METHOD DEVELOPMENT AND VALIDATION OF SEMAGLUTIDE BY UV SPECTROPHOTOMETRIC METHOD IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT: *The main aim and objective of the study was to develop and validate simple, precise and accurate UV-Visible spectrophotometric method for the determination of semaglutide in bulk and pharmaceutical dosage form. 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. All the parameters such as linearity, accuracy, precision, limit of detection and limit of quantification were chosen according to ICH guidelines and validated statistically. 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. This method can be employed for routine analysis of semaglutide in bulk and pharmaceutical dosage form.*

Key Words: *Semaglutide, Zero order, First-order, UV spectroscopy.*

INTRODUCTION

Method development is the process of selecting an accurate assay procedure to determine the composition and providing that an analytical method is acceptable for use in laboratory. Analytical methods must be developed using the protocols and acceptance criteria [Ashish et al, 2015]. Validation is the process of establishing documentary evidence demonstrating that a procedure, process or activity carried out in testing and then the production maintains the desired level of compliance at all stages [Lavanya et al., 2013]. It is used in achieving the quality and safety of the final product especially in pharmaceutical industry [Sibel, 2018].

The drug semaglutide is a once-daily glucagon-like peptide-1 analog that differs to others by the presence of an acyl group with a steric diacid at Lys26. It is a large synthetic spacer and modified by the presence of α-aminobutyric acid in position 8 which gives stability against the dipeptidylpeptidase-4 [Gotfredsen et al, 2014]. The IUPAC name of semaglutide is polypeptide derivatives joined between indole with imidazole derivative. It reduces blood glucose through a mechanism where it stimulates insulin secretion and lowers the glucagon secretion [Hjerpsted et al, 2018]. It is indicated to improve glycemic control in adults with type 2 diabetes mellitus as an adjunct of diet and exercise [Hjerpsted et al, 2018]. In clinical trials, reduction of glycated hemoglobin (HbA1c) compared to other medications like sitagliptin, exenatide and insulin glargine U100 were noticed. The HbA1c protein is a standard measure of high glucose as in normal conditions the hemoglobin forms 1-deoxyfructose which reduces the body weight. The stability of semaglutide by acylation permits high- affinity albumin binding and gives a long plasma half-life which allows the once daily dosage [Hjerpsted et al, 2018]. However, there was no work in the literature reported about UV spectrophotometric method for the analysis of semaglutide using 0.01N potassium dihydrogen ortho phosphate and sodium acetate buffer pH-5 as solvent. Thus, the main objective of the present study was to develop and validate UV spectroscopic method for the determination of semaglutide in bulk and pharmaceutical dosage form.

MATERIALS AND METHODS

Chemicals and Reagents

Semaglutide was marketed by Danish company Novo Nordisk under the trade name of ozempic which was obtained as a gift sample from Spectrum pharma research solutions, Hyderabad, India. Acetonitrile used was supplied by Thermo Fischer Scientific India Private Limited. All the chemicals used were of analytical grade. The tablet formulations were procured from a local pharmacy.

Instrumentation

UV 1800 double beam UV Visible Spectrophotometer (shimadzu) with a pair of 10mm path length matched quartz cells were used for the study. The UV solutions 2.42 software was used. An electronic balance used for weighing purpose was Shimadzu. Volumetric flasks and pipettes used in the study were of borosilicate glass. All the statistical calculations were carried out by using Microsoft Excel 2007.

PREPARATION OF SOLUTIONS

Preparation of stock solution

Accurately weighed 10mg of semaglutide and dissolved in 10 ml of buffer: acetonitrile (5:5) in a 10ml of volumetric flask. The solution was sonicated for 10mins. The prepared standard solution was scanned in the range of 200 - 400 nm for determination of the wavelength of maximum absorption.

Preparation of working standard solution

1ml of stock solution was transferred in to 10ml volumetric flask. Then, it was diluted with mixture of acetonitrile : water (5:5) and made up the volume to 10ml (working standard solution of 100µg/ml).

Preparation of 0.01N potassium dihydrogen ortho phosphate

Accurately weighed 1.36 gm of potassium dihydrogen ortho phosphate in a 1000 ml of volumetric flask. To that about 900 ml of milli-q water was added and sonicated. Then the volume was made up with water till mark.

