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ORIGINAL ARTICLE **OPEN ACCESS**

Analytical Method Development and Validation for the Estimation of Sitagliptin Phosphate Monohydrate in Bulk and Formulation by UFLC

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ABSTRACT

A simple and precise ultra-fast liquid chromatographic method was developed and successively validated for the estimation of Sitagliptin phosphate monohydrate in bulk and pharmaceutical dosage form. Sitagliptin separation was carried out by the Shim-pack GIST C18 column (250×4.6mm,5 µm) using a mobile phase composition of Acetonitrile and Water in the ratio of 80:20% v/v at ambient temperature with flow rate 1.0ml/min . Sitagliptin was determined at 267 nm using UV detection and the compound was eluted at retention time of 2.9 min and run time 6 min. As per ICH guidelines, the method was validated and parameters were linearity, precision , accuracy, LOD,LOQ, robustness, system suitability, specificity. The described method was found to be linear over the range of 10-50µg/ml and correlation coefficient was found to be 0.997. The LOD and LOQ was found to be 0.3181 µg/ml and 0.9639 µg/ml respectively. The results of the study showed that the proposed UFLC method is simple, rapid, accurate, precise, linear, reliable, useful and economical for the routine determination of Sitagliptin phosphate in bulk and its pharmaceutical dosage form.

Keywords: *Sitagliptin phosphate monohydrate, UFLC, ICH guidelines*

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INTRODUCTION

Sitagliptin Phosphate Monohydrate is chemically denoted as (R)-3-Amino-1-(3-(trifluoromethyl)-5,6-dihydro-[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl)-4-(2,4,5-trifluorophenyl)butan-1-one phosphate hydrate. The empirical formula is $C_{16}H_{15}F_6N_5O \bullet H_3PO_4 \bullet H_2O$ and the molecular weight is 523.32 g/mol. Sitagliptin phosphate is an oral anti hyperglycaemic of the dipeptidyl peptidase-4 (DPP-4) inhibitor class. This enzyme-inhibiting drug is used either alone or in combination with other oral anti hyperglycemic agents (such as metformin or a thiazolidinedione) for treatment of diabetes mellitus type 2. It rapidly and completely inhibits the activity of DPP- 4 enzymes this results in increase of the two incretin hormones available in our body, they are glucose-like peptide-1(GLP-1) and glucose dependent insulinotropic peptide (GIP). The activation of these two hormones results in decrease of the blood glucose level by decreasing the glucagon secretion and increase of insulin sensitivity. GLP-1 activation enhances the β-cell sensitivity and reduces the α-cell sensitivity of islets of Langerhans in the pancreas which results in increase in amount of insulin and decreases the amount of glucagon and reduces the glucose level in blood [1-5]. Literature survey revealed that there were few methods reported for estimation of sitagliptin individually and in combination with other drugs using spectrophotometric and RP-HPLC method. Here is an attempt made to develop an economical UFLC method for estimation of sitagliptin in bulk & tablet dosage forms [6,7].

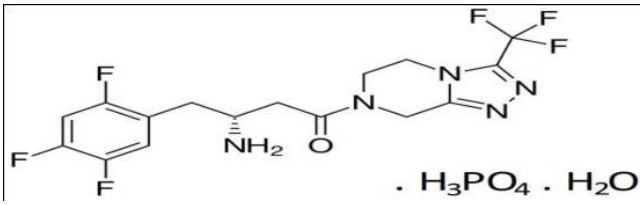


Fig. 1 - Structure of Sitagliptin Phosphate Monohydrate

MATERIAL AND METHODS

Materials and Reagents

A gift sample of Sitagliptin from Lee Pharma, Telangana used as standard drug. JANUVIA (Sitagliptin tablet 25 mg) film coated tablet dosage form bought from the local market . Other chemicals like HPLC grade Methanol, Acetonitrile, Water were bought from Research-lab Fine Chem Industries, Mumbai used as solvent for preparation of mobile phase , standard and sample solutions.

