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Development of a New Related Substance by HPLC Method for Vildagliptin for Quantification of Purity

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Using a simple, quick, precise, and cost-effective approach, a new method for measurement of Vildagliptin in Active Pharma Ingredient (API) besides its relative substance was developed and validated. This method is simple, rapid, exact and cost-effective. With isocratic elution of buffer acetonitrile and methanol in the proportions of (87:10:3 v/v/v), the chromatographic separation was done on an ODS-4 C18 column (3 m 250 4.6 mm) using a 4.6-millimeter ODS-4 C18 column. Using a photodiode array (PDA) detector, we measured the flow rate of one milliliter per minute (ml/min), the column temperature of fifty degrees Celsius, and the detection wavelength of two hundred and ten nanometers (nm). Vildagliptin has a theoretical plate of 8000 and a tailing factor of 1.38, making it one of the most potent drugs available. The approach was validated in compliance with the International Conference on Harmonization (ICH) and the Food and Drug Administration (FDA) requirements. Precision, accuracy, and resilience were all high on the list of desirable characteristics.

Keywords: Vildagliptin; RP-HPLC; validation; assay by HPLC; FDA and ICH standards.

1. INTRODUCTION

Vildagliptin is an oral hypoglycemic medicine that is taken once a day (anti-diabetic drug). (S)-1-[N-(3-hydroxy-1-adamantyl)glycyl] DIP-IV inhibitor pyrrolidine-2-carbonitrile has the chemical formula (S)-1-[N-3-hydroxy-1-adamantyl]glycyl, which is derived from the amino acid pyrrolidine-

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2-carbonitrile. pyrrolidine-2-carbonitrile. DPP-IV inhibitors are a novel family of oral anti-hyperglycemic medications that are used to treat individuals with type-2 diabetes. With DPP IV inhibitors, fasting and postprandial glycemic control are improved without the occurrence of hypoglycemia or weight gain. Because Vildagliptin prevents DPP IV from inactivating GLP-1 and GIP, these hormones are able to increase insulin synthesis in beta cells while reducing glucagon release in the islets of Langerhans in the pancreatic islets of Langerhans (pancreatic islets of Langerhans) [1-5].

It is a rapid, new, cost-effective, exact, and accurate strategy when compared to the existing chromatographic methods [6-11] for determining the Vildagliptin active pharmaceutical ingredient (API) suggested and developed. Analytical methods evolve over time to meet changing needs, resulting in a process that is simple, trustworthy, cost-effective, reproducible, and, above all, accurate and exact. Analytical methods have evolved over time to meet changing needs. Validation of the assay method was carried out in accordance with USP (United States Pharmacopeial Convention) or ICH requirements [12-15].

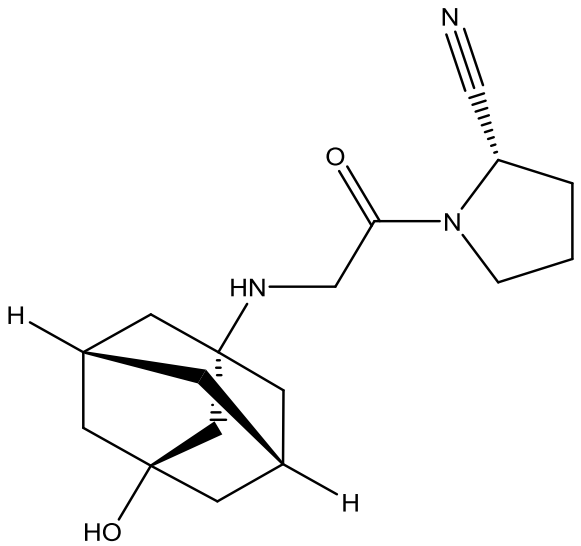
2. EXPERIMENITAL METHODOLOGY

2.1 Chemicals and Reagents

Vildagliptin working standard (99.88% potency) was obtained from GLP pharma standards in India. Perchloric acid, water (HPLC grade) and acetonitrile (HPLC grade) were procured from Merck.

