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Review Article

DEVELOPMENT OF NOVEL ACCURATE METHOD OF ESTIMATION OF GIMAGLIPTIN USING REVERSE PHASE HPLC

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ABSTRACT

Chromatography with high performance consists basically of a column filled with packing material (stationary phase), a pump that moves the mobile phase(s) through the column, and a detector that detects the molecule's retention time. Gemigliptin, sold as Zemiglo, is an oral hypoglycemic agent (anti-diabetic drug) that is a member of the dipeptidyl peptidase-4 (DPP-4) inhibitor class of medications. Thus, the goal of this work is to develop a novel analytical approach for determining Gimagliptin concentrations using RPHPLC. As the mobile phase, a mixture of acetonitrile and water with various compositions was utilised at a flow rate of 1,1.2ml/min. The stationary phase was a phenomenex C18 column. Numerous parameters such as accuracy, precision, linearity, system suitability, and formulation assay were examined, and an analytical method for qualitative and quantitative drug estimation was established.

Keywords: Gimagliptin, Hplc, Analytical Method, Stationary Phase.

INTRODUCTION

Chromatography with high performance is primarily composed of a column that contains packing material (stationary phase), a pump that drives the mobile phase(s) through the column, and a detector that measures the molecule's retention time. The retention duration depends on the interactions between the stationary phase, the molecules under investigation, and the solvent(s) utilised [1, 2].

Gemigliptin, marketed under the trade name Zemiglo, is an oral hypoglycemic agent (anti-diabetic medication) that belongs to the dipeptidyl peptidase-4 (DPP-4) inhibitor family of medicines. The glucose-lowering effects of DPP-4 inhibitors are mostly due to the inactivation of the incretin hormones GLP-1 and gastric inhibitory polypeptide (GIP) by DPP-4 [3, 4]. An extensive study of the literature indicated that few analytical procedures for determining Gimagliptinin commercial formulations have been documented.

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Thus, the purpose of this study is to create a novel analytical technique for estimating Gimagliptin using RPHPLC.

MATERIALS AND METHODS

Column Parameters

PhenomenexC_{18} (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.

This column was found to be stable at the desired pH and temperature. It offer good peak symmetry. Columns with 5µm particle size give the best compromise of efficiency.

Mobile phase Parameters

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 with pKa of Gimagliptin (9.86 ± 0.2) which ensures greater selectivity and interaction with the analyte. Water acts as non-selective strength adjusting solvent in RP-HPLC.

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.

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 Gimagliptin standard stock solution- I (1000 µg/ml):

10 mg of Gimagliptin 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 Gimagliptin 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. The chromatograms for the calibration set were then obtained and recorded.

Method Validation:

System suitability parameters

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

Specificity

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

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 Gimagliptin and also calculate the individual recovery and mean recovery values.

Assay of Formulation

20 tablets were weighed accurately and average weight of tablet was noted and was finely powdered.The tablet powder equivalent to 20 mg of Gimagliptin was accurately weighed and transferred to 10 ml volumetric flask and dissolved in about 5 ml of the solvent (Phosphate buffer ph-8(0.02M): Acetonitrile 40:60).It was then vortexed for 60 minutes to enhance maximum extraction of the active pharmaceutical ingredient from the dosage form and filtered through Whatmann No 1

filter paper to remove insoluble excipients to the maximum extent.It was then made up to the volume with the same solvent. This constitutes 100 µg/ ml of Gimagliptin.From the stock solution, aliquot corresponding to medium concentration of standard curve (30µg/ml) was prepared and made up to the mark with the solvent.The peak area was noted and the corresponding concentration was then determined from the standard calibration curve [Michael et al., 2005; IP, 2010].

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.

Specificity:

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

Linearity and Range:

The calibration set was linear with regression coefficient of 0.9995.

Precision

Intraday precision (Repeatability)

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

LOD and LOQ of Gimagliptin:

The obtained results were satisfactory.

