Sudharsanacharyulu S et al /J. Pharm. Sci. & Res. Vol. 13(11), 2021, 649-652

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Development and validation of a new HPLC analytical assay method for Vildagliptin

Sudharsanacharyulu S,[1,4] B. Govindh,[2] P mahesh,[3] Shanmukha kumar J. V[4*]

[1] *Dr. Reddy's Laboratories Ltd. API, CTO-2, Bollaram, Hyderabad-502325, Telangana, India.*
[2] *Department of H&S, Raghu Institue of Technology,Visakhapatnam, Andhra Pradesh, India;*
[3] *Therapiva Pvt Ltd, IDA Pashamylaram, Hyderabad, Telangana, India.*
[4] *Koneru Lakshmayya Educational Foundation, Guntur-522502, Andhra Pradesh, India.*

* govindhbdt@gmail.com

Abstract:

For the determination of Vildagliptin in API, a simple, quick, precise, and cost-effective approach was devised and validated. The chromatographic separation was performed on a 250 x 4.6 mm, 3 m analytical column with an Inertsil ODS-4 mobile phase containing 1% perchloric acid and buffer acetonitrile and methanol at a ratio of (870:100:30 v/v/v). A photodiode array detector was used with a flow rate of 1.0 ml/min, a column temperature of 50oC, and a detection wavelength of 210nm. Vildagliptin has a theoretical plate of 8000 and a tailing factor of 1.38. The approach was validated in accordance with ICH and FDA standards. Specificity, linearity, accuracy, precision, and robustness were all tested.

Keywords: Vildagliptin, RP-HPLC, validation, Assay by HPLC, FDA standards

INTRODUCTION

Vildagliptin is an anti-hyperglycemic medication taken orally (anti-diabetic drug). (S)-1-[N-(3-hydroxy-1-adamantyl)glycyl]pyrrolidine-2-carbonitrile is a dipeptidyl peptidase IV (dip-IV) inhibitor with a chemical formula of (S)-1-[N-(3-hydroxy-1-adamantyl) glycyl] pyrrolidine-2-carbonitrile. DPP-IV inhibitors are a novel class of oral anti-hyperglycemic drugs used to treat type-2 diabetic patients. Fasting and postprandial glycemic controls are improved with DPP IV inhibitors without hypoglycemia or weight gain. Vildagliptin prevents DPP IV from inactivating GLP-1 and GIP, allowing GLP-1 and GIP to potentiate insulin production de beta cells while suppressing glucagon release in the pancreatic islets of Langerhans.[1-5]

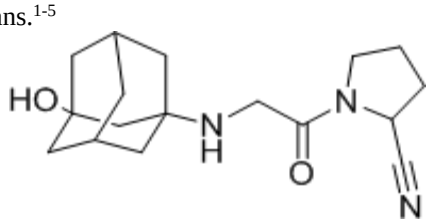

Figure 1: Structure of Vildagliptin

Because there is no published chromatographic method for determining Vildagliptin API and existing methods[6-11] are time consuming and difficult, the goal of this study was to design a fast, innovative, economical, precise, and accurate approach for determining Vildagliptin API. Analytical methods evolve over time to meet changing needs, resulting in a method that is simple, dependable, cost-effective, reproducible, and, above all, accurate and precise. USP 26 (United States Pharmacopeial Convention, 2003) or ICH guidelines were used to validate the assay method.[12-15]

EXPERIMENITAL

Materials and methods: Chemicals and reagents:

Vildagliptin working standard with 99.88 percent potency was obtained from GLP pharma standards in India. Merck provided HPLC grade acetonitrile and perchloric acid, while Merck provided HPLC grade water through a millipore water purification system.

HPLC instrumentation and chromatographic condition:

Liquid chromatography conditions:

The HPLC system utilised for the analysis was a Shimadzu module Shimadzu LC-2030 C plus equipped with an auto sampler and PDA detector. The data was collected on a C18 column (300 mm x 4.6 mm) using Lab solution. A Millipore Swinnex type filter (pore size = 0.45 m) was used as a mobile phase containing perchloric acid, acetonitrile, and methanol at a ratio of 870:100:30 (percent v/v/v) at a flow rate of 1.0 mL/min and a run period of 20 minutes. Filtration through a 0.45 m Millipore membrane filter and sonication for 10 minutes were used to degas the sample. The injection volume was 20 litres, and the detecting wavelength was 210 nanometers. The HPLC system was set to 50 degrees Celsius. Transfer 1.0 mL perchloric acid to 1000 mL water and thoroughly mix. Degas and filter through 0.45-micron filter paper. Prepare an 870:100:30 (percent v/v/v) degassed mixture of Buffer Acetonitrile and Methanol. Figures 2 and 4 show that the Vildagliptin peak has typical retention duration of about 10 minutes.

