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Development and Validation of A RP-HPLC-PDA Method for Simultaneous Determination of Linagliptin and Metformin in Pharmaceutical Dosage Form

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Abstract: A simple, accurate, precise, and stability-indicating reverse phase high-performance liquid chromatographic (RP-HPLC) method with photodiode array (PDA) detection was developed and validated for the simultaneous determination of Linagliptin and Metformin in pharmaceutical dosage forms. Chromatographic separation was achieved using a C18 column with an optimized mobile phase composition under isocratic conditions at a suitable flow rate. Detection was carried out using a PDA detector at an optimized wavelength, providing well-resolved and symmetrical peaks for both analytes within a reasonable run time.

The developed method was validated in accordance with ICH Q2(R1) guidelines. The method demonstrated good specificity with no interference from excipients. Linearity was observed over the selected concentration ranges with correlation coefficients close to unity. The method showed satisfactory accuracy, with percentage recovery within acceptable limits. Precision studies indicated low %RSD values, confirming repeatability and intermediate precision. The limits of detection (LOD) and quantification (LOQ) indicated adequate sensitivity of the method. Robustness studies revealed that small deliberate variations in chromatographic conditions did not significantly affect the results.

The validated method was successfully applied to the quantitative analysis of Linagliptin and Metformin in marketed tablet formulations. The results were found to be within acceptable limits of the labelled claim, confirming the suitability of the method for routine quality control analysis.

Keywords: RP-HPLC; PDA detector; Linagliptin; Metformin; Method development; Method validation; Simultaneous estimation; Pharmaceutical dosage form; ICH guidelines; Stability-indicating method.

1. INTRODUCTION

The recent advancements in RP-HPLC techniques, focusing on their application in the estimation and validation of pharmaceutical dosage forms. [1] High-performance Liquid Chromatography (HPLC) is a cornerstone in pharmaceutical analysis, with Reverse phase HPLC (RP-HPLC) being the most widely applied variant due to its versatility, reproducibility, and high resolution. As the demand for accurate and reliable drug quantification in varied dosage forms continues to grow, the development and refinement of RP-HPLC techniques have become essential. These advancements not only address the increasing complexity of modern pharmaceutical formulations but also ensure regulatory compliance, therapeutic efficacy, and patient safety. [2] By highlighting methodological improvements, analytical

challenges, and emerging trends, this work underscores the critical role of RP-HPLC in modern pharmaceutical analysis. Special attention is given to the application of RP-HPLC in various dosage forms such as tablets, capsules, injectables, and complex drug delivery systems, as well as its adaptability to regulatory frameworks and quality control practices.[3] Through this exploration, the review underscores RP-HPLC's pivotal role in ensuring drug safety, efficacy, and regulatory compliance across the pharmaceutical industry. Modern RP-HPLC techniques are characterized by enhanced sensitivity, reduced analysis time, and improved reproducibility. Innovations such as core-shell particles, ultra-high performance liquid chromatography (UHPLC), and the integration of diode-array and mass spectrometric detectors have significantly bolstered the capabilities of RP-HPLC.[4] These developments have made it possible to perform accurate estimation of active pharmaceutical ingredients (APIs), excipients, and impurities even in challenging matrices such as fixed-dose combinations, extended-release formulations, and biological samples. Method validation, as defined by International Council for Harmonisation (ICH) guidelines, is integral to RP-HPLC method development. Validation parameters including linearity, accuracy, precision, specificity, robustness, and detection limits must be rigorously established to ensure the analytical www.wjpr.net | Vol 14, Issue 11, 2025. | ISO 9001: 2015 Certified Journal | Pal et al. World Journal of Pharmaceutical method's reliability.[5] Modern validation strategies incorporate statistical tools and risk-based approaches, which help in achieving robust quality assurance in pharmaceutical analysis. Moreover, RP-HPLC continues to evolve in alignment with green analytical chemistry principles, emphasizing reduced solvent consumption, environmentally benign mobile phases, and miniaturized systems. The emergence of software-assisted method development, such as Quality by Design (QBD) approaches, has further revolutionized the analytical landscape by allowing the systematic optimization of chromatographic conditions with minimal experimental trials. [6]

