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RP-HPLC Method Validation, Greenness Evaluation and UV-Based Forced Degradation Studies of Metformin Hydrochloride and Vildagliptin

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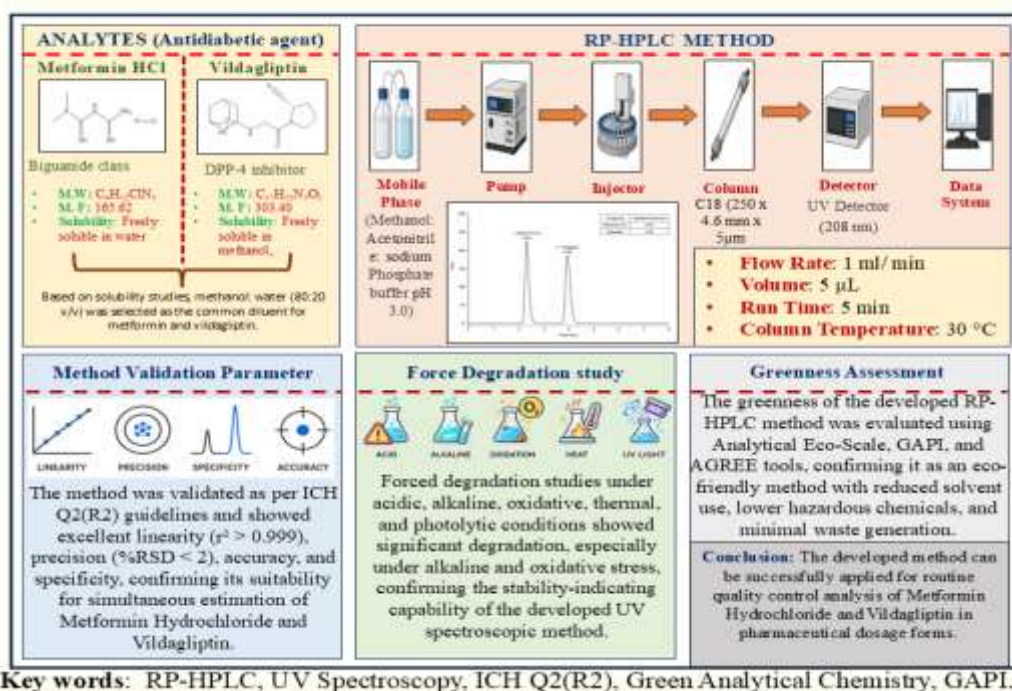
ABSTRACT:

An easy, sensitive, accurate, strong, and environmentally friendly RP-HPLC technique was established and validated for the concurrent measurement of Metformin Hydrochloride and Vildagliptin in drug formulations, including forced degradation analyses through UV spectroscopy. Chromatographic examination was conducted on a ZORBAX SB-C18 column utilizing phosphate buffer: methanol: acetonitrile (70:10:20 v/v/v, pH 3) as the mobile phase at a flow rate of 1.0 mL/min, with detection at 208 nm. Metformin Hydrochloride and Vildagliptin were released at retention times of 2.454 minutes and 3.750 minutes, respectively. The technique demonstrated outstanding linearity within concentration ranges of 50–150 µg/mL for Metformin Hydrochloride and 5–15 µg/mL for Vildagliptin, featuring correlation coefficients of 0.9999 and 0.9991, respectively. Validation according to ICH guidelines demonstrated acceptable linearity, accuracy, precision, robustness, specificity, LOD, and LOQ. The LOD/LOQ values for Metformin Hydrochloride were 1.15/3.50 µg/mL, while for Vildagliptin, they were 0.38/1.15 µg/mL. Studies on forced degradation in acidic, alkaline, oxidative, thermal, and photolytic environments showed considerable degradation of both medications, validating the method's stability-indicating properties. The assessment of greenness through AGREE, GAPI, and Analytical Eco-Scale confirmed its ecological profile by lowering solvent use and having a minimal environmental footprint. The technique is appropriate for standard quality control assessment of Metformin Hydrochloride and Vildagliptin in drug dosage forms.