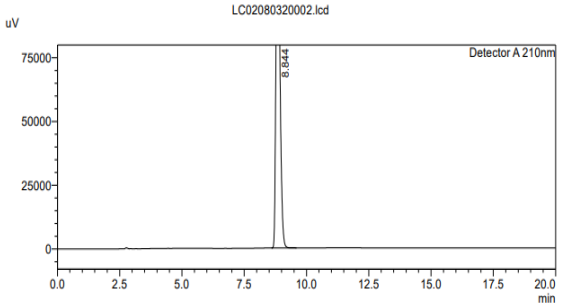

Figure 2: Chromatogram of Vildagliptin in standard solution

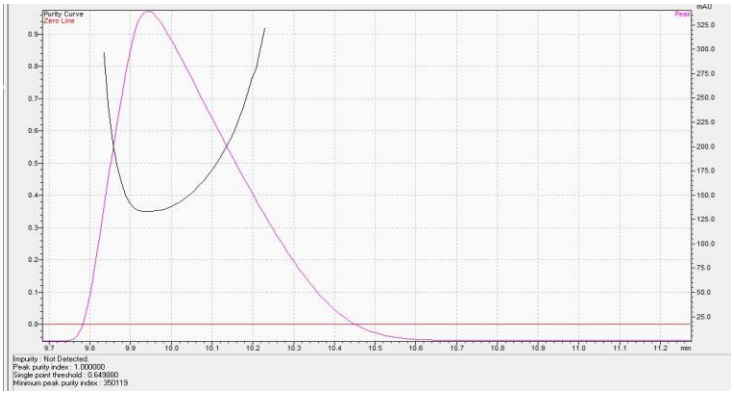

Figure 3: Purity curve of Vildagliptin in standard solution

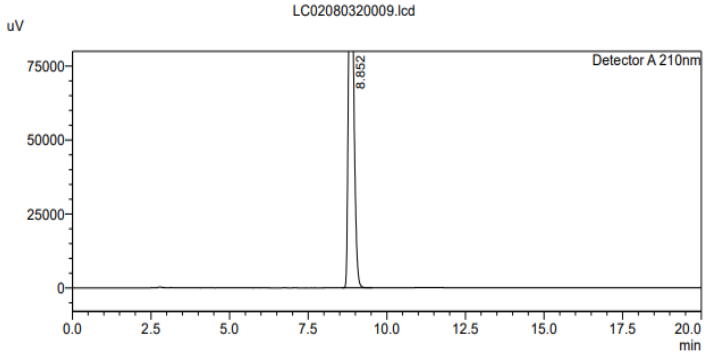

Figure 4: Chromatogram of Vildagliptin in sample solution

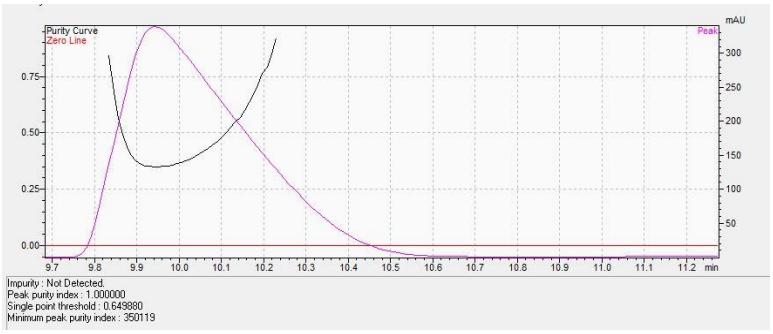

Figure 5: Purity curve of Vildagliptin in sample solution.

Preparation of standard and Sample solution:
In a 50 mL volumetric flask, weigh around 25 mg of Vildagliptin reference/working standard. Dissolve in mobile phase, make up the volume, and dilute 5.0 ml of the solution to 50 ml with mobile phase.

METHOD VALIDATION

The purpose of this technique of analysis was to develop a new, cost-effective, and convenient HPLC method for determining Vildagliptin. For factors such as specificity, system appropriateness, accuracy, linearity, precision, and robustness, the experimental procedure was validated according to the guidelines of ICH-1996 and USP-30.

System suitability:
The accuracy and precision of the chromatographic system were verified by injecting six replicates of standard solution at a 100 percent level to test system appropriateness. The percent relative standard deviation (percent RSD) for the peak area and retention timeframes for Vildagliptin had to be less than 2%.

