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A VALIDATED METHOD DEVELOPMENT FOR THE ESTIMATION OF SEMAGLUTIDE IN ITS BULK AND PHARMACEUTICAL DOSAGE FORM BY USING UV-VISIBLE SPECTROPHOTOMETER

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ABSTRACT

A rapid and precise UV-VISIBLE spectrophotometric method has been developed for the estimation of semaglutide in its bulk and pharmaceutical dosage form. 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. This method can be employed for routine analysis of semaglutide in bulk and pharmaceutical dosage form.

KEYWORDS: Semaglutide, estimation, Ozempic.

INTRODUCTION

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 and a large synthetic spacer and modified by the presence of a α-amino butyric acid in position 8 which gives stability against thedipeptidylpeptidase-4. The stability of semaglutide by the acylation permits a high-affinity albumin binding and gives it a long plasma half-life which allows the once-daily dosage. Drugs like semaglutide affect the glucose control through several different mechanisms like the increase of insulin secretion, slow of gastric emptying, and reduction of postprandial glucagon and food intake. The glucose homeostasis depends on hormones like insulin and amylin secreted in pancreatic beta cells, glucagon secreted in pancreatic alpha cells and gastrointestinal peptides like glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide. When glucose is administered orally, GLP-1 will stimulate the synthesis of insulin by stimulating pancreatic islets, it will also slow the gastric emptying, inhibit post-meal glucagon release and reduce food intake.

Brand name: Ozempic

MATERIALS AND METHOD

Semaglutide was a gift sample from Dr. Reddys Lab, Hyderabad. All chemicals (distilled water, methanol) and reagents used were of analytical grade and purchased from Qualigens Fine Chemicals, Mumbai, India.

A Labindia UV–visible spectrophotometer (UV-T60-India) was used for all absorbance measurements with matched quartz cells. The detection wavelength is 288nm.

Preparation of standard stock solution

Accurately weighed 10 mg of Semaglutide was transferred to a 100 ml volumetric flask, dissolved in 20 ml distilled water by shaking manually for 10 min. The volume was adjusted with the same up to the mark to give the final strength, i.e.100µg/ml.

Selection of wavelength for analysis of Semaglutide

Appropriate volume 0.5 ml of standard stock solution of Semaglutide was transferred into a 10ml volumetric flask, diluted to a mark with distilled water to give concentration of 5µg/ml(and also 10, 15µg/ml). The resulting solution was scanned in the UV range (200–400 nm). In spectrum Semaglutide showed absorbance maximum at 288 nm**.**

VALIDATION OF THE METHOD

The method was validated in terms of linearity, accuracy, precision, and ruggedness.

Linearity study
Different aliquots of Semaglutide in the range 0.5–3 ml were transferred into series of 10 ml volumetric flasks, and the volume was made up to the mark with distilled water to get concentrations 5, 10, 15, 20, 25, and 30 µg/ml, respectively. The solutions were scanned on a spectrophotometer in the UV range 200–400 nm. The spectrum was recorded at 288 nm. The calibration plot was constructed as concentration vs. absorbance

Accuracy: Accuracy describes the correctness of an experimental result. Accuracy is expressed in terms of either absolute error or relative error.

Precision: Precision describes reproducibility of results. Reproducibility of results is agreement between numerical values for two or more replicate measurements.

Sensitivity: The sensitivity of measurements of Semaglutide by the use of the proposed method was estimated in terms of the limit of quantification (LOQ) and limit of detection (LOD). The LOQ and LOD were calculated using equation LOD = 3 × N/B and LOQ = 10 × N/B, where 'N' is standard deviation of the peak areas of the drugs (n = 3), taken as a measure of noise, and 'B' is the slope of the corresponding calibration curve.

Repeatability: Repeatability was determined by analyzing 20 µg/ml concentration of Semaglutide solution for six times.
Ruggedness: Ruggedness of the proposed method is determined for 20 µg/ml concentration of Semaglutide by analysis of aliquots from a homogenous slot by two analysts using same operational and environmental conditions.

