



## Development and validation of stability indicating assay method of vildagliptin in bulk and tablet dosage form by RP-HPLC

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### Abstract

A novel, simple and economic reverse phase high performance liquid chromatography (RP-HPLC) method has been developed for the estimation of Vildagliptin in bulk and tablet dosage form with greater precision and accuracy. Separation was achieved on C18 column (250X4.6mm i.d.,5 $\mu$ m) in isocratic mode using Buffer :Acetonitrile in the ratio of 50:50(v/v) as mobile phase, pumped in to the column at flow rate of 1.0 mL min<sup>-1</sup> and the detection of eluent from the column was carried out using variable wavelength UV detector at 220 nm. The total run time was 10 min and the column was maintained at 30<sup>0</sup> C. The retention time of Vildagliptin was 5.017 min. The standard curves were linear over the concentration range of 10-60  $\mu$ g mL<sup>-1</sup> with R<sup>2</sup> 9996 and the LOD and LOQ values for Vildagliptin were 0.025  $\mu$ g mL<sup>-1</sup> and 0.054  $\mu$ g mL<sup>-1</sup> respectively. The percentage recovery was found to be 98.11 to 101.16, the % RSD of intra-day and inter day precision was found to be 0.68 and 0.61 respectively. The percentage amount of a marketed tablet formulation of Vildagliptin was found to be 99.46 %. The method was validated as per ICH guidelines. Validation studies demonstrated that the proposed RP-HPLC method is simple, specific, rapid, reliable and reproducible. Hence the proposed method can be applied for the routine quality control analysis of Vildagliptin in bulk and tablet dosage forms.

Key-Words: Vildagliptin, RP-HPLC, Method Development, Validation, ICH guideline

### Introduction

Vildagliptin is an oral anti-hyperglycemic agent (anti-diabetic drug) of the new dipeptidyl peptidase-4 (DPP-4) inhibitor class of drugs.<sup>[4]</sup> Chemically it is (2S)-[[[3-Hydroxyadamantan-1-yl)amino]acetyl]pyrrolidine-2-carbonitrile. The structure of Vildagliptin is shown in Fig.1. The drug is not official reported in pharmacopoeia. It is white to off-white solid powder that is soluble in water and Dimethyl sulfoxide (DMSO). Vildagliptin has been shown to reduce hyperglycemia in type 2 diabetes mellitus. Vildagliptin inhibits the inactivation of GLP-1 and GIP by DPP-4, 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.<sup>[3-4]</sup> Literature survey reveals HPLC methods have been reported for the estimation of Vildagliptin into tablet formulation.<sup>[5-9]</sup>

The objective of the present work was to develop simple, rapid, accurate, specific and economic RP-HPLC method for the estimation of Vildagliptin in bulk and tablet. The method was further validated as per ICH guidelines for the parameters like precision, accuracy, sensitivity, and linearity.<sup>[13]</sup> The results of analysis were validated statistically and by recovery studies.<sup>[2]</sup> These methods of estimation of Vildagliptin were found to be simple, precise, accurate and economic.

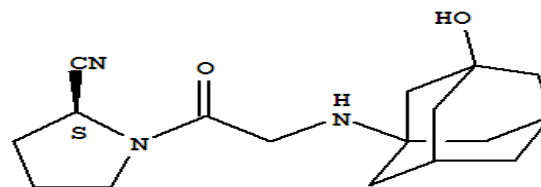


Fig. 1: Structure of Vildagliptin

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## Methodology

### Samples

(2S)-[[3-Hydroxyadamantan-1-yl)amino]acetyl]pyrrolidine-2-carbonitrile was kindly provided by Aristopharma Ltd (Bangladesh). A commercial tablet formulation named Galvus tablet from Novartis pharmaceuticals Ltd (Bangladesh) containing 50 mg of Vildagliptin within their shelf life period was purchased from local market and used.

### Reagents

Acetonitrile were of HPLC grade from Aristopharma Ltd (Bangladesh). Water used was bidistilled.

### Apparatus

HPLC machine (Agilent Technologies 1200 series) composed of a Binary Pump (Flow range: 0.001–5 mL/min for fast gradient analysis) equipped with High Performance Autosampler (Injection range: 0.1  $\mu$ L–100  $\mu$ L, extendable up to 1500  $\mu$ L) Sample container: Vials and well-plates, an Photodiode arrays with 1024 elements, programmable slit width (1, 2, 4, 8, 16 nm) plus UV–vis detector and a Agilent ChemStation software.

