Advances in Bioresearch
Adv. Biores., Vol 15 (6) November 2024: 353-363
©2024 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.15.6.353363

Advances in Bioresearch

ORIGINAL ARTICLE

Method Development and Validation for the Quantitation of Sitagliptin by RP-HPLC Dosage Forms

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ABSTRACT

A simple and selective LC method is described for the determination of Sitagliptin dosage forms. Chromatographic separation was achieved on a c_{18} column using mobile phase consisting of a mixture of Triethyl amine: ACN (60:40v/v/v), with detection of 295 nm. Linearity was observed in the range 75-150 µg /ml for Sitagliptin (r^2 =0.999) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim. The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.

KEYWORDS: Sitagliptin, Method development, Validation, RP-HPLC.

Received 09.10.2024 Revised 26.11.2024 Accepted 29.11.2024

How to cite this article:
Balaga K, Dasireddy S, Duvvada J, Sudheer Kumar D Nagoji K E V. Method Development and Validation for the Quantitation of Sitagliptin by RP-HPLC Dosage Forms. Adv. Biores. Vol 15 [6] November 2024. 353363

INTRODUCTION

Sitagliptin sold under the brand name Januvia among others, is an anti-diabetic medication used to treat type 2 diabetes. In the United Kingdom it is listed as less preferred than metformin or a sulfonylurea. It is taken by mouth.[7] It is also available in the fixed-dose combination medication sitagliptin/metformin (Janumet, Janumet XR) [1].

Common side effects include headaches, swelling of the legs, and upper respiratory tract infections [7]. Serious side effects may include angioedema, low blood sugar, kidney problems, pancreatitis, and joint pain. Whether use in pregnancy or breastfeeding is safe is unclear.[9] It is in the dipeptidyl peptidase-4 (DPP-4) inhibitor class and works by increasing the production of insulin and decreasing the production of glucagon by the pancreas[2,3].

Sitagliptin was developed by Merck & Co. and approved for medical use in the United States in 2006. In 2021, it was the 83rd most commonly prescribed medication in the United States, with more than 8 million prescriptions. It is available as a generic medication in Canada but not the United States [4].

Sitagliptin works to competitively inhibit the enzyme dipeptidyl peptidase 4 (DPP-4). This enzyme breaks down the incretins GLP-1 and GIP, gastrointestinal hormones released in response to a meal. By preventing breakdown of GLP-1 and GIP, they are able to increase the secretion of insulin and suppress the release of glucagon by the alpha cells of the pancreas. This drives blood glucose levels towards normal. As the blood glucose level approaches normal, the amounts of insulin released and glucagon suppressed diminishes, thus tending to prevent an "overshoot" and subsequent low blood sugar (hypoglycemia), which is seen with some other oral hypoglycemic agents [5].

Sitagliptin has been shown to lower HbA1c level by about 0.7% points versus placebo. It is slightly less effective than metformin when used as a monotherapy. It does not cause weight gain and has less

hypoglycemia compared to sulfonylureas. Sitagliptin is recommended as a second-line drug (in combination with other drugs) after the combination of diet/exercise and metformin fails [6].

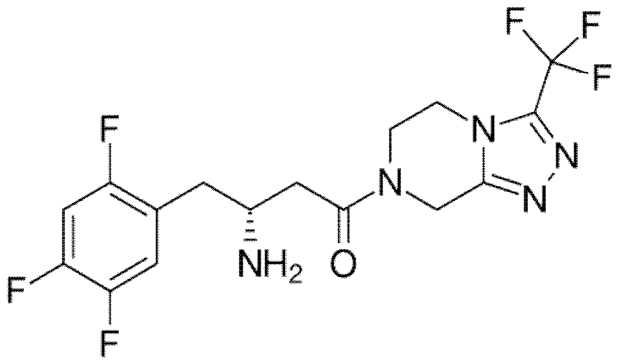

Fig. 1: Structure of Sitagliptin

MATERIAL AND METHODS

Chemicals and reagents

HPLC grade methanol, acetonitrile, water, triethyl amine and analytical grade sodium acetate, Potassium Dihydrogen ortho Phosphate, Ammonium acetate, Tetra Hydro Furan, Dipotassium hydrogen phosphate were purchased from Merck (Mumbai, India). Sitagliptin API gift Samples obtained from Hetero labs, Hyd. Sitagliptin dosage form was Obtained from local pharmacy.

