	Modify the surface of the column to provide improved selectivity and/or reduced non-specific adsorption	Improved selectivity and sensitivity
2D-LC	Combines two complementary separation modes (e.g., size exclusion and RP) to provide higher resolution and selectivity	Improved resolution and selectivity for complex samples

Table 1.8: Applications of RP HPLC method development in various industries [84,85]

Industry	Applications
Pharmaceutical	Drug development and quality control, impurity analysis, pharmacokinetic studies
Food and beverage	Analysis of additives, contaminants, and nutritional components
Environmental	Analysis of pollutants, toxins, and metabolites in air, water, and soil
Forensic	Analysis of drugs, toxins, and metabolites in biological samples
Biotechnology	Analysis of proteins, peptides, and nucleic acids in research and development

These tables provide a useful summary of the recent advances in RP HPLC method development, as well as the applications of RP HPLC in various industries.

D. Future directions for RP HPLC method development

Future directions for RP HPLC method development are centered around improving its efficiency, sensitivity, and selectivity, as well as expanding its capabilities for new and emerging applications. Some key areas of focus for future research and development include:

• Development of new stationary phases: Novel stationary phases with improved selectivity, stability, and durability are being developed to enhance the performance of RP HPLC. For example, hybrid stationary phases, such as core-shell and porous organic polymers, are being explored for their unique properties and potential applications.

• Development of new detection techniques: Advanced detection techniques, such as mass spectrometry, fluorescence, and electrochemical detection, are being integrated into RP HPLC systems to improve sensitivity, selectivity, and accuracy. The development of new detection technologies that can detect analytes at low concentrations and in complex matrices will be critical for future applications.

• Application to new areas: RP HPLC is already widely used in the pharmaceutical, food, and environmental industries, but its application to new areas, such as metabolomics, proteomics, and glycemic, is becoming increasingly important. RP HPLC is also being used for the analysis of natural products, synthetic polymers, and nanomaterials, which require specialized methods and techniques.

• Automation and miniaturization: Automation and miniaturization of RP HPLC systems are being developed to improve the efficiency and throughput of the method. Advances in microfluidics and lab-on-a-chip technologies are also enabling the development of portable and point-of-care RP HPLC systems for on-site analysis. [86-95]

Overall, the future of RP HPLC method development is exciting, with numerous opportunities for innovation, discovery, and application. Continued collaboration and interdisciplinary research among chemists, biologists, engineers, and data scientists will be critical in advancing the field and addressing the emerging challenges and opportunities in modern analytical chemistry.

Diabetes Mellitus (DM)

Diabetes Mellitus (DM) is a multi-factorial chronic health condition triggered by several genetic and/or environmental factors. There is no cure for diabetes so far, but it can be treated and controlled. Pharmacological therapy and/or insulin may be required to maintain the blood glucose level as near as possible to normal and to delay or possibly to prevent the development of diabetes-related health problems. However, disease management can also be helped by healthy eating and physical exercise. For determining the right therapy, the involved type of diabetes plays a key role, and in 2018 American Diabetes Association (ADA) proposed the following classification:

1. Type 1 diabetes mellitus (T1DM): due to autoimmune β-cell destruction, usually leading to absolute insulin deficiency;

2. Type 2 diabetes mellitus (T2DM): due to a progressive loss of β-cell insulin secretion frequently on the background of insulin resistance;

3. Gestational diabetes mellitus (GDM): diabetes diagnosed in the second or third trimester of pregnancy that was not overt before gestation;

4. Specific types of diabetes due to other causes, e.g., monogenic diabetes syndromes (such as neonatal diabetes and maturity-onset diabetes of the young (MODY)), diseases of the exocrine pancreas (such as cystic fibrosis and pancreatitis), and drug- or chemical-induced diabetes (such as with glucocorticoid use, in the treatment of HIV/AIDS, or after organ transplantation). [96,97]

Type 2 Diabetes Mellitus (T2DM)

Type 2 Diabetes Mellitus (T2DM) has been referred to for a long time as non-insulin-dependent diabetes, or adult-onset diabetes, characterized by insulin resistance, which could progressively worsen to absolute resistance. Still, in the past decade, reduced β-cell function has been recognized as a key problem in T2DM.

Anti-diabetic drugs

Anti-diabetic drugs are medications used to manage and control diabetes mellitus; a chronic condition characterized by elevated blood glucose levels. These drugs aim to regulate blood sugar levels and prevent complications associated with diabetes. There are several classes of anti-diabetic drugs, each with its mechanism of action. Here is an overview of the major classes of anti-diabetic drugs:

1. **Insulin:**

• Mechanism of Action: Insulin is a hormone that regulates glucose metabolism. It promotes the uptake of glucose by cells, reduces glucose production in the liver, and facilitates the storage of glucose in the form of glycogen.

• Administration: Injected subcutaneously or through an insulin pump.

2. **Biguanides:**

• Examples: Metformin

• Mechanism of Action: Reduces glucose production in the liver, improves insulin sensitivity in peripheral tissues, and decreases intestinal glucose absorption. Common Side Effects: Gastrointestinal symptoms (e.g., nausea, diarrhea).

3. **Sulfonylureas:**

• Examples: Glipalamide, Glipizide, Glimepiride

• Mechanism of Action: Stimulates insulin release from the beta cells of the pancreas, increasing insulin levels in the blood.

• Common Side Effects: Hypoglycemia, weight gain.

4. **Meglitinides:**

• Examples: Repaglinide, Nateglinide

• Mechanism of Action: Stimulates insulin secretion from the pancreas, particularly in response to meals.

• Common Side Effects: Hypoglycemia, weight gain.

5. **Thiazolidinediones (TZDs):**

• Examples: Pioglitazone, Rosiglitazone

• Mechanism of Action: Improves insulin sensitivity in peripheral tissues, such as muscle and adipose tissue.

• Common Side Effects: Fluid retention, weight gain, increased risk of heart failure.

6. **Alpha-Glucosidase Inhibitors:**

• Examples: Acarbose, Miglitol

• Mechanism of Action: Delays the absorption of glucose from the digestive tract by inhibiting enzymes that break down carbohydrates.

- Common Side Effects: Gastrointestinal symptoms (e.g., flatulence, diarrhea).

7. Dipeptidyl Peptidase-4 (DPP-4) Inhibitors:

- Examples: Sitagliptin, Sitagliptin, Linagliptin
- Mechanism of Action: Increases insulin secretion and reduces glucagon release by inhibiting the degradation of incretin hormones.
- Common Side Effects: Upper respiratory tract infections, headache.

