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# ANALYTICAL METHODS FOR ESTIMATION OF SEMAGLUTIDE IN PHARMACEUTICAL DOSAGEFORM - A REVIEW

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**ABSTRACT**

Semaglutide is a widely used medication for Type-2 diabetes mellitus and treats Obesity. It is administered in Oral as well as Subcutaneous Route.It is GLP-1 analogue with 94% sequence homology to human GLP-1. GLP-1 is a physiological hormone that has multiple actions in glucose and appetite regulation and in cardiovascular system. Semaglutide activates GLP receptor and increase insulin level which decrease glucose Production. It is highly lipophilic so absorb faster and produce its action quicker. There are many articles which have already been published describing analytical method and validation for the same. In Present to review account the disclosed analytical methods are outlined for the establishment of Semaglutide in its pharmaceutical preparation and Biological matrices. Analytical techniques such as UV, HPLC and UPLC Play a Pivotal role in validation of Semaglutide. HPLC Parameters like lambda max, stationary phase, mobile phase, composition, detection, wavelength, retention time are validated here. For UPLC parameters like solvent, method of detection, reports are noted. For UV spectroscopy parameters like , stationary phase, mobile phase, Run time, flow rate,(λmax), are validated. In this article, we will explore the importance of UV, HPLC & UPLC in the Validation Process and their applications in Assessing Semaglutide.

**Keywords:** Semaglutide, Analytical method & Validation technique, UV, HPLC & UPLC, Review article.

## INTRODUCTION

**Drug profile:**

Semaglutide (C187H291N45059) (M.W-4113.64) is a clear, colourless, white or half white substance with Iso electric point of 5.4 is chemically named as(IUPAC NAME- - (Rs) -1 - {4- [(2- Isopropoxyethoxy)methyl]phenoxy }-3 (isopropylamino) propan-2-ol. It is soluble in water, Dimethyl sulfoxide and in Buffers like Potassium Dihydrogen Ortho Phosphate and sodium acetate Buffer. Semaglutide is an oral and subcutaneously administered drug which belongs to newly developed class. It is a GLP receptor agonist. It contains SNAC (Sodium-N-(8-{2`-Hydroxy benzoyl}amino)Caprylate, which is an Absorption enhancer present in oral semaglutide which helps quick absorption across gastric mucosa.

Semaglutide is hygroscopic in nature.

Semaglutide, a polypeptide that contain linear Sequence of 31 aminoacids Joined together by Peptide linkage. Agonist of GLP-1 receptor which treats type-2 DM . It is RDNA Produced Polypeptide analogue of human GLP-1 which is used alone or in Combination with other Antidiabetics. There have been no published Reports of hepatotoxicity for Semaglutide therapy

**Brand Name;**

Oral Route - Rybelsus.

Subcutaneous Route - Wegovy, Ozempic (Prefilled injection pens).

Semaglutide characteristics in different dosage form;

**Mechanism of Action:**

**1) Anti Diabetic action**;

It improves efficiency of incretin function by activating GLP-1 receptors. It Acts by numerous Mechanism like Augmented insulin secretion, inhibition of glucagon release & suppressed hepatic gluconeogenesis thereby reducing Postprandial glucose and treats Type-2 DM.It stimulates the Pancreas and Secrets insulin which leads to decrease in blood glucose level.

GLP-1 is a physiological hormone that promotes glycemic control via several different mechanisms, including insulin secretion, slowing gastric emptying, and reducing postprandial glucagon secretion.

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Thehomeostasis of glucose is dependent on hormones such as insulin and amylin, which are secreted by thebeta cells of the pancreas. Semaglutide is 94% similar to human GLP-1. Analogs of this hormone such assemaglutide stimulate the synthesis of insulin by stimulating pancreatic islet cells and reducing glucagon secretion. They directly bind with selectivity to the GLP-1 receptor, causing various beneficial downstream effects that reduce blood glucose in a glucose-dependent function

**2) Mechanism of cardiovascular benefit and weight loss;**

GLP-1 RAS works by reducing the appetite and feelings of hunger, slowing the release of food from stomach and increase feeling of fullness. In hypercholesterolemia, semaglutide is believed to reduce the progression of atherosclerosis via decreased gut permeability and decreased inflammation. Weight loss is believed to occur via the reduction of appetite and food cravings after semaglutide administration.

