Original Research Article

Development and Validation of new RP-HPLC method for the estimation of Antidiabetic Drugs Metformin hydrochloride and Gemigliptin in combined pharmaceutical dosage form.

Abstract:

Metformin Hydrochloride and Gemigliptin is combination of Antidiabetic drug in tablet Zemimet SR ® Tablet (25/500 mg), a member Antidiabetic drug, is a recent drug developed by LG Life sciences for the treatment of Type 2 diabetes. A new sensitive and rapid HPLC method was developed for the determination of Metformin Hydrochloride and Gemigliptin in pharmaceutical dosage forms; it was validated according to International Conference on Harmonization and Food and Drug Administration guidelines. The analysis was performed on the HPLC system equipped with a using Gemni C18, (5 µm) (250 mm x 4.6 mm), with of Buffer (20mM Ammonium Acetate in water, pH 3.5) and Methanol: Acetonitrile 40:10 (%V/V) 60: 40 v/v with at a flow rate of 1.0 mL/min, column temperature 35°C, total run time was 10 min, injection volume 10 µl, and detection was performed at the wavelength (λ) of 265 nm. The calibration plot gave linear relationship over the concentration range of Metformin Hydrochloride 20, 40, 100, 200, 400 and 500 µg/ml, and Gemigliptin 1, 2, 5, 10, 20 and 25 µg/ml, respectively. The accuracy of the proposed method was determined by recovery studies and was found to be Metformin Hydrochloride 99.0 % to 101.0 % and Gemigliptin 98.0 % to 100.0 %.The Limit of Detection were 50.56 **and 14.21** µg/ml for Metformin Hydrochloride and Gemigliptin, respectively and the Limit of Quantitation were **166.85 and 43.90** µg/ml for Metformin Hydrochloride and Gemigliptin, respectively% Relative Standard Deviation of the determination of precision was <2%. The results of robustness and solutions stability studies were within the acceptable limits as well the main features of the developed method are low run time and retention time of around 2.9 min for Metformin Hydrochloride (Met) and 7.4 min for Gemigliptin.

Keywords: Metformin,Gemigliptin, Method Development and Validation, HPLC

1. Introduction

Drug Profile

Metformin Hydrochloride. Metformin, chemically 1-carbamimidamido-N, N dimethyl methanimidamide (Figure. 1) is a biguanide antihyperglycemic agent used for treating non-insulin dependent diabetes mellitus (NIDDM). It improves glycemic control by decreasing

hepatic glucose production, decreasing glucose absorption and increasing insulin-mediated glucose uptake. Metformin is the only oral antihyperglycemic agent that is not associated with weight gain. Molecular formula: $C_4H_{11}N_5$-HCl., Molecular Weight: 165.63 g/mol [1-3].

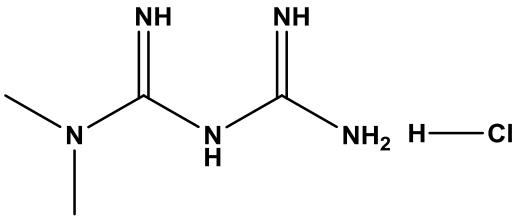

Figure. 1 Metformin Hydrochloride

Gemigliptin: - Gemigliptin is chemically 3(s)-3-amino-4- (5,5-difluro oxopiperidino [2,4 (triflurомethyl) -5,6,7,8 -tetra hydro [3,4-d] pyrimidine-7-yl] butan-1-one exhibits a unique structure. Gemigliptin (Zemiglo®, previously known as LC15-0444) has a different chemical structure compared to other DPP-4 inhibitors due to the presence of pyrimidine piperidine derivative as evident by X-ray crystallography. Gemigliptin binds to the S1, S2, and S2 extensive subsites of the DPP-4 enzyme. The piperidinone group of gemigliptin binds to the S1 subsite, where the upside F atom on the piperidin ring forms a hydrogen bond with the side chain of Tyr631 and the downside F atom makes a hydrophobic interaction with the side chain of Tyr666 and Tyr662. In addition, the key interaction occurs between the CF3 groups on the pyrimidinopiperidine and the S2 extensive subsite of the DPP-4 substrate, which enhances the potency of the drug and increases its selectivity as well. .For Gemigliptin Molecular formula:$C_{18}H_{19}F_8N_5$-O_2Molecular Weight: 489.36 g/mol . Gemigliptin L-tartrate Sesquihydrate Molecular formula: $C_{18}H_{19}F_8N_5$-O_2.$C_4H_6O_6$.1.5 H_2O., Molecular Weight: 666.4 g/mol.

