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Research Article

VALIDATED RP - HPLC METHOD FOR THE ESTIMATION OF LIRAGLUTIDE IN TABLET DOSAGE

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ABSTRACT

A simple, selective, linear, precise and accurate RP-HPLC method was developed and validated for rapid assay of Liraglutide in tablet dosage form. Isocratic elution at a flow rate of 1.0ml/min was employed on a symmetry C18 (250x4.6mm, 5µm in particle size) at ambient temperature. The mobile phase consisted of 0.1% Ortho Phosphoric Acid: Acetonitrile: Methanol 5:60:35 (V/V). The UV detection wavelength was 245nm and 20µl sample was injected. The retention time for Liraglutide was 4.447 min. The percentage RSD for precision and accuracy of the method was found to be less than 2%. The method was validated as per the ICH guidelines. The method was successfully applied for routine analysis of Liraglutide in tablet dosage form.

Key Words: Liraglutide, RP-HPLC, UV detection, recovery, precise.

INTRODUCTION

Liraglutide is a long-acting glucagon-like peptide-1 (GLP-1) analog that has been developed by Novo Nordisk for the treatment of type2 diabetes. The product was approved by the European Medicines Agency (EMA) on July 3, 2009, and by the U.S. Food and Drug Administration (FDA) on January 25, 2010.

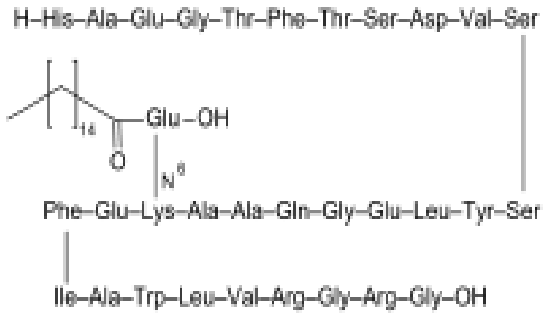

Figure 1: Stricture of Liraglutide

Liraglutide intended to help lower blood sugar levels along with diet, exercise, and selected other diabetes medicines. It is not recommended as initial therapy in patients who have not achieved adequate diabetes control on diet and exercise alone.

Liraglutide is an incretin mimetic. This means that it mimics the actions of incretin hormones in the body. As an incretin mimetic, liraglutide increases insulin production in response to meals and decreases the amount of glucose (sugar) that the liver produces. The medicine also slows the emptying of food from the stomach and decreases the amount of food that people eat.

EXPERIMENTAL

Chemicals and Reagents:

HPLC grade Actonitrile, Methanol and Ortho Phosphoric Acid (OPA)were purchased from Merck Specalities Pvt. Ltd.

Instrumentation and Analytical Conditions:

The analysis of drug was carried out on a PEAK HPLC system equipped with a reverse phase C18 column (250x4.6mm, 5µm in particle size), a LC-P7000 isocratic pump, a 20µl injection loop and a LC-UV7000 absorbance detector and running on PEAK Chromatographic Software version 1.06. Isocratic elution with 0.1% OPA: Acetonitrile: Methanol 30:40:30 (V/V) (P^H-5.5) was used at a flow rate of 1.0ml/min. The mobile

phase was prepared freshly and degassed by sonicating for 5 min before use.

Stock and Working Standard Solutions:

10mg of Liraglutide working standard was accurately weighed and transferred into a 10ml volumetric flask containing diluent. It was sonicated, dissolved completely and made volume up to the mark with the same solvent. 1ml of the above stock solution was pipetted into a 10ml volumetric flask and diluted up to the mark with diluent. The contents were mixed well and filtered through 0.45µm nylon filter paper and 60ppm was prepared finally. The calibration curve was plotted with the six concentrations of the 30 ppm-90 ppm working standard solutions. Calibration solutions were prepared daily and analyzed immediately after preparation.

Assay of Liraglutide Tablets:

20 Liraglutide tablets were weighed and the average weight was calculated. The sample equivalent to 10mg of Liraglutide was accurately weighed and transferred in to a 10ml volumetric flask. The diluent was added, sonicated for complete dissolution. The mixture wasmade volume up to the mark with diluents. They were mixed well and filterd through 0.45um filter. Further, 1ml of the above stock solution pipetted into a 10ml volumetric flask and diluted up to mark with diluents and finally 60 ppm were prepared. They were mixed well and filterd through 0.45um filter. An aliquot of this solution was injected into HPLC system. Peak area of Liraglutide was measured for the determination.

Validation Procedure:

The objective of the method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The method was validated for linearity, precision (repeatability and intermediate precision), accuracy, specificity, stability and system suitability. Standard plots were constructed with five concentrations in the range of 30 ppm to 90 ppm prepared in triplicates to test linearity. The peak area of Liraglutide was plotted against the concentration to obtain the calibration graph. The linearity was evaluated by linear regression analysis that was calculated by the least square regression method. The precision of the assay was studied with respect to both repeatability and intermediate precision. Repeatability was calculated from five replicate injections of freshly prepared Liraglutide test solution in the same equipment at a concentration value of 100% (60 ppm) of the intended test concentration value on the same day. The experiment was repeated by assaying freshly prepared solution at the same concentration additionally on two consecutive days to determine intermediate precision. Peak area of the Liraglutide was determined and precision was reported as %RSD.

