Research Article **CODEN: AJPAD7** **ISSN: 2321 - 0923**

Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry

Journal home page: www.ajpamc.com

METHOD DEVELOPMENT AND VALIDATION FOR THE VILDAGLIPTIN BY RP-HPLC METHOD

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ABSTRACT

A new simple, specific, precise and accurate RP-HPLC method has been for determination of vildagliptin in pharmaceutical dosage form. An Altima C18 column (150mm x 4.6mm, 5µm) as stationary phase. A mixture of dilute phosphoric acid solution pH 2.6±0.5 as buffer and acetonitrile in the ratio of 40:60 v/v was prepared and it is used as mobile phase. Injection volume was 10µL and flow rate was 0.5mL/min and run time was 6.0min. The column was maintained at ambient temperature and the eluent was monitored at 210nm. The retention time for vildagliptin was 3.05. The correlation coefficient value of Vildagliptin was 0.999. The validation method was developed as per ICH guidelines. The developed technique was validated for accuracy, precision, linearity, robustness, system suitability, specificity studies and result was analyzed according to the ICH guideline.

KEYWORDS

RP-HPLC method, Vildagliptin, Phosphoric acid, Acetonitrile and ICH guidelines.

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INTRODUCTION

Vildagliptin is a potent dipeptidyl peptidase-4 (DPP-4) inhibitor and used as oral potential anti-diabetic agent for treatment of type 2 diabetes mellitus[1]. IUPAC name of Vildagliptin is (1-[(3-Hydroxy-adamant-1-ylamino) acetyl]- pyrrolidine-2 (S)- carbonitrile[2] and is having molecular weight 303.40[2,3]. Pharmacologically, it acts as selective DPP-4 inhibitor. European Medicines Agency issued approval for use of vildagliptin in the European Union in February 2008 and is also listed on Australian Pharmaceutical Benefits Scheme[1].

Literature survey revealed that few analytical methods such as spectrophotometric, HPLC and LC-MS methods have been reported for the estimation of Vildagliptin in combination with other drug or in alone[4]. Hence a new accurate, sensitive and less time consuming HPLC method was developed and validated as per ICH guidelines for the estimation of Vildagliptin in bulk sample and in pharmaceutical dosage form[5].

MATERIAL AND METHODS

Chemicals and Reagents

Pharmaceutical grade Vildagliptin, gifted from Harman Finochem, Aurangabad. Galvus tablets nominally containing 50mg Vildagliptin per tablet purchased from local market. HPLC grade Acetronitrile, phosphoric acid and water procure from Shodh Advantech, Aurangabad.

Instrumentation

The liquid chromatographic system consisted of Waters HPLC system equipped with a reverse phase Altima C18 column (150mm x 4.6mm; 5µm), a 10µL injection loop, UV 1000 detector, running on Chrom Quest Software. Electronic balance was used for weighing purpose.

Chromatographic conditions

HPLC was connected with Altima C18 column (150mm x 4.6 mm, 5µm) as stationary phase. A mixture of dilute phosphoric acid solution pH 2.6±0.5 as buffer and acetonitrile in the ratio of 40:60 v/v was prepared and it is used as mobile phase. The phosphoric acid buffer solution was prepared by transferring about 1 ml of concentrated phosphoric acid into 1000ml standard flask, add 400ml of milli-Q water, mix and dilute to volume with milli-Q water, sonication was done for five minutes and cool to room temperature, pH of above solution was measure and adjusted the pH to 2.6 with phosphoric acid solution and filtered through 0.45µ nylon filter. Injection volume was 10µL and flow rate was 0.5mL/min and run time was 6.0 min. The column was kept at ambient temperature and the eluent was monitored at 210nm.

Preparation of standard stock solution

5.0mg of Vildagliptin powder dissolve in 50ml of mobile phase. For complete dissolution of drug sonication method was used. Then, pipette out 10ml of above stock solution into 100ml mobile phase to become 10µg/ml solution.

Prepation of sample solution

10 tablets were weighed and finely powdered. An accurately weighed portion of powder sample equivalent to 5mg of Vildagliptin was transferred to volumetric flask and is dissolved in 50ml of the mobile phase. For complete dissolution of drug sonication method was used. Then filtered it. Then, pipette out 10ml of above stock solution into 100ml mobile phase to become 10µg/ml solution.