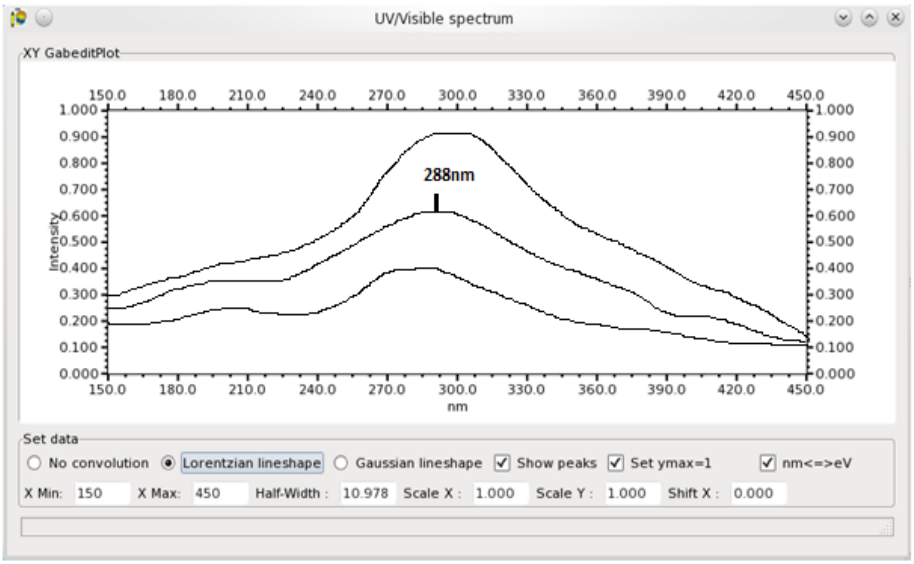
RESULTS AND DISCUSSION

Table.no. 1: Solubility studies.

Solvent	Solubility(mg/ml)
Ehanol	1.5
Methanol	0.9
Alcohol	1.2
Chloroform	1.6
Acetone	1.3
Distilled water	0.4

The pure API of Semaglutide is freely soluble in in organic solvents and sparingly soluble in aqueous solutions.

Selection of wavelength for analysis of Semaglutide
During the development phase, the use of ethanol as the diluent resulted in preferable outcome in UV analysis. The pre-determined wavelength of maximum absorption (λmax) was 288 nm.



Uv –visible spectrum

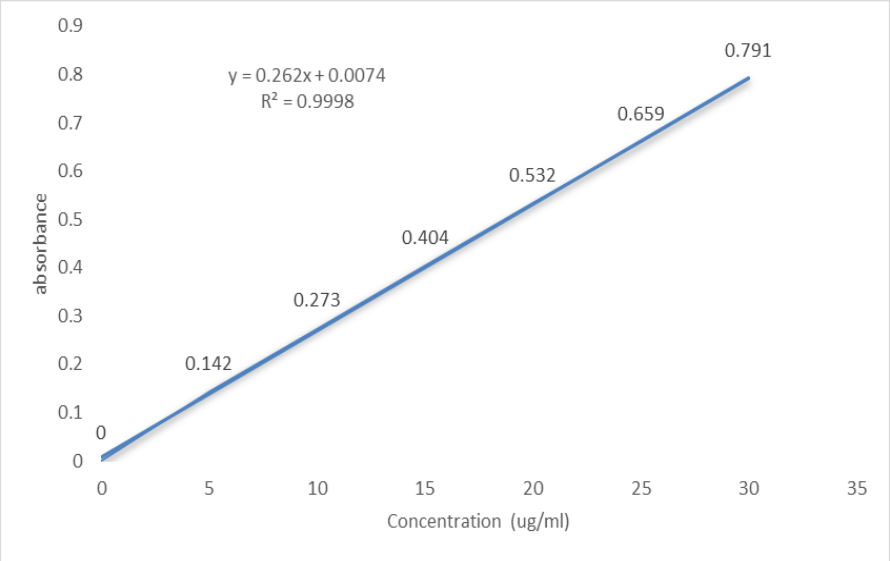
stocks	Wavelentgth of stocks	Absorbance
5 µg/ml	288	0.356
10 µg/ml	288	0.594
15 µg/ml	288	0.912