Method accuracy was tested (% recovery and %RSD of individual measurements) by analyzing sample of Liraglutide at three different levels in pure solutions using three preparations for each level. The results were expressed as the percentage of Liraglutide recovered in the samples. Sample solution short term stability was tested at ambient temperature (20±10^{0}C) for three days.

RESULT AND DISCUSSION

Optimization of the Chromatographic Conditions:

Proper selection of the stationary phase depends up on the nature of the sample, molecular weight and solubility. Among C8 and C18, C18 column was selected. Non-polar compound is very attractive with reverse phase columns. So the elution of the compound from the column was influenced by polar mobile phase. Mixture of 0.1% OPA, Methanol and Acetonitrile was selected as mobile phase and the effect of composition of mobile phase on the retention time of Liraglutide was thoroughly investigated. The concentration of the 0.1% OPA, Methanol and Acetonitrile were optimized to give symmetric peak with short run time (Fig.2).

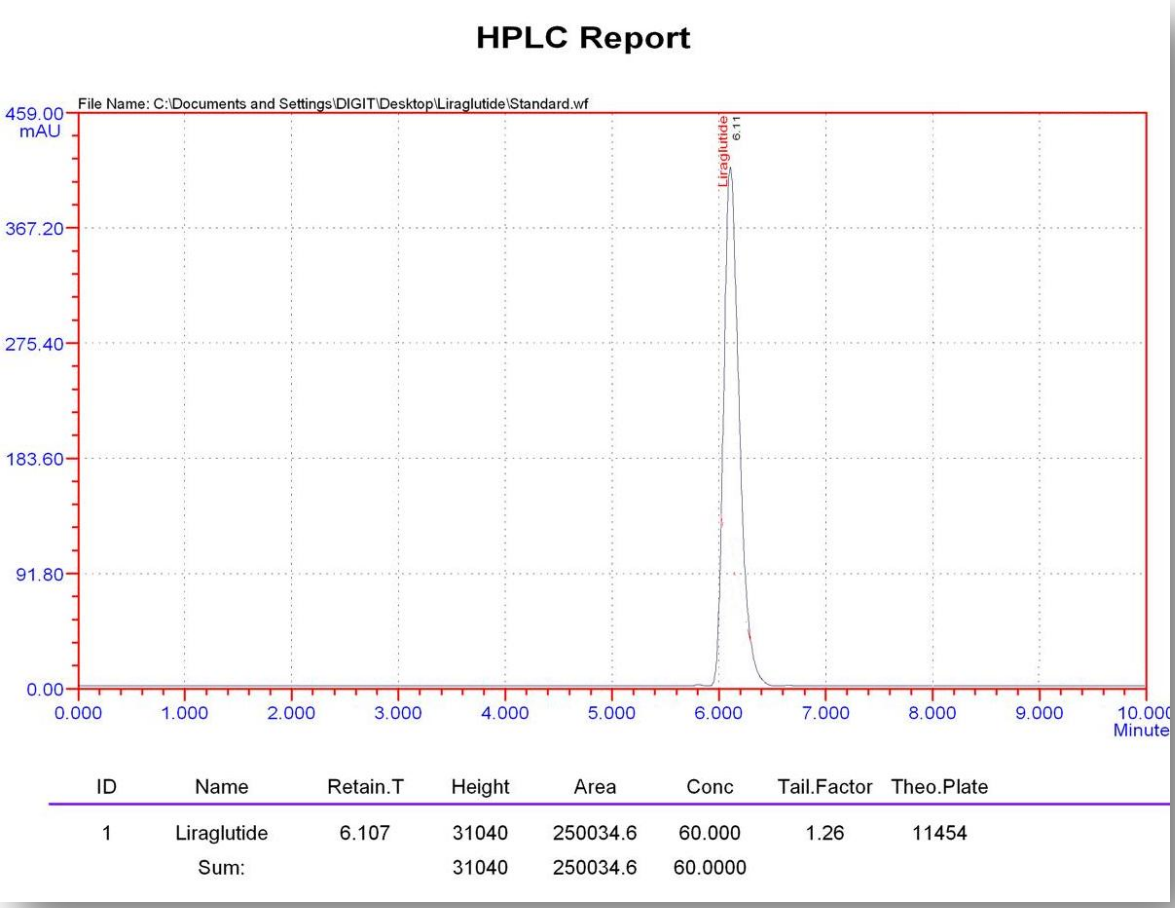

Fig 2: Typical chromatogram of Liraglutide standard

Validation of Method

Linearity:

Five points graphs was constructed covering a concentration range 3-18ppm (Three independent determinations were performed at each concentration). Linear relationships between the peak area signal of Liraglutide the corresponding drug concentraton was observed. The standard deviation of the slope and intercept were low. The statistical analysis of calibration is shown in Table 1.