8. Sodium-Glucose Co-Transporter 2 (SGLT2) Inhibitors:

- Examples: Canagliflozin, Dapagliflozin, Empagliflozin
- Mechanism of Action: Inhibits the reabsorption of glucose in the kidneys, leading to increased glucose excretion in urine.

9. Glucagon-Like Peptide-1 (GLP-1) Receptor Agonists:

- Examples: Exenatide, Liraglutide, Dulaglutide
- Mechanism of Action: Increases insulin secretion, decreases glucagon secretion, slows gastric emptying, and promotes satiety.
- Common Side Effects: Nausea, vomiting, pancreatitis (rare).

MATERIAL & METHODS

Materials Used:

Table 6.1: Material Used

Material	Description
Sitagliptin	Pharmaceutical compound used as the analyte
Sodium Hexanesulphonate	Chemical reagent used in the preparation of the mobile phase
Methanol	Solvent used in the preparation of standard solutions and mobile phase
Water	Solvent used in the preparation of standard solutions and mobile phase
Acetonitrile	Solvent used in the preparation of the mobile phase
Glacial Acetic Acid	Reagent used for pH adjustment
0.45µm Membrane Filter	Filter used for filtration of mobile phase
Volumetric Flask	Laboratory glassware used for precise volume measurements and preparation of standard solutions
UV-Visible Spectrophotometer	Instrument used for scanning drug solutions in the UV spectrum to determine optimal wavelength
HPLC System	High-Performance Liquid Chromatography system used for separation and quantification of analytes
10 ml Volumetric Flask	Laboratory glassware used for the preparation of stock solutions
0.45 µm Filter Paper	Filter paper used for filtration of stock solutions

Chemical Used

Table 6.2: Chemicals Used

Chemical	Description
Sodium Hexanesulphonate	Chemical reagent used in the preparation of the mobile phase
Methanol	Solvent used in the preparation of standard solutions and mobile phase
Water	Solvent used in the preparation of standard solutions and mobile phase
Acetonitrile	Solvent used in the preparation of the mobile phase
Glacial Acetic Acid	Reagent used for pH adjustment

Instrument Used

Table 6.3: Instrument used

Instrument	Description
UV-Visible Spectrophotometer	Instrument used for scanning drug solutions in the UV spectrum to determine optimal wavelength
High-Performance Liquid Chromatography (HPLC) System	Instrument used for separation and quantification of analytes using liquid chromatography techniques
Analytical Balance	Instrument used for precise weighing of chemicals and samples
Volumetric Flask	Laboratory glassware used for precise volume measurements and preparation of standard

	solutions
10 ml Volumetric Flask	Laboratory glassware used for the preparation of stock solutions
pH Meter	Instrument used for measuring the pH of solutions
Syringe	Instrument used for accurate dispensing of liquids

Selection of Wavelength

The selection of the optimal wavelength for analyzing a solution containing 50 µg/ml of Sitagliptin involves preparing blank solutions using water and methanol, followed by the creation of the drug solution through a 50-50 ratio mixture of water and methanol. Using a UV spectrophotometer, the drug solution is scanned from 200 to 400 nanometers, with the spectra overlaid against the blank solutions. The wavelength providing the highest absorbance or a satisfactory response is identified as the optimal wavelength for further analysis. This selection is validated through multiple analyses to ensure reproducibility, and the chosen wavelength is documented for future reference in the analysis of Sitagliptin solutions.

Preparation of Stock and Standard Solutions:

1. *Stock Solution of Sodium Hexanesulphonate:*

Weigh out 5.65 grams of sodium hexanesulphonate and dissolve it in a mixture consisting of equal volumes of acetonitrile and water. Bring the volume up to 1000 millilitres using the same solvent mixture. Adjust the pH to 4.5 ± 0.05 using glacial acetic acid, and thoroughly mix the solution. Filter the solution through a 0.45µm membrane filter, and degas the solution to remove any dissolved gases.

2. *Preparation of Standard Solution of Sitagliptin*:

Take a volumetric flask with a capacity of 100 millilitres. Add approximately 25.0 milligrams of Sitagliptin to the flask. Dissolve the Sitagliptin in 5 mL of methanol, then dilute the solution with the solvent mixture (water and methanol in equal proportions) to reach a final volume of 100 mL. The concentration of the standard solution should be 250 parts per million (ppm).

PREPARATION OF MOBILE PHASE

You should weigh 5.65 grams of sodium hexanesulphonate and dissolve it in a combination consisting of equal volumes of acetonitrile and water. The volume should be brought up to 1000 millilitres using the same mixture solvent. Adjust the pH to 4.5±0.05 using glacial acetic acid and mix the mixture. The filter is a 0.45µm membrane filter, and the degassing is performed.

Preparation of Solutions:

The compendium of solvents consists of a mixture of water and methanol in equal proportions. The procedure for preparing the standard solution: A volumetric flask with a capacity of 100 millilitres should be filled with approximately 25.0 milligrams of Sitagliptin. The material was dissolved in 5 mL of methanol and subsequently diluted with 100 mL of the solvent mixture. The concentration is 250 parts per million.

Table 6.4: Preparation of Standard Solution

Volume of Stock Solution Taken (ml)	Volume of Diluent Added (ml)	Final Concentration of Sitagliptin (µg/ml)
0.5	9.5	50
1.0	9.0	100
1.5	8.5	150
2.0	8.0	200
2.5	7.5	250

LINEARITY

Linearity Stock Solution

Precisely measured approximately 10mg of Sitagliptin and placed it into a 10 ml volumetric flask. The flask was then filled with water up to the mark, resulting in a stock solution of Sitagliptin with a concentration of 1000µg/ml. The flask was agitated, and the resultant solution was passed through a 0.45 µm filter paper.

Linearity Standard Solutions

A set of standard solutions with concentrations ranging from 50 to 250µg/ml for Sitagliptin was produced to establish linearity. Generating calibration plots for Sitagliptin: Sitagliptin standard solutions with concentrations ranging from 50 to 250 µg/ml were developed. The devised approach was used to measure the peak area of the medicines in each linearity level solution. A calibration curve was constructed by plotting the peak area against the concentration. The correlation coefficient and regression line equation for Sitagliptin were calculated as well.

