International Journal of Web of Multidisciplinary Studies
(Peer-Reviewed, Open Access, Fully Refereed International Journal)
website: https://ijwos.com OPEN ACCESS E-ISSN : 3049-2424
Volume 3 Issue 9, September 2026 DOI: https://doi.org/10.71366/ijwos03092605048

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF CAGRILINTIDE AND SEMAGLUTIDE BY RP-HPLC

Sonali[1], Dr. Mukesh Bansal[2], Dr. Dilip Agrawal[3]
[1] *Research Scholar, Mahatma Gandhi College of Pharmaceutical Sciences, Jaipur- 302022.*
[2] *Professor, Mahatma Gandhi College of Pharmaceutical Sciences, Jaipur- 302022.*
[3] *Professor & principal, Mahatma Gandhi College of Pharmaceutical Sciences, Jaipur- 302022.*

Article Info

Article History:
Published: 22 Sept 2026

Publication Issue:
Volume 3, Issue 9
September-2026

Page Number:
365-375

Corresponding Author:
Sonali

Abstract:

The present study focuses on the development and validation of a simple, accurate, precise, and robust reverse-phase high-performance liquid chromatography (RP-HPLC) method for the simultaneous estimation of cagrilintide and semaglutide, in pharmaceutical dosage forms. These drugs are widely used in the management of type 2 diabetes mellitus and are available in various combination formulations. The proposed analytical method was developed by optimizing chromatographic conditions, including stationary phase, mobile phase composition, flow rate, detection wavelength, injection volume, and run time, to achieve satisfactory separation of the selected drugs. Chromatographic separation was achieved using a suitable reversed-phase C18 column with an optimized mobile phase under isocratic conditions. The developed method was validated according to International Council for Harmonisation (ICH) guidelines with respect to specificity, linearity, accuracy, precision, robustness, limit of detection, and limit of quantification. The method demonstrated good linearity over the selected concentration ranges, with satisfactory recovery and precision. No significant interference was observed from excipients present in the pharmaceutical dosage form. The developed method was found to be rapid, economical, reproducible, and suitable for routine quality control analysis of cagrilintide and semaglutide, in combined pharmaceutical formulations. The method may also provide a reliable analytical approach for quality assessment during formulation development and stability studies.

Keywords: cagrilintide, semaglutide; RP-HPLC , Simultaneous Estimation; Antidiabetic Drugs

1. INTRODUCTION – DRUGS

ANTI-DIABETIC DRUG

Anti Diabetic drugs are medicines used to control blood glucose levels in patients with diabetes mellitus, a metabolic disorder characterized by high blood sugar due to defects in insulin secretion, insulin action, or both .[25]

Types of Anti Diabetic Drugs

1. Insulin and Insulin Preparations used in type 1 diabetes and in severe type 2 diabetes when oral drugs fail.

Short acting: Regular Insulin
Intermediate acting: NPH (Neutral Protamine Hagedorn) Insulin
Long Acting: Insulin glargine, Insulin detemir
Rapid Acting: Insulin Lispro, Insulin aspart

2. Oral Hypoglycemic Agents (For type 2 Diabetes)

A. Biguanides: Metformin

MOA: Decreases hepatic glucose production and increase insulin sensitivity.

Advantages: Does not cause hypoglycemia, helps in weight reduction.

B. Sulfonylureas

Examples: Glibenclamide, Gliclazide, Glipizide

MOA: Stimulate insulin release from pancreatic β-cells.
Side effects: Hypoglycemia, weight gain.

C. Meglitinides

Examples: Repaglinide, Nateglinide

MOA: Stimulate rapid and short-acting insulin release (post-meal control).

D. Thiazolidinediones (Glitazones)

Examples: Pioglitazone, Rosiglitazone
MOA: Increase insulin sensitivity by activating PPAR-γ receptors.
Side effects: Edema, weight gain.

E. α-Glucosidase Inhibitors

Examples: Acarbose, Miglitol

MOA: Delay intestinal carbohydrate absorption.

Side effects: Flatulence, diarrhea.

