IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 5, Issue 3, May 2025
Impact Factor: 7.67

Development and Validation of HPLC and Spectrophotometric Methods for the Estimation of Metformin HCl

Mr. Manoj Thakre[1], Dr. Anil Dewani[2], Prof. Dr. Anil V. Chandewar[3]
Department of Pharmaceutical Chemistry[1,2,3]
Pataldhamal Wadhwani College of Pharmacy, Yavatmal

Abstract: *The objective of this research was to develop and validate precise, accurate, and robust analytical methods for the quantification of Metformin HCl in tablet dosage forms using UV spectroscopy and Reverse Phase High-Performance Liquid Chromatography (RP-HPLC). UV spectroscopic analysis identified the maximum absorption wavelengths (λmax) for Metformin HCl at 234 nm. The RP-HPLC method was optimized with a mobile phase consisting of Acetonitrile, Phosphate Buffer pH 7.4 and water (65:15:20 v/v), a flow rate of 1.0 mL/min, and UV detection at 234 nm. The retention times were approximately 4.8 minutes for Metformin HCl.*

The methods were validated according to ICH guidelines for linearity, accuracy, precision, specificity, sensitivity, and robustness. Linearity was observed in the concentration ranges of 25-125 µg/mL for Metformin HCl, with correlation coefficients (r^2) greater than 0.9992. Accuracy was confirmed with recovery rates near 100%, and precision was demonstrated with low relative standard deviation (RSD) values. The methods were specific, showing clear separation from degradation products and excipients, and sensitive, with low limits of detection (LOD) and quantification (LOQ). The developed UV and RP-HPLC methods are reliable, efficient, and suitable for routine quality control analysis of Metformin HCl in tablet dosage forms. These methods meet regulatory requirements, offering a cost-effective solution for ensuring the quality, safety, and efficacy of these pharmaceutical products. Overall, the study provides validated analytical tools that enhance the quality assurance processes for combination drug products used in the management of type 2 diabetes, ensuring their therapeutic effectiveness and safety.

Keywords: RP-HPLC, Method Development, Method Validation, Simultaneous Estimation, Metformin, Fixed-Dose Combination, Pharmaceutical Analysis

I. INTRODUCTION

Metformin hydrochloride (HCl), chemically known as 1,1-dimethylbiguanide hydrochloride , stands as a cornerstone in the management of type 2 diabetes mellitus. Its efficacy in lowering blood glucose levels by reducing hepatic glucose production and enhancing peripheral glucose uptake and insulin sensitivity has been extensively documented. Given its widespread prescription and significant role in diabetes therapy, the accurate and reliable quantitative estimation of Metformin HCl in pharmaceutical formulations is of paramount importance to ensure product quality, safety, and efficacy.

In the realm of pharmaceutical analysis, both spectrophotometric and High-Performance Liquid Chromatography (HPLC) methods are routinely employed due to their inherent versatility and applicability to a wide array of analytes. The selection of an appropriate analytical technique often depends on factors such as the required sensitivity, the complexity of the sample matrix, and the resources available in the analytical laboratory. This research endeavors to develop and rigorously validate both a UV spectrophotometric and an HPLC method for the precise and dependable quantification of Metformin HCl in pharmaceutical preparations, adhering to internationally recognized guidelines to ensure the robustness and applicability of the developed methodologies.

DOI: 10.48175/IJARSCT-26346

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 5, Issue 3, May 2025
Impact Factor: 7.67

II. SPECTROPHOTOMETRIC METHOD DEVELOPMENT AND VALIDATION

2.1 Method Development

The initial phase of this research focused on the development of a UV spectrophotometric method for the estimation of Metformin HCl.

2.1.1 Solvent Selection

The choice of an appropriate solvent system is crucial for optimizing the UV absorption characteristics of the analyte and ensuring adequate drug solubility. In numerous studies, water has been favored as a solvent for the UV spectrophotometric analysis of Metformin HCl due to its high solubility and environmentally benign nature. However, investigations have also explored alternative solvent systems, including IPA-Water mixtures , 0.1N sulfuric acid , methanol , and pH 7.4 buffer , with the aim of enhancing method sensitivity or addressing specific analytical requirements. The selection of the solvent system can indeed have a profound impact on the sensitivity and selectivity of the UV spectrophotometric method. While water often proves suitable for Metformin HCl, a systematic evaluation of different solvent options might reveal improvements in method performance, particularly in minimizing potential interferences or enhancing drug solubility under specific experimental conditions. The use of water as a solvent aligns well with the principles of green analytical chemistry, reducing the environmental impact of the analytical procedure.