2.2 Instrumentation and Chromatographic Condition

The analysis was carried out using a high-performance liquid chromatography (HPLC) system (make: Shimadzu, model: LC-2030 C plus) equipped with an auto sampler and a PDA detector. The data was gathered on an ODS-4, C18 column (300 mm x 4.6 mm, 3 m) with Labsolution software on a 300 mm × 4.6 mm, 3 m resolution. The filter utilised was a Millipore Swinnex type filter with a pore size of 0.45 m. Mobile phase containing perchloric acid, acetonitrile, and methanol in the proportions 87:10:3 (percent v/v/v) was used in the present devised method with a flow rate of one microliter per minute (mL/min). The run will last 20 minutes, according to the schedule. The temperature of the HPLC system was set at 50°C. Transfer one millilitre of perchloric acid to one thousand millilitres of water and thoroughly mix. Degassing the sample was accomplished through filtration through a 0.45 micron Millipore membrane filter and sonication for 10 minutes. This experiment was carried out with a 20-liter injection volume and a detection wavelength of 210 nanometers. Figs 2 and 4 show that the Vildagliptin peak has a normal retention length of around 8.8 minutes, which is consistent with previous studies.



(*S*)-1-(((1*r*,3*S*,5*R*,7*S*)-3-hydroxyadamantan-1-yl)glycyl)pyrrolidine-2-carbonitrile

