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Research article Analytical Research

A New Analytical Method Development And Validation For The Quantitative Estimation of Anti-Diabetic Drug Vildagliptin In Pure Form And Marketed Combined Pharmaceutical Dosage Form

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ABSTRACT

Objective: The current investigation was pointed at developing and progressively validating novel, simple, responsive and stable RP-HPLC method for the Quantitative Determination of Vildagliptin in active pharmaceutical ingredient and Marketed Pharmaceutical Dosage form.

Methods: A simple, selective, validated and well-defined stability that shows isocratic RP-HPLC methodology for the quantitative determination of Vildagliptin. The chromatographic strategy utilized Symmetry C18, 250 mm x 4.6 mm i.d.5µm particle size, using isocratic elution with a mobile phase consists of Methanol and Phosphate Buffer (0.02M) (pH-3.8) was taken in the ratio of 70: 30% v/v. A flow rate of 1.0 ml/min and a detector wavelength of 245nm utilizing the UV detector were given in the instrumental settings. Validation of the proposed method was carried out according to an international conference on harmonization (ICH) guidelines.

Results: LOD and LOQ for the active ingredients were established with respect to test concentration. The calibration charts plotted were linear with a regression coefficient of R2>0.999, means the linearity was within the limit. Recovery, specificity, linearity, accuracy, robustness, ruggedness were determined as a part of method validation and the results were found to be within the acceptable range.

Conclusion: The proposed method to be fast, simple, feasible and affordable in assay condition. During stability tests, it can be used for routine analysis of the selected drugs.

Keywords: Vildagliptin, RP-HPLC, Method Development, Validation, Accuracy, Precision.

INTRODUCTION

Vildagliptin is a cyanopyrrolidine-based, orally bioavailable inhibitor of dipeptidyl peptidase 4 (DPP-4), with hypoglycemic activity. Vildagliptin's cyano moiety undergoes hydrolysis and this inactive metabolite is excreted mainly via the urine. Vildagliptin[1] (LAF237) is an orally active antihyperglycemic agent that selectively inhibits the dipeptidyl peptidase-4 (DPP-4) enzyme. It is used to manage type II diabetes mellitus, where GLP-1 secretion and insulinotropic effects are impaired. By inhibiting DPP-4, Vildagliptin prevents the degradation of glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), which are incretin hormones that promote insulin secretion and regulate blood glucose levels. Elevated levels of GLP-1 and GIP consequently results in improved glycemic control. In clinical trials, Vildagliptin[2] has a relatively low risk of hypoglycemia. Oral Vildagliptin was approved by the European Medicines Agency in 2008 for the treatment of type II diabetes mellitus in adults as monotherapy or in combination with [metformin], a sulfonylurea, or a thiazolidinedione in patients with inadequate glycemic control following monotherapy. It is marketed as Galvus. Vildagliptin[3] is also available as Eucreas, a fixed-dose formulation with metformin for adults in who do not adequately glycemic control from

monotherapy. Vildagliptin is currently under investigation in the US. The IUPAC Name of Vildagliptin is (2S)-1-[2-[(3-hydroxy-1-adamantyl) amino] acetyl] pyrrolidine-2-carbonitrile. The Chemical Structure of Vildagliptin is showing in following

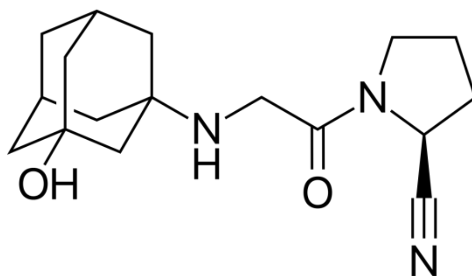

Fig 1: Chemical Structure of Vildagliptin

MATERIALS AND METHODS

Equipments

Table 1: List of Equipments

S.No.	Instruments/Equipments/Apparatus
1.	HPLC WATERS with Empower2 Software with Isocratic with UV-Visible Detector.
2.	T60-LABINDIA UV – Vis spectrophotometer
3.	High Precision Electronic Balance
4.	Ultra Sonicator (Wensar wuc-2L)
5.	Thermal Oven
6.	Symmetry C_{18} Column, 250 mm x 4.6 mm and 5µm particle size
7.	P^H Analyser (ELICO)
8.	Vaccum Filtration Kit (Labindia)

