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A VALIDATED REVERSED-PHASE HPLC METHOD FOR THE DETERMINATION OF VILDAGLIPTIN FROM TABLET DOSAGE FORM

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Abstract: A simple, rapid, precise and cost effective method has been developed and validated for determination of Vildagliptin in pharmaceutical tablet dosage form. The chromatographic separation was carried out with Shimpack VP-ODS, 150 × 4.6 mm, 5µm analytical column and mobile phase containing 0.02M phosphate buffer (pH 4.6) and acetonitrile at the ratio (80:20% v/v). pH of the buffer solution was adjusted with orthophosphoric acid. The instrumental settings include flow rate 0.7 ml/min, column temperature at 25ºC and detector wavelength of 210nm using a photodiode array detector. Theoretical plate for Vildagliptin was 6219 and tailing factor was 1.38.

Keywords: Vildagliptin, RP-HPLC, validation, tablet dosage form.

Introduction

Vildagliptin is an oral anti-hyperglycemic agent (antidiabetic drug). Chemically it is (S)-1-[N-(3-hydroxy-1-adamantyl) glycyl] pyrrolidine-2-carbonitrile, is a potent dipeptidyl peptidase IV (dip-IV) inhibitor. DPP-IV inhibitors represent a new class of oral antihyperglycemic agents to treat patients with type 2 diabetes. DPP IV inhibitors improve fasting and postprandial glycemic control without hypoglycemia or weight gain. Vildagliptin inhibits the inactivation of GLP-1 and GIP by DPP IV, allowing GLP-1 and GIP to potentiate the secretion of insulin in the beta cells and suppress glucagon release by the alpha cells of the islets of Langerhans in the pancreas. [1-5]

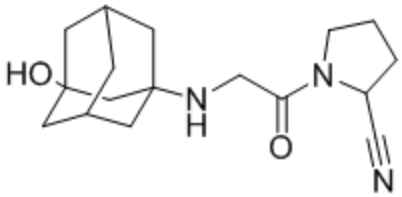

Figure 1: Structure of Vildagliptin

Literature survey reveals that few analytical methods are available for estimation of Vildagliptin. But there is no analytical method for the determination of Vildagliptin from its pharmaceutical dosage form [6].

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Due to lack of published chromatographic method and existing methods are time consuming and complex, the present work was aimed to develop a rapid, new, economical, precise and accurate method for the determination of Vildagliptin from its tablet dosage form.

Materials and methods

Chemicals and reagents

Working standard of Vildagliptin with a potency of 99.78% was collected from Beijing Huikang Boyuan Chemical Tech. Co. Ltd. China. Market sample of Vildagliptin (Galvus) containing 50 mg Vildagliptin were collected from Novartis Eurofarm limited company. HPLC grade Acetonitrile was purchased from Merck, Darmstadt, Germany, Potassium dihydrogen phosphate from Scharlab, Spain and Orthophosphoric acid were purchased from Merck, Darmstadt, Germany. HPLC grade water was obtained through millipore water purification system (Model- Arium 611DI, Sartorious).

HPLC instrumentation and chromatographic condition

High performance liquid chromatographic system consisted of a Shimadzu LC-20 AT, prominence, equipped with an auto sampler (SIL-20AC HT, Shimadzu, Japan) and PDA detector (SPD- M20A, Japan) was used for the analysis. The data was recorded using LC-solution software. A shimpack VP-ODS, Shimadzu (150mm x 4.6mm, 5µm) column was used for the analysis. A powersonic 505 ultrasonic bath (Hwashin technology, Seoul, Korea) was used for degassing of the mobile phase. In addition a pH meter (Mettler Toledo, Switzerland) and an electronic balance (Model- CP224S, Sartorious, Germany) were used in the present work.

The separation was carried out using a mobile phase consisting of 0.02M phosphate buffer and acetonitrile with pH 4.6, (adjusted with orthophosphoric acid) in the ratio of 80:20% v/v. The mobile phase was filtered, sonicated and degassed before use. The column was maintained at a temperature of 25ºC with column oven (CTO-20AC) and the flow rate was 0.7 ml/min. Analysis was performed with injection volume of 10µl using PDA detection at 210nm.The run time was set for 10 minutes. The typical retention time of Vildagliptin peak is about 3.6 min which is shown in figure 2 and figure 4.