Robustness

The developed method was robust

Accuracy

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

Acceptance criteria:

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

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 Inter day 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 Gimagliptin by RP-HPLC

Parameter	Gimagliptin (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			

Figure 1: Chromatogram of the Optimized Method (40 ug/ ml)

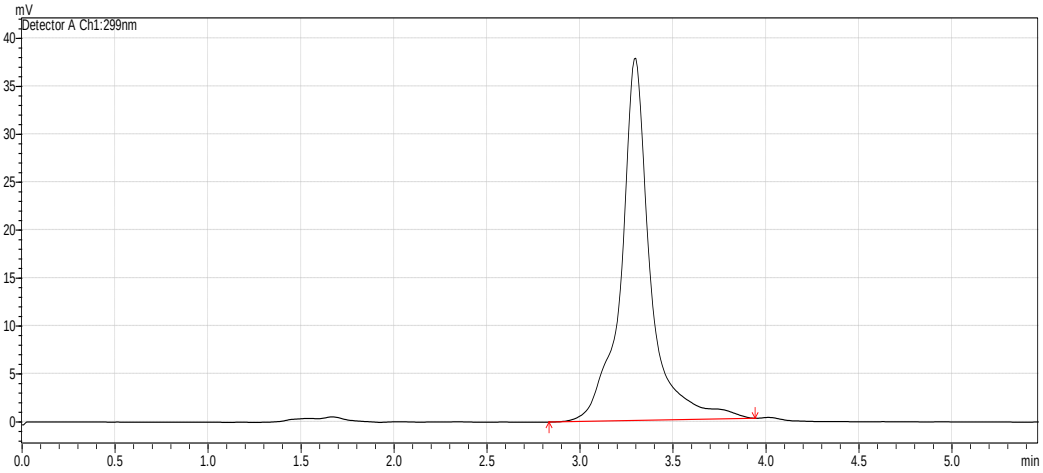


Figure 2: Chromatogram of Blank and Drug

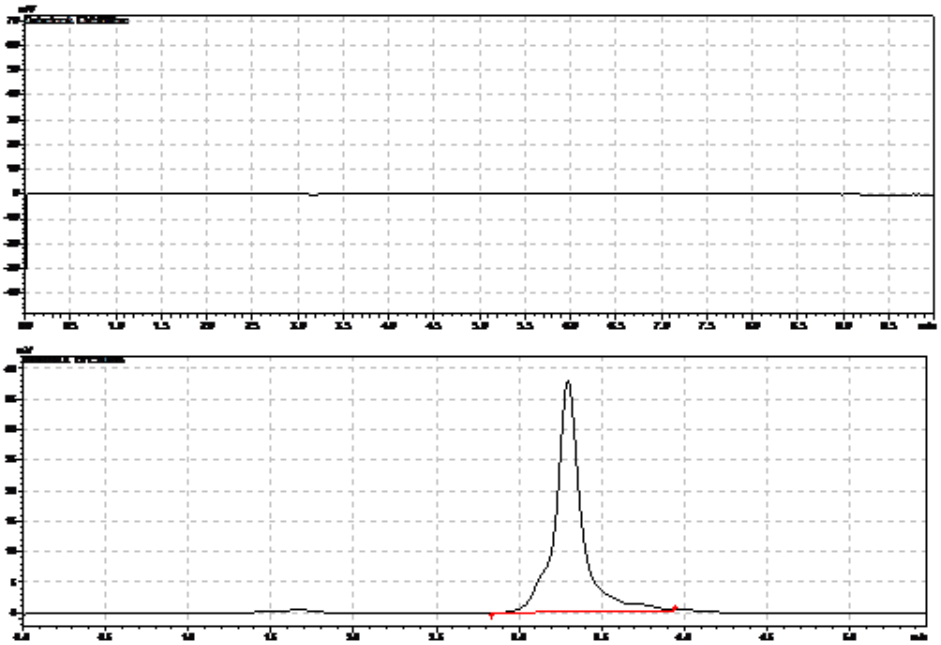
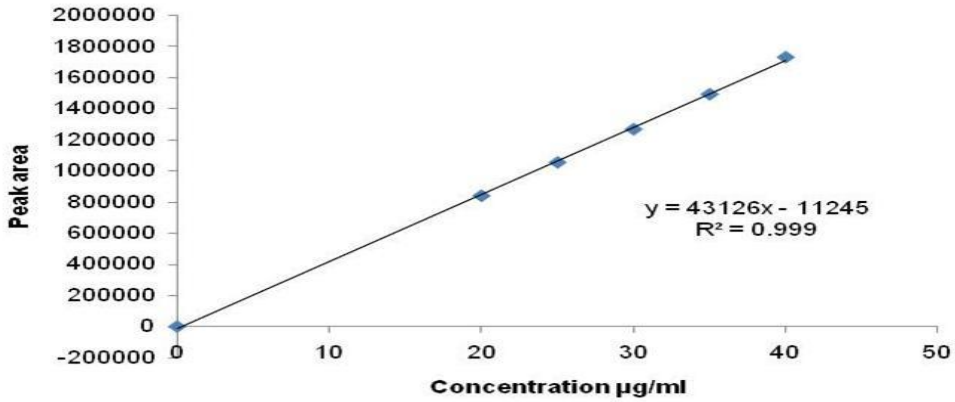


Figure 3: Calibration Curve for the Linearity Set by RP- HPLC



DISCUSSION

Gimagliptin is a more powerful DPP-4 inhibitor than metformin. The purpose of this study was to determine the amount of medication contained in tablets by developing and verifying a novel RP-HPLC technique.

