A Quantitative RP-HPLC Approach for the Method Development and Validation for the Simultaneous Quantification of Semaglutide and Liraglutide in Pharmaceutical Dosage Forms

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Abstract

Semaglutide and Liraglutide are two GLP-1 RA approved for treatment of type 2 diabetes. The main aim of the present work is to develop a simple, precise, valid, speedy and decisive chromatographic strategy for the estimation of Semaglutide and Liraglutide quantitatively in fixed dosage form. Effective Chromatographic separation was achieved by using a Discovery C18 column of dimensions of (4.6 x 250 mm) and a particle size of 5µm. The mobile phase used was Phosphate buffer ph 4.0: ACN (pH 4.0) in the proportion of (30:70 % v/v). The mobile phase was pumped at 1.0 ml/min at an ambient temperature and the eluted analyte was identified at 254 nm. Semaglutide and Liraglutide were eluted with a mean retention time of 2.507 min and 3.233 min. The intended method was validated as per ICH (International Council for Harmonisation) guidelines, indicating a high degree of accuracy, precision, robustness, specificity and system suitability. The LOD (Limit of detection) and the limit of measurement did not exceed prescribed limit. The method linearity was found to be 0.992. The acceptance criteria of precision and Relative variance should be less than 2.0 which indicates that the method can be performed repeatedly. Reliability of the proposed method was assessed by evaluation of validation parameters like linearity, precision, specificity, accuracy, LOD, LOQ values as per ICH guidelines. The results obtained on the validation parameters met ICH and USP requirements. The proposed method of chromatography has been applied to dosage form without additives interference and is specific for the estimation of Semaglutide and Liraglutide.

Keywords: RP-HPLC; Quantification; Semaglutide; Liraglutide; Pharmaceutical Dosage Forms; Validation.

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Introduction

Semaglutide and Liraglutide are two GLP-1 RA approved for treatment of type 2 diabetes. The drug profiles of these two drugs are characterized by mild dose-related weight loss of 2-6 kg [1]. Currently, liraglutide is the only GLP-1 RA approved for the treatment of obesity in a dose higher than that approved for the treatment of type 2 diabetes [2]. Semaglutide is another GLP-1 RA that has been approved for the treatment of type 2 diabetes in doses ranging from 0.5 to 1.0 mg given subcutaneously OW and as an oral formulation in doses ranging from 0.5 to 14 mg once daily [2]. Semaglutide is currently being evaluated for future approval for the treatment of obesity. A development programme was launched on Semaglutide Treatment Effect in People with Obesity (STEP), which included 5 phase 3 clinical trials (STEP 1–5) to evaluate the efficacy and safety of OW semaglutide at this high dose of 2.4 mg for the treatment of patients with obesity with and without diabetes [3]. The mechanisms of liraglutide and semaglutide weight loss are similar in general. Both medications have been shown to reduce appetite and hunger while increasing fullness and satiety [4, 5]. Furthermore, OW semaglutide 2.4 mg, but not liraglutide, has been shown to reduce food cravings [5]. Animal studies have shown that GLP-1 receptors in the hypothalamus and hindbrain mediate the anorexigenic effect of semaglutide [6, 7]. Delay in gastric emptying, a class effect of all GLP-1 RAs, may contribute to the sensation of early fullness [8]. In the meantime, one study with a relatively long follow-up period (52 weeks) discovered that improvements in hunger and fullness with OD liraglutide 3.0 mg peak after 4 weeks, then gradually decline and return to baseline after 40 weeks. [6] There have been no comparable follow-up studies for semaglutide.

The current strategy is primarily concerned with the development and validation of a novel reversed-phase chromatographic method for the estimation of Semaglutide and Liraglutide in bulk and pharmaceutical dosage forms. Following a thorough review of the literature on chromatographic analysis of various dosage forms, an attempt was made to develop a new rapid, valid, quick, and accurate method for quantitative estimation Semaglutide and Liraglutide. According to the literature, no method has been developed for simultaneous quantification of Semaglutide and Liraglutide in combined dosage forms. Only a few mono component analysis have been developed. [9] This is the first method that has been developed with this combination. There have been few reports of HPLC methods for determining and estimating Semaglutide and Liraglutide alone and in combination with other drugs. Two methods for estimating Semaglutide and Liraglutide alone with metabolites were reported using LCMS/MS. [10, 11]

Methodology

Instrument used

HPLC Alliance Waters 2695 Empower UV double beam UV 3000 UV Win 5 Lab India.