2.1 Fundamental Principles of RP-HPLC

Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) is a widely adopted chromatographic technique used in pharmaceutical analysis for the separation, identification, and quantification of compounds, particularly those with moderate to low polarity. The technique operates on the principle of partitioning between a polar mobile phase and a nonpolar stationary phase, making it particularly suitable for the analysis of hydrophobic and moderately polar substances. [7] In RP-HPLC, the stationary phase is typically composed of silica particles bonded with non-polar groups, most commonly C18 (octadecyl), although shorter alkyl chains like C8 or phenyl groups may also be used depending on the application. The mobile phase, in contrast, is relatively polar and usually consists of water or aqueous buffers in combination with organic solvents such as methanol or acetonitrile. [8] The polarity contrast between the two phases enables the effective separation of analytes based on their hydrophobic interactions with the stationary phase. The retention of compounds in RP-HPLC is governed by their hydrophobicity: more hydrophobic molecules interact strongly with the stationary phase and elute later, while more polar molecules elute faster. Adjusting the mobile phase composition, pH, flow rate, and temperature allows fine-tuning of retention times and peak resolution, making the technique highly flexible.[9] Detection in RP-HPLC is commonly performed using UV-Vis detectors, although other detection systems such as photodiode array (PDA), fluorescence, or mass spectrometry (MS) may be employed for enhanced sensitivity or structural identification-HPLC is distinguished by its high resolution, reproducibility, and efficiency, which are critical for meeting stringent regulatory requirements in pharmaceutical analysis. Its adaptability for analysing a wide range of dosage forms and chemical entities makes it a cornerstone technique in quality control, stability testing, and bioanalytical studies. [10, 11]

2.2 Chromatographic Performance Parameters

RP-HPLC methods are characterized by several essential chromatographic parameters that determine their efficiency and reliability. One fundamental measure of separation quality is the resolution between two peaks, which indicates the degree of distinction between them with minimal or no overlap. A resolution value of ≥ 1.5 is typically considered sufficient for accurate compound identification. [12] Another critical parameter is the number of theoretical plates, which reflects column efficiency; higher values indicate better separation, with values above 2000 generally required for high-quality analytical results. The selectivity factor measures how effectively two closely eluting compounds can be distinguished, and values greater than 1.2 suggest satisfactory separation. Peak symmetry, assessed by the asymmetry factor, is also crucial for accurate quantification. Ideally, asymmetry values should range between 0.8 and 1.5, as significant tailing or fronting can compromise analytical accuracy. [13]

3. Mobile Phase Selection and Optimization

The selection and optimization of the mobile phase are crucial for achieving effective separation in RP-HPLC. The mobile phase must be compatible with both the analyse and the stationary phase to ensure adequate solubility, retention, and resolution. [16]Typically, mixtures of water (or aqueous buffers) and organic solvents like acetonitrile or methanol are used. The pH of the mobile phase can significantly influence analyte ionization, retention time, and peak shape, especially for compounds with ionisable functional groups. Buffer systems such as phosphate or acetate buffers are commonly employed to maintain a stable pH. [17] The proportion of organic solvent is optimized to balance retention time and resolution higher organic content usually reduces retention time but may compromise separation. Gradient elution, where the mobile phase composition is varied during the run, is often used for complex mixtures to improve peak separation and reduce analysis time. Careful optimization of these parameters enhances reproducibility, sensitivity, and overall chromatographic performance. [18]

2.4 Column Selection and Temperature Control

The choice of column in RP-HPLC significantly influences separation efficiency, selectivity, and reproducibility. Columns are typically packed with silica-based stationary phases modified with hydrophobic groups, such as C18 (octadecyl), C8, or phenyl groups, depending on the polarity and chemical nature of the analyses. C18 columns are the most widely used due to their strong hydrophobic interactions and broad applicability. [19] The particle size and pore size of the column packing material also affect resolution and backpressure; smaller particles (e.g., 3–5 µm) offer higher efficiency but may require higher system pressure. In addition to column selection, temperature control plays a vital role in chromatographic performance. Elevated column temperatures can reduce mobile phase viscosity, improve mass transfer, and lead to sharper peaks with shorter retention times. [20] However, excessive temperatures may compromise column stability and analyse integrity. Typically, column temperatures are maintained between 25–40°C for most analyses, with optimization based on the thermal sensitivity of the analyse and desired resolution.

