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Original Article

STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS DETERMINATION OF VILDAGLIPTIN AND METFORMIN IN PHARMACEUTICAL DOSAGE FORM

RAMESH JAYAPRAKASH*, SENTHIL KUMAR NATESAN

Department of Pharmaceutical Analysis, JKK Munirajah Medical Research Foundation's-Annai JKK Sampoorani Ammal College of Pharmacy, Komarapalayam, Namakkal-DT, Tamilnadu, India
Email: rameshj1974@gmail.com

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ABSTRACT

Objective: The present study was aimed to develop a rapid, accurate, linear, sensitive and validate stability-indicating high performance liquid chromatographic [RP-HPLC] method for determination of vildagliptin and metformin in pharmaceutical dosage form.

Methods: The chromatographic separation was performed on kromasil-C_{18} column [4.5 x 250 mm; 5 µm] using a mobile phase consisting of 0.05 mmol potassium dihydrogen phosphate buffer: acetonitrile [80:20 v/v], [pH adjusted to 3.5 using orthophosphoric acid]. The flow rate is 0.9 ml/min and the detection was carried out at 263 nm.

Results: The chromatographic condition, the peak retention time of metformin and vildagliptin were found to be 2.215 min and 2.600 min respectively. Stress testing was performed in accordance with an international conference on harmonization [ICH] Q1A R2 guidelines. The method was validated as per ICH Q2 R1 guidelines. The calibration curve was found to be linear in the concentration range of 5-17.5 µg/ml and 50-175 µg/ml for vildagliptin and metformin. The limit of detection and quantification was found to be 0.0182 µg/ml and 0.0553 µg/ml for vildagliptin and 0.4451 µg/ml and 1.3490 µg/ml for metformin respectively.

Conclusion: A new sensitive, simple and stability indicating reverse-phase high-performance liquid chromatography [RP-HPLC] method has been developed and validated for the determination of vildagliptin and metformin. The proposed method can be used for routine determination of vildagliptin and metformin.

Keywords: Metformin, Method validation, RP-HPLC, Stability indicating method, Vildagliptin

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INTRODUCTION

Vildagliptin [fig. 1] is chemically [S]-1-[N-[3-hydroxy-1-adamantyl] glycyl] pyrrolidine-2-carbonitrile, is a potent di-peptidyl peptidase IV [dip-IV] inhibitor, a drug for the treatment of diabetes. DPP-IV inhibitor represents a new class of oral antihyperglycemic agents to treat patients with type 2 diabetes [1-4].

Metformin HCl [fig. 2] chemically 3-[diaminomethylidene]-1,1-dimethylguanidine HCl is an orally administered biguanide widely used in the treatment of type 2 [non-insulin dependent] diabetes mellitus [5-6].

Literature survey revealed that RP-HPLC [7-16] have been reported for the estimation of vildagliptin and metformin.

To the best of our knowledge, no stability indicating reverse-phase high-performance liquid chromatography [RP-HPLC] method has been reported for vildagliptin and metformin. The present work involves stress degradation as per ICH Q1A [R2] and Q1B [17-18] for the developing new, simple, sensitive stability indicating RP-HPLC method; the method was validated as per the ICH guidelines Q2 R1 [19].

The reported methods were not cost effective due to use costly solvents and in some methods the retention time vildagliptin was found to be more [7-16]. So in the present work a simple, precise, sensitive and stability indicating method was developed by using low-cost solvent acetonitrile with buffer in the ratio of 80:20, detected by using a photodiode array detector which was highly sensitive to detect at a lower concentration. The developed method was used for estimation of vildagliptin and metformin in pharmaceutical dosage form.

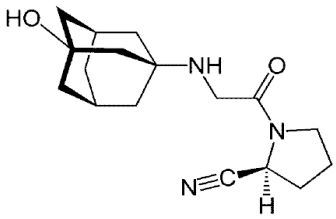

Fig. 1: Chemical structure of vildagliptin

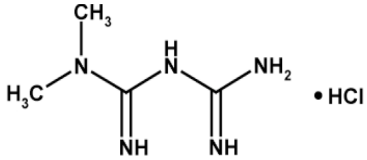

Fig. 2: Chemical structure of metformin Hcl

MATERIALS AND METHODS

Chemicals and reagents

Vildagliptin was provided as a gift sample by Aurobindo pharmaceuticals limited [Hyderabad, India]. Metformin HCl was provided as a gift sample by Ranbaxy laboratories limited [Gurgaon, India]. Acetonitrile [HPLC grade] purchased from E-Merck specialities private. Limited [Mumbai, India], and Potassium

dihydrogen phosphate [KH_2PO_4] purchased from Qualigens fine chemicals limited [Mumbai, India].

