A novel RP-HPLC method development and forced degradation studies for semaglutide in active pharmaceutical ingredients and pharmaceutical dosage forms

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**ABSTRACT**
This research objective is for the development of a specific and simple method to trace Semaglutide presence in active pharmaceutical ingredient and pharmaceutical dosages. As part of a study on Semaglutide drug, solvents of HPLC grade waters HPLC instrument (Empower software) with PDA detector, ultrasonicator (Make: Labman) and pH meter (Make: Adwa) are used. The Method was optimized with mobile phase with a composition of buffer and solvent were of 60:40%v/v, flow maintained was 1.0ml/min, the injection volume of 10μl, run time was 5min. All separations were performed with PDA detector and column used was Discovery C18 150 x 4.6mm, 5μ. Results for the developed method are accurate and specific. The detection wavelength was 292 nm, the retention time for Semaglutide was 2.689min, linearity resulted with r²= 0.9998, % RSD for precision was 1.0; %mean recovery for accuracy was in the range of 99.73 to 100.29. This study report is for industrial application for determining Semaglutide presence in pharmaceutical ingredient and dosages.

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**INTRODUCTION**
In type-2 diabetes (Novo Nordisk, 2016) treatment Semaglutide is used. It is a glucagon-like peptide-1 receptor agonist (Marso et al, 1834). It lowers the blood sugar level by increasing the production of insulin. Glucagon-like peptide-1 has 2-aminoisobutyric acid and arginine whereas Semaglutide has two amino acid substitutions at positions 8 and 34 (Lau et al, 2015). Semaglutide was approved by US FDA in 2017. It can be used as an injection-type or oral-type drug (Davies et al, 2017). Researchers at the University of Leeds and Novo Nordisk reported in 2017 that it could also be used for the treatment of obesity (Blundell et al, 2017).

Semaglutide Figure 1 is chemically known as 17-\{[(1R)-3-{2-[2-{2-{2-[2-{2-[2-{2-{2-{2-[2-{2-[2-{2-[2-[(2S,3R)\-2-

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From the literature survey, it was known that there were no methods developed and validated for the determination of Semaglutide with RP-HPLC. So this study on RP-HPLC was developed with an aim to keep this research method simple, sensitive and accurate. This method was developed and validated with accuracy, linearity, Limit of Detection, Limit of Quantification, precision, specificity, other validations and degradation related studies.

MATERIAL AND METHODS

Materials: The drug Semaglutide was kindly gifted by Spectrum Laboratories, Hyderabad. HPLC water, acetonitrile and OPA used are of Merck grade. Instruments like Waters HPLC (Empower software), ultrasonicator (Make: Labman) and pH meter (Make: Adwa) are used for developing this method.

Instrument and its conditions

A study performed on HPLC (Make: Waters) which has detector PDA. Empower software was used for analysis of data derived as a part of the study. Chromatographic separation was performed on column Discovery C18 150 x 4.6mm, 5µ. Gradient binary pump was used with ambient column temperature. The components of the mobile phase used for this gradient elution are Buffer (0.1% OPA): Acetonitrile (60:40 %v/v). Volume considered for injection was 10 µl. Detection performed at 292nm by the help of a detector.

Selection of method and Mobile phase

Solvent and buffer composition were 40:60%v/v for this study. 0.1% Ortho Phosphoric Acid was prepared by considering 1ml makeup to 1000ml with the help of Milli-Q Water in-order to make-up the mobile phase.

Standard preparation

2.5mg Semaglutide working Standards was measured and transferred into 25 ml clean and dried glass flask, diluents 10ml was added, sonication done for 10 min. And volume was made with diluents (100µg/ml Semaglutide).

Sample preparation

1.5ml injection volume (drug) equivalent for 2.0 mg of Semaglutide taken to a 50ml analytical glass flask, 10ml diluent added and mixing performed for 25 minutes. Derived the required quantity of volume with diluents HPLC filter used for the filtration process. 0.5ml of above-prepared solution transferred to the 10ml analytical flask for volume make with diluents (10µg/ml Semaglutide).

Details of Validation

Specificity: Drug quantities were determined with this study by the support of dilutions in appropriate quantities for determining the chromatographs. The drug was pre-weighed and used for spiking during the study.

Suitability of System: Method developed with this parameter to observe the performance-related factor. It is used to ensure adequate performance of the chromatographic system.