### Chromatographic conditions

The separation was performed on a ZORBAX Rapid Resolution HT C<sub>18</sub> columns (150 mm x 4.6 mm). The flow rate was 1.0 mL min<sup>-1</sup>. The injection volume was 20  $\mu$ L. The detection wavelength was set at 220 nm. The mobile phase consisted of Buffer :Acetonitrile in the ratio of 50:50 (v/v). Buffer solution was prepared by dissolving about 13.78 g of Sodium dihydrogen phosphate into 900 ml distilled water. The pH of solution was adjusted at 6.5 with Sodium Hydroxide or dilute phosphoric acid. Then, the buffer solution was adjusted upto 1000 ml with distilled water. The solution was degassed with 0.20 micron filter paper. The run time was set at 10 min and column temperature was maintained at 30<sup>0</sup>C. Prior to injection of analyte, the column was equilibrated for 30 min with mobile phase. The mobile phase was premixed, filtered through 0.45  $\mu$ m membrane filter and degassed by suction pump with negative pressure.

### Method Validation

#### Linearity

A stock solution of (1000  $\mu$ g mL<sup>-1</sup>) was prepared by dissolving 100 mg Vildagliptin in 100 ml mobile phase then solutions of different concentrations (10–60  $\mu$ g mL<sup>-1</sup>) for construction of calibration plots were prepared from this stock solution. The mobile phase was filtered through a 0.45  $\mu$ m membrane filter and delivered at 1.0 mL min<sup>-1</sup> for column equilibration; the baseline was monitored continuously during this process. The detection wavelength was 220 nm. The prepared dilutions were injected in series, peak area

was calculated for each dilution and concentration was plotted against peak area.

#### Accuracy

Accuracy was determined by the standard addition method. Previously analyzed samples of Vildagliptin (10  $\mu$ g mL<sup>-1</sup>) were spiked with 80, 100 and 120% extra Vildagliptin standard and the mixtures were analyzed by the proposed method. The experiment was performed in triplicate. Recovery (%), RSD (%) and standard error (SE) were calculated for each concentration.

#### Precision

Precision was determined as both repeatability and intermediate precision in accordance with ICH recommendations. Repeatability of sample injection was determined as intra-day variation and intermediate precision was determined by measurement of inter-day variation. For both intra-day and inter-day variation, solutions of Vildagliptin at single concentrations were determined.

#### Reproducibility

The reproducibility of the method was checked by determining precision on a different column, analysis being performed by another analyst. For both intra-day and inter-day variation, solutions of Vildagliptin at single concentrations (10 $\mu$ g mL<sup>-1</sup>) were determined six times.

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

LOD and LOQ were determined by the standard deviation (Sy/x) method. LOD and LOQ were determined from the slope, S, of the calibration plot, Sy/x, by use of the formulae LOD = 3.3  $\times$  Sy/x/S and LOQ = 10  $\times$  Sy/x/S.

#### Robustness

The robustness of the method was determined to assess the effect of small but deliberate variation of the chromatographic conditions on the determination of Vildagliptin. Robustness was determined by changing the mobile phase flow rate to 0.9 and 1.1 mL min<sup>-1</sup> and the concentration of Acetonitrile in the mobile phase to 48 and 52%.

#### Stability

The stability of the drug in solution during analysis was determined by repeated analysis of samples during the course of experimentation on the same day and also after storage of the drug solution for 48 hrs., under laboratory bench conditions (33  $\pm$  1 $^{\circ}$ C) and under refrigeration (8  $\pm$  0.5 $^{\circ}$ C).

#### Procedure for pharmaceutical formulation

For tablets, 20 units were weighed and finely powdered. An accurately weighed amount of the powder equivalent to 100 mg of Vildagliptin was



transferred into a 100 ml volumetric flask and sonicated for 20 min with 70 ml of mobile phase. The resulting suspension was filtered through 0.22 µm membrane filter and diluted upto 100 ml with mobile phase. A suitable aliquot of this filtrate was diluted with mobile phase in order to obtain a final concentration of 10 to 60 µg mL<sup>-1</sup>. A 20 µl of the obtained solution was chromatographed.