Instruments

Chromatography was performed on Waters HPLC 2695 equipped with quaternary pumps with PDA detector. The chromatographic separation was performed using kromasil-C_{18} column [4.5 x 250 mmx5 µm particle sizes]. Data acquisition and integration were performed using empower 2 software.

Methods

Chromatographic conditions

The method development for analysis of vildagliptin and metformin Hcl was performed using various solvents finally the separation was achieved using a mobile phase consisting of 0.05 mmol potassium dihydrogen phosphate buffer: acetonitrile [80:20 v/v], pH adjusted to 3.5 using orthophosphoric acid, pumped at a flow rate of 0.9 ml/min. The eluent was monitored using a PDA detector at a wavelength of 263 nm. The mobile phase was vacuum filtered through 0.22 µm nylon membrane filter followed by degassing in an ultrasonic bath prior to use.

Preparation of solutions

Preparation of buffer solution

The buffer solution was prepared by dissolving 0.68g of potassium dihydrogen orthophosphate in 1000 ml of water, and the pH was adjusted to 3.5 by adding orthophosphoric acid dropwise.

Preparation of standard solutions

The quantity of powder equivalent to 50 mg of vildagliptin and 500 mg of metformin were weighed and transferred into a 100 ml volumetric flask, 30 ml of diluent was added and sonicated for 15 min and the volume was made up to the mark with diluent. From this solution, further dilution was made to get the final concentration of 12.5 µg/ml and 125 µg/ml of vildagliptin and metformin respectively.

Validation of the developed method

The optimized analytical method was validated for system suitability, linearity and range, precision, limit of detection [LOD], limit of quantitation [LOQ] and accuracy in accordance with ICH guidelines for analytical procedures Q2[R1].

System suitability

System suitability parameters were studied to verify the system performance. Six replicate samples containing vildagliptin [12.5 µg/ml] and metformin [125 µg/ml] were analyzed using the developed method. Factors such as theoretical plate count, tailing factor, percent relative standard deviation [%RSD] of peak area and retention time were taken into consideration for testing system suitability.

Linearity and range

The linearity was evaluated at six concentration levels in the range between 5-17.5 µg/ml for vildagliptin and 50-175 µg/ml for metformin. A calibration curve was plotted by plotting concentration against corresponding peak area and linearity was determined using least square regression analysis. The analytical range was established by the highest and lowest concentrations of analyte were acceptable linearity obtained.

Precision

The precision of the developed analytical method carried out for same concentration level; six determinations were performed, both intra-day and inter-day variation were expressed in term of percent relative standard deviation [% RSD].

LOD and LOQ

Limit of detection and quantification of the developed method were calculated from the standard deviation of the y-intercepts and slope of the calibration curve of vildagliptin and metformin using the following formula:

$$\text{Limit of detection} = 3.3\ \alpha/S$$

$$\text{Limit of quantitation} = 10\ \alpha/S$$

Where α is the standard deviation of the y–intercepts and S is the slope of the calibration curve.

Accuracy

The accuracy of the developed analytical method was determined by calculating recovery of the analyte of interest. A fixed amount of pre-analyzed sample was taken and the standard drug was added at 80%, 100% and 120% levels. The standard concentration was fixed as 12.5 µg/ml of vildagliptin and 125 µg/ml of metformin, and three concentration levels of 10 µg/ml, 12.5 µg/ml and 15 µg/ml for vildagliptin and 100 µg/ml, 125 µg/ml and 150 µg/ml for metformin were added to the standard concentration. Each level was repeated three times. The percentage recovery and percentage relative standard deviation [% RSD] were taken into consideration for testing accuracy.