Preparation of sodium acetate buffer (pH-5)

Dissolved 13.6 gm of sodium acetate and 6 ml of glacial acetic acid in sufficient water to make up the volume to 1000 ml. Then pH was adjusted to 5 if necessary.

METHOD VALIDATION

The method was validated according to ICH guidelines to determine the linearity, accuracy, precision, LOD and LOQ of the analyte [Gilmartin and Gingrich, 2018].

Linearity

The linearity of the proposed method was determined by plotting concentration against corresponding absorbance [Mohammad et al, 2015]. A standard stock solution was further diluted with buffers to obtain 1µg/ml - 15µg/ml solutions. The calibration curve was obtained by plotting absorbance versus concentration and linear regression analysis was calculated to get linear equation [Acharjya et al, 2010].

Precision

The precision of the assay was determined by repeatability (intraday) and intermediate precision (interday) which was reported as % relative standard deviation (%RSD) [Choudhari et al, 2011].

Intra-day precision

Standard stock solutions (0.05 ml, 0.1 ml, and 0.15 ml) were taken in a 10 ml volumetric flasks and the final volume was made up to the mark with buffer. The absorbances of these solutions were individually measured thrice within a day and recorded [Shrinivas and Revanasiddappa, 2015].

Inter-day precision

Standard stock solutions (0.05 ml, 0.1 ml, and 0.15 ml) were taken in 10 ml volumetric flasks and volume were made up to the mark with buffer. The absorbances of these solutions were individually measured thrice in three days and recorded.

Accuracy

Accuracy of the method was estimated by standard addition recovery method with three different levels i.e 50 %, 100% and 150%. In this, known amount of standard drug was added to tablet samples at three different concentration levels [Arora et al, 2011]. The absorbance was recorded and % recovery was calculated.

Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) were determined by using standard deviation of response and slope of corresponding curve using following equation 3.3 σ/ S (LOD) and 10 σ/ S (LOQ) [Argekar and Sawant, 1999].

Assay of semaglutide tablets

The marketed formulation of semaglutide was analysed by this method. An amount equivalent to 10mg/ml of semaglutide (injection) was transferred to 100ml volumetric flask and the contents of the flask were dissolved in 50 ml of buffer and ultra sonicated for 10 min. The solution was filtered and then the final volume of the solution was made up to 100 ml with same solvent to get a stock solution containing 100µg/ml of semaglutide. After appropriate dilutions, the absorbance was measured and the concentration

of each analyte was determined with the equations obtained from calibration curve.

RESULTS AND DISCUSSION

The developed method was validated as per ICH guidelines with respect to linearity, accuracy, precision, LOD and LOQ. The standard solution was individually scanned and spectra of semaglutide was recorded between 200 - 400 nm. The spectral analysis showed the λ max of semaglutide to be at 293.80nm for 0.01N potassium dihydrogen ortho phosphate (method A) and 293.20nm for sodium acetate (method B). The overlain first order derivative spectrum of semaglutide at different concentrations revealed at 240.55 (method A), 254.27 (method B) for maxima and 254.28 (method A), 213.45 (method B) for minima .The overlain spectrum was depicted in fig 5-8 and the summary of validation parameters was represented in Table1.

Table 1: Summary of validation parameters

PARAMETERS	OBTAINED VALUES			
	0.01N Potassium dihydrogen ortho phosphate	**0.01N Potassium dihydrogen ortho phosphate**	**Sodium acetate buffer pH-5**	**Sodium acetate buffer pH-5**
	D_0	D_1	D_0	D_1
λ_{max}	293.80	254.28	293.20	254.27
Beer's Law limit (µg/ml)	1-15 µg/ml	1-15 µg/ml	1-15 µg/ml	1-15 µg/ml
Slope	0.1639	0.0102	0.1075	0.0117
Intercept	0.0161	0.0005	0.0006	0.0019
Correlation coefficient	0.9996	0.9992	0.9996	0.9996
% Recovery	99.8% - 102%		98% - 100.8%	
Precision (%RSD) Intraday (n=3)	0.14 - 0.99	0.32 - 1.07	0.18 - 0.74	0.62 - 1.65
Precision (%RSD) Interday (n=3)	0.10 - 0.71	0.28 - 1.48	0.15 - 0.85	0.52 - 1.18
LOD (µg/ml)	0.01	0.26	0.03	0.13
LOQ (µg/ml)	0.03	0.78	0.09	0.42
Sandell's sensitivity	0.0060	0.0952	0.0092	0.0862
Molar absorptivity	689955.75	41335.5	438034.5	47684.3

Linearity

The linearity of the semaglutide was determined by plotting calibration curve of absorbance versus concentration for zero order (D^0) and amplitude versus concentration for first order (D^1). The correlation coefficient R^2 value for semaglutide was found to be 0.9996 and 0.9992 in method A while 0.9996 and 0.9996 in method B, respectively. The following equation for straight line was observed.

