Quantitation of Sitagliptin in Drug Product by Validated Reversed Phase Liquid Chromatographic Technique

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ABSTRACT: A novel reversed phase ultra-high performance liquid chromatographic (RP-UHPLC) method was developed for the estimation of sitagliptin in pharmaceutical dosage form. Separation was done by a X-bridge C_{18} column (4.6 i.d.× 150 mm, 5 µm particle size) with a flow rate of 1 ml/min using phosphate buffer (pH 6) and acetonitrile (70:30, v/v) as mobile phase at 268 nm using photodiode array plus (PDA+) detector. The retention time was found at 4.607 min. The developed method was validated as per the requirements of ICH-Q2B guidelines for specificity, system suitability, linearity, precision, accuracy, sensitivity and robustness. The linear regression analysis data for the linearity plot showed correlation coefficient values of 0.999 with LOD value of 0.06 µg/ml and LOQ of 0.225 µg/ml. The relative standard deviation (%RSD) for inter-day and intra- day precision was not more than 2.0%. The method was found to be accurate with percentages recovery of 98.50±0.03 to 99.70±0.05 and the % RSD was less than 2. The results showed that the proposed method is highly convenient for routine analysis of sitagliptin.

Key words: Sitagliptin, UHPLC, validation.

INTRODUCTION

Sitagliptin, chemically [(R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a] pyrizine-7(8H)-yl]-1- (2,4,5-trifloro phenyl)butan-2-amine] (Figure 1), is a long-acting pyrizine-based drug. It is one of the promising drugs used for the treatment of type II diabetes.[1,2]

Since the inception in 2006 as first dipeptidyl peptidase-4 (DPP-4) inhibitor, it is a well-known hypoglycemic drug concurrently administered with lifestyle changes.[3] By enhancing the effect of incretins, it reduces blood glucose concentration and finally causes significant increase in insulin secretion. According to the global report on diabetes in 2014, 422 million adults were living with diabetes globally, compared to 108 million in 1980.[4] So, the demand of antidiabetic drugs especially DPP-4 inhibiting drugs have been rising in pharmaceutical market day by day.[5] To ensure the quality of the drug products in a cost effective way, it is helpful to use a fast, precise, sensitive, and robust HPLC method for the quantitation of sitagliptin. In continuation of our research work in the field of analysis of food and

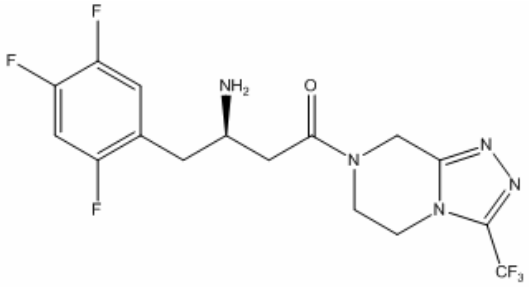

Figure 1. Structure of sitagliptin.

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drug products e.g. Sultana *et al.*[6-8], Alam *et al.*[9], Hossain *et al.*[10], Islam *et al.*[11], Hossain *et al.*[12], here, we report a simple, robust, economic and validated method for the estimation of sitagliptin.

Literature review revealed the determination of sitagliptin in dosage form either alone or combined form by UV spectrophotometry[13-15], RP-HPLC[5,16-21], UPLC[22], tandem mass spectrometry[23,24] and capillary electrophoresis.[25] However, UV spectrophotometry is the simplest technique but it is deficient in accuracy and precision and needs relatively higher amount of analytes for detection. HPLC is the best option for analysis because of its simplicity and sensitivity. Most of the reported methods for the estimation of sitagliptin contained complex mixture in mobile phase[5,16], higher percentages of organic solvent in mobile phase[15,19,20] and relatively long retention time.[17,18,22] Therefore, we aimed to establish a validated cost-effective, simple, fast and precise UHPLC method to determine sitagliptin in oral tablet dosage form.

MATERIALS AND METHODS

Chemicals and reagents. Sitagliptin standards (purity >99.87%; Aurobindo Pharma, Hyderabad, India) were obtained from Incepta Pharmaceutical Ltd. Bangladesh as a generous gift sample for research. HPLC-grade acetonitrile was obtained from RCI Labscan, Thailand, analytical potassium dihydrogen phosphate was obtained from Daejung Chemicals & Metal Co. Ltd., Korea and nano pure water was obtained from Evoqua Water Technologies. All other chemicals used were of analytical grade. Gilipta 50 tablet manufactured by Beximco Pharmaceuticals Ltd. Bangladesh was purchased from the local pharmaceutical market.

