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RP-HPLC Based Analytical Method: Development and Validation of Vildagliptin in Bulk Drug and Finished Product

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ABSTRACT

The present study was undertaken with an objective of developing suitable, sensitive and simple analytical RP-HPLC method for Bulk drug and its pharmaceutical dosage form. In RP-HPLC method was resolved using Acetonitrile: Phosphate Buffer (60:40) pH 3.6 at a flow rate of 1.0 ml/min, UV- visible detector with Data Ace Software and Inertsil C18 column. The detection was carried out at 215 nm. The retention times of Vildagliptin 3.924 minutes, the different analytical parameters such as accuracy, linearity, precision, robustness, ruggedness were determined according to the ICH Q2B guidelines. Due to its simplicity, rapidness, high precision and accuracy, the proposed RP-HPLC method may be used for determining vildagliptin in pure form and in tablet formulation.

Keywords: Vildagliptin, Acetonitrile, Accuracy, RP-HPLC.

Introduction

Vidagliptin (VILD), S-1-[N-(3-hydroxy-1- adamantyl) glycyl] pyrrolidine-2- carbonitrile is an oral antihyperglycemic agent of the new dipeptidyl peptidase-4 (DPP4) inhibitor class of drugs[1].

It is also called as antidiabetic agent used for the acute treatment of diabetes. Vildagliptin inhibits dipeptidyl peptidase-4 (DPP-4). This in turn inhibits the inactivation of GLP-1 by DPP-4, allowing GLP-1 to potentiate the secretion of insulin in the beta cells. Dipeptidyl peptidase-4's role in blood glucose regulation is thought to be through degradation of GIP and the degradation of GLP-1[2]. Literature survey revealed that few analytical methods such as spectrophotometric6-8, HPLC9-17 and LC-MS18,19 methods have been reported for the estimation of Vildagliptin in alone or in combination with other drugs.[3]

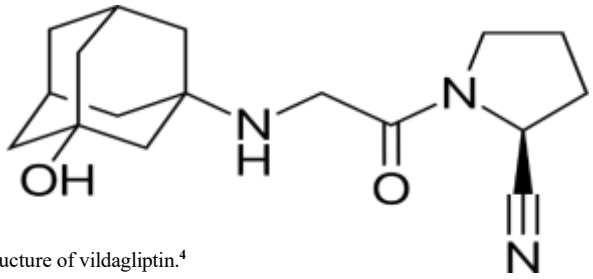

Fig. 1: Chemical structure of vildagliptin.[4]

Experimental Methods

Chemicals and materials

The drug used for present study was obtained from Arch Pharmalabs Ltd. Thane, Maharashtra India as gift sample.All reagents and chemicals used were of AR grade and HPLC grade from Merck Ltd., India , Acetonitrile HPLC Grade, Methanol HPLC grade, Ortho-phosphoric acid & Water HPLC grade.

Identification by FTIR-Spectroscopy:

Vildagliptin API: The IR absorbance spectrum of Vildagliptin was recorded using FTIR 8400S spectrometer (Shimadzu) over range of 4000 to 400 cm-1. The sample 1mg of Vildagliptin API was mixed properly with 200 mg of dried KBr and then carefully triturated in a mortar pestle[5]. At last this mixture was kept on a die and IR spectrum was taken using the Diffused Attachment reflectance mode.

