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Research Article

Stability Indicating Spectroscopic and Chromatographic Estimation of Semaglutide

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Abstract

New simple, economic and stable methods were developed and validated to estimate the Semaglutide using UV-Visible spectroscopy, HPLC and UPLC. 0.01N Potassium dihydrogen orthophosphate:Acetonitrile(61:39) used as solvent system. Method-I: At the maximum absorption wavelength of 230nm method was developed. Method-II: Kromasil C18 column selected as stationary phase. The flow rate and detector wavelength selected were 0.9 mL/min and 230nm respectively. Study conducted by 5µL volume of injection at column temperature of 25°C. RT was found to be 2.518min. Method-III: Acquity BEH-C18 (1.7 µ, 100×2.1mm) column selected as stationary phase. with the run time of 1.2min at 0.5ml/min flow rate method was optimized. The detection wavelength selected as 230nm.Rt was found to be 0.89min. Validation studies were conducted as per ICH guidelines. From the linearity studies the range and correlation co-efficient were found be 1.5-9.0µg/ml&0.999 respectively for developed methods. The %RSD calculated in the intra-day and inter-day precision were less than 2. . From accuracy studies %recovery of drug were found be 98-102%. Under the accelerated conditions the drug degradation was less than 10%. Hence the developed methods were stable as pe the guidelines. From assay studies the % of dug present in the sample solution was between 99-102%. Hence the developed methods were suitable to separate the analyte present in the formulations.

Key Words: UV-Visible spectroscopy, HPLC, UPLC Semaglutide, Potassium dihydrogen ortho phosphate, accelerated conditions, degradation, formulations.

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Introduction

Diabetes mellitus is a metabolic disease due to the lack of insulin secretion or insulin action or both, which leads to hyperglycemia.[1] Diabetes mellitus is two types, type-I diabetes mellitus and type-II diabetis mellitus. Type-II diabetes mellitus is treated with glucagon-like peptide-1 receptor agonist (GLP-1 RA) drug. Semaglutide is a drug used to treat with type-2 diabetes mellitus.[2-3] Chemical name of Semaglutide 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,3R)-2-[(2S)-2-[(2S,3R)-2-{2-[(2S)-2- {2-[(2S)-2 -amino-3- (1H-imidazol-4-yl) propanamido]-2- methyl propanamido} -4- carboxybutanamido] 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}-3-carboxypropyl] carbamoyl} pentyl] carbamoyl} methoxy) ethoxy]ethyl}carbamoyl)methoxy]ethoxy}ethyl)carbamoyl]-1-carboxy propyl] carbamoyl} heptadecanoic acid.[3] molecular weight is 4113.641 and molecular formula is C187H291N45O59. Structure was shown in fig 1.

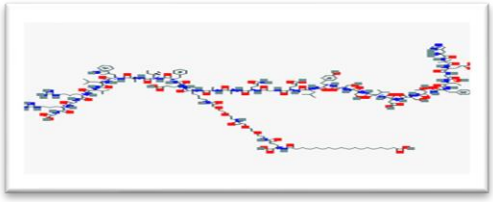

Figure no 1: Semaglutide Structure

Literature review [4-8] revealed that only few studies were reported to estimate the Semaglutide. The current research explicates about the stability indicating method development and validation of Semaglutide present in the bulk and pharmaceutical dosage forms by using UV-Visible spectroscopy, HPLC and UPLC.

Material and Methods

Chemicals and Reagents

Pure Semaglutide was procured from Spectrum pharma pvt ltd (Hyderabad). Hydrochloric acid AR grade (HCL) and sodium hydroxide AR grade (NAOH) were obtained from Merck India Pvt Ltd. Hydrogen Peroxide (H_2O_2) was purchased from Qauligens. 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.

Equipment

The UV-Visible spectrophotometer used for the method development and method validation was shimadzu-1800, japan. Other equipments used throughout the experimental work are hot air oven (Yorco

scientific), thermostat dry air equipment Thermo scientific and pH meter (Eutech instruments pH tutor, pH meter, India).

The LC system used for method development and method validation. Detection was carried by Waters with a diode array detector (model: 2996 detector 2487 separation module). The output signal was supervised and processed using Waters Empower 2 Software.

For method development and method validation was Acquity-UPLC of Waters, PDA-Detector, 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 and pH meter (Eutech instruments pH tutor, pH meter, India).

