International Journal of
Medicinal Chemistry & Analysis
www.ijmca.com
e ISSN 2249 - 7587
Print ISSN 2249 - 7595

NOVEL RP-HPLC TECHNIQUE FOR PRECISE ESTIMATION OF SITAGLIPTIN AND DOSAGE FORM

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ABSTRACT
Analytical method development and validation plays important role in the discovery, development and manufacture of pharmaceuticals. HPLC mainly utilizes a column that holds packing material (stationary phase), a pump that moves the mobile phase(s) through the column, and a detector that shows the retention times of the molecules. Sitagliptin is used for the treatment of type 2 diabetes mellitus. It is prescribed as an adjunct to diet and exercise to improve glycemic control in adults. Sitagliptin is anti-diabetic drug is more potent inhibitor of DPP-4. The present study was planned to estimate the drug present in the tablets by developing and validating new RP-HPLC method. In chromatographic analysis of substances RP-HPLC is normally advocated because of faster elution. The proposed RP-HPLC methods in construction with dissolution studies were developed for the quantitative estimation of the Sitagliptin was found to be accurate, rapid, sensitive and economical.

Keywords: High-performance liquid chromatography(HPLC), Diabetes mellitus, Sitagliptin etc.

INTRODUCTION

Analytical chemistry is a fundamental branch of chemistry devoted to the study of separation, identification, and quantification of substances within a sample. [1] In pharmaceutical sciences, analytical methods are crucial for ensuring the quality, safety, and efficacy of drugs. High-performance liquid chromatography (HPLC), a powerful analytical technique, plays a pivotal role in pharmaceutical analysis due to its ability to separate and quantify complex mixtures of compounds with high sensitivity and precision.[2] HPLC operates by passing a sample mixture through a column packed with a stationary phase material, where the interaction between the sample components and the stationary phase, as well as the mobile phase (solvent), determines their retention times. [3] The retention time is critical as it indicates the time taken for a specific compound to exit the column, providing information about its identity and quantity.

Sitagliptin, a medication used in the treatment of type 2 diabetes mellitus, exemplifies the application of HPLC in pharmaceutical analysis. [4] It functions as a potent inhibitor of dipeptidyl peptidase-4 (DPP-4), an enzyme responsible for degrading incretin hormones such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). By inhibiting DPP-4, sitagliptin enhances the secretion of insulin and reduces the release of glucagon, thereby helping to regulate blood glucose levels in diabetic patients when used adjunctively with diet and exercise. [5] This introduction underscores the critical role of analytical method development and validation in pharmaceutical research, development, and manufacturing. [6] Accurate and reliable analytical techniques like HPLC are essential for ensuring the potency, purity, and consistency of pharmaceutical products, thereby contributing to their efficacy and safety profiles.

METHODOLOGY

Choice of column

Phenomenex C18 (25 cm × 4.6 mm i.d., 5-µm particle size) is selected as the column owing to its robustness, reproducibility and reliability among diverse RP-HPLC columns. Columns with 5µm particle size give the best compromise of efficiency.

Choice of mobile phase

The preferred mobile phase binary mixture is Phosphate buffer (pH8.0, (0.02M)): Acetonitrile (40: 60) as the polarity index of Acetonitrile is 5.8 that correlates

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with pKa of Sitagliptin (9.86 ± 0.2) which ensures greater selectivity and interaction with the analyte.

Choice of solvent

Owing to free solubility of the analyte in mobile phase it is used as solvent as it accomplishes enhanced miscibility with mobile phase.

Choice of wavelength for detection

Analysis of the analyte in solvent by UV spectrophotometry revealed the wavelength of detection as 299 nm.

Assay

Preparation of Standard solutions:

Preparation of Mobile Phase:

40 ml of Phosphate buffer and 60 ml of HPLC grade Acetonitrile were mixed by continuous agitation and vacuum filtered using 0.45 um Millipore membrane.

Preparation of Sitagliptin standard stock solution- I (1000 µg/ml):

10 mg of Sitagliptin API was accurately weighed into 10 ml volumetric flask and dissolved in freshly prepared solvent (mobile phase) and made up to the volume to get concentration of 1000 µg/ ml.