METHOD VALIDATION

LINEARITY

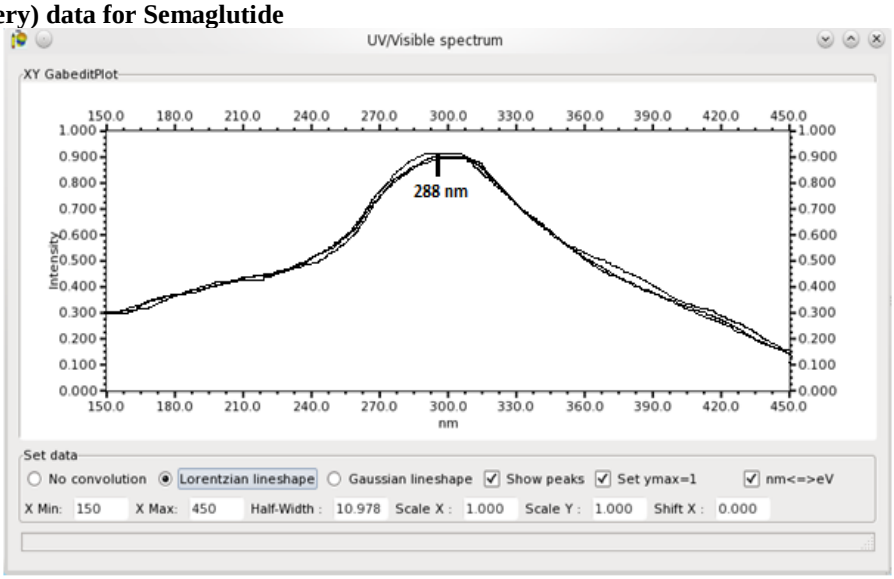
Results of Linearity

Concentration (ug/ml)	Absorbance(nm)
0	0
5	0.142
10	0.273
15	0.404
20	0.532
25	0.659
30	0.791