Chemicals and Reagents

Table 2: List of Chemicals used

S.No.	Name	Grade	Manufacturer/Supplier
1.	HPLC grade water	HPLC	Sd fine-Chem ltd; Mumbai
2.	Methanol	HPLC	Loba Chem; Mumbai.
3.	Ethanol	A.R.	Sd fine-Chem ltd; Mumbai
4.	Acetonitrile	HPLC	Loba Chem; Mumbai.
5.	DMSO	A.R.	Sd fine-Chem ltd; Mumbai
6.	DMF	A.R.	Sd fine-Chem ltd; Mumbai

HPLC Instrumentation & Condition

The HPLC system[4] employed was HPLC WATERS with Empower2 Software with Isocratic with UV-Visible Detector.

Standard Preparation for UV-Spectrophotometer Analysis

The standard stock solutions – 10 mg of Vildagliptin standard was transferred into 10 ml volumetric flask, dissolved & make up to volume with Methanol. Further dilutions were done by transferring 1 ml of the above solution into a 10ml volumetric flask and make up to volume with methanol to get 10ppm concentration. It scanned in the UV spectrum in the range of 200 to 400nm. This has been performed to know the maxima of Vildagliptin, so that the same wave number can be utilized in HPLC UV detector[5] for estimating the Vildagliptin.

Different trials for chromatographic conditions

Table 3: Different Chromatographic Conditions

Column Used	Mobile Phase	Flow Rate	Wave length	Observation	Result
Develosil C_{18}, 250 mm x 4.6 mm and 5µm Column	Acetonitrile : Water = 65 : 35	0.8 ml/min	245nm	Base line noise is high	Method rejected

Develosil C_{18}, 250 mm x 4.6 mm and 5µm Column	Acetonitrile : Water = 55 : 45	0.8ml/min	245nm	Tailing is more	Method rejected
Zorbax C_{18}, 250 mm x 4.6 mm and 5µm Column	Methanol : Acetonitrile = 30 : 70	0.9 ml/min	245nm	Extra peaks	Method rejected
Phenomenex C_{18}, 250 mm x 4.6 mm and 5µm Column	Methanol : Acetonitrile = 60 : 40	1.0 ml/min	245nm	Good sharp peak	Method accepted
Symmetry C_{18}, 250 mm x 4.6 mm and 5µm Column	Methanol : Acetonitrile = 50 : 50	1.0 ml/min	245nm	Improper peak separation	Method rejected
Symmetry C_{18}, 250 mm x 4.6 mm and 5µm Column	Methanol : Phosphate Buffer (0.01M) (pH-2.8) = 40 : 60	1.0 ml/min	245nm	Tailing peaks	Method rejected
Symmetry C_{18}, 250 mm x 4.6 mm and 5µm Column	Methanol : Phosphate Buffer (0.02M) (pH-3.2) = 60 : 40	1.0 ml/min	245nm	Tailing peaks	Method rejected
Symmetry C_{18}, 250 mm x 4.6 mm and 5µm Column	Methanol : Phosphate Buffer (0.02M) (pH-3.8) = 70 : 30	1.0 ml/min	245nm	Proper Peak	Method Accepted

Preparation of 0.02M Phosphate Buffer (pH-3.8)

Prepare 800 mL of distilled water in a suitable container. Add 2.72172g of Potassium dihydrogen Phosphate to the solution to the solution. Adjust solution to final desired pH 3.8 using diluted solution of orthophosphoric acid and add distilled water until volume is 1 Litre.

Preparation of Mobile Phase

Mix a mixture of 0.02M Phosphate Buffer (pH-3.8) 700 ml (70%) and 300 ml Methanol HPLC (30%) and degas in ultrasonic water bath for 15 minutes. Filter through 4.5 µ filter under vacuum filtration[6].