Preparation of Sitagliptin standard stock solution- II (100 µg/ml):

1 ml from the stock solution- I was pipetted into 10 ml volumetric flask and made up to volume with freshly prepared solvent to get 100 µg/ ml concentration.

Preparation of standards for calibration curve (20- 40 ug/ ml):

From stock solution- II 1ml, 1.25 ml, 1.5 ml, 1.75 ml and 2 ml were accurately transferred to respective 5 ml volumetric flasks and made up to volume with freshly prepared 0.1M Hydrochloric acid which corresponds to concentrations of 20, 25, 30, 35, 40 ug/ ml respectively. [7] The chromatograms for the calibration set were then obtained and recorded.

Method validation

System suitability parameters [25-31]

System suitability parameters including USP Theoretical Plate Count, USP Tailing factor, % RSD were assessed from 5 injections of Sitagliptin (30 µg/ ml).

Specificity

The interference of the blank with the chromatogram of Sitagliptin was checked by recording and comparing the chromatograms of blank and that of Sitagliptin.

Linearity and Range

Linearity for the concentration range 20- 40 µg/ml was established by plotting concentrations on X-axis and corresponding peak area on Y- axis. Statistical parameters like correlation coefficient (R^2), line equation including slope (m), y- intercept (C) were determined.

The specified range was derived from linearity studies by determining the difference between highest and lowest concentrations.

Precision

Intraday precision (Repeatability):

Repeatability of the developed method was assessed by 9 determinations covering 3 concentrations each of 3 replicates. % RSD was calculated for the results obtained.

Interday precision:

Variations in the results for the developed method was assessed amidst 3 different days (n=6). % RSD was calculated for the results obtained.

Robustness:

Typical variations including change in flow rate (± 0.2ml of optimized flow rate), change in the organic phase composition of mobile phase (± 10 ml) and change in wavelength (± 5 nm) were assessed.

Accuracy:

Preparation of 50% solution:

1ml of sample stock solution (10 µg/ ml) and 0.25ml of above standard stock solution- II were pipetted out into a 5ml volumetric flask and diluted up to the mark with diluent.

Preparation of 100% solution:

1ml of sample stock solution (10 µg/ ml) and 0.5ml of above standard stock solution- II were pipetted out into a 5ml volumetric flask and diluted up to the mark with diluent.

Preparation of 150% solution:

1ml of sample stock solution (10 µg/ ml) and 0.75ml of above standard stock solution- II were pipetted out into a 5ml volumetric flask and diluted up to the mark with diluent.

Calculate the amount found and amount added for Sitagliptin and also calculate the individual recovery and mean recovery values.

Table 1: Optimized Parameters for RP- HPLC.

Column	Phenomenex C18 packed with Octadecylsilane
Mobile phase	Acetonitrile: Phosphate buffer-8(0.002 M) (60: 40)

Solvent/ diluent	Acetonitrile: Phosphate buffer-8(0.002 M) (60: 40)
Flow rate	1 ml/ min
Injection volume	20µl
Pump mode	Isocratic
Temperature of column	Ambient
UV detection	299 nm

Table 2: Summary of System Suitability Parameters.

Inj.No	RT	Peak Area	Theoretical Plates	USP Tailing Factor
1	3.911	1270806	3565	1.047
2	3.905	1270815	3563	1.029
3	3.911	1270811	3593	1.032
4	3.930	1269705	3620	1.040
5	3.916	1262796	3562	1.028
Mean		1268987	3580.53	1.036
SD		3124.91	22.529	0.0072
%RSD		0.246	0.629	0.701

Table 3: Linearity Profile by RP- HPLC.

S.NO	Concentration(µg/ml)	Peak area
1	20	842312.874
2	25	1057315.245
3	30	1270806.147
4	35	1496328.659
5	40	1734695.645

Table 4: Intraday and Interday Precision Day- I by RP- HPLC

Conc (ng/ ml)	Peak area			Average	SD	% RSD
	Set 1	Set 2	Set 3			
20	842071	842076	842062	842069	5.804	0.001
30	1270426	1270431	1270435	1270430	3.689	0.000
40	1734176	1734179	1732067	1733474	995.000	0.057

