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A VALIDATED RP-HPLC METHOD FOR THE ESTIMATION OF SITAGLIPTIN IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

A new sensitive, specific, linear, precise and accurate RP-HPLC method was developed and validated for estimation of Sitagliptin in Bulk and Tablet dosage form. An isocratic, reversed phase HPLC method was developed. Shimadzu shim pack C18 (250mm x 4.5 μ m, x 5 μ) column. Shimadzu Prominence-I LC-2030C plus equipped with Auto sampler as the instrument model. Mobile phase consists of mixture of Methanol: 0.01M Phosphate buffer pH 2.5 in the ratio (60:40 v/v) at a flow rate of 1.0mL /min with injection volume of 10 μ L. UV detection was performed at 270nm. The Linearity was established for Sitagliptin in the range of 10-60 μ g/ml with correlation coefficient of 0.9998. LOD and LOQ were found to be 0.197 μ g/ml and 0.602 μ g/ml respectively. Retention time of Sitagliptin were found to be 3.837mins. % Recovery was found to be 99.75-101.10 and %RSD was found with in \pm 2. The method has been validated according to ICH guidelines for linearity, precision, accuracy, robustness, ruggedness, LOD and LOQ. The developed validated method was successfully applied for reliable quantification of Sitagliptin in bulk and pharmaceutical dosage form.

KEYWORDS

Sitagliptin, RP- HPLC, Validation and Pharmaceutical formulations.

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INTRODUCTION

Sitagliptin is a anti-diabetic medication used to treat type 2 diabetes. Sitagliptin is a dipeptidyl peptidase-4 inhibitor which is used in the combination with diet and exercise, either alone or in the combination with other oral hypoglycemic agents¹.

Literature survey revealed that there were few analytical methods have been reported for the determination of the Sitagliptin in pure drug and pharmaceutical dosage form by using UV-

Spectrophotometric²⁻⁸, RP-HPLC⁹⁻²⁰ and HPTLC²¹ so far.

The aim of the present work is to develop and validate a novel, rapid, precise and specific Area under curve UV spectrophotometric method for estimation of Sitagliptin in bulk and tablet dosage form.

MATERIAL AND METHODS

Material and reagents

The Sitagliptin was obtained as a gift sample from the pharmaceutical industry and Januvia tablet obtained from Pharmacy store. Phosphate buffer Methanol and distilled water were obtained Bharathi College of pharmacy, Bharathinagara, KM Doddi, Maddur Taluk, Mandya District, India. All chemicals used are of HPLC grade. Distilled water was used throughout the experiment.

Instrumentation

Chromatographic separation was performed on a Shimadzu Prominence-i LC-2030 plus equipped with Auto sampler comprising a variable wavelength programmable UV detector. Shimadzu shim pack C18 (250mm x 4.5 μ m x 5 μ) column is used.

Preparation of solutions Mobile phase preparation

The Mobile phase consisted of a mixture of 0.01M Phosphate buffer Ph 2.5(40%) Methanol (60%), in the ratio of 40:60v/v, which was filtered through a membrane and degassed before use. Ph adjusted with ortho phosphoric acid in Millipore water.

Preparation of sample Standard Solution

The formulation tablets of Sitagliptin (Januvia 100mg) were crushed to give finely powdered material. From the Powder prepared a 100mg of Sitagliptin was accurately weighed, transferred in a 100ml volumetric flask, add 30ml of diluents and sonicate to dissolve and dilute to volume with diluent. Transfer 10ml of standard stock solution into 100ml volumetric flask and dilute to volume with diluent. And an appropriate concentration of sample was prepared at the time of analysis. 10 μ l of these solutions were injected in triplicate into HPLC system and the peak areas were recorded.

Preparation of Standard solution

10mg of Sitagliptin was dissolved in 10ml of methanol in 10ml volumetric flask (1000 μ g/ml). Further dilution was made from above in such a way that the final concentration consist of 10, 20, 30, 40, 50, and 60 μ g/ml.