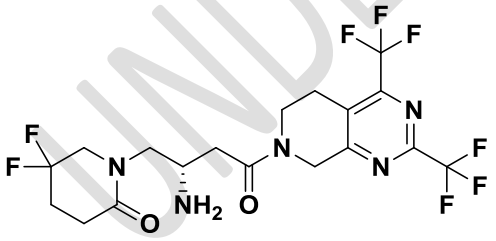

Figure-2 Gemigliptin

Analytical method validation ensures that various HPLC analytical techniques shall give reliable and repeatable results; it is a crucial step in developing new dosage forms as it provides information about accuracy, linearity, precision, detection, and quantitation limits. According to the ICH guideline, "the objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose." It is now obligatory in the process of

drug development to supply the validation data for the responsible authorities. Guidelines for analysis method validation include ICH and USP guidelines [**9–12**].

Literature survey revealed a few methods reported which are costly for routine testing for determination for Gemigliptin and Metformin in Pharmaceutical preparation.

In this research, a new sensitive and rapid HPLC method was developed for the determination of Gemigliptin and Metformin HCl in pharmaceutical dosage forms, and this method was validated according to ICH and FDA guidelines.

2. Materials and Methods

2.1. Instrumentation. Chromatographic HPLC system equipped with a Gemni C18, (5 µm) (250 mm x 4.6 mm) column.

2.2. Chemicals and Reagents.

Acetonitrile, Ammonium Acetate, Methanol, Water were of HPLC Grade.

2.3. Chromatographic Conditions.

Mobile Phase (Buffer 20mM Ammonium Acetate in water, pH 3.5 and Methanol : Acetonitrile 40:10 (%V/V) in the ratio 60: 40 v/v with a flow rate of 1.0 mL/min. the detection was performed at the wavelength (λ) of 265 nm, injection volume 10 µl, run time 10 min, and column temperature 35°C Diluent –HPLC Grade water.

2.4. Preparation of Standard Solution.

Weigh accurately and transfer about 25 mg of Gemigliptinand 500 mg of Metformin Hydrochloride standard into 100 ml volumetric flask, add 70 ml of diluent and sonicate to dissolve, cool. Dilute to volume with diluent and mix. Transfer 4 ml of this solution to a 50 ml volumetric flask and dilute with diluent to volume and mix well.

2.5. Preparation of Sample Solution.

Weigh accurately and transfer Approx. 595 mg of synthetic mixture (Equivalent to 25 mg of Gemigliptin and 500 mg of Metformin Hydrochloride) into 100 ml volumetric flask, add 50 ml of diluent and sonicate for15 min with intermittent shaking. Dilute to volume with diluent and mix. Filter a portion of this solution using 0.45 µ PVDF Syringe filter, transfer 4 ml of this solution to a 50 ml volumetric flask and dilute with diluent to volume and mix well.

2.6. Method Validation. The method was validated as per ICH and FDA guidelines, and the validation parameters included specificity, linearity, range, accuracy, precision, sensitivity (LOQ and LOD) robustness, and solution stability [5-7].

2.6.1. Specificity. Specificity is one of the significant features of HPLC, and it refers to the ability of the analytical method to discriminate between the analyte and the other components in the complex mixture [7]. Specificity of the method was evaluated by injecting 10 µL solutions of standard, sample, blank, and placebo separately.

2.6.2.Linearity. To evaluate the linearity and range of the method, Direct standard solutions were prepared by diluting the standard stock solution with the diluent in different concentrations Metformin Hydrochloride: 20, 40, 100, 200, 400 and 500 µg/ml, and Gemigliptin1, 2, 5, 10, 20 and 25 µg/ml which cover 70%, 90%, 100%, 110% and 120%, of the target concentration, respectively. Three replicate injections from each concentration were analysed under the same conditions. Linear regression analysis was used to evaluate the linearity of the calibration curve by using the least square linear regression method.

2.6.3. Sensitivity. Limit of detection (LOD)/limit of quantitation (LOQ) of Metformin Hydrochloride and Gemigliptin were determined by measuring the signal-to-noise ratio. limit of detection (LOD) is the concentration that gives a signal-to-noise ratio of approximately 3: 1, while the limit of quantification (LOQ) is the concentration that gives a signal-to-noise ratio of approximately 10 :1 with %RSD (n = 3) of less than 10%.

2.6.4. Accuracy. The accuracy of the assay method was determined by recovery studies at three concentration levels (80%, 100%, and 120%), i.e., 320, 400, and 480 µg/ml for Metformin Hydrochloride and Gemigliptin16, 20 and 24 µg/ml and three samples from each concentration were injected. percentage recovery of Metformin Hydrochloride and Gemigliptin added and RSD were calculated for each of the three replicate samples.