**Analytical Determination:**

1. **High Performance Liquid Chromatography (HPLC):**

HPLC is the advanced analytical technique in the pharmaceutical analysis, which is predominantly used in pharmaceutical industries for the large variety of samples. It is the method of choice for determining the purity of new drug candidates, monitoring changes or scale-ups of synthetic procedures, evaluating new formulations, and scrutinizing quality control of final drug products.

**Works Done Are;**

**1.**A simple, Accurate, precise method was developed for the Estimation of the Semaglutide in API form. Chromatogram was run through inertsil ODS C18 (250 x4.6mm, 5□). Mobile phase containing Methanol : Water in the ratio 70:30 was pumped through column at a flow rate of 1.0 ml/min in the room temperature. Optimized wavelength selected was 274 nm. Retention time of Semaglutide was found to be 3.237mins . %RSD of the Semaglutide was found to be 0.9 . LOD, LOQ values obtained from regression equations of Semaglutide was 0.57 ,1.74 respectively.

**2.**A New specific, economic and selective, accurate, precise and robust Reverse Phase High Performance Liquid Chromatography was developed for the quantification of Semaglutide in pharmaceutical substance and product. Chromatographic separation was achieved by C18 column (Azilent C18 150x 4.6, 5mm) is used as stationary phase and 0.01N Potassium dihyrogen ortho phosphate: Acetonitrile (50:50) used as a mobile phase at a flow rate of 1.0 mL/min and monitored at 230nm. The run time was 5min. The retention time of Semaglutide was found to be 2.222min. LOD and LOQ were found to be 0.007 µg/ml and 0.022 µg/ml respectively.

An accurate, precise, robust and stability-indicating RP-HPLC method was developed for the estimation of Semaglutide using QbD approach. After conducting several trials using CCD method, one desirable method was optimized. Stationary phase selected was Kromasil C18 (250×4.6 mm, 5 µm) and potassium dihydrogen orthophosphate (pH2) and Methanol used as mobile phase in the ratio of 61.2: 38.8. Detection was carried out at the wavelength 230nm. Flow rate selected for separation was 0.98ml/min and the temperature of 29.150C. The retention time was found to be 2.518 at the run time of 5min. LOD and LOQ were found to be 0.019µg/ml and 0.056µg/ml respectively.

**2. Ultra-performance liquid chromatography (UPLC):**

Advanced version of HPLC that offer advanced Seperation and higher Resolution and provideimproved sensitivity and Shorter analysis time when compared with HPLC . It provides faster and more detailed analysis of polymer molecular weight, size and structure.

**Works Done Are;**

1. A rapid, accurate, precise and stability indicating RP-UPLC method was developed to determine the Semaglutide present in the bulk and dosage forms. BEH-C18 (1.7 µ, 100×2.1mm) column selected as stationary phase and 0.01N Potassium dihydrogen ortho phosphate: Acetonitrile (60:40) selected as mobile phase. The run time is 1.2min and 0.5ml/min flow rate selected to optimize the method. The retention time of Semaglutide recorded as 0.89min at the detection wavelength of 230nm. Concentration range of linearity recorded 1.5-9.0 µg/ml and correlation co-efficient of 0.999.

2. The chromatographic separation was achieved with Acquity BEH C18 (50mm x 1.6 mm) 1.8 µm column thermostated at 30degree with mobile phases containing 0.01N potassium dihydrogen phosphate (3.2 pH): acetonitrile in the ratio of 50:50 v/v. The flow rate was maintained at 0.4ml/min and injection volume was found to be 0.50µl. The detection was done at 292 nm using TUV detector. The retention time was found to be 1.026min

**3)UV Spectrophotometric method ;**

Non destructive analytical technique that measures the absorbance or transmittance of UV light. It is a quantitative technique used to measure how much a chemical substance absorbs light. This is done by measuring the intensity of light that passes through a sample with respect to the intensity of light through a reference sample or blank.It is used for quantitative & qualitative determination of API.

**Works Done Are;**

1) The method development of semaglutide was carried out using Shimadzu 1800 UV Visible Spectrophotometer with a pair of 10mm path length

matched quartz cells. The solutions were scanned in the range of 200-400 nm with medium scanning speed. The method A was carried out with 0.01N potassium dihydrogen ortho phosphate and method B was accomplished with sodium acetate buffer (pH 5). The absorption maximum of semaglutide was found to be at 293nm. The drug obeyed Beer-Lambert's law over the concentration range of 1-15µg/ml. The accuracy retrieved by recovery studies was found to be 99.8% - 102% for method A and 98% - 100.8% for method B.