System suitability requirements from stock and standard solutions

Tailing factor: NMT 2.0

Theoretical Plates: NLT 2000

RESULTS AND DISCUSSION

Validation of the proposed method

The proposed method was validated as per ICH guidelines²²⁻²⁴. The parameters studied for validation were specificity, linearity, precision, accuracy, robustness, system suitability, limit of detection, limit of quantification, and solution stability.

Specificity

From the chromatograms of blank, standard (Prepared from Formulation). It was found that there is no interference due to excipients in the tablet formulation and also found good correlation between the retention time. The specificity results are shown in Table No.2.

Linearity

The linearity of the response of the drug was verified at six concentration levels, ranging from 10-60 μ g/ml of Sitagliptin in each linearity level were prepared. 10 μ l of each concentration was injected into the HPLC system. The response was read at 270nm and the corresponding chromatograms were recorded. From these chromatograms, the mean peak areas were presented in Table No.3.

Precision

Precision of the method was performed as intraday precision, Inter day precision. To study the intraday precision, six replicate standard solutions (20 μ g/ml) of Sitagliptin were injected. % RSD was calculated and it was found to be 0.516 and interday precision done same as intraday, six replicate standard solutions (20 μ g/ml) of Sitagliptin were injected. %RSD was calculated and it was found to be 0.797 which are well within the acceptable criteria of not more than 2.0. Results of system precision studies are shown in Table No.4.

Accuracy

Accuracy of the method was studied by recovery experiments. The recovery experiments were performed by adding known amounts of the drugs in the placebo. The recovery was performed at three levels, 50, 100 and 150% of the label claim of the tablet (100mg of Sitagliptin). The recovery values for Sitagliptin ranged from 98.0 to 102.0%. The average recoveries of three levels of Sitagliptin were found to be 99.75-101.10%. The results are shown in the Table No.5.

Limit of detection and Limit of quantification

The limit of detection is an analytical method is the smallest amount of analyte in a sample which can be reliable detected by the analytical method. The limit of quantitation is an individual analytical procedure is the smallest amount of the analyte in sample which can be quantitatively determined. LOD and LOQ were calculated using formula $LOD = 3.3(SD)/S$ and $LOQ = 10(SD)/S$. Results were shown in Table No.6.

Ruggedness

The ruggedness of test method was demonstrated by carrying out precision study in six preparations of sample on a single batch sample by different analysts, the results of the precision study are tabulated as below Table No.7. The % RSD values are less than 2.

Robustness

Robustness is the measure of the capacity of the analytical method to remain unaffected by small but deliberate variation in the procedure. The robustness of the method was evaluated by analysing the system suitability standard and evaluating system suitability parameter data after varying, individually, the HPLC pump flow rate ($\pm 0.2\text{ml/min}$), column temperature ($\pm 5\text{C}$) and detection wavelength ($\pm 2\text{nm}$) shown in Table No.8.

Acceptance criteria

System suitability should pass as per test method at variable conditions.

CHROMATOGRAPHIC CONDITIONS

Table No.1: HPLC method development parameters

S.No	HPLC method development parameters	
1	Column	C18, 250nm X 4.5µm, 5µ
2	Flow rate	1.0ml /min
3	Wavelength	270nm
4	Column temperature	Ambient
5	Injection volume	10µL
6	Run time	8minutes
7	Diluents	Mobile phase
8	Elution	Isocratic

Table No.2: Specificity of Sitagliptin

S.No	Name of the solution	Retention time in min
1	Blank	0
2	Sitagliptin (Standard)	3.837

Table No.3: Linearity of sitagliptin

S.No	Concentration (µg/ml)	Peak area* (mv)
1	10	50435
2	20	127436
3	30	196403
4	40	265917
5	50	339867
6	60	409563