Entry	Injection	RT	Area
1	Standard Inj-1	8.853	1504306
2	Standard Inj-2	8.854	1503806
3	Standard Inj-3	8.854	1503888
4	Standard Inj-4	8.856	1504178
5	Standard Inj-5	8.858	1504620
Average		**8.855**	**1504160**
Std Deviation		**0.002**	**329**
% RSD		**0.023**	**0.022**

Linearity:
The capacity to get test findings that are directly proportional to the concentration area of the Vildagliptin standard, as well as determining the correlation coefficient, is referred to as linearity (R2). Three injections of five different vildagliptin concentrations were used to

test linearity. For Vildagliptin standard, the detector response was shown to be linear from 50% to 150 percent of test concentration. The column was equilibrated with the mobile phase for at least 45 minutes before injection of the solutions. Each measurement was repeated five times to ensure that the detector response was consistent at each concentration level. A correlation coefficient (r2) of more than 0.998 indicates a linear relationship between analyte concentration and area under the peak. Figure 6 depicts the linearity curve, whereas table 1 contains the data.

Table 1: Linearity of Vildagliptin in Standard preparation from 50% to 150% of test concentration:

Entry	Injection	RT	Area	Average	Weights
1	50% Pre-1	8.884	767720	769181	100.4
2	50% Pre-2	8.886	769859		100.7
3	50% Pre-3	8.887	769965		100.4
4	75% Pre-1	8.876	1157026	1156273	100.4
5	75% Pre-2	8.877	1155965		100.7
6	75% Pre-3	8.877	1155827		100.4
7	100% Pre-1	8.867	1511902	1512019	100.4
8	100% Pre-2	8.866	1512124		100.7
9	100% Pre-3	8.865	1512030		100.4
10	125% Pre-1	8.853	1969025	1947841	100.4
11	125% Pre-2	8.852	1959163		100.7
12	125% Pre-3	8.853	1915336		100.4
13	150% Pre-1	8.844	2290048	2291686	100.4
14	150% Pre-2	8.845	2297484		100.7
15	150% Pre-3	8.844	2287527		100.4
Regression co-efficient=					0.999

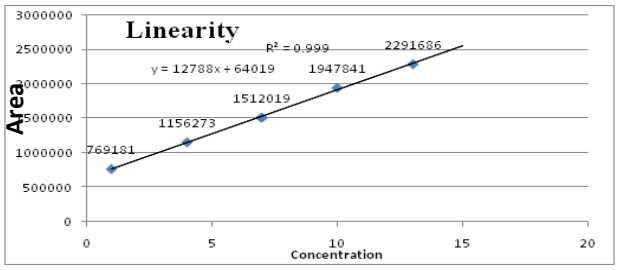

Figure 6: Calibration curve of Vildagliptin.

Accuracy

The method's accuracy is measured by how close the result is to the true value. Recovery tests were used to determine the method's accuracy. The recovery was calculated by adding the working standard test concentrations of Vildagliptin (80%, 100%, and 120%) and expressed as a percentage (%) recovered. For each recovery level, three samples were prepared. Table 2 shows the results.

Table 2: Results of Accuracy:

Entry	Injection	Area	Average	Accuracy
1	80% Pre-1	1221486	1221712	80.8
2	80% Pre-2	1221956		
3	80% Pre-3	1221693		
4	100% Pre-1	1503578	1508152	99.8
5	100% Pre-2	1510602		
6	100% Pre-3	1510275		
7	120% Pre-1	1829444	1829183	121.0
8	120% Pre-2	1830203		
9	120% Pre-3	1827901		

Stability of Analytical solution

By injecting the standard solution and sample solution at varied time intervals up to 24 hours (0, 4, 8, 12, 16, 18, and 24 hours) while keeping the auto sampler temperature at room temperature (25oC), the stability of analytical solutions was established. The response of the standard and sample solutions was measured, and the percent difference in peak area was calculated. The results are listed in table 3.

Table 3: Stability of standard and sample solution of Vildagliptin

Time Interval	Standard		Sample	
	Standard peak area	% Difference	Sample peak Area	% Difference
0 hour	1504306	-	1504406	-
4 hours	1504898	-0.003	1503306	0.07
8 hours	1503482	0.050	1502306	0.14
12 hours	1501100	0.210	1506208	-0.12
16 hours	1503520	0.050	1504303	0.01
18 hours	1504306	-0.010	1504201	0.01
24 hours	1501306	0.200	1504308	0.01

Precision

The degree of agreement among individual test results when the procedure is applied repeatedly to various samplings is the precision of an analytical method. By estimating the assay for six distinct sample preparations from the same batch, the repeatability, reproducibility, and intermediate precision of the assay were tested.Table 4 shows the results of the analysis for repeatability, intermediate precision, and reproducibility.

Table 4: Statistical analysis for repeatability, intermediate precision, and reproducibility of Vildagliptin.

