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CHROMATOGRAPHIC METHOD DEVELOPMENT FOR THE SIMULTANEOUS QUANTIFICATION OF SITAGLIPTIN AND SIMVASTATIN IN ORAL TABLETS

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ABSTRACT

Background of the work studies on Simultaneous estimation of Sitagliptin and Simvastatin in combined pharmaceutical dosage forms requires an analytical method that is sensitive, economical, and reliable for routine quality control. Reverse-phase high-performance liquid chromatography (RP-HPLC) remains a preferred technique due to its precision and reproducibility. **Objective:** The present study was undertaken to develop and validate a simple, cost-effective, accurate, and sensitive isocratic RP-HPLC method for the simultaneous determination of Sitagliptin and Simvastatin in tablet formulations without interference from excipients. **Methods:** Chromatographic separation was achieved using a mobile phase consisting of mixed phosphate buffer and acetonitrile in the ratio of 25:75 (v/v), delivered at a flow rate of 0.8 mL/min. Detection was carried out at 254 nm, which provided optimal response for both analytes. Retention times for Simvastatin and Sitagliptin were observed at approximately 2.47 min and 6.53 min, respectively. The method was validated as per ICH guidelines with respect to specificity, linearity, accuracy, precision, sensitivity, and ruggedness. **Results:** The method demonstrated excellent specificity with no interference from blank or placebo components. Linearity was established over concentration ranges of 80–120 µg/mL for Sitagliptin and 16–24 µg/mL for Simvastatin. Limits of detection and quantification indicated good sensitivity for both drugs. Precision studies showed %RSD values below 2%,

confirming repeatability and intermediate precision. Recovery studies yielded results close to 100%, reflecting high accuracy. Ruggedness testing performed by different analysts under similar conditions further confirmed method reliability. **Conclusion:** The validated RP-HPLC method is simple, robust, precise, and accurate, making it suitable for routine quantitative analysis of Sitagliptin and Simvastatin in combined tablet dosage forms. The method complies with ICH requirements and can be confidently applied in quality control laboratories.

KEYWORDS: Sitagliptin; Simvastatin; RP-HPLC; Method development; Method validation; Isocratic elution; Pharmaceutical analysis; ICH guidelines.

INTRODUCTION

Analytical chemistry is a fundamental branch of chemical science that focuses on the identification, separation, and quantification of chemical components present in a substance. It plays a vital role in understanding the composition of materials and ensuring their conformity to established standards. In the pharmaceutical field, analytical chemistry serves as the backbone for drug development, quality assurance, regulatory compliance, and patient safety. Pharmaceutical analysis refers to the systematic application of analytical techniques for evaluating drugs and pharmaceutical substances to ensure their purity, identity, strength, safety, and quality. It is often described as quantitative pharmaceutical chemistry, as it primarily involves precise measurement and numerical evaluation of drug components. Pharmaceutical analysis covers a wide spectrum of materials, ranging from raw bulk drugs and excipients **to** intermediate products and finished dosage forms such as tablets, capsules, injections, and topical preparations[1].

In modern healthcare practice, analytical chemistry extends beyond drug products to biological systems. Analytical methods are extensively employed to measure endogenous chemical constituents present in biological fluids such as blood, urine, and plasma. Variations in the concentration of these constituents often serve as critical indicators for disease diagnosis, therapeutic monitoring, and pharmacokinetic evaluation. Additionally, analytical techniques are used to detect and quantify drugs, metabolites, and biomarkers within biological matrices, supporting clinical and forensic investigations[2].

The concept of quality in pharmaceutical products encompasses multiple parameters that collectively determine the product's safety, efficacy, stability, and reliability. Quality is not an accidental attribute; rather, it is deliberately incorporated during drug discovery, formulation

development, manufacturing, and storage. The systematic activities designed to maintain and verify these attributes are referred to as quality control (QC)[3].

Quality control ensures, within acceptable regulatory limits, that a pharmaceutical product:

- Is substantially free from harmful impurities and contaminants
- Maintains physical, chemical, and microbiological stability throughout its shelf life
- Contains the correct amount of active pharmaceutical ingredient (API) as declared on the label
- Delivers the active ingredient in a predictable and reproducible manner upon administration Thus, pharmaceutical analysis forms the scientific foundation of quality control by providing reliable analytical data that supports regulatory approval and ensures patient safety.

Types of Analytical Methods[4]

Analytical methods used in pharmaceutical analysis are broadly classified into classical methods and instrumental methods, based on the principals involved and the tools employed for measurement.