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INTRODUCTION:

Diabetes mellitus is a chronic metabolic disease characterized by persistent hyperglycemia carried on by problems with insulin action, release, or both. About 90–95% of cases of diabetes are type 2 diabetes mellitus (T2DM), which is associated with insulin resistance, decreased insulin production, obesity, inactivity, genetics, and unhealthy eating patterns. Effective glycemic management is crucial since uncontrolled blood glucose levels can lead to serious consequences such as nephropathy, neuropathy, retinopathy, cardiovascular problems, and stroke..[1,2]

Combination therapy is widely used in T2DM management to target multiple pathophysiological mechanisms. Among the available combinations, Metformin Hydrochloride and Vildagliptin are extensively prescribed due to their complementary mechanisms of action and enhanced therapeutic efficacy[3]. Metformin, a biguanide, reduces hepatic glucose production, improves insulin sensitivity, and decreases intestinal glucose absorption[4], whereas Vildagliptin, a DPP-4 inhibitor,[5] enhances glucose-dependent insulin secretion and suppresses glucagon release by preventing incretin degradation.

Simultaneous estimation of combined dosage forms is essential for ensuring the quality, safety, efficacy, and stability of pharmaceutical products. High-Performance Liquid Chromatography (HPLC)[6–8], particularly Reverse-Phase HPLC (RP-HPLC), is widely employed in pharmaceutical analysis because of its excellent separation efficiency, sensitivity, accuracy, and reproducibility. UV spectroscopy is also commonly used due to its simplicity, rapidity, cost-effectiveness, and suitability for quantitative analysis and degradation studies.[9,10] Method validation is a critical step in analytical method development to ensure reliability and consistency of results. According to ICH guidelines, validation parameters such as system suitability, linearity, accuracy, precision, specificity, robustness, limit of detection (LOD), and limit of quantification (LOQ) are evaluated to confirm the method's suitability for intended analytical applications and routine quality control analysis.[11] Forced degradation studies are important for evaluating the intrinsic stability of pharmaceutical compounds under stress conditions such as acidic, alkaline, oxidative, thermal, and photolytic degradation[12]. These studies aid in identifying degradation pathways and developing stability-indicating analytical methods as recommended by ICH guidelines.[13]

Growing environmental concerns have encouraged the adoption of Green Analytical Chemistry (GAC) principles in pharmaceutical analysis. Green analytical methods aim to reduce solvent consumption, hazardous reagent

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usage, waste generation, and environmental impact while maintaining analytical performance[14]. Greenness assessment tools such as Analytical Eco-Scale, GAPI, and AGREE are commonly used to evaluate the environmental sustainability of analytical procedures.[15–18]

The present study focuses on the development and validation of a simple, rapid, accurate, precise, robust, stability-indicating, and eco-friendly RP-HPLC method for the simultaneous estimation of Metformin Hydrochloride and Vildagliptin in pharmaceutical dosage forms. The method was validated according to ICH guidelines, subjected to forced degradation studies using UV spectroscopy, and evaluated for environmental sustainability using Analytical Eco-Scale, GAPI, and AGREE assessment tools.

MATERIALS AND METHODS:

Materials:

Metformin HCl and Vildagliptin were procured from Dhamtec Pharma and Consultants, Navi Mumbai, India. HPLC-grade methanol and acetonitrile were obtained from ANA Lab Fine Chemicals. Sodium phosphate buffer was prepared using analytical-grade reagents from the same supplier. All chemicals used were of HPLC grade for chromatographic analysis and analytical grade for other experimental work.

Instrumentation and Chromatographic Conditions:

The analysis was performed using a Waters Alliance 2695 HPLC system equipped with an integrated autosampler and UV detector, with data acquisition and processing carried out using Empower 2 software. Separation was achieved on an Agilent ZORBAX SB-C18 column (250 × 4.6 mm, 5 µm). An Equiptronics Digital pH Meter (Model EQ-610) was used for pH adjustment of the mobile phase.

The chromatographic method was optimized through several trials employing different combinations of methanol, acetonitrile, and buffer to obtain suitable resolution, peak symmetry, and retention times for the simultaneous estimation of Metformin HCl and Vildagliptin. The optimum separation was achieved using a mobile phase comprising 0.01 M sodium phosphate monobasic anhydrous buffer (pH 3), methanol, and acetonitrile in the ratio of 70:10:20 (v/v/v). The analysis was carried out at a flow rate of 1.0 mL/min with UV detection at 208 nm. These optimized conditions provided satisfactory peak resolution, good peak symmetry, and appropriate retention times for both analytes.

Selection of Analytical Wavelength, Standard and Sample Preparation:

For HPLC analysis, Metformin HCl and Vildagliptin were individually evaluated at different wavelengths. Metformin HCl showed suitable absorbance at 235 nm, while Vildagliptin exhibited good absorbance at 210 nm. Analysis of the drug mixture at dual wavelengths (210/235 nm) did not produce satisfactory peaks. Further evaluation at 210/208 nm showed that 208 nm provided sharp, well-resolved, and accurate peaks for both drugs; therefore, 208 nm was selected as the optimized wavelength for simultaneous estimation.