## Results and Discussion

### Method Development

The HPLC procedure was optimized with a view to developing a method. From several solvents and solvent mixtures investigated Buffer : Acetonitrile in the ratio of 50:50 (v/v) was found to furnish sharp, well-defined peak with very good symmetry and low t<sub>R</sub> (5.017 min) (Fig. 2). Various other mobile phases tried earlier either did not give well defined peak in a short time, therefore were not considered. The final selection on mobile phase composition and flow rate was made on the basis of peak shape (peak area, peak asymmetry & tailing factor), baseline drift, time required for analysis, and cost of solvent and Buffer : Acetonitrile in the ratio of 50:50 (v/v) was selected as the optimum mobile phase. Under these conditions the retention time was 5.017 ± 0.01 min.

**Table 1: Optimized Chromatographic Conditions**

Parameters	Conditions
Stationary phase (column)	ZORBAX Rapid Resolution HT C <sub>18</sub> columns (150 mm x 4.6 mm)
Mobile Phase	Buffer : Acetonitrile in the ratio of 50:50 (v/v)
Runtime (min)	10
Volume of Injection (µl)	20
Detection wavelength (nm)	220
Drug Retention Time(min.)	5.017

### Validation of the Method

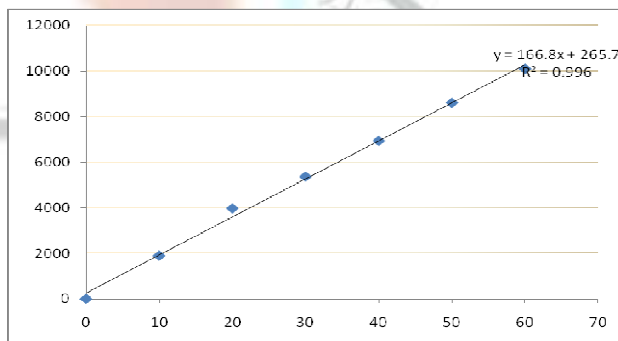
#### Linearity<sup>[13]</sup>

The calibration plot of peak area against concentration was linear in the range investigated (10–60 µg mL<sup>-1</sup>). The low values of RSD and standard error show the method is precise. Statistical calculations were performed at the 5% level of significance. The linear regression data for the calibration plot are indicative of a good linear relationship between peak area and concentration over a wide range. The linear regression equation was  $y = 166.8x + 265.7$  and the regression coefficient was 0.996. The correlation coefficient was

indicative of high significance. The low values of the standard deviation, the standard error of slope, and the intercept of the ordinate showed the calibration plot did not deviate from linearity. There were no significant differences between the slopes of standard curves constructed on different days.

$$y = 166.8x + 265.7$$

$$R^2 = 0.996$$



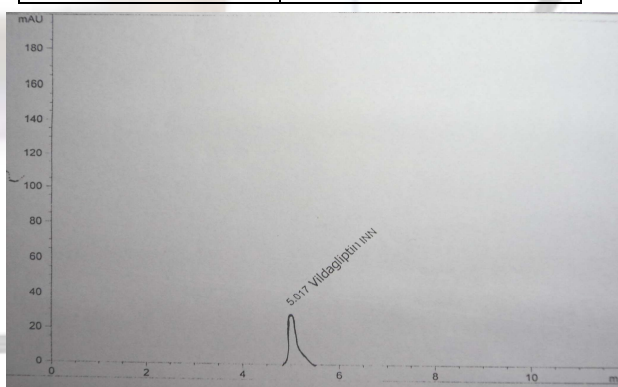
**Fig. 2: Calibration Curve of Vildagliptin**

**Table 2: Statistical data of calibration curves of Vildagliptin**

Parameters	Obtained Results
Linearity (µg mL <sup>-1</sup> )	10 - 60
Regression equation	$y = 166.8x + 265.7$
Correlation coefficient (R <sup>2</sup> )	0.996

**Table 3: System Suitability Parameters**

Parameters	Obtained Results
Theoretical plates (N)	5790
Tailing Factor	0.07
LOD (µg mL <sup>-1</sup> )	0.025
LOQ (µg mL <sup>-1</sup> )	0.054



**Fig. 3: Chromatogram of Vildagliptin (standard)**

**Accuracy**<sup>[13]</sup>

The recovery of the method, determined by spiking a previously analyzed test solution with additional drug standard solution, was 98.11-101.16. The values of recovery (%), RSD (%), indicate the method is accurate.