Chromatographic conditions and Instrumentation:

Shimadzu UFLC equipped with SPD -20A UV-visible detector and Shim-pack GIST C18 (250 mm × 4.6 mm i.d.,5 µm Particle size) column was used. Mobile phase Comprising of Acetonitrile and Water in a ratio 80:20 % v/v at a flow rate of 1.0ml/min and the effluent were detected by UV detectorat 267 nm. The Column temperature was maintained at ambient and the volume of injection is 20µl [8].

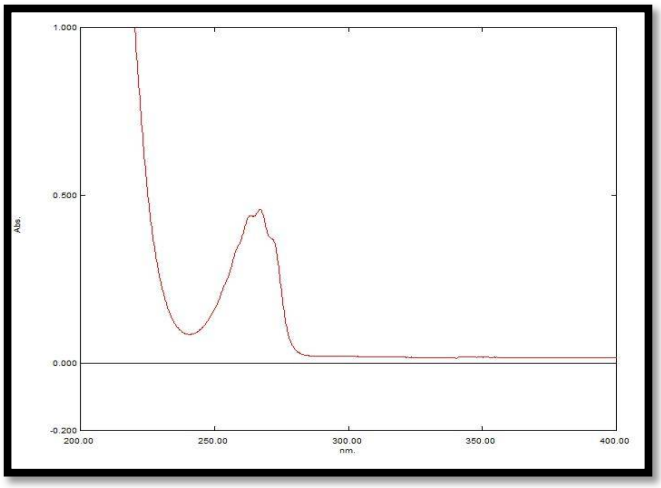
Table 1 - Chromatographic conditions for Sitagliptin

Stationary phase	Shim-pack GIST C18 (250× 4.6mm i.d.,5µm) column
Mobile Phase	Acetonitrile :Water (80:20%v/v)
Wavelength	267nm
Column temperature	Ambient
Flow rate	1.0ml/min
Injection volume	20 µL
Run time	6min
Retention time	2.9min

Selection of analytical wavelength:

Λmax of the Sitagliptin was determined by scanning the standard solution in the range of 200-400nm. Sitagliptin showed maximum absorbance at 267nm in methanol and water as solvent which is shown in fig no.2

Fig.no.2 - UV spectrum of Sitagliptin phosphate monohydrate in water (30ppm)



Preparation of Mobile Phase:

Mobile phase was prepared by mixing the Acetonitrile and Water in the ratio of 80:20 v/v and the mixture filtered using 0.45µ filter and sonicated.

Diluent Preparation :

Diluent 1: Methanol
Diluent 2: Water

Preparation of solutions :

Standard stock solution [9-10]

25 mg of Sitagliptin was accurately weighed and dissolved in 10ml Methanol (diluent1) in a 25 ml volumetric flask. Solution was sonicated for 2min for drug dissolving and finally the solution was made up to 25mL with Water (diluent 2) to obtained concentration of 1000 µg/ml.

Working Standard solution

10mL of standard stock solution was pipetted out into 100mL volumetric flask and diluted up to the mark with Water (diluent 2) and filtered through 0.45µ Millipore Nylonfilter to obtained concentration of 1g/ml. From the above 100 µg/ml solution 30ppm of final standard solution was prepared by serial dilutions.

Preparation of Sample Solution

10 tablets of Sitagliptin (JANUVIA) were weighed and average weight of tablet was calculated. The tablets were crushed into a fine powder using mortar and pestle. 25 mg of tablet powder equivalent to 25 mg of Sitagliptin phosphatemonohydrate was weighed accurately and transferred into a 25 mL clean and dry volumetric flask. Then 10 ml of Acetonitrile (diluent 1) was added, sonicated for 5 min. and then volume was made up to the mark with the Water (diluent 2) to obtained concentration of 1000 µg/ml. Further 10 mL of above sample stock solution was pipetted into a 100 mL volumetric flask and diluted up to the mark with Water (diluent 2), filtered through 0.45 µ Millipore Nylon filter. From the filtrate30µg/ml of final sample solution was prepared by serial dilution by using water (diluent 2) [10].