Instrumentation. Perkin Elmer Flexar series (FX-15 binary pump, PDA plus detector, autosampler, vacuum degasser and column oven) with Chromera Manager Software was used for analysis in reverse phase ultra-high performance liquid chromatography (RP-UHPLC) system. The isocratic elution was done with the mobile phase consisting of mixture of phosphate buffer at pH 6.0 and acetonitrile (70:30, v/v) (Table 1). The mobile phase was degassed in an ultrasonic bath (Human Lab Instrument Co. Ltd., Korea) and then filtered through 0.45-µm, 47mm nylon membrane filter (Restek, USA).

Table 1. Chromatographic conditions.

Equipment	UHPLC with PDA+ Detector
Column	X-bridge C_{18} (4.6 i.d. × 150 mm, 5 µm particle size)
Flow rate	1.0 ml per min
Wavelength	268 nm
Injection volume	20 µL
Column temperature	25 °C
Run time	10 min

Preparation of standard solution. Stock solution with a concentration of 1mg/ml of sitagliptin was prepared in mobile phase. A working standard solution with a concentration of 50 µg/ml was prepared by suitable dilution with mobile phase from the stock solution.

Preparation of sample solution. Glipita 50 tablets were crushed into fine powder. By transferring a weighed amount of the powder, stock sample solution was prepared with a concentration of 1 mg/ml by mobile phase which was equivalent to 100 mg sitagliptin in a 100 ml volumetric flask. 50 ml mobile phase was added to the sample and sonicated for 10 min. Finally, the volume was adjusted with mobile phase up to the mark. Then the solution was filtered (Whatman filter paper, Grade 1, 110 mm diameter). For further use the solution was stored in suitable container. A sample solution of 50 µg/ml was prepared for assay of tablet from the stock solution by dilution with the mobile phase and then filtered through 0.22 µm disc filter (Filter-Bio).

Method validation. The developed method for the determination and validation of sitagliptin was carried out as per ICH guidelines.[26]

Specificity. Separate injection of blank, standard and sample solution of sitagliptin in triplicate was used for the determination of specificity. By the value of peak purity, the results were confirmed (Figure 2).

System suitability test. By injecting six replicate injections of standard solution (50µg/ml) system suitability was established. The relative standard deviation (% RSD) and mean tailing factor of responses were calculated.

Linearity. Different concentrations of sitagliptin (10, 20, 30, 40 and 50 µg/ml) were prepared by suitable dilutions of standard stock solution to check the linearity of the developed method. The limit of detection (LOD) was determined at the signal to noise ratio of 3:1and limit of quantitation (LOQ) was determined at the signal to noise ratio 10:1.

Precision. Precision was assessed by intermediate precision and repeatability or intra-assay precision. Repeatability was determined from six replicate injections of 20 µl each of nominal standard solution (50 µg/ml). The nominal standard solution was analyzed for a period of six days with six replicate injections of 20 µl each on daily basis. The results of both studies were compared (intermediate precision) and expressed as %RSD of the measurements.

Accuracy. To check for accuracy or recovery of the developed method as well as studying the interference of formulation additives on analysis the standard drug substance was spiked with the sample solution at 80%, 100% and 120%. Triplicate injections were carried out for all determinations.

Robustness. By insignificant variations of the technique robustness study of the method was carried out such as altering flow rate, column temperature and changes in composition of the mobile phase.

RESULTS AND DISCUSSION

Optimization of chromatographic condition. During method development, different chromatographic conditions were used to achieve good separation of sitagliptin. Several columns (C_8 and C_{18}) and columns lengths (150 and 250 mm), various mobile phase components including (methanol, acetonitrile, phosphate buffer and water), different pH levels (between 3 and 7) and flow rates (0.5–2 ml/min) were tried. Finally, it was found better to use the C_{18} column (4.6 i.d. × 150 mm, 5 µm particle size) with a simple isocratic mobile phase composed of acetonitrile and phosphate buffer at a pH of 6.0 with the ratio of 30 and 70 and flow rate of 1 ml/min.

