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METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF VILDAGLIPTIN & METFORMIN HCL IN BULK DRUG AND DOSAGE FORM BY USING RP-HPLC

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ABSTRACT

A new simple, specific, precise and accurate RP-HPLC method has been developed for determination of Vildagliptin and Metformin Hydrochloride in pharmaceutical dosage form. The mobile phase used in this study was composed of Acetonitrile: 0.05% TFAA in water at the ratio of 15:85 *v/v*, with isocratic elution using Phenomenex C18 column (250 mm X 4.6 mm i.e., Volume 20µL. Injection volume was 20µL and flow rate was 1.0 mL/min. The column was maintained at ambient temperature and the eluent was monitored at 210nm. The retention time for Metformin was 2.02 and for Vildagliptin 3.37 with run time of 7 min. Metformin hydrochloride and Vildagliptin Standard and Sample solution was found stable for 24 hours Hence both solutions can be used up to 24 Hrs. The validation method was developed as per ICH Q2R1guidelines. The developed technique was validated for accuracy, precision, linearity, robustness, LOD, LOQ, system suitability, specificity studies and result were analyzed according to the ICH Q2R1 guideline.

Keywords: Vildagliptin, Metformin Hydrochloride, method development, validation, RP-HPLC, Pharmaceutical dosage form

1. INTRODUCTION:

Type 2 diabetes (T2DM) is a chronic disease that requires the combination of antidiabetic drugs with different mechanisms of action to achieve glycemic targets. Commonly used bidirectional therapy with metformin and sulfonylurea (SU) does not improve glycemic control and requires the addition of a third hypoglycemic drug. (3-Hydroxy- 1-

adamantyl) glycyl] pyrrolidine-2 carbonitrile] a new oral anticancer drug, is a dipeptidyl peptidase-4 inhibitor (reduces glucose-induced pancreatic hyperplasia Glucagonlike peptide 1 and gastric inhibitory peptide) and is used in as monotherapy in the treatment of adults with type 2 diabetes, especially in patients who cannot control their diabetes with diet and exercise alone. Vildagliptin may be used as a bi-oral therapy in combination with: Metformin in patients who fail to achieve adequate glycemic control even with the highest dose of metformin monotherapy. When used with metformin, it has similar effects compared to sulfonylureas and may reduce the risk of hypoglycemia without increasing weight. Vildagliptin, in addition to metformin, also has pharmacological properties that improve glucose-dependent insulin secretion and inhibit glucagon release, thereby improving glycemic and weight control and reducing blood sugar. 1-carbamidamido-N,N-dimethyl formamide] is an oral biguanide antibiotic. It is used as the first drug in the treatment of non-insulin dependent diabetes. It improves glycemic control properties by reducing liver glucose, reducing glucose absorption, and increasing insulin-mediated glucose uptake. The clinical indications for metformin are as second-line therapy in adults with type2 diabetes, especially in severely ill patients who cannot achieve adequate glycemic control with the maximum oral dose of metformin alone. The mechanism by which metformin hydrochloride reduces blood sugar and blood lipid concentration is through regulation of AMPK by activating AMP-activated protein kinase (AMK) and Peutz-Jeghers protein LKB1 **[1-8]**.

A broad literature survey is based on varied techniques used as HPLC, RP-HPLC **[9-14]**. According to the information from literature survey, there is no method developed with Acetonitrile: 0.05% TFAA in water at the ratio of 15:85 *v/v as* mobile phase. So, research was focus to develop a new RP-HPLC method with good accuracy, simple, economical mobile phase.

2. MATERIALS AND METHOD:

2.1. MATERIAL:

Table 1: Active Pharmaceutical Drug

Sr. No.	Name	Description
1.	Vildagliptin	Vildagliptin is a white crystalline powder, used to control blood sugar levels in patients with type 2 diabetes mellitus.
2.	Metformin HCl.	Metformin is a white crystalline powder, used to treat Type 2 DM.

Table 2: List of Chemicals use in Research work

Sr. No.	Chemicals/ Reagents/ Solvents	Supplier	Grade
1	Methanol	Merck	HPLC grade
2	Acetonitrile	Merck	HPLC grade
3	Water	Siddhi Lab	HPLC grade

2.2. METHOD:

2.2.1. Preliminary Analysis of Drug

a) Description

Color and texture of Vildagliptin & Metformin was compared with reported characters mentioned in drug bank.

b) Solubility

Solubility of Vildagliptin & Metformin was determined in various solvents like DMSO, methanol, ethanol and Acetonitrile.

c) UV Analysis

UV analysis was carried out by scanning the solution of Vildagliptin & Metformin at 200-400 nm.