Preparation of Solutions

Preparation of Standard Solution

Weigh accurately about 3mg of Semaglutide was dissolved in 0.01N Potassium dihydrogen orthophosphate:Acetonitrile (61:39). Sonicated the solutions for 5 min and made to 50ml.

Preparation of Sample Solution

Weight accurately about equivalent to 3mg of Semaglutide tablet powder was dissolved in 0.01N Potassium dihydrogen orthophosphate:Acetonitrile (61:39). Made up the volume to 50ml with solvent system.

Preparation of Potassium dihydrogen ortho phosphate buffer(pH 2)

Weigh accurately about 1.36g of Potassium dihydrogen ortho phosphate and dissolved in distilled water. Made the volume 1000ml with distilled water. A 1ml of Triethylamine was added. Using diluted ortho phosphoric acid solution pH was adjusted to 2.

Methodology

Method-I: Using standard solution of 6µg/mL of Semaglutide spectrum was produced between 200-400 nm wavelength in UV-Visible spectrophotometer. 0.01N Potassium dihydrogen orthophosphate:Acetonitrile (61:39) is used as blank.

Method-II: To select the stationary phase and mobile phase various trials were conducted. The selected stationary phase and mobile phase were Kromasil C18 column and 0.01N Potassium dihydrogen orthophosphate:Acetonitrile (61:39) respectively. The flow rate and detector wavelength selected were 0.9 mL/min and 230nm respectively. Study conducted by 5 µLvolume of injection at column temperature of 25 °C.

Method-III: 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 orthophosphate:Acetonitrile (61:39) respectively. The flow rate and detector wavelength selected were 0.5 mL/min and 230nm respectively. Study conducted by 5 µLvolume of injection at column temperature of 25 °C.

System Suitability

The proposed method was validated under selected system conditions as per ICH guidelines. Six replicate standard solutions of 6µg/mL Semaglutide were injected and %RSD was calculated (method-II & III).

Linearity

Different concentration levels of (1.5 µg/mL, 3 µg/mL, 4.5 µg/mL, 6 µg/mL, 7.5 µg/mL, 9 µg/mL) Semaglutide solutions were prepared. Using the results calibration curve was plotted against concentration vs absorbance (Method-I) and concentration vs area (Method-II & III).

Precision

Semaglutide solution of 6µg/mL six replicate standard solutions were prepared and measured the absorbance (Intra-day precision). Repeat the same procedure in different days (Inter-day precision).

Accuracy

The %recovery of drug was determined in three concentration levels. The concentration levels were 50% (3 µg/mL), 100% (6 µg/mL) and 150% (9 µg/mL).

Robustness

Robustness referred as, capacity of method to remain unaffected by small or deliberate changes in chromatographic conditions like organic content in mobile phase organic phase ration (± 10), flow rate (± 0.1ml/min) and temperature (± 5) (method-II & III).

LOD and LOQ

Limit of Detection (LOD) and Limit of Quantitation (LOQ) were determined by using the below mentioned formulas.

LOD = (3.3 × Standard deviation) ÷ Slope

LOD = (10× Standard deviation)÷ Slope

Stability

The solutions of 6µg/mL Semaglutide were exposed to stress conditions and percentage of dug degradation was determined. The accelerated conditions were acid(2N HCl), base(2N NaOH), neutral(Humidity), peroxide(20% H_2O_2), thermal degradation(Hot air oven) and photolytic degradation(UV radiation).

Assay

Using Rybelsus 14 mg tablets 6µg/mL solutions of six replicates samples prepared and percentage purity of Semaglutide present in the marketed formulation was calculated.

Result and Discussion

UV-Visible Spectroscopic Method development

Several trails were conducted by suing different solvent systems. Semaglutide spectrum was produced between 200-400 nm wavelength in UV-Visible spectrophotometer. Solvent is used as blank. The maximum absorption was occurred at 230nm wavelength. Semaglutide spectrum was shown in fig. 2.

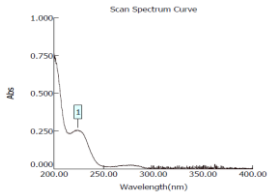

Figure no 2: Spectrum of Semaglutide

HPLC Method Development

Various trials were conducted to select mobile phase and stationary phase. The choice of a Kromasil C18 column and 0.01N Potassium dihydrogen orthophosphate : Acetonitrile (61:39) respectively. at wavelength detection of 230nm. The flow rate and detector wavelength selected were 0.9 mL/min and 230nm respectively. Study conducted by 5 µLvolume of injection at column temperature of 25 °C. Retention time was found to be 2.518min. Optimized chromatogram was shown in fig no 3.