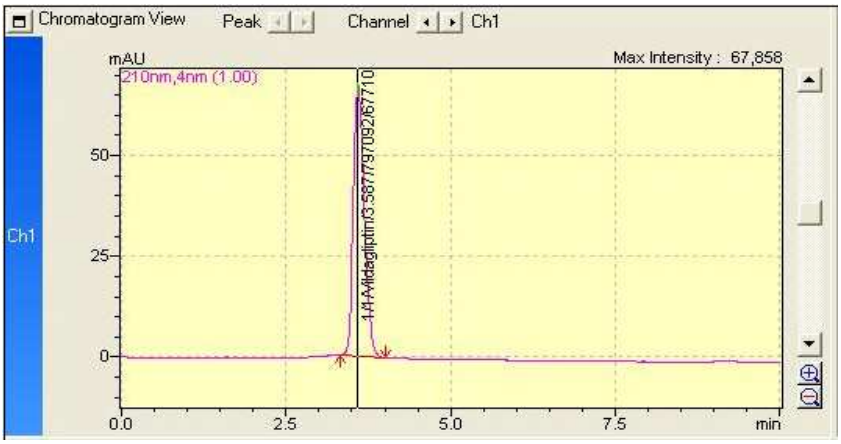

Figure 2: Chromatogram of Vildagliptin in standard solution

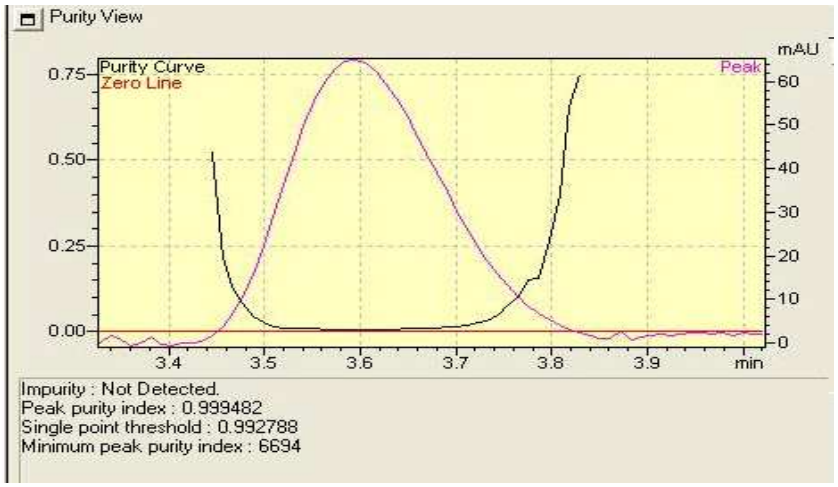

Figure 3: Purity curve of Vildagliptin in standard solution

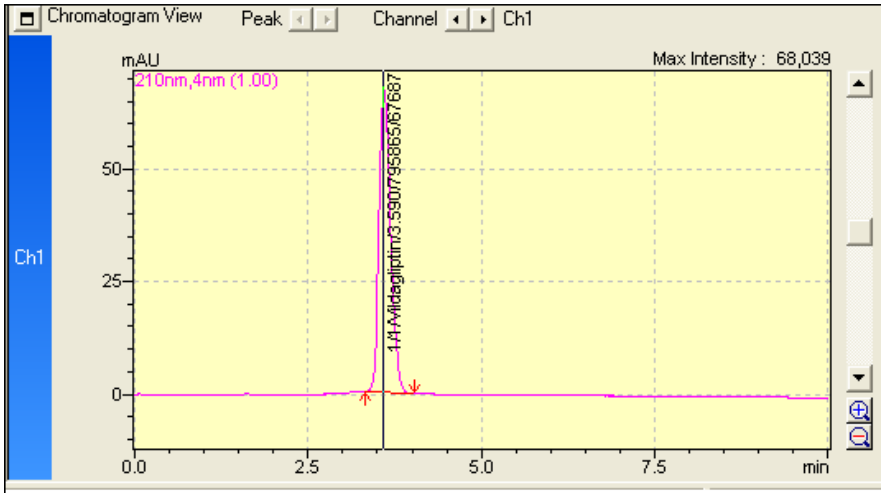

Figure 4: Chromatogram of Vildagliptin in sample solution

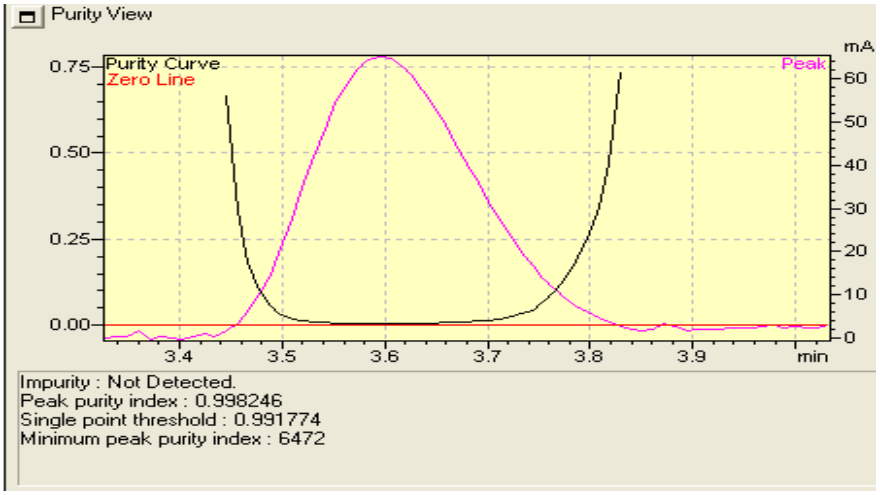

Figure 5: Purity curve of Vildagliptin in sample solution.