2.2.2. VALIDATION

The HPLC Validation of the Optimized Vildagliptin & Metformin result was performed at a Mobile Phase of Acetonitrile: 0.05% TFAA in Water (15:85), pH of buffer: 6.5 at a Maximum Wavelength of 210 nm. According to the International Conference on Harmonization (ICH) guidelines, the proposed HPLC method was validated in terms of system suitability, specificity, precision, accuracy, and robustness.

A. Validation:

According to the International Conference on Harmonization (ICH) guidelines, the proposed HPLC method was validated in terms of system suitability, specificity, precision, accuracy, and robustness.

1. Linearity:

The linearity of the peak area response for Vildagliptin & Metformin was determined from 80% to 120% of the working concentration of Vildagliptin & Metformin.

2. System Suitability:

System-suitability tests are an essential part of method development and are used to ensure that the chromatographic system is performing properly. For six replicate injections of the drug at a concentration of 20 g/ml, the retention time (Rt), number of theoretical plates (N), and tailing factor (T) were measured.

3. Specificity:

Lamivudine chromatogram revealed a peak at 3.943 min retention time. The transitional phase designed for the drug was successfully resolved using this method.

4. Sensitivity:

The proposed method's sensitivity for measuring Vildagliptin & Metformin was estimated in terms of the limit of detection (LOD) and the limit of quantification (LOQ) (LOQ). The signal-to-noise ratio was used to calculate the LOD and LOQ.

5. Precision:

Precision demonstrations were performed in two categories. The injection repeatability (System Precision) was evaluated by administering six injections of the standard Vildagliptin & Metformin solution and calculating the percent RSD of the replicate injections.

6. Accuracy:

Recovery studies using the standard addition method were carried out in order to validate the proposed method's accuracy.

7. Robustness:

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Robustness was assessed by injecting blank and stock standard solution samples under slightly varied chromatographic conditions. These conditions included variations in flow rate by ± 10%. (±0.1ml/min), column oven temperature by ± 2 °C (42 ºC, 38 ºC), and wavelength by ± 3 nm (212 nm, 208 nm).Blank and standard solution were injected under different chromatographic conditions as shown below.

3. RESULT AND DISCUSSION:

3.1. Method Validation:

1) Linearity and Range

Linearity of an analytical method is its ability to elicit test results that are proportional to the concentration of analyte in samples within a given range.

Table 3: Linearity Data for Metformin hydrochloride

Level	Conc (µg/mL)	Area	Mean	% RSD
80%	160.0	129956245	129947911	0.233
		129640753		
		130246734		
90%	180.0	145459073	145887814	0.271
		146239461		
		145964907		
100%	200.0	162260153	161788366	0.268
		161407642		
		161697304		
110%	220.0	177420984	177131083	0.212
		176707364		
		177264900		
120%	240.0	193954391	193856050	0.292
		194367054		
		193246706		