Instruments

The list of instruments used in the research were given in Table 1.

Table 1: Instruments used

UV-Visible Spectrophotometer	Nicolet evolution 100
HPLC	Shimadzu(LC 20 AT VP)
HPLC	Agilent 1200 series
Ultra sonicator	Citizen, Digital Ultrasonic Cleaner
pH meter	Global digital
Electronic balance	Shimadzu
Syringe	Hamilton
HPLC Column	INERTSIL column,C18(150x4.6 ID) 5µm

Mobile Phase

A mixture of 60 volumes of Triethylamine Buffer pH5.1:40 volumes of Acetonitrile were prepared. The mobile phase was sonicated for 10min to remove gases.

Preparation of standard stock solution of Sitagliptin

25mg of Sitagliptin was weighed and transferred in to 100ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 10 µg /ml of solution by diluting 0.4ml to 10ml with methanol.

Wavelength of maximum

The wavelength of maximum absorption (λ_{max}) of the drug, 10 µg/ml solution of the drugs in methanol were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against methanol as blank. The resulting spectra are shown in the fig. no. 8.1 and the absorption curve shows characteristic absorption maxima at 295 nm for Sitagliptin. λ_{max} was found to be 295 nm for Sitagliptin.

Table 2: Optimized chromatographic conditions

Mobile phase	Triethyl amine: ACN (60:40)
pH	5.1
Column	INERTSIL column,C18(150x4.6 ID) 5µm
Flow rate	1.0 ml/min
Column temperature	Room temperature(20-25◦C)
Sample temperature	Room temperature(20-25◦C)
Wavelength	295nm
Injection volume	20 µl
Run time	6min
Retention time	About 3.317min for Sitagliptin

Assay

Preparation of samples for Assay

Preparation of mixed standard solution

Weigh accurately 25mg of Sitagliptin in 25ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 100µg/ml of Sitagliptin is prepared by diluting 1ml of Sitagliptin to 10ml with mobile phase. This solution is used for recording chromatogram.

Preparation of sample solution

5 tablets (each tablet contains 400mg of Sitagliptin) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of 100µg/ml were prepared by dissolving weight equivalent to 100mg of Sitagliptin dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 100ml with mobile phase. Further dilutions are prepared in 5 replicates of 100µg/ml of Sitagliptin was made by adding 1ml of stock solution to 10 ml of mobile phase.

Calculation

The amount of Sitagliptin present in the formulation by using the formula given below, and results shown in above table:

$$\%\,Assay = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{AW}{LC} \times 100$$

Where, AS: Average peak area due to standard preparation, AT: Peak area due to assay preparation, WS: Weight of Sitagliptin in mg, WT: Weight of sample in assay preparation, DT: Dilution of assay preparation.

RESULTS AND DISCUSSION

Method development of sitagliptin

Trial - 1

Chromatographic conditions

Mobile phase : Water: Methanol
Ratio : 50:50
Column : BDS (295×4.6× 5µ)
Wavelength : 295nm
Flow rate : 1ml/min
pH : 3.0

Preparation of standard solution

Weigh accurately 25mg of Sitagliptin in 25ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 295µg/ml of Sitagliptin is prepared by diluting 2.5ml of Sitagliptin to 10ml with mobile phase. This solution is used for recording chromatogram.