Chemicals and Reagents

Semaglutide and Liraglutide API were obtained as a gift sample from Dr Reddy's labs, Hyderabad. Water HPLC Grade was procured from Merck, Methanol HPLC Grade was procured from Merck, Acetonitrile HPLC Grade procured from Merck, Potassium Dihydrogen procured from Merck A.R.

Preparation of mobile phase

A mixture of Phosphate buffer pH 4.0, 30.0 mL (30%) and 70.0 mL of ACN 70%) were taken and degassed in ultrasonic water

bath for 5 minutes. Then this solution was filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation

Mobile phase was used as Diluent**.**

Preparation of Semaglutide standard

The working standard semaglutide 10 mg was precisely weighed and transferred to a 10 ml volumetric flask, to which approximately 2 ml of DMF was added. The solution was then sonicated to completely dissolve it before being diluted to the desired volume with the diluent. 10.0 mL of the above stock solution was transferred into a 100 mL volumetric flask and the diluent was used for adjusting the volume.

Preparation of Liraglutide standard

The working standard was prepared by exactly taking Liraglutide 10 mg and transferred to a 10 ml volumetric flask, to which 2 ml of DMF was added. Then it was sonicated to completely dissolve it and diluted to the desired volume with the diluent. The stock solution of 10.0 ml was taken using a pipette into a 100 ml volumetric flask and the diluent was used for making up the volume.

Preparation of Sample Solution:

Accurately, 10 tablets were weighed and crushed in a mortar and pestle, and the weight equivalent to 10 mg of Semaglutide and Liraglutide (marketed formulation) sample was placed in a 10 mL volumetric flask, and approximately 7 mL of Diluent was added and sonicated to dissolve the contents completely, and the volume was increased with the same solvent. The above stock solution 3 ml was pipetted into a 10 ml volumetric flask and altered with diluent upto the mark.

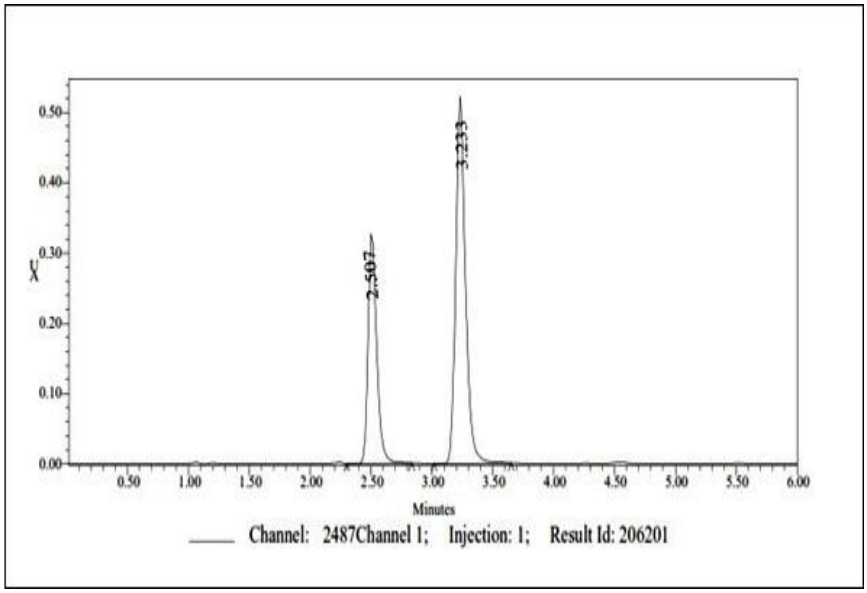

Fig 1 Optimized Chromatogram

Linearity

Linearity can be evaluated by introducing a series of standard stock solutions/diluted stock solution into the mobile phase/solvent with a minimum of five different concentrations ranging from 50–150 % of the expected working range.

Accuracy

The accuracy of the method was established by spiking the sample matrix of interest with a known concentration of analyte standard and then evaluating the sample using the "method being validated."

Preparation of standard solution (Semaglutide and Liraglutide)

The weight equivalent to 10 mg of Semaglutide and Liraglutide (marketed formulation) sample was placed in a 10 mL volumetric flask, and approximately 7 mL of Diluent was added and sonicated to dissolve it completely, and the volume is increased with the same solvent. (Stock solution) An additional 3 ml of the above stock solution was pipetted into a 10 ml volumetric flask and diluted to the mark with diluent.

Precision

Repeatability

The HPLC system was injected five consecutive times with the standard solution and the area of each injection was measured. The % RSD of the area of five replicate injections was found to be within the prescribed acceptable range.

