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Original Article

RP-HPLC determination of vildagliptin in pure and in tablet formulation

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ABSTRACT

Aim: The present work aims to a simple, rapid and reproducible reverse phase high performance liquid chromatographic (RP-HPLC) method for the estimation of vildagliptin in pure form and in tablet dosage form using Agilent XDB C18, 150 × 4.6 mm, 5 μm, column.
Methods: The mobile phase consists of 0.1 M Phosphate buffer and acetonitrile in the ratio of 85:15% v/v, and was pumped at rate of 1.0 mL/min. The detection was carried out at 210 nm and the calibration curve was linear in the concentration range of 10–150 μg/mL.
Results: The method was statistically validated for its linearity, precision, accuracy, stability, specificity, LOD and LOQ. 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.
Conclusion: The proposed method was found to be simple, precise, accurate and rapid for determination of vildagliptin from pure form and tablet dosage form.

1. Introduction

Vildagliptin chemically (S)-1-[N-(3-hydroxy-1-adamantyl) glycyl] pyrrolidine-2-carbonitrile, is a potent dipeptidyl peptidase IV (dip-IV) inhibitor, a drug for the treatment of diabetes. DPP IV inhibitors represent a new class of oral antihyperglycemic agents to treat patients with type 2 diabetes. DPP IV inhibitors improve fasting and postprandial glycemic control without hypoglycemia or weight gain. Vildagliptin inhibits the inactivation of GLP-1 and GIP by DPP IV, allowing GLP-1 and GIP to potentiate the secretion of insulin in the beta cells and suppress glucagon release by the alpha cells of the islets of Langerhans in the pancreas.[1–4] Literature survey reveals that vildagliptin can be estimated by UV spectroscopic method,[5] RP-HPLC method, which is a time consuming method being the retention time is more than 10 min,[6] RP-LC/MS method, requires mass spectroscopy detection and the LOD and LOQ are more than the present method, and has a narrow

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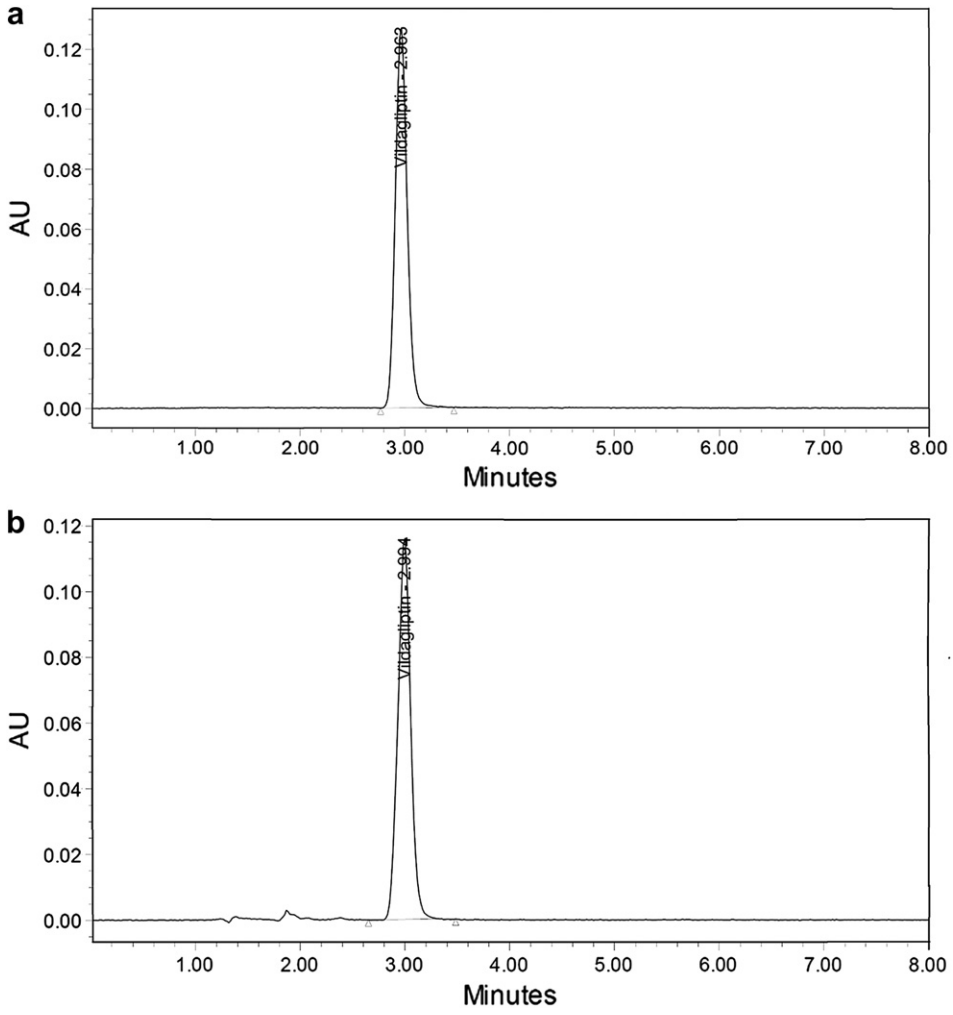