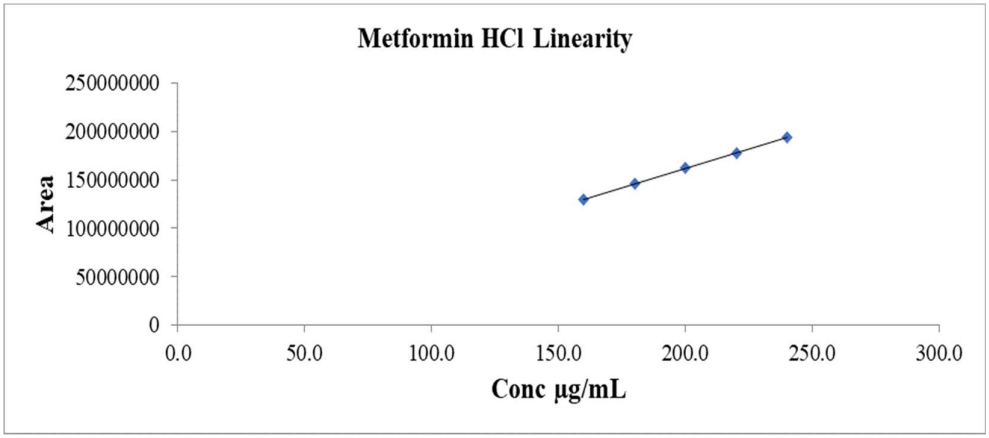


Figure 1: Calibration curve of Metformin hydrochloride

Table 4: Result of data of linearity of Metformin hydrochloride

Sr no.	Parameter	Result value	Acceptance criteria
1	Beer's linearity range	160.0 – 240.0 µg/mL	NA
2	Correlation coefficient (R^2)	0.99993	NLT 0.98
3	Intercept	2662697.80	To be report
4	Slope	795297.74	To be report
5	% RSD for area at each level	NA	NMT 2.0

Table 5: Linearity Data for Vildagliptin:

Level	Conc (µg/mL)	Area	Mean	% RSD
80%	16.00	10002562	10016779	0.383
		9987524		
		10060251		
90%	18.00	11246643	11256720	0.407
		11306726		
		11216790		
100%	20.00	12488635	12483943	0.281
		12446790		
		12516403		
110%	22.00	13731278	13735051	0.365
		13786940		
		13686934		
120%	24.00	14958137	14902150	0.378
		14845620		
		14902692		

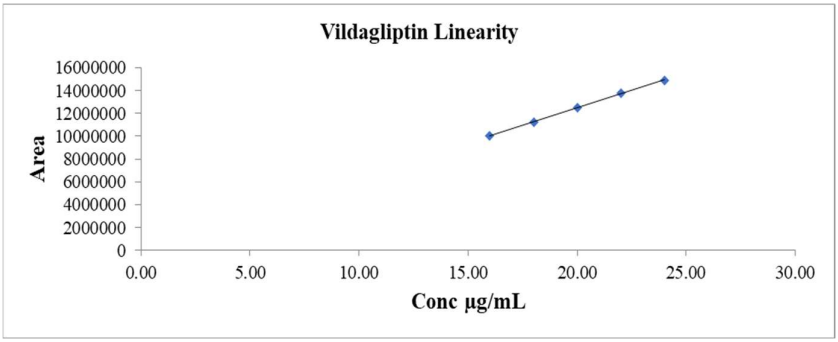


Figure 2: Calibration curve of Vildagliptin

Table 6: Result of data of linearity of Vildagliptin

Sr no.	Parameter	Result value	Acceptance criteria
1	Beer's linearity range	16.0-24.0 µg/mL	NA
2	Correlation coefficient (R^2)	0.99994	NLT 0.98
3	Intercept	229855.60	To be report
4	Slope	612453.65	To be report
5	% RSD for area at each level	NA	NMT 2.0

