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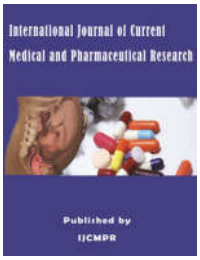

Research Article

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF VILDAGLIPTIN

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ABSTRACT

In the present work, a rapid, accurate and precise RP-HPLC method was developed for the estimation of Vildagliptin in tablet dosage form 50mg by selecting various chromatographic parameters.

A new method was developed using 250×4.6mm, 5µm, reverse phase C18 column with mobile phase of 80 volumes of Buffer and 20 volumes of Acetonitrile and 80 volumes of methanol and 20 volumes of water as diluent. Flow rate was 1.1ml/min with PDA detection at 210nm and the injection volume was set at 10µL with about 7.0 minutes of runtime.

The method was validated by using various validation parameters like accuracy, precision, linearity, specificity and stability in analytical solution and robustness. These results show the method could find practical application as a quality control tool for analysis of the drug in its tablet dosage forms in quality control laboratories.

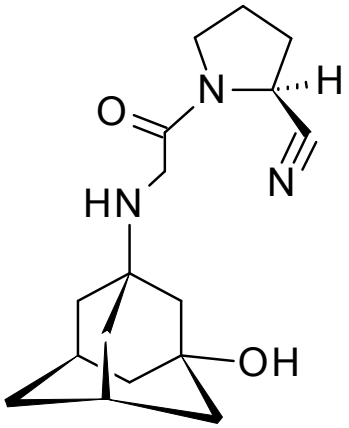
INTRODUCTION

Vildagliptin is a dipeptidyl peptidase-4 (CD26) inhibitor used in treatment of Diabetes. [1] It is an oral antidiabetic agent, prescribed for type 2 diabetes mellitus along with other medications. It is chemically (S)-1-[2-(3-Hydroxyadamantan-1-ylamino) acetyl] pyrrolidine-2-carbonitrile. [2] Vildagliptin binds covalently to the catalytic site of DPP-4, eliciting prolonged enzyme inhibition. This raises intact GLP-1 levels, both after meal ingestion and in the fasting state. Vildagliptin has been shown to stimulate insulin secretion and inhibit glucagon secretion in a glucose-dependent manner. At hypoglycemic levels, the counter regulatory glucagon response is enhanced relative to baseline by Vildagliptin. The proposed method is simple, accurate, reproducible and suitable for routine determination of Vildagliptin from its pharmaceutical dosage form. [3] Literature survey revealed that many analytical methods were developed using combination of drugs along with Vildagliptin. Simultaneous estimation of Vildagliptin and Meformin was been developed using RP - HPLC [4], Simultaneous determination of novel gliptins in their binary mixtures with Metformin was developed using Vildagliptin, Metformin and Saxagliptin. [5] But there are few methods developed using single drug.

MATERIALS AND METHODS

Chemicals and Reagents

Vildagliptin (Galvus) tablets 50mg were kindly supplied by Novartis distributers, Vijayawada. Vildagliptin API gift sample was given by Spectrum pharma research solutions, Hyderabad. HPLC grade water was obtained from RFCL Limited, Gujarat. HPLC methanol was obtained from S d Chem limited, Mumbai. Potassium dihydrogen phosphate, Triethylene amine, and Orthophosphoric acid were obtained from Savan Pharmaceuticals, Hyderabad. Acetonitrile was obtained by RANKEM.



Vildagliptin

Instruments

The HPLC system consisted of Waters 2695 Separation module with Empower Pro software using Hiber purospher C_{18} (250 × .6MM, 5µm) column. The system consists of PDA detector and an autosampler. An Utlrasonic sonicator is used for degassing the mobile phase. In addition Sartorius model

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EQMK VI Micro balance, Thermo scientific P^H meter and Nylon 0.45µm Filters used for filtration are used in this study.

Chromatographic conditions

Chromatographic separation was achieved on a Hiber purospher C18 column (250mm×4.6mm, 5µm) with PDA detection at 210 nm. Mobile phase was prepared by using buffer and acetonitrile in the ratio of 80: 20 was degassed for 20 mins. Buffer solution was prepared by weighing 2.72gm of Potassium dihydrogen phosphate in 1000ml of HPLC-grade water and adjusting the P^H to 4.35 using Triethylene amine and Orthophosphoric acid solution. Methanol and water in the ratio of 80: 20 was used as diluent. The mobile phase was pumped through the column at a flow rate of 1.1 ml /min. Analyses was performed at 30ºC and the injection volume was 10 µL.