Application of validated method for assay of vildagliptin and metformin in pharmaceutical dosage form

Tablet powder equivalent to 50 mg of vildagliptin and 500 mg of metformin were weighed and transferred into a 100 ml volumetric flask, 30 ml of diluent was added and sonicated for 15 min and the volume was made up to the mark with diluent. From this solution further dilution was made to get the final concentration of 12.5 µg/ml of vildagliptin and 125 µg/ml of metformin, ten microliters of final solutions were injected into the HPLC system; chromatograms were recorded and from the peak areas of vildagliptin and metformin, the amount of drug present in sample was computed.

Forced degradation study

Acid hydrolysis

Optimization trial

Initially, trials were conducted by exposing the sample solution to 0.1N hydrochloric acid for 8 h followed by 1N hydrochloric acid for 12 h and no degradation was observed.

Optimized trial

A quantity tablet powder equivalent to 50 mg of vildagliptin and 500 mg of metformin was accurately weighed and transferred to 100 ml volumetric flask and it is dissolved in 30 ml of the diluent. The solution was sonicated for a few minutes to dissolve the drug completely. Then it is filtered through 0.45 µ filter and the volume was made up to 100 ml with diluent. Further pipette 1 ml of the above stock solution and transferred to 10 ml volumetric flask and made up to 10 ml with diluent, from the above solution pipette 2.5 ml and transferred to 10 ml volumetric flask to that 2.5 ml of 2N hydrochloric acid was added and it was diluted to get the final concentration of 12.5 µg/ml of vildagliptin and 125 µg/ml of metformin and refluxed for 30 min at 80 °C, 10 µl of the refluxed solutions were injected into the system, and the chromatograms were recorded.

Alkaline hydrolysis

Optimization trial

Initially, trials were conducted by exposing the sample solution to 0.1N sodium hydroxide for 8 h followed by 1N sodium hydroxide for 12 h and no degradation was observed.

Optimized trial

A quantity tablet powder equivalent to 50 mg of vildagliptin and 500 mg of metformin was accurately weighed and transferred to a 100 ml volumetric flask and it is dissolved in 30 ml of the diluent. The solution was sonicated for a few minutes to dissolve the drug completely. Then it is filtered through 0.45 µ filter and the volume was made up to 100 ml with diluent. Further pipette 1 ml of the

above stock solution and transferred to 10 ml volumetric flask and made up to 10 ml with diluent, from the above solution pipette 2.5 ml and transferred to 10 ml volumetric flask to that 2.5 ml of 2N sodium hydroxide was added and it was diluted to get the final concentration of 12.5 µg/ml of vildagliptin and 125 µg/ml of metformin and refluxed for 30 min at 80 °C, 10 µl of the refluxed solutions were injected into the system and the chromatograms were recorded.

Degradation under neutral condition

A quantity tablet powder equivalent to 50 mg of vildagliptin and 500 mg of metformin was accurately weighed and transferred to a 100 ml volumetric flask and it is dissolved in 30 ml of the diluent. The solution was sonicated for a few minutes to dissolve the drug completely. Then it is filtered through 0.45 µ filter and the volume was made up to 100 ml with diluent.

Further pipette 1 ml of the above stock solution and transferred to 10 ml volumetric flask and made up to 10 ml with diluent, from the above solution pipette 2.5 ml and transferred to 10 ml volumetric flask and made up to 10 ml with diluent to get the final concentration of 12.5 µg/ml of vildagliptin and 125 µg/ml of metformin and the solution was refluxed in water bath for 30 min at 80 °C and 10 µl of the refluxed solutions were injected into the system and the chromatograms were recorded.

Degradation under oxidative condition

A quantity tablet powder equivalent to 50 mg of vildagliptin and 500 mg of metformin was accurately weighed and transferred to a 100 ml volumetric flask and it is dissolved in 30 ml of the diluent. The solution was sonicated for a few minutes to dissolve the drug completely. Then it is filtered through 0.45 µ filter and the volume was made up to 100 ml with diluent.

