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Analytical Method Validation of Vildagliptin in Bulk Drug and Dosage Form by RP HPLC Method

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ABSTRACT

The present analytical research work was to develop and validate RP-HPLC method for the estimation of Vildagliptin in bulk and dosage form. The method of analysis was validated for the parameters like Accuracy, Linearity, Precision, Recovery, and Robustness. By calculating percentage recovery of Vildagliptin accuracy of the method was determined. For the drug, recovery study was carried out by applying the method to known label claim tablet solution known amount of Vildagliptin corresponding to 50%, 100% and 150% of drug sample had been added (standard addition method). At each level performed and the results obtained were compared. Interday precision study of Vildagliptin was carried out by estimating the corresponding responses intraday. RP-HPLC method for Vildagliptin was developed using column Shimadzu C18 column (5µm, 250mm × 4.6mm) as stationary phase and Acetonitrile: Buffer (85:15 v/v) (pH 3.5 adjusted with OPA) as mobile phase. The mobile phase was maintained at a flow rate of 0.8 ml/min and volume of injection is 25 µl detection was carried out at 210 nm. Vildagliptin was found to be linear in the concentration rang of 30, 50, 60, 70, 80 and 100 µg/ml respectively. Accuracy of the method was determined by performing recovery study and the results were found in the range of 96.2 -99.3% w/w for Vildagliptin % RSD of area obtained in precision study of these drugs were found less than 2% which indicated good precision of the developed method.

Keywords: *Vildagliptin, Acetonitrile, OPA, RP-HPLC, Method Development, Validation, Antidiabetic*

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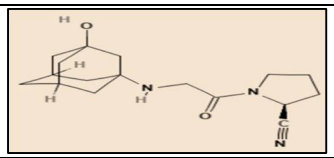
INTRODUCTION

Vildagliptin (VGT) [(S)-1-[N-(3-hydroxy-1-adamantyl) glycyl] pyrrolidine-2-carbonitrile], Fig. 1, is a brand new oral anti-diabetic drug belonging to the magnificence of dipeptidyl peptidase-4 inhibitor (reduces glucose-brought about glucagon-like peptide 1 and gastric inhibitory polypeptide secretion)[1] and is used as mono therapy in adults with type 2 diabetes mellitus treatment specifically in patients inadequately managed by means of weight-reduction plan and workout by myself[2][3]. Vildagliptin can be used as dual oral therapy in patients with insufficient glycemic control [4][5][6][7]. Vildagliptin has a supplement pharmacological effect as metformin, where it improves the glucose dependent insulin secretion and inhibits glucagon release, as a result increasing the glycemic manipulate and weight manage and reduced hypoglycemia [8][9].

Numerous strategies were evolved for the analysis of both vildagliptin and metformin in mixture which include UV-Vis spectroscopies, HPLC and LCMS/ MS methods [10][11][12][13]. Instant estimation of those compounds via RP-HPLC methods had been displaying more time of evaluation and complicated strategies; subsequently the existing look at became targeted on chromatographic analysis of vildagliptin and metformin in a much less time ingesting simultaneous evaluation of these compounds inactive element (API) and pharmaceutical dosage form which found in the pharmaceutical market[14][15][16][17][18].

Table 1: Vildagliptin Drug Profile

Drug	**Vildagliptin**
Structure	
Description	Vildagliptin (VDG), S-1-[N-(3-hydroxy-1- adamantyl) glycyl] pyrrolidine-2- carbonitrile is an oral anti- hyperglycemic agent (anti-diabetic drug) of the brand new dipeptidyl peptidase-4 (DPP-four) inhibitor Magnificence of medicine. Vildagliptin inhibits the inactivation of GLP- 1 and GIP by means of DPP-4, permitting GLP-1 and GIP to potentiate the secretion of insulin inside the beta cells and suppress glucagon release by way of the alpha cells of The islets of Langerhans inside the pancreas. Vildagliptin has been shown to reduce hyperglycemia in kind 2 diabetes mellitus.
IUPAC Name	(2S)-1-{2-[(3-hydroxyadamantan-1-yl)amino]acetyl}pyrrolidine-2-carbonitrile
Chemical Formula	C17H25N3O2
Molecular Mass	303.3993 g/mole
Physical State	Solid
Solubility	Soluble in Water and Methanol
pKa	14.71 & 9.03 Strongest acidic and basic respectively
$t_{1/2}$	90 minutes
Therapeutic Use	Used to reduce hyperglycemia in type 2 diabetes mellitus.[19]