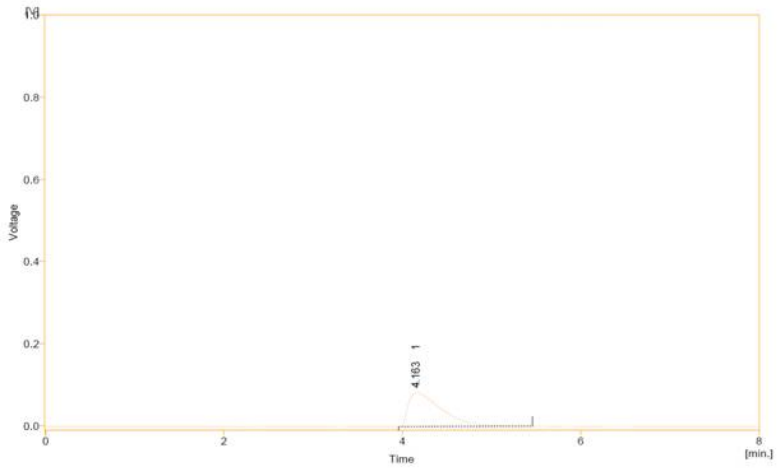

Fig. 2: Chromatogram of Sitagliptin

Observation:
Shape of the peak was not good and efficiency was not satisfactory.

Trial- 2

Chromatographic conditions

Mobile phase : Orthophosporic acid: Methanol
Ratio : 40:60
Column : Zodiac C18Column (295×4.6 ×5µ)
Wavelength : 295nm
Flow rate : 1.2 ml/min
pH : 2.4

Preparation of mixed standard solution

Weigh accurately 25mg of Sitagliptin in 25ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 295µg/ml of Sitagliptin is prepared by diluting 2.5ml of Sitagliptin to 10ml with mobile phase. This solution is used for recording chromatogram.

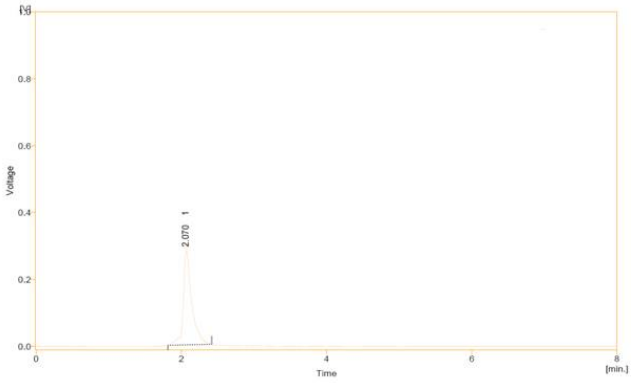

Fig. 3: Chromatogram of Sitagliptin

Observation:
Fronting and tailing of the peak was occurred.

Trial- 3:

Chromatographic conditions

Mobile phase : Phosphate buffer:ACN: Methanol
Ratio : 40:20:40
Column : Zodiac, C18 (295×4.6× 5µ)
Wavelength : 295nm
Flow rate : 1ml/min
pH :6.5

Preparation of mixed standard solution

Weigh accurately 25mg of Sitagliptin in 25ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 295µg/ml of Sitagliptin is prepared by diluting 2.5ml of Sitagliptin to 10ml with mobile phase. This solution is used for recording chromatogram.

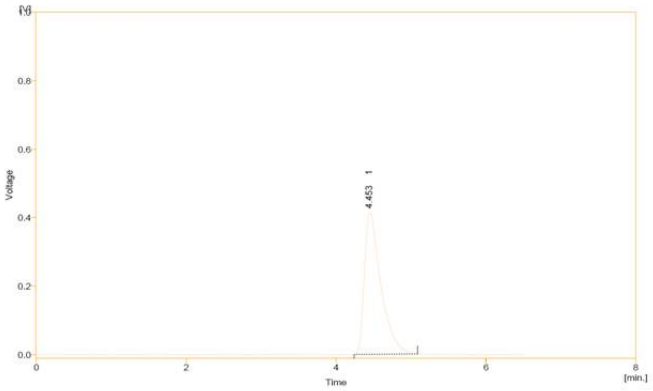

Fig. 4: Chromatogram of Sitagliptin

Observation:

Tailing of the peak was appears.