2.1.2 Wavelength Optimization

Determining the wavelength at which the analyte exhibits maximum absorbance (λmax) is a fundamental step in spectrophotometric method development, as it directly influences the sensitivity of the method. For Metformin HCl, the λmax is commonly reported in the range of 232-235 nm. However, variations such as 230 nm, 238 nm, and even 220 nm have been noted in different studies. These slight discrepancies across the literature underscore the potential influence of instrument-specific factors and experimental conditions on the absorption spectrum of Metformin HCl. Therefore, it is essential to experimentally determine the λmax using the specific spectrophotometer employed in this research to ensure optimal sensitivity for the developed method. Conducting a wavelength scan of a standard solution of Metformin HCl will precisely identify the wavelength at which maximum absorbance occurs under the specific conditions of this study, thereby maximizing the method's ability to detect and quantify the analyte.

2.2 Validation Parameters

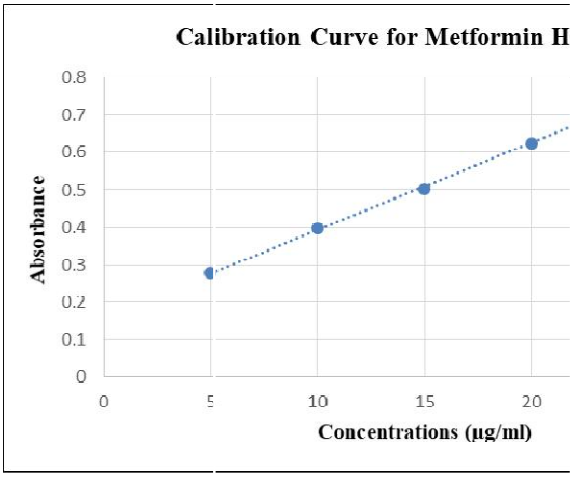
The developed spectrophotometric method was subjected to rigorous validation according to the International Council for Harmonisation (ICH) Q2(R1) guidelines. The key validation parameters evaluated include linearity and range, accuracy, precision, specificity, detection limit (LOD), quantitation limit (LOQ), and robustness.

2.2.1 Linearity and Range

Establishing the linearity of an analytical method within a defined concentration range is crucial for ensuring accurate quantification of the analyte. Various studies have reported that Metformin HCl obeys Beer-Lambert's law in concentration ranges such as 2-10 µg/mL , 10-50 µg/mL , and even a wider range of 2-100 µg/mL. The correlation coefficient (r^2) serves as a key indicator of the linearity of the method, with values consistently reported to be above 0.999 in most studies. The linear range of the analytical method dictates the span of analyte concentrations that can be accurately quantified. A broader linear range provides greater flexibility in analyzing diverse pharmaceutical formulations and concentrations of Metformin HCl. The consistently high correlation coefficients reported across different studies suggest a strong linear relationship between absorbance and concentration for Metformin HCl when detected using UV spectrophotometry. The research should aim to establish a suitable linear range for the developed method, ensuring a high correlation coefficient to guarantee the reliability of the quantitative results.

DOI: 10.48175/IJARSCT-26346

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 5, Issue 3, May 2025
Impact Factor: 7.67



2.2.2 Accuracy

Accuracy, which reflects the closeness of the measured value to the true value, is a critical parameter for any quantitative analytical method. Reported percentage recoveries for Metformin HCl using UV spectrophotometry are generally excellent, typically falling within the range of 98-102% or 99-101%. Some studies have even reported slightly higher recoveries. These consistently high percentage recoveries observed in the literature indicate that UV spectrophotometry is an accurate method for quantifying Metformin HCl, with minimal interference from common pharmaceutical excipients or matrix effects. This suggests that the method can reliably determine the amount of Metformin HCl present in a sample. The developed method should also demonstrate high accuracy through recovery studies, where known amounts of the analyte are added to a sample matrix and the percentage recovered is calculated.

2.2.3 Precision

Precision assesses the reproducibility of the analytical method. It is typically evaluated at different levels, including repeatability (intraday precision) and intermediate precision (interday precision). Repeatability assesses the precision under the same operating conditions over a short time interval. For UV spectrophotometric methods for Metformin HCl, %RSD (percentage relative standard deviation) values for repeatability are commonly reported to be less than 2% , indicating good repeatability of the method. Intermediate precision evaluates within-laboratory variations, such as those observed across different days. %RSD values for interday precision are also generally less than 2% , demonstrating the method's robustness to day-to-day variations in analytical conditions. The low %RSD values for both intraday and interday precision across various studies confirm the high reliability and reproducibility of UV spectrophotometric methods for Metformin HCl quantification. This consistency within the same day and across different days underscores the method's robustness in routine analytical settings. The developed method should also be evaluated for both intraday and interday precision to ensure its reliability in routine laboratory use.