EXPERIMENTAL METHODS

INSTRUMENTS USED

HPLC	WATERS Alliance 2695 separation Module, Software: Empower 2, 996 PDA detector.
pH meter	Lab India
Weighing machine	Sartorius
Volumetric flasks	Borosil
Pipettes and Burettes	Borosil
Beakers	Borosil
Digital ultra sonicator	Lab man

CHEMICALS USED:

Linagliptin	Tradjenta
Metformin	Glucophage
Methanol for HPLC	LICHROSOLV (MERCK)
Acetonitrile for HPLC	Merck

HPLC METHOD DEVELOPMENT:

TRAILS

Preparation of standard solution:

Accurately weigh and transfer 10 mg of Linagliptin and Metformin working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 2.25ml of the above Linagliptin and 0.45ml of the Metformin stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure:

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization:

Initially the mobile phase tried was Methanol: Water, Acetonitrile: Water with varying proportions. Finally, the mobile phase was optimized to Acetonitrile: Phosphate Buffer in proportion (35:65) v/v respectively.

Optimization of Column:

The method was performed with various columns like C18 column, X- bridge column, Altima C18, 150mm x 4.6 mm, 5µ was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

VALIDATION

PREPARATION OF MOBILE PHASE:

Preparation of mobile phase:

Accurately measured 350ml (35%) of HPLC Acetonitrile and 650ml of Phosphate Buffer (65%) were mixed and degassed in a digital ultra sonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION

(Optimized chromatogram):

Column	:	Altima C18, 150mm x 4.6 mm, 5µ
Column temperature	:	35˚C
Wavelength	:	210nm
Mobile phase ratio	:	Acetonitrile: Phosphate Buffer (35:65 v/v)
Flow rate	:	1ml/min
Injection volume	:	10µl
Run time	:	7 minutes

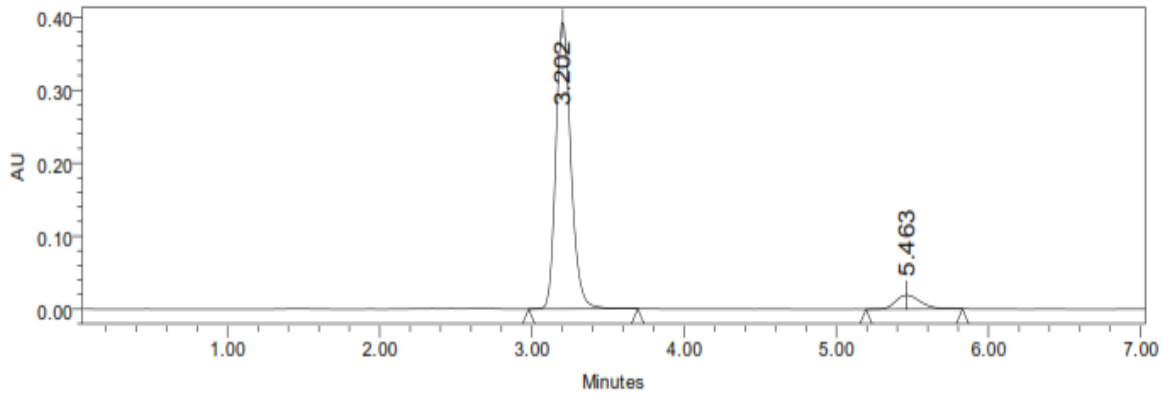


Figure: Optimized Chromatogram (Standard)

Table: Optimized Chromatogram (Standard)

S. No	Name	RT	Area	Height	USP Tailing	USP Plate Count	USP Resolution
1	Linagliptin	3.202	2391746	39726	1.2	9028	
2	Metformin	5.463	194627	8497	1.1	7398	7.4

Observation:

This trial shows improper separation sample peaks, baseline and show very less plate count in the chromatogram. So, it's required more trials to obtain good peaks.