Intermediate Precision (Ruggedness)

Precision is estimated by introducing a series of standards or evaluating a series of samples taken from a homogeneous lot. Precision is calculated as the relative standard deviation (% RSD) of the measured standard deviation (SD) and Mean values. To estimate the intermediate precision (Ruggedness) of the method, Precision studies were conducted on different day by using same dimensions of different make columns.

Specificity

The specificity was evaluated by injecting blank. The system suitability for specificity was carried out to estimate whether there is any interference of any impurities in retention time of analytical peak.

Range

It can be concluded that the assay method is precise, linear and accurate in the range of 1µg-5µg and 100µg- 500µg of Semaglutide and Liraglutide respectively based on precision, linearity and accuracy data.

Robustness

Robustness studies were evaluated by making deliberate changes in the flow rate, composition of mobile phase and variation in temperature was made to evaluate the impact on the method. Flow rates ranged from 0.8 ml/min to 1.2 ml/min. A standard solution containing 3 ppm Semaglutide and 300 ppm Liraglutide was prepared and analyzed using the different flow rates as well as the method flow rate. The organic composition of the mobile phase was varied from 65 to 75 % standard solution. 3 µg/ml Semaglutide and 300 µg/ml Liraglutide were prepared and analyzed in the method using the varied mobile phase composition as well as the actual mobile phase composition.

LOD

LOD's can be measured based on the standard deviation of the response (SD) and the slope of the calibration curve (S) at levels approximating the LOD according to the formula. The standard deviation of the response can be evaluated based on the standard deviation of y-intercepts of regression lines.

LOQ

LOQ's can be measured based on the standard deviation of the response (SD) and the slope of the calibration curve (S) according to the formula. The standard deviation of the response can be evaluated based on the standard deviation of y- intercepts of regression lines.

RESULTS AND DISCUSSION

Linearity

The chromatographic HPLC system was injected with each level and the peak area was observed. The correlation coefficient was measured by plotting a graph of peak area versus concentration by taking concentration on X-axis and peak area on Y-axis. The linearity results for Semaglutide and Liraglutide are shown in table 1 and table 2 and chromatograms are depicted in figure 2 & figure 3

Table 1 Linearity Results for Semaglutide

S. No	Linearity Level	Concentration	Area
1	I	20 ppm	471543
2	II	40 ppm	656277
3	III	60 ppm	794999
4	IV	80 ppm	946124
5	V	100 ppm	1002139
Correlation Coefficient			0.999

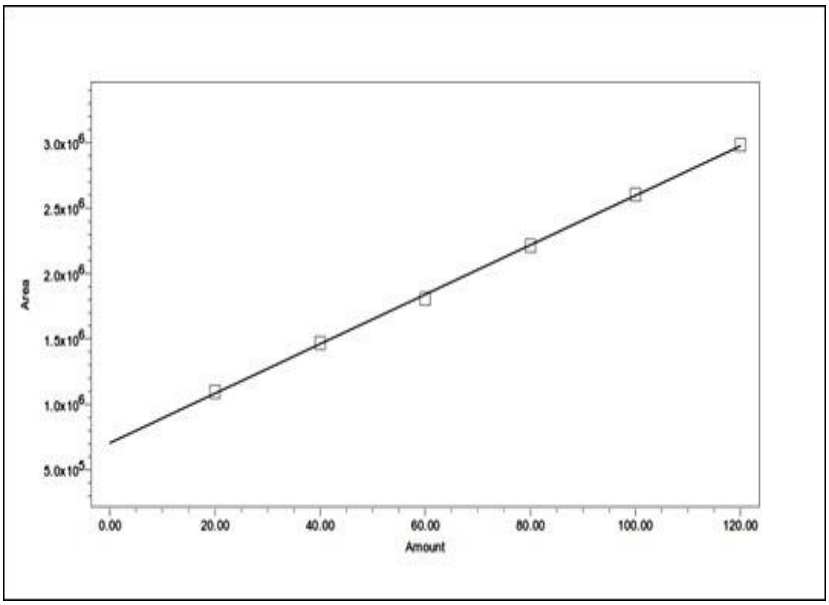

Fig.2 Calibration curve of Semaglutide

Table 2 Linearity Results for Liraglutide

S. No	Linearity Level	Concentration	Area

1	I	20 ppm	471543
2	II	40 ppm	656277
3	III	60 ppm	794999
4	IV	80 ppm	946124
5	V	100 ppm	1002139
Correlation Coefficient			0.999

