Development and Validation of a new robust RP-HPLC Method for the simultaneous quantitation of Semaglutide and Liraglutide in Bulk and Pharmaceutical Dosage Form

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Abstract:

The present work was mainly focussed on developing and validating a new isocratic, simple, rapid, precise, accurate and stable reverse phase high performance liquid chromatography method for a combination of Semaglutide and Liraglutide. The separation was achieved on a C_{18} µ-bondopak column (250 mm*4.6 mm) using a mobile phase consisting of buffer, Water and Acetonitrile in the ratio of 40:20:40 (buffer-0.1% v/v triethylamine pH-3.0 followed by 1 ml/min flow rate. The Wavelength detection was at 260 nm. The Chromatographic retentions were stable at

2.507 min for Semaglutide and 3.233 min for Liraglutide. Linearity concentrations were established in the range of 20-100 µg/ml for Semaglutide and 20-100 µg/ml for Leraglutide and correlation coefficient for the above drugs was 0.999. The Relative Standard Deviation of Inter and Intra Day Precision was less than 2%. The proposed method provides a useful tool for quantification of Semaglutide and Liraglutide for their assay. This method is simple, accurate and reproducible and can be successfully employed for routine quality control analysis of drugs in pure as well as in pharmaceutical Dosage form. The main advantage of the developed method was its high Specifity for the estimation of Semaglutide and Liraglutide.

Key Words: Semaglutide, Leraglutide, Validation, Accuracy

1. Introduction:

Analytical chemistry plays an important role for development of drugs in pure and also in marketed formulation[1] and also ensures the amount of particular drugs can be easily determined[2]. Semaglutide is chemically known as L-histidyl-2-methylalanyl-L-α-glutamylglycyl-L-threonyl-L-phenylalanyl-L-threonyl-L-seryl-L-α-aspartyl-L-valyl-L-seryl-L-seryl-L-tyrosyl-L-leucyl-L-α-glutamylglycyl-L-glutaminyl-L-alanyl-L-alanyl-N^6-((*N*-(17-carboxyheptadecanoyl)-D-γ-glutamyl)amino-10-oxo-3,6,12,15-tetraoxa-9-azaheptadecanan-1-oyl)-L-lysyl-L-α-glutamyl-L-phenylalanyl-L-isoleucyl-L-alanyl-L-tryptophyl-L-leucyl-L-valyl-L-arginylglycyl-L-arginylglycine with chemical formula $C_{187}H_{291}N_{45}O_{59}$ and molecular mass of 4113.64 g/mol is a hormonal medication and is used in a number of birth control methods[3]. The Chemical structure of Semaglutide was shown in Figure 1. It mainly works by Semaglutide injection (Ozempic) is used along with a diet and exercise program to control blood sugar levels in adults with type 2 diabetes (condition in which the body does not use insulin normally and therefore cannot control the amount of sugar in the blood) when other medications did not control the sugar levels)[4].

Leraglutide is chemically (8R,9S,13S,14S,17R)-17-ethynyl-13-methyl-7,8,9,11,12,14,15,16-octahydro-6H-cyclopenta[a]phenanthrene-3,17-diol with a chemical formula $C_{172}H_{265}N_{43}O_{51}$. Veeran[5] and its molecular mass was found to be 3751 g/mol. Liraglutide injection (Victoza) is used with a diet and exercise program to control blood sugar levels in adults and children 10 years of age and older with type 2 diabetes (condition in which the body does not use insulin normally and therefore cannot control the amount of sugar in the blood) with other medications[6] The Chemical structure of Leraglutide was shown in figure 2.

The present work mainly focuses on developing and validating a reproducible, less cost, simple and rapid liquid chromatography method for determining Semaglutide and Liraglutide in marketed formulations. Instead of routine analysis, the use of a rapid and uncomplicated method is matter of highly importance[7]. The present strategy mainly focuses on developing a novel method having a shorter run time and also symmetrical peaks for both of the drugs. The Liquid Chromatography method was designed and subsequently validated to assess the performance characteristics. [8] - [16]

2. Experimental

2.1 Chemicals and Materials:

Semaglutide and Liraglutide references standards were obtained as gift samples from Spectrum Labs, Hyderabad, Telangana and the marketed formulation produced by Cipla Pharmaceuticals Pvt Ltd was obtained from local

market. Acetonitrile and phosphate buffer were purchased from Merck Ltd. (Mumbai, India). Milli-Q water was used throughout the study.