Calibration curve

Appropriate dilutions of standard Vildagliptin stock solution were taken in different volumetric flasks and resultant solution was diluted up to the mark with mobile phase to obtain final concentration of 5, 10, 15, 20, 25 and 30µg/ml of Vildagliptin. These solutions were injected into chromatographic system injector, peak area ratio was determined for each concentration of drug solution with the help of obtained chromatogram. Linearity curve of Vildagliptin was constructed by plotting peak area ratio versus applied concentration of Vildagliptin and linear regression model can be fitted. Similarly, the sample solution was chromatographed and concentration of Vildagliptin in tablet sample was found out using linear regression model.

METHOD VALIDATION

The validation method was developed as per ICH guidelines and accordingly the parameter evaluated were accuracy, precision, linearity, robustness, system suitability, specificity studies. For all parameters %RSD were calculated[6-8].

Accuracy: (Recovery studies)

The accuracy of the method was determined by calculating recovery of Vildagliptin by the method of standard addition[5]. Recovery studies were performed by application of method to pre-analyzed sample by adding known amount of vildagliptin which corresponds to 50, 100 and 150 % label claim. From the above analysis, percentage recovery and standard deviation of percentage recovery were calculated.

Precision

The precision of an analytical procedure describes the closeness of agreement between series of measurements obtained from multiple sampling of same homogeneous sample under the prescribed conditions.

Intra-day precision

It was determined by analyzing vildagliptin standard solutions at three different concentrations in linearity range for thrice on the same day. Each concentration was spotted in triplicate and % R.S.D. was determined.

Inter-day precision

Standard drug solutions at three different concentrations on different three days over a period of one week were analyzed and % R.S.D. was calculated.

Linearity

The linearity of an analytical is its ability to obtain test result, which are directly proportional to the concentration of analyte in the sample. Calibration curves is used to determine the linearity. For HPLC methods, the linear relationship between detector response and analyte concentration is determined. The linearity of the method was determined at six concentration levels ranging from 5 to 30ppm for Vildagliptin. Evaluation of the drug was performed with UV detector at 210nm, peak area was recorded for all the peaks. The correlation coefficient value of Vildagliptin was 0.99987.

Robustness

The robustness of an analytical procedure is measure of its capacity to remain unaffected by small, but deliberate variation in method parameters. It provide an information of its reliability during normal usage. The robustness of the method was evaluated, during method development by analyzing the effects of small variation in the change in wavelength (±1nm) and change in flow rate (±0.1ml /min).

System suitability

System suitability study of the method was carried out by six replicate analysis of solution containing 100% target concentration of Vildagliptin. Various chromatographic parameters such as retention time, peak area, tailing factor, USP therotical plate, asymmetry factor and resolution between the peaks were determined and the method was evaluated by analyzing these parameters.

Specificity

Specificity is the ability of method to measure the analyte in the presence of other relevant components those are expected to be present in sample. To determine the specificity of the method, standard sample of Vildagliptin were injected first. Then marketed product, blank and excipients solution were run in the instrument one after another.

Limit of detection and Limit of quantification

The limit of detection of an individual analytical procedure is the lowest amount of analyte in a sample which can be find out but not necessarily described as an exact value. The limit of quantification of an individual analytical procedure is the lowest amount of analyte in sample which can be quantitatively determined.

LOD and LOQ were calculated by using ICH guidelines.

LOD = 3.3 × SD/SLOPE

LOQ = 10 × SD/SLOPE

And SD (Standard Deviation) is calculated by using R Studio software and MS -Excel.

RESULTS AND DISCUSSION

The procedure was reform with a view to develop an accurate, specific and precise HPLC method in tablet dosage form using Altima C18 column (150mm x 4.6mm, 5µm). The use of dilute phosphoric acid and acetonitrile as a mobile phase in the ratio of 40:60 v/v resulted in peak with good shape and resolution. The flow rate was 0.5ml/min and the drug component was measured with UV detector at 210nm.

Results of system suitability are summarized in Table No.2. Six consecutive injections of the standard solution showed uniform retention time, plate USP count, tailing facto, linearity range, slope for both the drugs which indicate a good system for analysis.

Chromatograms shown in Figure No.2 and Figure No.3 explain that retention time for standard sample and commercial product of Vildagliptin are same.

This proves that, analytical method was not affected by excipients and also blank peak did not overlap drug peak. So the method is highly selective. A linear relationship between peak areas versus concentrations was observed for Vildagliptin in the range of 5% to 30% of nominal concentration. The correlation coefficient is 0.999 which shows that method is linear. Linearity curve of Vildagliptin is shown in Figure No.4.

Results of Intra-day and inter-day variability were summarized in Table No.4. The % RSD of peak areas was calculated for various trial. The method is highly precise as % RSD of peak area was less than 1% in all tests.

Results of recovery study are presented in Table No.5. The measured value was obtained by recovery test. Peaked amount of both the drug were compared against the recovery amount. % Recovery was 98.97% for Vildagliptin.