Analytical Method Validation :

The developed method was validated as per ICH guidelines Q-2, R-1. The typical parameters were Specificity, Linearity and Range, Accuracy, Precision, LOD, LOQ, Robustness and System suitability [9,11].

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc.

This parameter performed by injecting a volume of 20µl blank, standard and sample solutions separately and the chromatograms were recorded.

Linearity and Range

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

These parameters were studied by injecting 10,20,30,40 and 50µg/ml solutions (prepared from standard stock solution) into UFLC system. A calibration graph is plotted between concentration of Sitagliptin (µg/mL) and chromatographic peak area (mV) and observed the linear relationship between concentration and peak area in the concentration range of 10-50µg/ml.

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This parameter studied by preparing 50%, 100% and 150% concentration solutions of Sitagliptin in triplicate by spiking the solution and calculated the percentage recovery of Sitagliptin.

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision was done by carrying out analysis of standard drug solution in the linearity range and %RSD was calculated. Low RSD value indicates that the method is precise. System precision carried out to determine whether the UFLC instrument working within limit.

Limit of Detection (LOD)

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

A signal-to-noise ratio between 3 or 2:1.**LOD = 3.3 × S.D./Slope**

Limit of Quantitation (LOQ)

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

A typical signal-to-noise ratio is 10:1.**LOQ = 10× S.D./Slope**

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

System Suitability

The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analysed constitute an integral system that can be evaluated as such. System suitability test parameters to be established for a particular procedure depend on the type of procedure being validated.

RESULTS AND DISCUSSION

Assay:

20µL of Sitagliptin standard and sample solutions were injected through auto sampler into chromatographic system and peak areas were measured. The percentage of assay of tablets calculated and results were given in the table 2.

Table 2 - Assay of Sitagliptin marketed tablet

Sitagliptin	Peak Area	JANUVIA label claim	% label claim
Standard	156300		
Sample	156085	25mg	**99.86%**

Specificity :

Specificity was evaluated by observing the chromatograms of blank , standard and sample of Sitagliptin. The chromatograms of blank, standard and sample showed no peaks were interfering with a drug retention time of 2.93 min. Chromatograms of blank and 30ppm standard and sample Sitagliptin were given in fig.no. 3,4 and 5.