Preparation of Buffer

Accurately weighed and transferred 2.72gm of 0.02M Potassium dihydrogen Orthophosphate in a 1000ml of Volumetric flask and 900ml of milli-Q water and 1ml of triethylamine were added and degassed to sonicate and finally made up the volume with water, then pH adjusted to 4.35 with dil. Ortho phosphoric acid solution.

Preparation of Stock solution

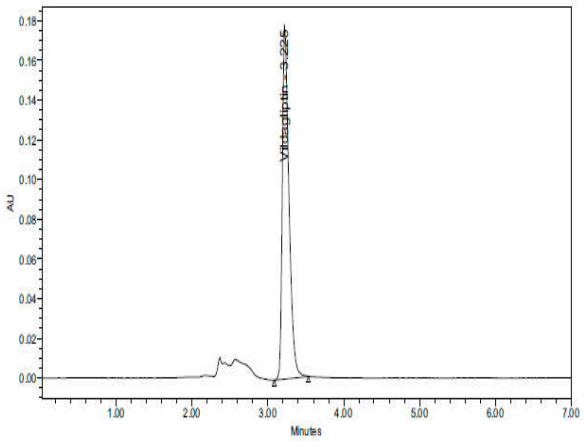
Accurately weighed and transferred 50mg of Vildagliptin WS into 10ml clean dry volumetric flask, add 7ml of diluent, sonicated for 5 minutes and made up to the final volume with diluent to obtain a concentration of 1µg/ml. This solution is used as a stock solution.

Calibration curve of Vildagliptin

The appropriate stock solutions were taken into different 10ml volumetric flasks and diluted upto the mark with diluent to obtain the concentrations of 50 - 375µg/ml. The solutions were injected into HPLC with 10µl injection volume and chromatograms were recorded. Calibration curve was obtained by plotting average peak area versus concentration.

Analysis of Marketed Formulations

5 tablets were weighed and calculated the average weight of each tablet, then the weight equivalent to 5 tablets was transferred into a 100 mL volumetric flask, 50mL of diluent added and sonicated for 30 min, further the volume made up with diluent and filtered using 0.45µm Nylon filters. From the filtered solution 1ml was pippeted out into a 10 ml volumetric flask and made up to 10ml with diluent. Final sample solutions were injected into HPLC and peak areas were measured under



Chromatogram – 2 sample

PROCEDURE

METHOD VALIDATION

The method of analysis was validated as per the recommendations of ICH for the parameters like accuracy, linearity, precision, limit of detection, limit of quantitation, robustness and degradation studies. [6, 7] The accuracy of the method was determined by calculating percentage recovery of Vildagliptin. [8,9,10] For the drug, recovery study was carried out by applying the method to drug sample to which known amount of Vildagliptin corresponding to 50%, 100% and 150% of label claim had been added (standard addition method). At each level of the amount three determinations were performed and the results obtained were compared. Precision studies of Vildagliptin were carried out by estimating the corresponding responses. Six samples solutions were prepared individually from Vildagliptin stock solution, as per methodology and each solutions were injected into HPLC. The limit of detection (LOD) and limit of quantitation (LOQ) were calculated using following formulae: LOD= 3.3(SD)/S and LOQ= 10 (SD)/S, where SD=standard deviation of response (peak area) and S= average of the slope of the calibration curve (see table – 2). System suitability parameters were checked to improve the effectiveness, by injecting the drug sample. For robustness evaluation certain parameters like Flow rate, Temperature, Mobile phase were deliberately changed. Flow rate was changed from 1.1 ml/min to 0.9 ml/min and 1.0 ml/min. Temperature was changed from 30ºc to 29ºc and 31ºc. Mobile phase ratio was changed from 80: 20 to 70: 30 and 90: 10. Degradation studies were also conducted to determine the stability of the drug. Degradation studies were carried out using Acid stress studies using 0.01N hydrochloric acid, Base stress studies using 0.01N sodium hydroxide, Peroxide stress studies using 20% Hydrogen peroxide, Thermal stress studies by keeping it at 105º c for 6hrs and UV studies using 200 Watt hours/m^2. See table – 1