*Average of six determinations

Table No.4: Results of precision of sitagliptin

S.No	Intraday Names	Studies Peak area	Interday Names	Studies Peak area
1	Injection-1	127493	Injection-1	127483
2	Injection-2	126464	Injection-2	126490
3	Injection-3	127159	Injection-3	127318
4	Injection-4	127345	Injection-4	127106
5	Injection-5	128501	Injection-5	125783
6	Injection-6	127497	Injection-6	128793
7	AVG	127409.833	AVG	127162.166
8	STDEV	658.3131	STDEV	1014.2878
9	%RSD	0.516	%RSD	0.797

Table No.5: Results of recovery of sitagliptin

S.No	Level of addition/ %	Amount added (µg/ml)	Amount found	%Recovery ±Standard deviation*	%RSD
1	50	20	58.09	99.75±0.717	0.718
			59.90		
			60.34		
2	100	40	81.01	101.10±0.702	0.694
			80.21		
			81.92		
3	150	60	100.7	100.20±0.69	0.688
			99.22		
			100.7		

*Average of three determinations.

Table No.6: System suitability parameters

S.No	Parameters	Sitagliptin
1	Linearity	10-60µg/ml
2	Regression equation	y = 7149.8x-18641
3	Correlation coefficient	R ² = 0.9998
4	Retention time	3.837min
5	Run time	8min
6	Limit of detection(LOD)	0.197µg/ml

Table No.7: Results of ruggedness of sitagliptin

By changing the Analysts

S.No	Concentration	T1	T2	Mean	SD	%RSD
1	10	50341	50856	50598.5	364.159	0.791
2	20	126349	127481	126915	800.444	0.630
3	30	197302	199018	198160	1213.39	0.612
4	40	267873	267105	267489	543.058	0.203
5	50	329894	334097	331995.5	2971.969	0.895
6	60	408675	411095	409885	1711.198	0.417

* Average of three determinations.

By changing the instrument

S.No	Concentration	T1	T2	Mean	SD	%RSD
1	10	50341	50913	50627	404.465	0.79
2	20	126958	129813	128385.5	2018.789	1.57
3	30	191058	188991	190024.5	1461.589	0.76
4	40	263413	257844	260628.5	3937.877	1.51
5	50	329076	330738	329907	1175.211	0.35
6	60	409742	419304	414523	6761.355	1.63

* Average of three determinations.

Table No.8: Robustness results for sitagliptin

S.No	Parameters	Conditions	Tailing Factor	% RSD
1	Column Temperature	Decreased (-5°C)	1.23	0.4
		Increased (+ 5°C)	1.078	
2	Flow rate (ml/min)	Decreased (-2min/min)	1.09	0.63
		Increased (+2 min/min)	1.17	
3	Wavelength	Decreased(-2nm)	1.077	1.18
		Decreased(+2nm)	1.08	