Sitagliptin UV spectrum was determined by PDA plus detector which showed that the peak purity value is 1.03 and the maximum absorption wavelength was found at 268 nm (Figure 3). The analyte eluted at 4.607 min with a total analytical run time of 10 min.

Specificity. From figure 3, the UHPLC chromatogram for the blank, standard and sample separately revealed that there was no interfering peak with sitagliptin.

System suitability test. After six replicate injection of the nominal concentration (50µg/ml) the %RSD of obtained data such as peak area, theoretical plate, tailing factor and retention time were not more than 2% (Table 2).[27]

Table 2. Results of system suitability test.

Parameters	Value (Mean ± % RSD)
Peak area	2,116,867 ± 0.41
Theoretical plate	6938 ± 0.35
Tailing factor	1.025 ± 0.14
Retention time	4.607 ± 0.12 min

Linearity. The regression curve was constructed by linear regression and its mathematical expression was y = 42127x + 2099.3, where y is the peak area and x is the concentration of sitagliptin (Figure 4). Linearity parameters of sitagliptin are described in table 3.

Table 3. Linearity parameters of sitagliptin.

Linearity Parameters	Obtained value
Regression Correlation Coefficient	0.999
Y-intercept	2099
Slope of Regression Line	42127
LOD	0.06 µg/ml
LOQ	0.225 µg/ml