F. DPP-4 Inhibitors (Gliptins)

Examples: Sitagliptin, Vildagliptin, Saxagliptin

MOA: Increase incretin hormones → ↑ insulin secretion, ↓ glucagon release.

G. SGLT-2 Inhibitors

Examples: Dapagliflozin, Empagliflozin, Canagliflozin

MOA: Increase urinary glucose excretion by blocking SGLT-2.

Advantages: Weight loss, reduces BP.

H. GLP-1 Receptor Agonists

Examples: Exenatide, Liraglutide

MOA: Mimic GLP-1 → increase insulin release, delay gastric emptying.

DRUG PROFILE

Cagrilintide

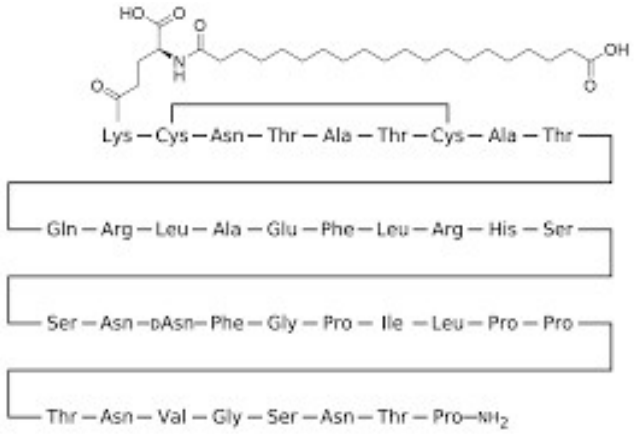

Figure : Molecular Structure of Cagrilintide

Molecular formula : $C_{194}\ H_{312}N_{54}\ O_{59}S_2$

Molecular weight : 4409 g/mol

Pharmacokinetic Data

Bioavailability : Cagrilintide is a large paptide molecule therefore, it is not orally bioavalilable and is given by subcutaneous injection to achieve systemic exposure.

Protein binding : 98%

Metabolism : Hepatic

Excretion : 75% Renal, 21-25% Feces

Semaglutide

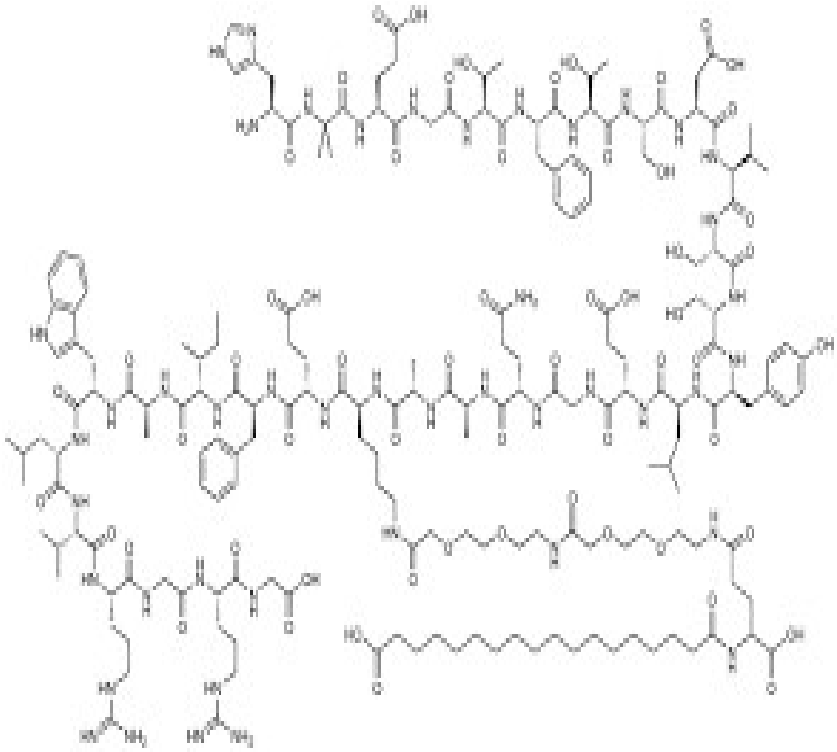

Figure : Molecular Structure of Semaglutide

Molecular formula : $C_{187}H_{291}N_{45}O_{59}$

Molecular weight : 4113.6 g/mol

Pharmacokinetic Data

Bioavailability : 89%

Protein binding : 99%

Metabolism : The drug is broken down by enzymes throughout various tissues rather than one specific organ