2.2 Wavelength Detection

Stock solutions of Semaglutide and Liraglutide of 1mg/ml were prepared in mobile phase and subsequent dilutions were done in order to get the final concentrations of 10µg/ml. Both the solutions were scanned in the range of 200 to 400 nm by UV Spectrophotometer and the spectra was recorded at 260nm and overlay UV spectrum of Semaglutide and Liraglutide was shown in figure 3.

2.3 Preparation of Mobile Phase

Mixture of water and Acetonitrile in the ratio of 20:80(200 ml of water, 800 ml of Acetonitrile) was transferred into 1000 ml volumetric flask and kept under sonication for 10 minutes, degassed and filtered through 0.45um membrane filter.

Diluent Preparation: Mobile phase is used as diluent

2.4 Preparation of standard solution of Semaglutide

10mg of Semaglutide working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and add about 7 ml of diluents is added and sonicated to dissolve completely and volume was made up to the mark with the same solvent. Further 0.3 ml of above stock solution was pipetted into a 10 ml volumetric flask and diluted up to the mark with diluent

2.5 Preparation of standard solution of Leraglutide

10 mg of Leraglutide working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and add about 7 ml of diluents is added and sonicated to dissolve completely and volume was made up to the mark with the same solvent. Further 0.3 ml of above stock solution was pipetted into a volumetric flask and diluted up to the mark with diluent

2.6 Preparation of Sample Solution

Weigh accurately 10 tablets and crushed in mortar and pestle and weight equivalent to 10 mg of Semaglutide and Liraglutide samples into a 10 ml clean dry volumetric flask and add about 7 ml of diluent and sonicate the above solution in order to dissolve completely the above samples and made up to the mark with the same solvent. From this pipette out 0.3 ml of above stock solution and transfer into 10 ml volumetric flask and fill the diluent up to the mark.

2.7 Chromatographic Conditions:

The liquid chromatographic analysis was performed using a Shimadzu HPLC system equipped with PDA Detector. Chromatographic separation of both the drugs was achieved by using a C_{18} µ-bondopak column having dimensions of 250*4.6 mm, 5 µm particle size and in an isocratic mode with mobile containing a mixture of water, Acetonitrile in the ratio of 20:80 (v/v). Elution of drugs was carried out at room temperature with a flow rate of 1 ml/min, injection volume of 20 µl and for a total run time of 12 min. Before injecting blank and drug solution the chromatographic system was equilibrated for 80 min with the degassed mobile phase. Blank solution was filtered through 0.22 µm nylon filter and injected to check the solvent interference.

Procedure: 20µl of the standard, sample are injected into the chromatographic system and the areas for Semaglutide and Liraglutide peaks are measured and the % assay is calculated by using the formulae.

3. Results

3.1 Method Development

Initially mobile phase optimization was carried out initially with Shimadzu C_{18} µ-bondopak column using buffer, Acetonitrile and water at different concentrations. During these trials the peaks are not eluted properly. Finally the mobile ratio was changed to 20:80 v/v at flow rate of 1 ml/min. With this composition of mobile phase the peaks were eluted satisfactorily. The peaks were well separated by following chromatographic parameters. The run time of the method was 10 minutes. Detection wavelength was at 260 nm. The prescribed method was validated as per the ICH guidelines.