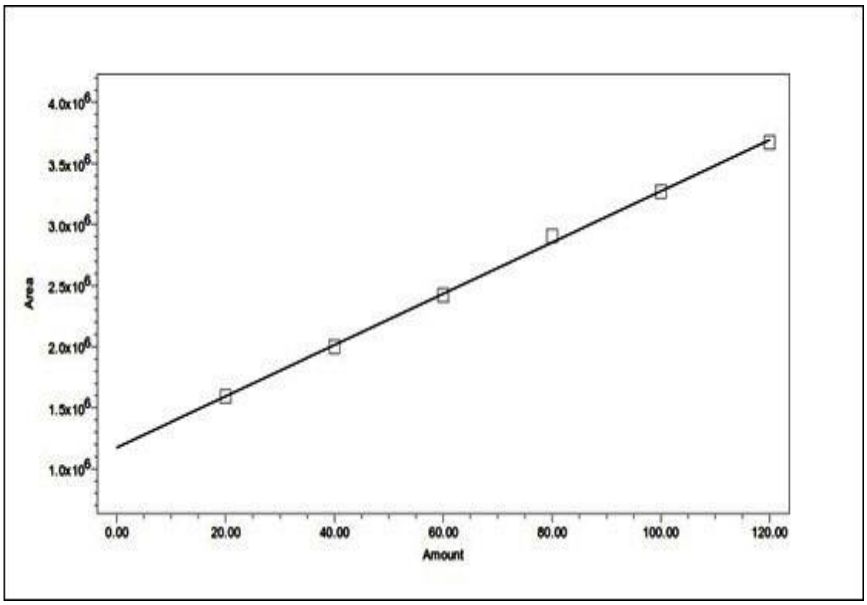

Fig. 3 Calibration curve of Liraglutide

Accuracy

Injections were made with the standard solution, Accuracy -50 %, Accuracy -100 %, and Accuracy -150 % solutions. The Amount discovered and Amount added for Semaglutide and Liraglutide, as well as the individual and mean recovery values, were calculated. The accuracy results for Semaglutide and Liraglutide are shown in table 3 and table 4

Table 3 Accuracy results of Semaglutide

% Concentration (at specification Level)	Area	Amount added (m)	Amount found (m)	% Recovery	Mean Recovery
50%	1426646	5	4.9	101.8%	102.5%
100%	2551005	10	9.98	99.9%	
150%	2139845	15	15.0	100.0%	

Table 4 Accuracy results of Liraglutide

% Concentration (at specification level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	975578	5	5.0	101.3%	101.0%
100%	1718370	10	9.96	99.6%	
150%	1465857	15	14.9	99.3%	

Precision

Repeatability

The standard solution was injected five times, and the areas of all five injections were measured in HPLC. The % RSD for the area of five replicate injections was found to be within the limits specified. Repeatability results of Semaglutide and Liraglutide are tabulated in Table 5 & 6

Table 5 Repeatability results of Semaglutide

	Name	RT	Area	Height (µV)	USP Plate count	USP Tailing
1	Semaglutide	2.506	1553631	316525	6346.5	1.3
2	Semaglutide	2.516	1508002	296974	6197.1	1.2
3	Semaglutide	2.519	1545624	307327	6184.0	1.3
4	Semaglutide	2.531	1542374	302327	6176.0	1.2
5	Semaglutide	2.544	1561368	302525	6382.1	1.3
Mean			1542200			
Std Dev			20490.0			
% RSD			1.33			

Table 6 Repeatability results of Liraglutide

	Name	RT	Area	Height (µV)	USP Plate count	USP Tailing
1	Liraglutide	3.230	2790686	497608	7950.1	1.2
2	Liraglutide	3.239	2661482	468477	8046.5	1.2
3	Liraglutide	3.246	2706096	474632	8054.1	1.2
4	Liraglutide	3.257	2703419	473234	8171.8	1.2
5	Liraglutide	3.271	2695932	474830	8068.3	1.2
Mean			2711560			
Std Dev			47796.3			
% RSD			1.76			

Limit of Detection and Limit of Quantification for Semaglutide and Liraglutide

The term LOD refers to the lowest concentration that the instrument can detect but not quantify, and the noise to signal ratio for LOD should be 1:3. The term LOQ refers to the lowest concentration that the instrument can detect and quantify. For LOQ, the noise to signal ratio should be 1:10. The LOD & LOQ results for Semaglutide and Liraglutide are shown in table 5 and table 6 and chromatograms are depicted in figure 6 & figure 7