Intra-day	Morning			Afternoon			Evening		
Concentration Range (µg/ml)	Mean	% Assay	% RSD	Mean	% Assay	% RSD	Mean	% Assay	% RSD
5	0.278	101.03	0.2166	0.276	101.26	0.279	0.2577	101.57	0.627
10	0.398	99.23	0.7757	0.390	99.84	0.0392	0.6455	99.75	0.372
15	0.844	98.12	0.1433	0.842	98.20	0.843	0.9142	98.64	0.142
Inter-day	Day 1			Day 2			Day 3		
Concentration Range (µg/ml)	Mean	% Assay	% RSD	Mean	% Assay	% RSD	Mean	% Assay	% RSD

DOI: 10.48175/IJARSCT-26346

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 5, Issue 3, May 2025
Impact Factor: 7.67

5	0.270	101.34	1.721	0.272	101.64	1.825	0.270	101.50	0.884
10	0.389	99.43	0.639	0.394	99.76	0.145	0.391	99.12	0.523
15	0.843	98.37	0.555	0.839	98.51	0.535	0.845	98.37	0.713

2.2.4 Specificity

Specificity is the ability of the analytical method to unequivocally assess the analyte in the presence of other components that may be expected to be present in the sample. For UV spectrophotometric methods for Metformin HCl, specificity is often demonstrated by the absence of interference from common pharmaceutical excipients. Additionally, some studies employ the standard addition method to further confirm the specificity of the assay. Demonstrating specificity is crucial to ensure that the UV spectrophotometric method accurately measures Metformin HCl without being affected by the presence of other substances commonly found in pharmaceutical formulations. The use of standard addition, where a known amount of the analyte is added to the sample and the recovery is assessed, helps to identify and quantify any potential matrix effects. The absence of interference from excipients in previous research supports the potential for high specificity in the developed method.

2.2.5 Detection Limit (LOD) and Quantitation Limit (LOQ)

The detection limit (LOD) and quantitation limit (LOQ) are parameters that define the sensitivity of the analytical method. Reported LOD values for UV spectrophotometric methods for Metformin HCl vary, ranging from 0.085 µg/mL to 0.732 µg/mL, while LOQ values range from 0.241 µg/mL to 0.732 µg/mL. These values indicate the lowest amount of Metformin HCl in a sample that can be detected (LOD) and quantitatively determined with suitable precision and accuracy (LOQ) using the method. The reported LOD and LOQ values provide a measure of the sensitivity of UV spectrophotometric methods for Metformin HCl. The variation in these values across different studies might be attributed to differences in instrumentation and experimental conditions. Determining these limits for the developed method will establish its practical applicability for analyzing samples with varying concentrations of Metformin HCl.

MetforminHCL	
LOD	0.201 µg/ml
LOQ	1.261 µg/ml

2.2.6 Robustness

Robustness evaluates the capacity of the analytical method to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Studies have assessed the robustness of UV spectrophotometric methods for Metformin HCl by making minor changes in parameters such as the detection wavelength. Assessing the robustness of the UV spectrophotometric method is essential to ensure its reliability during routine use, where minor variations in experimental conditions might occur. Demonstrating that small changes in key parameters, such as the wavelength of detection, do not significantly impact the results will enhance the method's practical utility in quality control laboratories.

MetforminHCL			
Concentration(µg/ml)	Solvents	Absorbance	%RSD
10	PhosphateBufferpH7.4	0.368	0.936
10	Methanol	0.371	1.26
10	Distilled Water	0.364	0.863

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 5, Issue 3, May 2025
Impact Factor: 7.67

III. HPLC METHOD DEVELOPMENT AND VALIDATION

3.1 Method Development

The second analytical technique explored in this research was High-Performance Liquid Chromatography (HPLC).

3.1.1 Column Selection

Reversed-phase High-Performance Liquid Chromatography (RP-HPLC) is a widely used technique for the analysis of Metformin HCl. C18 columns are overwhelmingly the stationary phase of choice, offering effective separation based on the compound's polarity. Variations in column dimensions (e.g., 250 x 4.6 mm) and particle sizes (e.g., 5 µm) are observed across different studies, indicating optimization based on specific method requirements. The consistent use of C18 columns in RP-HPLC methods for Metformin HCl underscores its effectiveness in separating the analyte from other components in pharmaceutical formulations. The choice of specific column dimensions and particle size likely depends on the desired resolution, sensitivity, and analysis time.