ANALYTICAL METHOD VALIDATION

Specificity

The specificity of the RP-HPLC method for the analysis of Sitagliptin was evaluated to ensure the method's ability to separate the target analyte from potential interfering components. Initially, the chromatographic system was equilibrated according to the method parameters. Standard solutions of Sitagliptin were prepared at a known concentration and injected into the HPLC system. The separation was carried out using a suitable stationary phase, mobile phase, and gradient program optimised for the analysis. The retention time (Rt) of the peak corresponding to Sitagliptin was noted, along with parameters such as resolution (Rs) and tailing factor (T). The chromatogram was examined to confirm that the peak for Sitagliptin exhibited a distinct and well-defined divergence from the baseline, indicating its specific elution from the column without interference from other compounds.

Accuracy (% recovery)

To assess the accuracy of the analytical method for the determination of Sitagliptin (% recovery), preparations were made at concentrations of 50%, 100%, and 150% of the pre-analyzed sample concentration. For each concentration level, three replicates of solutions were prepared. Duplicate injections of these three replicates were then analyzed using the established method.

Firstly, standard solutions of Sitagliptin were prepared at concentrations equivalent to 50%, 100%, and 150% of the expected concentration. These solutions were thoroughly mixed to ensure homogeneity. Subsequently, three replicates of each prepared solution were made to account for variability.

Next, duplicate injections of each of the three replicates were performed using the analytical method under the same conditions. The chromatographic system was equilibrated, and the samples were injected into the HPLC system. The peak areas corresponding to Sitagliptin were recorded for each injection.

The accuracy (% recovery) was calculated by comparing the experimentally determined concentrations of Sitagliptin in the prepared samples to their expected concentrations. The recovery (%) for each concentration level was calculated as follows:

Recovery (%) = Expected Concentration Experimentally determined concentration x100%

This process was repeated for all three concentration levels, and the average recovery (%) along with its standard deviation was determined to evaluate the accuracy and precision of the analytical method for the determination of Sitagliptin.

Precision

To evaluate the precision of the analytical method for Sitagliptin, solutions with concentrations ranging from lower to higher levels within the linearity range (50, 100, and 150 µg/ml) were prepared. Chromatograms were obtained, specifically documenting the peak region corresponding to Sitagliptin. The experiment was conducted three times within a single day to assess intra-day precision (repeatability), and repeated on three separate days to evaluate inter-day precision. Relative standard deviation (RSD) was calculated using the measured concentrations of Sitagliptin across the replicates for each concentration level and for both intra-day and inter-day assessments. This comprehensive analysis allowed for the determination of both the repeatability and reproducibility of the analytical method for the quantification of Sitagliptin.

LOD & LOQ

The Limit of Detection (LOD) and Limit of Quantification (LOQ) are important parameters in analytical chemistry, indicating the lowest concentration of an analyte that can be reliably detected and quantified, respectively. Here's how they are calculated based on the provided equations:

1. *Limit of Detection (LOD):*

- Formula: LOD = 3.3 × (SD/Slope)
- SD: Standard deviation of the Y-intercepts of the 5 calibration curves.
- Slope: Mean slope of the 5 calibration curves.

2. *Limit of Quantification (LOQ):*

- Formula: LOQ = 10 × (SD/Slope)
- SD: Standard deviation of the Y-intercepts of the 5 calibration curves.
- Slope: Mean slope of the 5 calibration curves.

These formulas essentially quantify the sensitivity of the analytical method being used. The LOD is the lowest concentration at which a signal can be distinguished from background noise, while the LOQ is the lowest concentration at which the analyte can be reliably quantified with acceptable accuracy and precision.

Robustness

A study on the robustness of the chromatographic conditions was conducted, with alterations made to the flow rate (±0.2 ml/min), pH (±0.2 units), and wavelength (±2 nm).

Analyzed were the replicated injections of a sample solution containing 250 µg/ml Sitagliptin under different conditions, following the specified protocol, and documented were the chromatograms. The relative standard deviation (RSD) of the test percentage of Sitagliptin was determined.

Calibration curve for Sitagliptin:

A set of functional reference solutions indicated previously was taken into account, and 20 µl of each solution was automatically injected into the column. The highest points were identified at a wavelength of 239 nm. The calibration curve was generated by graphing the concentration on the X-axis and the ratio of the peak area of the standard on the Y-axis.

A chromatogram was obtained for each solution, and the total quantity of the medication was determined. From this, the percentage recovery was computed. The data collected in the aforementioned investigation fall within the established limit, thereby leading to the conclusion that the provided analytical method is accurate within the stated range.

% Assay of Sitagliptin:

Criteria for acceptance: the relative standard deviation (RSD) for the peak area resulting from five repeated injections of the standard preparation containing Sitagliptin should not exceed 2.0%. The tailing for the peak caused by Sitagliptin: in 5 duplicate injectable preparations should fall within the range of 0.8 to 2.0%. The number of theoretical plates for the peak attributed to Sitagliptin, in each of the 5 repeated injections of the standard formulation, should exceed 2000. The relative standard deviation (RSD) for the percentage assay of six samples should not exceed 2.0%. The analysis of each sample should yield a mean value that falls within the range of 90 to 110%. Based on the data provided, it can be inferred that the analytical method is very accurate under the specified analytical conditions.

% Assay of Sitagliptin:

Criteria for acceptance

The relative standard deviation (RSD) for the percentage assay of the six samples should not exceed 2.0%. The analysis of each sample should yield a mean value that falls within the range of 90 to 110%. The relative standard deviation (RSD) of twelve results, consisting of six measurements each for procedure precision and intermediate accuracy, must not exceed 2.0%. The results obtained fall well within the acceptability requirements. Therefore, it can be inferred that the provided analytical procedure is robust. A study on the robustness of the chromatographic conditions was conducted by altering the flow rate (±0.2 ml/min), pH (±0.2 units), and wavelength (±2 nm).

Acceptance Criteria:

RSD stands for Relative Standard Deviation, expressed as a percentage. The assay of each of the six samples must not exceed 2.0%. The acceptable range for tailing caused by Sitagliptin, in 3 duplicate injectable preparations, should be between 0.8% and 2.0%. The number of theoretical plates for the peak attributed to Sitagliptin, in three repeated injections of the standard preparation, should exceed 2000. The results obtained fall well within the acceptability requirements. Therefore, it can be inferred that the provided analytical method is resilient to variations in flow rate, pH, and wavelength.