Further pipette 1 ml of the above stock solution and transferred to 10 ml volumetric flask and made up to 10 ml with diluent, from the above solution pipette 2.5 ml and transferred to 10 ml volumetric flask to that 2.5 ml of 3% hydrogen peroxide [H_2O_2] was added and it was diluted to get the final concentration of 12.5 µg/ml of vildagliptin and 125 µg/ml of metformin and refluxed for 30 min at 80 °C, 10 µl of the refluxed solutions were injected into the system and the chromatograms were recorded.

Degradation under wet heat condition

A quantity tablet powder equivalent to 50 mg of vildagliptin and 500 mg of metformin was accurately weighed and transferred to a 100 ml volumetric flask and it is dissolved in 30 ml of the diluent. The solution was sonicated for a few minutes to dissolve the drug completely. Then it is filtered through 0.45 µ filter and the volume was made up to 100 ml with diluent. Further pipette 1 ml of the above stock solution and transferred to 10 ml volumetric flask and made up to 10 ml with diluent, from the above solution pipette 2.5 ml and transferred to 10 ml volumetric flask and made up to 10 ml with diluent to get the final concentration of 12.5 µg/ml of vildagliptin and 125 µg/ml of metformin and the solution was placed in oven at 80 °C for 48 h, 10 µl of the solutions were injected into the system and the chromatograms were recorded.

Photo-degradation studies

A quantity tablet powder equivalent to 50 mg of vildagliptin and 500 mg of metformin was accurately weighed and transferred to a 100 ml volumetric flask and it is dissolved in 30 ml of the diluent. The solution was sonicated for a few minutes to dissolve the drug completely. Then it is filtered through 0.45 µ filter and the volume was made up to 100 ml with diluent. Further pipette 1 ml of the above stock solution and transferred to 10 ml volumetric flask and made up to 10 ml with diluent, from the above solution pipette 2.5 ml and transferred to 10 ml volumetric flask and made up to 10 ml with diluent to get the final concentration of 12.5 µg/ml of vildagliptin and 125 µg/ml of metformin and the solution was exposed to UV light by keeping the volumetric flask in UV chamber for 7 d, 10 µl of the solutions were injected into the system and the chromatograms were recorded.

RESULTS AND DISCUSSION

Method development

Optimization of mobile phase

Method development for vildagliptin and metformin was started with a different combination of solvents with different ratios. We have tried various buffer-solvent ratios [50:50, 60:40, 70:30, 80:20, and 90:10]. However, finally a combination of 0.05 mmol potassium dihydrogen phosphate buffer [pH adjusted to 3.5 using orthophosphoric acid]: acetonitrile 80:20 v/v has shown good resolution for vildagliptin and metformin. Initially, 1.0 ml/min flow rate was tried it has not shown adequate separation between vildagliptin and metformin, but further increase and decrease in the flow rate 0.9 ml/min shows good resolution between the peaks of vildagliptin and metformin. Some reported methods use costly solvents and were found to be less sensitive [7-9]. So the present work was developed by using a low-cost solvent buffer with acetonitrile in ratio 80:20.

Chromatographic conditions

The analytical conditions were selected, keeping in mind the chemical nature of vildagliptin and metformin. The development trails were taken using different conditions. The column selection has been done on the basis of back pressure, peak shape, theoretical plates and day-to-day reproducibility of the retention time. After evaluating all these factors, the chromatographic separation was carried out on kromasil-C_{18} column [4.5 x 250 mm; 5 µm] using a mobile phase consisting of 0.05 mmol potassium dihydrogen phosphate buffer [pH adjusted to 3.5 using orthophosphoric acid]: acetonitrile [80:20 v/v], the flow rate 0.9 ml/min and the injection volume was 10 µl, the detection was carried out at 263 nm.

The peak retention time of metformin and vildagliptin were found to be 2.215 min and 2.600 min respectively. The retention times in all the reported methods were more when compared to the developed method [10-14]. Hence this method was finalised as an optimized method for the estimation of vildagliptin and metformin. The optimised chromatographic condition was shown in table 1 and the typical HPLC chromatogram of standard and sample were shown in fig. 1 and 2.

