Original Article

Stability Indicating RP-UPLC Method Development Validation and Dissolution Testing of Semaglutide

Merugu Manasa[1], Vijey Aanandhi M[2*]

[1] Research Scholar, Department of Pharmaceutical Chemistry and Analysis, School of Pharmaceutical Sciences, Vels Institute of Science, Technology and Advanced Studies (VISTAS), Pallavaram, Chennai, Tamil Nadu, India.

[2] Department of Pharmaceutical Chemistry and Analysis, School of Pharmaceutical Sciences, Vels Institute of Science Technology and Advanced Studies (VISTAS), Chennai, India.

Email: hodpchemistry@velsuniv.ac.in

Abstract

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. %RSD found in intra-day and inter-day precisions were 0.2. The % recovery of drug at the concentration levels of 50%, 100% and 150% were 100.02%, 99.98% and 99.99% respectively. The developed method was robust. Under the stress conditions like acid, base, neutral, oxidative, thermal and UV light the drug degradation was less than 10% and the analyte peak can separate under this stress condition without interaction of degradation products. The developed method can able to separate the active ingredients present in the formulation without interference of excipient.

Keywords: Semaglutide, RP-UPLC, ICH guidelines, Stability, dissolution, accurate, precise.

Introduction

Semaglutide is an anti-diabetic drug. Type 2 diabetes mellitus is treated by Semaglutide. It acts as glucagon-like peptide 1 receptor agonist1. Semaglutide molecular weight is 4113.641 and molecular formula is C187H291N45O59. The IUPAC name is 17-{[(1R)-3-[(2-{2-[({2-[2-({[(5S)-5-[(2S) -2- [(2S) -2- [(2S) -2- {2- [(2S) -2- [(2S) -2- [(2S) -2- [(2S) -2- [(2S) -2-[(2S)-2-[(2S)-2-[(2S)-2-[(2S)-2-[(2S,3R)-2-[(2S)-2-[(2S,3R)-2-{2-[(2S)-2- {2-[(2S)-2 -amino-3- (1H-imidazol-4-yl) propanamido]-2- methyl propanamido} -4- carboxy butanamido] acetamido}-3-hydroxybutanamido]-3-phenylpropanamido]-3-hydroxy butanamido]-3-hydroxypropanamido]-3-carboxy propanamido]-3-methyl butanamido]-3-hydroxy propanamido] -3- hydroxyl propanamido] -3- (4-hydroxyphenyl) propanamido] -4-methyl pentanamido]-4-carboxybutanamido]acetamido}-4-carbamoyl butanamido] propanamido] propanamido] -5 -{[(1S) -1 -{[(1S) -1 -{[(1S,2S) -1 -{[(1S) -1- {[(1S) -1-{[(1S)-1-{[(1S)-1-{[(1S)-4-carbamimidamido-1-[({[(1S)-4-carbamimidamido-1-[(carboxymethyl) carbamoyl] butyl] carbamoyl}methyl) carbamoyl]butyl]carbamoyl}-2-methylpropyl]carbamoyl}-3-methylbutyl] carbamoyl}-2-(1H-indol-3-yl)ethyl]carbamoyl}ethyl]carbamoyl}-2-methylbutyl]carbamoyl}-2-phenylethyl]carbamoyl}

Address for correspondence: Vijey Aanandhi M, Department of Pharmaceutical Chemistry and Analysis, School of Pharmaceutical Sciences, Vels Institute of Science Technology and Advanced Studies (VISTAS), Chennai, India, Email: hodpchemistry@velsuniv.ac.in

Received date: 11 August 2022 Accepted: 13 September, 2022

Published: 10 October, 2022

How to cite this article Manasa M, Aanandhi M V, Stability Indicating RP-UPLC Method Development Validation and Dissolution Testing of Semaglutide , J Pharm Negative Results 2022;13(4):1459-1464

Access this article online

Quick Response Code:

Website: www.pnrjournal.com

DOI: 10.47750/pnr.2022.13.04.205

-3-carboxypropyl] carbamoyl} pentyl] carbamoyl} methoxy)
ethoxy]ethyl}carbamoyl)methoxy]ethoxy}ethyl)carbamoyl]-1-carboxy propyl] carbamoyl} heptadecanoic acid.2 Structure was shown in fig 1.

