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Development of a Novel Method for Determination of Semaglutide in Tablet Dosage Form using RP-HPLC

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Abstract

A simple and precise RP-HPLC method for the estimation of Semaglutide in tablet dosage form was developed and validated. The chromatographic separation of the drug was achieved with a Ascen tis® C-18 HPLC column (250mm x 4.6mm i. d., particle size 5µ) using 0.1% TFA and acetonitrile (35:65v/v) as the mobile phase.

The instrument was set at flow rate of 1.2 $mLmin^{-1}$at ambient temperature and the wavelength of UV-visible detector at 225nm. The method showed excellent linearity over a range of 15-120 μgmL^{-1} for the drug. The correlation coefficient for Semaglutide was noted to be 0.9998. The mean recovery values were found to be 99.53% and 100.09%.

The proposed method could be suitable for quantitative determination of Semaglutide in pharmaceutical dosage forms and also for quality control in bulk manufacturing. The *f*-test and *t*-test at 95% confidence level were subjected on data for statistical analysis.

Keywords: RP-HPLC TFA, Acetonitrile, Semaglutide.

Introduction

Semaglutide (SG) is a long-acting glucagon-like peptide-1 (GLP-1) receptor agonist developed for the management of type 2 diabetes mellitus and chronic weight management.

It is a synthetic peptide comprising 31 amino acids with approximately 94% structural homology to native human GLP-1.

Structural modifications, including amino acid substitutions and attachment of a C18 fatty diacid side chain, enhance albumin binding, increase resistance to degradation by dipeptidyl peptidase-4 (DPP-4), and prolong its elimination half-life to approximately one week, allowing once-weekly administration. SG exerts its pharmacological effects by selectively activating GLP-1 receptors, thereby stimulating glucose-dependent insulin secretion, suppressing glucagon release, delaying gastric emptying, and promoting satiety.

These actions contribute to improved glycaemic control while reducing the risk of hypoglycemia and facilitating clinically significant weight loss. In addition to its efficacy in type 2 diabetes, SG has demonstrated cardiovascular and metabolic benefits in patients with obesity and related co morbidities, leading to its widespread clinical acceptance.

It is currently available as a once-weekly subcutaneous injection and as an oral tablet formulation for selected indications. The increasing therapeutic use of SG has created a growing demand for robust analytical methods to ensure its identity, purity, potency, and quality throughout pharmaceutical development, manufacturing, and routine quality control.

The objective of this work was to develop a simple liquid chromatography method that could serve as an assay method for SG in marketed dosage forms.

A survey of literature revealed the availability of a few analytical methods for the quantitative determination of SG in biological fluids and marketed formulations. The reported methods were mainly based on liquid chromatographic estimation using fluorescence, electrochemical, or mass spectrometry detectors[1-9]. Hence, an attempt was made to develop a simple, precise, and accurate method for the estimation of SG in pharma ceuticaldos age forms. The manuscript describes the development and validation of an isocratic reversed-phase HPLC method with a UV-Vis detector for the assay of SG in accordance with ICH guidelines.

Experimental

Chemicals and Reagents

Methanol and acetonitrile were procured from Thermo Fisher Scientific India Pvt. Ltd. New Delhi, India. Trifluoroacetic acid (TFA) was procured from Central Drug House (P) Limited, New Delhi, India. Mili Q water was used throughout the study. Other chemicals used in this study were of analytical or HPLC grade.

Instrumentation

The analysis was carried out on Waters Alliance e-2695 separating module (Waters Co., MA, USA) using photo diode array detector (waters 2998) with auto sampler and column oven. The instrument was controlled by Empower software (version 6.00.00.00) installed with equipment for data collection and acquisition. Ascentis® C-18 HPLC column (250mm x 4.6mm i. d., particle size 5µ) was used for separation with mobile phase at the flow rate of 1.2mLmin^{-1} was used.

Chromatographic Conditions

The mobile phase consisted of 0.1% TFA and acetonitrile (35:65 v/v). Measurements were made with injection volume 10 µL and UV detection at 225nm. All analyses were performed at ambient temperature.