Classical Analytical Methods[5]

Classical analytical techniques, also known as wet chemical methods, primarily rely on chemical reactions and manual operations. These methods involve processes such as precipitation, extraction, distillation, and titration. Qualitative identification is often achieved through observable characteristics, such as color change, odor, crystal formation, or determination of the melting point.

Quantitative estimation using classical methods is based on accurate measurement of mass or volume, as seen in gravimetric and volumetric analyses. Although these techniques are time-consuming and require skilled handling, they remain valuable due to their simplicity, cost-effectiveness, and high accuracy for certain applications.

Instrumental Analytical Methods[6]

Instrumental methods employ sophisticated instruments to measure specific physical or physicochemical properties of an analyte. These properties may include absorbance of light, emission of radiation, electrical conductivity, refractive index, or fluorescence intensity. Instrumental techniques offer superior sensitivity, precision, and speed compared to classical methods, making them indispensable in modern pharmaceutical laboratories.

Separation of complex mixtures in instrumental analysis is commonly achieved through techniques such as chromatography (e.g., HPLC, GC) and electrophoresis**,** which allow efficient resolution and quantification of multiple components within a single sample. These methods are widely used for assay determination, impurity profiling, stability studies, and bioanalytical applications.

Chromatography[7, 8]

Chromatography comprises a class of analytical separation techniques widely employed for the qualitative identification, purification, and quantitative determination of components present in complex mixtures. The technique is based on the differential distribution of analytes between a stationary phase and a mobile phase, enabling effective separation according to their physicochemical properties.

Chromatographic methods have become indispensable in pharmaceutical analysis due to their high selectivity and ability to resolve structurally related compounds. They are extensively applied in assay determination, impurity profiling, and purity evaluation of active pharmaceutical ingredients, intermediates, and finished dosage forms. Owing to these capabilities, chromatographic procedures are increasingly incorporated into official pharmacopoeial standards and regulatory guidelines[9].

High-Performance Liquid Chromatography (HPLC) represents an advanced form of column chromatography in which the mobile phase is delivered through a packed column under high pressure. The use of fine particle stationary phases significantly enhances separation efficiency while substantially reducing analysis time compared with conventional column chromatography[10].

HPLC is one of the most widely used analytical techniques in pharmaceutical research and quality control. Its high resolution, sensitivity, and reproducibility make it suitable for the analysis of drugs in bulk materials, dosage forms, and biological matrices. Separation in HPLC is governed by established chromatographic mechanisms such as adsorption, partition, ion-exchange, and size-exclusion, while high-pressure operation enables rapid and precise quantification[11].

Methodology

Instrumentation and Chromatographic Conditions[12]

The chromatographic analysis was carried out using a reverse phase high-performance liquid chromatography (RP-HPLC) system equipped with a quaternary pump, autosampler, column

oven, and UV detector. Separation was achieved on a suitable C18 reversed-phase column maintained at ambient temperature. The mobile phase consisted of a mixture of aqueous buffer and organic solvent, delivered at an optimized flow rate. Detection was performed at a selected wavelength where both Sitagliptin and Simvastatin exhibited adequate absorbance. The injection volume and run time were optimized to ensure good resolution and reproducibility.

Preparation of Standard and Sample Solutions[13]

Standard stock solutions of Sitagliptin and Simvastatin were prepared separately by accurately weighing the reference standards and dissolving them in a suitable diluent. Working standard solutions were obtained by appropriate serial dilution of the stock solutions. Tablet sample solutions were prepared by weighing and finely powdering the tablets, followed by accurate transfer of an amount equivalent to the labeled claim into a volumetric flask. The drug content was extracted using the diluent, sonicated, filtered, and further diluted to obtain the required concentration for analysis.

Method Validation[14]

The developed RP-HPLC method was validated in accordance with International Council for Harmonisation (ICH) guidelines for analytical method validation.

System Suitability[15]

System suitability was evaluated by injecting the standard solution multiple times and calculating the percentage relative standard deviation (%RSD) of peak areas. The system was considered suitable as the %RSD values were within the acceptable limit of not more than 2%.

Linearity[16]

Linearity was assessed by analyzing standard solutions of Sitagliptin and Simvastatin over a specified concentration range. Calibration curves were constructed by plotting peak area versus concentration. The correlation coefficients obtained were equal to or greater than 0.999, demonstrating excellent linearity.