The results of robustness of the present method showed that small changes were made in the flow rate and wavelength did not produce significant changes in analytical results which determines that the method is robust. All the results describes that the method is accurate.

Table No.1: Optimized chromatographic conditions of Vildagliptin

S.No	Parameter	Condition
1	Mobile phase	0.1 % phosphoric acid : Acetonitrile (40:60)
2	pH	2.6
3	Diluent	Water
4	Column	Altima C18 column (150mm x 4.6mm, 5µm)
5	Column temperature	Ambient
6	Wavelength	210nm
7	Injection volume	10µl
8	Flow rate	0.5ml/min
9	Retention time	3.05
10	Run time	6 min

Table No.2: Result of system suitability tests of Vildagliptin

S.No	Parameter	Range
1	Linearity range (µg/ml)	5-30
2	Slope	16409
3	Intercept	18037
4	Retention time	3.05
5	Correlation coefficient	0.999
6	Theoretical plates (N)	5892
7	Tailing factor	1.42
8	LOD (µg/ml)	1.36
9	LOQ (µg/ml)	4.12

Table No.3: Linearity results of Vildagliptin

S.No	Concentration	Area
1	5	97097
2	10	183194
3	15	265653
4	20	348775
5	25	429412
6	30	507067

Table No.4: Intra-day and inter-day precision result of Vildagliptin

S.No	Concentration (µg/ml)	Intra-day precision (%RSD)	Inter-day precision (%RSD)
1	7 (µg/ml)	0.53	0.79

Table No.5: Recovery results of Vildagliptin

S.No	Level	Standard concentration (µg/ml)	Concentration added (µg/ml)	Concentration found (µg/ml)	% Recovery	Mean recovery
1	50%	10	5	14.91	99.40	98.97
2	100%	10	10	19.6	98	
3	150%	10	15	24.88	99.52	

Table No.6: Assay results of Vildagliptin

S.No	Formulation	Label claim	Amount found	% Assay
1	Galvus	50mg	49.96	99.92

Table No.7: Results for robustness test of Vildagliptin

S.No	Parameter	Flow change		Wavelength Change	
1	Condition	0.4	0.6	209nm	211nm
2	Area	174323	162004	160083	161796
3	Retention time	3.60	2.70	3.08	3.08