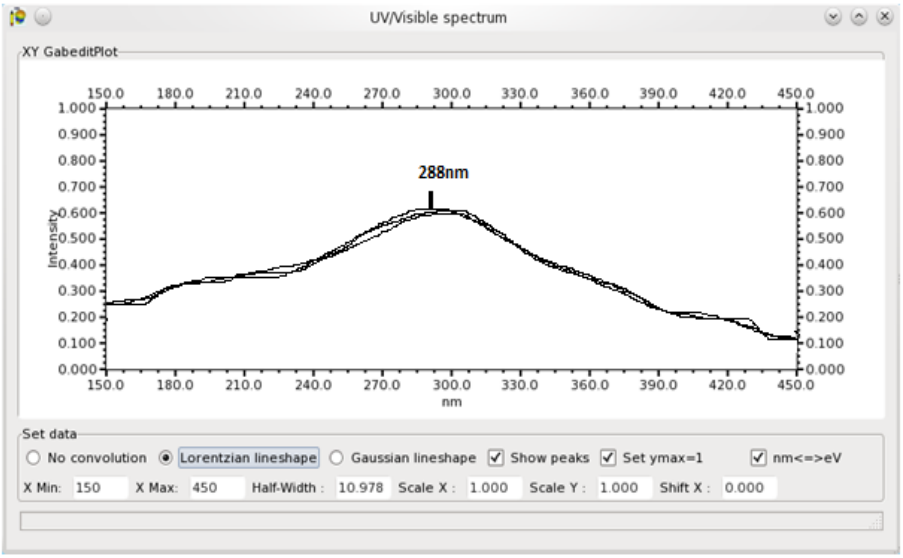


RESULTS OF CALIBRATION GRAPH

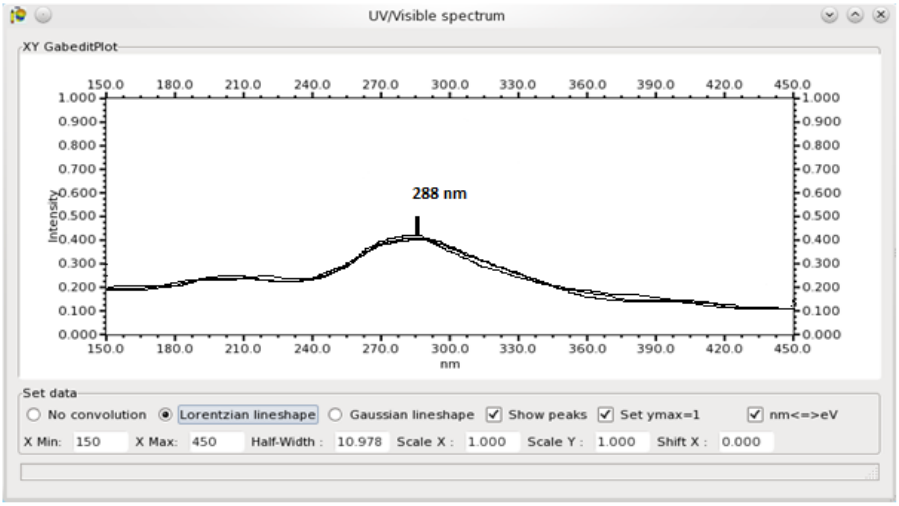
Accuracy (recovery) data for Semaglutide