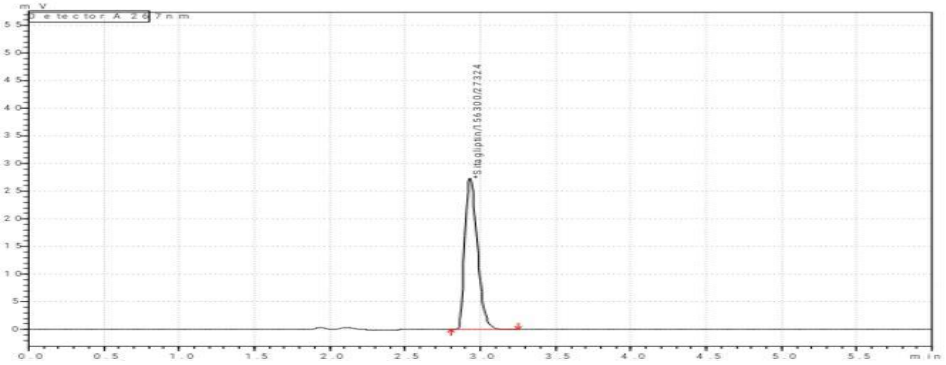


Fig.3 – Blank Chromatogram of Sitagliptin

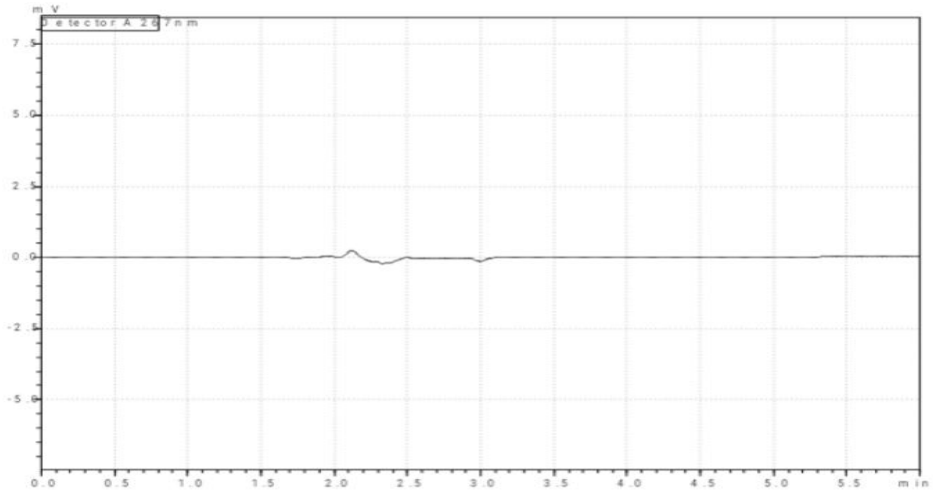


Fig. 4 – Standard Chromatogram of Sitagliptin

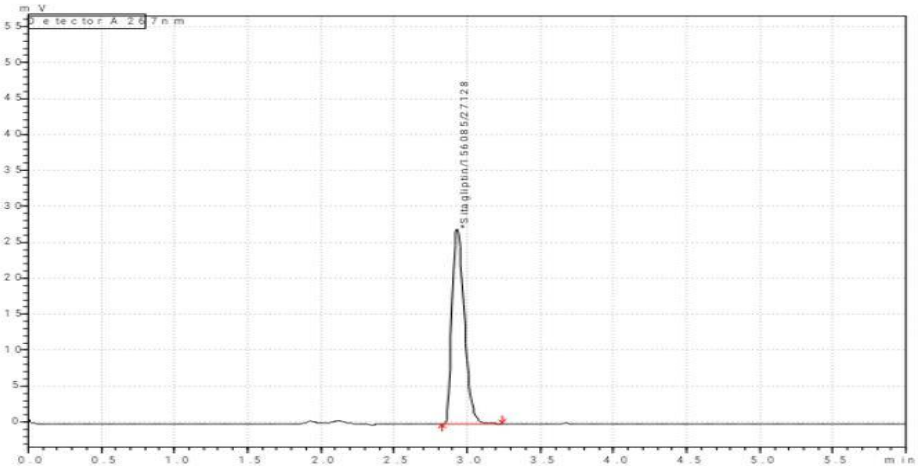


Fig. 5- Sample Chromatogram of Sitagliptin

Linearity and Range :

Linearity and range estimated by constructing the calibration curve by taking concentration on x-axis and peak area on y-axis of 10,20,30,40 and 50µg/ml solutions. From the curve y-intercept is 11304 and slope is 5733. Linearity values tabulated in table no.3 . Calibration curve and concentration vs peak area in concentration range of 10-50µg/ml shown in fig. 6,7,8,9,10 and 11 respectively.

Table 3- Linearity data for Sitagliptin

Concentration (µg/ml)	Peak Area
10	50089
20	102145
30	156547
40	213669
50	280976
Slope	**5733**
Intercept	**11304**
Correlation coefficient (R^2)	**0.9974**