Preparation of Standard Solution

Accurately weigh and transfer 10 mg of Vildagliptin working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette out 0.1ml of Vildagliptin from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

RESULTS AND DISCUSSION

Method Development

Optimization of the Method for Vildagliptin

The method was developed using HPLC WATERS equipped with Auto Sampler. Initially the solubility of Vildagliptin in various solvents was tested. Then, suitable column for separation was selected for the proposed method. To achieve a suitable separation of eluted compound, the chromatographic conditions were optimized. Initially different diluent was tested to elute the drug. Flow rate and mobile phase choice is determined based on peak parameters like tailing factor or asymmetry, run time, resolution. Symmetry C18, 250 mm x 4.6 mm i.d.5µm particle size was used for separation at a column temperature of Ambient, using Methanol and Phosphate Buffer (0.02M) (pH-3.8) in ratio of 70:30% (v/v) as mobile phase at a flow rate of 1.0ml/min for a run time of 7.0 mins. The injection volume was maintained at 10µL and the wavelength was set to 245nm for detection using UV Vis Spectrophotometer. The retention time for Vildagliptin was found to be 2.768min.

Optimized Chromatographic Conditions

Column : Symmetry C18, 250 mm x 4.6 mm i.d.5µm particle size
Mobile Phase : Methanol: Phosphate Buffer (0.02M) (pH-3.8) (70: 30% v/v)
Flow Rate : 1.0ml/minute
Wave length : 245 nm
Injection volume : 10 µl
Run time : 7 minutes
Column temperature : Ambient

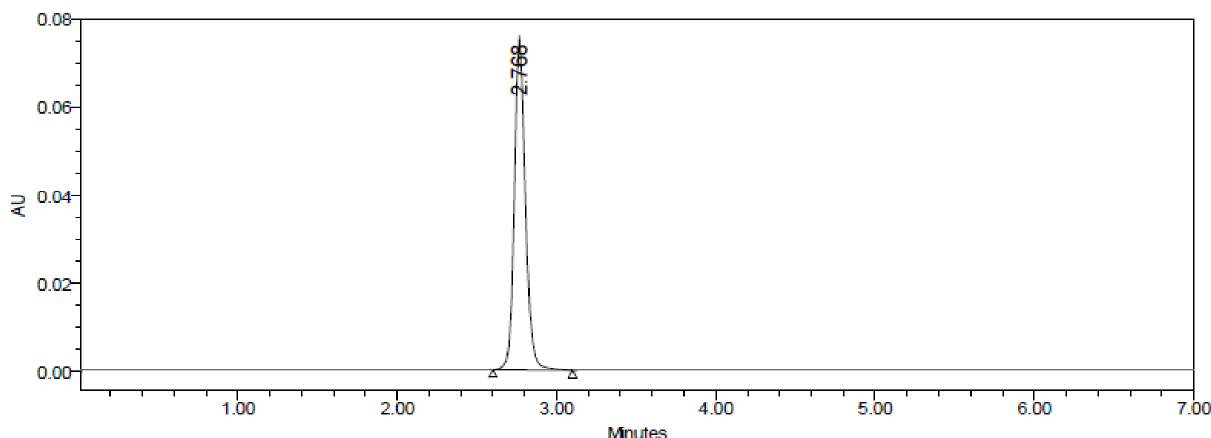

Fig 2: Optimized Chromatogram for Vildagliptin

The selected and optimized mobile phase[7] was Methanol: Phosphate Buffer (70: 30% v/v) and conditions optimized were flow rate (1.0 ml/minute), wavelength (245nm), Run time was 07 mins. Here the peak has shown better theoretical plate count and symmetry. The proposed chromatographic conditions were found appropriate for the quantitative determination of the Vildagliptin drug.

Method Validation

System Suitability Test

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analysed constitute an integral system that can be evaluated as such. Following system suitability test[8] parameters were established. The data are shown in Table-4.

Table 4: Data of System Suitability Test

S.No.	Injection No.	RT	Area	Height	USP Plate Count	USP Tailing
1	Injection 1	2.786	715268	47844	5857	1.36
2	Injection 2	2.784	716584	46985	5986	1.38
3	Injection 3	2.768	715364	47258	5784	1.35
4	Injection 4	2.789	714895	47152	5896	1.34
5	Injection 5	2.784	716587	47258	5749	1.36
6	Injection 6	2.781	718549	47985	5657	1.39
Mean			**716207.8**		**5821.5**	**1.36**
S.D			**1347.976**			
%RSD			**0.18821**			

Table 5: Acceptance Criteria and Result

S.No.	Parameter	Limit	Result
1	Tailing factor	$T \leq 2$	1.36
2	Theoretical plate	$N > 2000$	5821.5

Accuracy

Recovery Study

To determine the accuracy of the proposed method, recovery studies[9] were carried out by adding different amounts (80%, 100%, and 120%) of pure drug of Vildagliptin were taken and 3 replications of each has been injected to HPLC system. From that percentage recovery values were calculated from the linearity equation[10] $y = 74143x+7294.9$. The results were shown in table-6.