Preparation of standard solution

About 50 mg of Vildagliptin WS was accurately weighed into a 100 ml volumetric flask and dissolved in about 60 ml of diluting solution (20% acetonitrile in water) and shaked by hand for 5 minutes. Then keep it for sonicate for 10 minutes. Cooled to room temperature and the volume made up with the diluting solution. 5 ml of this solution was diluted to 50 ml with diluting solution and mixed well. The final concentration was 50µg/ml and this was used as stock solution.

Preparation of sample solution

About 20 tablets were weighed and powdered. Powders equivalent to 50 mg of Vildagliptin were taken into a 100 ml volumetric flask. About 3 ml of water was added to disperse the powders, and then drugs were dissolved in 60 ml of diluting solution and shaked at 250 rpm for 10 minutes by shaker. The volume was made up to the mark with diluting solution and sonicated for 10 minutes. Cooled to room temperature and the resultant solution were filtered through Whatman 1 filter paper. 5 ml of this solution was diluted to 50 ml with diluting solution. Final solution was filtered through 0.2µ-disc filter.

Method validation

The present method of analysis was conducted to obtain a new, cost effective, convenient method for HPLC determination of Vildagliptin in tablet formulation**.** The experimental method was validated according to the recommendations of ICH-1996 and USP-30 for the parameters like specificity, system suitability, accuracy, linearity, precision, robustness.

Specificity

The specificity of the method was evaluated to ensure that there is no interference of excipeints, diluting solution in the chromatogram of Vildagliptin. The specificity was studied by injecting the excipients, diluting solution and standard solution of Vildagliptin.

System suitability

System suitability was performed by injecting six replicates of standards and two replicates of sample preparation at a 100% level to verify the accuracy and precision of the chromatographic system. This method was evaluated by analyzing the repeatability of retention time, tailing factor, theoretical plates of the column.

Linearity

The linearity of the chromatographic method was established by plotting a graph to concentration area of Vildagliptin standard and determining the correlation coefficient (R^2). Linearity of Vildagliptin standard solution at a concentration level of 40%, 60%, 80%, 100%, 120%,140% were injected into the HPLC system. The detector response was found to be linear form 40% to 140% of test concentration for Vildagliptin standard. Before injection of the solutions, the column was equilibrated for at least 45 minutes with the mobile phase. Each measurement was carried out in six replicates to verify the

repeatability of the detector response at each concentration level. Linearity curve is shown in figure 6 and data are shown in table 1.

Table 1: Linearity of Vildagliptin in Standard preparation from 40% to 140% of test concentration

Sl. no.	% test concentration	Concentration (µg/ml)	Average peak area
1	40	20	319634
2	60	30	480627
3	80	40	639798
4	100	50	797112
5	120	60	953983
6	140	70	1117742
		Regression co-efficient =	1

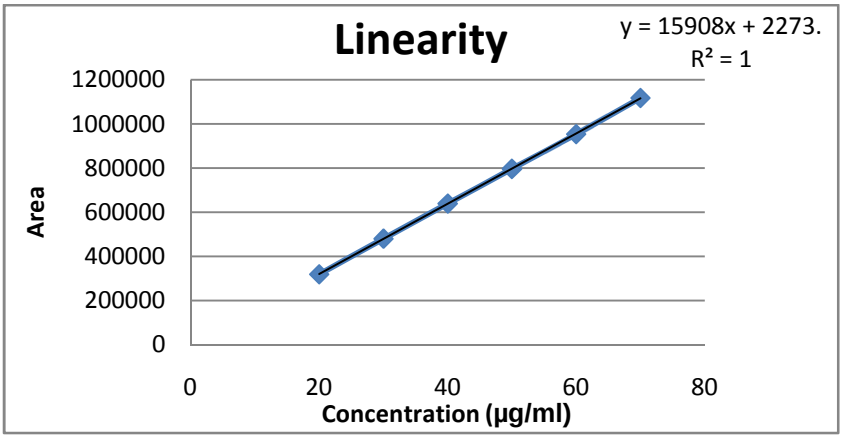

Figure 6: Calibration curve of Vildagliptin.