Trial- 4:

Chromatographic conditions

Mobile phase : Mixed phosphate buffer: Methanol
Ratio : 70:30
Column : Zodiac, C18 (295×4.6× 5µ)
Wavelength : 295nm
Flow rate : 1ml/min
pH :4.0

Preparation of mixed standard solution

Weigh accurately 25mg of Sitagliptin in 25ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 295µg/ml of Sitagliptin is prepared by diluting 2.5ml of Sitagliptin to 10ml with mobile phase. This solution is used for recording chromatogram.

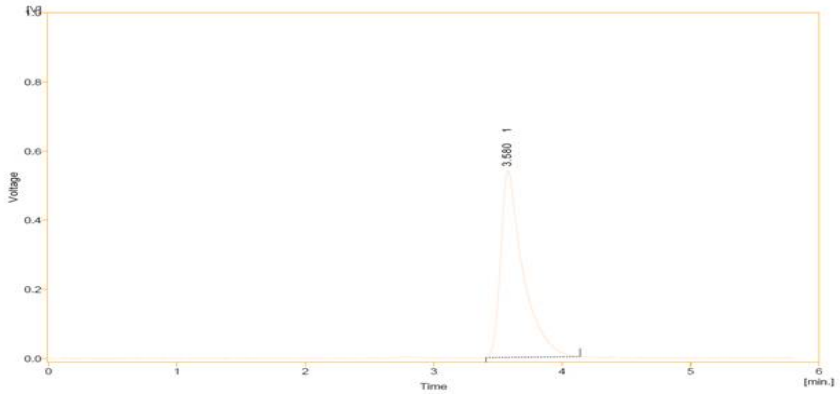

Fig. 5: Chromatogram of Sitagliptin

Observation:

Tailing of the peak was appears.

Trial - 5:

Chromatographic conditions

Mobile phase : Triethylamine: Acetonitrile
Ratio : 60:40
Column : Zodiac, C18 (295×4.6× 5µ)
Wavelength : 295nm
Flow rate : 1ml/min
pH : 5.1

Preparation of mixed standard solution

Weigh accurately 25mg of Sitagliptin in 25ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 295µg/ml of Sitagliptin is prepared by diluting 2.5ml of Sitagliptin to 10ml with mobile phase. This solution is used for recording chromatogram.

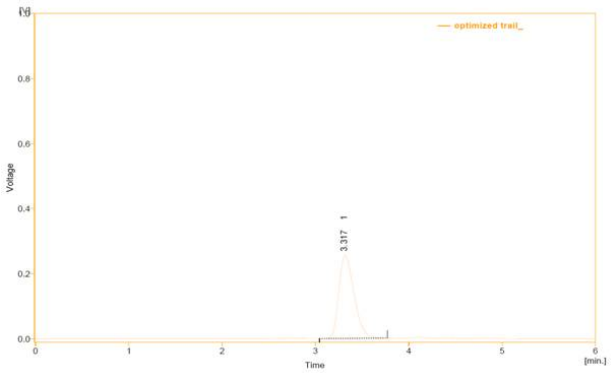

Fig. 6: Chromatogram of Sitagliptin

Observation:

Efficiency of the peaks was more. Hence this method was optimized.

Table 3: Optimized chromatographic conditions

Mobile phase	Triethyl amine:ACN (60:40)
pH	5.1
Column	INERTSIL column,C18(150x4.6 ID) 5µm
Flow rate	1.0 ml/min
Column temperature	Room temperature(20-25°C)
Sample temperature	Room temperature(20-25°C)
Wavelength	295nm
Injection volume	20 µl
Run time	6min
Retention time	About 3.317min for Sitagliptin

Assay method

Table 4: Assay results.