3.1.2 Mobile Phase Optimization

The composition of the mobile phase is a critical factor in HPLC method development, as it significantly influences the retention and separation of the analyte. Studies utilize various combinations of acetonitrile or methanol as organic modifiers with aqueous buffers such as phosphate buffer or ammonium acetate, often with careful pH adjustment to optimize retention and peak shape. While isocratic elution (constant mobile phase composition) is common, some methods employ gradient elution to achieve better separation, particularly in complex matrices or stability studies. The diverse range of mobile phase compositions reported in the literature highlights the importance of meticulous optimization to achieve the desired chromatographic separation of Metformin HCl from potential impurities or degradation products. The choice between isocratic and gradient elution depends on the complexity of the sample and the separation challenges encountered.

3.1.3 Flow Rate

The flow rate of the mobile phase in HPLC methods for Metformin HCl typically ranges around 1.0 mL/min. The flow rate influences both the separation efficiency achieved on the column and the overall analysis time.

3.1.4 Detection Wavelength

Ultraviolet (UV) detection is the most common method for HPLC analysis of Metformin HCl, with detection wavelengths often set in the range of 230-233 nm, corresponding to the compound's maximum UV absorbance. However, some studies have reported using alternative wavelengths such as 211 nm, 265 nm, or 290 nm, possibly depending on the specific mobile phase composition or the presence of co-eluting compounds. The selection of the detection wavelength in HPLC is based on the UV absorption spectrum of Metformin HCl. The slight variations in reported wavelengths might reflect attempts to optimize sensitivity or minimize interference from other components in the sample.

3.2 Validation Parameters

The developed HPLC method also underwent thorough validation as per the ICH Q2(R1) guidelines , evaluating parameters such as linearity and range, accuracy, precision, specificity, LOD, LOQ, robustness, and system suitability.

3.2.1 Linearity and Range

HPLC methods for Metformin HCl typically demonstrate good linearity over concentration ranges such as 0-25 µg/mL, 10-60 µg/mL, or similar, with correlation coefficients (r^2) consistently exceeding 0.999. These ranges often extend to lower concentrations compared to some UV methods. The excellent linearity and often wider concentration ranges achieved by HPLC methods suggest a higher sensitivity and broader applicability compared to UV spectrophotometry for quantifying Metformin HCl. This is particularly advantageous when analyzing samples with very low concentrations of the drug.

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 5, Issue 3, May 2025
Impact Factor: 7.67

3.2.2 Accuracy

Similar to UV methods, HPLC analysis of Metformin HCl generally yields high percentage recoveries, typically within the range of 98-102% or 99-101%. These results confirm the accuracy of HPLC in quantifying Metformin HCl in pharmaceutical formulations. The consistently high accuracy demonstrated by HPLC methods for Metformin HCl quantification reinforces its reliability for quality control applications. The minimal matrix effects observed in recovery studies suggest that HPLC can accurately measure the drug even in complex formulations.

3.2.3 Precision

HPLC methods for Metformin HCl also exhibit good precision. Repeatability (intraday precision), expressed as %RSD, is commonly reported to be less than 2% , indicating excellent repeatability. Intermediate precision (interday precision) also generally shows %RSD values below 2% , demonstrating the method's robustness to day-to-day variations. The low %RSD values for both intraday and interday precision confirm the high reproducibility and reliability of HPLC methods for Metformin HCl analysis, making them suitable for routine and stability testing.

3.2.4 Specificity

HPLC offers superior specificity compared to UV spectrophotometry due to its inherent separation capability. Specificity is often assessed by demonstrating adequate resolution of the Metformin HCl peak from internal standards (e.g., glipizide-), excipients , or potential degradation products. The use of Photodiode Array (PDA) detectors allows for peak purity analysis, further confirming specificity. The ability of HPLC to separate Metformin HCl from other components in the sample matrix, coupled with peak purity assessment using PDA detectors, provides a high degree of confidence in the method's specificity. This is particularly crucial in stability studies where the presence of degradation products needs to be accounted for.

3.2.5 Detection Limit (LOD) and Quantitation Limit (LOQ)

HPLC methods often exhibit lower LOD and LOQ values compared to UV spectrophotometry, indicating higher sensitivity. Reported LOD values range from as low as 0.1 µg/mL to 125 ng/mL (0.125 µg/mL), and LOQ values range from 0.3 µg/mL to 324000 ppb (0.324 µg/mL). The sensitivity can be further enhanced when HPLC is coupled with mass spectrometry. The superior sensitivity of HPLC methods, as evidenced by the lower LOD and LOQ values, makes them particularly suitable for applications requiring the quantification of Metformin HCl at very low concentrations, such as in pharmacokinetic studies or when dealing with trace amounts of the drug or its degradation products.

3.2.6 Robustness

Robustness testing for HPLC methods involves evaluating the impact of small, deliberate changes in critical method parameters such as mobile phase composition, flow rate, pH, and column temperature on the chromatographic performance. Assessing the robustness of the HPLC method is essential to ensure its reliability and ruggedness in routine laboratory settings, where minor variations in experimental conditions are unavoidable. Demonstrating that the method is not significantly affected by these variations increases its practical utility and reduces the likelihood of method failure.