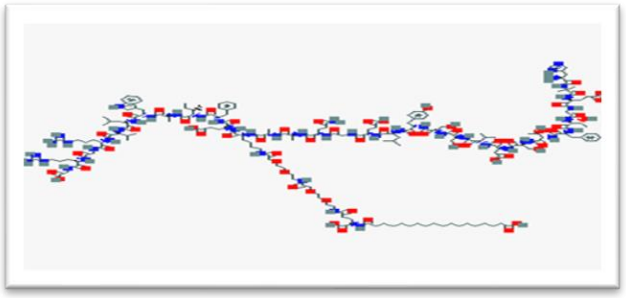

Figure 1. Semaglutide Structure

Literature review 3-7 revealed that only one study reported for determination of Semaglutide by using UPLC method. UPLC method is a advanced, efficient and highly resolute technique. By using UPLC method separation time will reduce. The current study explains a new spectroscopic and chromatographic stability indicating method development and validation of Semaglutide in bulk and dosage form according to ICH guidelines. 8 Dissolution studies were performed to determine the percentage drug release of Semaglutide present in the formulation. The drug present in the aliquots was determined by using developed RP-UPLC method.

Materials And Method

Chemicals and reagents

Pure Semaglutide was procured from Spectrum pharma pvt ltd (Hyderabad). From Merck India Pvt Ltd, AR grade Hydrochloric acid (HCL) and sodium hydroxide (NAOH) were obtained. From Qauligens, Hydrogen Peroxide (H2O2) was purchased. Acetic acid AR grade was purchased from Fisher scientific, India and S.D. Fine chem Ltd. Respectively. Potassium dihydrogen phosphate obtained from S.D. Fine chem Ltd. HPLC grade Acetonitrile (ACN) and methanol (MeOH) were purchased from Fischer scientific. Using Merck milli-Q water purification unit, HPLC grade water was prepared.

Equipment

For method development and method validation was Acquity-UPLC of Waters, PDA-Detector, and auto-sampling system was used. Using Waters Empower software signals monitoring was done. Other equipments used throughout the experimental work are hot air oven (Yorco scientific), thermostat dry air equipment Thermo scientific, USP type-II apparatus (Agilent technologies) and pH meter (Eutech instruments pH tutor, pH meter, India).

Preparation of solutions

Preparation of standard solution

A 3 mg of Semaglutide was weighed accurately and dissolved in solvent system of 0.01N Potassium dihydrogen ortho phosphate: Acetonitrile (60:40). Sonicated the solutions for 5 min and made to 50ml.

Preparation of sample solution

Equivalent to 3 mg of Semaglutide tablet powder was weighed accurately and dissolved in solvent system of 0.01N Potassium dihydrogen ortho phosphate: Acetonitrile (60:40). Sonicated the solutions for 5 min and made to 50ml.

Preparation of potassium dihydrogen ortho phosphate buffer(pH 2)

In 900ml milli-Q water 1.36g of accurately weighed potassium dihydrogen ortho phosphate was dissolved. This solution was sonicated for degassing. The volume was made up to 1000ml and 1ml of Triethylamine was added. Diluted ortho phosporic acid was added to adjust the pH to 2.

Preparation of Phosphate buffer (pH 6.8)

Weigh accurately about 28.2g of disodium hydrogen phosphate and 11.45g of potassium dihydrogen phosphate. Dissolve in sufficient amount of water and make up the volume to 1000ml.

Chromatographic Conditions

To select the stationary phase and mobile phase various trials were conducted. The selected stationary phase and mobile phase were Acquity BEH-C18 (1.7 µ, 100×2.1mm) column and 0.01N Potassium dihydrogen ortho phosphate : Acetonitrile (60:40) respectively. The flow rate and detector wavelength selected were 0.5 mL/min and 230nm respectively. Study conducted by 5 µL volume of injection at column temperature of 25 °C.