2.6.5. Precision. The system precision and method precision (repeatability) of the proposed methods were determined by several measurements of standard solution and sample solution, respectively [7-9]. System precision was established by six measurements of the standard solution at the 100% concentration levels on the same day. Method precision was established

by six assay determinations of the sample solution at the 100% concentration levels on the same day [9-12]. The RSD of obtained results was calculated to evaluate repeatability results.

2.6.6. Robustness. Robustness of the method was verified by applying minor and deliberate changes in the experimental parameters, for example:

(i) Column temperature: ±3°C

(ii) Flow rate: ±0.2 mL/min.

(iii) Change in pH.: ± 1

Change was made to evaluate its effect on the method. Obtained data for each case was evaluated by calculating % RSD and percent of recovery.

2.6.7. Stability of Analytical Solutions. The stability of analytical solutions was determined by analysing the standard and sample preparations at Initial, 12 Hr and 24 Hr at ambient room temperature 25°C. Three injections from each solution were analysed, and the average of the peak and the RSD were calculated.

3. Results and Discussion

3.1. Method Development and Optimization. Several physical and chemical properties of Metformin Hydrochloride and Gemigliptin were obtained from the literature. The analytical method was developed to select preliminary reversed phase HPLC method chromatographic conditions, including detection wavelength, mobile phase, stationary phase, and sample preparation procedure.For this purpose, a series of trials were performed by varying the ratio of include trials.

Table 1. Results of method optimization

Column Used	Mobile Phase	Flow Rate	Wavelength	Observation
Gemni C18 (5 µm) (250 mm x 4.6 mm)	Water : Methanol (60:40)	1.0 ml/min	265 nm	Improper Peak Shape observed for both drugs
Gemni C18 (5 µm) (250 mm x 4.6 mm)	MeOH : Acetonitrile : 20 mM Ammonium Formate, pH 3.5 15:15: 70 v/v)	0.6ml/min	265 nm	Improvement was needed

Gemni C18 (5 µm) (250 mm x 4.6 mm)	MeOH : Acetonitrile : 20 mM Ammonium Formate, pH 3.5 25:15: 60 v/v)	0.6 ml/min	265	Improvement was needed

Optimizing the chromatographic conditions on the Gemni C18 (5 µm),(250 mm x 4.6 mm column. the results of method optimization are summarized in Table 1. the mobile phase consisting of (Buffer 20mM Ammonium Acetate in water , pH 3.5 and Methanol : Acetonitrile 40:10 (%V/V) in the ratio 60: 40 v/v with a flow rate of 1.0 mL/min, injection volume 10 µl, run time 10 min, and column temperature 35°Cat wavelength (λ) 265was optimized as the best chromatographic conditions for the entire study where Metformin Hydrochloride and Gemigliptin was eluted forming symmetrical peak shape, resolution and suitable analysis time with retention time about 2.9 min for Metformin Hydrochloride (Met) and 7.4 min for Gemigliptin (Figure. 3) and Table 2.

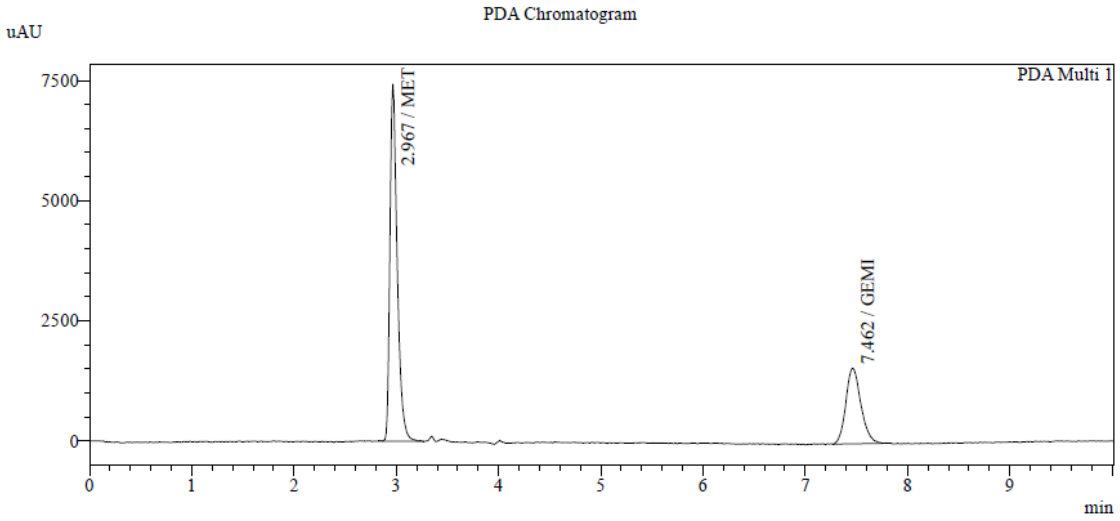

Figure 3. Chromatogram of Metformin Hydrochloride and Gemigliptin standard solution