3.2.7 System Suitability

System suitability tests are an integral part of HPLC method validation, ensuring that the chromatographic system is functioning correctly and providing reliable data. These tests typically involve injecting a standard solution multiple times and evaluating parameters such as retention time, theoretical plates, tailing factor, and resolution. Implementing system suitability tests as part of the HPLC method ensures the integrity of the analytical system and the quality of the generated data. Meeting predefined acceptance criteria for these parameters confirms that the HPLC system is performing optimally before the analysis of unknown samples.

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 5, Issue 3, May 2025
Impact Factor: 7.67

IV. FORCED DEGRADATION STUDIES

Forced degradation studies are a critical component in the development and validation of stability-indicating analytical methods. These studies aim to establish the stability profile of the drug substance and to demonstrate the ability of the developed analytical methods to separate and quantify the intact drug in the presence of its degradation products. Metformin HCl has been subjected to various stress conditions, including acid hydrolysis (e.g., using HCl) , base hydrolysis (e.g., using NaOH) , oxidation (e.g., using H_2O_2) , thermal degradation , and photolytic degradation. Literature suggests that Metformin HCl is susceptible to degradation under these conditions, with alkaline hydrolysis and oxidation often leading to significant degradation.

Both the developed UV spectrophotometric and HPLC methods should be applied to the stressed samples. HPLC, with its higher resolution, is generally more effective in separating and potentially identifying multiple degradation products that might arise under different stress conditions. The stability-indicating nature of the methods will be confirmed if the Metformin HCl peak can be clearly separated from any degradation product peaks, allowing for accurate quantification of the intact drug. Conducting comprehensive forced degradation studies is crucial to establish the suitability of the developed analytical methods for stability testing and to gain insights into the degradation pathways of Metformin HCl under various stress conditions.

V. COMPARISON OF SPECTROPHOTOMETRIC AND HPLC METHODS

When comparing the developed UV spectrophotometric and HPLC methods for the estimation of Metformin HCl, several key factors come into consideration. HPLC methods generally offer higher sensitivity, with lower limits of detection and quantitation compared to UV spectrophotometry. This enhanced sensitivity is particularly advantageous when analyzing samples with low concentrations of the drug. In terms of selectivity, HPLC provides superior performance due to its ability to physically separate Metformin HCl from other components in the sample matrix, including pharmaceutical excipients and degradation products, before detection. This is a significant advantage, especially in complex formulations and stability studies.

On the other hand, UV spectrophotometry is generally more cost-effective than HPLC due to the lower initial investment and maintenance costs associated with the instrumentation. Furthermore, UV spectrophotometric methods are typically simpler and faster to develop and perform compared to HPLC methods, which often require more extensive method optimization and analyst training. The suitability of each method depends on the specific application. UV spectrophotometry can be a suitable choice for routine quality control assays of Metformin HCl in simpler pharmaceutical formulations where high sensitivity and selectivity are not critical. In contrast, HPLC is generally preferred for more complex formulations, stability studies involving the identification and quantification of degradation products, and applications requiring higher sensitivity and selectivity, such as pharmacokinetic studies or analyses in biological matrices. The choice between these two analytical techniques involves a trade-off between sensitivity and selectivity (higher with HPLC) and cost-effectiveness and ease of use (better with UV spectrophotometry).

VI. CONCLUSION

This research has focused on the development and validation of both UV spectrophotometric and HPLC methods for the quantitative estimation of Metformin HCl in pharmaceutical formulations. The UV spectrophotometric method developed offers a simple, cost-effective, and reasonably accurate approach suitable for routine quality control analysis of Metformin HCl in less complex formulations. The validation parameters evaluated, including linearity, accuracy, precision, specificity, LOD, LOQ, and robustness, consistently met acceptable criteria.

The HPLC method developed provides a more sensitive and selective alternative for Metformin HCl estimation. Its superior separation capability makes it particularly advantageous for analyzing complex pharmaceutical formulations and for stability studies where the identification and quantification of degradation products are crucial. The HPLC method also demonstrated good linearity, accuracy, precision, and robustness, along with satisfactory system suitability parameters.

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 5, Issue 3, May 2025
Impact Factor: 7.67

Forced degradation studies were conducted to establish the stability-indicating nature of both developed methods. The results from these studies demonstrated the ability of both UV and HPLC methods to quantify Metformin HCl in the presence of its degradation products, with HPLC showing better resolution of the degradants.