RESULTS AND DISCUSSION

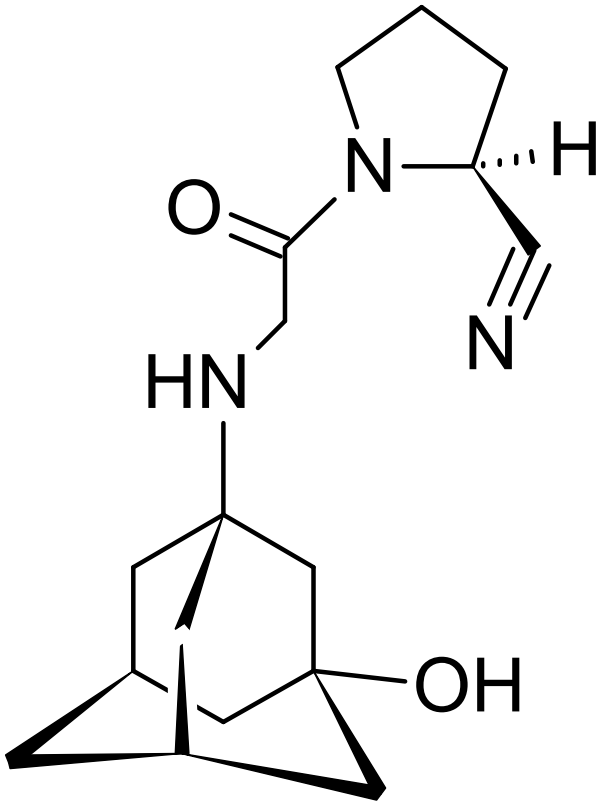
The RP-HPLC method was developed by using Hibar Purospher C18, 250 x 4.6 mm, 5µ column with mobile phase of 80 volumes of 0.02M Potassium dihydrogen Orthophosphate and 20 volumes of Acetonitrile which is sonicated to degas. And 80 volumes of 100% Methanol and 20 volumes of 20% HPLC grade water are used as diluent. Flow rate was 1.1ml/min with UV detection at 210nm and injection volume was set to be 10µl with 7 minutes of run time. Mobile phase used has sufficient polarity to elute the drug. All the system suitability parameters like theoretical plates, resolution, tailing factor were optimal. The developed LC method was found to be specific for estimation of Vildagliptin in its tablet dosage form 50mg.

System Suitability

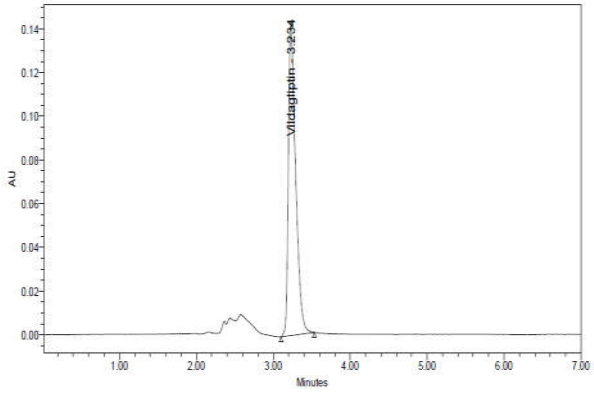
The RSD for area response obtained for six replicate injections was 0.5%, the tailing factor was found to be 1.52 which were in the acceptance criteria of NMT 2.0% and NMT 2.0% respectively. The no.of theoretical plates were found to be 5296 for Vildagliptin which is in the acceptance criteria of NLT 2000

Precision

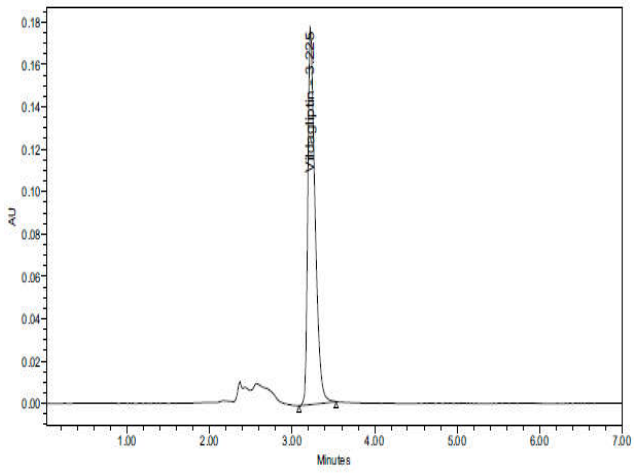
The RSD obtained from six replicate injections were well in the acceptance limit of NMT 2.0%.Method precision was