Table 2. Result of optimized method

Sr. No	Name	Area	Retention Time	Resolution	Tailing Factor	Theoretical Plate	Peak Purity Index
1	Metformin HCl	36996	2.9 Min	NA	1.3	6377	0.999995
2	Gemigliptin	16009	7.4 Min	20.9	1.1	11379	0.999915

3.2. Method Validation

3.2.1 System Suitability: The % RSD for each parameter was found to be less than 2 %. This indicates the suitability of the system.

3.2.2. Specificity. Specificity was evaluated by comparing the chromatograms of mobile phase blank, placebo solution, standard solution, and sample solution (Metformin Hydrochloride and Gemigliptin). For this purpose, 10 µl from solutions mobile phase blank, standard solution (API) and sample solution were injected into the HPLC system separately, and the chromatogram results are shown in Figures 4–5a, 5b, 5c, and 5d. It can be observed that there no coeluting peaks at the retention time of Metformin Hydrochloride and Gemigliptin interference. This result indicates that the peak of the analyte was pure and this confirmed the Specificity of the method.

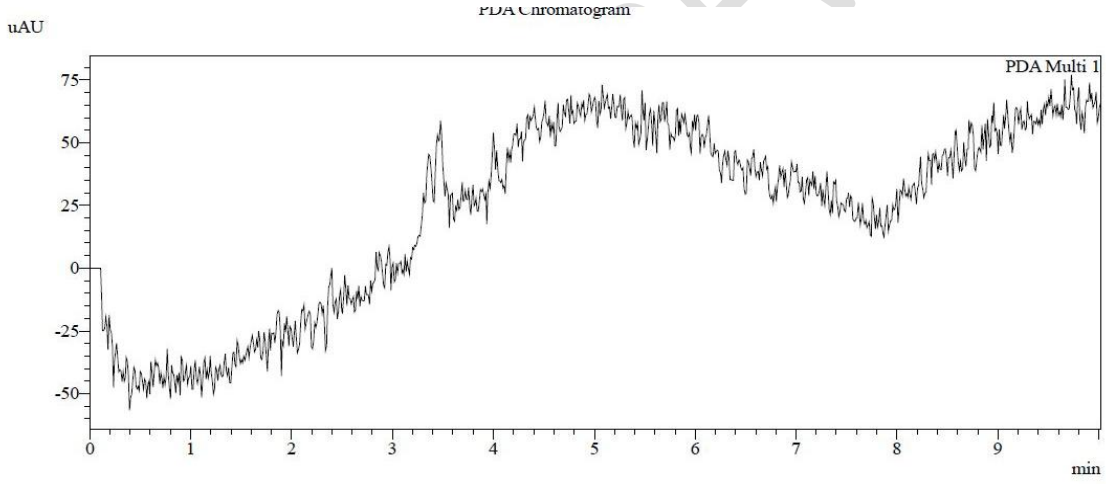

Figure 4. Chromatogram of Blank solution

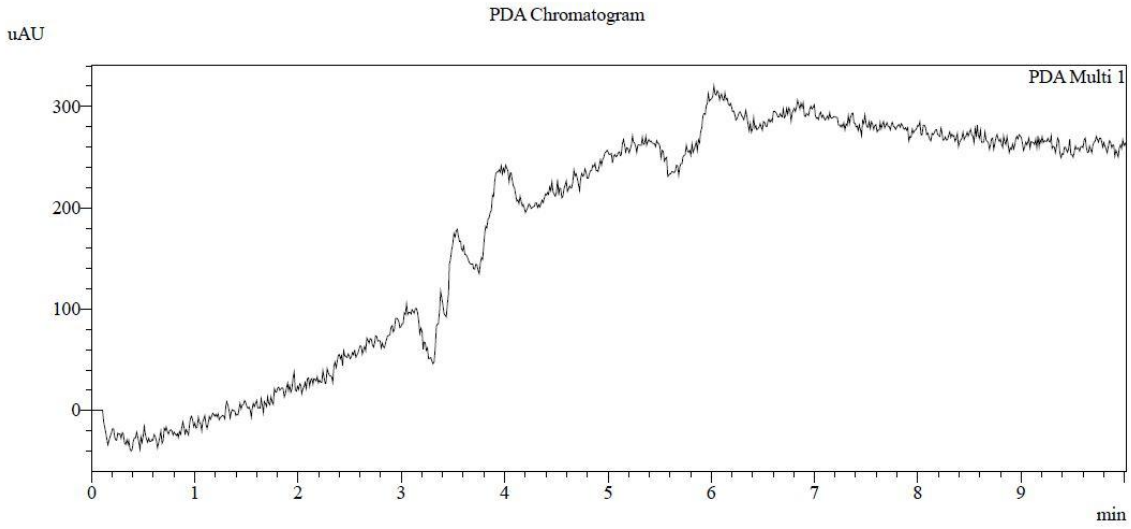

Figure 5a: Chromatogram of placebo solution.

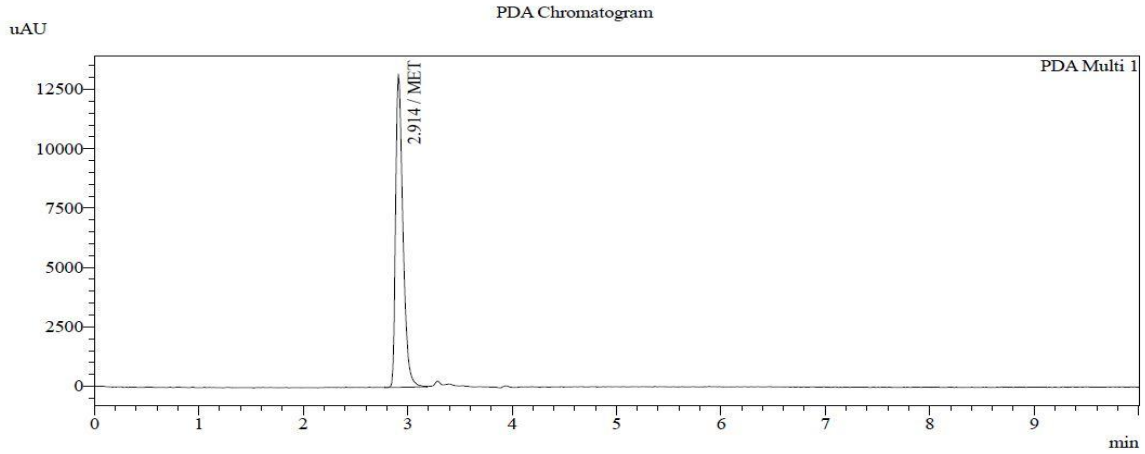

Figure 5b: Chromatogram of Metformin API solution.