3.2 System Suitability

The retention times of Semaglutide and Liraglutide were found to be 2.53 minutes and 3.26 minutes respectively. No major deviations are observed in the retention times during the analysis. The Percentage of related standard deviation (% RSD) of lowest concentration of individual six peaks was less than 2%. System suitability parameters are summarized. The chromatogram of standard solution was shown in figure 4 and the chromatogram of sample solution was shown in figure 5

3.3 Linearity

The peaks were eluted at different concentrations of standard solution and the calibration curve was plotted using peak area against concentration. Regression coefficient was found to be 0.999 for the above two drugs. The linear concentrations of Semaglutide and Liraglutide are in the range of 20-100 µg/ml for Leraglutide and 20-100 µg/ml for Semaglutide respectively. Linearity results of Semaglutide are tabulated below in table 1 and linearity results of Leraglutide are tabulated below in table 2. Calibration graphs of Semaglutide and Liraglutide were shown in figure 6 and Figure 7.

3.4 Accuracy

The accuracy of a method mainly depends on the recovery estimation study. The Actual, Ascending and Descending concentrations of standard solutions, blank are spiked at 50 and 150% against 100 %. Recovery studies were found to be satisfactory and are in the range of 98-102%. The results of accuracy for Semaglutide are tabulated in Table 3 and for Leraglutide accuracy results are tabulated in Table 4. The result has shown that the proposed method was accurate. Hence the developed method can be adopted in industry and as well as academics for the assay of Semaglutide and Liraglutide.

3.5 Precision

Precision studies indicate that the developed method has accepted values of validation for the estimation of above drugs in combination. The repeatability results of Semaglutide are tabulated in Table 5 and for Leraglutide are tabulated in Table 6. %RSD value was found to be less than 2. Deviations were not observed in inter and intraday analysis which reveals that the proposed method is more precise.

3.6 Specificity

The most important aspect of this method was its usage in the formulation analysis. Hence the marketed formulations were collected and analyzed by employing the above method. The test samples are prepared by weighing known amount of sample and diluted with the mobile phase. Later the solution was filtered through membrane filter and the solution was further diluted to get the equivalent concentration like that of standard solution. The two samples are injected separately and the concentration was calculated as per the standard formula. The results were found to be within the acceptable range

3.7 Limit of Detection and Limit of Quantification

LOD and LOQ was calculated for sensitivity measurement by dividing K* Standard deviation of peak response area with slope. Where k =3.3 and 10 for LOD and LOQ. Sensitivity means single to signal to noise ratio i.e., 3:1 and 10:1, calculated in terms of % RSD should not be more than 10%. LOD Chromatogram for Semaglutide and Liraglutide was shown in Figure 8 and LOQ chromatograms for Semaglutide and Liraglutide was shown in Figure 9.

4. Discussion

The marketed pharmaceutical dosage forms contain the above mentioned drugs and hence it is most important to have a mono method for the determination of the above mentioned ingredients. By keeping in mind the above criteria a simple , rapid method has been developed for the estimation of the above drugs. The Results obtained clearly reflects that the method is rugged and sensitive. The method developed has cleared all the validation parameters indicating that the method can be employed for routine quality control analysis

5. Conclusion

There are few liquid chromatography methods were available in the literature for the determination of the Semaglutide and Liraglutide. But majority of them are reported with single or with other combinations. The Proposed method was a simple, sensitive, accurate and rapid method for the assay of these drugs. The method developed shows lesser run time and an isocratic method has been developed for separation of drugs. The proposed Liquid Chromatography method simultaneously determines the amount of these drugs in pure and marketed formulations. The method is simple, accurate, precise and cost effective. This method can be employed for routine quality control analysis of the above mentioned drugs.

Conflict of Interest

The authors declare that they have no conflict of interest

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Figure 1: Structure of Semaglutide