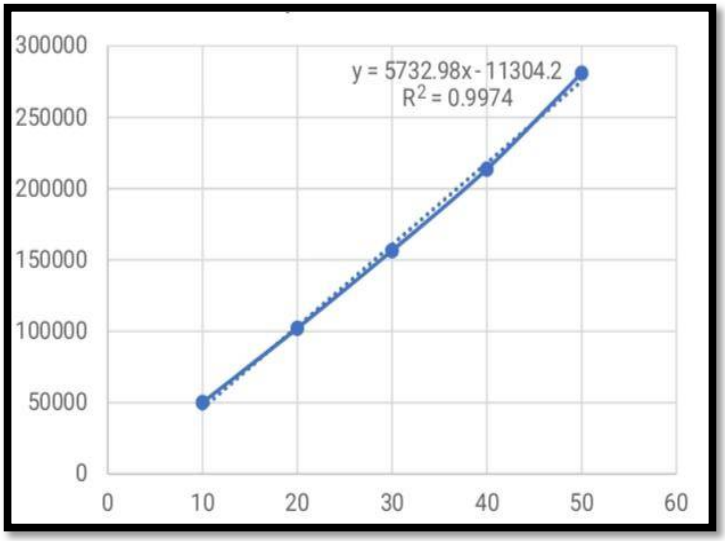


Fig. 6 – Calibration Curve of Sitagliptin

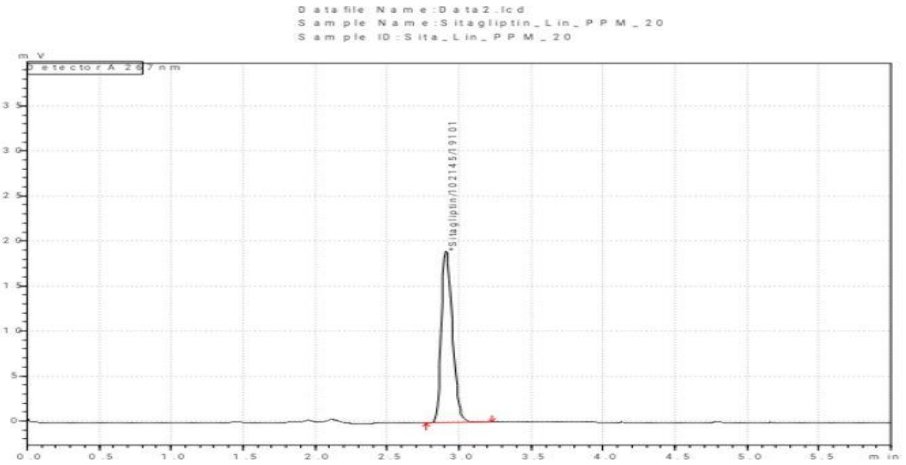


Fig. 7 – Linearity of Sitagliptin 10ppm

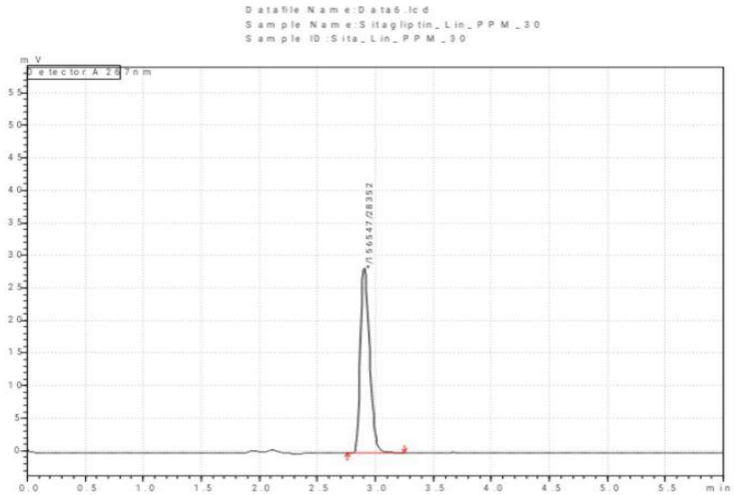


Fig. 8 – Linearity of Sitagliptin 20ppm

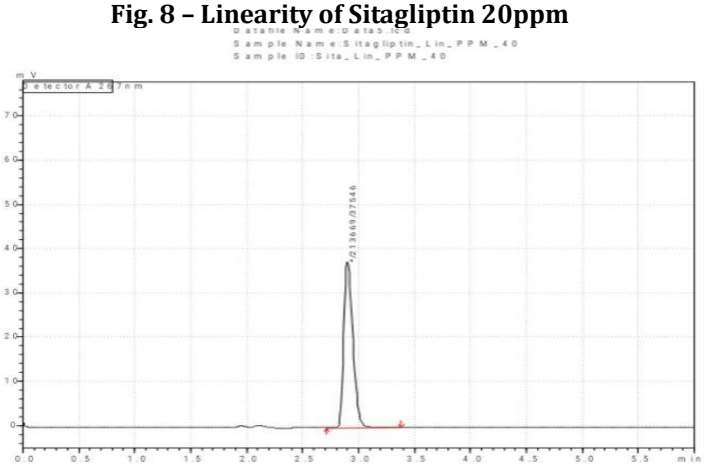


Fig. 9- Linearity of Sitagliptin 30ppm

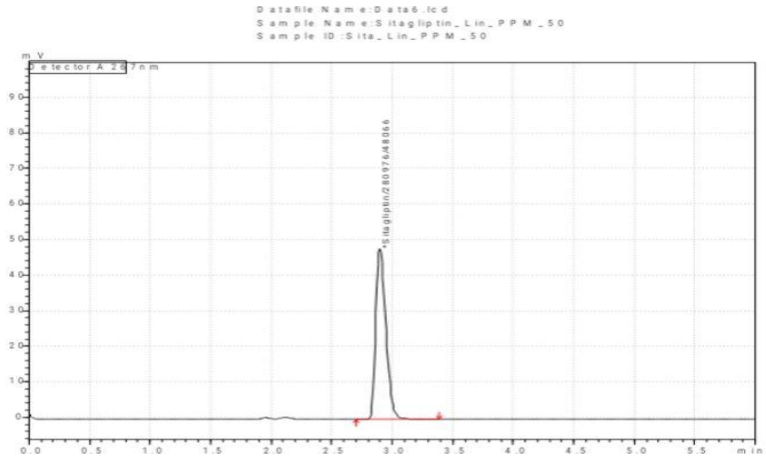