Standard and Sample Solutions Preparation

- Preparation of Standard Stock Solution

Standard solution was prepared by dissolving the standard drug in the solvent and diluting to the desired concentration by mobile phase as solvent system. Accurately weighed 50mg of SG was transferred into a 50mL volumetric flask and dissolved in the mobile

phase. The solution was further diluted with mobile phase to obtain the desired concentration [10-15].

- Preparation of Sample Solutions

The method was used for quantitation of SG in the marketed formulation (Ry belsus®). For sample preparation mobile phase was used as solvent. Powder equivalent to 25mg of SG was transferred in to25mL volumetric flask and dissolved in 10mL of mobile phase. Volume was made up to the mark.

The solution was ultra solicited for 30 min and filtered through a 0.45-micron membrane filter. The solution was further diluted with mobile phase to obtain desired concentration and was subjected to HPLC analysis as described earlier. From the peak area of SG the amount of drugs in samples was computed[16-18].

Method Validation

The optimized chromatographic conditions were validated by evaluating specificity, range, linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), robustness and system suitability parameters in accordance with the ICH guidelines Q2 (R1). To assess the linearity and range of the developed method, eight different standard concentrations (15, 30, 45, 60, 75, 90, 105, and 120 μgmL^{-1}) of SG were prepared. The analyses were performed in triplicate. The peak area values were plotted against corresponding concentrations.

The accuracy and precision were measured by performing the assay of samples (spiked placebos) prepared at three concentration levels of 80%, 100% and 120% of the standard concentration, with 3 replicates for each concentration. The %recovery and % relative standard deviation (RSD) were calculated for each replicate sample.

The limit of detection (LOD) and limit of quantification (LOQ) of the method were calculated based on the standard deviation of the response (σ) and slope approach as defined in ICH guidelines. The LOD was calculated using the formula 3.3*σ/slope, and the LOQ was calculated using the formula 10*σ/slope.

Robustness of the method was investigated under a variety of conditions, including flow rate, wavelength, and percentage of solvent in the mobile phase (10-19).

Results and Discussion

In this study, a HPLC method for the determination of SG in bulk drug and pharmaceutical formulations with UV detection was developed and validated as per ICH guidelines for analytical method validation[10-19].

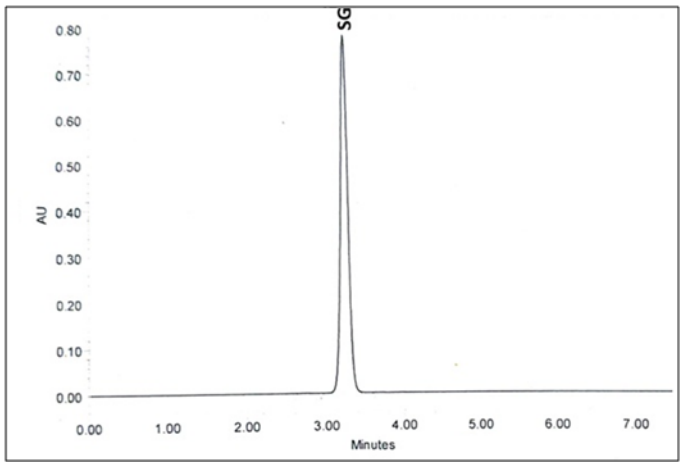
Method Development

The main objective of this work was to develop a HPLC method for determination of SG within a short run time below 7 min and symmetry between 0.80 and 1.20. The stationary and mobile phases play an important role on theoretical plates, peak shape, symmetry and resolution. To obtain symmetrical peaks with better resolution and peak purity, various chromatographic conditions were investigated and optimized for the determination of SG; such as mobile phases with different composition, and stationary phases with different packing material etc. Finally, the mobile phase containing0.1% TFA and acetonitrile (35:65 v/v) was selected and found to be optimal with more theoretical plates (≥ 24908) and short retention time (3.4, below7 min).

Based on the optimal mobile phase, a highly symmetrical and sharp characteristic peak of SG was further obtained on Ascent is® C-18 HPLC column (250mm x 4.6mm i. d., particle size 5µ) with 1.2 $mLmin^{-1}$flow rate.

A typical HPLC chromatogram of standard solution of SG is given in Figure 1[10-19].

Figure 1: HPLC chromatogram of the standard solution of SG.