MATERIAL AND METHODS

Details of Chemicals used-

Table No. 2 Chemicals used

Sr.No.	**Chemicals/ Solvents used**	**Grade**	**Make**
1	Acetonitrile	HPLC Gradient	Rankem
2	Water	HPLC	Molychem
3	Ortho Phosphoric acid	SQ	Qualigens
4	Potassium dihydrogen orthophosphate	AR	Merck
5	Methanol	HPLC	Rankem

EXPERIMENTAL WORK:

Chromatographic conditions:

Table No. 3: Chromatographic conditions

Mobile Phase	Acetonitrile : Phosphate buffer pH 3.50 (85 : 15)
Column	C18, 4.6 mm x 2.5 cm , 0.5 µm (Make- Shimadzu)
Flow rate	0.80 ml/min
Injection volume	25µl
Wavelength	210 nm
Oven temperature	25°c
Run time	10 min

Solution Preparation

Buffer preparation: Dissolved 13.6 g of Potassium dihydrogen phosphate in 1000 ml of HPLC grade water. Adjusted pH of this solution to 3.50 + 0.10 with ortho phosphoric acid. Sonicated for 5 minutes and filter through 0.45 µ filter paper.

Mobile phase: Mixed 850 ml of Acetonitrile and 150 ml of Phosphate buffer pH 3.50.Sonicated for 15 minutes.
Preparation of Stock Solution: About 100 mg of Vildagliptin was weighed accurately and transferred into a 100mlvolumetric flask and dissolved and diluted to volume with mobile phase to get 1000 ppmsolution.
Preparation of Sample Solution: 5 ml of stock solution was pipetted and diluted to 100 ml with mobile phase to get 50 ppm solution.

Method development: A new simple, specific, precise and accurate reversed-phase liquid chromatography method has been developed for the determination of Vildagliptin (VLG) API. The estimation was achieved on a Shimadzu C18 column (250mm×4.6mm, 5µm) using mobile phase consisting of a mixture of aqueous phase (13.6 g of Monobasic Potassium Phosphate was dissolved in 1000 ml of water for chromatography, pH of the solution was adjusted to the value of 3.50 using orthophosphoric acid) and organic phase (Acetonitrile) in the ratio of 15:85 v/v at a flow rate of 0.8 ml/min. The mobile phase was degassed for 15 min and filtered through 0.45µm membrane filters before use. The mobile phase was pumped through the column at a flow rate of 0.80 mL per min. Analysis was performed at 25 0 C and the injection volume was 25 µL of 50 ppm solution. Detection was carried out at 210nm. The retention time of Vildagliptin was found to be 2.972 min.

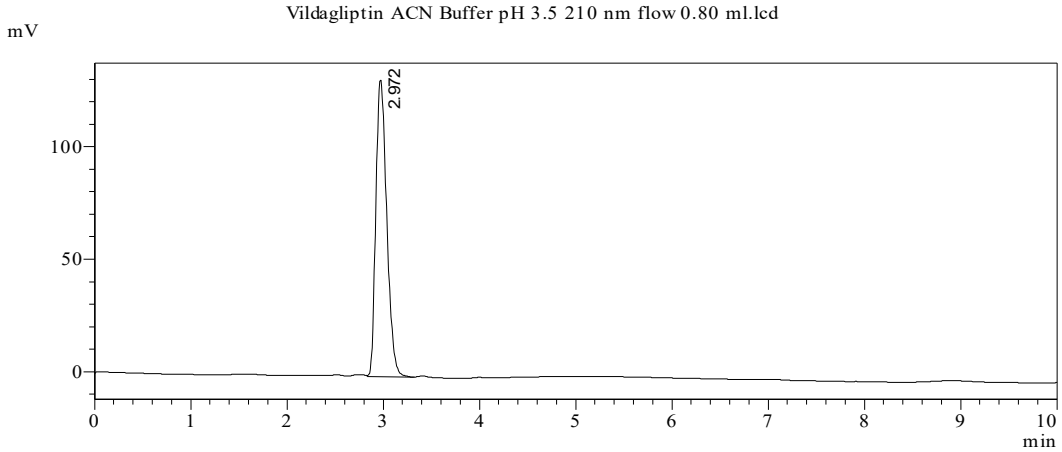


Fig 1:A typical chromatogram showing the separation of the drug.