Sample ID	Assay		
	Repeatability (Analyst 1)	Intermediate precision (Analyst 2)	Reproducibility (Analyst 3)
Sample-1	99.23	100.12	98.82
Sample-2	99.85	99.00	99.23
Sample-3	100.10	99.47	99.14
Sample-4	98.55	99.30	100.87
Sample-5	99.15	99.92	99.89
Sample-6	99.12	99.37	99.97
Average	99.00	99.80	99.87
SD	0.52494	0.392786	0.684832
% RSD	0.528716	0.394488	0.687001

ROBUSTNESS

The ability of a procedure to remain unaffected by slight changes in parameters is known as robustness. The method's robustness was determined by purposefully changing experimental conditions and calculating percent assay of Vildagliptin, peak tailing, theoretical plates, and percent RSD. The flow rate was reduced by 0.2 units from 1 ml/min to 0.8 ml/min and 1.2 ml/min to investigate the influence of flow rate. Instead of 50oC, the influence of column temperature was investigated at 48oC and 52oC, with alterations made to evaluate its effect on method. Table 5 shows the information gathered.

Table 5: Results of robustness study:

SI. No.	Parameter	Variation	Assay % (n=3)
1.	Flow rate (± 20% of the set flow)	a) at 0.8ml/min b) at 1.5ml/min	a) 99.01 b) 99.28
2.	Column oven temperature (± 2ºC of set temperature)	a) at 48ºC b) at 52ºC	a) 99.36 b) 99.89

RESULTS AND DISCUSSION

The devised method was unique since there were no additional contaminants, diluting solution, or impurity in the Vildagliptin chromatogram (purity curve shown in figure 3 and 5). The method demonstrated detector linearity and produced a linear calibration curve in the 50-150 percent range (Figure 6). Table 2 demonstrates the correctness of the results, and the percent RSD is 0.464, which is within the acceptable range. The percent difference between the peak areas of the Standard and Sample solutions injected at regular intervals was found to be within the specified range (Table3). Vildagliptin's robustness evaluation (Table 5) yielded positive results.

CONCLUSION

The proposed method was found to be exact, accurate, simple, time-saving, cost-effective, and easy to apply. All of the analytical method's validation parameters yielded appropriate results, including an adequate correlation coefficient and a decreased percent RSD. As a result, the suggested method can be easily used to quality control, stability, and future research.

Acknowledgement

The authors would like to thank Faculty of Chemistry, University of KL.

REFERENCES

1. Gupta, S. C., Patchva, S., Koh, W., Aggarwal, B. B., *Clin. Exp. Pharmacol. Physiol.* 2012, *39(3)*, 283.
2. Hassaninasab, A., Hashimoto, Y., Tomita-Yokotani, K., Kobayashi, M., *Proc. Natl. Acad. Sci.* 2011, *108(16)*, 6615.
3. Okada, K., Wangpoengtrakul, C., Tanaka, T., Toyokuni, S., Uchida, K., Osawa, T., *J Nutr*. 2001, *131(8)*, 2090.
4. Mondal, NK., Behera, J., Kelly, KE., George, AK., Tyagi, PK., Tyagi, N., *Neurochem Int*. 2019, *122*, 120.
5. Murugan, P., Pari, L., Rao, CA., *J Biosci*., 2008, *33(1)*, 63.
6. Uçaktürk, E., *J. Anal. Methods. Chem*, 2015, 707414.
7. Patil, K.R., Dr. T, Deshmukh., V. Patil., *World Journal of Pharmacy and Pharmaceutical Sciences*, 2015, *4(09)*, 1151.
8. Satheesh, kumar., Pradeep, Kumar, M., Rao, V.J., *Int. J. Pharm. Pharm.Sci*, 2012, *4(3)*, 119.
9. Pragati, R.S., Mohan, G., Sharma, S., *World Journal of Pharmacy and Pharmaceutical Sciences*, 2014, *3(2)*, 2303.
10. Lakshmana, Rao., Ramesh., *IJPCBS*, 2014, *4(2)*, 361.
11. Shrikrishna, B., Baokar., *Research J. Pharma. Dosage Forms and Tech*, 2013, *5(2)*, 95.
12. Malakar, A., Biswaji, Bokshi., D, Nasrin., *International Journal of Pharmaceutical and Life Sciences*, 2012, *1(1)*, 1.
13. Subhakar, Nandipati.,V, Krishna, R., *Int. Res J Pharm. App Sci.* 2012, *2(3)*, 44.
14. Santhakumari, B., A. B Pharne., H. K. Jain., *Int. J. Pharm. Pharm. Sci.*, 2012, *4(3)*, 119.
15. EI, Bagary R. I., Elkady, EF., Ayoub, BM., *Int. J. Biomed. Sci.*, 2011, *7(3)*, 201.
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