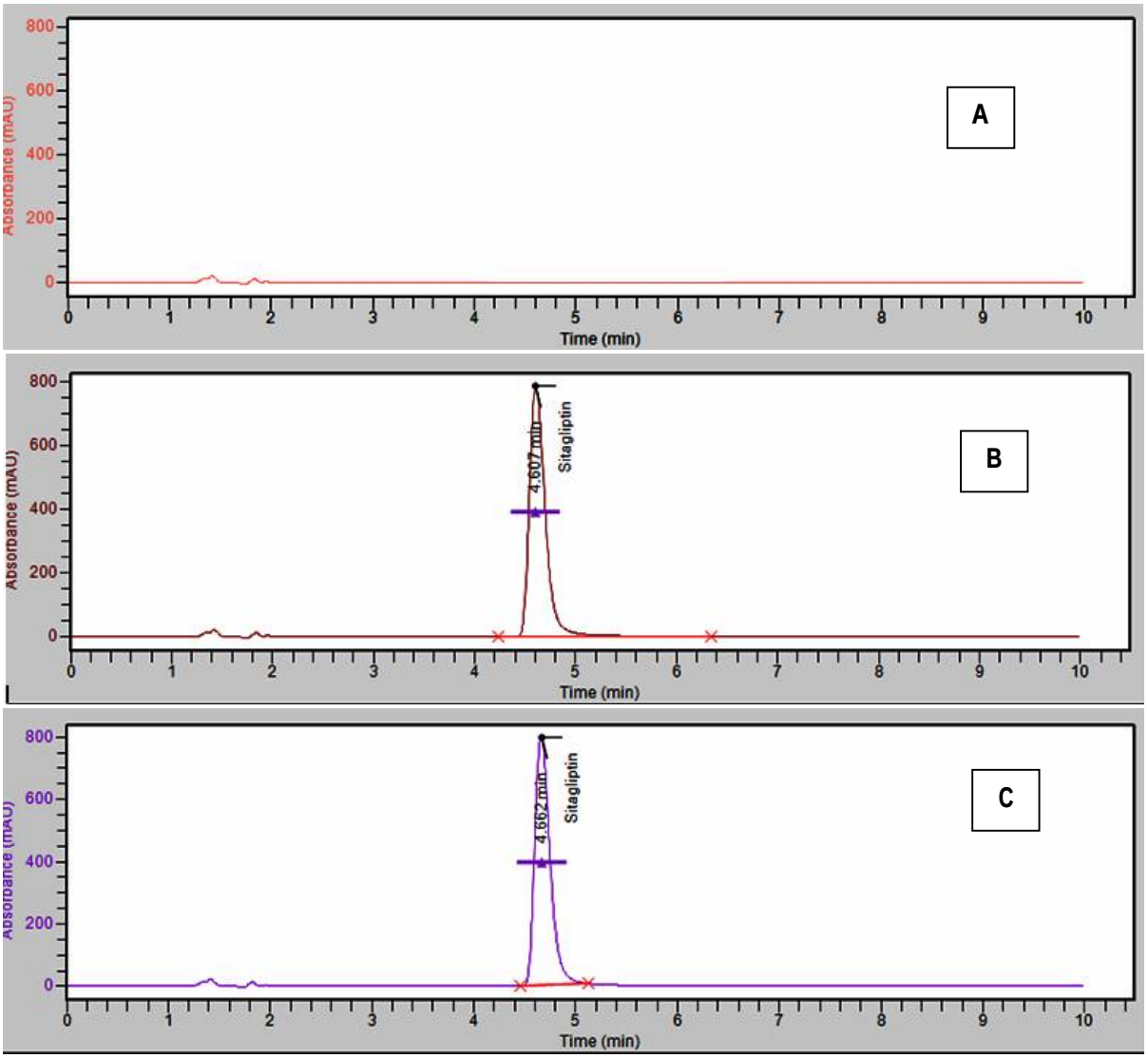

Figure 2. UHPLC chromatogram of blank (A), sitagliptin standard solution (B) and sample solution (C).

Table 4. Inter-day and intra-day precision for sitagliptin.

Inter-day	Time (hr)	0	1	2	4	8	12
	Assay (%) Mean ± SD	99.56 ± 0.06	99.25 ± 0.23	99.03 ± 0.28	99.45 ± 0.41	99.88 ± 0.55	99.60 ± 0.63
Intra-day	Day	1	2	3	4	5	6
	Assay (%) Mean ± SD	99.85 ± 0.33	99.55 ± 0.25	99.06 ± 0.39	98.87 ± 0.74	98.54 ± 0.24	98.24 ± 0.44

Accuracy. The result of recovery study (Table 5) showed that the method was found accurate and the % RSD was not more than 2%.

Robustness. The variation for robustness study performed by changing flow rate (±0.2 ml/min), column temperature (±3◦C), composition of mobile

phase (±5% of acetonitrile) resulted in SD and %RSD NMT 2%, which indicated good and satisfactory robustness of the proposed method (Table 6).

The developed method could elute the drug molecule at 4.607 minute which is shorter than the method described by Prasad *et al.*[19], S. Patil *et al.*[21], Chellu *et al.*[22] which indicated the rapid quantification capacity. In addition, the method was found to be free from cumbersome mobile phase composition with additional advantages of less consumption of organic solvents that of reported methods of Konari and Jane[5], Shyamala *et al.*[16], Bhende *et al.*[17], Rao *et al.*[18] and Patil *et al.*[21]