RESULTS AND DISCUSSION
System suitability: System suitability tests are an integral part of chromatographic method which is used to verify reproducibility of the chromatographic system. Before proceeding for Method Validation to ascertain its effectiveness, certain system suitability test parameters were checked by injecting the drug solution at the concentration level of 50µg/ml and verified the results against acceptance limits. Results are tabulated as shown in Table 4.

Table No. 4: System Suitability - 50 ppm solution

Peak Name	Ret. Time	Area	Area%	Asymmetry	Theoretical Plates
Vildagliptin	2.998	1492812	100.000	0.997	3109

Linearity: The linearity of a test procedure is its ability (within a given range) to obtain test results proportional to the concentration (amount) of analyte in the sample. Linearity explores the directly proportional relationship between two different variables; concentration and area in this case. Linearity was studied by diluting the volume of standard stock solution (1000 µg/ml) equivalent to3.0, 5.0, 6.0, 7.0, 8.0 and 10.0ml to 100ml with same composition of mobile phase to obtain30, 50, 60, 70, 80, and 100µg/ml working solutions respectively. All of these solutions were injected to aforesaid chromatographic conditions to quantitatively estimate the area and equivalent concentration. From the results obtained, calibration curve was plotted by taking concentration on X axis and mean area on Y axis. From the calibration curve drawn between concentration versus mean area, the equation of straight line, slope, intercept and regression coefficient was determined. The equation line resulted was as given herein below.

Where, Y =area of chromatogram; $y = 30448x - 151226$ $R^2 = 0.9991$

Linear relationship was established between concentration and mean area and the same was confirmed by regression coefficient obtained viz. 0.9991. Therefore, it was stated from those results of the present study that the linearity experiment was successful for Vildagliptin within a range of 30 to 100µg/ml.

Table No. 5: Linearity Parameter

Sr. No.	Peak Name	Solution Conc	Ret. Time	Area
1	Vildagliptin	30 ppm	3.031	765859
2	Vildagliptin	40 ppm	3.058	1053296
3	Vildagliptin	50 ppm	3.065	1412095
4	Vildagliptin	60 ppm	3.122	1667085
5	Vildagliptin	70 ppm	3.122	1956989
6	Vildagliptin	100 ppm	3.124	2908126

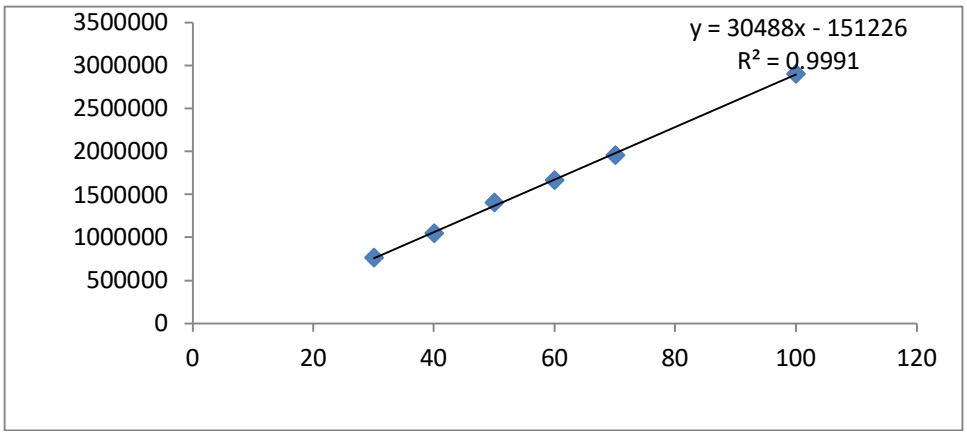


Figure 2: Calibration curve resulted from the linearity study of Vildagliptin.

LOD and LOQ-The lowest concentration of an analyte in a sample that can be consistently detected with a stated probability (typically at 95% certainty) is defined as the limit of detection (LOD). Limit of quantification, is defined as the lowest concentration of a substance that is possible to be determined by means of a given analytical procedure with the established accuracy, precision, and uncertainty.

Table No 6 : Results obtained for LOD and LOQ.