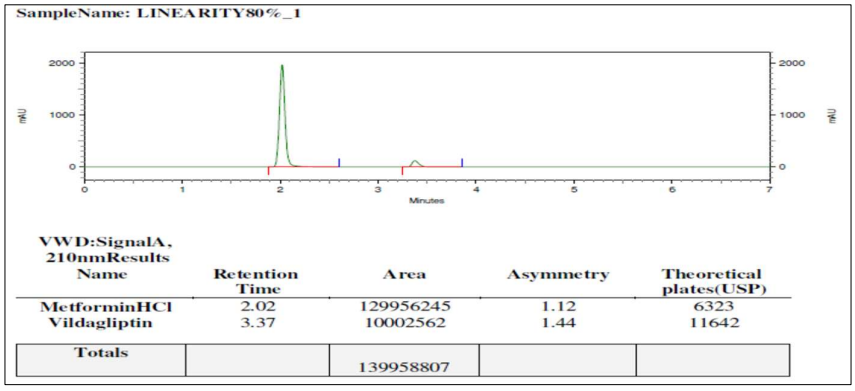


Figure 3: Typical chromatogram of Linearity 80%

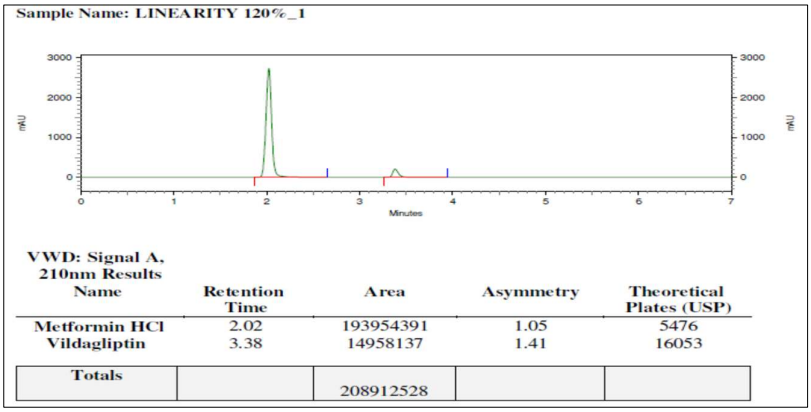


Figure 4: Typical chromatogram of Linearity 120%

Conclusion: From the calibration curve it was concluded that the Metformin hydrochloride and Vildagliptin shows linear response in the proposed range. The Regression value was found well within the limit.

2) Limit of Detection (LOD) and Limit of Quantitation (LOQ):

A) LOD and LOQ for Metformin hydrochloride:

Table 7: Result Summary of LOD and LOQ

Drug	LOD (µg/mL)	LOQ (µg/mL)
Metformin hydrochloride	1.248	3.781
Vildagliptin	0.116	0.353

3) ACCURACY (RECOVERY)

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. The accuracy of an analytical method is determined by applying the method to analyzed samples to which known amounts of analyte have been added.

Table 8: Result and statistical data of Accuracy of Metformin hydrochloride

Level (%)	Area	Metformin hydrochloride Recovered conc (µg/mL)	Metformin hydrochloride Added conc (µg/mL)	% Recovery	Mean % Recovery	% RSD
80	128436023	157.81	160.00	98.63	99.42	0.849
	130784613	160.70	160.20	100.31		
	129430724	159.03	160.10	99.33		
100	160346705	197.02	200.10	98.46	98.80	0.676
	160357924	197.03	200.30	98.37		
	162074057	199.14	200.00	99.57		
120	193403760	237.64	240.10	98.97	99.85	1.062
	197427564	242.58	240.10	101.03		
	194637054	239.15	240.20	99.56		

Overall Recovery of Metformin hydrochloride: 99.36 %
% RSD for Overall Recovery Metformin hydrochloride: 0.890

Table 9: Result and statistical data of Accuracy of Vildagliptin

Level (%)	Area	Vildagliptin Recovered conc (µg/mL)	Vildagliptin Added Conc (µg/mL)	% Recovery	Mean % Recovery	% RSD
80	10198025	16.32	16.30	100.12	99.22	0.824
	9901083	15.84	16.00	99.03		
	9973025	15.96	16.20	98.52		
100	12460148	19.94	20.10	99.20	99.16	0.369
	12530254	20.05	20.30	98.78		
	12498047	20.00	20.10	99.51		
120	14903690	23.85	24.20	98.56	99.58	0.959
	15020747	24.04	24.10	99.74		
	15190147	24.31	24.20	100.45		

Overall Recovery of Vildagliptin: 99.32 %
% RSD for Overall Recovery Vildagliptin: 0.688