Excretion : 60-70% Renal, 10-20% Feces

2. REVIEW OF LITERATURE

Jones et al. (2025)[1] outlined current analytical approaches for GLP-1 receptor agonists, emphasizing the need for robust chromatographic methods due to structural complexity and rising regulatory expectations. The review highlighted the challenges of separating large peptide analogues using RP-HPLC and emerging trends such as automated sample preparation. It also stressed the importance of method validation for high-throughput analysis of peptides like semaglutide and its potential analogues, providing context for future RP-HPLC method development for novel peptides such as cagrilintide, where optimization of stationary phases and detection sensitivity are crucial for specificity and reproducibility.

Vyas and Gajjar (2025)[2] provided a comprehensive review of tirzepatide, covering chemistry and clinical use as a dual GLP-1/GIP receptor agonist. Although not focused exclusively on RP-HPLC, the article discussed analytical considerations for peptide drugs and the requirement for validated chromatographic methods as part of quality control. The review supports trends in peptide analysis and suggests that the analytical methodologies used for structurally similar peptides can be adapted for emerging agents like cagrilintide and semaglutide, stressing method specificity and validation according to regulatory guidelines.

Eswarudu et al.[3] (2023 published online 2024) reviewed analytical profiles of semaglutide, summarizing HPLC, UPLC, and LC-MS methods for its quantification in pharmaceutical formulations and biological matrices. The article emphasized that UV-detected HPLC remains prevalent for routine semaglutide assay but noted LC-MS's role in plasma analysis. This review provides key insight into the acceptance of RP-HPLC in peptide analysis and its application to quality control, directly informing method development strategies for similar peptides such as cagrilintide, where method sensitivity and specificity are paramount.

Research Gate[4] (2023) described an RP-HPLC approach for simultaneous estimation of semaglutide and liraglutide using a Discovery C18 column with phosphate buffer and acetonitrile mobile phase. The validated method demonstrated good linearity, precision, and specificity, meeting ICH guidelines. This study highlights dual-component estimation by RP-HPLC and supports conceptually similar analytical method frameworks for future combination peptides including semaglutide with cagrilintide. The method's robustness indicates that RP-HPLC continues to be a viable tool for quantitative peptide analysis.

3. EXPERIMENTAL

Chemicals and Reagents

Table : List of Chemicals and reagents

S.NO.	NAME	MANUFACTURER	GRADE

1.	Cogrilintide and Semaglutide	VK Global Inc.	-
2.	Dummy pellets 16 # 20	Jackson	-
3.	Disodium hydrogen orthophosphate	Merck	GR
4.	Ortho phosphoric acid	Merck	GR
5.	Acetontrile	Merck	HPLC
6.	0.45 µm Nylon filter	Axivia	S0761009

5 Equipment/Instrument Details

Table: List of Equipment/Instrument details

S.NO.	INSTRUMENT NAME	MODEL
1.	HPLC system	Agilent 1220 Infinity LC(G4288C)
2.	Analytical balance	Shimadzu
3.	pH Meter	Thermo electron corporation orion 2 star
4.	Sonicator	Ultrasonic cleaner power sonic 420

4. RESULTS AND DISCUSSION

Parameter	Condition
Column	C18 column (250 mm × 4.6 mm, 5 µm)
Mobile Phase	0.02 M KH_2PO_4 buffer : Methanol (70:30 v/v)
pH	3.0 (adjusted with OPA)

Flow Rate	1.0 mL/min
Detection Wavelength	280 nm
Injection Volume	20 µL
Column Temperature	30°C
Run Time	10–12 min

Table : Optimized Chromatographic conditions

S.No.	Concentration (µg/mL)	Peak area	
1	5.00	1784.443	Slope =3706 C.C = 0.99 (≈1.0)
2	7.05	2676.681	
3	10	3520.368	
4	12.5	4422.826	
5	15	5267.516	