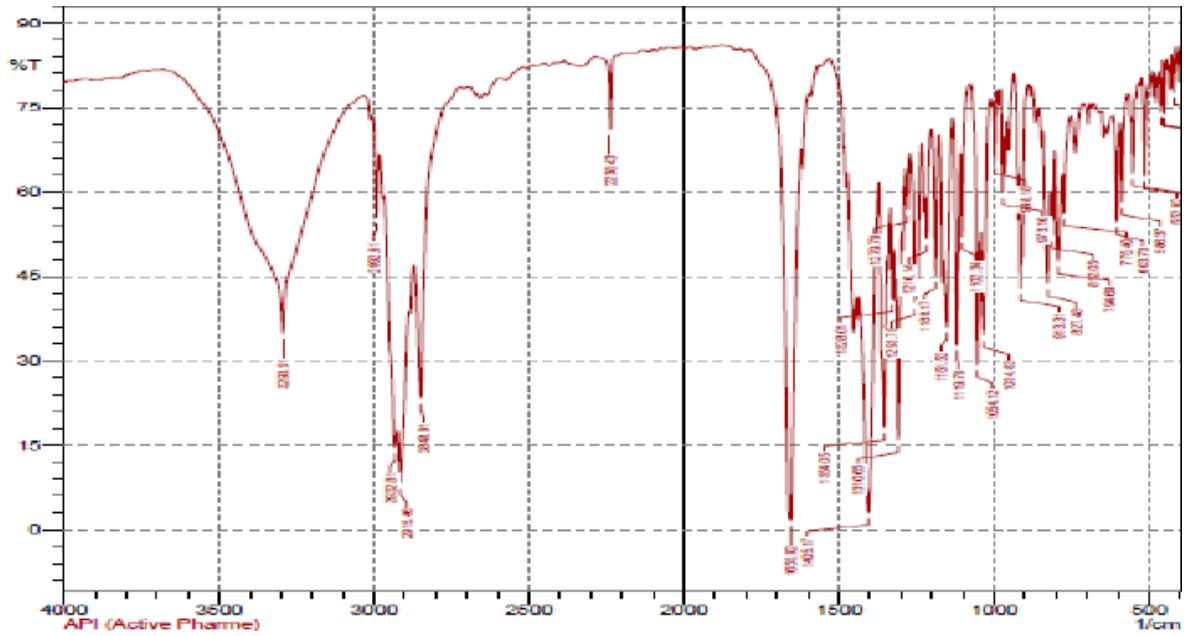

Figure 2: IR spectra of Vildagliptin

Determination of Max wavelength: The ultraviolet absorption spectrum of VILD was obtained using Shimadzu1800- UV visible spectrophotometer and 1cm quartz cells, over a wavelength range of 400 to 200 nm. The wavelength maxima (λmax) were analyzed & observed value (λmax) nm 215[6].

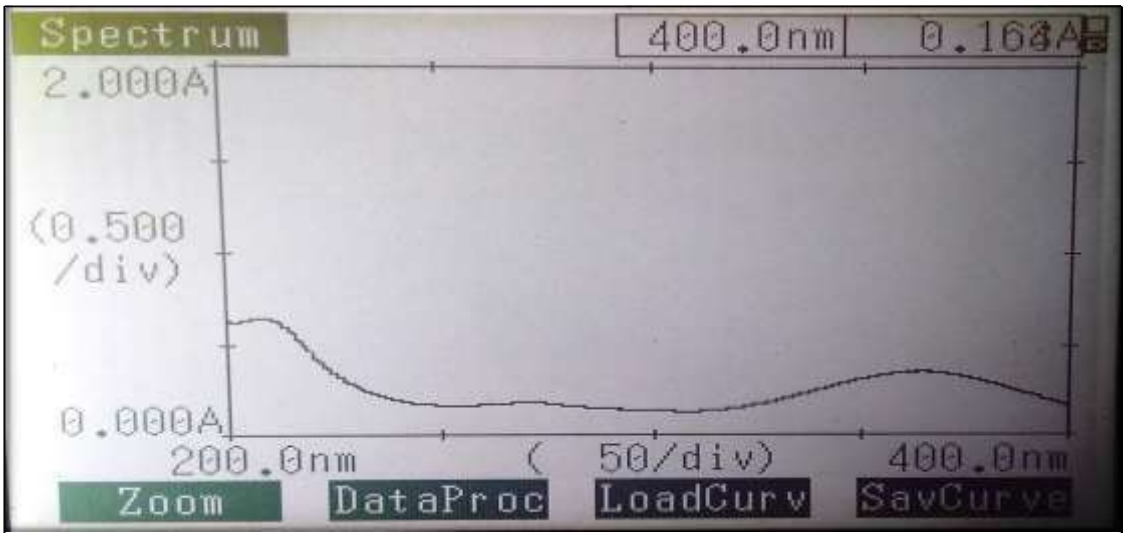

Figure 3: Determination of Wavelength for Vildagliptin.