Sitagliptin		
	Standard Area	**Sample Area**
Injection-1	2929.483	2915.223
Injection-2	2925.543	2928.592
Injection-3	2946.561	2945.457
Injection-4	2925.890	2923.218
Injection-5	2900.370	2915.166
Average Area	2933.862	2925.531
Tablet average weight	399.80mg	
Standard weight	25mg	
Sample weight	25.12mg	
Label amount	400 mg	
Std. purity	99.8%	
Amount found in mg	248.79	
Assay(%purity)	99.52%	

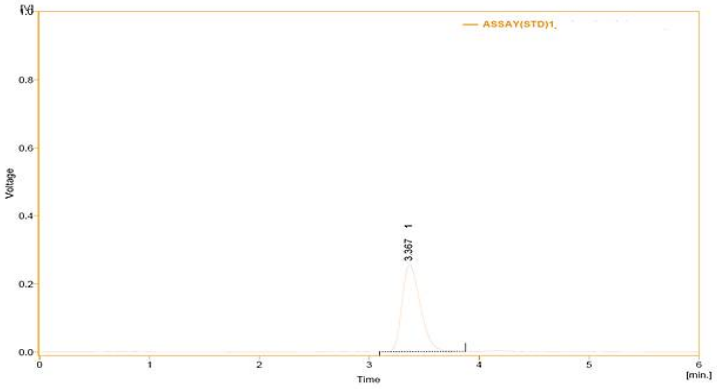

Fig. 7: Chromatogram of Assay standard preparation.

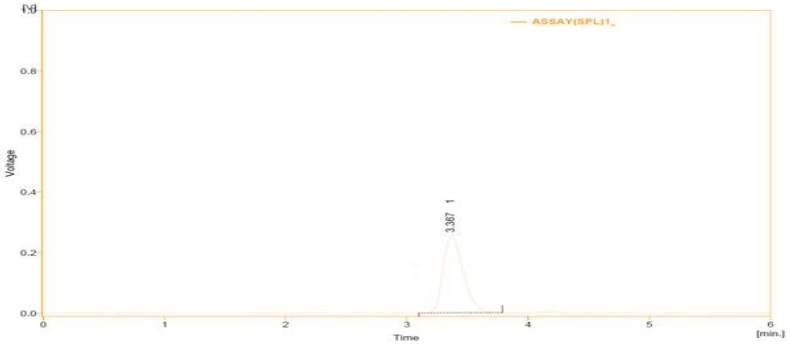

Fig.8: Chromatogram of Assay sample preparation.

Observation

The amount of Sitagliptin present in the taken dosage form was found to be 99.52%.

Validation

Specificity by direct comparison method

There is no interference of mobile phase, solvent and placebo with the analyte peak and also the peak purity of analyte peak which indicate that the method is specific for the analysis of analytes in their dosage form [7-13].