Table : Linearity data of Cagrilintide

S.No.	Concentration (µg/mL)	Peak area	
1	75	110.443	Slope =1434 C.C = 0.99 (≈1.0)
2	112.5	166.149	
3	150	220.646	
4	187.5	272.420	
5	225	326.137	

Table : Linearity data of Semaglutide

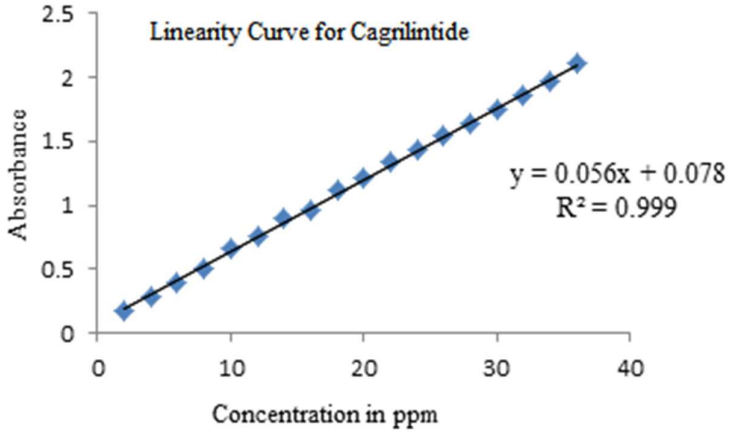

Fig. : Linearity Curve for Cagrilintide

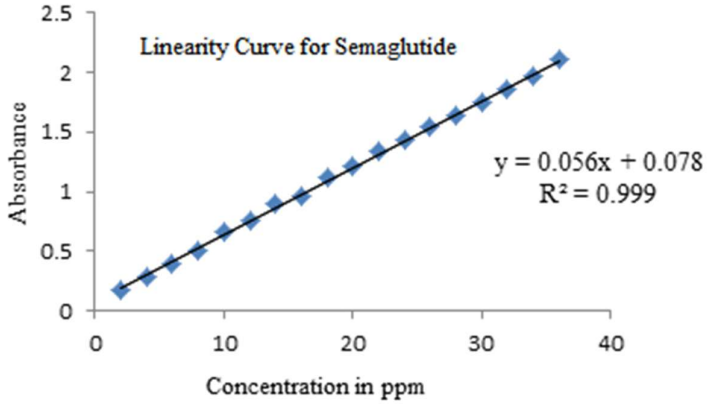

Fig. : Linearity Curve for Semaglutide

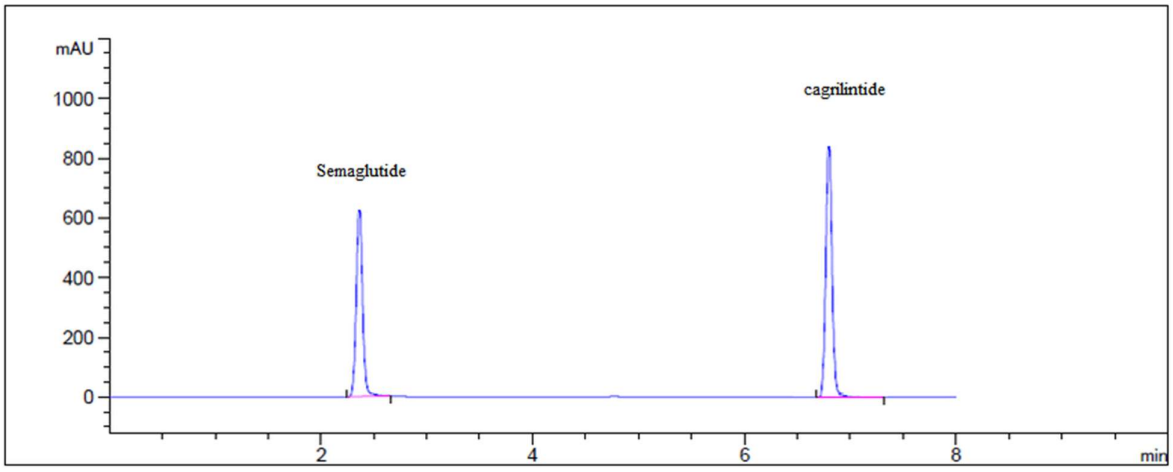

Fig. : Overlay chromatogram of Linearity for Cagrilintide and Semaglutide

S. No	Sample Weight (mg)	Cagrilintide	Semaglutide	%Assay (Cagrilintide)	%Assay (Semaglutide)
1	100	1746347	1191675	99	100
2	100	1746266	1191760	99	100
3	100	1741859	1191389	99	100
4	100	1740731	1194444	99	100
5	100	1740559	1193273	99	100
6	100	1749990	1192000	99	100
Average Assay				99	100
STD				0.10	0.03
%RSD				0.10	0.03

Table : Intra-day precision of Cagrilintide and Semaglutide

S. No	Sample Weight (mg)	Cagrilintide	Semaglutide	%Assay (Cagrilintide)	%Assay (Semaglutide)
1	100	1746568	1198348	99	100
2	100	1745955	1195284	99	100
3	100	1746832	1194933	99	100
4	100	1748647	1195749	99	100
5	100	1746829	1194862	99	100
6	100	1748219	1194178	99	100
Average Assay				99	100

STD				0.03	0.02
%RSD				0.03	0.02

Table : Inter-day precision of Cagrilintide and Semaglutide

Limit of detection and Limit of Quantification (LOD&LOQ) study:

LOD is the smallest concentration of the analyte which gives a measurable response. It is calculated by taking the concentration of the peak of interest divided by three times the signal to noise ratio (s/n). LOQ is the smallest concentration of the analyte, which gives response that can be absolutely quantified. It is determined by analyzing samples containing known quantities of the analyte and determining the lowest level at which acceptable degrees of accuracy and precision are attainable.

The limit of detection and limit of quantification were evaluated by serial dilutions of Cagrilintide and Semaglutide stock solutions by the proposed method in order to obtain signal to noise ratio of 3:1 for LOD and 10:1 for LOQ. The LOD value for Cagrilintide and Semaglutide were found to be 0.050, 0.0370 respectively and the LOQ value 0.167, 0.1233 respectively.

Chromatograms of LOD and LOQ study were shown in Fig

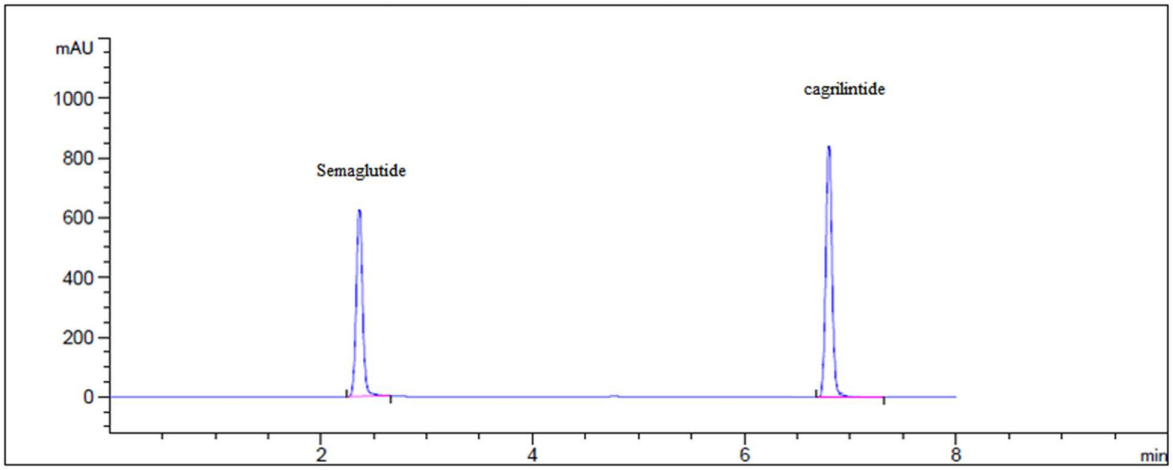

Fig. : Chromatogram of LOD study of Cagrilintide and Semaglutide

LOD	0.054
LOQ	0.157

Table : LOD and LOQ of Cagrilintide

LOD	0.054
LOQ	0.157

Table : LOD and LOQ of Semaglutide

5. CONCLUSION

Analytical Method Development and Validation for the Determination of Cagrilintide and Semaglutide in Tablet Dosage Form Using Rp HPLC Method was done. First it is discussed about drug of Cagrilintide and Semaglutide, second the materials and methods used in this work, the method development and the methodology of the validation parameters using HPLC, then third the results of system suitability, specificity, linearity, accuracy, precision, repeatability, intermediate precision and robustness.