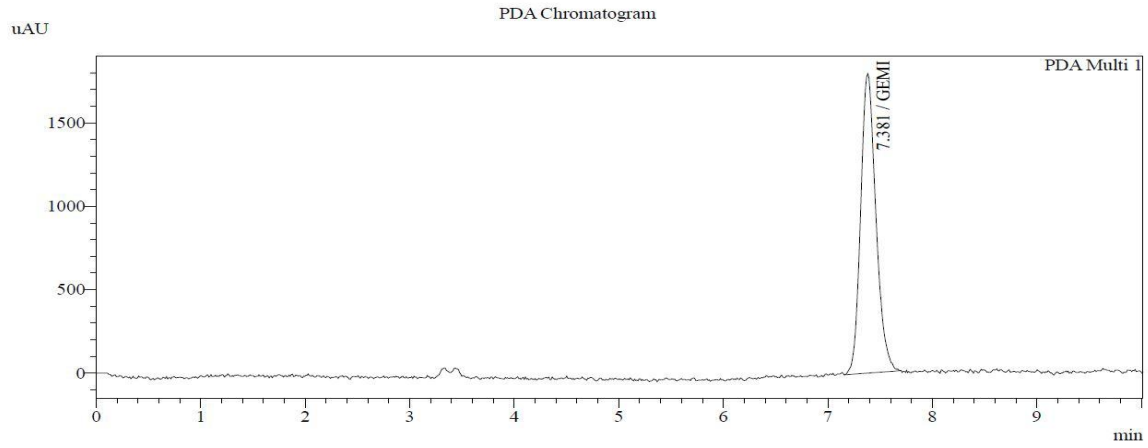

Figure 5c. Chromatogram of Gemigliptin API solution

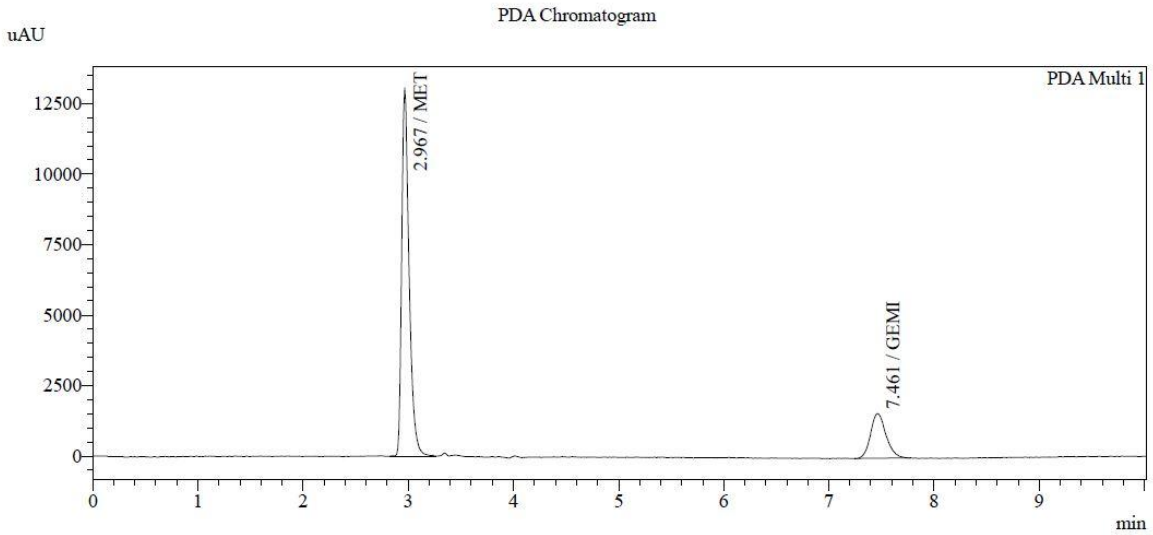

Figure 5d. Chromatogram of Sample Solution

3.2.3. Linearity and Range. Analytical method linearity is defined as the ability of the method to obtain test results that are directly proportional to the analyte concentration, within a specific range. The mean peak area obtained from the HPLC was plotted against corresponding concentrations to obtain the calibration graph. The results of linearity study (Figures 6 and 7) gave linear relationship over the concentration range of Metformin Hydrochloride: 20, 40, 100, 200, 400 and 500 µg/ml and Gemigliptin1, 2, 5, 10, 20 and 25 µg/ml. From the regression analysis, a linear equation was obtained and the goodness-of-fit (r2) was found to be 0.99, indicating a linear relationship between the concentration of analyte and area under the peak.