Table 5: Intraday and Interday Precision Day- II by RP- HPLC

Conc (ng/ ml)	Peak area			Average	SD	% RSD
	Set 1	Set 2	Set 3			
20	841970	843153	842061	842394	537.641	0.064
30	1269325	1270426	1270426	1270059	519.110	0.041
40	1733074	1733374	1734176	1733541	465.328	0.027

Table 6: Intraday and Interday Precision Day- III by RP- HPLC

Conc (ng/ ml)	Peak area			Average	SD	% RSD
	Set 1	Set 2	Set 3			
20	840907	840669	842962	841513	1029.137	0.122
30	1262418	1275466	1272361	1270081	5565.308	0.438
40	1722750	1752397	1734657	1736601	12181.209	0.701

Table 7: LOD and LOQ of Sitagliptin by RP-HPLC

Parameter	Sitagliptin (µg/ml)
LOD (µg/ml)	0.365864
LOQ (µg/ml)	1.10869

Table 8: Summary of Robustness Data.

Parameter	Condition	System suitability parameters	
		Theoretical plates	USP Tailing factor
Change in flow rate(± 0.2 ml/ min)	0.8 ml/ min	2727058	1.017
	1.2 ml/ min	2739935	1.867
Change in organic phase composition (± 10 ml)	Acetonitrile: phosphate buffer-8 (0.002 M) (50:50)	2552146	1.610
	Acetonitrile: phosphate buffer-8 (0.002 M) (70:30)	1915312	1.159
Change in detector wavelength (± 5 nm)	294nm	3647349	2.602
	304nm	3428680	2.683

Table 9: Recovery from Formulation (Tablets) by RP-HPLC

% addition of label claimed	Label claimed µg/ml	Spiked Conc. µg/ml	Obtained Amount µg/ml	% Recovery
50%	10	5	15.154	101.0267
100%	10	10	20.069	100.345
150%	10	15	24.981	99.924

Table 10: Assay of Marketed Formulation by RP-HPLC

Formulation	Peak area	Label claim	Amount found	% Assay±SD
Tablets	1271048	5 mg	4.851 mg	97.02
	1271152			
	1275442			

Acceptance criteria: 95- 105% w/v; Assay results were satisfactory and within limits.

Figure 1: HPLC Chromatograms of the trials

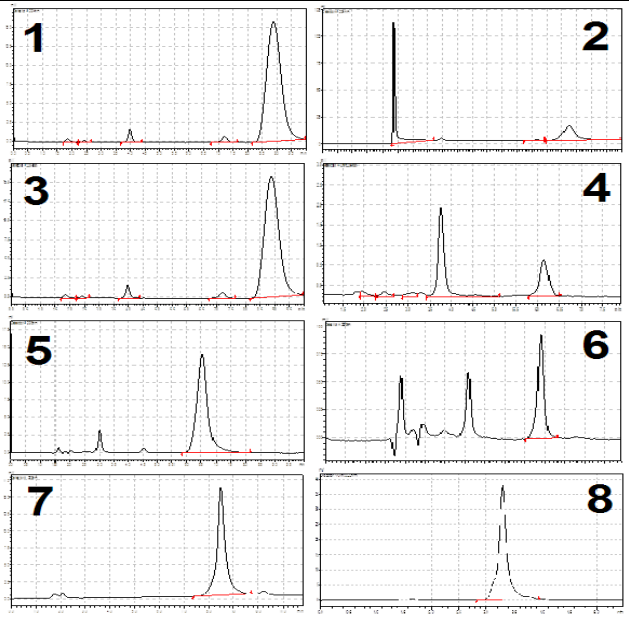


Figure 2: Chromatogram of the Optimized Method (40 ug/ ml)