RP-HPLC is often preferred for chromatographic examination of compounds due to its quicker elution time. Polar chemicals elute more rapidly than nonpolar compounds. The current work established and validated an isocratic mode RP HPLC technique that was simple, less costly, and faster. The wave length was chosen by scanning the medication (pure) in the ultraviolet range (200-400nm), and the greatest absorption of Gimagliptin was determined to be at 299nm. The analysis's sensitivity range was determined to be 20-40g/ml for the drug components.

Nonpolar chemicals are analysed using a variety of columns such as C8, C14, C16, and C18, whereas polar compounds are analysed using silicon columns. The approach was developed in this paper using the phenomenex C18 column. In RP HPLC, the mobile phase is often polar. The mobile phase in this study is composed of Acetonitrile and Phosphate buffer-pH-8(0.02M) (60:40 percent v/v), which results in a reasonably inexpensive and quick method. The chemical was dissolved in the same mobile phase and injected into the port in a concentration of 20l.

Numerous experiments were conducted and reported using the technique devised, which resulted in the optimal chromatographic conditions for the measurement of Gimagliptin in tablet dosage form. A combination of acetonitrile and water with varying compositions was used as the mobile phase at a flow rate of 1,1.2ml/min. A phenomenex C18 column was used as the stationary phase, and detection of the analyte at numerous wavelengths was utilised to determine the concentration of Gimagliptin. Numerous Interferences have been detected.

In the subsequent trial, a mobile phase of acetonitrile: phosphate buffer pH-8 (0.002M) (50:50 percent v/v) was utilised, and a phenomenex C18 column was used. Retention periods of 7 minutes were recorded, and more trials were undertaken for improved results. The Trail-XV was operated at a flow rate of 1ml/min with a mobile phase of acetonitrile: phosphate buffer pH-8 (0.002M) (60:40 percent v/v) on a phenomenex C18 column. The Peaks were appropriately eluted, resulting in shorter retention durations. As a result, the experiment was deemed optimal for the chromatographic separation of Gimagliptin in tablet form.

Assays were run using the pure compound and tablet dosage forms, and the results were computed using the provided equations. If the results were determined to be within the specified limits, they were tabulated, together with the absorption spectrum and chromatographs.

The purpose of the method validation report was to offer documented proof for Gimagliptin in tablet dosage form based on the performance of the validation research parameters. The validation research confirms the method's validity for the purpose of determining Gimagliptin in tablet dose forms. The current technique has been validated in compliance with the USP assay determination requirements, which include specificity, precision, accuracy, linearity, and robustness.

Specificity testing was performed to verify the analytical method's capacity to quantify the analyte correctly and precisely in the presence of components that were predicted to be present in the sample matrix and were within the limits. Precision of the system and technique were determined to indicate that the analytical method is capable of producing close agreement between data values acquired from multiple sampling of the same homogenous sample and that the findings obtained were within the limitations. Accuracy was checked to determine the influence of random occurrences on the analytical procedure's precision. The accuracy of the system was verified, and the acquired findings were within acceptable limits.

It was established that analytical procedures were capable of obtaining test findings that were directly proportional to the concentration (quantity) of analytic in the sample (linearity). The linearity graphs for related substances were discovered and calibrated. Analytical techniques were capable of producing data values that were near to true values, which were regarded as conventional true values (accuracy).

CONCLUSION

The suggested RP-HPLC techniques in conjunction with dissolution experiments were shown to be accurate, quick, sensitive, and inexpensive for the quantitative quantification of Gimagliptin. The described methodologies can be used in normal quality control laboratory analysis. It has been verified in accordance with ICH requirements. Percent RSD values for intraday and interday precision were determined to be less than 2 for three techniques. The values attest to the precision of the approaches. Percent Recovery readings were more than 96 for this procedure, indicating that it was accurate and devoid of influence from excipients used in formulation. The percent recovery values for formulation analysis were found to be between 96 and 104 percent, indicating that the procedures are appropriate to the examination of commercial formulations.

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