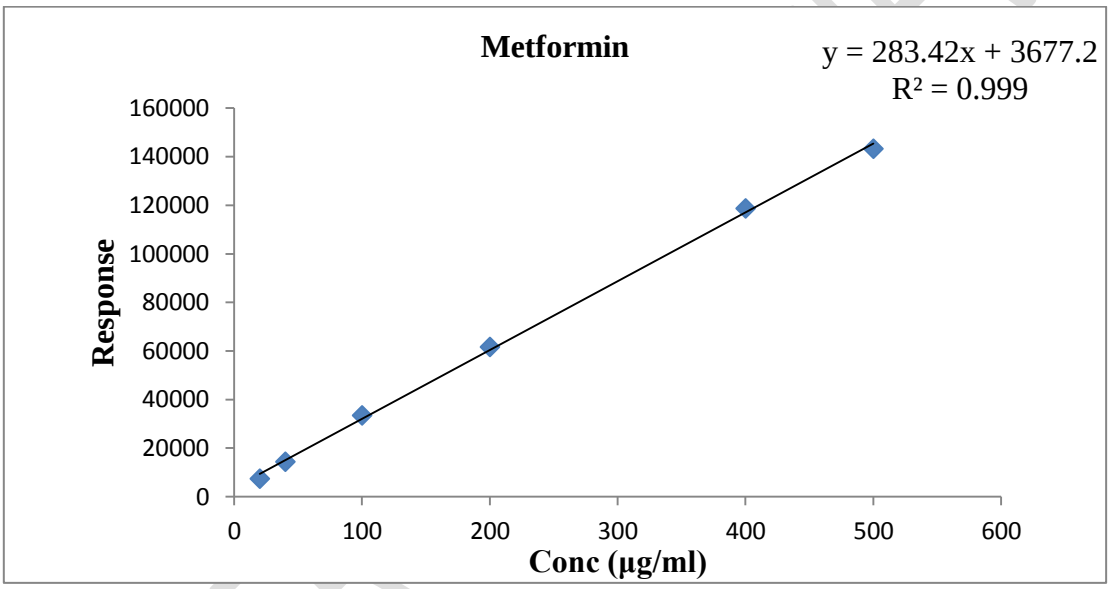

Figure 6. Standard calibration curve of Metformin Hydrochloride

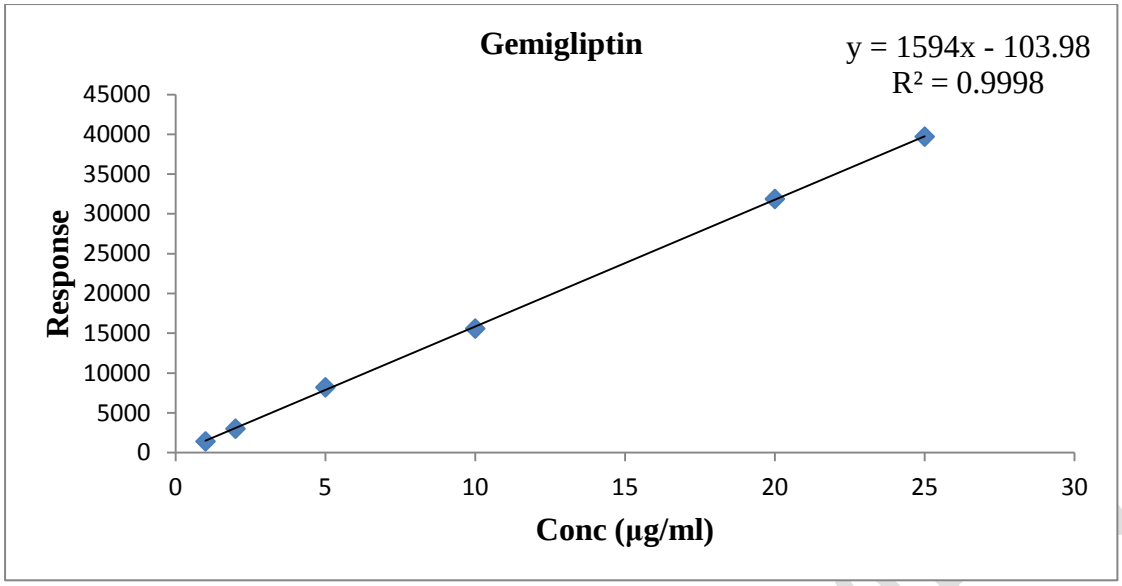

Figure 7. Standard calibration curve of Gemigliptin

3.2.4. Accuracy. The accuracy of an analytical procedure expresses the closeness of results obtained by that method to the true value. The results of accuracy showed percentage recovery at all three levels in the range of for Metformin Hydrochloride99.0 % to 101.0 % and Gemigliptin98.0 % to 100.0%, and % RSD values were in the range of 0.70 - 0.93 % as shown in **Table 3.1 and 3.2**. The results of percentage recovery and %RSD were within the accepted limits from 98.0% to 102.0% and not more than 2.0%, respectively, which indicates the applicability of the method for routine drug analysis.

Table 3.1Metformin Hydrochloride Recovery data of the proposed HPLC method

% Level	Peak Area	Mean Area	Amount found	Amount added	% recovery
80	95532	96029	321.01	320.200	100
	96354		323.78	320.288	101
	96201		323.26	320.448	101
100	119416	119316	401.27	400.176	100
	118463		398.07	400.704	99
	120071		403.47	400.184	101
120	143299	143796	481.52	480.264	100
	144731		486.34	480.184	101
	143360		481.73	480.704	100

Acceptance: - 98.0 to 102.0 **% RSD: - NMT 2.0 %**	**Min**	99
	Max	101
	Mean	100
	SD	0.707
	% RSD	**0.70**

Table 3.2. Gemigliptin Recovery data of the proposed HPLC method

% Level	Peak Area	Mean Area	Amount found	Amount added	% recovery
80	26511	26254	16.05	16.088	100
	26012		15.75	16.064	98
	26239		15.89	16.176	98
100	32479	32733	19.67	20.040	98
	32583		19.73	20.192	98
	33139		20.07	20.048	100
120	39766	39582	24.08	24.040	100
	39403		23.86	24.096	99
	39579		23.97	24.128	99
Acceptance: - 98.0 to 102.0 **% RSD: - NMT 2.0 %**				**Min**	98
				Max	100
				Mean	98.88
				SD	0.92
				% RSD	**0.93**