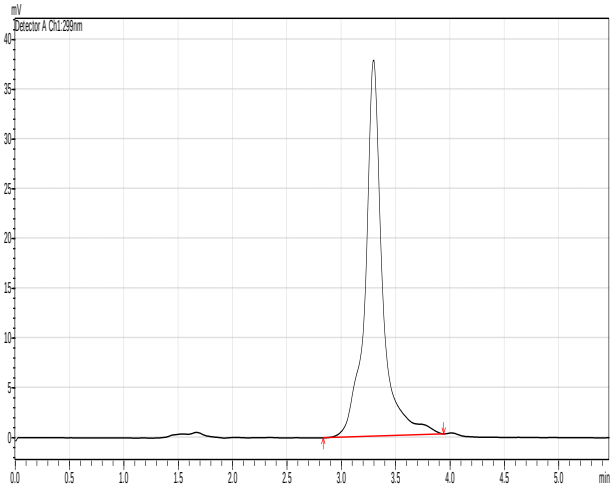


Figure 3: Chromatogram of Blank

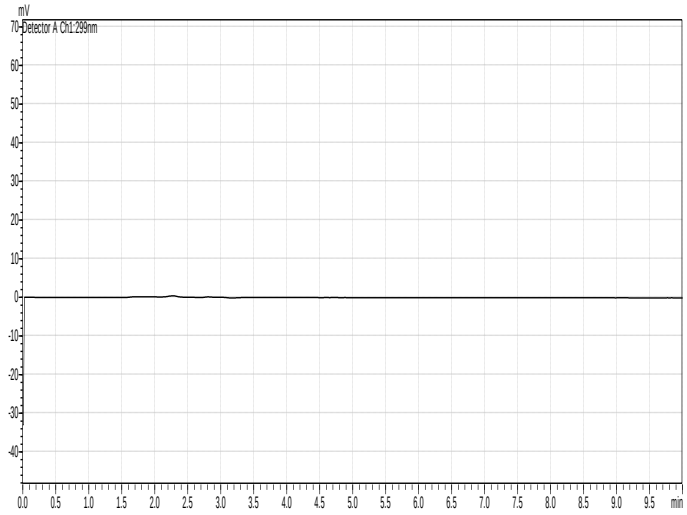


Figure 4: Chromatogram of Sitagliptin

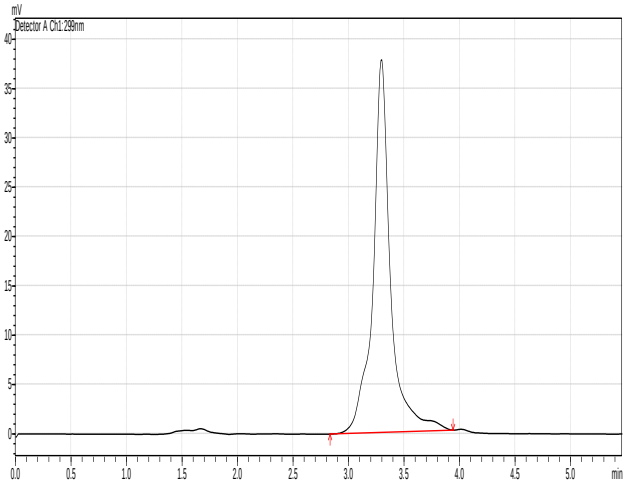
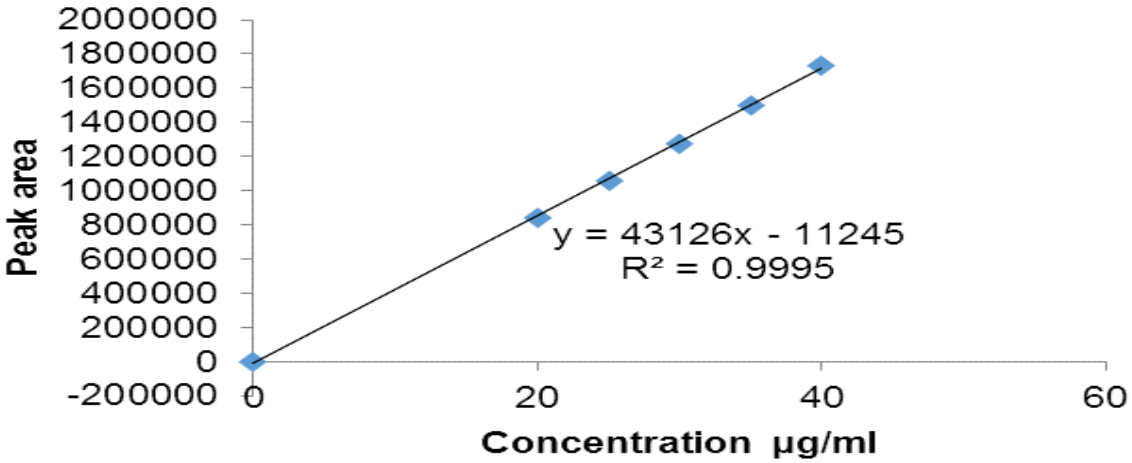


Figure 5: Calibration Curve for the Linearity Set by RP- HPLC



RESULTS

Method Validation:

System Suitability Parameters (30 ug/ ml)

Acceptance criteria:

- Theoretical Plates- NLT 2000; USP Tailing factor- NMT 2.0; % RSD- NMT 2.0

The system suitability parameters were within limits and hence the parameters for the optimized method could be applicable for the method to be validated.

