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ORIGINAL ARTICLE **OPEN ACCESS**

Development and Validation of RP-HPLC Method for The Estimation of Impurity from Sitagliptin Bulk and formulation

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ABSTRACT
Sitagliptin is a widely used antidiabetic drug belonging to the class of dipeptidyl peptidase-4 (DPP-4) inhibitors. In the manufacturing process of Sitagliptin, several impurities may arise which can affect the quality, safety, and efficacy of the drug. Hence, the accurate determination of process-related impurities in both bulk drug substance and pharmaceutical formulations is of paramount importance. In this study, a robust and validated reverse-phase high-performance liquid chromatography (RP-HPLC) method has been developed for the quantitative estimation of process-related impurities of Sitagliptin. The method was validated as per International Council for Harmonization (ICH) guidelines and found to be accurate, precise, specific, and sensitive. The developed method can be effectively utilized for routine analysis in quality control laboratories for ensuring the quality and safety of Sitagliptin formulations.
Keywords: *Sitagliptin, process-related impurities, RP-HPLC, method development, method validation, antidiabetic drug.*

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INTRODUCTION

Sitagliptin, chemically known as (R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine, is an orally active antidiabetic agent that functions by inhibiting the enzyme dipeptidyl peptidase-4 (DPP-4). Sitagliptin enhances the levels of incretin hormones such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), thereby increasing insulin secretion and decreasing glucagon secretion, leading to improved glycemic control in patients with type 2 diabetes mellitus [1]. During the manufacturing process of Sitagliptin, various impurities may be formed due to chemical reactions, degradation, or impurities in starting materials. These impurities can adversely affect the safety, efficacy, and quality of the drug product. Therefore, it is essential to establish analytical methods for the accurate quantification of process-related impurities to ensure the quality and safety of Sitagliptin formulations [2]. High-performance liquid chromatography (HPLC) is one of the most widely used analytical techniques for the analysis of pharmaceutical compounds due to its high selectivity, sensitivity, and reproducibility. Reverse-phase HPLC (RP-HPLC) is particularly suitable for the separation and quantification of hydrophobic compounds such as Sitagliptin and its impurities [3]. In this study, we report the development and validation of an RP-HPLC method for the quantitative determination of process-related impurities of Sitagliptin in bulk drug substance and pharmaceutical formulations.

MATERIAL AND METHODS

Chemicals

The HPLC grade acetonitrile, methanol and water are used as solvents other chemicals are of AR grade.

Drug

The Sitagliptin bulk was obtained as a gift sample for research purpose.

HPLC Method Development [4-12]

The method development procedure for HPLC analysis of the process-related impurity of Sitagliptin using an acetonitrile: water mobile phase (65:35) on a Japan-based LC20AD Prominence Liquid Chromatography system coupled with SPD20-A Schimadzu UV-Vis detector at a wavelength of 290 nm is outlined as follows:

Preparation of Standard Solution:

Prepare a stock solution of the process-related impurity of Sitagliptin by accurately weighing a suitable amount of the impurity reference standard and dissolving it in HPLC-grade acetonitrile or water to achieve a concentration within the linear range of the detector. Ensure thorough mixing and sonication if necessary to ensure complete dissolution.

Optimization of Chromatographic Conditions:

a. Mobile Phase Composition: Prepare the acetonitrile: water mobile phase in the ratio of 65:35. Evaluate different ratios of acetonitrile and water to achieve optimal peak shape, resolution, and retention time of the process-related impurity.

b. Column Selection: Choose an analytical column appropriate for the separation of the impurity. A C18 column with dimensions of 4.6 mm × 250 mm and a particle size of 5 µm is commonly used for HPLC analysis.

c. Flow Rate: Set the flow rate to an appropriate value to ensure efficient separation and elution of the impurity without causing backpressure issues. Typical flow rates range from 0.8 to 1.2 mL/min.

d. Detection Wavelength: Configure the SPD20-A Schimadzu UV-Vis detector to monitor the elution of the process-related impurity at a wavelength of 290 nm, which corresponds to the maximum absorbance of the impurity.

e. Column Temperature: Maintain the column temperature at ambient conditions, typically around 25°C, to ensure consistent chromatographic performance.

Method Optimization and Validation:

Perform test injections of the prepared standard solution under various chromatographic conditions, including different mobile phase compositions, flow rates, and injection volumes. Optimize the chromatographic parameters to achieve sharp, well-resolved peaks with good symmetry and minimal tailing. Validate the method according to international guidelines, such as specificity, linearity, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ), and robustness.