Chromatographic condition: C18 [250mm x 4.6m, 5µm] ODS Hypersil column was used at ambient temperature. Mobile phase consisted of Acetonitrile, 10mM Ammonium acetate buffer (pH 4.5, adjusted with OPA) in the ratio of 70:30 V/V, was pumped at a flow rate of 1.5 ml/min. The mobile phase was filtered through 0.45 µm nylon membrane filter and degassed before used[7]. The elution was monitored at 218 nm and run time was 10 min.

Table 2: Optimized chromatographic conditions of vildagliptin.

Parameters	**Conditions**
Mobile phase	Acetonitrile: Phosphate Buffer (60:40) pH 3.6
Column	Inertsil 4.6 (id) x 250 mm

Particle size packing	5 µm
Stationary phases	: C18 Inertsil
Detection wavelength	: 215 nm
Flow rate	: 1 ml/min.
Temperature	: Ambient
Sample size	: 20 µL

Preparation of Mobile phase : Accurately weighed 0.77gm of phosphste dissolved and diluted with HPLC grade water. pH of buffer solution was adjusted to 3.6 with OPA. The mobile phase comprised of Acetonitrile Phosphate buffer 60:40 V/V. It was degassed for 15min before used.

Standard stock solution: Accurately weighed quantity of VILD 10 mg was transferred to 100 ml volumetric flask, shaken vigorously for five minutes and volume was made up to mark with mobile phase. The standard solution of VILD was mixed and diluted with mobile phase properly to obtain laboratory mixtures containing a concentration 50 µg/ml of VILD.

Sample preparation: For the estimation of drug from commercial formulation twenty tablets were weighed accurately. The average weight was determined, finely powdered and powder equivalent to 10 mg of drug was transferred in to a dry and well stopper 25 ml volumetric flask and mobile phase was added. It was shaken vigorously for 5-10 min and volume was made up to mark with mobile phase. The solution was filtered through Whatman filter paper no. 41. Further dilution was made to get final concentration of 50 µg/ml of VILD**.**

System suitability: System suitability is a pharmacopoeial requirement and is used to verify, whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be done. The tests were performed by collecting data from five replicate injections of standard solutions[8].

Table No. 3: Result of System Suitability Study

Sr. No	**Retention time (Rt) (Min)**	**Peak Area**	**Capacity Factor (K')**	**Tailing Factor (T)**	**No. of Theoretical plates (N)**
1	3.923	550611.8	6.38	1.12	6992.0
2	3.888	540713.0	6.38	1.12	6992.0
3	3.923	551279.0	6.36	1.11	6931.6
4	3.967	567124.5	6.36	1.11	6931.6
5	3.912	541983.5	6.38	1.12	6992.0
Statistics					
Mean	3.922	550611.8	6.372	1.12	6967.84
S.D.	0.028	1054.7	0.0109	0.008	33.08
C.V.	0.217	0.191	0.171	0.714	0.474

Validation parameters:

Accuracy: It was ascertained on the basis of recovery studies performed by standard addition method[9]. The results of recovery studies and statistical data are recorded in Table No. 4.

Table No.4: Results and statistical data for Recovery study of VILD

Sr. No.	**Wt. of tablet powder taken (mg)**	**Amount of Drug Added in**	**Wt of Std Drug**	**Peak Area of stand**	**Peak Area of sample**	**% Recovery**
1	38	2	10	550611.8	550666.86	100.01
2	38	4			545325.92	99.04
3	38	6			544775.31	98.94
					Mean	99.33

S.D.	0.48
C.V.	0.23

Precision:

Precision of an analytical method is expressed as S.D or R.S.D of series of measurements. It was ascertained by replicate estimation of the drugs by proposed method.