Vildagliptin



Chromatogram - 1 Vildagliptin Standard



Chromatogram- 2 Vildagliptin sample

Table 1 Degradation studies

Stress Condition		Drug Product			
		Purity angle	Threshold angle	Peak response	% Degradation
Exposed to UV light for about 200 Watt hours/m^2	Vildagliptin	3.630	1.330	1327864	3.2%
Exposed to Dry heat in oven at 105ºc for 6hrs	Vildagliptin	0.245	0.383	1241612	9.4%
Refluxed with 0.01N HCl for 30 mins at 60ºc	Vildagliptin	0.263	0.337	1224064	10.7%
Refluxed with 2N Sodium hydroxide for 30mins at 60ºc	Vildagliptin	0.212	0.311	1249630	8.9%
Refluxed with 20% sodium peroxide for 30mins at 60ºc	Vildagliptin	0.212	0.345	1197815	12.6%

Table 2 summary of present study (rp-hplc)

Validation Parameters	Vildagliptin
Mobile phase	0.02M Potassium dihydrogen Orthophosphate buffer : Acetonitrile (80:20v/v)
Flow rate	1.1ml/min
Detection wavelength	210 nm
Retention time	3.234 min
Run time	7 mins
Theoretical plates	5296
LOD	0.946
LOQ	2.850
Linearity	$R^2 = 0.9998$
Precision	% RSD < 2
Recovery	98 – 102%

carried out by the same analyst on the same day with same instrument repeatedly for six times to ensure that the method gives consistent results. The RSD was calculated for standard and sample on six determinations and was found to be in the acceptance limit of NMT 2.0%.

Specificity

Specificity of the method was determined and the peaks of diluent, mobile phase and excipients of tablets did not interfere with the peaks of Vildagliptin.

Linearity

The response was linear over the concentration range 20% to 150%.The correlation coefficient was found to be 0.9998 with the acceptance criteria limit of NLT 0.999.

Accuracy

The mean % recoveries at concentrations ranging from 20 to 150% were found to be 99.7 to 100.5% which were in the acceptance limit of 98.0 to 102.0% and the RSD was within the limit of NMT 2.0%.

Robustness

Robustness of the method was determined to ensure its capacity to remain unaffected by small deliberate variation in the method parameters like flow rate of mobile phase, mobile phase ratio, pH and column oven temperature. The system suitability parameters obtained from normal experimental conditions and that obtained from small and deliberately changed conditions for each of the system suitability parameters like RSD, Tailing factor and Theoretical plates passes for all the conditions.

Degradation studies

It is observed that the percentage of degradation at UV was 3.2%, at Thermal it was 9.4%. When it was refluxed with

0.01N HCl, 2N NaOH and 20% sodium peroxide the percentage of degradation were 10.7%, 8.9% and 12.6% respectively.The proposed method was validated and met the requirements as per ICH guidelines. Hence, the method can be conveniently adopted for the routine analysis in quality control laboratories. It was shown that the method was accurate, precise, linear, selective, reproducible, repeatable proving the reliability of the method.

CONCLUSION

Vildagliptin is the drug used in the treatment of Diabetes. It is a Dipeptidyl Peptidase – IV inhibitor. An efficient HPLC method was developed and validated for estimation of Vildagliptin in its tablet dosage form 50mg. From literature review and solubility analysis initial chromatographic conditions were set and different trials were run to Vildagliptin got eluted with good peak symmetric properties. The HPLC method was developed using buffer and Acetonitrile 80:20 ratio as mobile phase, Hibar Purospher C18, 250 x 4.6 mm, 5µm, flow rate 1.1 ml/min detection wave length 210 nm, column temperature 30^{o}C and diluent Mobile phase, conditions were finalized as optimized method. 0.01M Potassium dihydrogen phosphate was used as buffer, volume of injection was 10µl with 7 minutes of runtime. System suitability parameters were studied by injecting the standard six times and results were well under the acceptance criteria.

The method was validated by using various validation parameters like Accuracy, Linearity, Precision, Robustness and Ruggedness, and stability in analytical solution. All the validation parameters were found to be within the acceptance criteria. Linearity study was carried out between 20% (50ppm) to 150% (375ppm) levels, R^2 value found 0.9998. By using above method assay of marketed formulation was carried out, and it is found to be between 98.00% and 101.00% were within the limits. It was shown that the method was accurate, precise, linear, selective, reproducible, repeatable proving the reliability of the method.

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