Y= 0.1639x + 0.0161 for zero order (Method A)
Y= 0.0102x + 0.0005 for first order derivative. (Method A)
Y= 0.1075x - 0.0006 for zero order (Method B)
Y= 0.0117x - 0.0019 for first order (Method B)

Linearity obeyed Beer Lambert's law in the concentration range of 1-15µg/ml. Calibration curves were shown in fig 1- 4

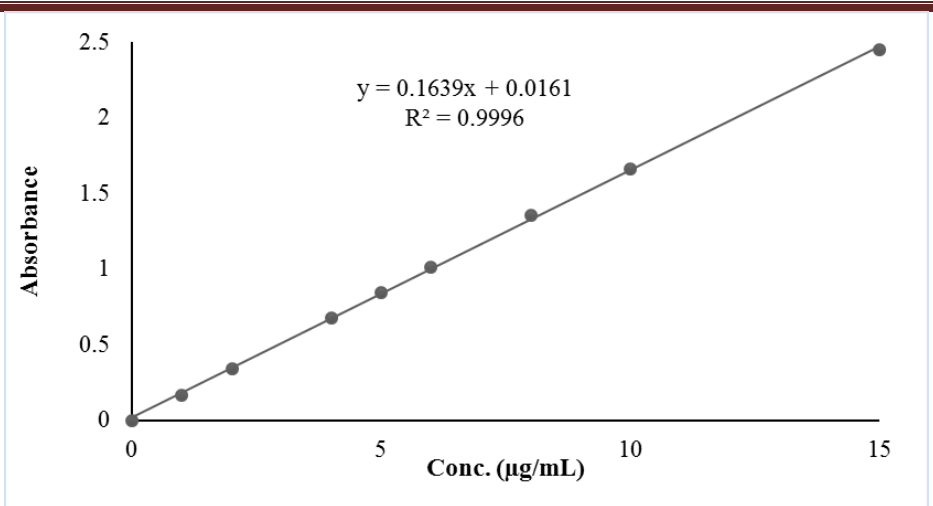

Fig.1: Calibration curve of zero order 0.01N Potassium dihydrogen ortho phosphate

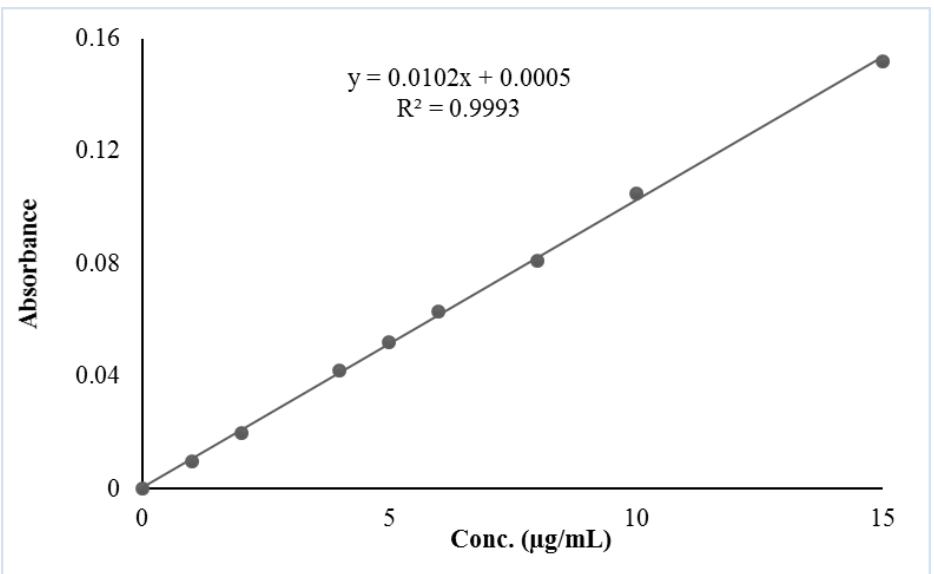

Fig.2: Calibration curve of First order 0.01N Potassium dihydrogen ortho phosphate