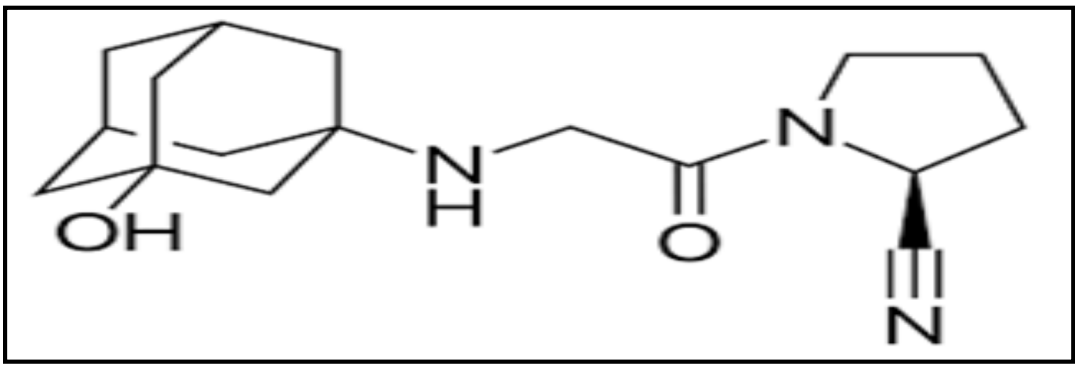

Figure No.1: Structure of Vildagliptin

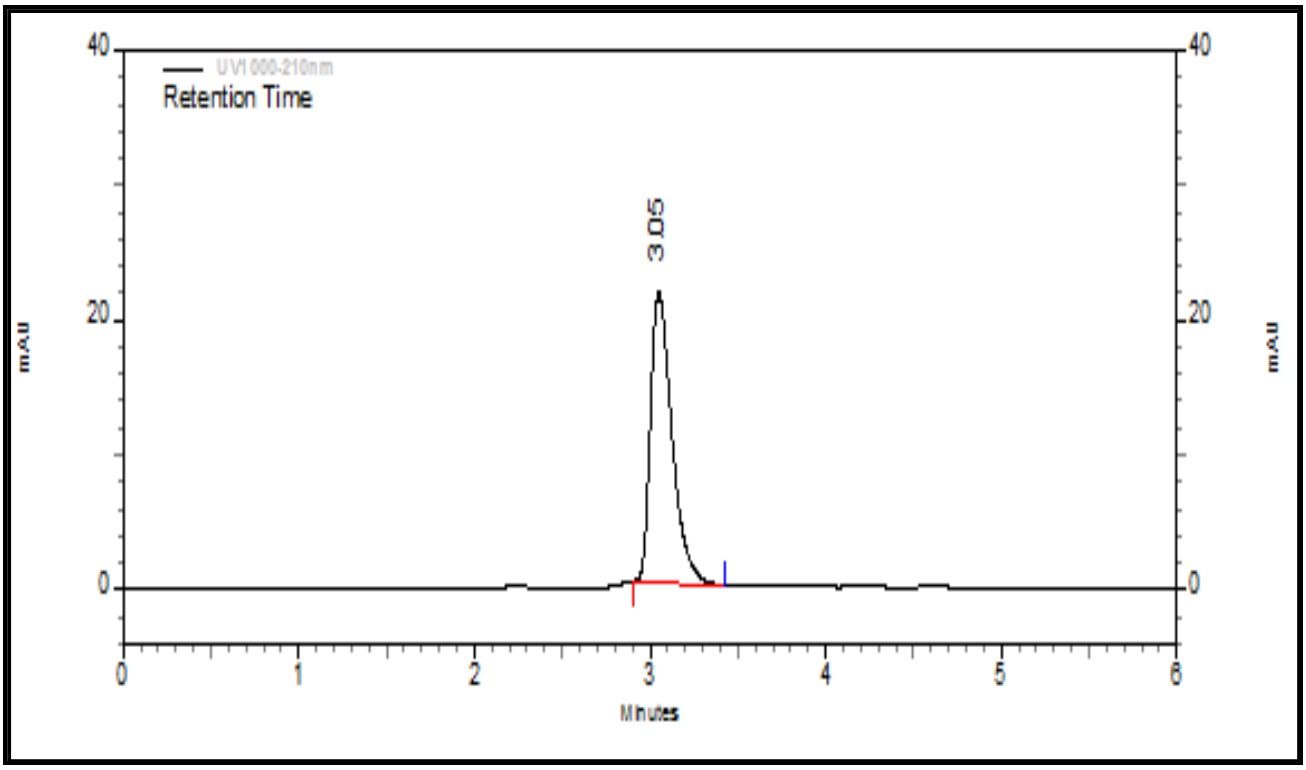

Figure No.2: Typical Chromatogram of standard Vildagliptin

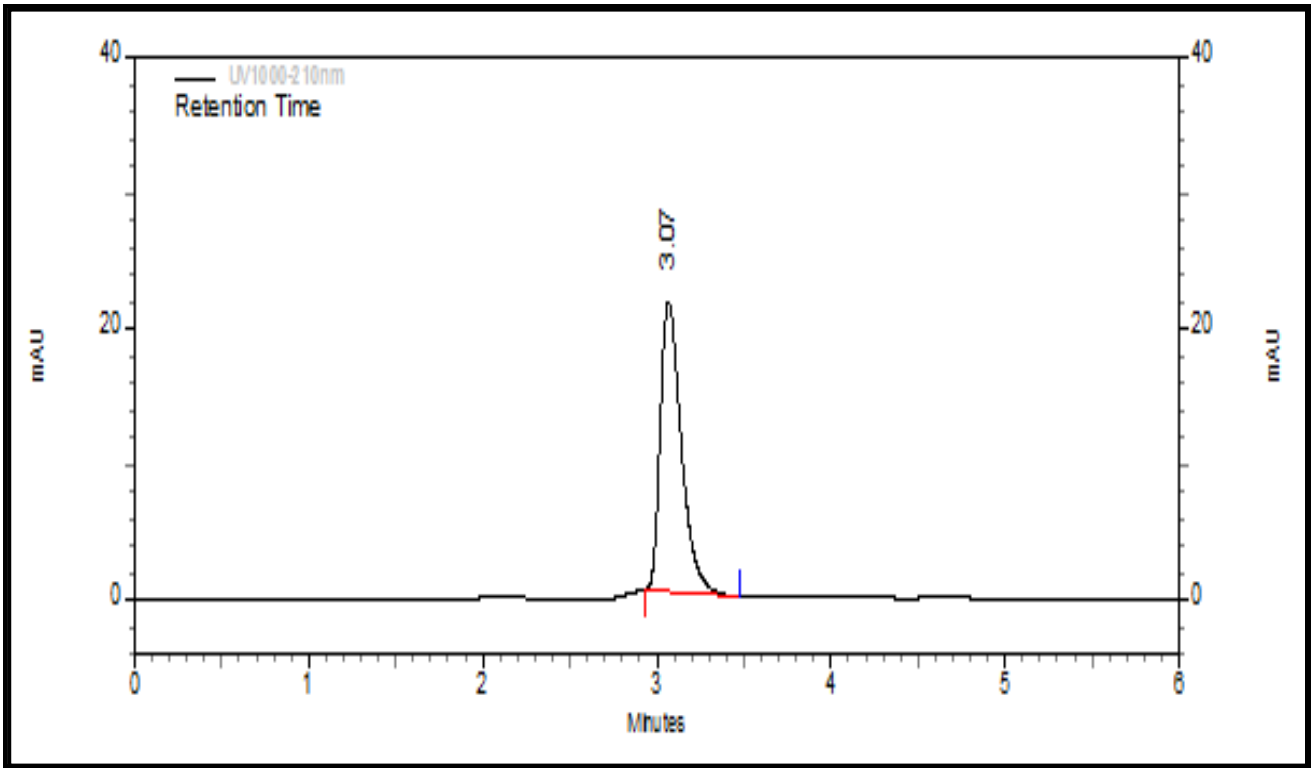

Figure No.3: Typical chromatogram of Vildagliptin in marketed formulation

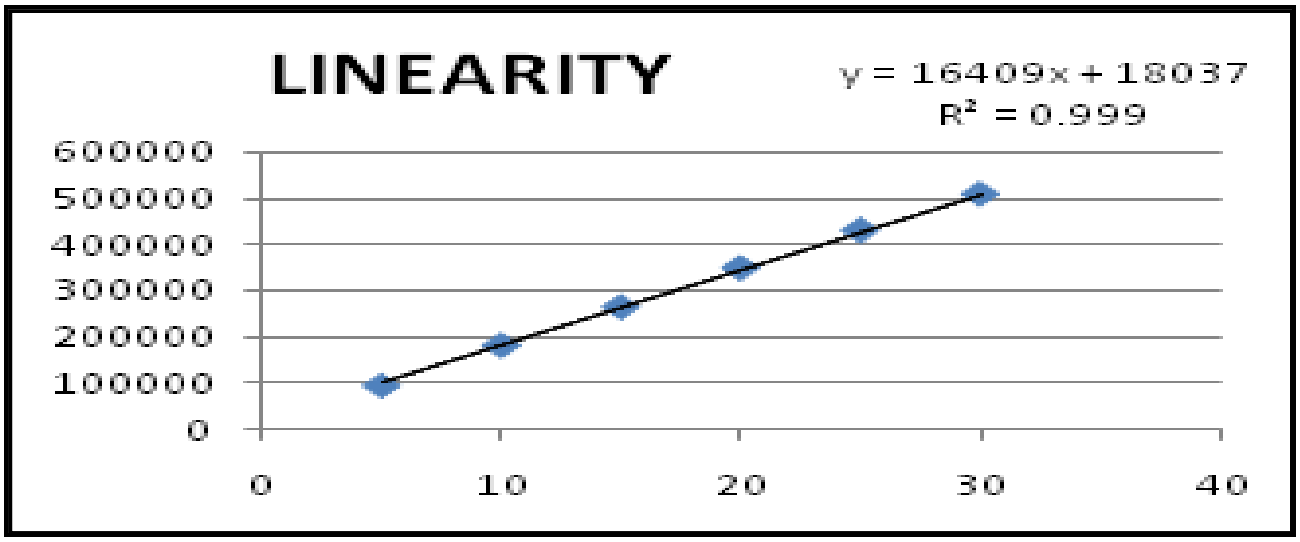

Figure No.4: Linearity curve of Vildagliptin

CONCLUSION

The proposed RP-HPLC has been validated as per the of ICH guidelines. The method was found to be very simple, sensitive, accurate and precise. Percentage of recovery shows that the method is free from interference of the excipients used in the formulation and shows acceptable correlation coefficient. The advantages of proposed method are short analysis time and a simple procedure for sample preparation. Therefore, the above method can be used for analysis of estimation of Vildagliptin in its tablet dosage form.

ACKNOWLEDGEMENT

I am very thankful to Department of Quality Assurance, faculty of Dr. Vedprakash Patil Pharmacy College, Aurangabad. I would also like to thank the Management of College, for providing the necessary facilities to carry out this work.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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Please cite this article in press as: Vijay Govindrao Napate and Pratik Anantrao Napate. Method development and validation for the vildagliptin by RP-HPLC method, *Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry,* 8(1), 2020, 24-31.