Table 6: Accuracy Readings

Sample ID	Concentration (µg/ml)		Peak Area	% Recovery of Pure drug	Mean % Recovery	
	Amount Injected	Amount Recovered				
S_1 : 80 %	8	8.013	601425	100.162	Mean = 100.195%	% Mean Recovery = 100.364%
S_2 : 80 %	8	8.012	601396	100.150		
S_3 : 80 %	8	8.022	602123	100.275		
S_4 : 100 %	10	10.038	751584	100.380	Mean = 100.356	
S_5 : 100 %	10	10.039	751642	100.390		
S_6 : 100 %	10	10.030	750969	100.300		
S_7 : 120 %	12	12.057	901253	100.475	Mean = 100.541	
S_8 : 120 %	12	12.073	902431	100.608		
S_9 : 120 %	12	12.065	901864	100.541		

From the Accuracy Method, we observed that the mean %Recovery of the drug is 99.686 which are within the range of 98-102%.

Precision

Repeatability

The precision[11] of each method was ascertained separately from the peak areas & retention times obtained by actual determination of six replicates of a fixed amount of drug Vildagliptin (API). The percent relative standard deviation[12] was calculated for Vildagliptin.

Table 7: Results of Repeatability readings

HPLC Injection Replicates of Vildagliptin	Retention Time	Peak Area	Theoretical Plates	Tailing Factor
Replicate – 1	2.777	716984	5986	1.36
Replicate – 2	2.795	715698	5897	1.37
Replicate – 3	2.789	716859	5869	1.39
Replicate – 4	2.797	718548	5967	1.37
Replicate – 5	2.797	714895	5984	1.35
Replicate – 6	2.799	715986	5879	1.38
Average		**716495**	**5930.333**	**1.37**
Standard Deviation		**1268.126**		
% RSD		**0.17699**		

From the Precision method, we observed that the %RSD of the Peak Area is 0.176 which are within the acceptable range as per ICH guidelines[30].

Intermediate Precision

The Intermediate Precision[13-14] consists of two methods:-

Intra Day: In Intra Day process, the 80%, 100% and 120% concentration are injected at different intervals of time in same day.

Inter Day: In Inter Day process, the 80%, 100% and 120% concentration are injected at same intervals of time in different days.

Intra-Day

Table 8: Peak results for Intra-Day Precision

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Vildagliptin	2.784	716587	48685	1.38	5954	1
2	Vildagliptin	2.768	717845	48698	1.39	5935	2
3	Vildagliptin	2.786	716857	46989	1.36	5798	3
4	**Average**		**717096.3**	**48124**	**1.376**	**5895.66**	
5	**S.D**		**662.2698**				
6	**% RSD**		**0.092354**				

Inter-Day

Table-9: Peak results for Inter-Day Precision

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Vildagliptin	2.780	716987	49867	1.34	5968	1
2	Vildagliptin	2.794	718695	48574	1.33	5998	2
3	Vildagliptin	2.775	718542	48569	1.39	5859	3
4	**Average**		**718074.7**	**49003.33**	**1.353333**	**5941.667**	
5	**S.D**		**945.0483**				
6	**% RSD**		**0.131609**				

The intra & inter day variation of the method was carried out for standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Vildagliptin revealed that the proposed method is precise.

Linearity & Range

To evaluate the linearity, serial dilution of analyte were prepared from the stock solution was diluted with mobile phase to get a series of concentration ranging from 6-14μg/ml. The prepared solutions were sonicated. From these solutions, 10μl injections of each concentration were injected into the HPLC system[15] and chromatographed under the optimized conditions. Calibration curve was constructed by plotting the mean peak area (Y-axis) against the concentration (X-axis).