System Suitability:

The establishment of system appropriateness parameters is crucial to ensure the ongoing validity of the analytical method whenever it is employed. Common variables include the stability of the analytical solution, variances in equipment, and variations in the analyzer. Common alterations in liquid chromatography include adjustments to the pH of the mobile phase, changes in wavelength, and modifications to the flow rate. The method was formulated utilizing conventional Sitagliptin and verified following the ICH guideline (Q2 (A)). Linearity, accuracy, precision, and robustness were quantified to validate. The linearity of Sitagliptin was observed within the concentration range of 50-250 µg/ml. Accuracy was verified through the implementation of recovery experiments. The mean percentage recovery for Sitagliptin was determined to be 99.2%. During precision studies, the relative standard deviation (% RSD) for Sitagliptin was determined to be less than 2. The robustness studies revealed that the relative standard deviation (% RSD) was below 2.0%, the tailing factor was within the range of 0.8% to 2.0%, and the number of theoretical plates was less than 2000. This method is characterized by its higher reliability and reproducibility in comparison to the existing method.

RESULT & DISCUSSION

Selection of Wavelength

Identifying the most effective wavelength for detecting Sitagliptin by UV spectroscopy. In order to accomplish this, solutions were made bearing a uniform concentration of 50 µg/ml of the drug in a solvent mixture of water and methanol with a ratio of 50:50. This concentration selection is characteristic of UV spectroscopy, guaranteeing that the medication is present in detectable quantities without overpowering the detector. Afterwards, the solutions underwent UV scanning from 200 to 400 nm, covering the UV area where organic compounds commonly absorb light. While scanning, the spectra of the solutions were documented, using the water: methanol mixture as a reference to adjust the baseline. The identification of relevant absorbance wavelengths for Sitagliptin can be achieved by superimposing these spectra. The wavelength(s) exhibiting the maximum absorbance would be regarded as the optimal wavelength(s) for detecting the drug in subsequent studies. This methodical approach guarantees that the UV spectroscopy technique is fine-tuned to maximize sensitivity and precision in detecting Sitagliptin given the specified experimental circumstances.

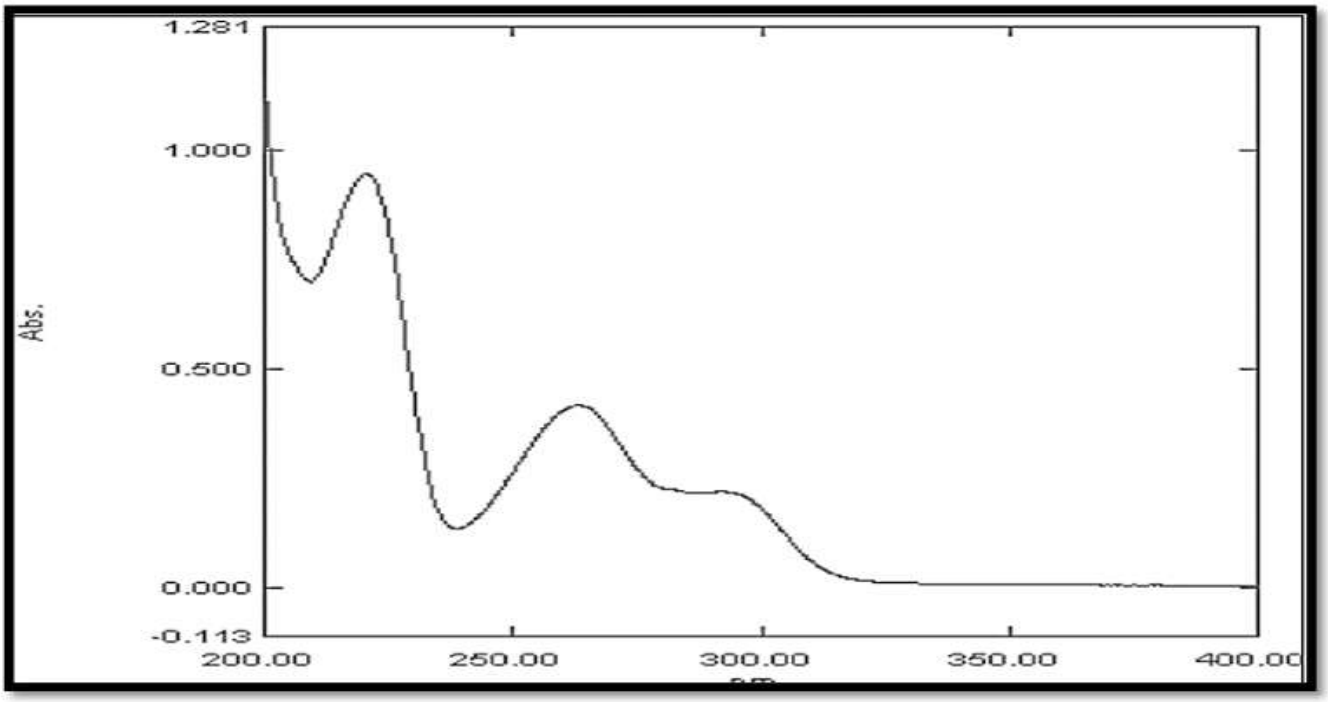

Fig 7.1: UV-Spectrum for Sitagliptin (239nm)

Calibration curve for Sitagliptin:

To create a calibration curve for Sitagliptin, we can plot the concentration (µg/ml) on the x-axis and the corresponding peak area on the y-axis.

Table 7.1: Calibration Curve Data

Concentration (µg/ml)	Peak Area of Sitagliptin
50	21429
100	44857
150	67288
200	89717
250	112147

From the plot, we observe a clear linear relationship between concentration and peak area. As the concentration of Sitagliptin increases, the peak area also increases linearly. We can use this calibration curve to determine the concentration of Sitagliptin in unknown samples by comparing their peak areas to the calibration curve. Typically, a linear regression analysis would be performed on this data to obtain the equation of the line, which can then be used to calculate the concentration of Sitagliptin in samples based on their peak areas.