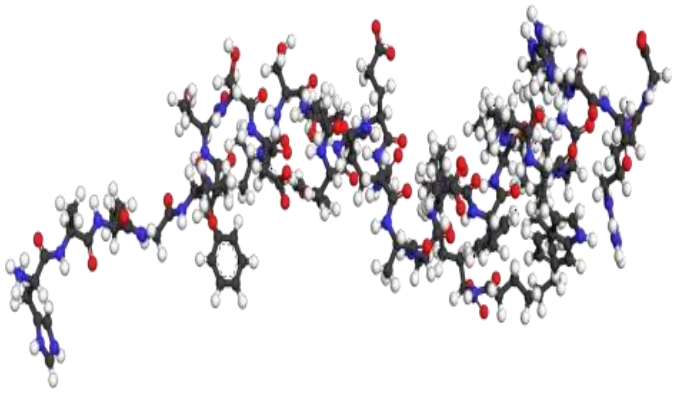

Figure 2: Structure of Leraglutide

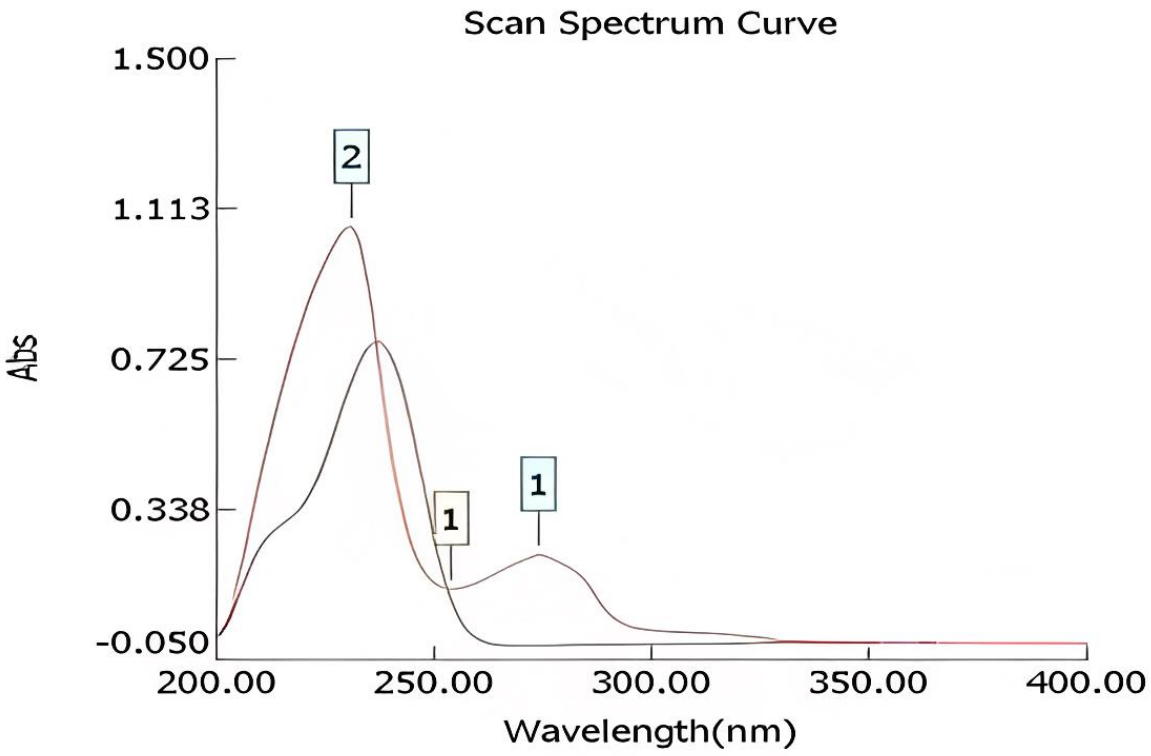

Figure 3: Overlay Spectrum of Semaglutide and Liraglutide at 260nm

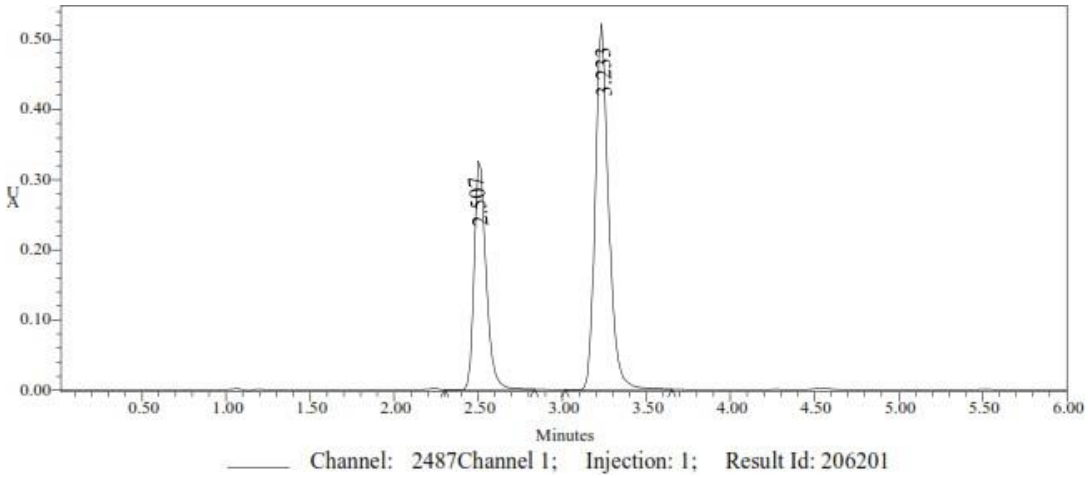

Figure 4: Chromatogram of Standard Solution

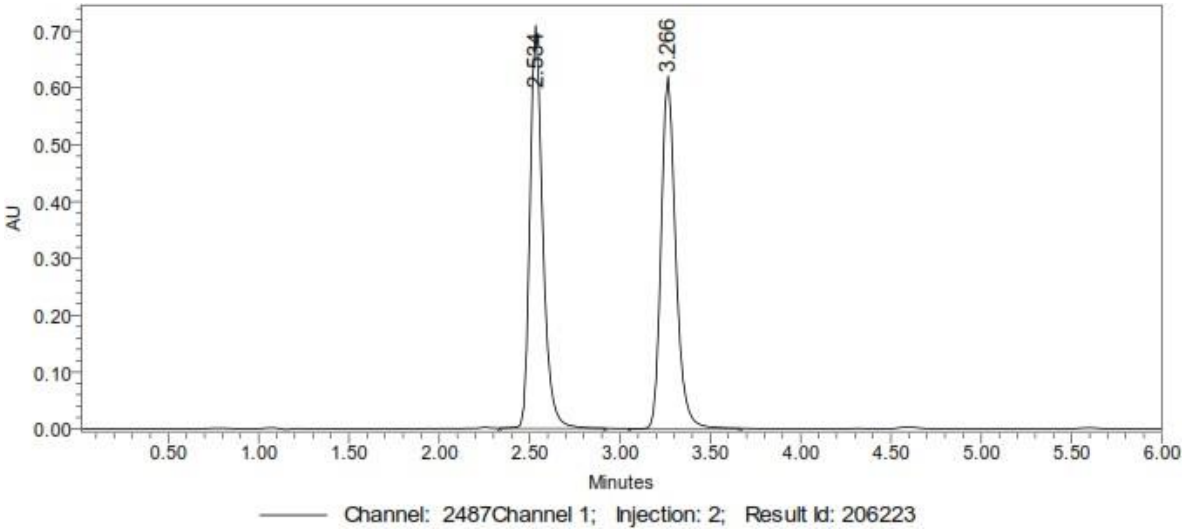

Figure 5: Chromatogram of Sample Solution

Table 1: Linearity Values of Semaglutide

Injection No	Concentration (µg/ml)	Peak Area
1	20	756892
2	40	1583785
3	60	2241771
4	80	2997573
5	100	3744464

Table 2: Linearity Values of Leraglutide

Injection No	Concentration (µg/ml)	Peak Area
1	20	496854
2	40	993761
3	60	1490625
4	80	2017587
5	100	248445