Fig. 1. Structure of Vildagliptin

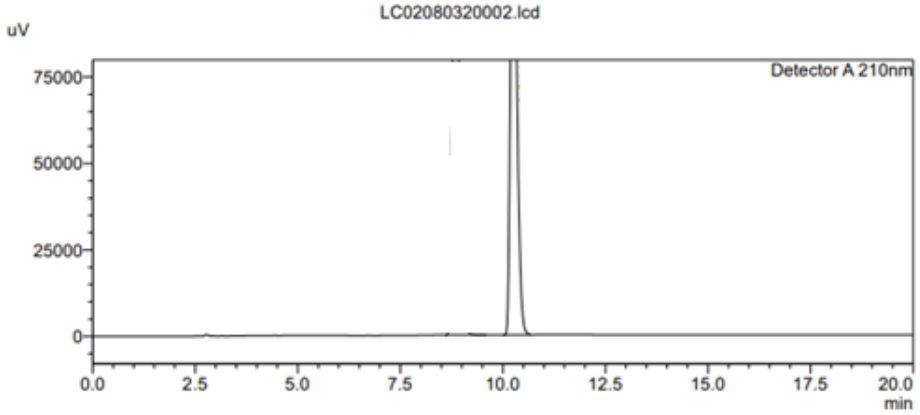


Fig. 2. Chromatogram of Vildagliptin in standard solution

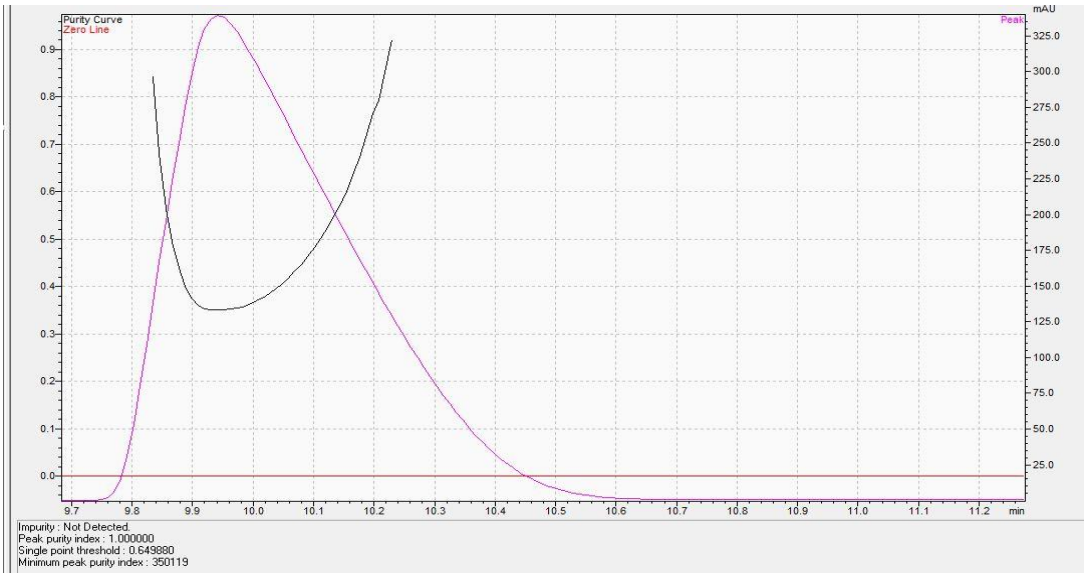


Fig. 3. Purity curve of Vildagliptin in standard solution

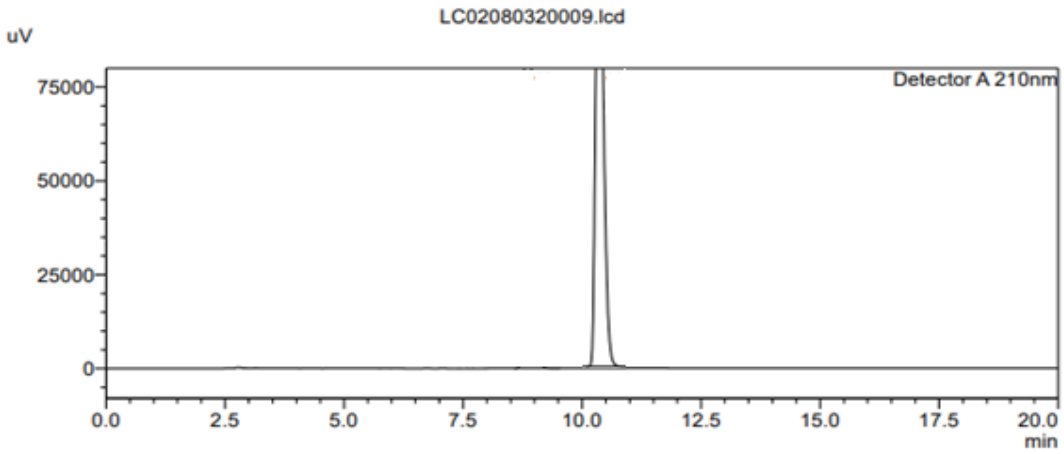


Fig. 4. Chromatogram of Vildagliptin in sample solution

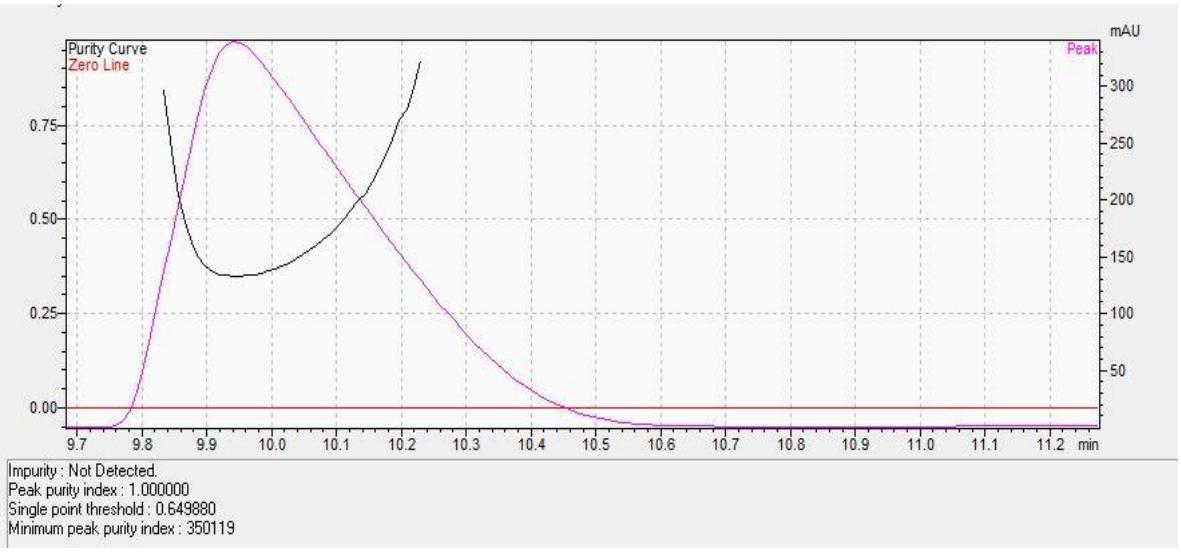


Fig. 5. Purity curve of Vildagliptin in sample solution

2.3 Preparation of Standard and Sample Solution

In a 50 mL volumetric flask, weigh around 25 mg of Vildagliptin working standard/sample. Dissolve in Diluent and make up the volume with diluent and take 5.0 ml of the above solution to 50 ml of volumetric flask and dilute up to the mark with diluent and mix well.

2.3.1 Impurity stock solution-I

Weigh and transfer about 7.5 mg each of Impurity-a, Impurity-b, Impurity-c standards in to 25 mL volumetric flask, add about 15-16 mL of diluent, mix well and sonicate to dissolve. Then make up to the mark with same diluent and mix well.