Table 10: Linearity Concentrations of Vildagliptin

S.No.	Concentration (in ppm)	Peak Area
1	0	0
2	6	457896

3	8	607574
4	10	752268
5	12	896587
6	14	1036579

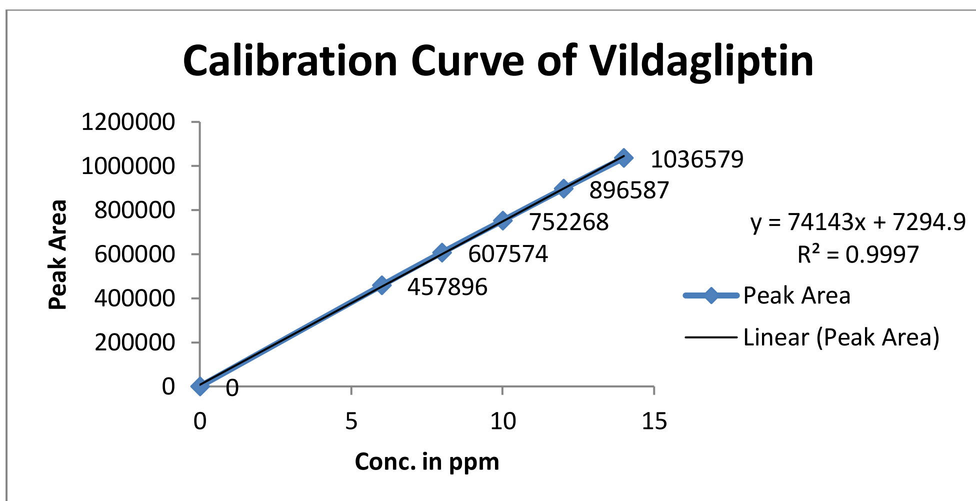

Fig 3: Calibration Curve of Vildagliptin

We observed that the calibration curve[16] showed good linearity in the range of 6-14 µg/ml, for Vildagliptin with correlation coefficient (R^2) of 0.9997. A typical calibration curve has the regression equation[17] of y = 74143x + 7294.9 for Vildagliptin.

Specificity

Specificity[18] of the pharmaceutical analysis is the ability to measure accurately and specifically the concentration of API, without interference from other active ingredients, diluents, mobile phase. Solutions of mobile phase, sample solution, standard solution were injected into liquid chromatography. Retention times of samples and standard were compared.

Method Robustness

Influence of small changes in chromatographic conditions such as change in flow rate 1ml (± 0.1ml/min), Wavelength of detection 245nm (±2nm) & organic phase content in mobile phase 60 (±5%) studied to determine the robustness[19] of the method are also in favour of (Table-11, % RSD <2%) the developed RP-HPLC method for the analysis of Vildagliptin (API).

Table 11: Results of Method Robustness Test

Change in Parameter	Theoretical Plates	Tailing Factors
Flow (1.1 ml/min)	5954	1.35
Flow (0.8 ml/min)	6188	1.39
More Organic (70+5)	5748	1.41
Less Organic (70-5)	6185	1.48
Wavelength of Detection (250 nm)	6184	1.69
Wavelength of detection (240nm)	6247	1.47
Temperature (30 ^{0}C)	6324	1.34
Temperature (20 ^{0}C)	6985	1.32

LOD & LOQ

The detection limit[20] (LOD) and quantization limit (LOQ) may be expressed as:

L.O.D. = 3.3(SD/S).
L.O.Q. = 10(SD/S)

Where,
SD = Standard deviation of the response
S = Slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.
The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified[22] (LOQ) were found to be 0.507 & 1.539 µg/ml respectively.

Estimation of Vildagliptin in Pharmaceutical Dosage Form

Twenty tablets were taken and the I.P. method was followed to determine the average weight. Above weighed tablets were finally powdered and triturated well. A quantity of powder equivalent[23] to 10 mg of drug were transferred to 10

ml volumetric flask, and 8 ml of mobile phase was added and solution was sonicated for 15 minutes, there after volume was made up to 10 ml with same solvent. Then 1ml of the above solution was diluted to 10 ml with HPLC grade methanol. The solution was filtered through a membrane filter (0.45 µm) and sonicated to degas[24]. From this stock solution (1.0 ml) was transferred to five different 10 ml volumetric flasks and volume was made up to 10 ml with same solvent system.