S.NO	Concentration in ppm	Area
1	30	125697
2	40	167238
3	50	213028
4	60	250034
5	70	298632
6	80	342413
7	90	377098
Range 30 – 60 ppm	Slope	4226
	Intercept	-653
	Correlation coefficient	0.999

Table 1: Linearity of Liraglutide

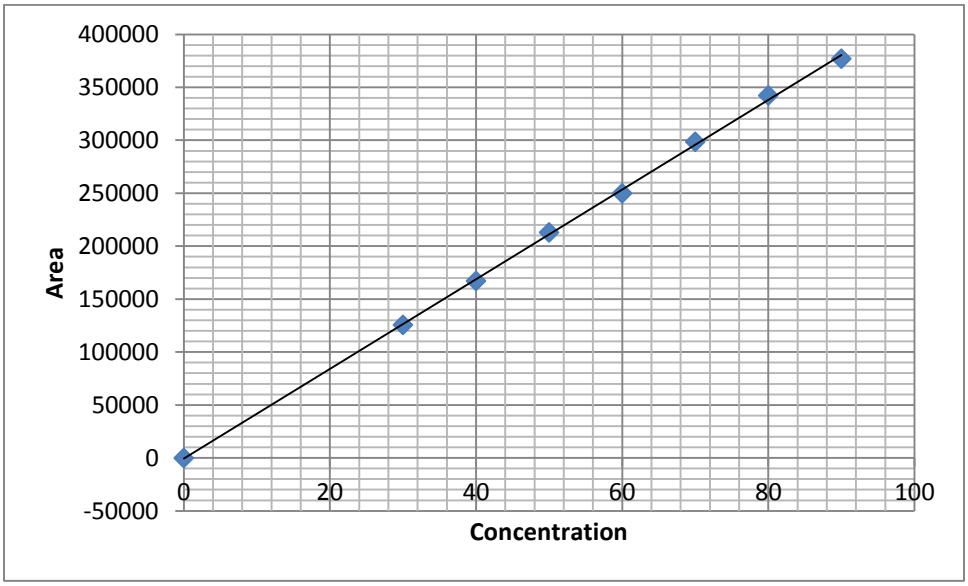

Fig 3: Calibration curve of Liraglutide

Precision:

The validated method was applied for the assay of commercial tablets containing Liraglutide. Sample was analyzed for five times after extracting the drug as mentioned in assay sample preparation of the experimental section. The results presented good agreement with the labeled content. Low values of standard

deviation denoted very good repeatability of the measurement. Thus it was showing that the equipment used for the study was correctly and hence the developed analytical method is highly repetitive. For the intermediate precision a study carried out by the same analyst working on the same day on two consecutive days indicated a RSD of 0.3135. This indicates good method precision.

% Concentration	% Recovery	Mean Recovery
50%	99.67	99.31 %
100%	99.29	
150%	98.97	

Table 2: Recovery studies of Liraglutide

System Suitability:

The system suitability parameter like capacity factor, asymmetry factor, tailing factor and number of theoretical plates were also calculated. It was observed that all the values are within the limits (Table.3). The statistical evaluation of the proposed method was revealed its good linearity, reproducibility and its validation for different parameters and let us to the conclusion that it could be used for the rapid and reliable determination of Liraglutide in tablet formulation. The results are furnished in Table 3.

Parameters	Values
λ max (nm)	245nm
Beer's law limit (ppm)	30 – 90 ppm
Correlation coefficient	0.999
Retention time	6.11
Theoretical plates	11453
Tailing factor	1.26
Limit of detection	0.06ppm
Limit of quantification	0.2 ppm
Slope	4226
Intercept	-653
Accuracy	99.31
R.S.D.	1.13
% Estimation of Liraglutide in formulation	98.97%

Table 3: System stability parameters

Formulation	Label claim (mg)	% Amount found
Victoza	1.8 mg	98.97%

Table 4: Assay

Mobile phase	Methanol:Acetonitrile:0.1% OPA (35:60:5)
PH	5.1
UV detection	245nm
Analytical column	C18
Flow rate	1.0ml/min
Temperature	ambient
Injection volume	20µl
Runtime	10 min
Retention time	6.11 min

Table 5: Chromatographic condition

CONCLUSION

A validated RP-HPLC method has been developed for the determination of Liraglutide in tablet dosage form. The proposed method is simple, rapid, accurate, precise and specific. Its chromatographic run time of 10 min allows the analysis of a large number of samples in short period of time. Therefore, it is suitable for the routine analysis of Liraglutide in pharmaceutical dosage form. The limit of detection for Liraglutide was found to be 0.06 ppm and the limit of quantification was found to be 0.2 ppm. It proves the sensitivity of method.

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