**Table 4: Result of Recovery Studies of Vildagliptin**

Level of Recovery	Amount Present in formulation ( $\mu\text{g mL}^{-1}$ )	Amount of pure drug added ( $\mu\text{g mL}^{-1}$ )	% Recovery*	R.S.D.	S.E
80	50	8	98.48	0.266	0.64
100	50	10	100.09	0.0754	0.35
120	50	12	99.88	0.264	0.46

\* Indicates mean of three determination, R.S.D. =Relative Standard Deviation, S.E. =Standard Error

**Precision**<sup>[13]</sup>

Intra-day and inter-day precision were carried out for the various concentrations of the sample at different time intervals in the same day and at same time on different days. The concentration of the sample solution was determined as per the procedure given for the tablet formulation by determining peak area at selected analytical wavelength 220 nm. The variation of the results within the same day was analysed and statistically validated.

**Table 5: Results Analysis of Precision Studies**

Concentration ( $\mu\text{g mL}^{-1}$ )	Repeatability (intra day precision) *		Intermediate precision (inter day) *	
	% RSD	SE	% RSD	SE
	50	0.68	0.58	0.61

\* Indicates mean of six determinations, R.S.D. =relative Standard Deviation, S.E. =Standard Error

**Reproducibility**<sup>[13]</sup>

Reproducibility was checked by measuring the precision of the method on another column with analysis performed by another person. Both intra-day and inter-day precision were determined. There were no significant differences between RSD (%) values for intra-day and inter-day precision, which indicates the method, is reproducible.

**Limit of Detection (LOD) and Limit of Quantification (LOQ)**<sup>[13]</sup>

The LOD and LOQ of the method, determined by the standard deviation method, as described above, were 0.025 and 0.054  $\mu\text{g mL}^{-1}$ , respectively, which indicated the method can be used for detection and quantification of Vildagliptin over a very wide range of concentrations.

**Robustness**<sup>[13]</sup>

There was no significant change in the retention time of Vildagliptin when the composition and flow rate of the mobile phase were changed. The low values of the RSD indicated the robustness of the method.

**Table 6: Robustness of the method**

System suitability parameters	Normal condition	Change in condition	Change in % RSD
Flow Rate	1.0 mL min <sup>-1</sup>	0.9 mL min <sup>-1</sup> 1.1 mL min <sup>-1</sup>	0.031 0.028
Mobile phase ratio (Buffer :Acetonitrile)	50:50	50:48 50:52	0.011 0.0041

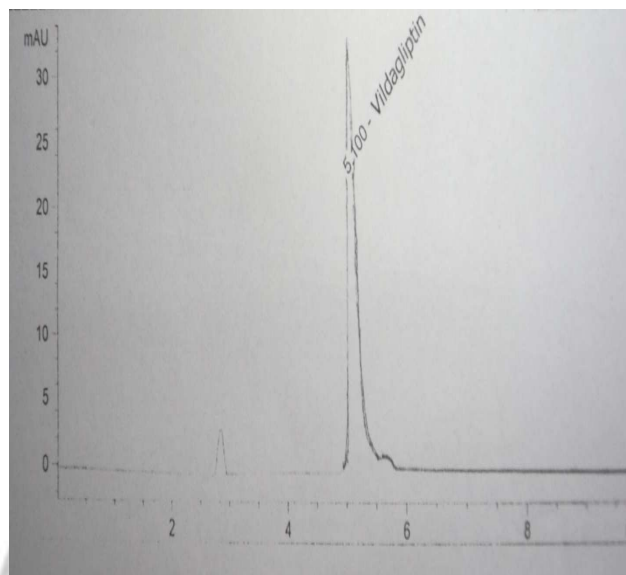
**Analysis of Vildagliptin from tablet formulation**<sup>[13]</sup>

The proposed method was applied to the determination of Vildagliptin in tablets formulation (50 mg). The mean average (six replicates) was found to be 49.73 mg corresponding to a mean recovery of 99.46 % with an R.S.D. of 0.314%. This result was in good agreement with the label value. It should be pointed out that the chromatogram of the solution of excipients is absolutely free of any peak indicating thus that no interference from the excipients is encountered.

**Table7: Analysis of commercial formulation**

Commercial formulation	% Label claim estimated (average)	* S.D.	%RSD
Tablet (Galvus)	49.73	0.135	0.270

SD= Standard deviation, RSD = Relative standard deviation, \*Average of six determinations



**Fig. 4: A chromatogram of Vildagliptin formulation (Galvus Tablet)**

### Conclusion

A simple and rapid HPLC method has been developed for the determination of Vildagliptin. Statistical analysis of the results has been carried out revealing high accuracy and good precision. The method is reliable and convenient for routine control and stability assays of Vildagliptin in both raw material and tablets.

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