Fig. 10 – Linearity of Sitagliptin 40ppm

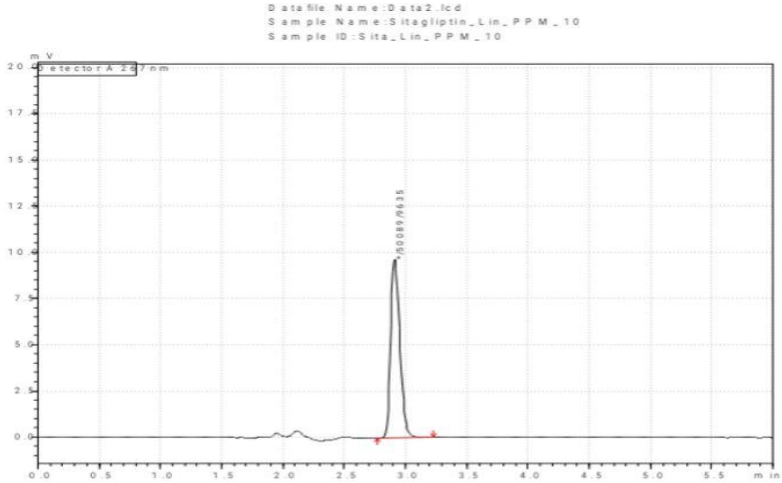


Fig.11- Linearity of Sitagliptin 50ppm

Accuracy:

Triplicate solutions of 50%,100% and 150% concentration of Sitagliptin was prepared by spiking the standard solution to sample and performed assay method. The results were depicted in the table no.4 and the %RSD was less than 2.