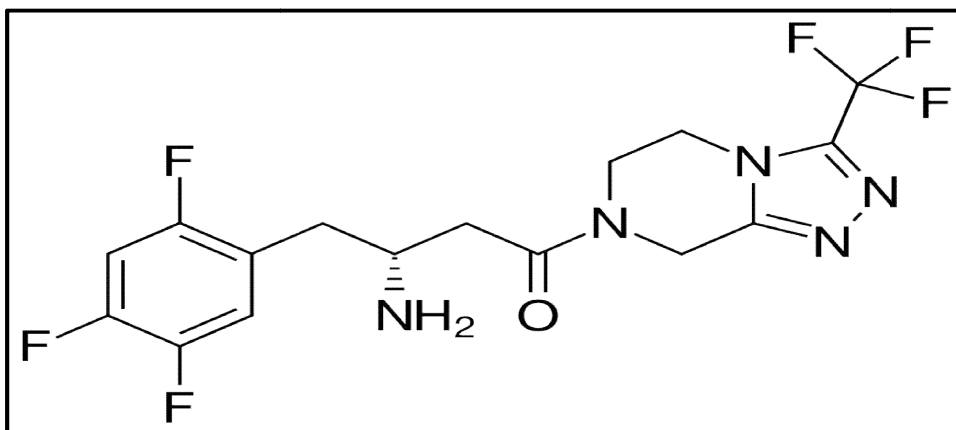


Figure No.1: Chemical structure of Sitagliptin

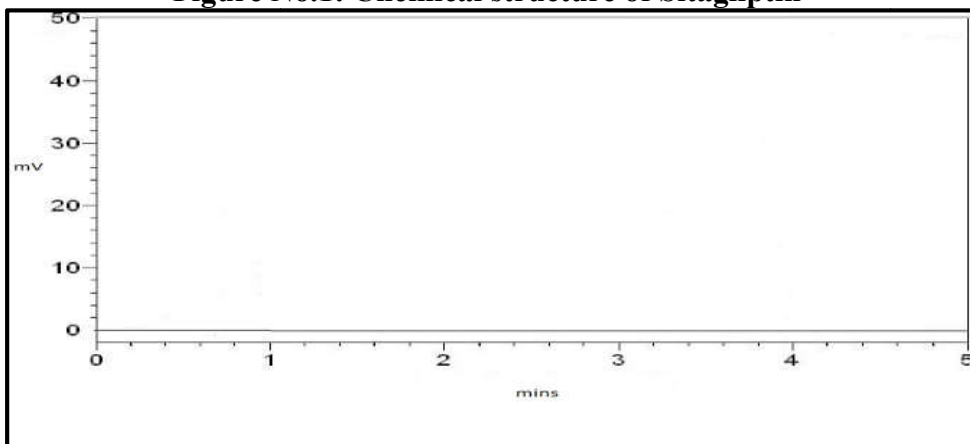


Figure No.2: Chromatogram of blank solution of sitagliptin

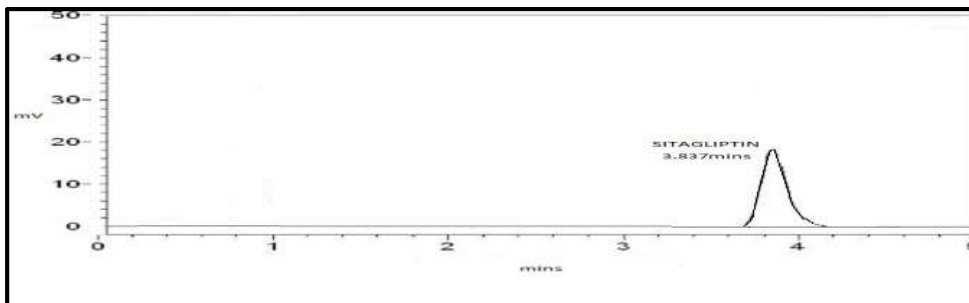


Figure No.3: Chromatogram of standard solution of sitagliptin

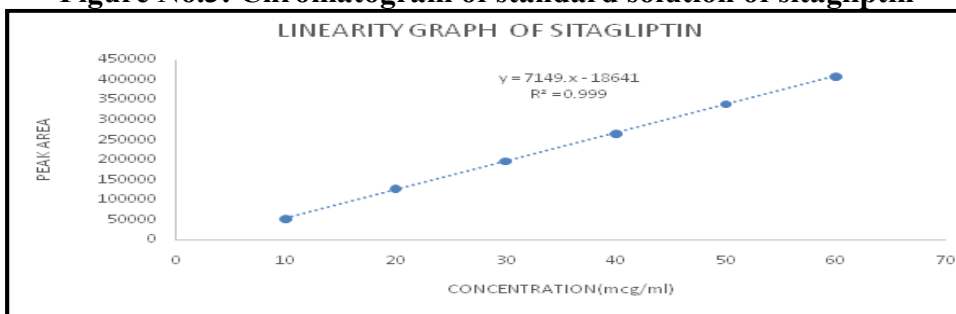


Figure No.4: Linearity of Sitagliptin

CONCLUSION

The present analytical method was validated as per ICH guidelines and met the acceptance criteria. It was concluded that the developed analytical method was simple, accurate, economical and sensitive, and can be used for routine analysis of Sitagliptin bulk drug and pharmaceutical dosage forms.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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