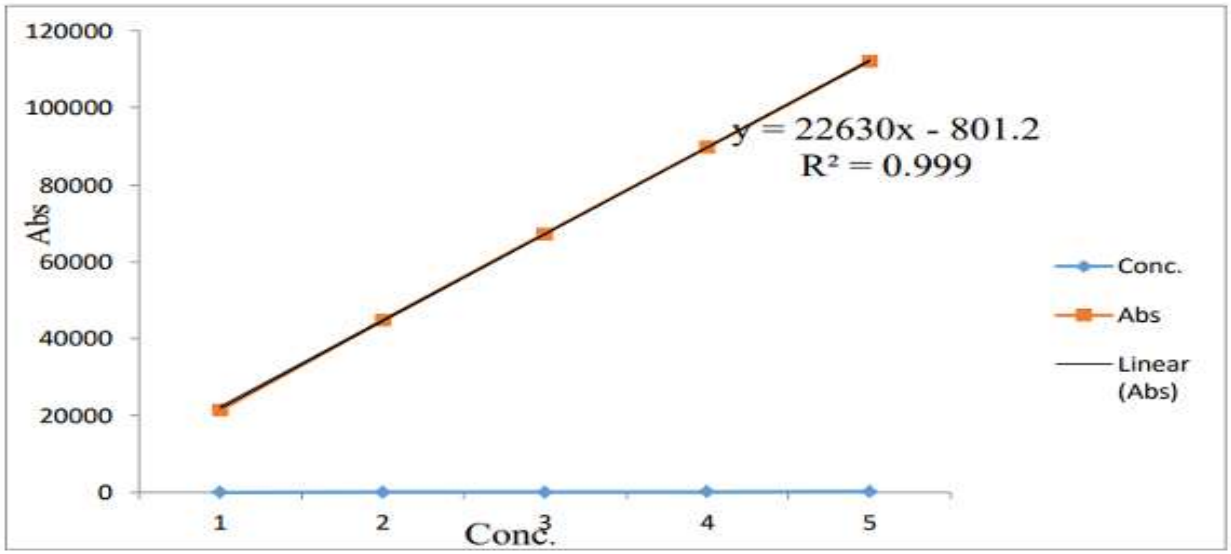

Figure 7.2: Calibration Curve for Sitagliptin

Method development:

The developed method for the determination of Sitagliptin by RP-HPLC using a Waters, Inertial ODS C18 column demonstrated robustness and reliability. The chromatographic conditions, including the column type, mobile phase composition, flow rate, and detection wavelength, were optimised to achieve efficient separation within a short analysis time of 10 minutes. The mobile phase consisted of Acetonitrile: Water (50:50 v/v) with the addition of Sodium hexanesulphonate, delivered isostatically at a flow rate of 2.0 ml/minute. UV detection at 239 nm enabled sensitive and selective quantification over a concentration range of 50-250 µg/ml for Sitagliptin.

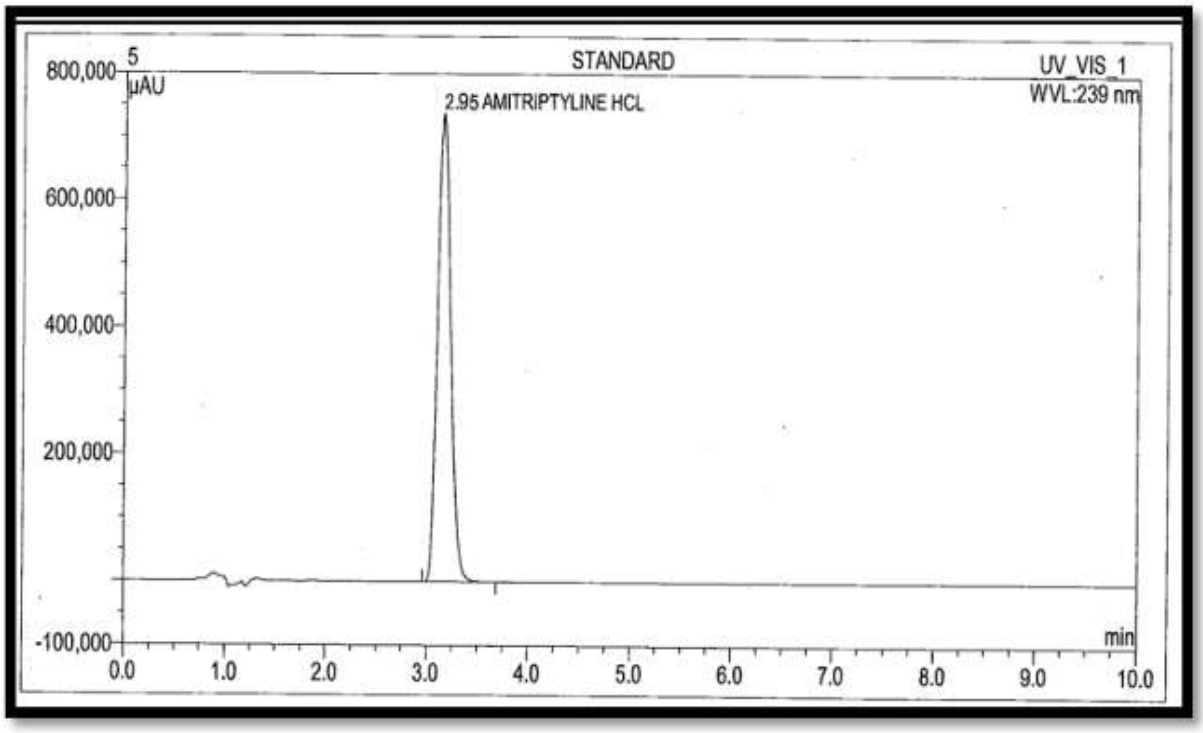

Figure 7.3: Specificity by injecting standard solution (Sitagliptin).

The method exhibited excellent performance characteristics, with mean recoveries of 99.2% w/w and a regression coefficient (R^2) of 0.999, indicating a high level of linearity.

The assay percentage of the bulk form was determined to be 100.3% w/w, demonstrating the accuracy of the method. Intra-day and inter-day precision data showed % RSD values below 2%, confirming the method's precision.

Robustness studies further validated the method's reliability under variations in flow rate, pH, and wavelength, with % RSD values consistently below 2%.

These results suggest that the method is robust and suitable for routine analysis.

Additionally, stress testing of Sitagliptin under various conditions revealed its stability profile. Degradation studies showed minimal degradation under neutral conditions, while oxidative stress led to moderate degradation in the presence of hydrogen peroxide. Acidic and basic conditions resulted in slight degradation due to hydrolysis, and thermal and photolytic stress conditions also induced minimal degradation. Overall, the developed RP-HPLC method proved to be simple, rapid, accurate, precise, and reliable for the quantitative estimation of Sitagliptin in solid tablet dosage forms. It demonstrated applicability for routine analysis and exhibited excellent performance in stability studies, making it a valuable tool for pharmaceutical quality control.

Accuracy Data

The accuracy data presented in Table 7.2 for the developed method of Sitagliptin reveals the precision and reliability of the method in quantifying the drug at different concentration levels.