From the above chromatogram it was observed that the Linagliptin and Metformin peaks are well separated and they show proper retention time, resolution, peak tail and plate count. So it's optimized trial.

Optimized Chromatogram (Sample)

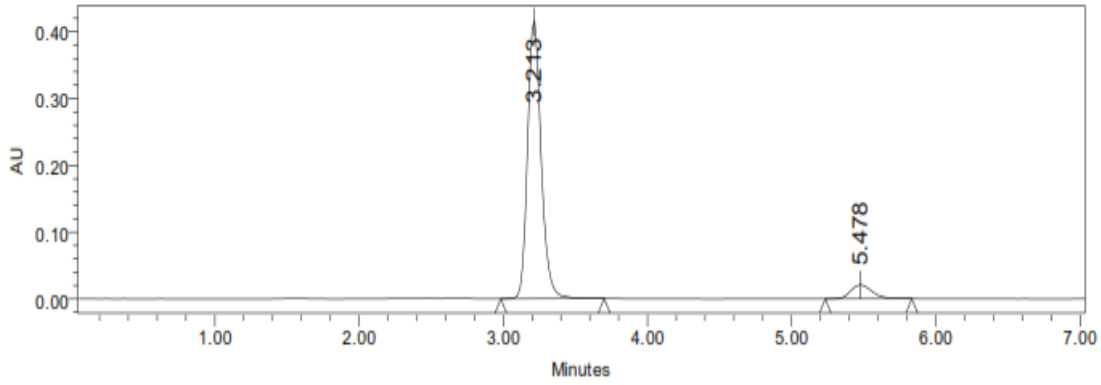


Table: Optimized Chromatogram (Sample)

S. No	Name	RT	Area	Height	USP Tailing	USP Plate Count	USP Resolution
1	Linagliptin	3.213	2381649	391846	1.2	9472	
2	Metformin	5.478	191057	8104	1.1	8936	7.5

Acceptance criteria:

Resolution between two drugs must be not less than 2

Theoretical plates must be not less than 2000

Tailing factor must be not less than 0.9 and not more than 2.

It was found from above data that all the system suitability parameters for developed method were within the limit.

VALIDATION

Blank:

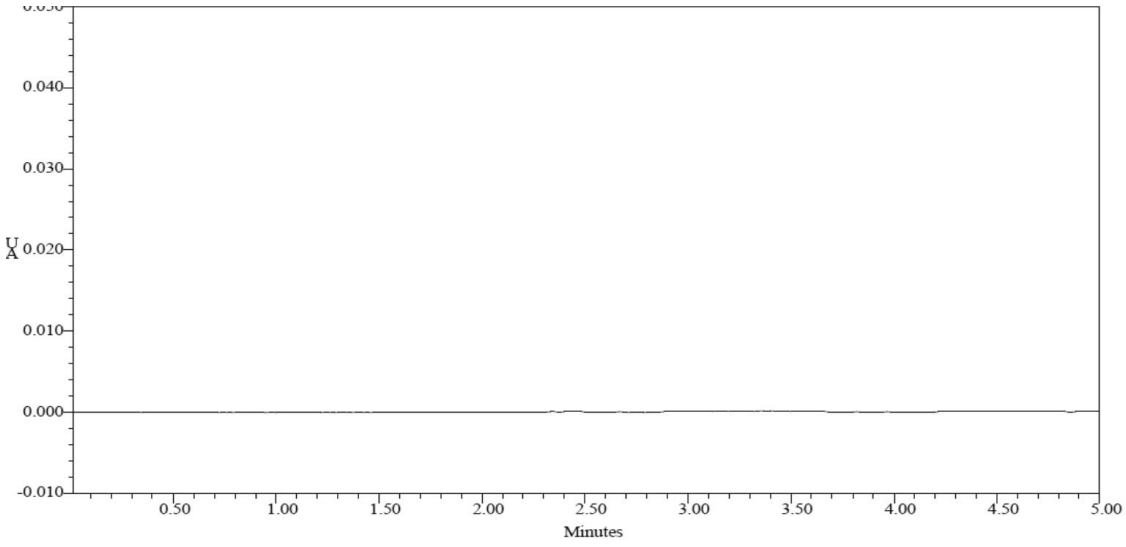


Fig: Chromatogram showing blank (mobile phase preparation)

System suitability:

Table: Results of system suitability for Linagliptin

S. No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Linagliptin	3.200	2391746	394171	8952	1.2
2	Linagliptin	3.248	2391647	381946	9561	1.2
3	Linagliptin	3.299	2381647	391746	6572	1.2
4	Linagliptin	3.297	2385631	386562	6452	1.2
5	Linagliptin	3.297	2385635	389164	7452	1.2
Mean			2387261			
Std. Dev.			4363.771			
% RSD			0.182794			

Acceptance criteria:

% RSD of five different sample solutions should not more than 2
The % RSD obtained is within the limit, hence the method is suitable.

Table: Results of system suitability for Metformin

S. No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Metformin	5.413	198362	7917	5272	1.1
2	Metformin	5.484	197486	7486	6291	1.1
3	Metformin	5.405	198354	7859	6184	1.1
4	Metformin	5.405	197352	7926	7145	1.1
5	Metformin	5.409	198453	7946	6946	1.1
Mean			198001.4			
Std. Dev.			535.1774			
% RSD			0.27029			

Acceptance criteria:

% RSD of five different sample solutions should not more than 2
The % RSD obtained is within the limit, hence the method is suitable.

SPECIFICITY

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components.

Analytical method was tested for specificity to measure accurately quantitate Linagliptin and Metformin in drug product.

Assay (Standard):

Table: Peak results for assay standard

Linagliptin

S. No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Linagliptin	3.211	2397162	397161	1.2	9472
2	Linagliptin	3.222	2394721	389173	1.2	9745
3	Linagliptin	3.254	2389461	391723	1.2	8917

Metformin

S. No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Resolution
1	Metformin	5.414	198462	7811	1.1	8492	7.49
2	Metformin	5.453	198472	8193	1.1	8916	7.52
3	Metformin	5.424	198735	7972	1.1	9372	7.44

Assay (Sample):

Table: Peak results for Assay sample

Linagliptin

S. No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Linagliptin	3.297	2391741	381612	1.2	9472
2	Linagliptin	3.294	2389166	391746	1.2	8927
3	Linagliptin	3.295	2361731	381634	1.2	9017

Metformin

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Resolution
1	Metformin	5.435	198641	8174	1.1	9284	7.18
2	Metformin	5.417	196547	8942	1.1	8974	7.44
3	Metformin	5.434	194027	7294	1.1	9017	7.38

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

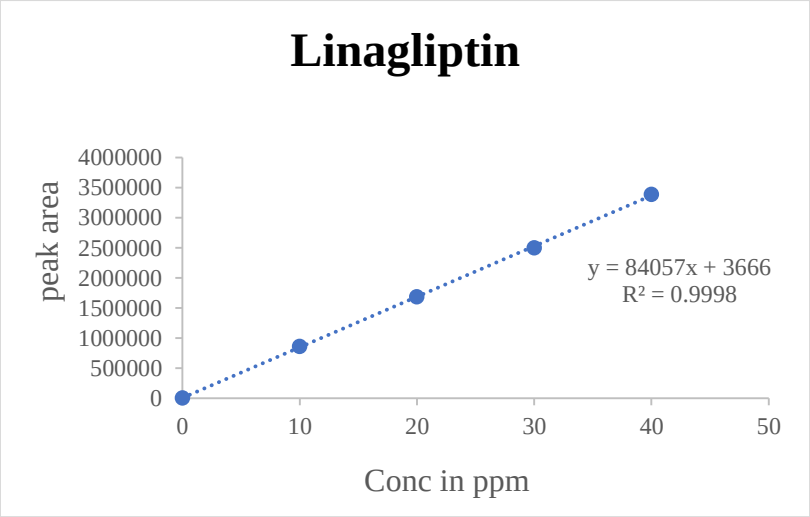
The % purity of Linagliptin and Metformin in pharmaceutical dosage form was found to be 99.2%.