REFERENCES

[1]. RavisankarP,Gowthami S,DevlalaRG.Areviewonanalyticalmethoddevelopment. Indian Journal of Research in Pharmacy and Biotechnology 2014; 2(3): 1183.
[2]. Masoom RS, Zeid AA, Nafisur R. Analytical techniques in pharmaceutical analysis:A review.Arabian Journal of Chemistry 2013; 1-7.
[3]. Rasmussen HT. Method Development. In Ahuja S, Scypinski Sa, editor. A handbook of Modern Pharmaceutical Analysis 1st edn. San Diego: Academics press; 2001.p. 2, 346, 383.
[4]. Sharma, B. K., Instrumental Methods of Chemical Analysis, 23rd edn. Meerut: Goel Publishing House; 2002. P. 7-8.
[5]. Miller JM. Chromatography Concepts & Contrasts, 2nd edn. New York: John Wiley &Sons Publication; 2004. p. 35-41.
[6]. Skoog DA, Holler FJ, Crouch SR. Principle of Instrumental Analysis, 6th edn. New Delhi: Cengage Learning India Private Limited; 2007. p. 145-47, 893-96.
[7]. Willard Hb, Merritt L, Dean J, Settle F. Instrumental methods of analysis. 7th edn.New Delhi: CBS distributors;1986. p. 625-63.
[8]. Josefsson M, Zackrisson AL, Norlander B. Sensitive high-performance liquid chromatographic analysis of amlodipine in human plasma with amperometric detection and a single-step solid-phase sample preparation. J Chromatogr B 1995; 672(2): 310-13.
[9]. Liu Y, Lee ML. Ultrahigh pressure liquid chromatography usingelevated temperature. J Chromatogr 2006; 1104(1-2): 198–202.
[10]. Skoog DA, Holler FJ, Nieman TA, Principle of Instrumental Analysis, 5th edn. Singapore: Brooks/Cole A division of Thomson Learning, Inc;1998. p. 674-85, 726- 41.
[11]. Sethi PD. High Performance Liquid Chromatography: Qualitative Analysis of Pharmaceutical Formulations 1st edn. New Delhi:CBS Publishers and Distributors; 2001. p. 116-20.
[12]. Abidi SL. High Performance Liquid Chromatography of phosphatidic acids and related polar lipids. J Chromatogr 1991; 587: 193-203.
[13]. Hearn MT. Ion-pair chromatography on normal and reversed-phase systems. Adv Chromatogr 1980; 18: 59-100.
[14]. Munson JW. High Performance Liquid Chromatography: Theory,Instrumentation, and Pharmaceutical Applications. In Munson JW, editor. Pharmaceutical Analysis- Modern Methods Part B, UK: Informa Healthcare; 2012.p. 51-73.
[15]. DayRA,Underwood AL,QuantitativeAnalysis6th Vol. NewDelhi PHILearning Pvt. Ltd. 2009; p. 1-2.
[16]. Gowthami S, Devlala RG. A review on analytical method development. IJRBP 2014; 2(3):1183.
[17]. BroadN, Graham P, Hailey P, Hardy A, Holland S, Hughes S, et al. Guidelines for the development and validation of near-infrared spectroscopic methods in the pharmaceutical industry. Chichester: John Wiley and Sons Ltd; 2002. p. 1-5.
[18]. Ashok K, Kishore L, Kaur N, Nair A. Method development and validation: Skills andtricks. Chronicles of Young Scientist 2012; 3(1): 1-5.
[19]. SnyderLR,KirlandJJ, Glajch JL. PracticalHPLCMethod Development2ndedn.New york: A Wiley-Interscience Publication, John Wiley & Sons, Inc; 1997. p. 3-4, 234-42, 25- 27, 46-53, 351-52.
[20]. Ahuja S and Michael WD. Hand book of Pharmaceutical Analysis by HPLC. Vol.6.1sted. Philadelphia: Elsevier Academic Press; 2005. p. 85-89.
[21]. Thompson M, Ellison SL, Wood R. Harmonized Guidelines for single Laboratory Validation of Method of Analysis. Pure Appl Chem2008; 74: 835-55.

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 5, Issue 3, May 2025
Impact Factor: 7.67