Standard Preparation:

Stock solutions of Metformin HCl and Vildagliptin were prepared separately by dissolving 25 mg of each drug in Methanol:Water (80:20, v/v) and diluting to 50 mL to obtain concentrations of 500 ppm. Working standard solutions were prepared by diluting 10 mL of Metformin HCl stock solution to 50 mL to obtain 100 ppm, and 1 mL of Vildagliptin stock solution to 50 mL to obtain 10 ppm.

Sample Preparation:

Twenty tablets containing Metformin HCl and Vildagliptin were accurately weighed, powdered, and a quantity equivalent to 500 mg of Metformin HCl and 50 mg of Vildagliptin was transferred to a 100 mL volumetric flask. About 70 mL of Methanol:Water (80:20, v/v) was added, and the mixture was sonicated for 15–20 minutes for complete drug extraction. After cooling, the volume was adjusted to 100 mL with the same diluent and filtered through a 0.45 µm nylon membrane filter. An aliquot of 2 mL of the filtrate was further diluted to 10 mL with the diluent to obtain the final sample solution containing 100 µg/mL (100 ppm) of Metformin HCl and 10 µg/mL (10 ppm) of Vildagliptin.

Validation of the Developed RP- HPLC Method:

The optimized RP- HPLC method was validated according to the following parameters:[11]

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1. Linearity

Linearity was evaluated using five concentration levels prepared in diluent. Metformin HCl was studied over the range of 50–150%, and Vildagliptin over 5–15% of the target concentration. Each level was injected in triplicate, and calibration curves were constructed by plotting peak area versus concentration.

2. Precision

Precision was assessed in terms of repeatability and intermediate precision. Repeatability was determined by triplicate analysis of Metformin HCl (100 ppm) and Vildagliptin (10 ppm) under identical conditions on the same day, with results expressed as %RSD of peak areas. Intermediate precision was evaluated by repeating the analysis on different days.

3. Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ were calculated from the standard deviation of the Y-intercept (σ) and the slope (S) of the calibration curve using the following equations:

$$LOD = \frac{3.3\sigma}{S}$$
$$LOQ = \frac{10\sigma}{S}$$

Where,

σ = Standard deviation of Y-intercept

S = Slope of calibration curve

4. Robustness

Robustness was evaluated by deliberately varying chromatographic parameters, including flow rate and detection wavelength, and assessing their effect on chromatographic performance.

5. Specificity

Specificity was determined by analyzing the marketed formulation in the presence of excipients. No interference was observed at the retention times of Metformin HCl and Vildagliptin, confirming method specificity.

6. Accuracy

Accuracy was evaluated through recovery studies at 80%, 100%, and 120% of the target concentration. Six determinations were performed at each level, and percentage recovery was calculated to assess method accuracy.

Degradation Analysis Through UV Spectroscopy

Forced Degradation Studies

Forced degradation studies were conducted to evaluate the stability of Metformin HCl and Vildagliptin under acidic, alkaline, oxidative, thermal, and photolytic stress conditions using UV spectroscopic analysis.

Preparation of Calibration Curve Solutions

Stock solutions of Metformin HCl and Vildagliptin (100 ppm) were prepared separately by dissolving 10 mg of each drug in 100 mL methanol. Calibration standards of 2, 4, 6, 8, and 10 ppm were prepared by diluting 0.2, 0.4, 0.6, 0.8, and 1.0 mL of stock solution to 10 mL with methanol.

Preparation of Working Standard Solution

Working standard solutions (10 ppm) were prepared by diluting 1 mL of each 100 ppm stock solution to 10 mL with methanol and were used for degradation studies.

Stress Degradation Studies[13,19]

1) Acidic Degradation:

Working standard solution (10 mL) was treated with 1 mL of 0.1 N HCl and heated at 60°C for 30 min. After cooling, the solution was neutralized with 0.1 N NaOH, diluted appropriately, and analyzed.

2) Alkaline Degradation:

Working standard solution (10 mL) was treated with 1 mL of 0.1 N NaOH and heated at 60°C for 30 min. After cooling, it was neutralized with 0.1 N HCl, diluted, and analyzed.

3) Oxidative Degradation:

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Working standard solution (10 mL) was treated with 1 mL of 3% H₂O₂, kept at room temperature for 30 min, suitably diluted, and analyzed.