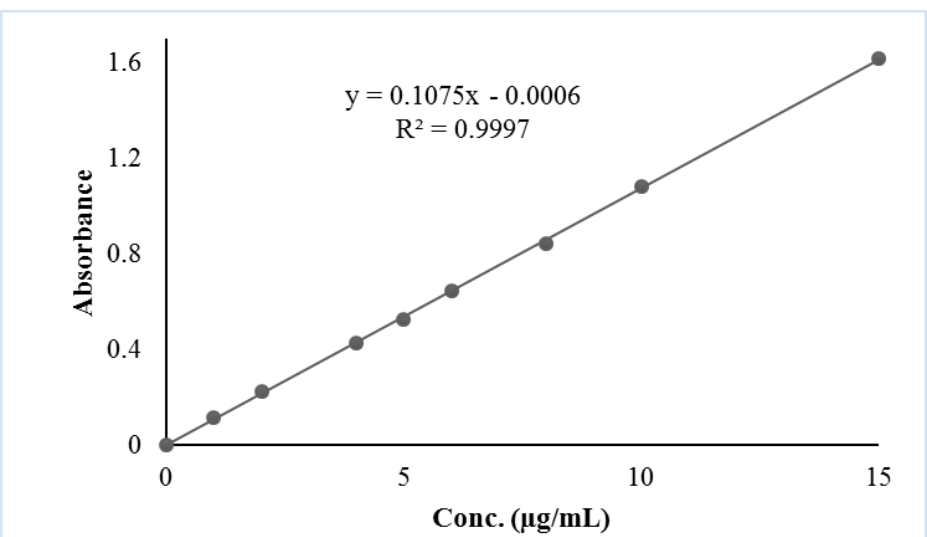

Fig.3: Calibration curve of Zero order Sodium acetate buffer pH-5

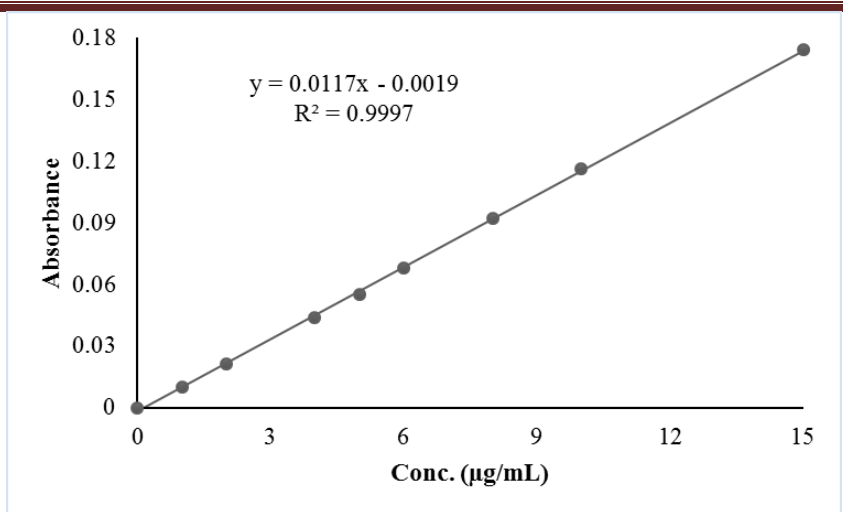

Fig.4: Calibration curve of First order Sodium acetate buffer pH-5