3.2.5. Precision. The precision of the method is deemed as "the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions," and it is normally expressed as the relative standard deviation. The results of both system and method precision showed that the method is precise within the acceptable limits. The RSD, tailing factor, and number of theoretical plats were calculated for both solutions; all the results are within limits. Acceptable precision was not more than 2.0% for the **RSD** as shown in **Tables 3.3 and Table 3.4.**

Table 3.3.System Precision data from standard solution of the proposed HPLC method

Replicate No.	Metformin HydrochlorideArea	GemigliptinArea

1	63297	16605
2	62576	16341
3	63124	16587
4	62846	16400
5	62868	16345
6	62755	16481
Mean	62911	16460
SD	260	117
% RSD	0.40	0.70

Table 3.4.Method Precision data from Sample solution of the proposed HPLC method

Replicate No.	**Metformin Hydrochloride Area**	**Gemigliptin Area**
1	63300	16605
2	62569	16441
3	63102	16587
4	62896	16469
5	62895	16496
6	62754	16489
Mean	62919	16514
SD	256.70	66.19
% RSD	0.40	0.40

3.2.6.Robustness. The analytical method robustness was tested by evaluating the influence of minor modifications in HPLC conditions on system suitability parameters of the proposed method, as mentioned in Section 2.6.6. The results of robustness testing showed that a minor

change of method conditions, such as the variation of the temperature and flow rate, is robust within the acceptable limits. The results are summarized in **Table 3.5**. In all modifications, good separation of Metformin Hydrochloride and Gemigliptin was achieved, and it was observed that the percent of recovery was within acceptable limits and the %RSD is within limit of not more than 2.0 %. Acceptable limits as well. The results are shown in Table 3.5.

Table3.5: Robustness data of the proposed HPLC method

Parameter	**Condition**	**Peak Area**		**% RSD**	
		Metformin HCl Area	**Gemigliptin Area**	**Metformin HCl**	**Gemigliptin**
Column Temperature ±3°C	**32 °C**	61952	16552	0.80	1.59
	35 °C	62919	16489	0.40	0.40
	38 °C	63409	16675	0.56	0.90
Flow Rate ±0.1 ml/min	**0.9 ml/min**	76058	19826	0.80	1.16
	1.0 ml/min	62919	16489	0.40	0.40
	1.1 ml/min	62040	16535	0.37	1.34
Change in pH ± 0.1 pH	**3.4**pH	69643	18290	0.51	1.20
	3.5pH	62919	16489	0.40	0.40
	3.6 pH	70591	18415	0.37	0.53

3.2.7. Solution Stability. The percent of recovery was within the range of 98.0% to 102.0% and RSD was not more than 2.0%, indicating a good stability of the sample and standard Solutions for 0 Hr, 12 Hr and 24 Hr at Room Temperature (RT) conditions. The peak area was as comparable to standard and percent of recovery was within acceptable limits, and the % RSD is within the limit of not more than 2.0%. The results are shown in **Table 4**.

Table 4. **Solution stability data of the proposed HPLC method**

Parameter	**Time Point**	**Peak Area**	
		Metformin Hydrochloride Area	**Gemigliptin**
Standard Solution	**0 Hr (Initial) at RT**	62911	16460
	After 12 Hr at RT	62093	16357
	After 24 Hr at RT	62061	16332

Parameter	**Time Point**	**Peak Area**		**% RSD**	
		Metformin Hydrochloride Area	**Gemigliptin**	**Metformin Hydrochl**	**Gemiglipti n**

				oride	
Sample Solution	**0 Hr (Initial) at RT**	62919	16489	0.40	0.40
	After 12 Hr at RT	63637	16747	0.21	0.22
	After 24 Hr at RT	71268	18593	0.50	0.59

4. Conclusion

In the present research, a fast, simple, accurate, precise, and linear HPLC method has been developed and validated for quantitative analysis of Zemimet SR ® Tablet (25/500 mg) combined dose and formulation, and hence it can be employed for routine quality control analysis for finished and stability sample analysis. The analytical method conditions and the mobile phase solvents provided good resolution for Metformin Hydrochloride and Gemigliptin. In addition, the main features of the developed method are short run time and retention time around 2.9 min for Metformin Hydrochloride (Met) and 7.4 min for Gemigliptin. The method was validated in accordance with ICH/FDA guidelines. The method is robust enough to reproduce accurate and precise results under different chromatographic conditions.

7. Data Availability

Data available through correspondence if required.

8. COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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