LINEARITY

CHROMATOGRAPHIC DATA FOR LINEARITY STUDY:

Linagliptin

Concentration	Average
µγ/µλ	Peak Area
0	0
10	859889
20	1683641
30	2495378
40	3385089



LINEARITY PLOT:

The plot of Concentration (x) versus the Average Peak Area (y) data of Linagliptin is a straight line.

Y = mx + c Slope (m) =11208

Intercept (c) = 3666

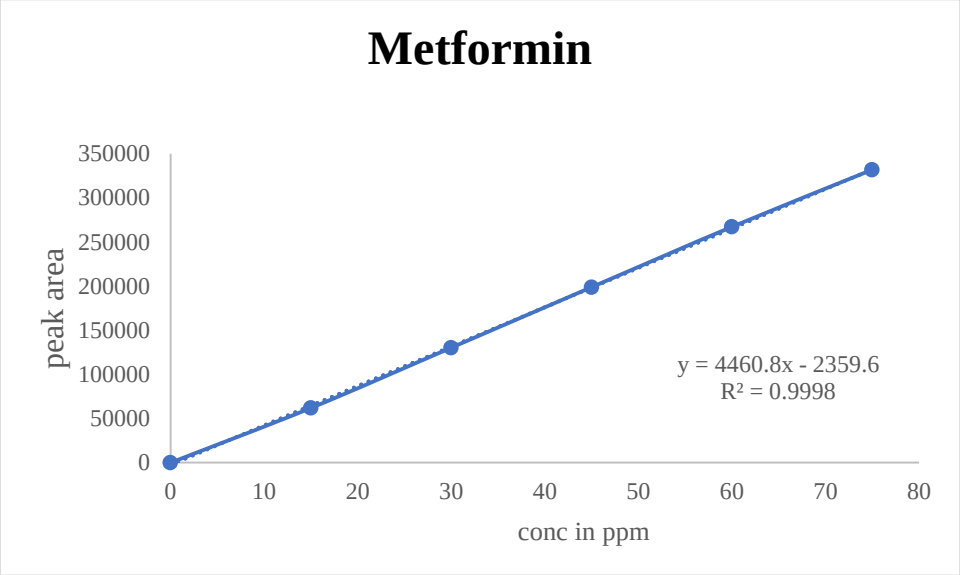
Correlation Coefficient (r) = 0.9998

VALIDATION CRITERIA: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

CONCLUSION: Correlation Coefficient (r) is 0.99, and the intercept is 48956. These values meet the validation criteria.

Metformin

Concentration	Average
μγ/μλ	Peak Area
0	0
15	61953
30	130213
45	198697
60	267002
76	331658



LINEARITY PLOT:

The plot of Concentration (x) versus the Average Peak Area (y) data of Metformin is a straight line.

Y = mx + c

Slope (m) = 4460.8

Intercept (c) = 2359.6

Correlation Coefficient (r) = 0.9998

VALIDATION CRITERIA: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

CONCLUSION: Correlation Coefficient (r) is 0.99, and the intercept is 454.8. These values meet the validation criteria.