2.3.2 Impurity stock solution-II

Transfer about 5ml of above stock solution-I in to 50 mL volumetric flask, add about 10-15 mL of diluent, mix well and sonicate to dissolve. Then make up to the mark with same diluent and mix well.

2.3.3 100% spiked solution

Weigh and transfer about 50.2 mg sample in to 50 mL volumetric flask Transfer Accurately 2.5 mL standard stock-II into a 50 mL volumetric flask, add 20-25 mL of diluent mix well and sonicate to dissolve. Then make up to the mark with diluent and mix well.

3. RESULTS AND DISCUSSION

The goal of this methodology was to provide a new HPLC method for determining Vildagliptin that was both cost-effective and convenient. The experimental method was validated according to ICH and USP guidelines for specificity, system suitability, accuracy, linearity, precision, and robustness.

3.1 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%.The results are listed in Table 1.

Table-1: System suitability Vildagliptin determination by HPLC was developed with the goal of developing a new, cost-effective, and convenient method for determining the drug Vildagliptin. The experimental technique was verified in accordance with the guidelines of the International Conference on Harmonization and the United States Pharmacopeia (ICH/USP) for factors such as specificity, system appropriateness, accuracy, linearity, precision, and robustness.

Suitability of the system: A total of six replicates of standard solution were injected into the chromatographic system at a 100 percent level to

ensure that the system was appropriate for the task at hand. In order for Vildagliptin to be approved, the percent relative standard deviation (percent RSD) for the peak area and retention periods had to be less than 2 percent. Table 1 is a summary of the findings.

3.2 Linearity

Linearity refers to the ability to obtain test results that are directly proportional to the concentration area of the Vildagliptin standard, as well as determining the correlation coefficient (R2). To demonstrate linearity, three injections of five different vildagliptin concentrations were employed. The detector response for Vildagliptin standard was found to be linear from 50% to 150% of the test concentration. Before injecting the solutions, the column was equilibrated with the mobile phase for at least 45 minutes. To ensure that the detector response was constant at each concentration level, each measurement was performed five times. A correlation coefficient (r^2) of more than 0.998 indicates a linear relationship between analyte concentration and area under the peak. Fig. 5 depicts the linearity curve, whereas Table 2 contains the data.

3.3 Accuracy

The accuracy of a method is determined by how near the outcome is to the true value of the procedure. The accuracy of the procedure was determined through the use of recovery tests. Calculated by combining the working standard test concentrations of Vildagliptin (80 percent, 100 percent, and 120 percent) and expressed as a percentage of the total amount of Vildagliptin recovered. Three samples were prepared for each of the three recovery levels. The results are shown in Table 3.

Table 1. System suitability

Entry	Injection	Retention Time (RT)	Area
1	Standard Inj-1	10.542	7412445
2	Standard Inj-2	10.542	7412341
3	Standard Inj-3	10.539	7413460
4	Standard Inj-4	10.541	7422611
5	Standard Inj-5	10.542	7411803
6	Standard Inj-5	10.542	7408677
Average		10.541	7413556
Std Deviation		0.001	4724
% RSD		0.011	0.064

Table 2. Linearity of Vildagliptin in Standard preparation from 50% to 150% of test concentration

Entry	Injection	RT	Area	Average
1	50% Pre-1	10.678	3725089	3737804
2	50% Pre-2	10.677	3749653	
3	50% Pre-3	10.676	3738671	
4	75% Pre-1	10.599	5560763	5566924
5	75% Pre-2	10.601	5578434	
6	75% Pre-3	10.602	5561575	
7	100% Pre-1	10.534	7367793	7348423
8	100% Pre-2	10.536	7334477	
9	100% Pre-3	10.533	7342999	
10	125% Pre-1	10.472	9153105	9174349
11	125% Pre-2	10.471	9197745	
12	125% Pre-3	10.469	9172196	
13	150% Pre-1	10.416	10948165	10926780
14	150% Pre-2	10.416	10917214	
15	150% Pre-3	10.418	10914960	

Table 3. 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		

3.4 Stability of Analytical Solution

The stability of analytical solutions was determined by injecting the standard solution and sample solution at various time intervals up to 24 hours (0, 4, 8, 12, 16, 18, and 24 hours) while maintaining the auto sampler temperature at room temperature (25°C). The response of the standard and sample solutions were measured, and the % difference in peak area between the two solutions was determined using this information. Table 4 is a summary of the findings.