Fig. 1 – (a) A typical chromatogram of vildagliptin standard solution. (b) A typical chromatogram of vildagliptin tablets sample solution.

linearity range,[7] HPLC method, which requires a solid-phase extraction and determination by high-performance liquid chromatography quadrupole time-of-flight mass spectrometry which requires a special attention throughout the study but the present work is a simple method.[8] So based on the above mentioned reasons the authors aim to develop a simple, sensitive and accurate RP-HPLC method for the estimation of vildagliptin in pure form and tablet dosage form.

2. Experimental

2.1. *Instrumentation*

Waters 2695 HPLC system equipped with Agilent Eclipse XDB C18, 150 × 4.6 mm, 5 μ column, Rheodyne injector with 25 μL loop, 2996 PDA detector and Empower-2 software was used.

2.2. *Reagents and chemicals*

Potassium dihydrogen orthophosphate of analytical grade, HPLC grade Milli-Q water and acetonitrile were used. Vildagliptin was a gift sample from Novartis, India. The tablets of vildagliptin were obtained from local pharmacy.

2.3. *Preparation of buffer solution*

0.1 M phosphate buffer was prepared by dissolving 13.6 g of potassium dihydrogen orthophosphate in 1000 mL of HPLC grade water.

2.4. *Chromatographic condition*

Vildagliptin was eluted in Agilent XDB C18, 150 × 4.6 mm, 5 μ, column using a mobile phase mixture of phosphate buffer and acetonitrile in the ratio of 85:15% v/v. The lambda max of the drug in mobile phase was 210 nm, so column outlet was monitored at 210 nm. The injection volume is 25 μL. The total runtime was 8 min.

2.5. *Procedure for standard solution preparation*

Hundred milligrams of pure vildagliptin was weighed accurately and transferred in to a 100 mL volumetric flask. The

content was dissolved by using HPLC grade water, after complete dissolution the volume was made up to the mark by using the same which gives 1000 μg/mL of the drug.

2.6. Construction of calibration curve

The standard vildagliptin solution was further diluted in 10 mL volumetric flask to get various concentrations ranging from 10 to 150 μg/mL of drug using mobile phase. From this each calibration standard solutions 25 μL was injected in to the HPLC system. The chromatograms were recorded. The concentration of the vildagliptin in μg/mL is taken in X axis and peak area of the individual concentrations of calibration standards was taken in Y axis. The calibration graph was plotted. This is used for the estimation of vildagliptin in tablets.

2.7. Estimation of vildagliptin

Twenty tablets of vildagliptin were weighed accurately; average weight was calculated and powdered well. The powder equivalent to 100 mg of the drug was transferred in to a 100 mL calibrated standard flask. 70 mL of HPLC grade water was added. The content of the flask was sonicated for 15 min to dissolve vildagliptin and made up to the volume with the same and the resulting mixture was filtered through 0.45 μm filter. Subsequent dilution of this solution was made with mobile phase to get concentration of 50 μg/mL. This solution (25 μL) was injected six times into the HPLC system. The mean value of peak areas of six such determinations was calculated and the drug content in the tablet was quantified.

Table 2 – Linear regression data for calibration curve.