4) Thermal Degradation:

Solid drug samples were exposed to 60–80°C in a hot air oven for 2 h. After cooling, samples were dissolved to obtain 10 µg/mL solutions and analyzed.

5) Photolytic Degradation:

Working standard solutions were exposed to UV light or direct sunlight for 24 h, diluted to 10 µg/mL, and analyzed.

UV Spectroscopic Analysis

After each stress treatment, samples were suitably diluted and analyzed using a UV spectrophotometer. Metformin HCl was measured at 234 nm and Vildagliptin at 208 nm. Percentage degradation and percentage drug remaining were calculated by comparing stressed samples with unstressed standards using:

$$\% \text{ Degradation} = \frac{A_0 - A_t}{A_0} \times 100$$

Where:

A₀ = Initial absorbance of standard drug

A_t = Absorbance after degradation

$$\% \text{ Drug Remaining} = 100 - \% \text{ Degradation}$$

The calculated values were used to determine percentage degradation, percentage drug remaining, and remaining drug concentration under each stress condition.

Greenness Evaluation of the Developed RP-HPLC Method

The greenness of the developed RP-HPLC method was assessed using three established tools: Analytical Eco-Scale, Green Analytical Procedure Index (GAPI), and Analytical GREENness (AGREE).

The Analytical Eco-Scale evaluated the method based on reagent type and quantity, energy consumption, occupational hazards, and waste generation. Penalty points were assigned according to environmental impact, and the final Eco-Scale score was calculated from the total penalty points.[17]

The GAPI tool was used to assess the environmental impact of the entire analytical procedure, including sample preparation, reagent selection, instrumentation, energy consumption, and waste generation. The results were represented using a color-coded system indicating low, medium, and high environmental impact.[18]

The AGREE tool evaluated the method according to the 12 principles of Green Analytical Chemistry, considering factors such as solvent toxicity, energy consumption, waste generation, sample size, automation, and analysis time.[20]

The combined use of Analytical Eco-Scale, GAPI, and AGREE provided a comprehensive assessment of the environmental sustainability and eco-friendliness of the developed analytical method.[21]

RESULT AND DISCUSSION:

Selection of Detection Wavelength.

Based on the dual wavelength approach, analysis was carried out at 208 nm, where both Metformin HCl and Vildagliptin showed adequate absorbance with suitable sensitivity for simultaneous estimation. Therefore, 208 nm was selected as the detection wavelength for HPLC analysis to achieve reliable quantification of both drugs.

Table. 1. System suitability parameters

Sr. no.	Parameters	Metformin hydrochloride	Vildagliptin
1.	Retention time	2.454	3.750
2.	No. of theoretical plate	>2500	>2000
3.	Tailing factor	1.54	1.38

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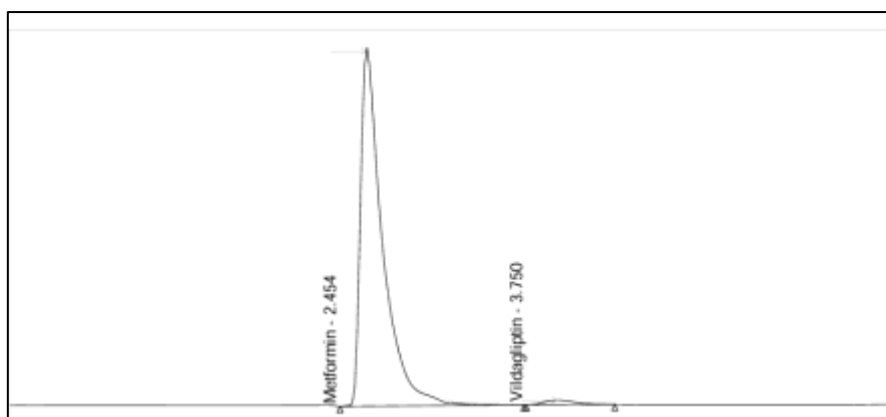


Fig. No. 1. Chromatogram of mixture of Metformin hydrochloride and Vildagliptin

Validation parameter through RP- HPLC

Validation of the analytical method for simultaneous estimation of Metformin Hydrochloride and Vildagliptin was carried out to ensure that the method is reliable, accurate, precise, and reproducible for pharmaceutical analysis. The validation parameters evaluated included linearity, precision, intermediate precision, and accuracy, in accordance with standard analytical guidelines.