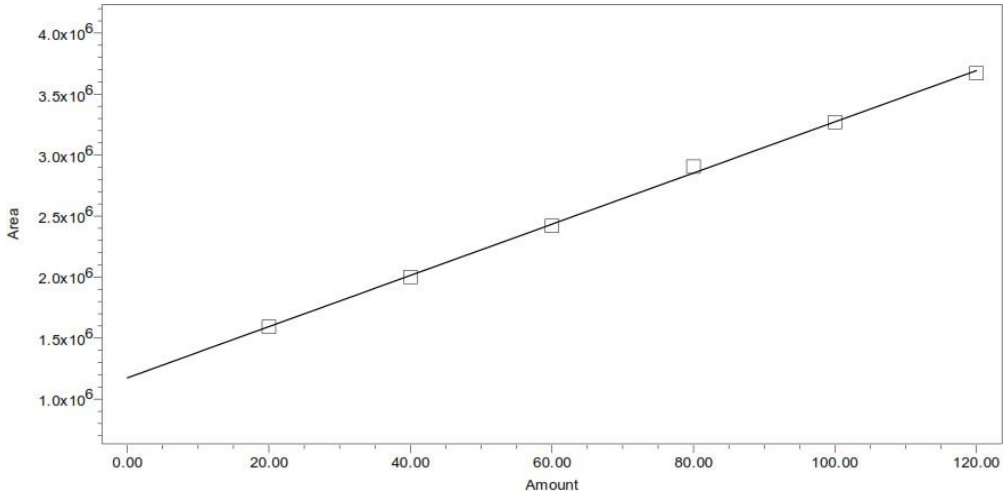

Figure 6: Calibration Graph of Semaglutide

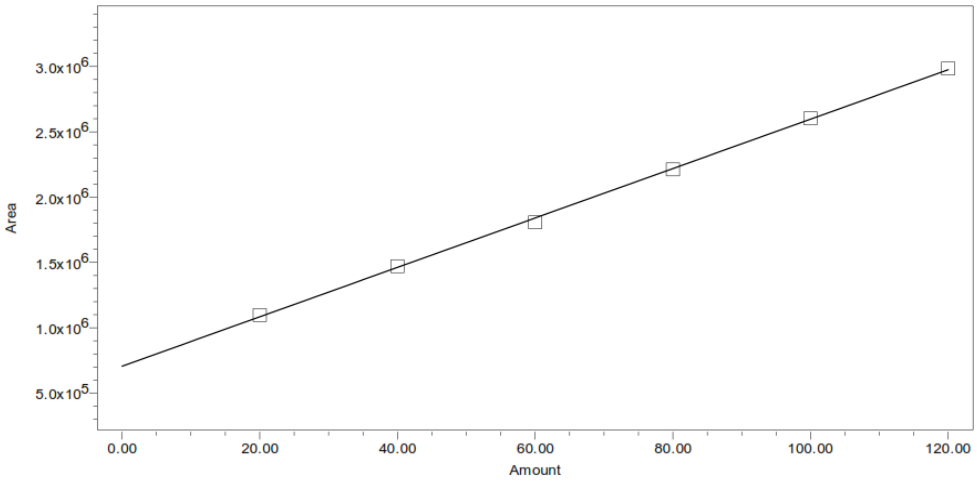

Figure 7: Calibration Graph of Leraglutide

Table 3: Accuracy Results of Semaglutide

% Con	Area	Amount added	Amount Found	% Recovery	Mean Recovery
50	3513866	5	5.10	101.3	100.5%
100	4735089	10	9.94	99.4	
150	5911797	15	14.8	99.2	

Table 4: Accuracy Results of Leraglutide

% Con	Area	Amount added	Amount Found	% Recovery	Mean Recovery
50	2332745	5	5.10	101.8	100.5%
100	3132694	10	9.99	99.9	
150	3918996	15	14.9	99.1	

Table 5: Repeatability Results of Semaglutide

Injection	Area
Injection-1	2235418
Injection-2	2240677
Injection-3	2249491
Injection-4	2245823
Injection-5	2251693
Average	2244605
Standard Deviation	6657.7
%RSD	0.33

Table 6: Repeatability Results of Leraglutide

Injection	Area
Injection-1	1501418

Injection-2	1486941
Injection-3	1490657
Injection-4	1487327
Injection-5	1490385
Average	1491347
Standard Deviation	5882.5
%RSD	0.39