At Level I, which represents 50% of the sample concentration, the recovery percentages ranged from 98.3% to 99.6%, with a mean recovery of 99.03%. The low % RSD values (0.006 to 0.005) indicate excellent precision and consistency in recovering the drug at this concentration level.

For Level II (100% of the sample concentration), the recovery percentages varied from 98.4% to 99.5%, with a mean recovery of 99.03%. Although the % RSD values are not provided for all samples, the ones provided are low (0.005), suggesting good precision in the recovery of Sitagliptin at this concentration level.

At Level III (150% of the sample concentration), the recovery percentages ranged from 99.4% to 99.7%, with a mean recovery of 99.6%. The % RSD values (0.002) are extremely low, indicating excellent precision and reproducibility in recovering the drug at this higher concentration level.

Table 7.2: Accuracy Data of Developed Method of Sitagliptin

Conc. Level (%)	Sample No.	Actual Amount Added (mg)	Amount Recovered (mg)	Recovery (%)	Mean Recovery (%)	RSD (%)
Level I	Sample solution 1_1	125.02	122.96	98.3	99.03	0.006
	Sample solution 1_2					
	Sample solution 2_1	124.96	124.56	99.6		
	Sample solution 2_2					
	Sample solution 3_1	124.83	123.85	99.2		
	Sample solution 3_2					
Level II	Sample solution 1_1	247.81	245.93	99.2	99.03	0.005
	Sample solution 1_2					
	Sample solution 2_1	250.01	248.86	99.5		
	Sample solution 2_2					
	Sample solution 3_1	249.93	245.85	98.4		
	Sample solution 3_2					
Level III	Sample solution 1_1	376.80	375.80	99.7	99.6	0.002
	Sample solution 1_2					
	Sample solution 2_1	374.85	372.56	99.4		
	Sample solution 2_2					
	Sample solution 3_1	375.06	373.96	99.7		
	Sample solution 3_2					
Overall				99.2		0.004

Overall, the mean recovery percentage across all concentration levels was found to be 99.2%, with a % RSD of 0.004%. These results demonstrate the accuracy and reliability of the developed method for quantifying Sitagliptin across a wide range of concentrations. The consistent and precise recovery of the drug at different levels confirms the suitability of the process for the accurate determination of Sitagliptin content in pharmaceutical formulations.

Precision

The precision data provided illustrates the consistency and reproducibility of the developed method for the analysis of Sitagliptin.

The retention time of Sitagliptin across five standard solutions ranged from 3.09 to 3.05 minutes, with a mean retention time of 3.05 minutes. The low standard deviation (0.016) indicates minimal variability in retention time among the samples.

Similarly, the % RSD values for the area, theoretical plates, and tailing factor are very low, indicating excellent precision in these parameters. The % RSD values for area, theoretical plates, and tailing are 0.003%, 0.042%, and 0.31%, respectively. These low % RSD values suggest that the method is highly precise in quantifying the area under the chromatographic peaks, determining the efficiency of the column, and assessing the symmetry of the peaks.

Table 7.3: Precision Data (%Rsd).

Sr. No.	Name	Retention Time	Area	Theoretical Plates	Tailing
1	Standard Solution 1	3.09	212147	3288	1.27
2	Standard Solution 2	3.05	212159	3286	1.26
3	Standard Solution 3	3.05	212140	3284	1.27
4	Standard Solution 4	3.05	212145	3285	1.27
5	Standard Solution 5	3.05	212145	3287	1.27
Mean		3.05	212147	3286	1.27
Std. Dev.		0.016	7.08	1.41	0.004
% RSD		0.52%	0.003%	0.042%	0.31%

The mean values for retention time, area, theoretical plates, and tailing factor are consistent across all standard solutions, further confirming the precision of the method. Overall, these results demonstrate the reliability and robustness of the developed method for the analysis of Sitagliptin, ensuring accurate and consistent results in routine analysis.

% Assay Data of Sitagliptin

The % assay data presented in Table 7.4 provides information on the potency of Sitagliptin (HCl) in various standard solutions, indicating the accuracy and reliability of the analytical method.

The % assay values for standard solutions 1 to 5 range from 98.9% to 99.8%, with a mean assay value of 99.4%. These values reflect the percentage of the active ingredient present in each standard solution relative to the labelled potency, demonstrating consistency in the assay result.

Table 7.4: % Assay Data of Sitagliptin.

Sample Solution	**% Assay**
Standard Solution 1	99.5
Standard Solution 2	99.8
Standard Solution 3	99.6
Standard Solution 4	99.0
Standard Solution 5	98.9
Mean	99.4%
Std. Dev.	0.40
% RSD	0.402%

The standard deviation (Std. Dev.) of 0.40 indicates the variability of the assay values around the mean. Additionally, the % RSD (relative standard deviation) of 0.402% provides a measure of the precision of the assay method. The low % RSD value suggests that the assay results are precise and reproducible, with minimal variability among the standard solutions.

Overall, the % assay data confirms the accuracy and precision of the developed method for quantifying Sitagliptin, ensuring reliable and consistent results in pharmaceutical quality control and dosage form analysis.

Intermediate Precision Observations.

The intermediate precision observations provided in Table 7.5 offer insights into the robustness and reliability of the analytical method for determining Sitagliptin (HCl) The retention time for Sitagliptin in five standard solutions remained consistent at 3.15 minutes, indicating the stability of the chromatographic system over time and across different runs. Similarly, the mean values for the area, theoretical plates, and tailing factor also remained stable across the standard solutions, with mean values of 246082 for area, 3068 for theoretical plates, and 1.23 for tailing.

Table 7.5: Intermediate Precision Observations.

Sr. No.	Name	Retention Time	Area	Theoretical Plates	Tailing
1	Standard Solution 1	3.15	246078	3066	1.23
2	Standard Solution 2	3.15	246108	3068	1.22
3	Standard Solution 3	3.15	246080	3070	1.24
4	Standard Solution 4	3.15	246075	3065	1.23
5	Standard Solution 5	3.15	246071	3070	1.23
Mean		3.15	246082	3068	1.23
Std. Dev.		0.000	14.7	2.28	0.007
% RSD		0.0%	0.006%	0.074%	0.56%

The standard deviation (Std. Dev.) values provide a measure of the variability of the observations around the mean. In this case, the Std. Dev. Values for area, theoretical plates, and tailing are 14.7, 2.28, and 0.007, respectively, indicating minimal variability in these parameters. The % RSD (relative standard deviation) values reflect the precision of the method. With % RSD values of 0.006% for area, 0.074% for theoretical plates, and 0.56% for tailing, the method demonstrates excellent precision and reproducibility in these parameters.