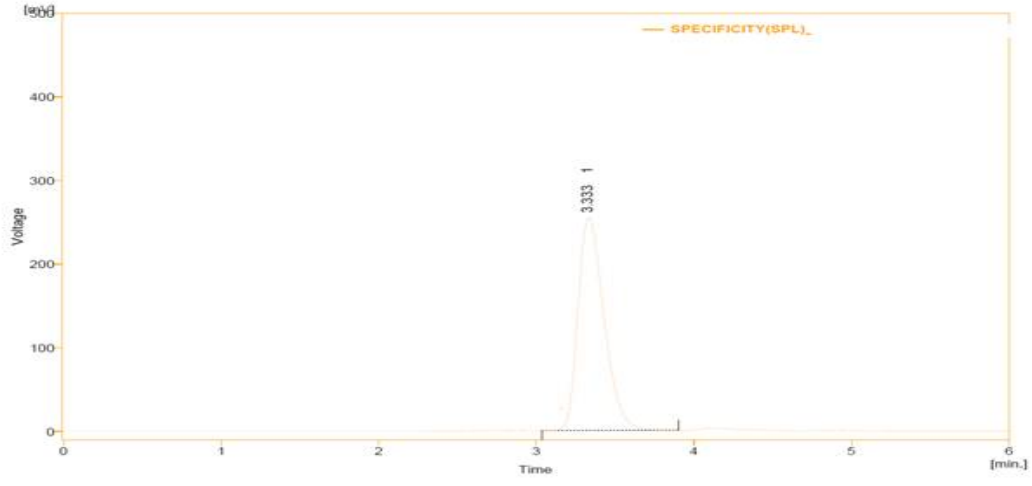

Fig.9: Chromatogram for specificity of Sitagliptin sample

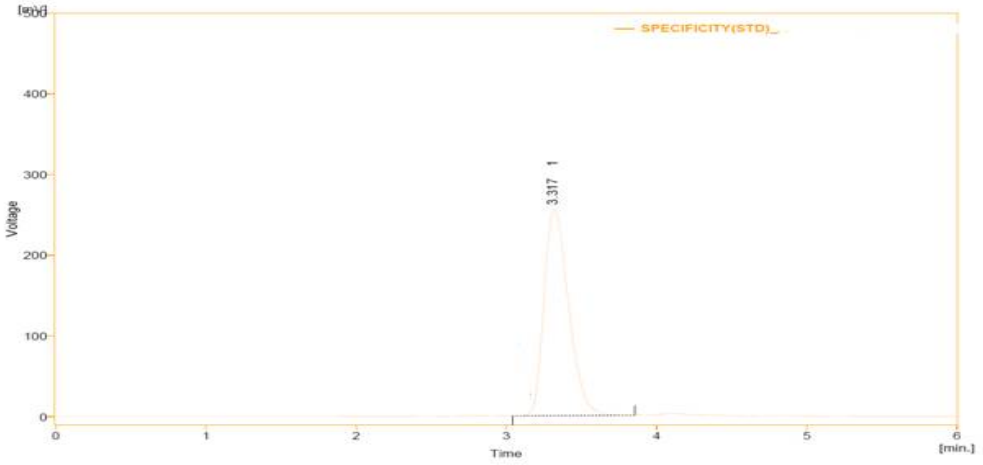

Fig.10: Chromatogram for Specificity of Sitagliptin standard.

It is observed from the above data, diluents or excipient peaks are not interfering with the Sitagliptin peaks.

Linearity and range

Preparation of mixed standard solution

Weigh accurately 25mg of Sitagliptin in 100 ml of volumetric flask and from this, 5ml dissolve in 10ml of mobile phase and make up the volume with mobile phase[14-16].

Table 5: Linearity Preparations.

Preparations	Volume from standard stock transferred in ml	Volume made up in ml (with mobile phase)	Concentration of solution(µg /ml)
Preparation 1	2	10	50
Preparation 2	3	10	75
Preparation 3	4	10	100
Preparation 4	5	10	125
Preparation 5	6	10	150

Table 6: linearity of Sitagliptin

S.No.	Conc.(µg/ml)	Area
1	50	1638.768
2	75	2197.919
3	100	2882.593
4	125	3550.790
5	150	4155.542

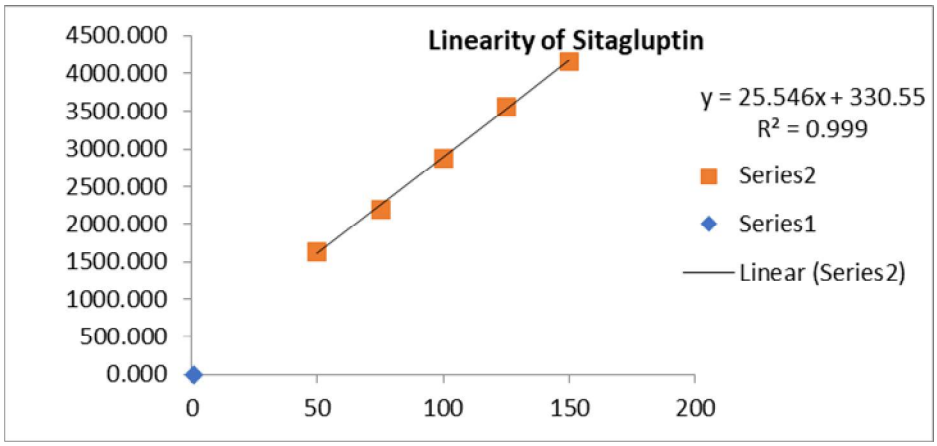

Fig.11: Linearity of Sitagluptin.