1) Linearity

Linearity studies were performed to evaluate the direct proportional relationship between drug concentration and detector response. For Metformin Hydrochloride, concentrations ranging from 50–150 ppm were analyzed, and a linear relationship between concentration and peak area was observed. The regression equation obtained was $y = 36462x + 369285$ with a correlation coefficient (R^2) of 0.9999, indicating excellent linearity of the method for Metformin Hydrochloride. Similarly, Vildagliptin concentrations ranging from 5–15 ppm were evaluated, and the regression equation obtained was $y = 8881.5x - 4921.2$ with a correlation coefficient (R^2) of 0.9991. These results demonstrated that the developed method showed a good linear response for both drugs within the selected concentration ranges.

Table. 2. Linearity parameters for Metformin HCL and Vildagliptin

Sr. no	Parameter	Metformin HCL	Vildagliptin
1.	Linearity Range	50- 150	5-15
2.	Regression Equation	$y=36462x+369285$	$y=8881.5x-4921.2$
3.	Correlation Coefficient (R^2)	0.9999	0.9991
4.	Slope	36462	8881.5
5.	Intercept	369285	4921.2

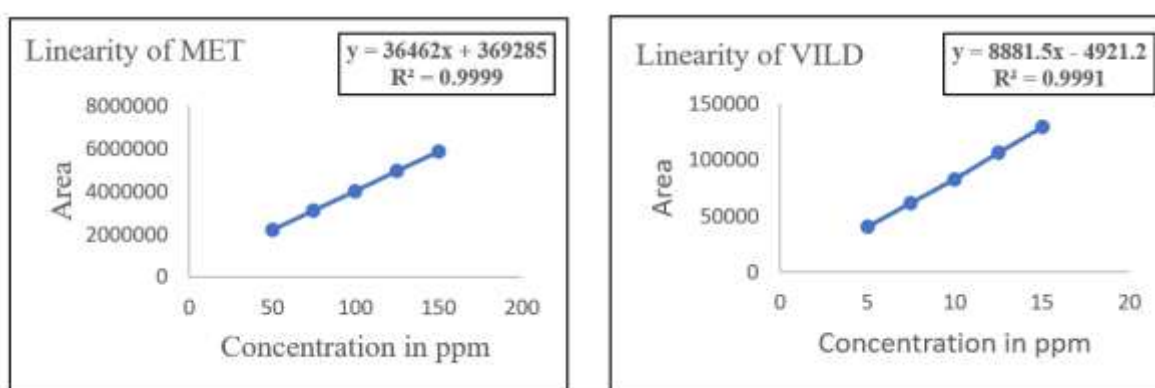


Fig. No. 2. Linearity curve for Metformin HCL and Vildagliptin

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2) Precision

Precision evaluates the agreement between repeated measurements and is expressed as %RSD. It includes intraday precision (same-day repeatability) and interday precision (day-to-day reproducibility). %RSD values below 2% indicate that the method is precise and suitable for routine analysis.

Intraday Precision

Intraday precision studies were performed to evaluate the repeatability of the method under the same experimental conditions on the same day. Metformin Hydrochloride showed an average peak area of 3887113 with %RSD of 0.62%, while Vildagliptin showed an average peak area of 76949.5 with %RSD of 1.46%. Since the %RSD values were less than 2%, the method was found to be precise and reproducible.

Table. 3. Intraday Precision parameters for Metformin HCL and Vildagliptin

Parameter	Metformin Hydrochloride	Vildagliptin
Injection 1	3,875,900	77,930
Injection 2	3,879,186	75,720
Injection 3	3,876,912	77,165
Injection 4	3,874,088	77,886
Injection 5	3,880,159	77,616
Injection 6	3,936,433	75,380
Average	3,887,113	76,949.5
SD	24,261.49	1,122.86
%RSD	0.62	1.46

Interday Precision

Interday precision studies were carried out to evaluate the reproducibility of the method on different days. Metformin Hydrochloride showed an average peak area of 3920519.833 with %RSD of 0.58%, while Vildagliptin showed an average peak area of 75691 with %RSD of 0.74%. Since the %RSD values for both drugs were less than 2%, the method was found to be reproducible, robust, and reliable for routine pharmaceutical analysis.

Table. 4. Interday Precision parameters for Metformin HCL and Vildagliptin

Parameter	Metformin Hydrochloride	Vildagliptin
Injection 1	3,934,220	75523
Injection 2	3,877,502	75122
Injection 3	3,918,452	75055
Injection 4	3,920,555	76255
Injection 5	3,938,756	76387
Injection 6	3,933,634	75802
Average	3,920,519.83	75691
SD	22,570.10	560.6
%RSD	0.58	0.74

3) Limit of Detection (LOD) and Limit of Quantification (LOQ)

The Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated according to ICH guidelines using the standard deviation of response (σ) and slope of the calibration curve (S).