Overall, the intermediate precision observations suggest that the developed method for analyzing Sitagliptin is robust and reliable, with consistent and reproducible results across different runs and conditions. These findings instill confidence in the accuracy and consistency of the method, making it suitable for routine analysis and quality control in pharmaceutical settings.

Comparison of Method Precision with Intermediate Precision

The comparison of method precision with intermediate precision, as shown in Table 7.6, provides insights into the consistency and reliability of the analytical method for quantifying Sitagliptin (HCl).

In the method precision data, the % assay values for standard solutions 1 to 5 range from 98.9% to 99.8%, with a mean assay value of 99.2%. These values represent the precision of the method in quantifying the concentration of Sitagliptin in individual standard solutions.

Table 7.6: Comparison of Method Precision with Intermediate Precision.

Sample Solution	Method Precision	Intermediate Precision
Standard Solution 1	99.5	100.5
Standard Solution 2	99.8	100.1
Standard Solution 3	99.6	100.3
Standard Solution 4	99.0	100.0
Standard Solution 5	98.9	100.9
Standard Solution 6	99.7	100.7
Mean		99.2%
Std. Dev.		0.043
% RSD		0.023%

On the other hand, in the intermediate precision data, the % assay values for the same standard solutions are slightly higher, ranging from 100.0% to 100.9%, with a mean assay value of 100.3%. These values represent the precision of the method under different conditions or by different analysts, indicating the reproducibility and robustness of the method.

Overall, both method precision and intermediate precision data demonstrate high levels of accuracy and precision, with % RSD values of 0.023% and 0.043%, respectively. The consistency of the assay results across different precision studies reaffirms the reliability and suitability of the developed method for routine analysis and quality control of Sitagliptin in pharmaceutical formulations.

Change in Flow Rate of Mobile Phase ± 0.2ml (I.E., 1.8ml and 2.2ml).

Table 7.7 presents the impact of changing the flow rate of the mobile phase by ± 0.2 ml/min (i.e., 1.8 ml/min and 2.2 ml/min) on various chromatographic parameters for the analysis of Sitagliptin (HCl).

The table illustrates that changing the flow rate of the mobile phase has a significant impact on the retention time of the analyte. At a lower flow rate of 1.8 ml/min, the retention time is longer (3.15 minutes) compared to the higher flow rate of 2.2 ml/min (2.97 minutes). This indicates that the flow rate directly affects the elution time of the analyte from the column.

Table 7.7: Change in Flow Rate of Mobile Phase ± 0.2ml (I.E. 1.8ml and 2.2ml).

Parameter	Flow rate 1.8 ml/min	Flow rate 2.2 ml/min
Retention Time	3.15	2.97
Area	215160	215066
Theoretical Plates	3435	3035
Tailing	1.28	1.25

Additionally, changes in the flow rate also influence other chromatographic parameters. The area under the chromatographic peak remains relatively consistent between the two flow rates, suggesting that the flow rate variation does not significantly affect the peak area. However, there is a noticeable difference in the theoretical plates, with a higher flow rate resulting in fewer theoretical plates. This indicates that a higher flow rate may compromise the efficiency of the chromatographic separation.
Furthermore, the tailing factor shows a decrease when the flow rate is increased from 1.8 ml/min to 2.2 ml/min. This suggests that a higher flow rate leads to better peak symmetry, which can improve the resolution and accuracy of the analysis.

Overall, these observations highlight the importance of optimizing the flow rate in HPLC analysis to achieve the desired chromatographic performance and ensure accurate and reproducible results.

Change in pH of Mobile Phase by ± 0.2 Units (I.E., pH 4.3 and pH 4.7.

Table 7.8 demonstrates the effect of changing the pH of the mobile phase by ± 0.2 units (i.e., pH 4.3 and pH 4.7) on various chromatographic parameters for the analysis of Sitagliptin (HCl).

The table illustrates that changing the pH of the mobile phase has a notable impact on several chromatographic parameters.

Table7.8: Change in pH Of Mobile Phase By ± 0.2 Units (I.E. pH 4.3 and pH 4.7.

Parameter	**pH 4.3**	**pH 4.7**
Retention Time	3.23	2.88
Area	221819	231144
Theoretical Plates	3212	2966
Tailing	1.25	1.26

Firstly, altering the pH results in significant changes in the retention time of the analyte. At pH 4.3, the retention time is 3.23 minutes, while at pH 4.7, it decreases to 2.88 minutes. This shift in retention time suggests that changes in pH can affect the interaction between the analyte and the stationary phase of the column, influencing its elution time.

Secondly, variations in pH also impact the area under the chromatographic peak. In this case, the area increases when the pH of the mobile phase shifts from 4.3 to 4.7, indicating changes in the analyte's concentration or its response to the detection method.

Furthermore, alterations in pH affect the theoretical plates, with a decrease observed when the pH increases from 4.3 to 4.7. This suggests that changes in pH may impact the efficiency of the chromatographic separation, potentially leading to broader peaks and reduced resolution.

Finally, the tailing factor shows a slight increase when the pH changes from 4.3 to 4.7, indicating a potential decrease in peak symmetry. This may affect the accuracy and precision of the analysis, especially in quantitative measurements.

Overall, these observations highlight the importance of controlling and optimizing the pH of the mobile phase in HPLC analysis to ensure robust and reproducible chromatographic performance and accurate quantification of analytes.

Change In Wavelength ±2 Nm (I.E., 237nm and 241nm).

Table 7.9 illustrates the impact of changing the wavelength of the UV detection by ±2 nm (i.e., 237 nm and 241 nm) on various chromatographic parameters for the analysis of Sitagliptin (HCl).

Table 7.9: Change In Wavelength ±2 Nm (I.E. 237nm And 241nm).

Parameter	Wavelength: 237 nm	Wavelength: 241 nm
Retention Time	3.03	3.01
Area	236455	211153
Theoretical Plates	3214	3207
Tailing	1.26	1.25

The table demonstrates that changing the wavelength of UV detection has noticeable effects on several chromatographic parameters.

Firstly, alterations in wavelength lead to changes in the retention time of the analyte. At a wavelength of 237 nm, the retention time is 3.03 minutes, while at 241 nm, it decreases slightly to 3.01 minutes. This suggests that variations in wavelength can influence the elution behavior of the analyte from the column.