Table 1: Optimised chromatographic condition for the estimation of vildagliptin and metformin

Parameter	Condition
Mobile phase	0.05 mmol potassium dihydrogen phosphate buffer: acetonitrile [80:20 v/v], [pH adjusted to 3.5 using orthophosphoric acid]
Diluent	Mobile phase
Column	Kromasil-C_{18} column [4.5 X 250 mm; 5 µm]
Column temperature	30 °C
Detection wavelength	263 nm
Injection volume	10 µl
Flow rate	0.9 ml/min
Runtime	6 min

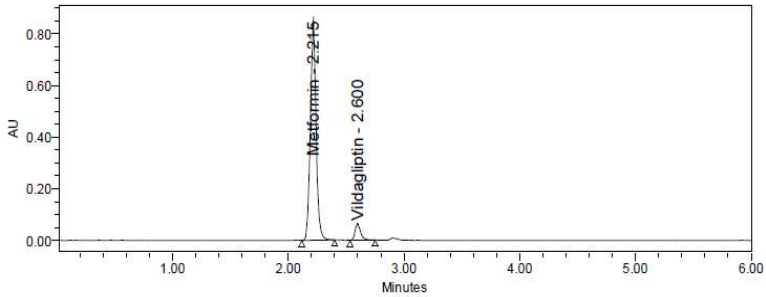

Fig. 1: Typical HPLC chromatogram of standard

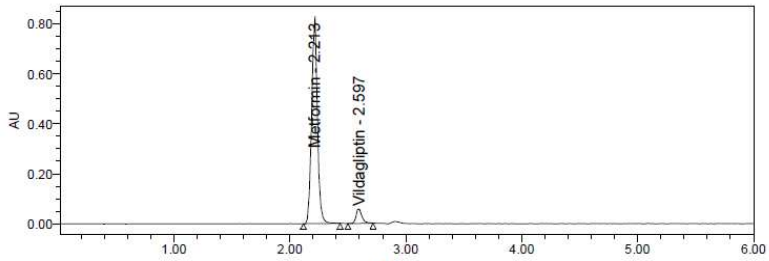

Fig. 2: Typical HPLC chromatogram of sample

Method validation

System suitability

The developed method has produced theoretical plate above 2000 for vildagliptin and metformin with tailing factor less than 2. Similarly, the percent relative standard deviation [% RSD] of peak area and retention time of vildagliptin and metformin were less than 2, which ensure the suitability of the developed method. The results of the system suitability study were summarised in table 2.

Acceptance criteria

1. The relative standard deviation of six replicate injections for peak area and retention time should not be more than 2.0%.
2. The tailing factor should not be more than 2.
3. The theoretical plates should not be less than 2000.

Linearity and range

For linearity of six point's calibration curve were obtained in concentration ranges from 5-17.5 µg/ml for vildagliptin and 50-175 µg/ml for metformin. The response of the drug was found to be linear in the selected concentration range the correlation coefficient for vildagliptin and metformin were 0.9935 and 0.9933 respectively.

The results of linearity of vildagliptin and metformin were summarised in table 3.

Precision

The developed method has shown percent relative standard deviation [% RSD] less than 2 for both intra-day and inter-day precision study, which ensures precision of the developed method. The results of the precision study were summarised in table 4 and 5.

Table 2: System suitability of the developed method

Parameters	Name of drug		Acceptance criteria
	Vildagliptin	Metformin	
Retention time	2.600 min	2.215 min	---
Theoretical plates [N]	7679	9373	>2000
Tailing factor	1.26	1.06	<2
Asymmetry factor	1.68	1.68	<2
Capacity factor	1.14	2.01	>1<10
Resolution	2.58		>2
% RSD of Peak area	0.1305	0.2023	<2
% RSD of Retention time	0.7206	0.2435	<2

Table 3: Linearity and range of the developed method

S. No.	Vildagliptin		Metformin	
	Concentration (µg/ml)	Mean peak area (n = 6)	Concentration (µg/ml)	Mean peak area (n = 6)
1	5	51147	50	765148
2	7.5	109332	75	1381755
3	10	160394	100	2055238
4	12.5	210773	125	2818825
5	15	259822	150	3376178
6	17.5	306751	175	4138245
Slope		18313.98	Slope	24270.89
y-intercept		-19710.41	y-intercept	-263923.11
Correlation coefficient		0.9935	Correlation coefficient	0.9933

Table 4: Intra-day and inter-day precision of the developed method for vildagliptin