Drug	LOD (µg/ml)	LOQ (µg/ml)
Vildagliptin	2.78628	8.44327

Precision: Precision is the assessment of how close the records are to each other for a number of measurements under the same experimental conditions. Three QC standards were selected as LQC, MQC and NQC on the basis of calibration range. LQC was concentration slightly more than lowest concentration of linearity. Lowest concentration in HPLC linearity study was 30µg/ml and next to it was 50µg/ml, consequently, 45µg/ml was selected LQC.MQC was concentration near to middle concentration, slightly more or less and it was decided as75µg/ml. NQC was concentration near to the highest concentration but less than highest concentration. It was selected as 75µg/ml across the range. Precision of the method was established for intra-day (repeatability) and inter-day Precision was studied by diluting the volume correspondent to 4.5, 7.5 and 9.5ml of standard stock solution (100µg/ml) to 100ml with mobile phase to obtain 45, 75 and 95µg/ml working solutions respectively. All of these solutions were injected in predetermined chromatographic conditions and observed for various parameters and found within limit. Mean area was subjected to statistical analysis to determine percent RSD and found within limit as per ICH guideline Q2R1.

Table 7: Results observed for intra and inter-day precision experiments

Conc.	INTER DAY Peak Area	INTER DAY %RSD	Intra-day Peak Area	Intra-day %RSD
45 ppm	1422687	0.21 %	142524	0.18 %
75 ppm	2145665	0.26 %	2140165	0.47 %
95 ppm	2499110	0.13 %	2500847	0.23 %

Accuracy: The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness. Accuracy was calculated from the results of precision experiment and the results obtained for percent accuracy at three levels across range are tabulated. According to ICH

guideline Q2R1, accuracy is determined at three concentration levels (QC standards) across the range. The area of injections was determined for corresponding concentration levels. From the measured concentrations and correspondent nominal concentrations, percent accuracy was determined. Results of the same are tabulated. Results attained were found within acceptable limit of pharmacopoeia standards for Vildagliptin (95-105 % w/w). Vildagliptin drug was purchased from Swapn roop Drugs Pvt. Ltd. Aurangabad% Assay of Marketed Formulation – Brand name- Zomelis 50mg, Mfd. By-Eris Lifesciences Ltd. Ten tablets were weighed. Avg. Wt. of tablets = 0.1902 g. An accurately weighed amount of the finely powdered Zomelis tablets equivalent to 100 mg of Vildagliptin was taken and transferred into a 100 ml volumetric flask; after addition of 60ml of mobile phase it was sonicated with occasional shaking for 10 min. To the room temperature solution was cooled and diluted to volume with the mobile phase. The resultant solution was filtered through 0.2 µl syringe filter. With mobile phase5 ml of this solution was diluted to 100 ml. Through the 0.20µm PTFE membrane filter final solution was filtered.25µl volume of final sample solution was injected in duplicate into HPLC and peak areas were measured under optimized chromatographic conditions.

RECOVERY

Preparation of Standard Stock Solution-About 100 mg of Vildagliptin was weighed accurately and transferred into a 100ml volumetric flaskand dissolved and diluted to volume with mobile phase to get 1000 ppm solution.

Table 8 – Result % Recovery of Vildagliptin

Parameter	**Spike concentration**		
Condition	50%	80%	120%
Spike area	1465280	2381250	3630234
AVG	1465280	2381250	3630234

Theoretical Conc. (t)	**y**	**c**	**x observed conc. in ppm**	**% recovery**
50 ppm	1465280	30448	48.12	96.24%
80 ppm	2381250	30448	78.21	97.76%
120 ppm	3630234	30448	119.23	99.36%

Calculations- x = ym

% Recovery= x * 100T

Robustness: The robustness of an analytical method is a assessment of its capability to stay unaffected by small, but purposeful variations in method parameters and provides an indication of its consistency throughout customary usage. Experiments were performed for 50µg/ml concentration of Vildagliptin by changing conditions such as flow rate ratio (±0.1ml /min) and wavelength (± 2nm). The mean area of 50µg/ml concentration of Vildagliptin solution was recorded for deliberate variations in method parameters viz, flow rate (0.7 and 0.9 ml/min) and wavelength variation (± 2nm)

Table9: Change in wavelength i.e.± 2 nm

	Wavelength	
WL	**208 nm**	**212 nm**
Area	1474628	1478330

Table 10: Change in Flow i.e.,± 0.1 ml/min

1. **0.7 ml/min 2. 0.9 ml/min.**

	Flow Rate	
Flow	**0.7 ml/min**	**0.9 ml/min**
Area	1444556	1419112

CONCLUSION

The proposed method is simple, accurate, reproducible and suitable for routine determination of Vildagliptin from its pharmaceutical dosage form as well. Literature survey revealed that few analytical methods are used for estimation of Vildagliptin. This method is also useful for the determination of

Vildagliptin from its pharmaceutical dosage form. The method was validated successfully using parameters like accuracy, precision, linearity, LOD, LOQ and robustness.

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