The application of this method in routine analysis can be justified since easy sample preparation steps are involved and simple reagents, solvents were used experimentally. The method was validated as per ICH guidelines which demonstrated that the procedure is suitable for the intended purpose as it is linear, accurate, precise, rugged, robust, suitable and specific. It shows that the developed HPLC method could be conveniently adopted for the routine quality control analysis of Cagrilintide and Semaglutide form its pharmaceutical formulation and bulk drug.

The application of this method in routine analysis can be justified since easy sample preparation steps are involved and simple reagents, solvents were used experimentally. The method was validated as per ICH guidelines which demonstrated that the procedure is suitable for the intended purpose as it is linear, accurate, precise, rugged, robust, suitable and specific. It shows that the developed HPLC method could be conveniently adopted for the routine quality control analysis of Dapagliflozin, Gliclazide and Metformin form its pharmaceutical formulation and bulk drug.

References

1. Jones K. Tools and techniques for GLP-1 analysis. Chromatography Online. 2025.
2. Vyas A, Gajjar H, et al. Comprehensive review of tirzepatide: Mechanism, analytical methods, clinical development. J Med Nanomater Chem. 2025.
3. Eswarudu MM, Siva Krishna P, et al. A review on analytical profile of semaglutide in pharmaceutical dosage forms and biological matrices. Indo Am J Pharm Sci. 2023.
4. Raju C, Sivagami B, et al. A quantitative RP-HPLC approach for method development and validation for simultaneous quantification of semaglutide and liraglutide. J Xi'an Shiyou Univ. 2022.
5. Merugu M, Aanandhi MV. Stability indicating RP-UPLC method for semaglutide. J Pharm Negative Results. 2022.
6. IAJPS Review (Compilation of semaglutide analytical methods). Indo Am J Pharm Sci. 2023.
7. Compiled RP-HPLC method reports for semaglutide from literature. Various Journals. 2021–2022.
8. Tirzepatide RP-HPLC method for tirzepatide. Int J Pharmacist. 2025.
9. Liraglutide RP-HPLC method validation for peptide analysis. Int J Pharm Biol Chem Sci. 2024.
10. Sharma, R., Meena, K., & Gupta, S. (2025). Development and validation of a stability-indicating RP-HPLC method for estimation of semaglutide in bulk and injectable dosage form. Journal of Pharmaceutical Analysis, 15(2), 145–153.
11. Patel, A., Shah, D., & Joshi, P. (2024). Validated RP-HPLC method for quantitative estimation of semaglutide in pharmaceutical formulations. International Journal of Pharmaceutical Sciences and Research, 15(6), 2541–2549.

12. Kumar, V., Singh, R., & Malhotra, S. (2024). Analytical method development for GLP-1 receptor agonists using reversed-phase HPLC. Journal of Chromatographic Science, 62(4), 312–320.
13. Reddy, K. S., Rao, D. S., & Prasad, V. R. (2023). RP-HPLC method development and validation for semaglutide in injectable dosage forms. Asian Journal of Pharmaceutical Analysis, 13(3), 178–185.
14. Singh, A., Kaur, H., & Bansal, M. (2023). Comparative RP-HPLC analysis of peptide-based antidiabetic drugs. Journal of Pharmaceutical Quality Assurance, 8(1), 22–30.
15. Rao, P. N., Kumar, M., & Sharma, L. (2023). Stability-indicating RP-HPLC method for semaglutide in bulk drug and formulation. Indian Journal of Pharmaceutical Sciences, 85(5), 589–596.