The solution prepared was injected in five replicates into the HPLC system[25] and the observations were recorded.

A duplicate injection of the standard solution was also injected into the HPLC system and the peak areas were recorded. The data are shown in Table-12.

ASSAY

$$\% \text{ Assay} = AT/AS \times WS/DS \times DT/WT \times P/100 \times AW/LC \times 100$$

Where:
AT = Peak Area of Vildagliptin obtained with test preparation
AS = Peak Area of Vildagliptin obtained with standard preparation
WS = Weight of working standard taken in mg
WT = Weight of sample taken in mg
DS = Dilution of Standard solution
DT = Dilution of sample solution
P = Percentage purity of working standard
Results obtained are tabulated below:

Table 12: Assay of Vildagliptin

Brand name of Tablets/Capsules	Labelled amount of Drug (mg)	Mean (±SD) amount (mg) found by the proposed method (n=5)	Assay + % RSD
Galvus 50 Tab	50mg	49.698 (± 0.598)	99.784 % (± 0.487)

The %Purity[26] of Galvus 50 Tablet containing Vildagliptin was found to be 99.769% (±0.746).

Stability Studies

Following stability study protocol was strictly used for forced degradation of Vildagliptin Active Pharmaceutical Ingredient (API).

The API (Vildagliptin) was subjected to some stress conditions[27-29] in various ways to observe the rate and amount of degradation that is likely to take place in the course of storage and/or after ingestion to body.

This is one type of accelerated stability studies that helps us determining the fate of the drug that is likely to happen after long time storage, within a very short time as compare to the real time or long term stability testing.

The various degradation pathways are studied is acid hydrolysis, basic hydrolysis, thermal degradation and oxidative degradation.

Results of Degradation Studies

The results of the stress studies indicated the specificity of the method that has been developed. Vildagliptin was stable in Acidic, Photolytic & Oxidative conditions. The result of forced degradation studies are given in the following table-13.

Table 13: Results of Forced Degradation Studies of Vildagliptin

Stress Condition	Time	Assay of Active Substance	Assay of Degraded Products	Mass Balance (%)
Acid Hydrolysis (0.1N HCl)	24Hrs.	87.635	12.365	100
Basic Hydrolysis (0.1N NaOH)	24Hrs.	94.154	5.846	100
Thermal Degradation (60^0C)	24Hrs.	90.311	9.689	100
UV (254nm)	24Hrs.	91.205	8.795	100
3% Hydrogen peroxide	24Hrs.	89.346	10.654	100

SUMMARY

The analytical method was developed by studying different parameters. First of all, maximum absorbance was found to be at 245nm and the peak purity was excellent. Injection volume was selected to be 10µl which gave a good peak area. The column used for study was Symmetry C18, 250 mm x 4.6 mm i.d.5µm particle size because it was giving good peak. Ambient temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area and satisfactory retention time. Mobile phase is Methanol: Phosphate Buffer (0.02M) (pH-3.8) (70: 30% v/v) was fixed due to good symmetrical peak. So this mobile phase was used for the proposed study. Methanol was selected because of maximum extraction sonication time was fixed to be 10min at which all the drug particles were completely soluble and showed good recovery. Run time was selected to be 7min because analyze

gave peak around 2.768min and also to reduce the total run time. The percent recovery was found to be 98.0-102 was linear and precise over the same range. Both system and method precision was found to be accurate and well within range. The analytical method was found linearity over the range of 6-14ppm of the Vildagliptin target concentration. The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Vildagliptin in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatization or purification steps. Vildagliptin was found to be soluble in organic solvents such as ethanol, DMSO, and dimethyl formamide, which should be purged with an inert gas, sparingly soluble in aqueous buffers; it is freely soluble in water, Acetonitrile, slightly soluble in methanol. Methanol and Phosphate Buffer (0.02M) (pH-3.8) in the ratio of 70: 30% v/v was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP-HPLC method and validation was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Vildagliptin in bulk drug and in Pharmaceutical dosage forms.

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