Table 4 –Recovery data for Sitagliptin

Spiked levels	Amount added (µg/ml)	Peak Area	Amount found (µg/ml)	%recovery	Mean +/-S.D.	%RSD
50%	15	79417	15.24	101.62	**100.50 +/- 0.9865**	**0.98**
	15	78264	15.02	100.14		
	15	77956	14.96	99.75		
100%	30	157421	30.21	100.71	**100.05 +/- 0.6871**	**0.68**
	30	155278	29.80	99.34		
	30	156494	30.03	100.12		
150%	45	236919	44.33	98.52	**99.11 +/- 0.6106**	**0.61**
	45	238265	44.58	99.08		
	45	239852	44.88	99.74		

Precision :

System Precision–Standard solution of Sitagliptin (30ppm) was injected six times and %RSD from peak areas was calculated which is shown in table no.5

Table 5 – System precision data for Sitagliptin

Concentration (µg/ml)	Peak Area	Retention time (min)
30	157394	2.932
30	156597	2.932
30	157199	2.932
30	156435	2.932
30	156106	2.931
30	156085	2.933

Avg	**156636**	**2.932**
SD	**550.9911**	**0.000632**
%RSD	**0.3517**	**0.021571**

Limit of Detection (LOD) and Limit of Quantitation (LOQ) :

The LOD and LOQ of present method were calculated based on the standard deviation of the response and slope of linearity curve. LOD and LOQ values found to be **0.3181 µg/ml** and **0.9639 µg/ml** respectively.

Robustness :

Robustness was done by changing the actual flow rate and mobile phase ratio. Results were mentioned in table no. 6 and calculated %RSD values were less than 2

Table 6- Robustness for Sitagliptin

Parameter	Variation	Peak area	%RSD
Change in flow rate (ml/min)	0.8ml/min	157035	0.29
	1.0ml/min	156300	
	1.2ml/min	156164	
Change in mobile phase	78:22%v/v	155844	0.31
	80:20%v/v	156300	
	82:18%v/v	156820	

System Suitability :

Six standard solutions of Sitagliptin were injected into chromatographic system and from the chromatograms %RSD , theoretical plates and tailing factor were calculated. Results were given in the table 7 [11].

Table 7 – System

Parameter	Result
Theoretical plates	5105
Retention time	2.93min
Tailing factor	1.388
%RSD	0.3517

Suitability data for Sitagliptin

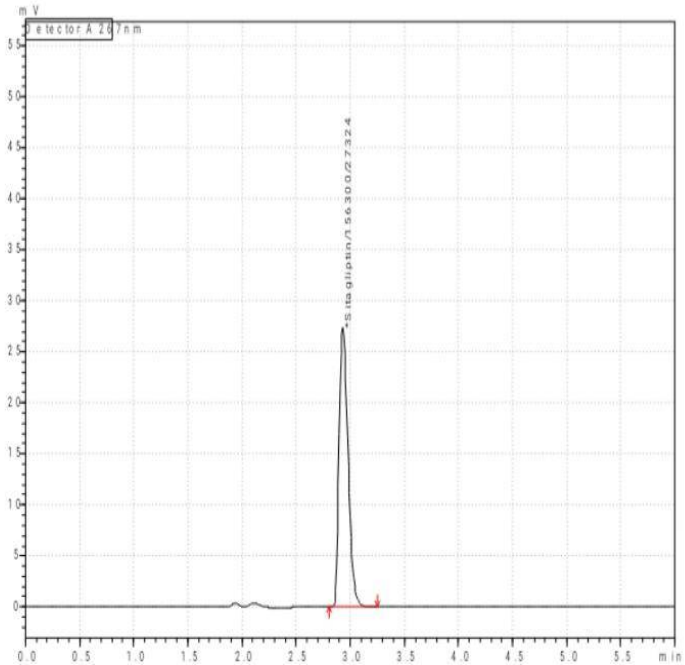


Fig.12 – Optimized chromatogram of Sitagliptin

CONCLUSION

The developed UFLC method is simple, rapid, accurate, precise, linear, reliable, useful and economical for the routine determination of Sitagliptin phosphate in bulk and its pharmaceutical dosage form. In the proposed method symmetrical peaks with good resolution were obtained and this method was validated

according to ICH guidelines. Hence, it can be applied for routine analysis quality control units of formulation.

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