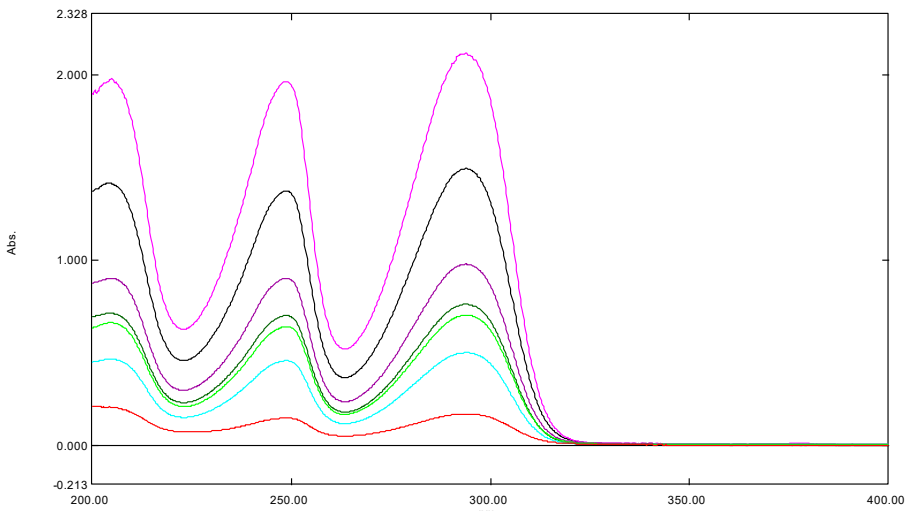

Fig.5: Overlain spectra of Zero order 0.01N Potassium dihydrogen ortho phosphate

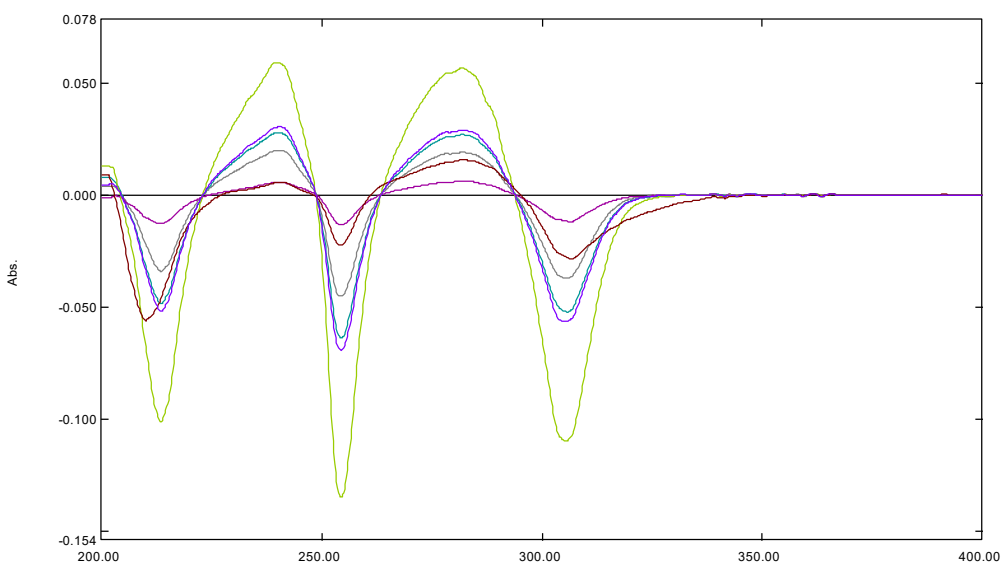

Fig.6: Overlain spectra of First order 0.01N Potassium dihydrogen ortho phosphate