3.5 Precision

When an analytical method is used repeatedly to different samplings, the precision of the method is defined as the degree of agreement among individual test findings when the process is followed. Using six different sample preparations from the same batch, the repeatability, reproducibility, and intermediate precision of the assay were all evaluated to determine whether the assay was repeatable. The findings of the analysis for repeatability, intermediate precision, and reproducibility are shown in Table 5 of this report.

3.6 Robustness

A procedure's robustness is defined as its capacity to remain unaffected by minor changes in its input or output parameters. When experimental circumstances were purposely changed, the method's robustness was tested by calculating percent assay of Vildagliptin, peak tailing, theoretical plates, and percent RSD under different conditions. In order to evaluate the influence of flow rate, the flow rate was reduced by 0.2 units from 1 ml/min to 0.8 ml/min and 1.2 ml/min, respectively. Instead of 50°C, the influence of column temperature was tested at 48°C and 52°C, with adjustments made to evaluate its effect on the approach to determine its effectiveness. The information gathered is shown in Table 6.

The developed method was unique since the Vildagliptin chromatogram had no extra impurities, diluting solution, or impurity (purity curve shown in Fig. 3 and 5). The procedure yielded a linear calibration curve in the 50-150 percent range, demonstrating detector linearity. Table 1 shows that the results are valid, and the % RSD is 0.464, which is within acceptable limits. Vildagliptin robustness evaluation (Table 6) yielded positive results.

3.7 Limit of Detection and Limit of Quantification

Established the limit of detection and limit of quantification for all the impurities and main compound mentioned in the specification and the details of LOD LOQ are presented in Table-7 and Table-8.

Table 4. 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

Table 5. Statistical analysis for repeatability, intermediate precision, and reproducibility of Vildagliptin

Sample ID	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

Table 6. Results of robustness study

Sl. 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

Table 7. LOD

Injection	RT	Area	S/N Ratio
LOD Inj-1	10.956	419	5.77
LOD Inj-2	10.924	561	8.36
Acceptance criteria	The Signal to noise ratio should be above 3.0.		

Table 8. LOQ

Injection	RT	Area	S/N Ratio
LOQ Inj-1	10.914	972	18.01
LOQ Inj-2	10.912	979	16.70
LOQ Inj-3	10.914	960	13.99
LOQ Inj-4	10.914	963	15.05
LOQ Inj-5	10.908	955	15.82
LOQ Inj-6	10.905	960	14.15
Average	10.911	965	
Std Deviation	0.004	9	
% RSD	0.035	0.926	
Limit of Quantification	The signal to noise ratio should be above 10.0.		

Table 9. Acceptance criteria: System suitability

Solution	Requirement	Acceptance Criteria
Standard solution	%RSD for six replicate inj. of standard solution	%RSD for six replicate injections of standard solution 2%

3.7.1 50% spiked solution

Weighed and transferred about 50.1 mg sample into 50 mL volumetric flask and transferred accurately 1.25 mL standard stock-II into a 50 mL volumetric flask, added 20-25 mL of diluent mixed well and sonicated to dissolve. Then made up to the mark with diluent and mixed well.

3.7.2 100% spiked solution

Weighed and transferred about 50.2 mg sample into 50 mL volumetric flask and transferred accurately 2.5 mL standard stock-II into a 50 mL volumetric flask, added 20-25 mL of diluent mixed well and sonicated to dissolve. Then made up to the mark with diluent and mixed well.

3.7.3 150% spiked solution

Weighed and transferred about 50.2 mg sample into 50 mL volumetric flask and transferred accurately 3.75 mL standard stock-II into a 50 mL volumetric flask, added 20-25 mL of diluent mixed well and sonicated to dissolve. Then made up to the mark with diluent and mixed well.

Accuracy study shall be performed in the range between 50% and 150% (50%, 100%, and 150%) of test concentration.

Injected the solutions into the HPLC system as per the chromatographic conditions, recorded the Chromatograms and measured the peak responses. Calculated the assay content at each level.