Parameter	Intra-day			Parameter	Inter-day	
	Concentration (µg/ml)	Peak area*	% amount found*		Peak area*	% amount found*
0 H	12.5	212897	101.00	Day-I	212960	101.03
3 H		212903	101.05	Day-II	212928	101.01
6 H		213027	101.06	Day-III	212811	100.96
SD			0.2421	SD		0.2349
% RSD			0.2398	% RSD		0.2327

* Mean of six determinations

Table 5: Intra-day and inter-day precision of the developed method for metformin

Parameter	Intra-day			Parameter	Inter-day	
	Concentration (µg/ml)	Peak area*	% Amount found*		Peak area*	% Amount found*
0 H	125	2807870	99.60	Day-I	2824936	100.21
3 H		2817655	99.95	Day-II	2809835	99.67
6 H		2813222	99.79	Day-III	2803669	99.45
SD			0.5391	SD		0.5684
% RSD			0.5403	% RSD		0.5696

* Mean of six determinations

Limit of detection and limit of quantification

Limit of detection [LOD] and limit of quantification [LOQ] was estimated from the standard deviation of the y-intercepts and slope of the calibration curve of vildagliptin and metformin. The LOD and LOQ were found to be 0.0182 and 0.0553 µg/ml for vildagliptin and 0.4451 and 1.3490 µg/ml for metformin. This showed that the developed method can detect and quantify at lower concentration was highly sensitive whereas other methods is less sensitive [7-14]

Accuracy

The percentage recovery of the spiked sample was within 100±2% which ensures the accuracy of the developed method. The results of recovery studies were summarised in table 6 and 7.

Robustness

As per ICH, the prepared solution was analysed as per the proposed method with a small but deliberate change in chromatographic conditions as listed below table 8.

- Change in flow rate
- Change in mobile phase composition
- Change in nanometer
- Change in temperature
- Change in pH

Table 6: Accuracy of the developed method for vildagliptin

Parameters	Amount present (µg/ml)	Amount spiked (µg/ml)	Peak area	Amount found (µg/ml)	Amount recovered (µg/ml)	% amount recovered
80%	12.5	10	379281	22.49	9.99	100.33
			378455	22.44	9.94	99.84
			377832	22.40	9.90	99.47
100%	12.5	12.5	421146	24.97	12.47	99.97
			421538	24.99	12.49	100.15
			421379	24.99	12.49	100.08
120%	12.5	15	464792	27.56	15.06	100.97
			464586	27.55	15.05	100.88
			464353	27.53	15.03	100.79
Average						100.27
SD						0.4831
% RSD						0.4818
SE						0.1708
CI (Confidence Interval 99%)						99.72-100.81

Table 7: Accuracy of the developed method for metformin

Parameters	Amount Present (µg/ml)	Amount spiked (µg/ml)	Peak Area	Amount Found (µg/ml)	Amount Recovered (µg/ml)	% Amount Recovered
80%	125	100	5068347	224.75	99.75	99.78
			5065973	224.64	99.64	99.67
			5096352	225.99	100.99	101.02
100%	125	125	5630324	249.67	124.67	99.80
			5629112	249.62	124.62	99.76
			5630984	249.70	124.70	99.82
120%	125	150	6199615	274.92	149.92	100.05
			6195824	274.75	149.75	99.94
			6198142	274.85	149.85	100.02
Average						99.98
SD						0.3845
% RSD						0.3846
SE						0.1360
CI (Confidence Interval 99%)						99.54-100.41

Table 8: Robustness of the developed method for vildagliptin and metformin

Parameters	Vildagliptin			Metformin		
	R. T	% Amount found	% RSD	R. T	% Amount found	% RSD
Flow minus (0.7 ml/min)	2.591	99.17	0.3103	2.200	99.58	0.5470
Flow plus (1.1 ml/min)	2.597	99.13	0.5143	2.209	99.53	0.5955
pH minus (-0.2)	2.579	98.99	0.3088	2.200	99.51	0.8448
pH plus (+0.2)	2.600	98.97	0.1975	2.212	99.63	0.4982
nm plus (265)	2.609	98.98	0.4710	2.213	99.58	0.6701
nm minus (261)	2.609	99.11	0.2459	2.215	98.94	0.4900
Temperature plus (32 ℃)	2.599	99.18	0.3548	2.212	99.18	0.3780
Temperature minus (28 ℃)	2.560	98.87	0.4392	2.213	99.15	0.5752
Acetonitrile (25)	2.564	99.20	0.4157	2.221	98.95	0.2893
Acetonitrile (15)	2.567	99.02	0.3977	2.219	99.09	0.4153