[22]. WoodR. HowtoValidate AnalyticalMethods.Trends Analyte Chem2005; 54: 149-58.
[23]. GeethaG,KaranamNaga,GanikaRaju,VigneshKB,RajaM.AnalyticalMethod Validation: An Updated Review. IJAPBC. 1(1): 64-71.
[24]. ValcarcelM,CardenasS,GallegoM.SampleScreeningsysteminanalyticalchemistry. Trends Analyt Chem1999; 23: 137-45.
[25]. Kallner A. 2005. Quality specification based on the uncertainty of measurement. Seand JLab Invest 2005; 59: 513-6.
[26]. Lindner W, Wainer IW. Requirements for initial assay validation and publication. J Chromatogr 2006; 707: 1-2.
[27]. Maithani M and Singh R. Development and Validation of a Stability indicating HPLC Method for the Simultaneous Determination of Salbutamol Sulphateand Theophylline in Pharmaceutical Dosage Forms. J Bioanalytical Tech 2011; 2(1): 1-5.
[28]. W. AbuDayyij, M. Hamad, E. Mallah, A. Abu Dayyih, R. Awad, Z.Zakaria And T.ArafatMethod Development And Validation Of Vildagliptin And Metformin Hcl In Pharmaceutical Dosage Form By Reverse Phase–High Performance LiquidChromatography(Rp-Hplc), International Journal of Pharmaceutical Sciences and Research 2018, 9(7): 2965-2972.
[29]. S. Pawar; J. Patel; R. Sharma; S. Khan; Dr. R. Patel, Method Development and Validation For Anti Diabetic Drugs ByRP-HPLC, International Journal of Pharmaceutical Sciences & Medicine, 2022, Vol.7(10),6-29.
[30]. M. Sha'at, A. F. Spac , I. Stoleriu, A. Bujor, M. S. Cretan, M. Hartan, L. Ochiuz, Implementationof QbD Approach to the Analytical Method Development and Validation forthe Estimation of Metformin Hydrochloride in Tablet Dosage Forms by HPLC, Pharmaceutics,2022, 14(6), 1-22.
[31]. P. B. N. Prasad, K. Satyanarayana, G. Krishnamohan. Development and Validation of a Method for Simultaneous Determination of Metformin Hydrochloride and Sitagliptin Phosphate in a Formulation by RP-HPLC. American Journal of Analytical Chemistry 05(11):737-742
[32]. Sai Lohit.Ch, Suman Pattanayak, T. Rama Rao. Development and Validation of RP-HPLC Method for the Simultaneous Estimation of Vildagliptin and Metformin In Tablet Dosage Form. American Journal of PharmTech Research, 2014; 4 (5): 138-145.
[33]. Pragati Sinha, Mukul Maurya and Manoj Kumar Mishra. Simultaneous Estimation of Sitagliptin Phosphate and Metformin Hydrochloride in Bulk and Tablet Dosage Form By FTIR Spectrophotometric. World Journal of Pharmaceutical Research, 2022; 11 (9): 1075-1084.
[34]. NSatheeshkumar,MPradeepkumar,SShanthikumar,VJRao.Developmentofvalidated stability indicating assay method for simultaneous estimation of metformin hydrochloride and vildagliptin by RP-HPLC. Drug Res (Stuttg), 2014; 64(3):124-9.
[35]. D Raju, P Karunakar, China Babu Jonnakuti and N Asha. Simultaneous estimation of vildagliptin and metformin hydrochloride by using RP-HPLC in bulk and pharmaceutical dosage form. The Pharma Innovation Journal. 2019; 8(6): 296-301.
[36]. Abdul Shakoora, Mahmood Ahmedb, Rabia Ikramc, Sajad Hussaina, Arifa Tahird , Badrul Mohamed Janc and Ahmad Adnan. Stability-Indicating RP-HPLC Method for Simultaneous Determination of Metformin Hydrochloride and Vildagliptin in Tablet and Biological Samples. Acta Chromatographica. 2020; 32 (1):39–43.
[37]. Usharani gundala, Chandra shekar Bhuvanagiri, Devanna nayakanti. Simultaneous Estimation of Vildagliptin and Metformin Hydrochloride In Bulk and Pharmaceutical Dosage Form By RP-HPLC. 2013; 3(2): 1554-1563.
[38]. Shrikrishna B. Baokar, Sugandha V. Mulgund, Nisharani S. Ranpise. Development and Validation of RP-HPLC Method for Simultaneous Estimation of Vildagliptin and Metformin. Research J. Pharma. Dosage Forms and Tech. 2013; 5(2): 95-98.
[39]. A. R. Shirode,P. D. Maduskar,M. S. Deodhar andV. J.Kadam. RP-HPLC and HPTLC Methods for Simultaneous Estimation of Metformin Hydrochloride and Vildagliptin from Bulk and Marketed

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 5, Issue 3, May 2025
Impact Factor: 7.67

Formulation: Development and Validation. British Journal of Pharmaceutical Research, 2014; 4(20):2370-2386.

[40]. K. Manohar, B. Thangabalan, S. Manohar Babu. Method Development and Validation for The Simultaneous Estimation of Vildagliptin And Metformin In Tablet Dosage Form By RP-HPLC. International Journal of Research in Pharmaceutical and Nano Sciences. 3(2), 2014, 80 – 87.