Observation

The correlation coefficient for linear curve obtained between concentrations vs. Area for standard preparations of Sitagliptin is 0.999. The relationship between the concentration of Sitagliptin and area of

Sitagliptin is linear in the range examined since all points lie in a straight line and the correlation coefficient is well within limits.

Accuracy

Accuracy of the method was determined by Recovery studies. To the formulation (pre analyzed sample), the reference standards of the drugs were added at the level of 50%, 100%, 150%[7,13]. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug is shown in table. To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 50%, 100% & 150%.

Table 7: **Recovery results for Sitagliptin**

Recovery level	Accuracy Sitagliptin					Average % Recovery
	Amount taken (mcg/ml)	Area	Average area	Amount recovered (mcg/ml)	%Recovery	
75%	75	2069.772	2060.856	74.69	99.58	99.89
	75	2054.232				
	75	2058.565				
100%	100	2882.593	2871.680	99.10	99.10	
	100	2875.659				
	100	2856.789				
125%	125	3535.008	3502.665	126.26	101.01	
	125	3421.987				
	125	3551.001				

Observation

The percentage mean recovery of Sitagliptin is 99.89%.

Precision

Method precision

Prepared sample preparations of Sitagliptin as per test method and injected 5 times in to the column.

Table 8: Results for Method precision of Sitagliptin.

Sitagliptin		
S.No.	Rt	Area
1	3.367	2912.410
2	3.367	2932.566
3	3.533	2946.873
4	3.333	2920.975
5	3.777	2911.577
Avg	3.3474	2924.978
Stdev	0.0220	14.819
%RSD	**0.66**	**0.51**

Observation

Test results for Sitagliptin are showing that the %RSD of Assay results are within limits. The results were shown in table 8.5.7.

Robustness

Chromatographic conditions variation

To demonstrate the robustness of the method, prepared solution as per test method and injected at different variable conditions like using different conditions like Temperature and wavelength. System suitability parameters were compared with that of method precision [17].

Table9: Result of Robustness study

Parameter	Sitagliptin	
	Retention time(min)	Tailing factor
Flow		
0.8ml/min	4.187	1.717
1.0 ml/min	3.367	1.632
1.2ml/min	2.830	1.656
Wavelength		
293nm	3.353	1.605
295nm	3.367	1.632
297nm	3.353	1.605

Observation

From the observation it was found that the system suitability parameters were within limit at all variable conditions.

Ruggedness

The ruggedness of the method was studied by the determining the analyst to analyst variation by performing the Assay by two different analysts

Acceptance criteria:

The % Relative standard deviation of Assay values between two analysts should be not more than 2.0%.

Table10: Results for Ruggedness.

Sitagliptin	%Assay
Analyst 01	99.67
Analyst 02	98.34
%RSD	0.94%

Observation

From the observation the %RSD between two analysts Assay values not greater than 2.0%, hence the method was rugged.

CONCLUSION

A simple and selective LC method is described for the determination of Sitagliptin dosage forms. Chromatographic separation was achieved on a c_{18} column using mobile phase consisting of a mixture of Triethyl amine: ACN (60:40v/v/v), with detection of 295 nm. Linearity was observed in the range 75-150 µg /ml for Sitagliptin (r^2 =0.999) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim. The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.