*Mean of six determinations, R. T = retention time

Application of validated method for assay of vildagliptin and metformin in pharmaceutical dosage form

Developed method was successfully implemented in the assay of vildagliptin and metformin in pharmaceutical dosage form. Assay of vildagliptin was found to be 99.75% and for metformin 99.80%. The results of the assay were summarised in table 8.

The method validation results were satisfactory as per ICH Q2R1 guidelines. The peak areas were found to be linear over the concentration range 5-17.5 µg/ml and 50-175 µg/ml with a correlation coefficient of 0.9935 and 0.9933 for vildagliptin and metformin respectively. Method specificity can be proved using "Peak purity" parameter in empower 2 software of HPLC. The unaffected assay of the drug in the blend confirms the non-interference by any excipient. Intra-day and inter-day precision were less than 2%. Percent recovery in accuracy study was within the limit of 98 to 102%. The results of validation were summarised in table 9.

Thus, the optimised HPLC method for vildagliptin and metformin is based on the use of the buffer and acetonitrile. The drug was found to be prone to acid and alkali-catalyzed hydrolysis. The degradation products observed under these conditions were well resolved from the drug peak. Thus the developed method is stability indicating.

Table 8: Assay of vildagliptin and metformin in pharmaceutical dosage form

Formulation	Vildagliptin			Metformin		
	Label claim (mg)	Amount found (mg)	% Assay	Label claim (mg)	Amount found (mg)	% assay
Galvusmet (vildagliptin 50 mg and metformin Hcl 500 mg)	50	49.82	98.78	500	502.43	100.48
		50.17	100.39		500.12	100.03
		49.53	99.19		493.22	98.65
		50.62	100.28		502.71	100.55
		49.47	98.82		500.37	100.07
		50.58	101.05		495.19	99.03
Average			99.75	Average		99.80
SD			0.8660	SD		0.7148
%RSD			0.8681	%RSD		0.7162
SE			0.4104	SE		0.3197
CI (Confidence Interval 99%)			98.32-101.17	CI (Confidence Interval 99%)		98.62-100.97

Table 9: Summery of validation parameters of the developed method

S. No.	Validation parameters	Vildagliptin	Metformin
1	Linearity range	5-17.5 µg/ml	50-175 µg/ml
2	Precision		
	• Intra-day precision [%RSD]	0.2398	0.5403
	• Inter-day precision [%RSD]	0.2327	0.5696
3	Accuracy [% Recovery]	100.27	99.98
4	LOD [µg/ml]	0.0182	0.4451
5	LOQ [µg/ml]	0.0553	1.3490
6	Specifity	Specific	Specific
7	Robustness	Robust	Robust

Forced degradation studies

Forced degradation studies were carried under a condition of acid/base/neutral hydrolysis, oxidation, dry heat and photolysis. For each study, samples were prepared. The blank subjected to stress in the same manner for the drug solution, working standard solution of vildagliptin and metformin subjected to stress degradation. Dry heat and photolytic degradation were carried out in a solid state. The concentration of degrading reagent and time of exposure was optimised to degradation within the range of 10%.

During optimisation of degradation conditions, if the higher percentage of degradation was observed, milder conditions were used for the lesser duration of exposure. If percent degradation is high, there are chances of formation of secondary products.

This was carefully avoided. Although percent assay reduced under all conditions; the separate peak for degradation product was observed only under acid and alkali conditions fig 3 and 4. Summary of stress degradation results is given in table 9 and 10.