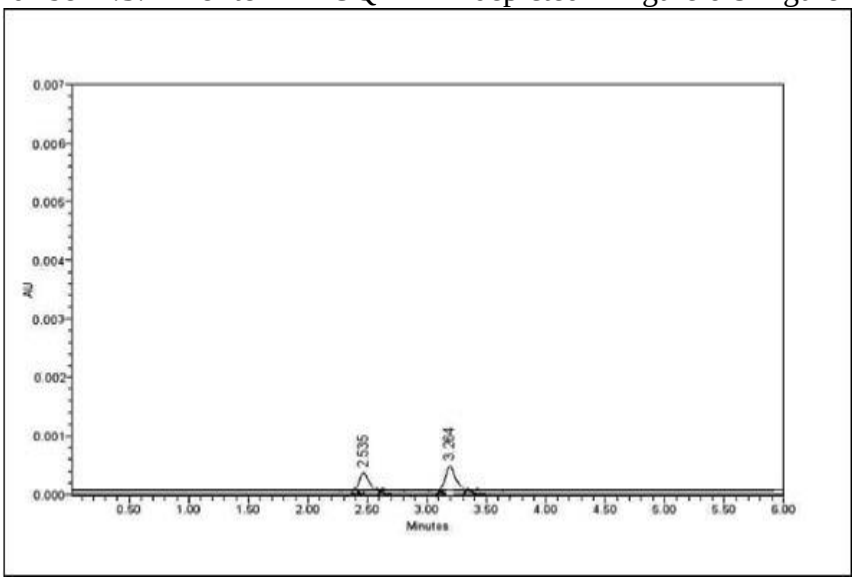

Fig. 4 Results of LOD

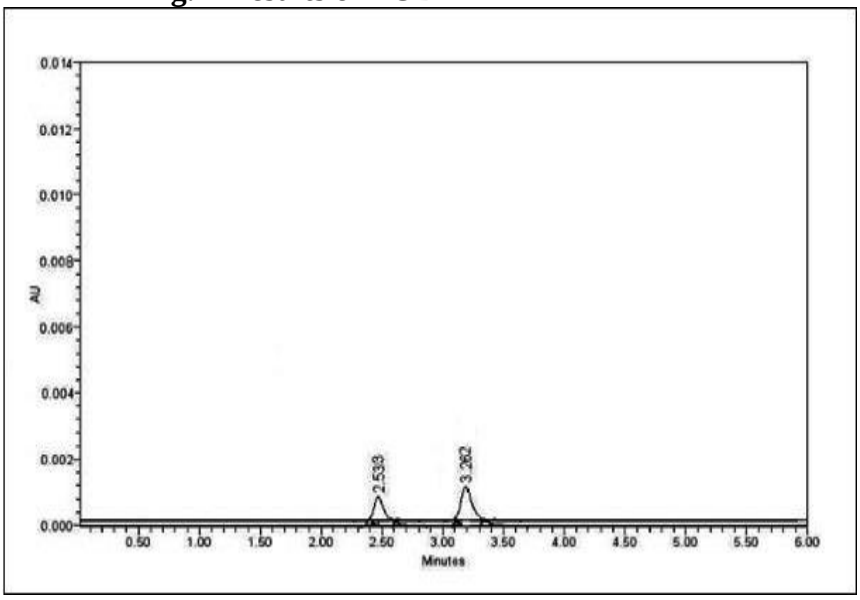

Fig. 5 Results of LOQ

Discussions

In RP-HPLC method, the conditions were optimized to obtain an adequate separation of eluted compounds. Initially, various mobile phase compositions were tried, to separate title ingredients. Mobile phase and flow rate selection was based on peak parameters (height, tailing, theoretical plates, capacity or symmetry factor), run time and resolution. The mobile phase containing mixture of orthophosphoric acid buffer solution: mobile phase ratio was Phosphate buffer (0.05M) pH 4.6: ACN (30:70%v/v) (pH was adjusted with orthophosphoric acid) with a flow rate of 1ml/min is quite robust. Robustness of the proposed method was determined by varying various parameters, the % RSD reported was found to be less than 2 %. The proposed method was validated in accordance with ICH parameters and the applied for analysis of the same in marketed formulations.

Conclusion

A systematic and practical approach was utilized to develop an efficient and robust RP-HPLC method for the separation of drugs. Different trials were carried out to determine the optimized chromatographic conditions and initial attempt was performed by utilizing low proportion of organic solvents for the elution of compounds by reducing retention time of the compounds. The acceptable results were achieved by the proposed chromatographic conditions. The proposed method was easy, speedy and measurably substantial. During the analysis of drug no interfering peak was found within the chromatogram indicating that there is no excipient interference.

Acknowledgements

Nil

Conflict of Interest

The authors do not declare any competing interest

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