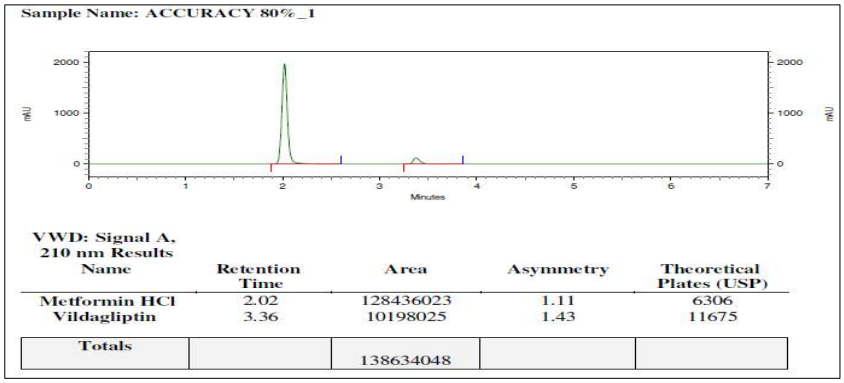


Figure 5: Typical chromatogram of Accuracy 80%

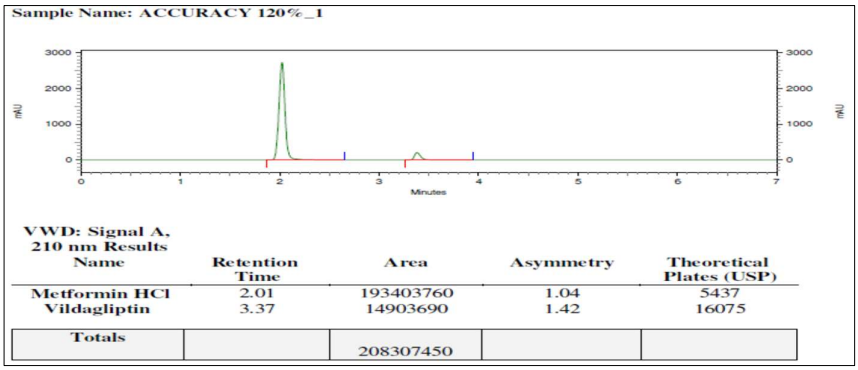


Figure 6: Typical chromatogram of Accuracy 120%.

Data interpretation: Recovery of analytical procedure was found well within acceptance criteria at all 3 levels. % Recovery not get hampered by changed in analyte concentration.

4) PRECISION

Precision of an analytical method is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings of a homogenous sample. Precision of an analytical method is usually expressed as standard deviation or relative standard deviation. Precision was performed on Test sample.

Table 10: Result for Metformin hydrochloride of Intra- day and Inter- Day Precision of test sample assay

	Sample	Test Sample (mg)	Area	% Assay
Repeatability	Sample 1	499.4	160352145	98.46
	Sample 2	498.9	163630204	100.57
	Sample 3	499.2	162035840	99.53
	Sample 4	499.1	158698201	97.50
	Sample 5	498.8	160481097	98.66
	Sample 6	499.6	162014852	99.44
	Mean			99.03
	STD DEV			1.058299
	% RSD			1.069
Intermediate precision (Inter-Day)	Sample 1	498.9	163402756	100.43
	Sample 2	499.2	159673401	98.08
	Sample 3	498.7	164324309	101.04
	Sample 4	499.4	157963042	96.99
	Sample 5	499.1	159902754	98.24
	Sample 6	499.5	161964612	99.43
	Mean			99.03
	STD DEV			1.538727
	% RSD			1.554
Repeatability Plus Inter-day	Mean			99.030
	STD DEV			1.25910
	% RSD			1.271

Table 11: Result for Vildagliptin of Intra- day and Inter- Day Precision of test sample assay

	Sample	Test Sample (mg)	Area	% Assay
Repeatability	Sample 1	499.4	12460143	99.64
	Sample 2	498.9	12132561	97.12
	Sample 3	499.2	12403256	99.23
	Sample 4	499.1	12654631	101.26
	Sample 5	498.8	12278569	98.31
	Sample 6	499.6	12345860	98.69
	Mean			99.04
	STD DEV			1.390317
	% RSD			1.404
Intermediate precision (Inter-Day)	Sample 1	498.9	12630254	101.11
	Sample 2	499.2	12303651	98.43
	Sample 3	498.7	12458601	99.77
	Sample 4	499.4	12600191	100.76
	Sample 5	499.1	12349781	98.82
	Sample 6	499.5	12196404	97.52
	Mean			99.40
	STD DEV			1.395140
	% RSD			1.404
Repeatability Plus Inter-day	Mean			99.222
	STD DEV			1.34109
	% RSD			1.352