Precision:

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

REPEATABILITY

Obtained Five (5) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

Table: Results of repeatability for Linagliptin:

S. No	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Linagliptin	3.213	2397164	381741	8155	1.2
2	Linagliptin	3.253	2391741	371742	9174	1.2
3	Linagliptin	3.297	2371846	391746	7154	1.2
4	Linagliptin	3.215	2361748	391847	9917	1.2
5	Linagliptin	3.254	2371649	384622	9247	1.2
Mean			2378830			
Std.dev			14958			
%RSD			0.628797			

Acceptance criteria:

% RSD for sample should be NMT 2
The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Table: Results of repeatability for Metformin:

S. No	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Metformin	5.441	198464	7291	6274	1.1
2	Metformin	5.442	193643	7219	6592	1.1
3	Metformin	5.409	196462	7194	6028	1.1
4	Metformin	5.520	194644	8174	6927	1.1
5	Metformin	5.424	198464	8653	5920	1.1
Mean			196335.4			
Std.dev			2190.191			
%RSD			1.115536			

Intermediate precision:

Day 1:

Table: Results of Intermediate precision for Linagliptin

S. No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USPTailing
1	Linagliptin	3.211	2389572	395275	9375	1.2
2	Linagliptin	3.211	2391847	392175	9275	1.2
3	Linagliptin	3.210	2319472	312947	8265	1.2
4	Linagliptin	3.212	2306842	310585	6254	1.2
5	Linagliptin	3.211	2375972	310694	9028	1.2
6	Linagliptin	3.297	2396746	358373	8928	1.2
Mean			2363409			
Std. Dev.			39730.83			
% RSD			1.681082			

Acceptance criteria:

% RSD of six different sample solutions should not more than 2

Table: Results of Intermediate precision for Metformin

S. No	Peak Name	RT	Area (µV*sec)	Height (µV)	USPPlate count	USPTailing
1	Metformin	5.411	197284	7194	8264	1.2
2	Metformin	5.410	197849	7294	9174	1.2
3	Metformin	5.420	196572	7147	9164	1.2
4	Metformin	5.423	195028	7927	9733	1.2
5	Metformin	5.419	199474	8238	9194	1.2
6	Metformin	5.409	197482	7638	8973	1.2
Mean			197281.5			

Std. Dev.			1466.354			
% RSD			0.74328			

Acceptance criteria:

% RSD of six different sample solutions should not more than 2

Day 2:

Table: Results of Intermediate precision Day 2 for Linagliptin

S. No	peak Name	RT	Area (µV*sec)	Height (µV)	USPPlate Count	USPTailing
1	Linagliptin	3.211	2389562	391741	9264	1.2
2	Linagliptin	3.233	2381654	391047	9746	1.2
3	Linagliptin	3.244	2381946	391748	9816	1.2
4	Linagliptin	3.297	2391741	391746	9917	1.2
5	Linagliptin	3.297	2386452	381641	9742	1.2
6	Linagliptin	3.202	2374763	381645	9017	1.2
Mean			2384353			
Std. Dev.			6183.339			
% RSD			0.25933			

Acceptance criteria:

% RSD of six different sample solutions should not more than 2

Table: Results of Intermediate precision Day 2 for Metformin

S. No	Peak Name	RT	Area (µV*sec)	Height (µV)	USPPlate Count	USPTailing
1	Metformin	5.411	197486	7582	6272	1.1
2	Metformin	5.410	197486	7184	6174	1.1
3	Metformin	5.420	196746	7456	5184	1.1
4	Metformin	5.405	195862	7814	6194	1.1
5	Metformin	5.409	196582	7194	6292	1.1
6	Metformin	5.463	198463	7745	6191	1.1
Mean			197104.2			
Std. Dev.			903.542			
% RSD			0.458408			

Acceptance criteria:

% RSD of six different sample solutions should not more than 2

ACCURACY:

Accuracy at different concentrations (50%, 100%, and 150%) were prepared and the % recovery was calculated.