Table. 5. LOD and LOQ for Metformin HCL and Vildagliptin

Sr.no	Parameter	Metformin HCL	Vildagliptin
1.	LOD	1.15 ppm	0.38 ppm
2.	LOQ	3.50 ppm	1.15 ppm

4) Robustness

Robustness was evaluated by varying the detection wavelength (204 and 210 nm) and flow rate (0.8 and 1.2 mL/min) from the optimized conditions. No significant changes in retention time, peak area, or chromatographic performance were observed, and %RSD values remained within acceptable limits, confirming the robustness of the method.

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Table. 6. Robustness parameter for Metformin HCL and Vildagliptin

Sr. No.	Parameter	Metformin HCL		Vildagliptin	
		204 nm	210 nm	204 nm	210 nm
1	Wavelength Variation				
a	%RSD	0.18%	0.07%	0.18%	0.11%
b	Retention Time (min)	2.454	2.452	3.782	3.781
c	Peak Area	3,880,559	3,880,979	77,019	77,507.5
d	Tailing Factor	1.24	1.22	1.61	1.20
		0.8 mL/min	1.2 mL/min	0.8 mL/min	1.2 mL/min
2	Flow Rate Variation				
a	%RSD	0.03%	0.02%	0.11%	0.09%
b	Retention Time (min)	2.236	2.714	3.442	4.169
c	Peak Area	3,481,262.67	4,361,144.00	69,399.00	86,995.33
d	Tailing Factor	1.19	1.40	1.25	1.31

5) Specificity

Specificity studies showed well-resolved peaks for Metformin Hydrochloride ($R_t = 2.502$ min, tailing factor = 1.69) and Vildagliptin ($R_t = 3.689$ min, tailing factor = 1.84) without interference from excipients. The results confirmed the specificity of the developed RP-HPLC method.

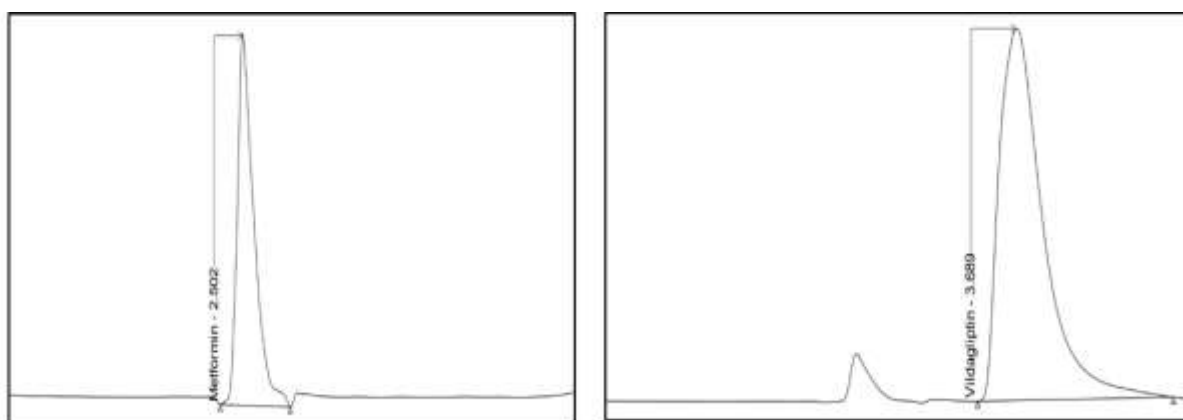


Fig. no. 3. Specificity for the Metformin Hydrochloride and Vildagliptin

6) Accuracy

Accuracy was assessed by recovery studies at 80%, 100%, and 120% concentration levels. Metformin Hydrochloride showed recoveries of 99.0%, 98.7%, and 100.1%, while Vildagliptin exhibited recoveries of 100.7%, 101.6%, and 101.8%, respectively. The recovery values were within acceptable limits, confirming the accuracy and reliability of the developed RP-HPLC method for simultaneous estimation of both drugs.