✓ The method was found to be specific since the interference of blank with the chromatogram of Sitagliptin was not observed.

Precision

Intraday precision (Repeatability)

Results obtained reveal that the developed method was precise and rugged.

LOD and LOQ of Sitagliptin:

Acceptance Criteria:

The % Recovery for each level should be between 97.0 and 103.0%. The accuracy data was found to be within limits.

Assay of tablets by RP-HPLC

DISCUSSION

Sitagliptin is anti-diabetic drug is more potent inhibitor of DPP-4. The present study was planned to estimate the drug present in the tablets by developing and validating new RP-HPLC method. [8] In chromatographic analysis of substances RP-HPLC is normally advocated because of faster elution. The polar compounds are eluted faster than nonpolar compounds. In the present work the RP HPLC method was developed and validated which was

simple, less expensive and more rapid with isocratic mode. [9] The wave length selection was done by scanning the drug (pure) in the U.V range (200-400nm) and maximum absorbance was found to be at 299nm in the case of Sitagliptin. The sensitivity range for the analysis was found to be 20-40µg/ml in the case of the drug components. A variety of columns like C8, C14, C16, C18 etc are used for the analysis of nonpolar compounds, while silicon columns are used for the analysis of polar compounds. [10] In the present work phenomenex C18 column was selected for development of the procedure.

From the method developed several trials were carried out and reported, which lead to the optimized chromatographic conditions for the determination of Sitagliptin in tablet dosage form. A mixture of Acetonitrile: Water in different compositions was used as mobile phase at flow rate of 1,1.2ml/min using phenomenex C18 column as stationary phase and detection of analyte was carried out at multiple wavelengths for estimation of Sitagliptin. Multiple Interferences were observed. [11] In the next trial, a mixture of Acetonitrile: Phosphate buffer pH-8 (0.002M) (50:50%v/v) used as mobile phase and phenomenex C18 column was used retention times was 7 minutes was observed and for better results further trial was conducted. In the Trail-XV, flow rate was 1ml/min, a mixture of Acetonitrile: Phosphate buffer pH-8 (0.002M) (60:40%v/v) used as the mobile phase and phenomenex C18 column was used. [12] The Peaks was properly eluted and earlier retention times were achieved. So the trial was considered as optimized for chromatographic separation of Sitagliptin in tablet dosage form. [13]

Specificity was done to demonstrate the ability of the analytical method to measure accurately and specifically the analyte in the presence of components that may be expected to be present in the sample matrix and was to be within the limits. [14] System precision and method precision was done to demonstrate that the analytical method is capable to yield closeness of data values between a series of measurements obtained from multiple sampling of the same homogeneous sample and the results obtained were within the limits. The precision was check to establish the effects of random events on the precision of the analytical procedure. [15] The system precision was checked and the results obtained were within the limits.

The analytical methods were demonstrated which was capable to obtained test results, which were directly proportional to the concentration (amount) of analytic in the sample (linearity). The linearity graph was identified for individual related compounds and the calibration was done. The analytical methods were capable to yield data values close to true values, which were accepted as a conventional true value (accuracy).

CONCLUSION

The proposed RP-HPLC methods in construction with dissolution studies were developed for the quantitative estimation of the Sitagliptin was found to be accurate, rapid, sensitive and economical. The developed methods can be utilized for routine analysis in quality control laboratories.. It was validated as per the ICH guidelines. The values of %RSD for intraday and interday precision for three methods were found to be less than 2. The values confirm that methods are precise. The values of % Recovery were greater than 96 for this methods which showed that the methods was accurate and free from the interference of excipients used in formulation. The values of % recovery for analysis of formulations were found within 96-104%, which shows that the methods are applicable for analysis of marketed formulation.

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