Method Validation

The proposed method was validated under selected system conditions as per ICH guidelines. Six replicate standard solutions of 6µg/mL Semaglutide were injected to UPLC. Calibration curve was plotted by taking concentration (1.5-9µg/ml range) on X-axis and area on Y- axis. The Correlation co-efficient (R2) value produced should be less than 1. Using triplicate standard solutions of 50%, 100% and 150% concentrations accuracy studies were conducted to determine the percentage recovery. Intra- day and inter-day precision

were determined by injecting 100% solution of Semaglutide

in 6 replicates. Calculated % RSD should be less than 2. Lowest level of concentration of Semaglutide resulting peak area noise of 3 times the baseline is Limit of Detection. The lowest concentration resulting peak area of 10 times the baseline is Limit of Quantification. Any small or deliberate changes like mobile phase ratio, flow rate and temperature should not affect the method, and then the method can called as robust.

Stress Studies

Sample solutions were treated with 2N HCl, 2N NaOH, 20% H2O2 solution, heat and sun light individually. Sampling should be done at multiple points and the percentage degradation was determined.

Dissolution

Rybelsus 7mg tablet immediate release dosage form was taken to conduct the dissolution method. Using Phospphate buffer pH 6.8 as dissolution media with the help of USP type-II apparatus at 70 rpm study was conducted. A 5mL of aliquots withdrawn at 5, 10, 15, 20, 25, 30, 45 and 60 min then injected in to RP-UPLC method. The percentage drug release was calculated.

Results And Discussion

Method development and Optimization

Using different combination of mobile phase and different columns used as stationary phase different trial were conducted. Optimized chromatographic conditions were Acquity BEH-C18 (1.7 µ, 100×2.1mm) stationary phase and 01N Potassium dihydrogen ortho phosphate : Acetonitrile (60:40) as mobile phase. Flow rate, run time and temperature were selected as 0.5 Ml/min, 1.2min and column temperature of 25 °C respectively. Peak developed at the retention time of 0.89min at detection wavelength of 230nm. The selected injection volume was 5 µL. The optimized chromatographic peaks were shown in fig 2.

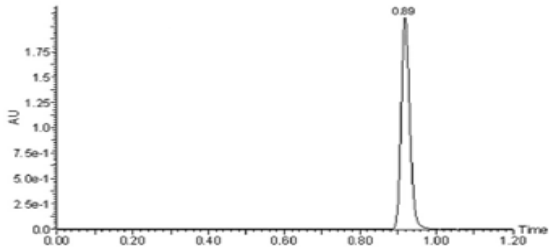

Figure 2: Optimized chromatogram of Semaglutide

Method Validation

System Suitability

Six replicate samples of 6µg/mL of Semaglutide were injected. All system parameters like retentions time, theoretical plates, area, standard deviation and %RSD were within the limits as per the ICH guidelines. The results were shown in the Table no 1.

Table 1: System suitability of Semaglutide

S.No	Name	Injection No	Area
1	Standard	1	763489
2	Standard	2	764593
3	Standard	3	763487
4	Standard	4	762674
5	Standard	5	761589
6	Standard	6	765451
Mean			763547
SD			1365.4
%RSD			0.2

Precision

System precision (Intra-day) was performed to ensure that the standard solutions are giving reproducible and proper results. After injecting six replicates of 100% solution the calculated %RSD was less than 2. Intermediate precision was determined by changing the day. Intermediate precision (Inter-day) reflects that by changing day or instrument the results were unaffected. After injecting six replicates of 100% solution the calculated %RSD was less than 2. The precision results were shown in table no 2.

Table 2: Intra-day and inter-day Precision of Semaglutide

S.No	Name	Injection No	Precision	Intermediate Precision
1	Standard	1	764487	763489
2	Standard	2	763694	765532
3	Standard	3	763762	762714
4	Standard	4	762714	761589
5	Standard	5	762283	762283
6	Standard	6	765532	764593
Mean			763745	763367
SD			1178.7	1481.1
%RSD			0.2	0.2

Linearity

To determine the proportionality between concentration range of analyte and results linearity was performed. The selected concentration range was 1.5 µg/mL to 9µg/mL. The obtained correlation co-efficient was 0.999 which is within the limits as per the ICH guidelines. The results were shown in Fig no 3 and Table no 3.