The accuracy results for Linagliptin

% Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	1217218	112.5	112.4	99.6	99.3
100%	2397141	225	225	100	
150%	3514547	337.5	332.5	98.5	

Acceptance Criteria:

The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

The accuracy results for Metformin

% Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery

50%	98598.67	22.5	22.4	99.9	99.6
100%	198359.7	45	45	100	
150%	291512.3	67.5	66.8	99	

Acceptance Criteria:

The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

LIMIT OF DETECTION

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

$$LOD = 3.3 \times \sigma / s$$

Were

σ = Standard deviation of the response

S = Slope of the calibration curve

Linagliptin:

Result:

=3.3×39762/10421

=12.5µg/ml

Metformin:

Result:

=3.3×5008/4365

=3.7µg/ml

Quantitation limit

The quantitation limit of an individual analytical procedure is the lowest amount of analyze in a sample which can be quantitatively determined.

$$LOQ=10\times\sigma/S$$

Were

σ = Standard deviation of the response

S = Slope of the calibration curve

Linagliptin:

Result:

=10×39762/10421

=38.1µg/ml

Metformin:

Result:

=10×5008/4365

=11.4µg/ml

ROBUSTNESS

The robustness was performed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Linagliptin and Metformin. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%. The standard sample of Linagliptin and Metformin were injected by changing the conditions of

chromatography. There was no significant change in the parameters like resolution, tailing factor and plate count.

Variation in flow

Table: Results for Robustness

Linagliptin

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0mL/min	2391746	3.202	9028	1.2
Less Flow rate of 0.9mL/min	2371831	3.639	7381	1.2
More Flow rate of 1.1mL/min	2218319	2.859	9311	1.1
Less organic phase (about 5 % decrease in organic phase)	2294821	3.460	7462	1.2
More organic phase (about 5 % Increase in organic phase)	2394811	3.022	6817	1.1

Acceptance criteria:

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

Table: Results for Robustness

Metformin

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.1mL/min	194627	5.463	7398	1.1
Less Flow rate of 0.9mL/min	183738	6.250	6883	1.1
More Flow rate of 0.8mL/min	198373	4.863	9917	1.2
Less organic phase (about 5 % decrease in organic phase)	178471	6.196	8372	1.1
More organic phase (about 5 % Increase in organic phase)	189462	5.010	7716	1.2

Acceptance criteria:

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

SUMMARY AND CONCLUSION

SUMMARY

The present study was aimed at developing and validating a simple, accurate, precise, and reliable RP-HPLC-PDA method for the simultaneous estimation of Linagliptin and Metformin in pharmaceutical dosage forms.

A reverse-phase high-performance liquid chromatographic method was successfully developed using an appropriate C18 column with a suitable mobile phase composition, optimized flow rate, and detection wavelength using a photodiode array (PDA) detector. The chromatographic conditions were optimized to obtain well-resolved, sharp, and symmetrical peaks for both drugs within a reasonable run time.

The developed method was validated according to ICH Q2 (R1) guidelines. The validation parameters demonstrated that:

- The method was specific, with no interference from excipients or degradation products.
- The method showed excellent linearity within the selected concentration range, with correlation coefficients close to 1.
- The accuracy of the method was confirmed by recovery studies, showing percentage recoveries within acceptable limits (98–102%).

- The method exhibited good precision, as indicated by low %RSD values for both intra-day and inter-day studies.
- The LOD and LOQ values indicated adequate sensitivity of the method.
- The method proved to be robust, as minor variations in chromatographic conditions did not significantly affect the results.
- System suitability parameters such as theoretical plates, resolution, and tailing factor were found within acceptable limits.

The validated method was successfully applied for the assay of Linagliptin and Metformin in marketed tablet formulations, and the assay results were found to be within acceptable limits of the labelled claim.

CONCLUSION

The developed RP-HPLC-PDA method for the simultaneous determination of Linagliptin and Metformin in pharmaceutical dosage forms is simple, rapid, accurate, precise, sensitive, and cost-effective.

The method complies with ICH validation requirements and demonstrates good specificity, linearity, accuracy, precision, robustness, and system suitability. Furthermore, the method is capable of separating the drugs from their degradation products, confirming its stability-indicating nature.

Therefore, the proposed method can be effectively employed for routine quality control analysis of Linagliptin and Metformin in bulk drugs and combined tablet dosage forms in pharmaceutical industries.

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