Table. 7. Recovery data for Metformin HCL and Vildagliptin

Sr.no	Name of Drug	Amount Added (mg)	Amount Added (mg)	% Recovery	Amount Recovered (mg)
1.	Metformin HCL	80%	400	99.0	396.0
2.		100%	500	98.7	493.5
3.		120%	600	100.1	600.6
4.	Vildagliptin	80%	40	100.7	40.28
5.		100%	50	101.6	50.80
6.		120%	60	101.8	61.08

GREENNESS EVALUATION OF DEVELOPED RP- HPLC METHOD

Overall Greenness Evaluation

The greenness of the developed RP-HPLC method for simultaneous estimation of Metformin Hydrochloride and Vildagliptin was evaluated using Analytical Eco-Scale, AGREE, and GAPI assessment tools.

In the Analytical Eco-Scale assessment, the total penalty points obtained were 15, and the overall Eco-Scale score achieved was 85, calculated as:

$$\text{Eco Scale Score} = 100 - 15 = 85$$

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The obtained Eco-Scale score of 85 indicates that the developed method qualifies as an excellent green analytical procedure due to low organic solvent consumption, higher aqueous buffer composition, reduced waste generation, and simple sample preparation.

The AGREE software assessment, based on the 12 principles of Green Analytical Chemistry, showed an overall greenness score of 0.69, indicating satisfactory environmental sustainability of the developed RP-HPLC method. High scores were obtained for integration of analytical processes, automation, derivatization avoidance, and energy minimization, while moderate scores were observed for waste reduction, renewable reagents, toxic reagent elimination, and operator safety.

The GAPI assessment showed predominantly green and yellow zones, confirming that the developed method is environmentally acceptable and suitable for routine pharmaceutical analysis with reduced solvent consumption and minimal waste generation.



Fig. No. 4. AGREE score (0.69) and GAPI assessment of the developed RP-HPLC method.

Forced Degradation Study of Metformin Hydrochloride and Vildagliptin by UV Spectroscopy

Forced degradation studies were conducted under acidic, alkaline, oxidative, thermal, and photolytic stress conditions using UV spectroscopy to evaluate the stability-indicating capability of the developed method. Both drugs exhibited degradation under all stress conditions, with maximum degradation observed under alkaline and oxidative stress and comparatively lower degradation under thermal and photolytic conditions.

Calibration Curve and Linearity

Linearity was evaluated over the concentration range of 2–10 ppm. Metformin Hydrochloride, analyzed at 234 nm, showed a linear response with the regression equation $y = 0.0907x + 0.0359$ and $R^2 = 0.9974$. Vildagliptin, analyzed at 208 nm, exhibited linearity with the regression equation $y = 0.0184x + 0.3025$ and $R^2 = 0.9935$. The high correlation coefficients confirmed the suitability of the developed UV spectroscopic method for quantitative estimation and degradation studies of both drugs.

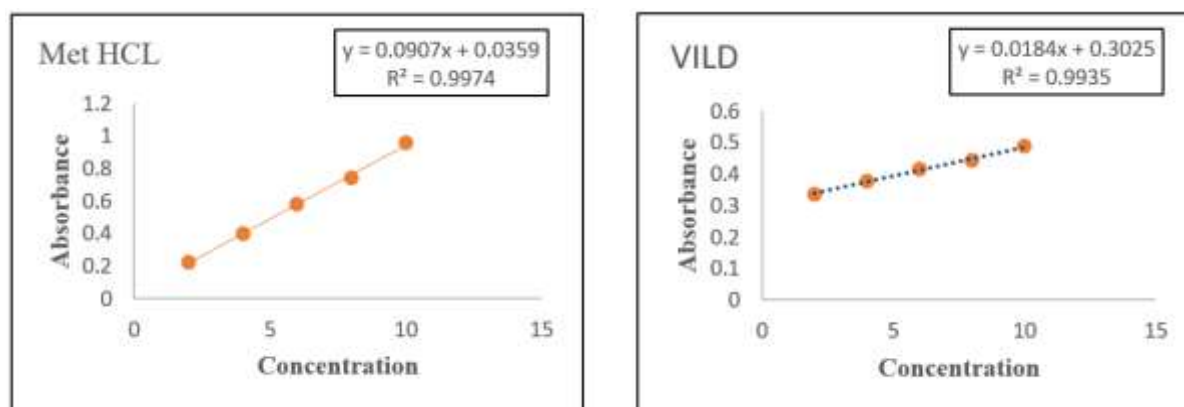


Fig. no. 5. Calibration curve of Metformin Hydrochloride and Vildagliptin

1) Acidic Degradation

Under acidic stress (0.1 N HCl), Metformin Hydrochloride and Vildagliptin exhibited moderate degradation. The percentage degradation was 10.20% and 10.43%, respectively, with 89.80% and 89.57% drug remaining. The remaining drug concentrations were 8.98 ppm for Metformin Hydrochloride and 8.96 ppm for Vildagliptin,

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demonstrating the suitability of the developed UV spectroscopic method for monitoring acidic degradation.