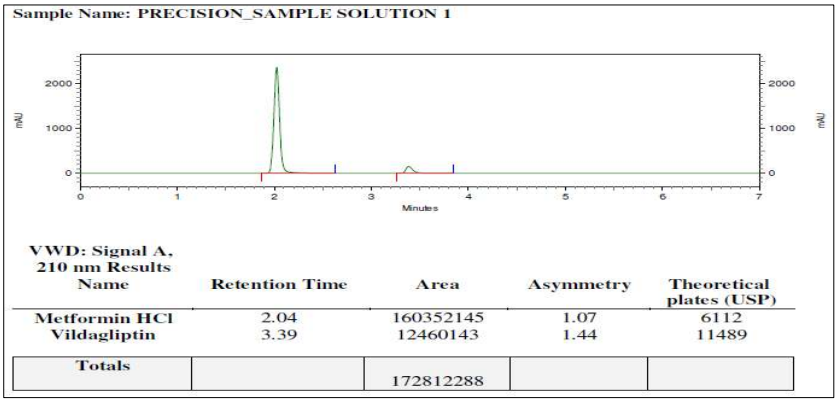
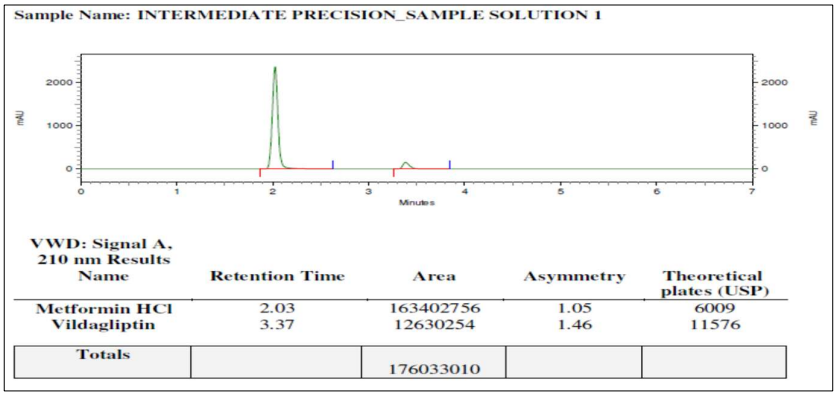


Figure 7: Typical chromatogram of Repeatability precision (Sample 1)



VWD: Signal A, 210 nm Results Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Metformin HCl	2.03	163402756	1.05	6009
Vildagliptin	3.37	12630254	1.46	11576
Totals		176033010		

Figure 8: Typical chromatogram of Inter-day precision (Sample 1)

% Assay: % Assay value for each sample (Individual sample) and mean assay value for precision (6 sample), mean assay value intermediate precision (6 sample) and mean assay value for precision plus intermediate precision sample (12 sample): 90-110%

% RSD: % RSD for precision study samples (6 sample), Intermediate precision study samples (6 sample) and precision plus intermediate precision sample (12 sample): NMT 2.0

Data interpretation: % Assay and% RSD was found well within acceptance limit and hence method is precise (Reproducible).

5) ROBUSTNESS:

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Following changes made under Robustness:

- Change in Wavelength
- Change in flow rate
- Change in column oven temperature

Result of Robustness study:

Metformin hydrochloride

Table 12: Results of Metformin hydrochloride Robustness study

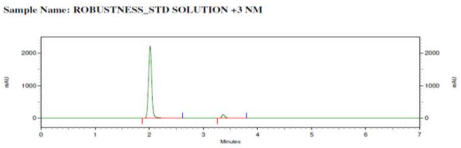
Change in Parameter	R.T.	Standard area	Asymmetry	Theoretical plates
Wavelength by +3 NM (213 NM)	2.02	150474952	1.07	5952
Wavelength by -3 NM (207 NM)	2.02	172767100	1.06	5975
Flow rate by +10% (1.1 mL/min)	1.83	147738898	1.08	5574
Flow rate by -10% (0.9 mL/min)	2.24	179898929	1.08	6393
Column oven temp by +2ºC (42 ºC)	2.04	161460480	1.08	5974
Column oven temp by -2ºC (38 ºC)	2.02	162163486	1.07	5996

A) Vildagliptin

Table 13: Results of Vildagliptin Robustness study

Change in Parameter	R.T.	Standard area	Asymmetry	Theoretical plates
Wavelength by +3 NM (213 NM)	3.37	8036226	1.38	16503
Wavelength by -3 NM (207 NM)	3.37	16981519	1.35	17110
Flow rate by +10% (1.1 mL/min)	3.06	11336433	1.34	15589
Flow rate by -10% (0.9 mL/min)	3.74	13869931	1.37	19434
Column oven temp by +2ºC (42 ºC)	3.37	12436907	1.43	11612
Column oven temp by -2ºC (38 ºC)	3.39	12459639	1.46	11508

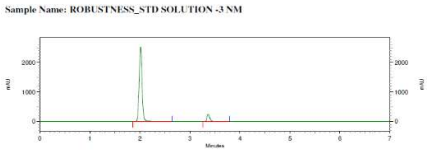
A. Change in Wavelength by +3 NM:



VWD: Signal A, 213 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Metformin HCl	2.02	150474952	1.07	5952
Vildagliptin	3.37	8036226	1.38	16503
Totals		158511178		

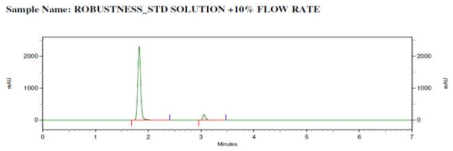
Typical chromatogram of Standard +3 NM.