2) Spectrophotometry was carried out on a UV-Visible spectrophotometer (UV-T60-India) in quartz cells using ethanol as suitable solvent, the detection is carried out at 288nm.The pre-determined wavelength of maximum absorption (λmax) was 288nm. The drug obeyed Beer-Lambert's law over the concentration range 5-30µg/ml. The accuracy retrived by recovery studies was found to be 99.92% for 50% concentration, 99.08% for 100% concentration, 99.95% for 150% concentration.

**Table-1 Characteristics;**

| Characteristics | Subcutaneous | Oral |
|---|---|---|
| Absolute bioavailability | 89% | 0.4-1% |
| Volume of distribution | 12.5 litre | 8litre |
| Protein binding | >99% | >99% |
| Elimination t1/2 | 01 week | 01 week |
| Rate of clearance | 0.05 litre/hr | 0.04 litre/hr |

**Table-2 High Performance Liquid Chromatography (HPLC);**

| Stationary phase | Mobile phase | Flow rate method of detection retention time | Results | Reference |
|---|---|---|---|---|
| 1) Inertsil ODS c18 (250×4.6mm)5µm | Methanol : water (70:30) | 1.0ml/min UV at 274nm3.237min | LOD-0.57µg/mlLOQ-1.74µg/ml | Varsha.Pet:al |
| 2)Azilent C18 column (150×4.6mm) 5mm | 0.01N KH2PO4 : acetonitrile(50:50) | 1.0ml/min UV at 230nm 2.222min | LOD-0.007µg/mlLOQ-0.022µg/ml | Merugu manasaet:al |
| 3) Kromasil c18column (250×4.6mm)5 µm | KH2PO4: Methanol (61.2:2:38.8) | 0.98ml/min UV at 230 nm 2.518 min | LOD-0.019µg/mlLOQ-0.056µg/ml | Merugumanasa&Vijay anadhi,int j. Respharm |

**LOD- limit of detection**
**LOQ- limit of quantitation**

**Table-3: Ultra-Performance liquid chromatography(UPLC);**

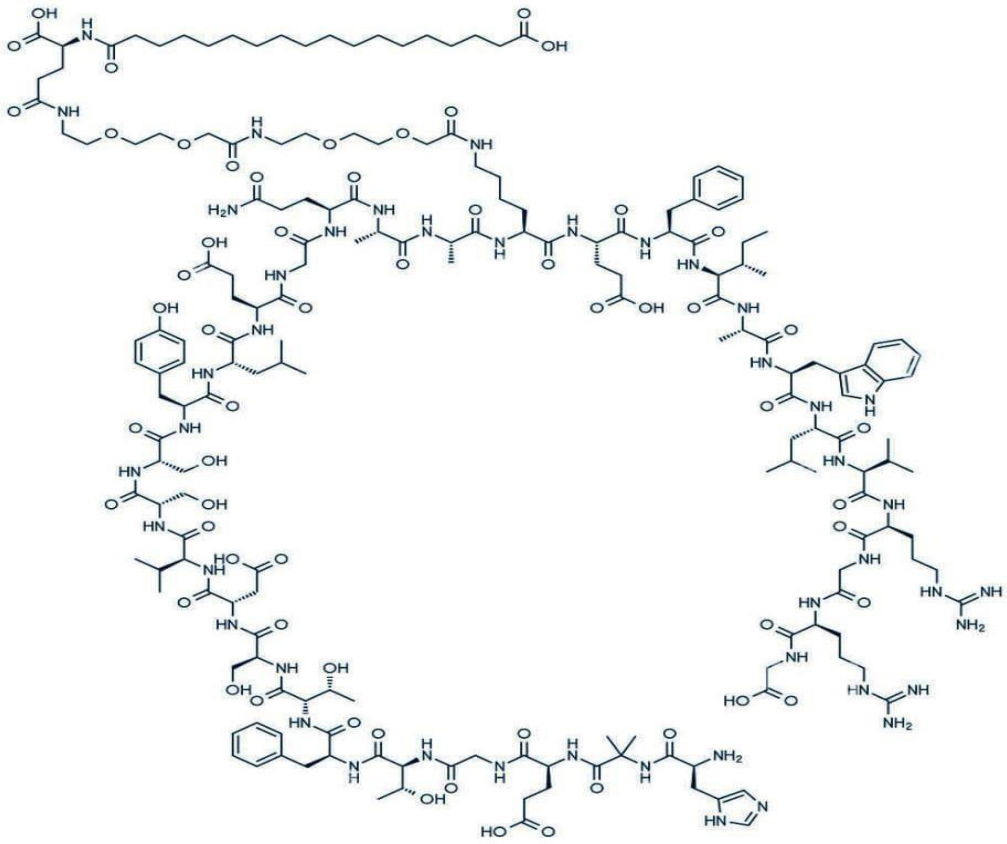
| Sample | Description | Detection | Reference |
|---|---|---|---|
| 1) Oral soliddosageform | **Stationary phase:** BEH-C18 (1.7 µ,100×2.1mm) **Mobile phase:** 0.01N KH2PO4: Acetonitrile (60:40) **Runtime**-1.2 min **Flow rate**-0.5ml/min | PDA detector | Subha harika penmetsa&Raja sundarajan (Ijrar) |
| 2) Parenteraldosage form | **Stationary phase** BEH- C18 (50mm×1.6mm)1.8 µm **Mobile phase**: 0.01N potassium dihydrogenortho phosphate(3.2 PH) : Acetonitrile (50:50)v/v **Flow rate** – 0.4ml/min **LOD**- 0.086 µg/ml **LOQ**- 0.261 µg/m | TUV detector | Subha Harika Penmetsa&Raja sundararajan (Ijrar) |