2) Alkaline Degradation

Under alkaline stress (0.1 N NaOH), Metformin Hydrochloride and Vildagliptin showed significant degradation, with percentage degradation of 23.75% and 27.20%, respectively. The percentage drug remaining was 76.25% for Metformin Hydrochloride and 72.80% for Vildagliptin, corresponding to remaining concentrations of 7.63 ppm and 7.28 ppm, respectively. These results indicated that alkaline conditions produced the highest degradation, and the developed UV spectroscopic method effectively monitored the degradation behavior of both drugs.

3) Oxidative Degradation

Under oxidative stress (3% H₂O₂), Metformin Hydrochloride and Vildagliptin exhibited significant degradation, with percentage degradation of 18.64% and 19.84%, respectively. The percentage drug remaining was 81.36% for Metformin Hydrochloride and 80.16% for Vildagliptin, corresponding to remaining concentrations of 8.14 ppm and 8.02 ppm, respectively. These results confirmed the suitability of the developed UV spectroscopic method for monitoring oxidative degradation of both drugs.

4) Thermal Degradation

Under thermal stress, Metformin Hydrochloride and Vildagliptin exhibited minimal degradation, with percentage degradation of 5.00% and 4.70%, respectively. The percentage drug remaining was 95.00% for Metformin Hydrochloride and 95.30% for Vildagliptin, corresponding to remaining concentrations of 9.50 ppm and 9.53 ppm, respectively. These results indicated good thermal stability of both drugs and demonstrated the suitability of the developed UV spectroscopic method for monitoring thermal degradation.

5) Photolytic Degradation

Under photolytic stress, Metformin Hydrochloride and Vildagliptin showed minimal degradation, with percentage degradation of 3.64% and 3.27%, respectively. The percentage drug remaining was 96.36% for Metformin Hydrochloride and 96.73% for Vildagliptin, corresponding to remaining concentrations of 9.64 ppm and 9.67 ppm, respectively. These results indicated good photolytic stability of both drugs and confirmed the suitability of the developed UV spectroscopic method for monitoring photolytic degradation.

Table. 8. Comparative Forced Degradation Study of Metformin Hydrochloride and Vildagliptin Under Different Stress Conditions Using UV Spectroscopy.

Stress Condition	Parameter	Metformin Hydrochloride	Vildagliptin
Acidic Degradation	Absorbance after degradation	0.862	0.438
	% Degradation	10.20%	10.43%
	% Drug Remaining	89.80%	89.57%
	Remaining Concentration	8.98 ppm	8.96 ppm
Alkaline Degradation	Absorbance after degradation	0.732	0.356
	% Degradation	23.75%	27.20%
	% Drug Remaining	76.25%	72.80%
	Remaining Concentration	7.63 ppm	7.28 ppm
Oxidative Degradation	Absorbance after degradation	0.781	0.392
	% Degradation	18.64%	19.84%
	% Drug Remaining	81.36%	80.16%
	Remaining Concentration	8.14 ppm	8.02 ppm
Thermal Degradation	Absorbance after degradation	0.912	0.466
	% Degradation	5.00%	4.70%
	% Drug Remaining	95.00%	95.30%
	Remaining Concentration	9.50 ppm	9.53 ppm
Photolytic Degradation	Absorbance after degradation	0.925	0.473
	% Degradation	3.64%	3.27%
	% Drug Remaining	96.36%	96.73%
	Remaining Concentration	9.64 ppm	9.67 ppm

CONCLUSION:

The present research work successfully developed and validated a simple, rapid, accurate, precise, cost-effective, stability-indicating, and eco-friendly RP-HPLC method for simultaneous estimation of Metformin HCl and Vildagliptin in pharmaceutical dosage form. The optimized method showed excellent chromatographic separation and was validated according to ICH guidelines for linearity, accuracy, precision, robustness, specificity, LOD, and LOQ. Forced degradation studies confirmed the stability-indicating nature of the method, with maximum

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degradation observed under alkaline and oxidative conditions. Green assessment using Analytical Eco-Scale, GAPI, and AGREE demonstrated the environmental sustainability of the method, with an Eco-Scale score of 85, penalty points of 15, and AGREE score of 0.69. Overall, the developed method was found to be reliable, environmentally sustainable, and suitable for routine pharmaceutical quality control and stability studies.

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