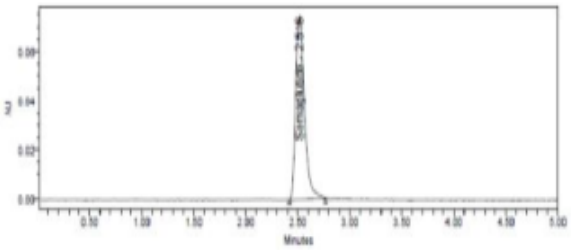

Figure no 3: HPLC chromatogram of Semaglutide

UPLC Method Development

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 (61:39) 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.

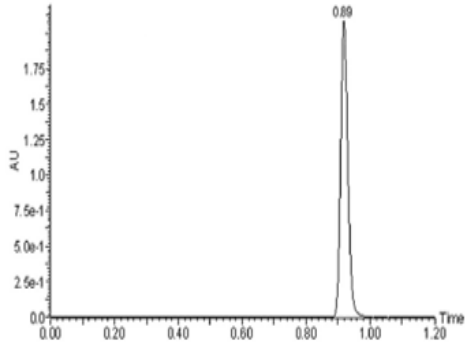

Figure 3: UPLC chromatogram of Semaglutide

Method Validation

Linearity

Different concentration levels of (1.5 µg/mL, 3 µg/mL, 4.5 µg/mL, 6 µg/mL, 7.5 µg/mL, 9 µg/mL) Semaglutide solutions were prepared. Using the results calibration curve was plotted against concentration vs absorbance/area. The obtained results were within the limits as per the ICH guidelines. The results were shown in Fig. 4&5 and Table1&2.

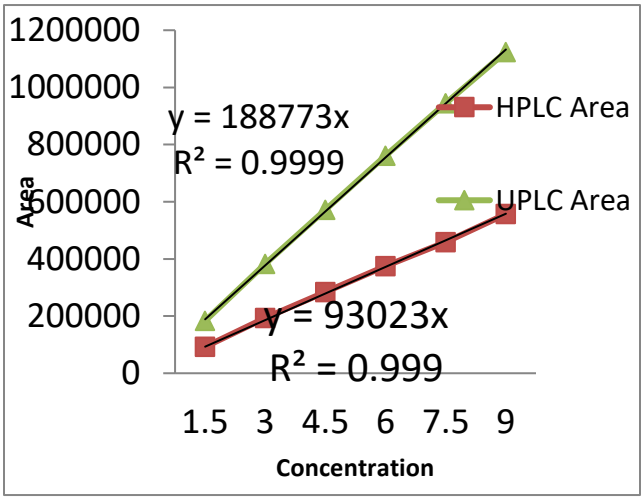

Figure no 4:Linearity of Semaglutide by HPLC&UPLC

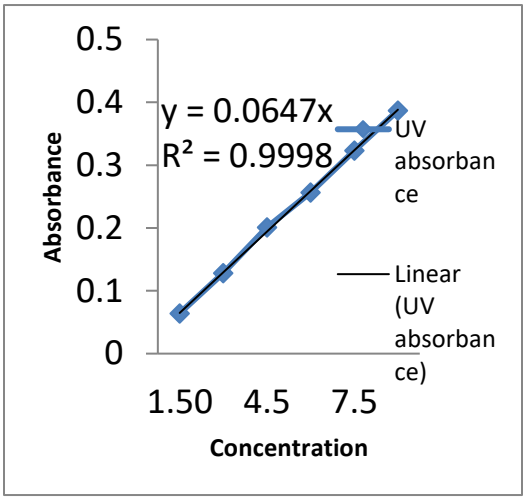

Figure no 5:Linearity of Semaglutide by UV

Table 1

Linearity of Semaglutide

S.No	Concentration	UV Absorbance	HPLC Area	HPLC Area
1	0	0	0	0
2	1.5	0.06421	92489	183362
3	3	0.12835	193079	382342
4	4.5	0.20126	283992	572412
5	6	0.2567	374706	761642
6	7.5	0.32318	458744	944342

7	9	0.38714	556985	1124123
Correlation Co-efficient		0.999	0.999	0.999

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 2.