Secondly, changes in wavelength impact the area under the chromatographic peak. In this instance, the area decreases when the wavelength shifts from 237 nm to 241 nm, indicating changes in the analyte's response to UV detection.

Furthermore, variations in wavelength affect the theoretical plates, with a slight decrease observed when the wavelength increases from 237 nm to 241 nm. This suggests that changes in wavelength may affect the efficiency of the chromatographic separation, potentially impacting resolution.

Lastly, the tailing factor shows a slight decrease when the wavelength changes from 237 nm to 241 nm, indicating a potential improvement in peak symmetry.

Overall, these observations emphasize the importance of selecting an appropriate wavelength for UV detection in HPLC analysis to achieve optimal sensitivity, selectivity, and chromatographic performance. Optimization of wavelength is crucial for accurate and reproducible quantification of analytes in pharmaceutical and analytical applications.

System Suitability Parameters Observation.

Table 7.10 provides observations on various system suitability parameters, which are essential for evaluating the performance of the chromatographic system and ensuring the reliability of the analytical method for the analysis of Sitagliptin (HCl).

Table 7.10: System Suitability Parameters Observation.

Sr. No.	Validation Parameter	Retention Time	% RSD of Area	Theoretical Plates	Tailing
1	Specificity by injecting blank	------	------	------	------
2	Specificity by injecting standard	2.97	0.08%	3262	1.34
3	Linearity and range	3.05	0.055%	2966	1.22
4	Accuracy (Recovery)	2.97	0.005%	3212	1.26
5	Method precision	3.05	0.003%	3285	1.27
6	Intermediate precision	3.15	0.006%	3068	1.23
7	Robustness - Change in flow (1.8 ml/min)	3.15	0.005%	3435	1.28

8	Robustness - Change in flow (2.2 ml/min)	2.97	0.005%	3035	1.25
9	Robustness - Change in pH of mobile phase (pH 4.3)	3.23	0.015%	3212	1.25
10	Robustness - Change in pH of mobile phase (pH 4.7)	2.88	0.012%	2966	1.26
11	Robustness - Change in wavelength (237nm)	3.03	0.001%	3214	1.26
12	Robustness - Change in wavelength (241nm)	3.01	0.023%	3207	1.25
Minimum		2.88	0.001%	2966	1.22
Maximum		3.23	0.08%	3435	1.34
Mean		3.04	0.017%	3165	1.25
Limit		Not more than 2.0%	More than 2000	Between 0.8 and 2.0	

The table presents a comprehensive assessment of system suitability parameters, including specificity, linearity and range, accuracy, precision, and robustness. These parameters evaluate the performance characteristics of the chromatographic system and ensure that it meets predefined acceptance criteria.

Overall, the observed values for retention time, % RSD of area, theoretical plates, and tailing factor fall within the specified limits, indicating that the developed method is suitable for the analysis of Sitagliptin. The minimum, maximum, and mean values provide insights into the range of variability observed across different validation parameters.

Furthermore, the limit column specifies the acceptance criteria for each parameter, ensuring that the chromatographic system meets predefined quality standards. By assessing these system suitability parameters, analysts can verify the reliability and robustness of the analytical method and ensure accurate and reproducible results in routine analysis.

CONCLUSION

SUMMARY:

The results and discussion of the study on Sitagliptin encompass a comprehensive evaluation of various analytical methods for detecting, quantifying, and assessing the stability of the drug. Here's a summary of the key findings and observations:

1. *Selection of Wavelength:*
The study identified the optimal wavelength for detecting Sitagliptin using UV spectroscopy. By scanning solutions at different wavelengths, the maximum absorbance was found to occur at 239 nm, providing the ideal conditions for subsequent analyses.
2. *Calibration Curve:*
A calibration curve was established to correlate the concentration of Sitagliptin with the peak area obtained through UV spectroscopy. The linear relationship between concentration and peak area allowed for accurate quantification of the drug in unknown samples.
3. *Method Development:*
A robust and reliable method for determining Sitagliptin by RP-HPLC was developed. The chromatographic conditions were optimised to achieve efficient separation and quantification of the drug within a short analysis time. The method demonstrated sensitivity and selectivity over a concentration range of 50-250 µg/ml.
4. *Specificity and Performance Characteristics:*
The developed method exhibited excellent performance characteristics, including high mean recoveries, a regression coefficient (R^2) of 0.999, and precise intra-day and inter-day precision. Robustness studies validated the method's reliability under varying conditions.
5. *Stability Studies:*
Stress testing of Sitagliptin under different conditions revealed its stability profile. Minimal degradation was observed under neutral conditions, while oxidative stress and hydrolysis led to moderate degradation. Overall, the drug exhibited stability under most tested conditions.
6. *Accuracy Data:*
The accuracy data demonstrated the precision and reliability of the developed method in quantifying the drug at different concentration levels. The mean recovery percentages across various concentration levels were consistent, indicating the method's accuracy.
7. *Precision:*
Precision data showed minimal variability in retention time, peak area, theoretical plates, and tailing factor, confirming the method's consistency and reproducibility across different samples and runs.
8. *System Suitability Parameters:*
Assessment of system suitability parameters confirmed the method's suitability for analyzing Sitagliptin. Specificity, linearity, accuracy, precision, and robustness were within acceptable limits, ensuring the reliability of the analytical method.

Overall, the study demonstrated the effectiveness of UV spectroscopy and RP-HPLC methods for detecting and quantifying Sitagliptin, along with providing insights into its stability under various conditions. The developed analytical methods offer valuable tools for pharmaceutical quality control and dosage form analysis, contributing to the broader understanding of this important drug.

CONCLUSION

In conclusion, the comprehensive study on Sitagliptin showcased the successful development and validation of analytical methods, including UV spectroscopy and RP-HPLC, for the detection, quantification, and assessment of the drug's stability. Through meticulous experimentation and analysis, optimal conditions were identified, leading to robust methods with high sensitivity, selectivity, and reliability. The accuracy and precision of the developed methods were demonstrated through calibration curves, accuracy data, and precision studies, highlighting their suitability for routine pharmaceutical analysis. Moreover, the stability profile of Sitagliptin under various stress conditions provided valuable insights into its behavior, enhancing our understanding of its pharmaceutical characteristics. Overall, this study contributes significantly to the body of knowledge surrounding Sitagliptin, offering valuable tools for quality control and dosage form analysis in pharmaceutical settings.

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