Table No.5: Results and statistical data of Precision Study

Sr. No.	**Wt. of tablet powder taken (mg)**	**Wt. of standard taken (mg)**	**Standard peak area for VILD**	**Sample peak area for VILD**	**Amount of drug estimated in tablet (mg)**	**Labeled claim %**
1	38	10	550611.8	552924.36	50.21	100.42
2	38			544279.76	49.42	98.85
3	38.01			545931.59	49.57	99.15
					Mean	99.47
					S.D.	0.83
					C.V.	0.82

Ruggedness:

The studies of ruggedness were carried out under two different conditions-

Interday (Different days):

Same procedure was performed as under marketed formulation analysis on different days. The % label claim was calculated. Data obtained for day 1, day 2, and day 3 is shown in Table No. 06. **Brand name** : Vildamac 50 **Avg wt -** 190 mg

Table No.6: Results and statistical data of Interday Study

Sr. No.	**Wt. of tablet powder taken (mg)**	**Wt. of standard taken (mg)**	**Standard peak area for VILD**	**Sample peak area for VILD**	**Amount of drug estimated in tablet (mg)**	**Labeled claim %**
1	38.1	10	550611.8	553750.28	50.2	100.57
2	38			544279.76	49.42	98.85
3	38			552924.36	50.21	100.42
					Mean	100.17
					S.D.	1.21
					C.V.	1.20

Intraday:

It was performed by using same procedure as under marketed formulation analysis and absorbance recorded at 3 hrs. interval within a day. The percent label claim was calculated using formula No. 4. Result and statistical data are shown in Table No. 7.

Table No.7: Result and statistical data for Intraday

Sr. No.	Wt. of tablet powder taken (mg)	Wt. of standard taken (mg)	Standard peak area for VILD	Sample peak area for VILD	Amount of drug estimated in tablet (mg)	Labeled claim %
1	38	10	550611.8	544279.76	49.42	98.85
2	38.01			545931.59	49.57	99.15
3	38			553915.47	50.30	100.60
					Mean	99.53
					S.D.	0.93
					C.V.	0.94

Different analyst:

The sample solution was prepared by two different analysts and same procedure was followed as described earlier. The % label claim was calculated as done in marketed formulation estimation[9-10].

Table No.9: Result and statistical data of Different analyst study

Sr. No	% Label claim	
	ANALYST I	**ANALYST II**
	VILD	**VILD**
1	99.85	100.3
2	100.15	99.77
3	99.66	99.61
4	100.2	99.8
5	99.60	100.18
Mean	99.89	99.93
S.D	0.2748	0.2933
C.V	0.2750	0.294

Specificity:

Specificity was measured as ability of the proposed method to obtain well separated peak for VILD without any interference from component of matrix.Mean retention time for VILD– 3.923.The values obtained were very close to that in standard laboratory mixture indicates no interference from the component of matrix.Typical chromatogram is shown in the Fig. No. 04

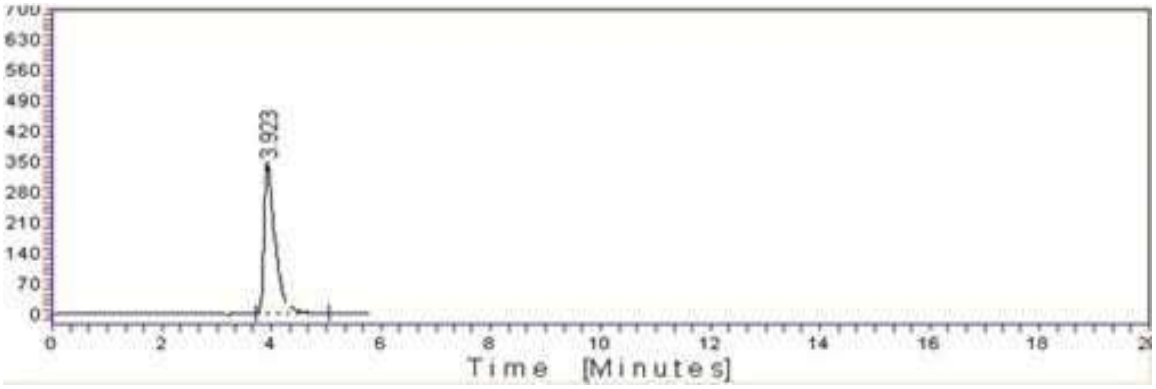

Fig. No.04: Chromatogram obtained by formulation of VILD in specificity

Linearity and range:

The plot showing linearity and range study for VILD is shown in the Fig. No. 05

Table No.10 : Observations of Linearity and range study for VILD

Sr.No.	%Label claim	Peak area
		VILD
1	80	440789
2	90	495551
3	100	550612
4	110	600673
5	120	661834

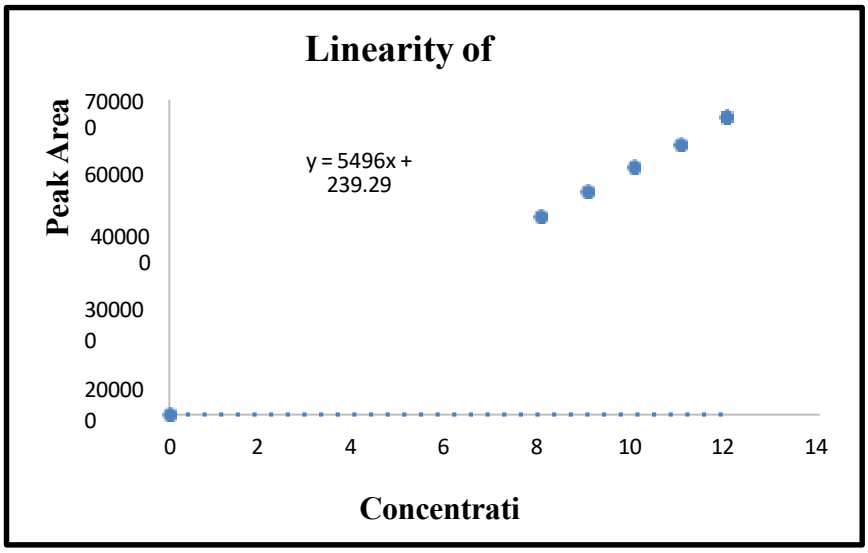

Fig. No.05: -Plot of linearity and range study for VILD

Limit of Detection (LOD) and Limit of Quantitation (LOQ):

As per ICH guideline both LOD & LOQ were performed on the basis of standard deviation of the response and slope and expressed by following formulae the results of LOD and LOQ are shown in table 11.

Table 11: LOD & LOQ of VILD

Sr. No.	Drug Name	LOD μg/ml	LOQ μg/ml
1	VILD	0.42	1.29

SUMMARY

The formulation containing Vildagliptin used in the treatment of type II diabetes mellitus in adults in the market.The present study was undertaken with an objective of developing suitable, sensitive and simple analytical RP-HPLC method for drug and its dosage form.In RP-HPLC method, the analyte were resolved using Acetonitrile: Phosphate Buffer (60:40) pH 3.6 at a flow rate of 1.0 ml/min, UV- visible detector with Data Ace Software and Inertsil C18 column {4.6 (id) x 250 mm}. The detection was carried out at 215 nm. The method gave the good resolution and suitable retention time.The results of analysis in all the method were validated in terms of accuracy, precision, ruggedness, linearity and range. The methods were found to be sensitive, reliable, reproducible, rapid and economic also.The RP-HPLC method is accurate, precise, specific, reproducible and sensitive. No interference of additives, matrix etc. is encountered in these methods. Further studies on other pharmaceutical formulations would throw more light on these studies.

1. The method provides selective quantification of Vildagliptin. This developed RP-HPLC method for estimation Vildagliptin is accurate, precise and robust.
2. The method has been found to be better because of its less retention time, isocratic mode and use of economical readily available mobile phase, readily available column, UV detection and better resolution of peaks.
3. The run time is relatively short, which will enable rapid quantification many samples in routine and quality controlled analysis of various formulations containing Vildagliptin. All these factors make this method suitable for quantification of Vildagliptin in bulk drug and in the pharmaceutical dosage forms without any interference.
4. The method was completely validated showing satisfactory data for all the method validation parameters tested. Hence this method can be introduced into routine use for determination of Vildagliptin.

Conclusion:

From the studies it can be concluded that RP-HPLC technique can be successfully used for the estimation of the Vildagliptin in bulk and its pharmaceutical dosage form. The RP-HPLC method is accurate, precise, specific, reproducible and sensitive.No interference of additives, matrix etc. is encountered in these methods. Further studies on other pharmaceutical formulations would throw more light on these studies.

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