Parameters	Values
Concentration range, μg/mL	10–150
Slope	9944
Intercept	5738.83
Correlation coefficient	0.999

3. Results and discussion

Vildagliptin pure drug is soluble in water and acetonitrile. Different mobile phase compositions were tried to elute the drug from the column and adequate resolution is achieved with phosphate buffer and acetonitrile in the ratio of 85:15% v/v with Agilent Eclipse XDB C18, 150 × 4.6 mm, 5 μ, column and this solvent system was found to be most suitable for method development and validation. Vildagliptin shows the maximum absorbance [λ-max] at 210 nm in mobile phase, so the column outlet was detected at 210 nm in the proposed method. A typical chromatogram of vildagliptin standard solution and tablets sample solution are shown in Fig. 1a and b respectively. Chromatogram of the excipients is shown in Fig. 2. The retention time was 3.04 min. The system suitability tests were carried out on freshly prepared standard stock solution and summery is given in Table 1. These parameters indicate good sensitivity and selectivity of the developed method. In the present developed HPLC method, the standard and sample preparation involves simple and rapid extraction procedure and requires less time. A good linear relationship was obtained in the concentration range of 10–150 μg/mL. Linear regression analysis report is given in Table 2. The proposed method was used to estimate the amount of

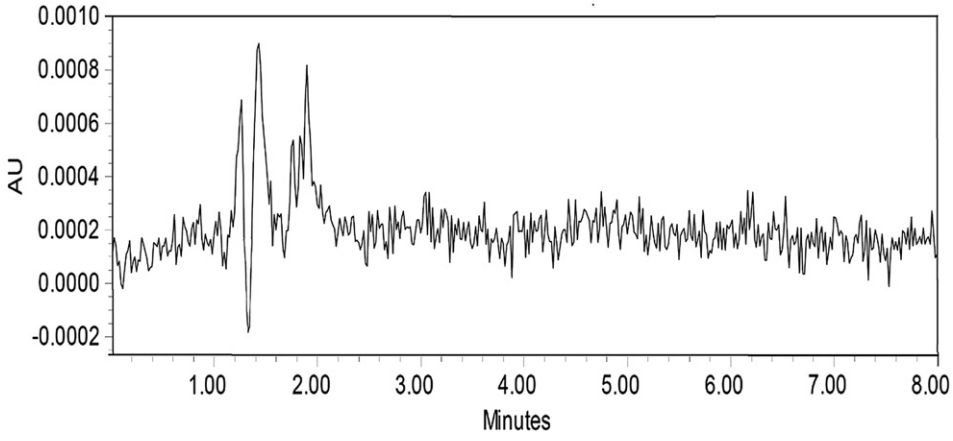

Fig. 2 – A chromatogram of excipients.

Table 1 – System suitability data.

Drug	Theoretical plates (N)	Tailing factor (T)	Retention time (min) (n = 6)		Peak area (n = 6)	
			Mean ± S.D	% RSD	Mean ± S.D	% RSD
Vildagliptin	3311	1.13	3.04 ± 0.0117	0.3837	486592 ± 216.6	0.0445

Table 3 – Assay, recovery results and precision studies.

Formulation	Labeled amount (mg/tablet)	(%) Label claim[a] ± S.D.	% Recovery	Precision (% RSD)	
				Inter-day ($n = 24$)	Intra-day ($n = 6$)
Vildagliptin tablets	50	99.92 ± 0.2125	99.47–100.83%	0.1668	0.1497

a Average of six determinations.

vildagliptin in tablets, assay results are in Table 3. Precision of the method was determined by repeatability (intra-day) and intermediate precision (inter-day). Repeatability refers to the use of the analytical procedure within a laboratory over a short period of time that was evaluated by analyzing six drug solutions, at the final concentration corresponding to 50 μg/mL of vildagliptin during the same day. Intermediate precision was assessed by comparing the estimation on different days by different analyst (Table 3). The vildagliptin concentrations were determined and the relative standard deviations (% RSD) were calculated. The accuracy of the developed method was carried out by adding the known amount of vildagliptin pure drug to placebo solution and subjected to the proposed method. Results of recovery study are shown in Table 3. The study was done at 50, 100 and 150% of test concentration (50 μg/mL) levels. The limit of detection (LOD) and limit of quantification (LOQ) for vildagliptin was found to be 0.0329 and 0.0998 μg/mL, respectively.

4. Conclusion

The proposed method was found to be simple, precise, accurate and rapid for determination of vildagliptin from pure form and tablet dosage form. The mobile phase used in this method is simple to prepare and the runtime was 8 min, so less time consuming method. The recovery study shows that there is no interference of additives used for the preparation of tablets. Hence, the method can be easily and conveniently applied for routine quality control of vildagliptin in its dosage form and can also be used for dissolution studies.

Conflicts of interest

All authors have none to declare.

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