Linearity and Range: In the chromatographic method, linearity generates data which helps to evaluate the correlation coefficients. These are related to the analyte concentration and proportionality with the given range. Analyte extents with respect to upper and lower intervals were determined in this range system. The linearity of Semaglutide was 2.5 µg/ml to 15 µg/ml. The correlation was derived as 0.9998.

Precision: This study has two parts; they are interring day (between days) and intraday (same day). The study considered by estimating the equivalent response five times for interday and intraday. These results are linked to the RSD (Relative Standard Deviation). Studies related to repeatability done by response estimation of concentrations of drug analysed. The generated results are reported in terms of percentage, termed as percentage relative standard deviation.

Accuracy: Recovery calculated for Semaglutide by the method of Spiking. Amount of known quantity drug was added for a solution sample which is pre-qualified. Estimations were done by measuring the curve. Curve derived by calibration with the help of peak area determination and applying the peak areas for deriving the straight line equation.

Detection/ Quantification limits

The level of quantification (LOQ) and detection (LOD) were conducted with the application of a signal to noise methodology.

Degradation studies

Oxidation: 1 ml of Semagludite stock solution, 1 ml hydrogen peroxide with a concentration of 20
percent added. For 30 minutes' solution was kept at 60°C. During this study, the solution was diluted in order so that 10ppm solution can be obtained. 10 µl of the obtained solution was considered for injection into HPLC, and recorded graphs were used for the analysis of samples stability. It is the stability of the prepared sample used for the study.

**Degradation Study of Acid**

1 ml of Semaglutide stock solution, 1 ml hydrogen peroxide with a concentration of 2N added. This preparation was refluxed for 30 minutes by considering 60°C. During this study, the solution was diluted in order so that 10ppm solution can be obtained. 10 µl of the obtained solution was considered for injection into HPLC, and recorded graphs were used for the analysis of samples stability.

**Alkali Studies**

To 1ml of 2N Sodium hydroxide, 1ml of a prepared stock solution of Semaglutide was added, and the obtained solution was diluted to 10ppm. Injection of 10 µl was taken into the HPLC system and recorded the chromatographs. Activities performed for 30min by considering 60°C.
Degradation Studies of dry heat

Maintained conditions are for 6 hours with 105°C. During this study, the solution was in over with stated conditions. Solution diluted to 10ppm. Injection of 10 µl was taken into the HPLC system and recorded the chromatographs.

Photo Stability studies

This study performed by considering 10ppm solution and it is exposed to UV light with the help of UV chamber for 7 days with photostability chamber. The derived solution was diluted for the study of 10ppm. Injected 10 µl into the HPLC system, recorded the chromatographs to determine the sample stability.

Degradation Study with Water

Stress testing at neutral condition was studied by refluxing Semaglutide in water for 6hrs by maintaining a temperature of 60°C. In this study, the resultant solution was diluted to the 10ppm solution, and 10 µl was injected. This determines the stability of the sample applied for the degradation study.

RESULTS AND DISCUSSION

Figure 2: Structure of Semaglutide

Specificity

This RP-HPLC method was specific. The retention time for Semaglutide was 2.689 min. Specificity results were represented in Table 1, Figure 2a for blank and Figure 2b for the drug.

Table 1: Specificity Data for RP-HPLC

<table>
<thead>
<tr>
<th>S. No</th>
<th>Peak Name</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Blank</td>
<td>Nil</td>
</tr>
<tr>
<td>2</td>
<td>Placebo</td>
<td>Nil</td>
</tr>
<tr>
<td>3</td>
<td>Standard</td>
<td>Rt = 2.689min (\lambda_{max} = 292\text{nm})</td>
</tr>
</tbody>
</table>

System suitability

Tailing factor was 1.2 (T), and a number of theoretical plates were found to be 3145 (N). System Suitability results were represented in Table 2.

Table 2: System Suitability data

<table>
<thead>
<tr>
<th>Factors</th>
<th>Result</th>
<th>Acceptance Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rt</td>
<td>2.689</td>
<td>--</td>
</tr>
<tr>
<td>Resolution factor</td>
<td>NA</td>
<td>--</td>
</tr>
<tr>
<td>Number of theoretical plates (N)</td>
<td>3145</td>
<td>More than 2000</td>
</tr>
<tr>
<td>Tailing factor (T)</td>
<td>1.2</td>
<td>Less than 2</td>
</tr>
</tbody>
</table>

Linearity and Range

Linearity was determined for a concentration of 2.5 to 15 µg/ml. The correlation coefficient was 0.9998. Linearity results were represented in Table 3 and co-relation graph in Figure 3.