RESULT AND DISCUSSION

HPLC Method Development

The ICH Q2B recommendations cover the HPLC-based analytical method validation. Currently, HPLC is used to determine the vast majority of process-related impurity measurements. It provided the desired sensitivity for highly automated trace level determinations. HPLC is applicable to all drug classes due to the wide range of stationary phases and operation modes. The typical HPLC detection limits for process-related impurities are 0.1% or lower, and in the vast majority of cases, this can be achieved using traditional UV detectors. These techniques involved anticipating potential impurities during the synthetic process, isolating them, and identifying them using the appropriate analytical methods. The optimised HPLC method was unique compared to the study's placebo. The chromatograms of every placebo lacked any interference.

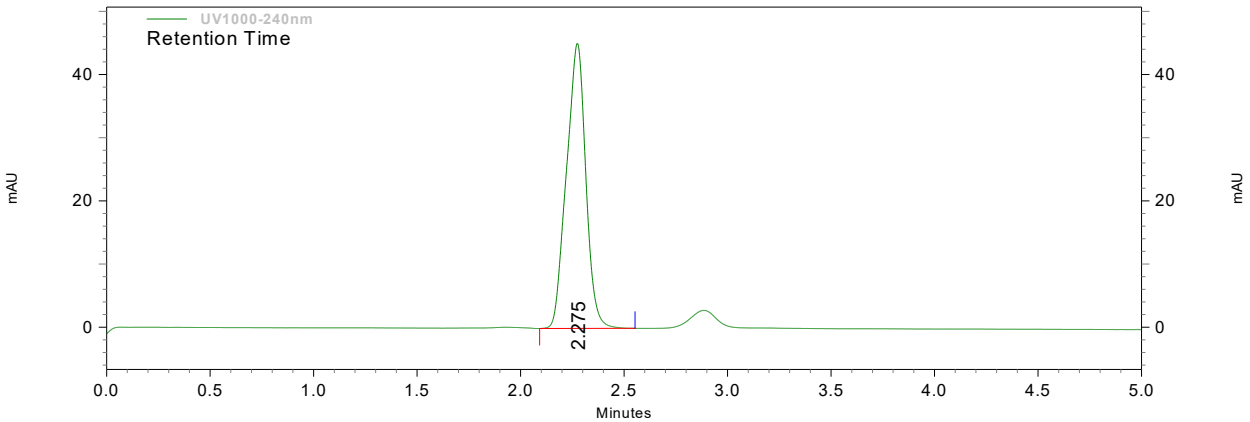

Fig No.1: HPLC chromatogram for Sitagliptin Impurity

Linearity

Regression analysis was employed to assess the linearity of the proposed method across six concentration levels within the intermediate range of 20-120 µg/mL. The quantitation results are tabulated in Table 1. Figure 2 illustrates the calibration curves' slope and intercept. An R-squared value (R^2) of 0.99 was obtained, indicating a high degree of linearity. Additionally, the linear intercept was determined to be y = 8020.5

Table no. 01 : Linearity data of Sitagliptin Impurity		
Sr. no.	**Conc in ppm**	**Area (mv-sec)**
1	20	151023
2	40	286557
3	60	456456
4	80	629865
5	100	775789
6	120	956134

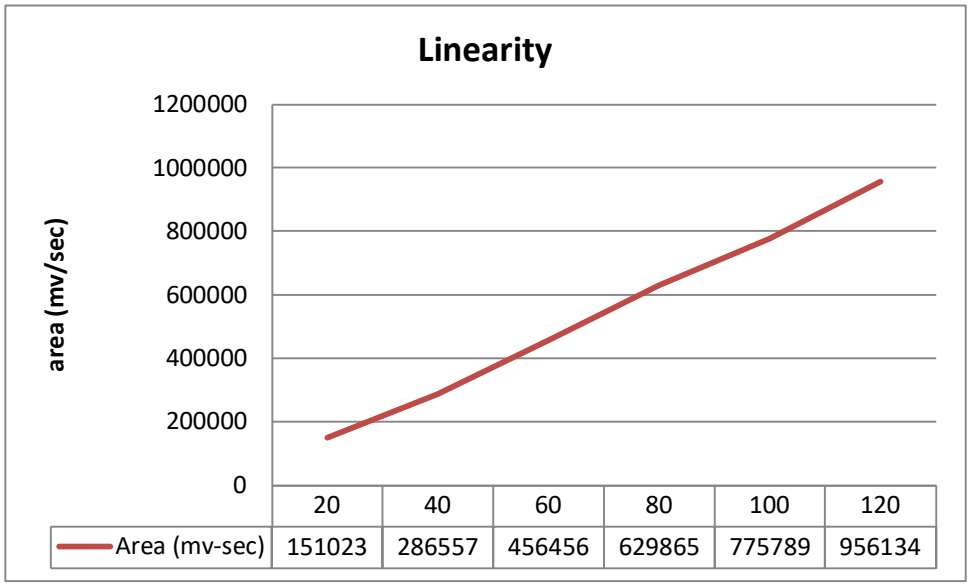

Fig No.2: Graph of peak area vs. conc. in ppm.