N=3 12 Accuracy 50%



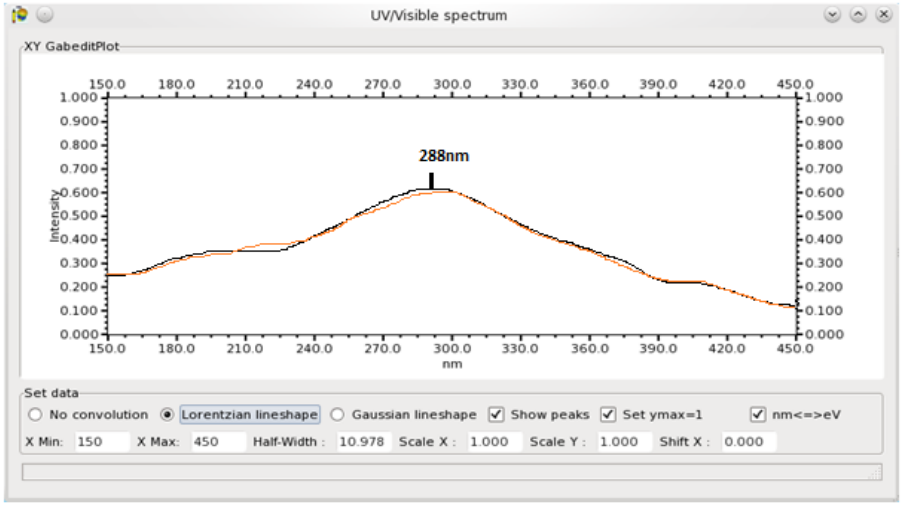
N=3 Accuracy 100%

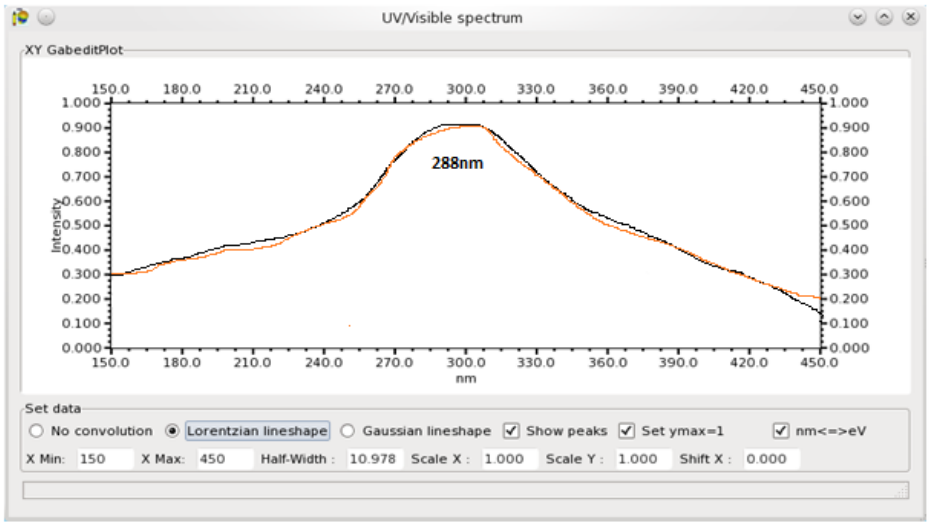


N=3 Accuracy 150%

Results of Accuracy

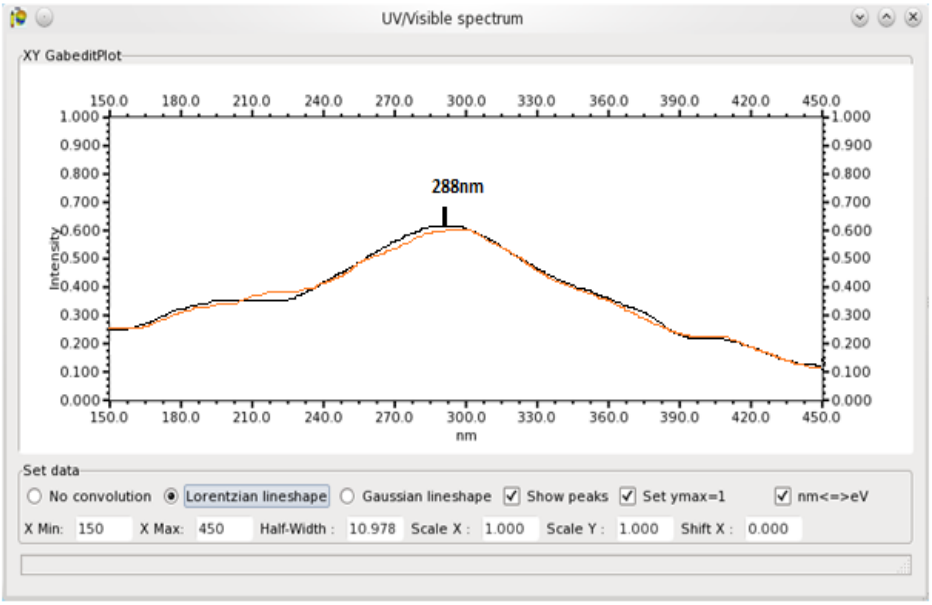
%Concentration (at specification Level)N=3	absorbance	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	0.4213	2.5	2.498	99.92	99.65
100%	0.6213	5.0	4.990	99.08	
150%	0.9199	10	9.995	99.95	