Table 10. Injection sequence

S No	Name of solution	No of injections
1	Diluent	Minimum 1
2	Working standard preparation-1	1
3	50% Precision solution 3 preparations	Each preparation one injection
4	100% Precision solution 3 preparations	Each preparation one injection
5	150% Precision solution 3 preparations	Each preparation one injection

Table 11. Area wise Injection distribution (Vildaglitin RT & Amide RT)

Injection	Vildaglitin RT	Area	Amide RT	Area
Spike 100% Inj-1	10.543	7359352	6.670	21495
Spike 100% Inj-2	10.546	7355966	6.672	21198
Spike 100% Inj-3	10.546	7352763	6.672	21210
Spike 100% Inj-4	10.546	7351387	6.673	21360
Spike 100% Inj-5	10.544	7349519	6.672	21170
Spike 100% Inj-6	10.545	7351343	6.674	21158
Average	10.545	7353388	6.672	21265
Std Deviation	0.001	3627	0.001	134
% RSD	0.012	0.049	0.020	0.631

Table 12. Area wise Injection distribution (Acid RT, Chloro RT, & Dimer RT)

Injection	Acid RT	Area	Chloro RT	Area	Dimer RT	Area
Spike 100% Inj-1	9.074	14046	11.696	63704	35.150	51077
Spike 100% Inj-2	9.078	13994	11.699	63624	35.320	51072
Spike 100% Inj-3	9.078	14027	11.699	63331	35.329	51746
Spike 100% Inj-4	9.079	13968	11.700	63424	35.338	51543
Spike 100% Inj-5	9.078	13990	11.698	63526	35.322	51165
Spike 100% Inj-6	9.080	13979	11.699	63420	35.333	51130
Average	9.078	14001	11.699	63505	35.299	51289
Std Deviation	0.002	30	0.001	140	0.073	285
% RSD	0.022	0.213	0.012	0.221	0.207	0.556

Table 13. Area wise Injection distribution with average (Vildaglitin RT & Amide RT)

Injection	Vildaglitin RT	Area	Average	Amide RT	Area	Average
Spike 50% Inj-1	10.538	7498645		6.675	10922	10944
Spike 50% Inj-2	10.537	7500380	7498087	6.674	10970	
Spike 50% Inj-3	10.535	7495236		6.675	10939	
Spike 100% Inj-1	10.540	7347140		6.676	21169	21177
Spike 100% Inj-2	10.540	7349684	7347023	6.677	21225	
Spike 100% Inj-3	10.538	7344245		6.677	21136	
Spike 150% Inj-1	10.535	7389051	7391723	6.677	31175	31207
Spike 150% Inj-2	10.534	7395849		6.677	31236	
Spike 150% Inj-3	10.536	7390268		6.679	31209	

Table 14. Area wise Injection distribution with average (Acid RT, Chloro RT, & Dimer RT)

Injection	Acid RT	Area	Average	Chloro RT	Area	Average	Dimer RT	Area	Average
Spike 50% Inj-1	9.082	7002	7019	11.699	32846	33013	35.331	26458	26765
Spike 50% Inj-2	9.081	7072		11.699	33052		35.337	26947	
Spike 50% Inj-3	9.080	6982		11.698	33141		35.320	26891	
Spike 100% Inj-1	9.082	13916	13982	11.699	63175	63298	35.323	50834	51196
Spike 100% Inj-2	9.084	14032		11.701	63402		35.332	51312	
Spike 100% Inj-3	9.084	13997		11.701	63318		35.332	51441	
Spike 150% Inj-1	9.084	20776	20835	11.700	94004	94018	35.332	74977	75413
Spike 150% Inj-2	9.084	20883		11.700	94064		35.328	75724	
Spike 150% Inj-3	9.086	20846		11.703	93985		35.336	75539	

Table 15. Calculate the % Accuracy at each level: %Recovery = (Area content at each level x 100) /Area of at 100% level

Preparation	Requirement	Acceptance Criteria
Sample solution at each level	%Accuracy	Between 90% and 110.0%

%Recovery	Impurity-A amide	Impurity-C Dimer	Impurity-B Acid	KSM- Chloro	Vildagliptin
50%	99.89	101.02	100.27	103.97	101.97
100%	99.57	99.81	99.86	99.67	99.91
150%	98.81	98.59	99.21	98.70	100.52

Table 16. Requirement and Acceptance Criteria

Study	Requirement	Acceptance Criteria	Results
Precision	%RSD for six preparation	Not more than 2.0%	0.064%%
Linearity	Correlation coefficient	Not less than 0.999	1.000
Accuracy	% Accuracy	Not less than 90.0 % and Not more than 110.0%	100.80

3.8 Range

Range is defined as the range of concentration in which method is linear, precise and accurate. For establishing range, data shall be considered from linearity, precision and accuracy study.

4. CONCLUSION

In this study a simple, accurate, time-saving, cost-effective, and easy to apply. All of the validation parameters for the analytical method produced satisfactory findings, including a good correlation coefficient and a lower percent RSD. As a result, the proposed method may be used for quality control, stability, and future study with ease.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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