**Table-4: UV Spectrophotometry;**

| Solvent | Method ofdetection | Result | Reference |
|---|---|---|---|
| 1)Method-A 0.01NKH2PO4 Method-B sodiumAcetateBuffer PH-5 | UV-293nm | LOD Method-ADO-0.01µg/mlD1- 0.26µg/mlMethod-B DO-0.03µg/mlD1- 0.13µg/mlLOQ Method– ADO - 0.03µg/ml D1- 0.78µg/mlMethod-B DO- 0.09µg/mlD1-0.42µg/ml | Subha Harika Penmetsa & Raja Sundararajan |
| 2) Ethanol | UV - 288nm | LOD- 2.99µg/mlLOQ- 0.51µg/ml | K.l.rajita,ejpmr |

**LOD- limit of detection**
**LOQ- limit of quantitation**

**Figure-1-Structure of Semaglutide.**




**Discussion and Report:**

In this above review article, we had investigated the analytical development Methods of Semaglutide in different Pharmaceutical dosage form. Semaglutide is a drug used for the treatment of Type-2DM and it is available in different pharmaceutical dosage forms.

(i.e- Tablet, injections). As stated in different article, we came to conclusion that the RP-HPLC along with UPLC had produced efficient Result in analytical development of Semaglutide. So, RP- HPLC, is the most Preferable method.

While comparing the results of UV Spectrophotometric method ,by the different solvents such as Potassium Dihydrogen Ortho Phosphate and Ethanol, Their results shows the absorbance of SEMAGLUTIDE was found Satisfied using Ethanol. While comparing the results of High Performance Liquid Chromatography (HPLC) by the different solvents such as Methanol:Water(70:30), Potassium Dihydrogen Ortho Phosphate:Acetonitrile (50:50) and Potassium Dihydrogen Ortho Phosphate:Methanol,Their Results shows the absorbance of SEMAGLUTIDE was found Satisfied using Methanol.

**CONCLUSION:**

SEMAGLUTIDE is a GLP-1 receptor agonist which is highly effective in treating Type-2 Diabetes Mellitus, administered orally as well as subcutaneous route. In this review article, we discussed about the analytical development method for estimation of semaglutide in pharmaceutical dosage form. The proposed technique accomplished forSEMAGLUTIDE are RP-HPLC, UPLC, UV- Spectrophotometry. A new precise accurate by simple HPLC and UV spectroscopy method was developed and validated for estimation of SEMAGLUTIDE tablet dosage form. This method is fast and accurate, precise and sensitive. These proposed methods were found to be highly effective and could be used for quantification of SEMAGLUTIDE in bulk and a tablet formulation for routine analysis. The Validation results were reaching the acceptance limit of ICH guidelines,This above data are helpful for further research studies in analysis of Semaglutide.

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