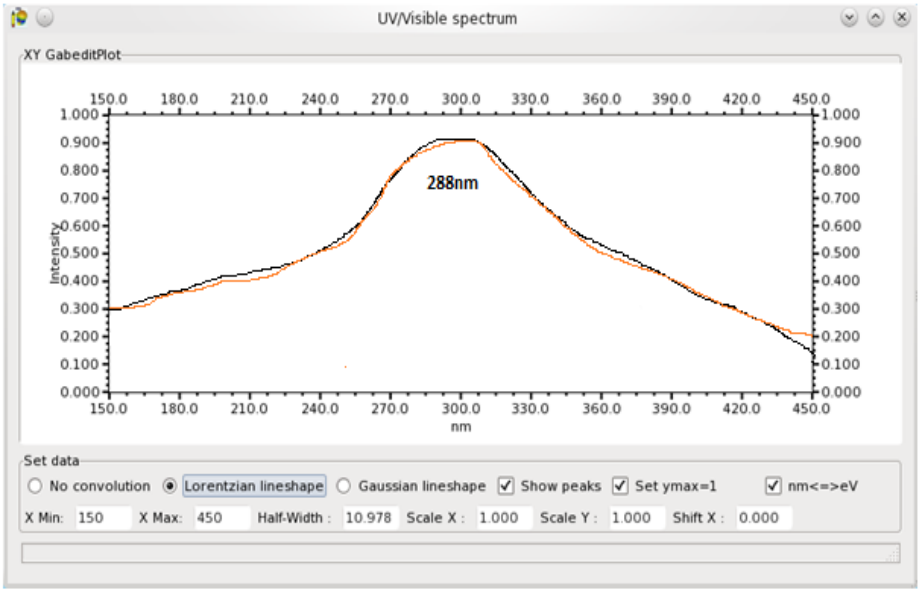




Intra-day and inter-day precision determined for three different concentrations of Semaglutide (*n*=3).

Concentration (µg/mL)	Intra-day precision			Inter-day precision		
	Absorbance measured	RSD (%)	Average (%)	Absorbance measured	RSD (%)	Average (%)
10	0.4113	0.140	98.96	0.4110	0.240	98.96





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Concentration (µg/mL)	Intra-day precision			Inter-day precision		
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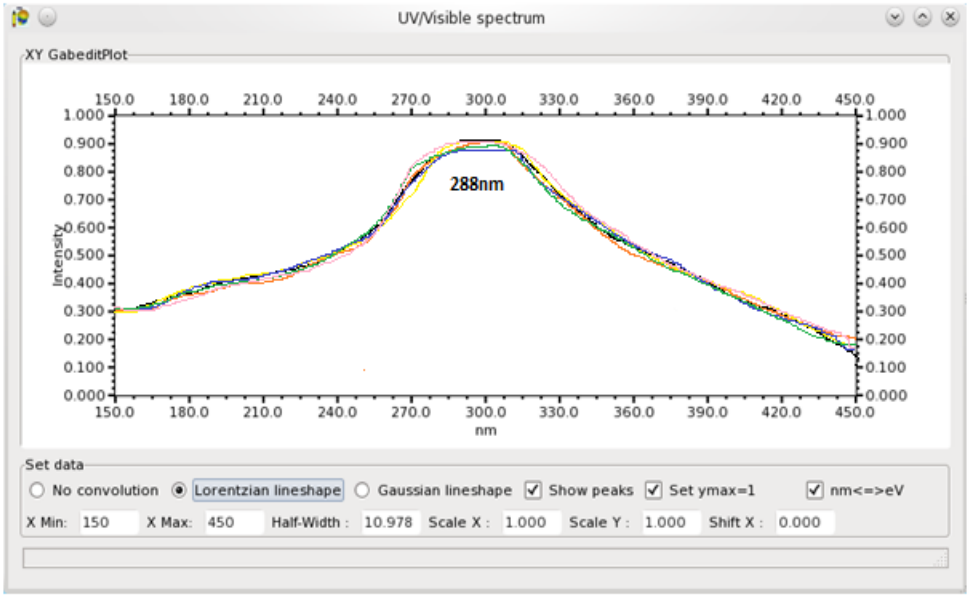
10	0.4113	0.140	98.96	0.4110	0.240	98.96
15	0.6147	0.094	98.60	0.6153	0.094	98.70
20	0.9210	0.122	98.77	0.8213	0.070	98.81

SENSITIVITY

The linearity equation was found to be Y = 0.262X + 0.0074. The LOQ and LOD for Semaglutide were found to be 0.51µg and 2.99µg, respectively.