Regression coefficient of the linearity was R2 – 0.99; indicates linear relationship

Precision

The area of the chromatogram for the sitagliptin Impurity, whose precision was measured for repeated concentrations in the 60 ug/ml range, is shown in table no 2. Both the relative standard deviation (RSD) and the standard deviation (SD) were determined to be precisely 8.174 and 0.84.

Table No.2: Standard and % relative standard deviation data for precision				
Sr. no.	**Concentration in ppm**	**Area in mv sec**	**SD**	**%RSD**
1	60	426756	8.174	0.84
2	60	426978		
3	60	425988		

Accuracy

The accuracy of each Impurity was determined using its known concentration. The analysis of a Sitagliptin formulation spiked with known concentrations of each impurity at three concentration levels in triplicate of 25%, 50%, and 100% was used to determine the accuracy of the recovery assessment, as shown in table no. 3. Although the increase in concentration recovery is low, a very good percentage recovery was achieved for 50%.

Table No.3: Percentage recovery data for intermediate										
Sr. no.	**Sample**	**Amount found in mg (n=3) in %**			**% Amount Recovered**			**SEM**	**SD**	**RSD**
		25	50	100	25	50	100			
1	A	167.54	251.45	305.45	100.46	95.78	91.48	1.15	2.54	0.011
2	B	169.65	228.65	305.64	98.65	92.56	91.54	1.56	2.95	0.009

Limit of Quantitation and detection

The LOD and LOQ values were calculated for Sitagliptin Impurity based on the noise level and the values are shown in table no 4.

Table No.4: Method validation summary for HPLC.		
Sr. no.	**Parameter**	**Standard**
1	Linearity range	20-120 µg/ml
2	Slope	8015.8
3	Intercept	8020.5
4	Correlation coefficient	0.99
5	LOD	0.897ug/ml
6	LOQ	3.015 ug/ ml

Robustness

The robustness of the Sitagliptin Impurity was carried out for three changes in analyst and method was found to be robust at standard deviation to 2.53 and percentage relative standard deviation to 1.98 as shown in the table no 5.

Ruggedness

The ruggedness of method were performed for change in flow rate up to 0.8ml/min and method was rugged with high standard deviation to 6.49 and % relative standard deviation to 3.89 as shown in table no 5.

Table No.5 Robustness, ruggedness and precision of the method			
Sr. no.	**Parameter**	**SD**	**%RSD**
1	Precision (n = 3)	**8.156**	**0.7854**
2	Intraday precision (n = 3)	8.174	0.85
3	Inter day precision (n = 5)	101.45	45.67
4	Ruggedness (change in flow rate; n =5)	6.49	3.89
5	Robustness (change in analyst; n = 3)	2.53	1.98

System suitability parameter

To know reproducibility of the method, system suitability test was carried out to determine the chromatographic parameter such theoretical plates, tailing factor, % RSD by analyzing sample solution shown in the table no 6. The values obtained demonstrated the suitability of the system for the analysis of Sitagliptin Impurity

Table No.6: Robustness and ruggedness of the method (System suitability parameters)					
Sr.no.	**Parameter**	**Theoretical Plate**	**Peak area**	**Tailing Factor**	**Retention time**
1	Flow rate 0.8ml/min	7156	156.86	2.9	3.85
2	Flow rate 1ml/min	6498	121.65	2.85	3.08
3	Analyst 1	7569	114.65	1.73	3.05

Formulation

As shown below in the table no.7 shows better peak and area for impurity present in the Sitagliptin formulation at the retention time of 2.860 minutes was reported accurately. The linearity for Sitagliptin formulation was determined and the graph conc. vs. area was linear and the regression coefficient was found to be 0.99 and the intercept was 4987.77 thus, we can determine the synthesized impurity is present in the Sitagliptin formulation.