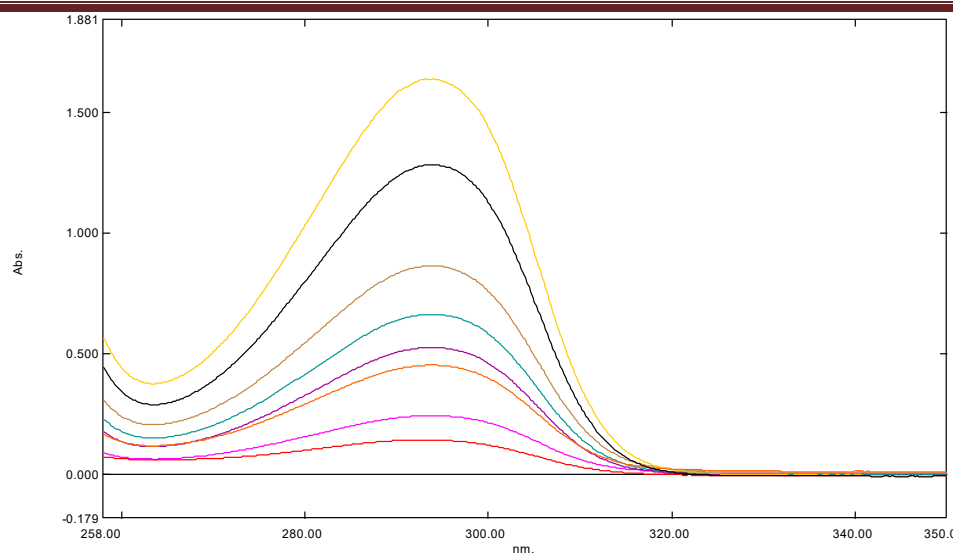

Fig.7: Overlain spectra of Zero order Sodium acetate buffer pH-5

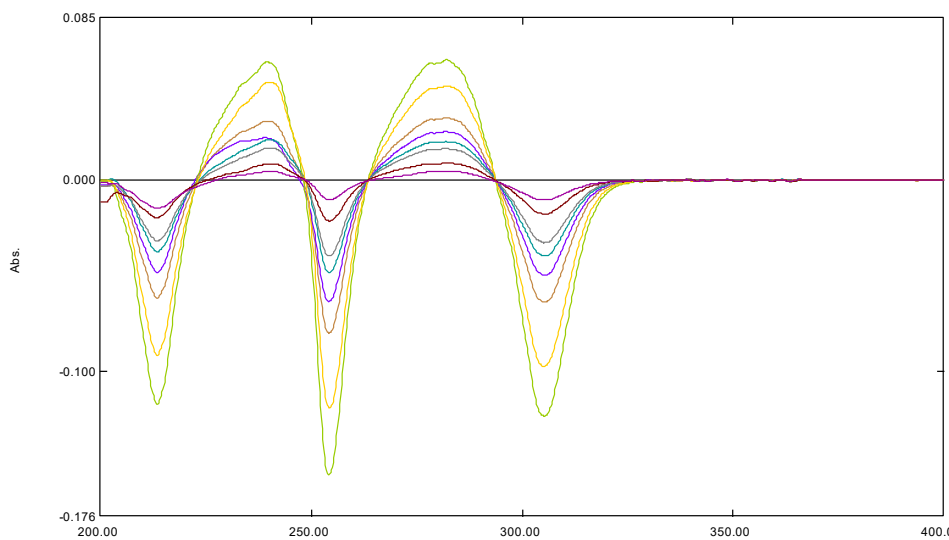

Fig.8: Overlain spectra of First order Sodium acetate buffer pH-5

Accuracy

The mean % recovery of concentrations ranging (spike level) 50%, 100%, 150% was found to be 99.8% - 102 % for method A and 98 % - 100.8 % for method B, respectively. The results of recovery studies were mentioned in Table 2 and 3.

Table 2: Accuracy data of method A (0.01N Potassium dihydrogen ortho phosphate)

Method A	Initial amount (µg/ml)	Amount added (µg/ml)	Amount recovered (µg/ml, n=3)	%Recovered ± standard deviation	%RSD
Zero-order	5	2.5	7.63	101.9±0.56	0.21
	5	5	10.21	102 ±0.16	0.95
	5	7.5	12.49	99.8±0.66	0.69
First-order	5	2.5	7.54	100.8±0.25	1.54
	5	5	10.04	100.8±0.63	1.54
	5	7.5	12.50	100±0.20	1.33

Table 3. Accuracy data of method B (Sodium acetate buffer pH-5)

Method B	Initial amount (µg/ml)	Amount added (µg/ml)	Amount recovered (µg/ml, n=3)	%Recovered ± standard deviation	%RSD
Zero-order	5	2.5	7.47	99.4±0.24	0.30
	5	5	9.95	99±0.204	0.19
	5	7.5	12.4	98±0.163	0.12
First-order	5	2.5	7.45	99±0.63	1.18
	5	5	10.04	100.8±0.36	1.42
	5	7.5	12.4	98±0.53	1.14

Precision

Precision defines closeness between a series of measurements from multiple sampling of same homogeneous sample under the prescribed conditions. Precision was determined by intra day and inter-day of semaglutide and it was expressed as %RSD. The %RSD of intra-day of method A was found to be 0.14 - 0.99 for zero order and 0.32 - 1.07 for first order. Similarly, %RSD of inter-day of method A was found to be 0.10 - 0.71 for zero order and 0.28 - 1.48 for first order. In the same way, the %RSD of intra-day of method B was found to be 0.18 - 0.74 for zero order and 0.62 - 1.65 for first order. Correspondingly, %RSD of inter-day of method B was found to be 0.15 - 0.85 for zero order and 0.52 - 1.18 for first order which were found to be <2. The results of precision were shown in table 4-7.