Table 3: Linearity of Semaglutide

S.No	Concentration	Area
1	0	0
2	1.5	193362
3	3	392342
4	4.5	582412
5	6	762642
6	7.5	943342
7	9	1144123
Correlation Co-efficient		0.999

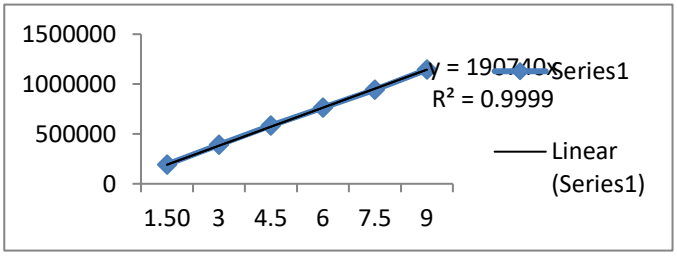

Figure 3: Linearity of Semaglutide

Accuracy

Accuracy is performed to determine the closeness between true values and measure values. At the concentration range of 50%, 100% and 150% the % percentage recovery of analyte was 98%-102% as per the ICH guidelines within the limits. The results were shown in the table no 4.

Table 4: Accuracy of Semaglutide

S.No	50% (3µg/ml)	100% (6 µg/ml).	150% (9µg/ml).
1	392348	762642	1143923
2	392493	762594	1144134
3	392425	762489	1144224
Average Area	392422	762575	1144094
Drug added	3	6	9
Drug recovery	3.0006	5.9992	8.99
% Accuracy	100.02	99.98	99.99

Robustness

By changing the parameters is there any effect on developed method was determined by robustness. The changed parameters were flow rate, mobile phase ratio and column temperature and %RSD were found to be 0.3, 0.33 and 0.4. The calculated % RSD were within the limits as per ICH guidelines.

Stability

Under various stress condition how much drug is stable and how much dug is degrading is determined by using stability studies. The stress conditions were acid hydrolysis, base hydrolysis, neutral hydrolysis, peroxide hydrolysis, thermal degradation and UV degradation. Under the stress conditions degradation percentage was less than 10% , indicates that the results were within the limits as per the ICH guidelines. The results were shown in the table no 5.

Table 5: Stability of Semaglutide

S. No.	Condition of degradation study	% drug recovery	% of drug degraded
1.	2N HCl	95.64	4.36
2.	2N NaOH	95.77	4.23
3.	Neutral hydrolysis	95.70	4.3
4.	Oxidative degradation	97.85	2.15
5.	Thermal degradation	98.88	1.12
6.	Photo degradation	99.53	0.47

Dissolution

After conducting several tests suitable dissolution method was selected for the dissolution studies. The finalized parameters were Medium: Phosphate buffer pH 6.8,Volume 900ml, Apparatus: UP type-II at 70 rpm. A 5mL of aliquots withdrawn at 5, 10, 15, 20, 25, 30, 45 and 60 min. The sample were analyzed by RP-UPLC method drug release was calculated. The graph of percentage dug release verses time is shown in figure. Drug products met the USP requirement of Q > 85% in 60min. The results were shown in Figure no. 4. And Table no. 6.

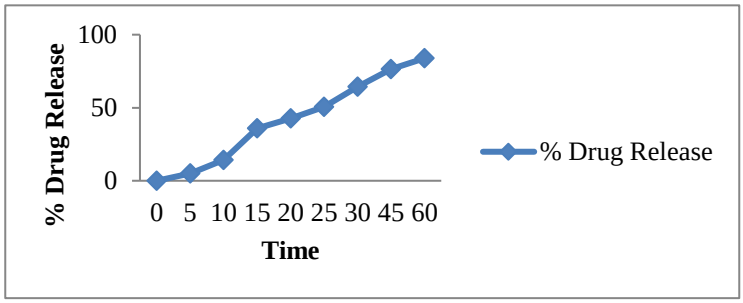

Figure 4: Dissolution profile of Semaglutide oral dosage form (Rybelsus)