Table 2

System suitability of Semaglutide

S.No	HPLC Area	UPLC Area
1	369765	764487
2	369582	761694
3	365226	761762
4	366412	761714
5	365262	761283
6	367302	761532
Mean	367258	762079
SD	2025.67	1088.824
%RSD	0.6	0.142876

Precision

Intra-day and inter-day precisions were performed and %RSD were calculated. As per the ICH guidelines %RSD should be less than 2. Hence the developed method was precise. The results were shown in table 3.

Table 3

Intra-day and inter-day Precision of Semaglutide

S.No	UV		HPLC		UPLC	
	Precision	Intermediate Precision	Precision	Intermediate Precision	Precision	Intermediate Precision
1	0.2574	0.2498	368190	345553	764487	763489
2	0.2569	0.2562	365689	341650	761694	763532
3	0.2529	0.2586	364421	344492	761762	763714
4	0.2487	0.2487	366696	340676	761714	762589
5	0.2498	0.2519	361043	339670	761283	763283
6	0.2489	0.2569	366406	337240	761532	761593
Mean	0.25244	0.25368	365408	341547	762079	763033

SD	0.00395	0.0041	2254.655	2815.543	1088.824	736.4253
%RSD	1.56515	1.61521	0.617025	0.82435	0.142876	0.096513

Accuracy

The % recovery of drug present in 50%, 100% and 150% solutions were between 98%-102%. As per the ICH guideline the obtained results were within the limits. The accuracy results were shown in the table 4.

Table 4

Accuracy of Semaglutide

S.No	Concentration	% recovery in UV	% recovery in HPLC	% recovery in UPLC
1	50% (3µg/ml)	100.3	99.8	100.01
2	100% (6 µg/ml)	99.89	100.3	99.97
3	150% (9µg/ml)	99.98	99.9	99.98

Robustness

Robustness studies were performed by altering the parameters like column temperature, flow rate and % organic concentration. The % RSD was calculated and found that the results within the limits. Results were shown in table no 5.

Table 5

Robustness of Semaglutide

CONDITION / METHOD	%RSD + Flow rate	%RSD - Flow rate	%RSD + Organic phase ratio in MP	%RSD -Organic phase ratio in MP	%RSD + Column Temperature	%RSD - Column Temperature
UV	1.42	1.63	1.31	1.52	1.67	1.41
HPLC	0.71	0.82	0.58	0.77	0.96	0.73
UPLC	0.3	0.32	0.25	0.31	0.4	0.6

LOD and LOQ

Limit of Detection (LOD) and Limit of Quantitation (LOQ) results were mentioned in table no 6.

Table 6

LOD and LOQ of Semaglutide

	UV	HPLC	UPLC
LOD	0.2	0.19	0.07
LOQ	0.61	0.57	0.24

Stability

The standard solutions of 100% concentration of Semaglutide were exposed to accelerated conditions. The percentage degradation should be less than 10%. As per the ICH guidelines the results were within the limits and shown in table 7.

Table 7

Stability of Semaglutide

S. No.	Condition of degradation study	UV		HPLC		UPLC	
		% drug recovery	% of drug degraded	% drug recovery	% of drug degraded	% drug recovery	% of drug degraded
1.	2N HCl	95.48	4.52	95.83	4.17%	95.65	4.35
2.	2N NaOH	95.56	4.44	94.73	5.27%	95.78	4.22
3.	Neutral hydrolysis	95.87	4.13	100	No degradation	95.71	4.29
4.	Oxidative degradation	97.86	2.14	93.74	6.26%	97.84	2.16
5.	Thermal degradation	98.75	1.25	95.84	4.16%	98.86	1.14
6.	Photo degradation	99.42	0.58	98.13	1.87%	99.54	0.46

Assay

Assay was performed by using Rybelsus 14 mg tablets. The percentage purity of Semaglutide present in the formulation was found to be 99.15% w/v, 99.99% w/v and 100.2% w/v for UV, HPLC and UPLC respectively.

Conclusion

The obtained results revealed that the developed method is an economic, simple, accurate, stable and precise method. Using the same solvent system estimation of Semaglutide done by UV, HPLC and UPLC. The assay results demonstrating that the proposed method can be used for the determination of commercial formulations without interference of the excipeints used in the preparation of formulations.

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Conflict of Interest

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

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