Accuracy

The accuracy of the method is the nearness of the result obtained to the true value. Accuracy of the method was determined by recovery experiments. The recovery was performed by adding Vildagliptin working standard to placebo (excipients mixture) in the range of test concentration (40%, 60%, 80%, 100%, 120%, and 140%) and expressed as percent (%) recovered. Three samples were prepared for each recovery level. The recovery value for Vildagliptin ranged from 99.20 to 100.30%. The average recovery of six levels was 99.73%. Results are shown in table 2.

Table 2: Results of Accuracy experiment using spiked placebo method

Level (%)	Amount of drug spiked (mg)	Found (mg)	Recovery (%) (n=3)
40	19.96	19.88	99.60
60	29.93	29.69	99.20
80	39.91	40.02	100.27
100	49.89	50.04	100.30
120	59.87	59.48	99.34
140	69.85	69.62	99.67
		Average	99.73
		SD	0.463
		% RSD	0.464

Stability of Analytical solution

The stability of analytical solutions was established by injecting the standard solution and sample solution at different time intervals up to 24 hours (0, 4, 8, 12, 16, 18 and 24 hours) by keeping the auto sampler temperature at room temperature (25ºC). The response of standard solution and sample solution were measured and % differences of peak area were calculated. The values are presented in the table 3.

Table 3: Stability of standard and sample solution of Vildagliptin

Time Interval	Standard		Sample	
	Standard peak area	% Difference	Sample peak area	% difference
0 hour	796544	-	795567	-
4 hour	797630	0.13	795273	0.04
8 hour	797082	0.07	793947	0.2
12 hour	796949	0.05	795120	0.06
16 hour	796953	0.05	795432	0.02
18 hour	797245	0.08	794021	0.2
24 hours	797895	0.2	795089	0.06

Precision

The precision of an analytical method is the degree of agreement among individual test results where the method is applied repeatedly to multiple samplings. Precision of the assay was assessed with respect to repeatability, reproducibility and intermediate precision by estimating the assay for six different sample preparations of same batch.

Results of analysis for repeatability, intermediate precision, and reproducibility are given in the table 4.

Table 4: Statistical analysis for repeatability, intermediate precision and reproducibility of Vildagliptin 50 mg Tablet

Sample ID	Assay (% labeled amount)		
	Repeatability (Analyst 1)	Intermediate precision (Analyst 2)	Reproducibility (Analyst 3)
Sample-1	99.83	100.04	98.32
Sample-2	99.67	99.59	99.85
Sample-3	100.13	99.41	99.02
Sample-4	98.99	99.20	100.53
Sample-5	99.29	98.97	99.08
Sample-6	99.31	99.17	99.26
Average	99.54	99.40	99.34
SD	0.416	0.380	0.760
% RSD	0.418	0.383	0.766

Robustness

The robustness is the ability of method to remain unaffected by small changes in parameters. The robustness of the method were determined by purposely altering experimental conditions and % assay of Vildagliptin, peak tailing, theoretical plates, % RSD were calculated. To study the effect of flow rate, it was changed to 0.2 units from 0.7ml/min to 0.5ml/min and 0.9ml/min. The effect of column temperature was studied at 23ºC and 27ºC instead of 25ºC. The effect of pH change was studied by changing the buffer pH from 4.6 to 4.4 and 4.8. Results are shown in the table 5.

Table 5: Results of robustness study

Sl. No.	Parameter	Variation	Assay % (n=3)
1.	Flow rate (± 20% of the set flow)	a) at 0.5 ml/min b) at 0.9 ml/min	a) 98.88 b) 99.23
2.	Mobile phase pH (± o.2 of set pH)	a) at pH 4.4 b) at pH 4.8	a) 99.06 b) 99.17
3.	Column oven temperature (± 2ºC of set temperature)	a) at 23ºC b) at 27ºC	a) 99.56 b) 99.19

Results and discussion

The developed method was specific because there was no interference of excipients, diluting solution and impurity in the chromatogram of Vildagliptin (purity curve shown in figure 3 and 5). The method showed linearity of detector response and produces a linear calibration curve over the range of 20-70µg/ml (Figure 6). Results of accuracy were proven by the table 2 and % RSD is 0.464, which is within the acceptable limit. The % difference of peak area of Standard solution and Sample solution that were injected at periodic intervals were found to be within the specified limit (Table3). Results for robustness evaluation for Vildagliptin (Table 5) were also satisfactory. So, this method is applicable for determination of Vildagliptin from its tablet dosage form.

Conclusion

The proposed RP-HPLC has been validated as per the recommendation of ICH guidelines. The method is accurate, precise, simple, less time consuming, cost effective and convenient for use. All the validation parameters of the analysis method showed satisfactory data with acceptable correlation co-efficient and lower % RSD. So the developed method can be used conveniently for analysis of quality control, stability and further studies.

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