[41]. Ramesh Jayaprakash, Senthil Kumar Natesan. Stability Indicating RP-HPLC Method Development and Validation For The Simultaneous Determination of Vildagliptin And Metformin In Pharmaceutical Dosage Form. Int J Pharm Pharm Sci., 2017;9(3):150-157.

[42]. Amit Chaudhary, Bhuvnesh Kumar Singh. Method Development and Validation for simultaneous Quantification of Remogliflozin and Metformin in Bulk and Tablets by RP- HPLC. Research Journal of Pharmacy and Technology 2022; 15(10):4709-4.

[43]. Santhosha B., Sundari C.h., Ravindranath A. Validated method for the simultaneous estimation of Metformin Hydrochloride and Vildagliptin by RP-HPLC in bulk and the pharmaceutical dosage form. International Research Journal of Pharmaceutical andApplied Sciences, 2(3), 22-28.

[44]. Hanan A. Merey, Nesrin K. Ramadan, Sherine S. Diab, Azza A. Moustafa. Chromatographic methods for the simultaneous determination of binary mixture of Saxagliptin HCl and Metformin HCl. Bulletin of Faculty of Pharmacy, Cairo University, 2017; (55): 311-317.

[45]. Mahesh Attimarad, Sree Harsha Nagaraja, Bandar E. Aldhubaib, Ahmed Al-Najjar. Development of a rapid reversed phase-high performance liquid chromatography method for simultaneous determination of metformin and vildagliptin in formulation and human plasma. Journal of Young Pharmacists, 2014; 6 (4): 40-46.

[46]. https://en.wikipedia.org/wiki/Metformin

[47]. Sweetman, S.C. (Ed.). (2009). Martindale: The Complete Drug Reference. Pharmaceutical Press.

[48]. He,Y.L.,etal.Absorption,metabolismandexcretionofvildagliptin,anoveldipeptidyl peptidase 4 inhibitor, in humans. Xenobiotica, 2012; 42(2): 208-219.

[49]. Martindale:TheCompleteDrugReference.(2009).Sweetman,S.C.(Ed.).Pharmaceutical Press.

[50]. DrugBank."Vildagliptin".CanadianInstitutesofHealthResearch,2024.DrugBank.

[51]. Sherif Mohamed Eid, Shymaa S. Soliman, Mohamed Refaat Elghobashy. ATR-FTIR coupled with Chemometrics for quantification of vildagliptin and metformin in pharmaceutical combinations having diverged concentration ranges. Vibrational Spectroscopy,2020; 106(C):102995

[52]. Dr.Safila Naveed, Hina Rehman, Fatima Qamar. Method development and validation of Vildagliptin using UV spectrophotometer. International Journal of Pharma Sciences and Research, 2014; 5(10): 714-717.

[53]. Y.D. Dange, Sandip Honmane, Somnath Bhinge, Development and Validation of UV- Spectrophotometric Method for Estimation of Metformin in Bulk and Tablet Dosage Form. Indian Journal of Pharmaceutical Education and Research, 2017; 51 (4S): S764-S760.

[54]. B.D.Musmade,S.G.Lokhande,M.S.Sable,R.R.Korhale,S.G.Bhope,M.Nagarand

[55]. K. S. Lohar. Impurity Profiling Method Development And Validation For Metformin Hcl and Vildagliptin From Combination Tablet Dosage Form By Rp-Hplc Coupled WithUv/Pda Detector. IJPSR, 2024; Vol. 15(2): 460-467.

[56]. Mahesh Attimarad, Anroop B. Nair, Nagaraja Sreeharsha, Bandar E. Al-Dhubiab, Katharigatta N. Venugopala and Pottathil Shinu. Development and Validation of Green UV Derivative Spectrophotometric Methods for Simultaneous Determination Metformin and Remogliflozinfrom Formulation:Evaluationof Greenness. Int J Environ ResPublicHealth, 2021;18(2): 448.

[57]. Ranjan Pragati, Satpathy, Mohan Goud. Development and validation of RP-HPLC method for the assay of Vildagliptin. World Journal of Pharmacy and Pharmaceutical Sciences, 2014; 3(2): 2303-2310.

[58]. K. Hanumantha Rao, A. Lakshmana Rao and KB. Chandra Sekhar. Development and Validation of Hplc Method for the Estimation of Vildagliptin In Pharmaceutical Dosage Form. International Journal of Pharmaceutical, Chemical and Biological Sciences. IJPCBS 2014, 4(2), 361-366.

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 5, Issue 3, May 2025
Impact Factor: 7.67

[59]. Subhakar Nandipati, Ravindranadh Reddy T, Krishna Reddy v. Development andValidation of RP-HPLC method for Simultaneous Determination of Vildagliptin and Metformin in Bulk and Formulation Dosage. International journal of pharmaceutical and applied sciences, 2012; 2(3): 1-12