Method Validation

An optimized method must be validated before actual use. System suitability testing was performed as per ICH guidelines for analytical method validation, Q2 (R1). The validation studies were performed as prescribed in the following sections. Linear regression data demonstrated an excellent relationship over a concentration range of 15-120 µgmL^{-1} for SG. The linear regression equation for SG was found to be y = 202111x -81003. The regression coefficient value (r^2) was found to be 0.9998 indicating a high degree of linearity. The linearity curve of SG is given in Figure 2 and linearity parameters for the SG are given Table 1. The specificity studies depicted the complete absence of any other excipients as no peak was reported during the retention time of SG. Standard deviation and slope-based method was adopted for determining the LOD and LOQ which was respectively found to be 1.2 and 3.8µgmL^{-1} for SG. The values indicate that the method is sensitive. The lower values of % RSD 0.72 and 1.09 for intra-day and inter-day precisions respectively (Table 2) indicate that the method is precise. Recovery study was carried out using standard addition method at three different levels of 80%, 100% and 120%.

The average % recoveries for SG in marketed formulation were found to be between 99.53and 100.09 (Table 3). The results revealed that there was no interference. The developed method was successfully applied to analyze SG in marketed formulation. The amounts recovered were expressed as percentage of the label claim. The average percentage of drug contents of formulation obtained by the proposed method for SG was noted to be 99.57%. These values comply with the official specifications (10-19).

Figure 2: Standard curve of SG

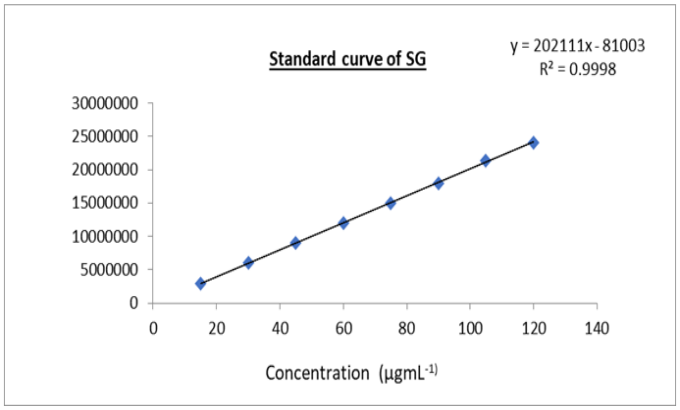


Table 1: Linearity parameters for SG.

Linearity Parameter	SG
Range	15-120 µgmL^{-1}
Slope	202111
Intercept	81003
Regression coefficient (r^2)	0.9998
f-test	1.80 (5.92)[a]
t-test	0.57 (3.99)[a]

Table 2: Statistical treatment of the precision data.

Parameter	SG
Intraday or Repeatability (%RSD)	0.72
Inter day (%RSD)	1.09
f-test	3.82 (6.27)[a]
t-test	1.89 (2.40)[a]

Table 3: Percent recovery data SG.

% simulated dosage nominal	% Mean (n=3)	RSD (%)
80	100.02	0.85
100	100.09	1.06
120	99.53	0.77

System Suitability Parameters

For system suitability parameters, six replicates of standard solution were injected. All critical parameters met the acceptance criteria on all days. Parameters such as resolution, capacity factor, tailing factor, theoretical plate and retention volume of the peaks were calculated. The results are shown in Table 4 (10-19).

Table 4: System suitability data for SG

Parameters	SG
Retention time (min)	3.4
Injection precision RSD (%)	0.39
Resolution	-
Tailing factor	1.10
Theoretical plates	24788
Capacity factor	0.76
Retention volume	4.08

Conclusion

A simple isocratic reversed phase HPLC method for estimation of SG was developed and validated as per ICH guidelines. Validation experiments proved that the HPLC method is linear inthe proposed working range as well as accurate, precise and specific. The good recovery percentage of marketed formulation suggests that the excipient have no interference in the determination. The RSD (%) was also less than 2showing a high degree of precision of the method. The proposed method was also found to be robust with respect to flow rate and composition of mobile phase. In addition, simple isocratic elution and easy extraction procedure offered rapid and cost-effective analysis of the drugs. F-test and *t*-test were applied to the data at 95% confidence level, and no statistically significant difference was observed. The proposed method can be used for routine analysis of SG in marketed dosage forms and in the quality control in bulk manufacturing as well.

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