Table 3: Results of Linearity and range

<table>
<thead>
<tr>
<th>Serial</th>
<th>Concentration (µg/ml)</th>
<th>Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.5</td>
<td>185200</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>347963</td>
</tr>
<tr>
<td>3</td>
<td>7.5</td>
<td>513667</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>688904</td>
</tr>
<tr>
<td>5</td>
<td>12.5</td>
<td>867268</td>
</tr>
<tr>
<td>6</td>
<td>15</td>
<td>1043533</td>
</tr>
</tbody>
</table>

Figure 3: Correlation graph

Precision

In method validation, intraday and interday precision determined. The %RSD for interday precision is 0.5 and intraday is 1.0. Precision results were represented in Table 4.

Table 4: Results for intraday and interday precision

<table>
<thead>
<tr>
<th>S. No</th>
<th>Intraday precision Area</th>
<th>Interday precision Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>682866</td>
<td>671865</td>
</tr>
<tr>
<td>2</td>
<td>679715</td>
<td>676730</td>
</tr>
<tr>
<td>3</td>
<td>685659</td>
<td>670652</td>
</tr>
<tr>
<td>4</td>
<td>684941</td>
<td>673626</td>
</tr>
<tr>
<td>5</td>
<td>698604</td>
<td>679618</td>
</tr>
<tr>
<td>6</td>
<td>691844</td>
<td>673981</td>
</tr>
<tr>
<td>Mean</td>
<td>687272</td>
<td>674412</td>
</tr>
<tr>
<td>Std Dev</td>
<td>6839.2</td>
<td>3283.4</td>
</tr>
<tr>
<td>%RSD</td>
<td>1.0</td>
<td>0.5</td>
</tr>
</tbody>
</table>
Figure 4: LOD Chromatograph

Figure 5: LOQ Chromatograph

Figure 6: Chromatograph of Acid degradation

Figure 7: Degradation purity plot of Acid
Accuracy
Percentage Mean recovery of accuracy was from 99.73 to 100.29. Accuracy results were represented in Table 5.

Table 6: LOD & LOQ

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Results</th>
<th>LOD</th>
<th>LOQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>RT</td>
<td></td>
<td>2.624</td>
<td>2.634</td>
</tr>
<tr>
<td>Area</td>
<td></td>
<td>23464</td>
<td>85711</td>
</tr>
<tr>
<td>Plate count</td>
<td></td>
<td>2571</td>
<td>2550</td>
</tr>
<tr>
<td>USP Tailing</td>
<td></td>
<td>1.5</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Detection and quantification limits: The LOD was found to be 0.08µg/ml, and the LOQ was determined to be 0.26µg/ml for Semaglutide. The level of quantification (LOQ) and detection (LOD) were represented in Table 6 and Figure 4, Figure 5. Detection sensitivity was determined through LOD, and the concentration that can be quantified at lowest is derived from LOQ.

Degradation studies
Degradation studies related to acid, base, peroxide, thermal, UV and water were represented in Table 7. Figure 6 represents acid degradation chromatograph, and Figure 7 represents the degradation of acid purity plot. Figure 8 represents degradation chromatograph of base and Figure 9 represents degradation purity plot of a base.
Figure 11: Degradation purity plot of Peroxide

Figure 12: Thermal degradation chromatograph

Figure 13: Thermal degradation purity plot

Figure 14: UV degradation Chromatograph
Degradation studies performed on Semaglutide drug in order to prove that drug considered for this study has stability and the same property is being exhibited during an experiment with the help of this study performed. Prepared and executed methods have contributed for more accurate determination of drug by HPLC study.

**CONCLUSION**

The method developed was simple, applicable and sensitive with a validated approach. This analytical method for determination and elution of Semaglutide can be applied for day to day analysis, routine analysis and experimental analysis. Results generated through this study indicate that this method is accurate.

**CONFLICT OF INTEREST**

No conflicts of interest.
Acknowledgment

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REFERENCES