Table No.07 Linearity of Sitagliptin formulation.		
Sr. no	**Conc. in ppm**	**Area in mv/s**
1	20	375467
2	40	733865
3	60	1100478
4	80	1489654
5	100	1785645

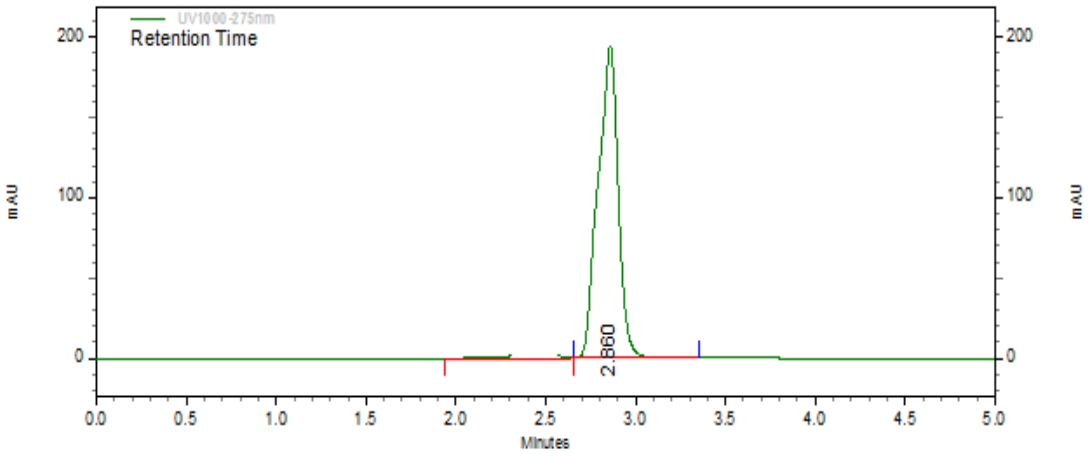

Fig No.3: HPLC chromatogram for Sitagliptin Formulation

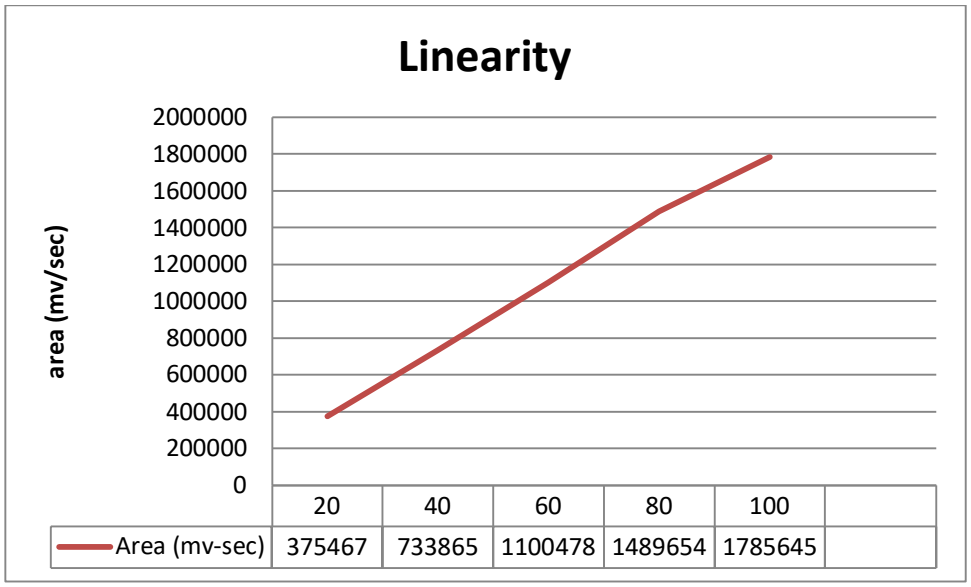

Fig no.4: Linearity graph for Sitagliptin formulation.

Quantitation of Impurity

The total amount of impurity present in the formulation of Sitagliptin is quantified. The 40 ug/ml and 20 ug/ml of both standard as well as sample solution was injected and the retention time was 2.860 minutes and shows the better area of chromatogram. Hence, the following data express the total amount of impurity present in formulation;

Table No.8: Data for Sitagliptin formulation.

Conc. (ppm)	Retention time in min.	Area in mv/s
20	2.860	378654

The synthesis, characterization and RP-HPLC method development of process-related impurity of Sitagliptin was found to be linear, precise, accurate, and robust. From the above stated we can conclude that the synthesized Impurity of the Sitagliptin was found to be 0.01296% in the Sitagliptin formulation.

CONCLUSION

A robust and validated RP-HPLC method has been successfully developed and validated for the estimation of process-related impurities of Sitagliptin in bulk drug substance and pharmaceutical formulations. The

method offers excellent specificity, sensitivity, accuracy, and precision and can be effectively used for routine analysis in quality control laboratories to ensure the quality and safety of Sitagliptin formulations.

FUTURE PERSPECTIVES

Future studies may involve the application of the developed method for the analysis of Sitagliptin in different matrices, such as biological samples and stability studies. Additionally, further research could focus on the investigation of degradation pathways and impurity profiling of Sitagliptin under various stress conditions to understand the degradation mechanisms and ensure the stability of the drug product.

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