In order to develop a suitable RP-HPLC method for the estimation of vildagliptin and metformin, different buffer ratios at different flow rate were applied. Some of the reported methods were costly due to the use of expensive solvents and it was replaced by buffer and acetonitrile in this study. The LOD and LOQ were found to be 0.0182 and 0.0553 µg/ml for vildagliptin and 0.4451 and 1.3490 µg/ml for metformin which indicates that the method was sensitive, and can detect and quantify at lower levels of vildagliptin and metformin. Linearity range was from 5-17.5 µg/ml for vildagliptin and 50-175 µg/ml for metformin. The response of the drug was found to be linear in the selected concentration range the correlation coefficient for vildagliptin and metformin were 0.9935 and 0.9933 respectively which indicates that at this concentration range both were highly linear. Present assay the amount of both the drugs recovered was found to be 99.75% for vildagliptin and 99.80% for metformin. The developed RP-HPLC stability indicating assay method was found to be appropriate for the analysis of drug in their pharmaceutical dosage form.

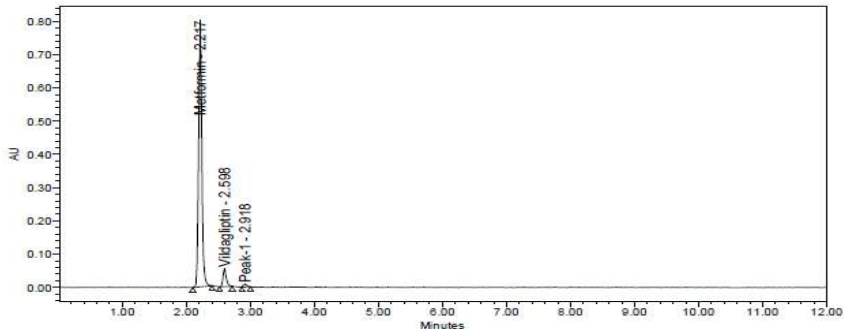

Fig. 3: Chromatogram of acid hydrolysis

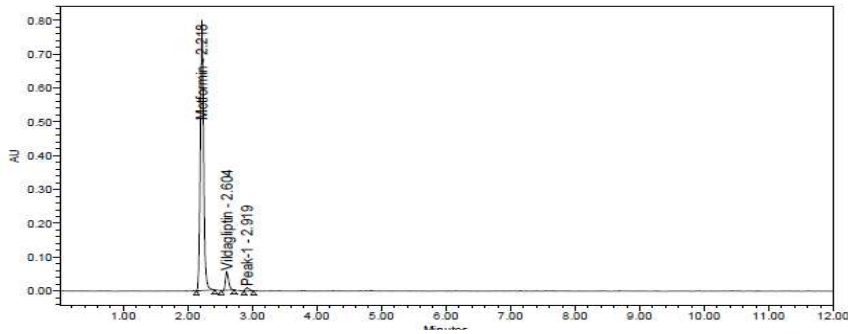

Fig. 4: Chromatogram of alkali hydrolysis

Table 9: Forced degradation study data of vildagliptin

Parameters	Degradation time	Peak area*	% Degradation	% of active drug present after degradation
Control sample	-	214581	-	-
Neutral sample	30 min	212693	0.89	99.98
Acidic degradation	30 min	205634	4.21	96.66
Alkaline degradation	30 min	207784	3.20	97.67
Oxidative degradation	30 min	208781	2.73	98.14
Thermal degradation	48 h	210938	1.72	99.15
Photolytic degradation	7 d	212693	0.95	99.92

*Mean of six determinations

Table 10: Forced degradation study data of metformin

Parameters	Degradation time	Peak area*	% degradation	% of active drug present after degradation
Control sample	-	2832156	-	-
Neutral sample	30 min	2799432	1.06	99.32
Acidic degradation	30 min	2716181	4.12	96.36
Alkaline degradation	30 min	2732399	3.54	96.94
Oxidative degradation	30 min	2777504	1.93	98.55
Thermal degradation	48 h	2784513	1.69	98.79
Photolytic degradation	7 d	2799432	1.16	99.22

*Mean of six determinations

CONCLUSION

The developed method is stability indicating where well-resolved peaks were observed for analyte and degradation product. The method is specific, accurate, precise, and robust and can be used for routine quality control as well as assessing the stability of vildagliptin and metformin in bulk and in pharmaceutical dosage form.

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CONFLICT OF INTERESTS

Declared none

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