VWD: Signal A, 207 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Metformin HCl	2.02	172767100	1.06	5975
Vildagliptin	3.37	16981519	1.35	17110
Totals		189748619		

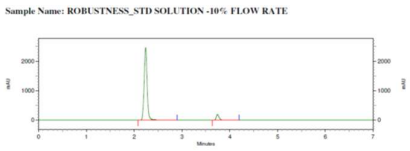
Change in Wavelength by -3 NM



VWD: Signal A, 210 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Metformin HCl	1.83	147738898	1.08	5574
Vildagliptin	3.06	11336433	1.34	15589
Totals		159075331		

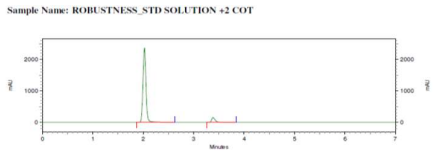
Change in Flow rate by + 10%



VWD: Signal A, 210 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Metformin HCl	2.24	179898929	1.08	6393
Vildagliptin	3.74	13869931	1.37	19434
Totals		193768860		

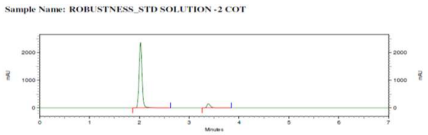
Change in Flow rate by - 10%



VWD: Signal A, 210 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Metformin HCl	2.04	161460480	1.08	5974
Vildagliptin	3.37	12436907	1.43	11612
Totals		173897387		

Change in Column temp by +2°C



VWD: Signal A, 210 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Metformin HCl	2.02	162163486	1.07	5996
Vildagliptin	3.39	12459639	1.46	11508
Totals		174623125		

Change in Column temp by -2°C

Acceptance criteria:

Chromatography (System suitability) acceptance criteria should not get failed.

Data interpretation: From the above results, it was concluded that the system suitability test result was found well within the limits and analytical method was robust.

3. CONCLUSION:

The present work involved the development of simple, accurate, precise and suitable RP-HPLC method. Literature survey revealed that several methods have been reported for determination of Vildagliptin in bulk drug or in pharmaceutical dosage forms. Hence, in the present study, a new, sensitive and suitable reversed-phase high performance liquid chromatography method was developed and validated for the determination of Metformin hydrochloride in bulk drug and pharmaceutical dosage form.

In developed RP-HPLC method, the analyte were resolved by using isocratic program and mobile phase was used Acetonitrile :0.05%TFAA in water (15:85 % v/v) at a flow rate of 1.0 ml/min, on HPLC system

containing UV- visible detector with Openlab EZChrome Software and Kromasil C18, 250 mm X 4.6 mm, 5 µm. The detection was carried out at 210 nm. The results of analysis in the developed method were validated in terms of linearity, accuracy, precision, robustness, limit of detection and limit of quantification.

The developed method has several advantages, including reproducibility of results, rapid analysis, simple sample preparation and improved selectivity as well as sensitivity. The regression coefficient (r2) for each analyte is not less than 0.999 which shows good linearity. The % recovery was in the acceptable range in tablet dosage form. The %RSD was also less than 2% showing high degree of precision of the proposed method. Since the developed method is robust and reproducible and also less time consuming, it can be performed for routine analysis in pharmaceutical industry for bulk drug of Vildagliptin and Metformin hydrochloride and also in pharmaceutical dosage form

4. REFERENCE:

[1] Shirode AR, Maduskar PD, Deodhar MS, Kadam VJ. RP-HPLC and HPTLC Methods for Simultaneous Estimation of Metformin Hydrochloride and Vildagliptin from Bulk and Marketed Formulation: Development and Validation.

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