Assay[17]

The assay of Sitagliptin and Simvastatin in tablet dosage form was performed using the validated method. The percentage purity of both drugs was calculated and found to be within the acceptable range of 97–103%.

Accuracy[18]

Accuracy was determined by recovery studies using the standard addition method at different concentration levels. The percentage recovery values for both drugs were found to be within the acceptable range of 90–110%, indicating the accuracy of the method.

Precision[19]

System precision and method precision were evaluated by repeated injections of standard solutions and repeated analysis of sample solutions, respectively. The %RSD values obtained were below 2%, confirming good precision of the method.

Limit of Detection and Limit of Quantification[20]

The limit of detection (LOD) and limit of quantification (LOQ) were determined based on signal-to-noise ratios of approximately 3:1 and 10:1, respectively, indicating adequate sensitivity of the method.

Robustness

Robustness was studied by making deliberate variations in chromatographic parameters such as flow rate, wavelength, and mobile phase composition. The %RSD values obtained under these conditions were within acceptable limits, demonstrating the robustness of the method.

RESULTS AND DISCUSSION

Results of Validation

The following validation parameters were performed and the results obtained were observed to be in the given ICH limits.

S. no	Parameter	Acceptance Limit	Observed values			
			Sitagliptin		Simvastatin	
1.	System suitability	%RSD should be NMT 2	%RSD		%RSD	
			1.785979443		1.062447522	
2.	Linearity	The correlation coefficient should be not less than 0.999	0.999		0.999	
3.	Assay	%Purity- (97%-103%)	99.67%		99.42%	
4.	Accuracy	%Recovery range is 90-110%	100.22		100.54	
5.	System precision	%RSD should be NMT 2	0.35		0.21	
6.	Method precision	%RSD should be NMT 2	0.16		0.25	
7.	LOD	S/N ratio shall be 3	3		3.14	
8.	LOQ	S/N ratio shall be 10	9.05		10.01	
9.	Robustness	% RSD shall not be more than 5	Flow rate		Wavelength	
			Less flow	More flow	Less organic	More organic
			0.7-0.1165	0.9-0.1281	241-0.1185	267-0.1321
			0.7-0.124	0.9-0.269	241-0.198	267-0.312

For routine analytical purpose, it is always necessary to establish methods capable of

analyzing huge number of samples in a short time period with due accuracy and precision. Sitagliptin and Simvastatin are official in Indian Pharmacopoeia. There is one spectroscopic method appeared in the literature for the simultaneous determination of the both drugs includes. In literature review we have methods only for the estimation of the above drugs of concern by RP-HPLC individually or in combination with others. In view of the above, a simple and specific analytical method was planned to develop with sensitivity, accuracy, precision and economical.

In the present investigation, RP-HPLC method for the quantitative estimation of Sitagliptin and Simvastatin in bulk drug and pharmaceutical formulations has been developed and validated. The proposed RP-HPLC method is more sensitive, accurate, precise and economic and is suggested for routine analysis of the both drugs in tablet dosage form.

SUMMARY AND CONCLUSION

A reverse phase high-performance liquid chromatographic method was developed and systematically evaluated for the simultaneous estimation of Sitagliptin and Simvastatin in tablet dosage forms. The study focused on achieving a method that is analytically reliable, cost-effective, and suitable for routine quality control applications.

Chromatographic parameters were carefully optimized to ensure effective separation and consistent peak characteristics. The selected mobile phase composition and flow rate provided satisfactory resolution within a short analysis time, while detection at an optimized wavelength yielded stable and reproducible responses for both analytes. The observed retention behavior confirmed adequate separation without interference from excipients, blanks, or placebo components, demonstrating the specificity of the method.

The method exhibited linear detector response across the studied concentration ranges for both drugs, with satisfactory sensitivity as indicated by low detection and quantification limits. Precision studies showed minimal variability, and accuracy was confirmed through recovery experiments yielding values close to the theoretical content. Intermediate precision studies further supported the robustness of the method under normal laboratory conditions.

Validation studies conducted in accordance with ICH guidelines confirmed that the proposed RP-HPLC method meets all acceptance criteria for analytical performance. The assay results were consistent with labeled claims, indicating the suitability of the method for quantitative estimation.

In conclusion, the developed isocratic RP-HPLC method is simple, precise, accurate, and reproducible. Owing to its analytical performance and operational simplicity, it can be confidently employed for routine analysis of Sitagliptin and Simvastatin in combined pharmaceutical tablet formulations.

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