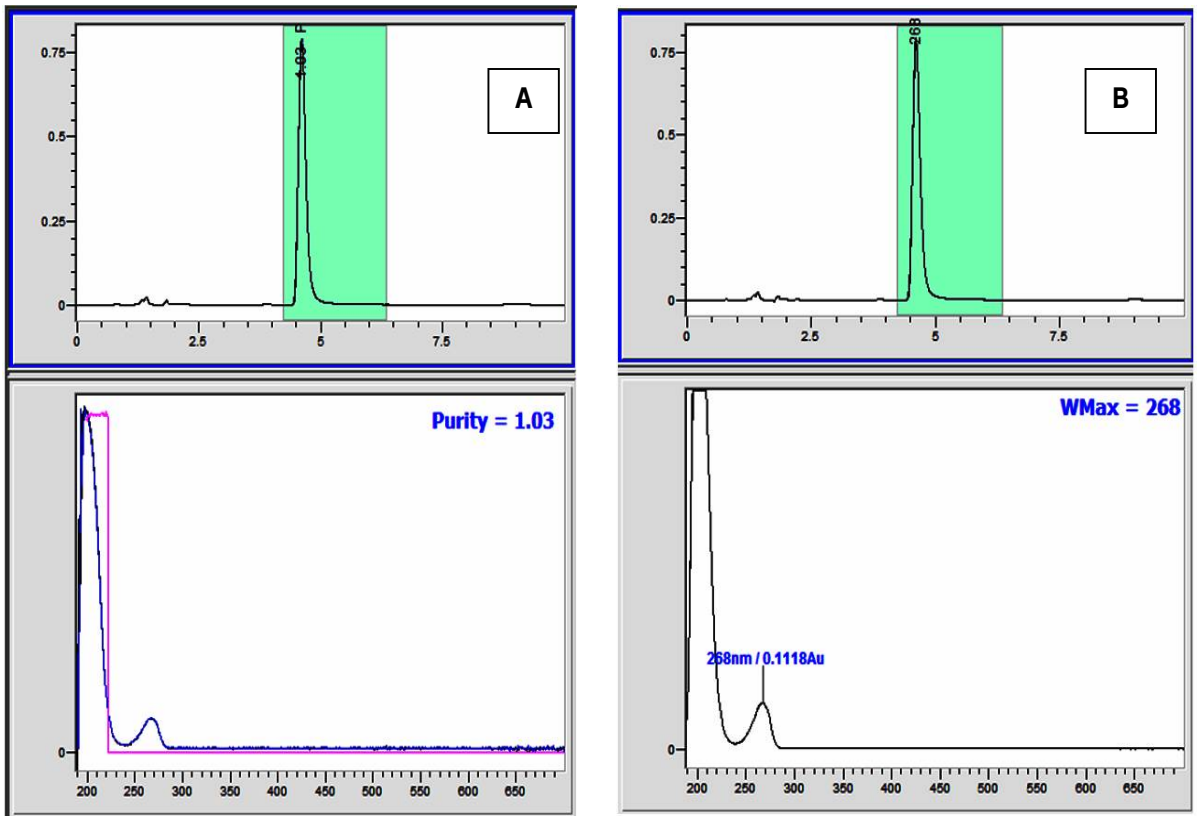

Figure 3. Peak purity (A) and maximum wavelength, 268 nm (B) of sitagliptin.

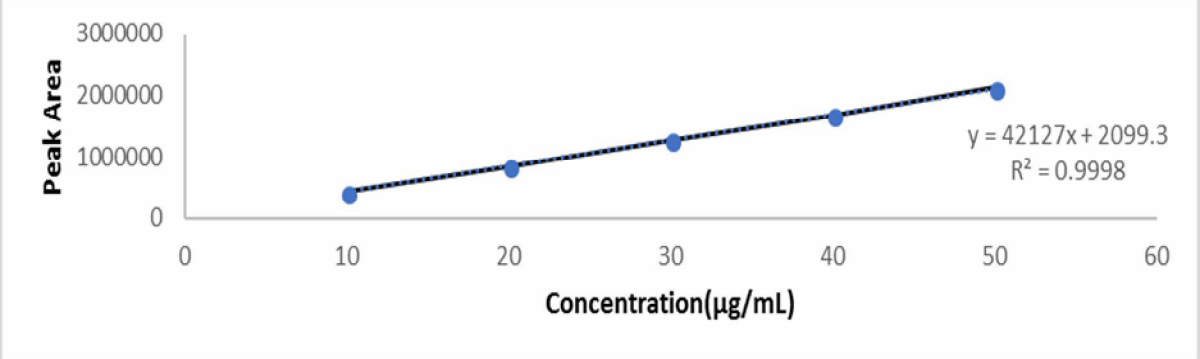

Figure 4. Linearity curve of sitagliptin.

Table 5. Recovery study for method validation of sitagliptin.

Sl#	Level (%)	% Recovered ± SD*
1	80	99.70 ± 0.05
2	100	98.50 ± 0.03
3	120	99.43 ± 0.09

*Mean of triplicate determinations.

Table 6. Robustness study for method validation of sitagliptin.

Parameters	Variations	Amount Added (µg/ml)	Amount Detected (µg/ml) (Mean ± SD*)
Flow rate (ml/min)	0.8	50	50.12 ± 0.09
	1	50	49.95 ± 0.16
	1.2	50	50.04 ± 0.24
Column temperature (◦C)	22	50	50.14 ± 0.15
	25	50	50.86 ± 0.19
	28	50	50.78 ± 0.26
Mobile phase composition (Acetonitrile %)	25	50	49.98 ± 0.43
	30	50	50.22 ± 0.40
	35	50	50.68 ± 0.25

*Mean of triplicate determinations

CONCLUSION

In pharmaceutical market, the demand of DPP-4 inhibitor products is growing. So, it was highly necessary to develop an easy and simple technique to quantitate the drug in the dosage forms. The reported method is time-saving, rapid, selective, linear, precise, accurate and robust. Thus, the method seems to be appropriate for the quality control in pharmaceutical industries as well as for drug substances quantitation in biological fluid during *in vivo* studies.

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