Development of a New RS by HPLC Method for Vildagliptin for Quantification of Purity

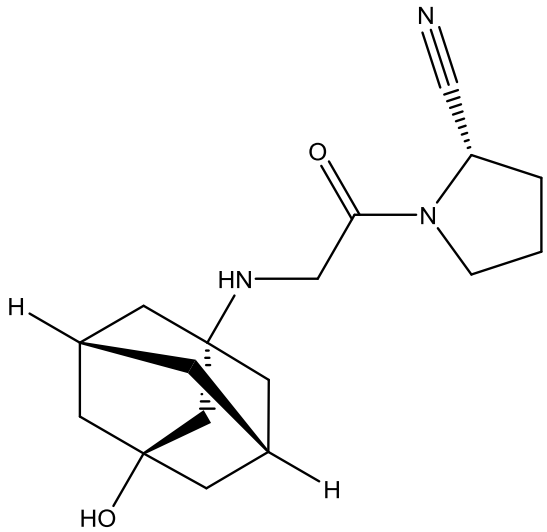
ABSTRACT

Development of a new method for quantification of Vildagliptin in Active Pharma Ingredient (API) besides its relative substance by employing simple, quick, precise, and cost-effective approach was devised and validated. The chromatographic separation was performed on ODS-4 C18 column (3 µm 250 × 4.6 mm) with isocratic elution of buffer acetonitrile and methanol at a ratio of (870:100:30 v/v/v). A photodiode array (PDA) detector was used with a flow rate of 1.0 ml/min, at column temperature 50°C, and detection wavelength at 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 well in agreement.

Keywords: Vildagliptin, RP-HPLC, validation, Assay by HPLC, FDA and ICH 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]



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

Figure 1: Structure of Vildagliptin

When compared to the existing chromatographic methods[6-11] for determining the Vildagliptin API proposed and developed is a fast, innovative, economical, precise, and accurate approach. 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 (United States Pharmacopeial Convention) or ICH guidelines were used to validate the assay method [12-15].

EXPERIMENITAL

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.

Instrumentation and chromatographic condition: Analysis was done by using HPLC (make: Shimadzu, model: LC-2030 C plus) equipped with an auto sampler and PDA detector. The data was collected on a ODS-4, C18 column (300 mm x 4.6 mm,3 µm) using Labsolution software. A Millipore Swinnex type filter (pore size = 0.45 m) was used. For the present developed method, mobile phase containing perchloric acid, acetonitrile and methanol at a ratio of 870:100:30 (percent v/v/v) with a flow rate of 1.0 mL/min. The time for the run is set to 20 minutes. The HPLC system was set to 50°C. Transfer 1.0 mL perchloric acid to 1000 mL water and thoroughly mix. Filtration through a 0.45µ Millipore membrane filter and sonication for 10 minutes were used to degas the sample. The injection volume was 20µL, and the detecting wavelength was set to 210 nanometers. Figures 2 and 4 show that the Vildagliptin peak has typical retention duration of about 8.8 minutes.