REPEATABILITY

Results of Repeatability



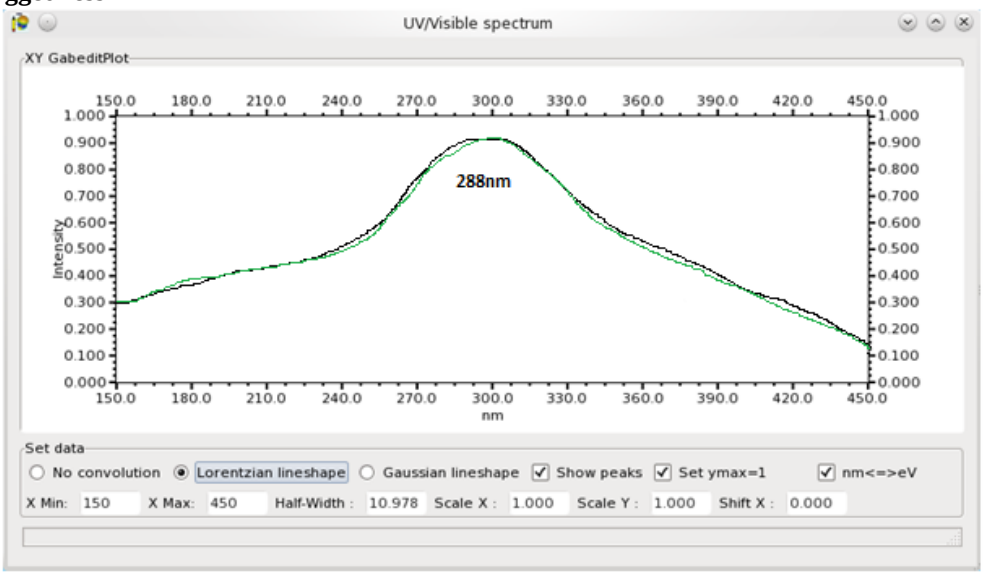
Results of Repeatability

Concentration (µg/mL)	Absorbance measured (Mean ± SD)	Amount Found (%)	RSD (%)
20	0.8310±0.0324	99.69	0.02

Repeatability was determined by analyzing 20µg/ml concentration of Semaglutide solution for six times and the % amount found was 99.69 % RSD < 2.

RUGGEDNESS

Results of Ruggedness



The peak area was measured for same concentration solutions, six times. The results are in the acceptable range for both the drugs. The result showed that the % RSD was less than 2%.

Results of Ruggedness

Analyst	Concentration (µg/mL)	Absorbance measured (Mean ± SD)	Amount Found (%)	RSD (%)
I	20	0.8116±0.0015	98.98	0.02
II	20	0.8214±0.0010	99.12	0.01

CONCLUSION

This UV-spectrophotometric technique is quite simple, accurate, precise, reproducible, and sensitive. The UV method has been developed for quantification of Semaglutide in tablet formulation. The validation procedure confirms that this is an appropriate method for their quantification in the formulation. It is also used in routine quality control of the formulations containing this entire compound.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBLIOGRAPHY

1. *Dudhe P.B* **et al.,** A simple, reproducible and economical two spectrophotometric methods have been developed for determination of Semaglutide in tablet dosage form. All solutions were prepared by using methanol as an solvent. Method A is area under curve (AUC), in which area was integrated in the range of 253.00 nm – 273.00 nm and in method B i.e. first order derivative spectroscopy.
2. **M. I. Walash et al**., A simple and sensitive spectrophotometric method was developed for the determination of semaglutide (ACM) in pure form and pharmaceutical preparations. The proposed method is based on the complex formation of semaglutide with Palladium (II) chloride in acetate buffer pH5.4 and measuring the absorbance at 308 nm.
3. **Dr. KK. Senthil kumar* et al.,** Analytical monitoring of a pharmaceutical product or of specific ingredients within the product is necessary to ensure its safety efficacy throughout all phases of its shelf life. Such monitoring is in accordance with the specifications elaborated during product development.
4. **Sravanthi B al.,** A simple, precise, rapid and accurate UV Spectrophotometric method has been developed and validated for the simultaneous estimation of Omeprazole (OMP) and Domperidone (DOM) in combined pharmaceutical dosage forms
5. **Kusuma Kumari, et al.,** A new, simple, sensitive and economical UV spectrophotometric method was developed for the simultaneous analysis of Sulfadiazine and Trimethoprim in pharmaceutical formulations. This UV method was developed with methanol as solvent. The wavelengths selected for analysis in the present method were 265 nm for TMP and 289 nm for SDA.
6. **Shalin K al.,** An UV spectrophotometric method for the quantitative determination of Itraconazole, a highly potent antimycotic, in capsule was developed in present work. The parameters linearity, precision, accuracy, limit of detection and limit of quantitation were studied according to International Conference on Harmonization guidelines.
7. **MahfuzaMaleque et al.,** A rapid, specific and economic UV spectrophotometric method has been developed using a solvent composed of water:methanol:acetonitrile (9:0.5:0.5) to determine the levofloxacin content in bulk and pharmaceutical dosage formulations. At a pre- determined λ_{max} of 292 nm, it was proved linear in the range of 1.0–12.0 µg/mL, and exhibited good correlation coefficient (R^2=0.9998) and excellent mean recovery (99.00–100.07%).
8. **B. Hemalatha Rathod et al.,** To develop a simple and cheap UV spectrophotometric method for the quantitative estimation of Indinavir sulphate (400mg) in capsules and validate as per ICH guidelines. The optimized method uses a diluent 100% potassium dihydrogen ortho phosphate buffer (pH 4.0) for the estimation of assay of Indinavir sulphate whose λmax is 259 nm.
9. **Kiran Sharma et al.,** A rapid, simple, selective and precise UV- Visible Spectrophotometric method has been developed for the determination of Cur cumin in bulk forms and solid dosage formulations. The spectrophotometric detection was carried out at an absorption maximum of 421 nm using methanol as solvent. The method was validated for specificity, linearity, accuracy, precision, robustness and ruggedness.
10. **Shalin K. Parikh et al.,** An UV spectrophotometric method for the quantitative determination of Itraconazole, a highly potent antimycotic, in capsule was developed in present work. The parameters linearity, precision, accuracy, limit of detection and limit of quantitation were studied according to International Conference on Harmonization guidelines.
11. **R.Valarmathiet** al., A simple and reproducible UV spectroscopic methods have been developed and validated for the determination of lacosamide (LCM) in bulk and tablet dosage forms of two different brands. The standard and sample solutions were prepared using methanol: water (50:50 % v/v) as solvent.
12. **Micheli Wrasse-Sangoi[I], et al.,** For determination of aliskiren in commercial samples, an analytical UV spectrophotometric method was developed and validate according to ICH guideline. The method was linear in the range between 40 and 100µg mL^{-1} (r^2 = 0.9997, n = 7) and exhibited suitable specificity, accuracy, precision, and robustness.
13. **Subha Harika Penmetsa et al.,** The objective of the study was to develop RP-UPLC method for the determination of purity of semaglutide in bulk and pharmaceutical dosage form. The chromatographic separation was achieved with Acquity BEH C18 (50mm x 1.6 mm) 1.8 m column thermo stated at 30oC with mobile phases containing 0.01N

potassium dihydrogen phosphate (3.2 pH): acetonitrile in the ratio of 50:50 v/v.