REFERENCES

1. Herman GA, Stevens C, van Dyck K, Bergman A, Yi B, De Smet M, et al. (2005). "Pharmacokinetics and pharmacodynamics of sitagliptin, an inhibitor of dipeptidyl peptidase IV, in healthy subjects: results from two randomized, double-blind, placebo-controlled studies with single oral doses". Clinical Pharmacology and Therapeutics. 78 (6): 675–688.
2. Zhao Q, Hong D, Zheng D, Xiao Y, Wu B (2014). "Risk of diarrhea in patients with type 2 diabetes mellitus treated with sitagliptin: a meta-analysis of 30 randomized clinical trials". Drug Design, Development and Therapy. 8: 2283–2294.
3. Stricklin SM, Stoecker WV, Rader RK, Hood AF, Litt JZ, Schuman TP (2012). "Persistent edematous-plaque photosensitivity observed with sitagliptin phosphate (Januvia®)". Dermatology Online Journal. 18 (2): 9.
4. Olansky L (January 2010). "Do incretin-based therapies cause acute pancreatitis?". Journal of Diabetes Science and Technology. 4 (1): 228–229.
5. Matveyenko AV, Dry S, Cox HI, Moshtaghian A, Gurlo T, Galasso R, et al. (2009). "Beneficial endocrine but adverse exocrine effects of sitagliptin in the human islet amyloid polypeptide transgenic rat model of type 2 diabetes: interactions with metformin". Diabetes. 58 (7): 1604–1615.

6. Herman GA, Bergman A, Liu F, Stevens C, Wang AQ, Zeng W, et al. (2006). "Pharmacokinetics and pharmacodynamic effects of the oral DPP-4 inhibitor sitagliptin in middle-aged obese subjects". Journal of Clinical Pharmacology. 46 (8): 876–886.
7. International Conference on Harmonization, ICH Guidelines, Validation of Analytical Procedures Technical Requirements for Registration of Pharmaceuticals for Human Use: Text and Methodology Q 2 (R1), International Conference on Harmonization, Geneva, Switzerland, November 2005.
8. Kiranmaie GS, Nagaraju P, Mounika V and Priyadarshini GI: (2016). Development and validation of stability indicating RPHPLC method for simultaneous estimation of darunavir and cobicistat in the pharmaceutical dosage form. European Journal of Pharmaceutical and Medical Research; 3(12): 405-10.
9. 13. Babu R, Sharma V and Singhvi PK: (2014). A new gradient liquid chromatographic method for simultaneous estimation of tenofovir disoproxil fumarate, cobicistat, emtricitabine and elvitegravir in bulk drug and tablet dosage form. Asian Journal of Chemistry; 26(18): 6233-37.
10. Olin JL, Spooner LM and Klibanov OM: (2012). lvitegravir/ Cobicistat/Emtricitabine/Tenofovir disoproxil fumarate single tablet for HIV-1 infection treatment. Annals of Pharmacotherapy; 46(12): 1671-77.
11. Runja C, Kumar PR and Avanapu SR: (2016). A validated stability-indicating RP-HPLC method for the determination of emtricitabine, tenofovir disoproxil fumarate, elvitegravir and cobicistat in the pharmaceutical dosage form. Journal of Chromatographic Science; 54(5): 759-64.
12. Sathish J, Prasad K and Babu KS: (2016). A stability-indicating RP-HPLC method for simultaneous estimation of darunavir and cobicistat in bulk and tablet dosage form. Der Pharmacia Letter 2016; 8(12): 89-96
13. Mallikarjuna N and Sankar DS: (2016). Development and validation of stability-indicating HPLC-DAD method for simultaneous determination of emtricitabine, elvitegravir, cobicistat and tenofovir in their tablet dosage forms. Indian Journal of Pharmaceutical Education and Research; 50(1): 205-11.
14. Nagaraju P, Richards MP, Chandrasekhar KB and Kumar S: (2016). RP-HPLC method development and validation for simultaneous estimation of attazanavir and cobicistat in tablet dosage form. World journal of pharmacy and pharmaceutical sciences 5(8): 650-71.
15. Gummaluri R, Parthasarathi N and Madhulika GA: (2016). Simultaneous method for determination of emtricitabine, tenofovir disoproxil fumarate, elvitegravir and cobicistat in tablets by HPLC. Indian Journal of Pharmaceutical Sciences; 78(4): 532-37.
16. Rao PP, Reddy DM and Ramachandran D: (2014). Stability indicating HPLC method for simultaneous estimation of emtricitabine, tenofovir disoproxyl fumarate, cobicistat and elvitegravir in pharmaceutical dosage. World Journal of Pharmaceutical Sciences; 2(12): 1822-29.