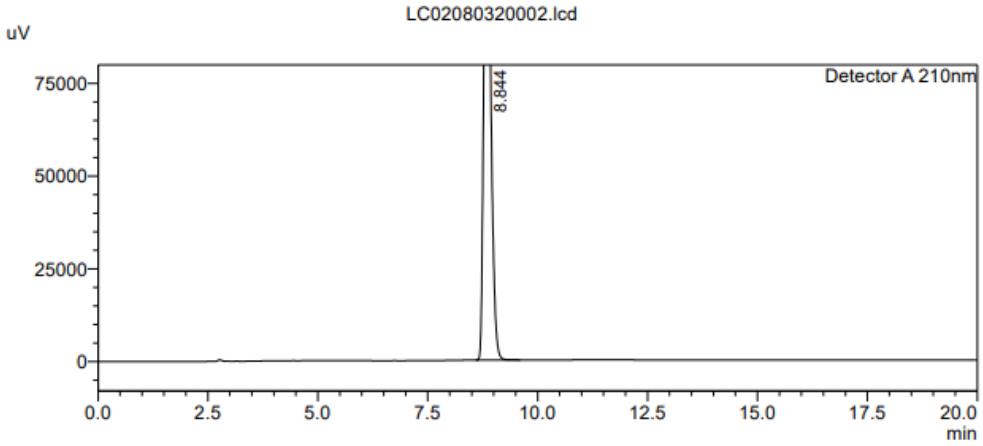


Figure 2: Chromatogram of Vildagliptin in standard solution

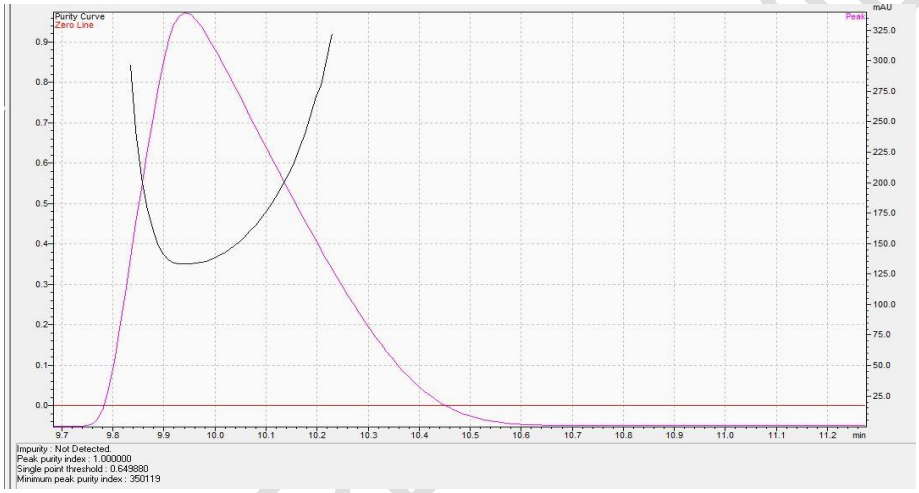


Figure 3: Purity curve of Vildagliptin in standard solution

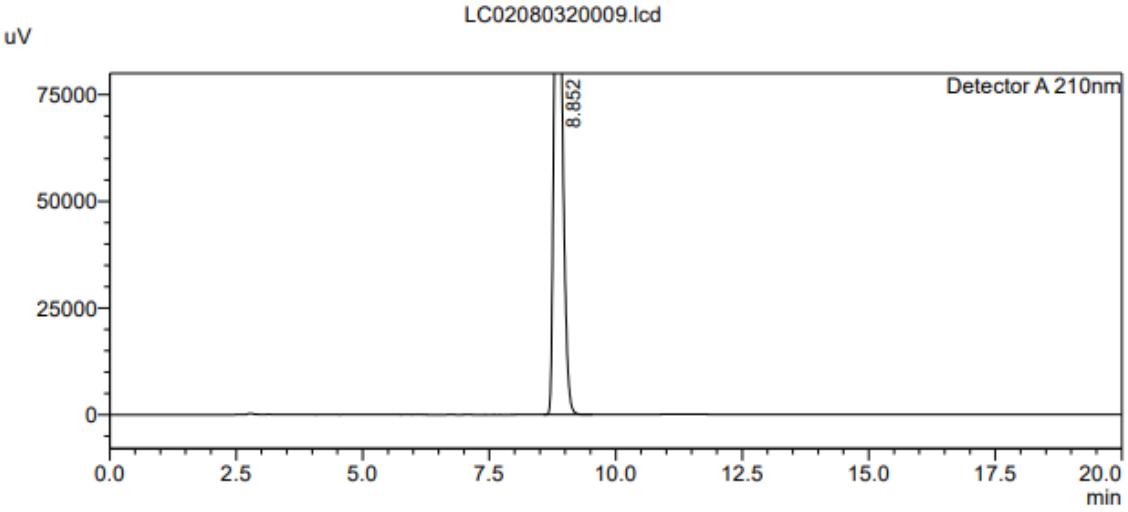


Figure 4: Chromatogram of Vildagliptin in sample solution

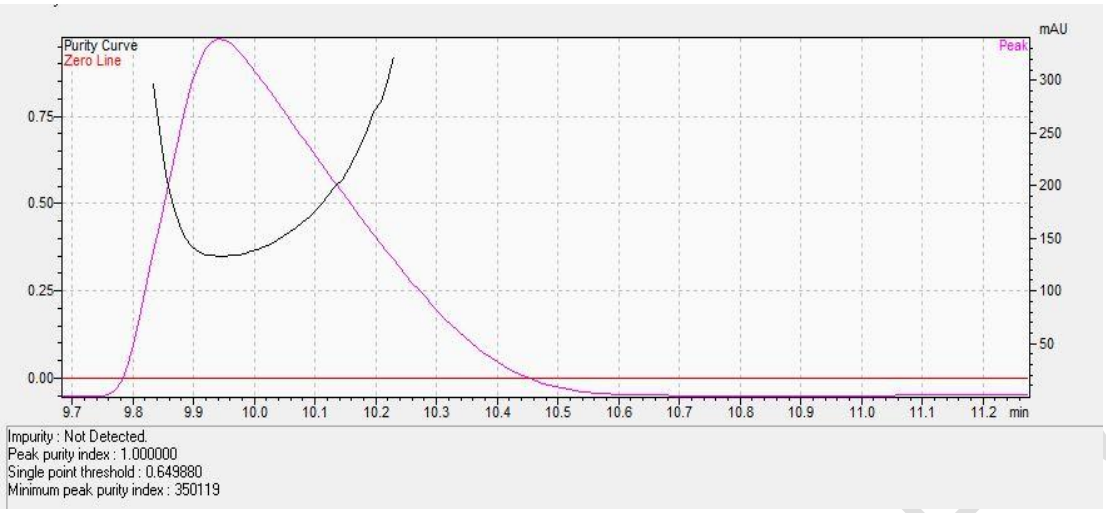


Figure 5: Purity curve of Vildagliptin in sample solution.

Preparation of standard and Sample solution:

In a 50 mL volumetric flask, weigh around 25 mg of Vildagliptin 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.

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.

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.

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.

RESULTS AND DISCUSSION

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 and USP.

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

Entry	Injection	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

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 (R^2). 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 (r^2) 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 2 contains the data.

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

Table-2: Linearity

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	

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 3 shows the results.

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		

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 (25°C), 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 4.

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

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 5 shows the results of the analysis for

repeatability, intermediate precision, and reproducibility.

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

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 50°C, the influence of column temperature was investigated at 48°C and 52°C, with alterations made to evaluate its effect on method. Table 6 shows the information gathered.

Table 6: 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

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 1 demonstrates the correctness of the results, and the percent RSD is 0.464, which is within the acceptable range. Vildagliptin robustness evaluation (Table 6) yielded positive results.

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-7: LOD

Injection	Data File No.	RT	Area	S/N Ratio
LOD Inj-1	LC0220032000 2	10.956	419	5.77
LOD Inj-2	LC0220032000 3	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%

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

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.

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	7498087	6.675	10922	10944
Spike 50% Inj-2	10.537	7500380		6.674	10970	
Spike 50% Inj-3	10.535	7495236		6.675	10939	
Spike 100% Inj-1	10.540	7347140	7347023	6.676	21169	21177
Spike 100% Inj-2	10.540	7349684		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	Vildaglipti n
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

08. 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.

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

CONCLUSION

In this study a simple, accurate, 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.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. 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.

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