Table 6: Dissolution profile of Semaglutide oral dosage form (Rybelsus)

S.No	Time (Min)	% Drug Release
1	0	0
2	5	6.14
3	10	18.27
4	15	27.87
5	20	42.67
6	25	54.56
7	30	66.36
8	45	76.48
9	60	83.85

Conclusion

The current experiment is performed to develop a rapid, simple, stable, accurate, linear, robust and precise method for determination of Semaglutide in bulk and dosage forms using UPLC. The validation results were reaching the acceptance limit of ICH guidelines. The developed method is capable to separate the drug from degradation products in stability studies. From the dissolution studies the drug can able to separate from excipient present in the formulation by developed UPLC method.

Acknowledgement

The authors are grateful for the management of Vels Institute of Science, Technology and Advanced Studies (VISTAS), for their support in current work. The authors wish to show their gratitude towards Spectrum labs, for providing facilities to conduct this work. They would also like to appreciate their colleagues for supporting to complete the experiment.

Conflict Of Interest

The authors declare that they have no conflict of interest for this study

Reference

Marso SP, Bain SC, Consoli A, Eliaschewitz FG, Jódar E, Leiter LA, Lingvay I, Rosenstock J, Seufert J, Warren ML, Woo V, Hansen O, Holst AG, Pettersson J and Vilsboll T. Semaglutide and Cardiovascular Outcomes in Patients with Type 2 Diabetes. New England Journal of Medicine. 2016 Nov 10; 375(19):1834-1844. Available on https://doi.org/10.1056/NEJMoa1607141 .

Arun Kumar Kuna, Ganapathi S, and Radha GV. A novel RP- HPLC method development and forced degradation studies for Semaglutide in active pharmaceutical ingredients and pharmaceutical dosage form. International Journal of Research in Pharmaceutical Sciences. 2019 Apr 29;10(2):865-873. doi:10.26452/ijrps.v10i2.263 .

Panagiotis Andreadis, Thomas Karagiannis, Konstantinos Malandris, Ioannis Avgerinos, ArisLiakos, ApostolosManolopoulos, EleniBekiari, David R Matthews, and ApostolosTsapas. Semaglutide for type 2 diabetes mellitus: A systematic review and meta-analysis. Diabetes, Obesity and Metabolism Journal of Pharmacology and Therapeutics. 2018 May 13; 20(9):2255-2263. doi:10.1111/dom.13361 .

MeruguManasa and VijeyAanandhi M. Stability Indicating Assay Method (Siam) For Determination of Semaglutide by HPLC Using Qbd Approach. International Journal Of Research In Pharmaceutical Sciences. 2021 Apr 7; 12(2):1367-1374. Available on https://doi.org/10.26452/ijrps.v12i2.4692 .

Merugu Manasa and VijeyAanandhi M. Stability Indicating Method Development and Validation of Semaglutide by RP-HPLC in Pharmaceutical substance and Pharmaceutical Product. Research Journal Of Pharmacy And Technology. 2021 March 18;14(3):1385-1389. doi: 10.5958/0974-360X.2021.00247.X

Subha Harika Penmetsa and Raja Sundararajan. Method development and validation of RP-UPLC method for the determination of semaglutide in bulk and pharmaceutical dosage form. International Journal of Research and Analytical Reviews 2018 Dec; 5(4): i534-i543. Available on http://ijrar.com/upload_issue/ijrar_issue_20542598.pdf .

Subha Harika Penmetsa and Raja Sundararajan. Method development and validation of semaglutide by UV Spectrophotometric method in bulk and pharmaceutical dosage form. International Journal of Research and Analytical Reviews. June 2019; 6(2):394-402. Available on http://ijrar.com/upload_issue/ijrar_issue_20543538.pdf .

US FDA, Center for Drug Evaluation and Research (CDER), Silver spring, USA (Updated on July 2015). Available on https://www.fda.gov/files/drugs/published/Analytical-Procedures-and-Methods-Validation-for-Drugs-and-Biologics.pdf