Limit of detection and limit of quantification

LOD is defined as the lowest amount of analyte which can be detected and LOQ is defined as the lowest amount of analyte which can be quantitatively determined. The LOD values of method A were found to be 0.01 for zero order and 0.26 for first order. Similarly, LOQ values of method A were establish to be 0.03 for zero order and 0.78 for first order. In the same way, the LOD values of method B were found to be 0.03 for zero order and 0.13 for first order. Likewise, LOQ values of method B were found to be 0.09 for zero order and 0.42 for first order. The LOD and LOQ were calculated as per ICH guidelines.

Table 4: **Intra-day precision data of method A (0.01N Potassium dihydrogen ortho phosphate)**

Method A	Concentration (µg/ml)	Amount found (µg/ml) (n=3)	Mean ± SD	%RSD
Zero-order	5	5.04	99.6 ± 0.0012	0.14
	10	9.90	97.7 ± 0.016	0.99
	15	14.97	98.4 ± 0.016	0.66
First-order	5	5.14	98 ± 0.0018	0.32
	10	10.39	98.5 ± 0.014	1.07
	15	14.02	98.1 ± 0.010	0.51

Table 5: Inter-day precision of method A (0.01N Potassium dihydrogen ortho phosphate)

Method A	Concentration (µg/ml)	Amount found (µg/ml) (n=3)	Mean ± SD	%RSD
Zero-order	5	5.14	99.6 ± 0.0012	0.10
	10	10.39	98.8 ± 0.0122	0.71
	15	14	101.6 ± 0.0122	0.48
First-order	5	5.44	98.18 ± 0.0013	0.28
	10	10.73	99 ± 0.0016	1.48
	15	15.63	98 ± 0.008	0.51

Table 6: Intra-day precision of method B (Sodium acetate buffer pH-5)

Method B	Concentration (µg/ml)	Amount found (µg/ml) (n=3)	Mean ± SD	%RSD
Zero-order	5	4.912	99.2 ± 0.0012	0.23
	10	10.05	99.6 ± 0.0020	0.18

	15	15.21	98.2 ± 0.0122	0.74
First-order	5	5.13	98.38 ± 0.0015	0.62
	10	10.09	98.3.8 ± 0.0021	1.65
	15	15.01	98.3 ± 0.0020	1.15

Table 7: Inter-day precision of method B (Sodium acetate buffer pH-5)

Method B	Concentration (µg/ml)	Amount found (µg/ml) (n=3)	Mean ± SD	%RSD
Zero-order	5	5.05	99.6± 0.0012	0.23
	10	10.36	99.7± 0.0016	0.15
	15	15.18	98.7± 0.014	0.85
First-order	5	5.13	98.33± 0.0010	0.52
	10	10.09	99.16± 0.0014	1.18
	15	15.01	98.3± 0.0020	1.14

Table 8: Assay results of semaglutide

	Method A	Method B
Formulation	Semaglutide	Semaglutide
Brand name	Omnicef	Omnicef
Label claim (mg/ml)	1.34	1.34
Amount found (mg/ml)	1.32	1.33
% Recovery	98.5	99.2

Assay results of marketed formulation

The marketed formulation was analyzed by the proposed method. In accordance with ICH guidelines the assay values for the formulation was found to be ranging in between 98.5% - 99.2%. The results were shown in table 8

CONCLUSION

The developed UV-spectrophotometric method for the estimation of semaglutide in bulk and pharmaceutical dosage form was found to be simple, precise, accurate and cost-effective. The method was found to be linear over a convenient range and the mean % recovery was stated. The %RSD for precision (inter-day and intra-day) were reported. The LOD and LOQ were determined by using standard deviation of response and slope. The above parameters were validated with respect to linearity, accuracy, precision, LOD and LOQ as per ICH guidelines.

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