International Journal of Medicine and Pharmaceutical Research
https://doi.org/10.30904/j.ijmpr.2026.4917 | E-ISSN: 2321-2624

Method Development and Validation for the Estimation of Liraglutide and Its Related Substances by using RP-HPLC

Tudi Avinash Reddy*, Dr. K. Chaitanya Prasad, Dr. K. Nagasree, Dr. K. Shravan Kumar

Samskruti College of Pharmacy, Kondapur, Ghatkesar, Medchal, Hyderabad-501301, Telangana, India

Received: 08 Sept 2025 | Revised: 14 Oct 2025 | Accepted: 12 Dec 2025 | Published: 6 Feb 2026

Abstract:
A robust and precise gradient reversed-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of Liraglutide and its related impurities Des-acetyl Liraglutide (Impurity A) and Glycosylated Liraglutide (Impurity B). The optimized chromatographic conditions employed an Inertsil C18 column (250×4.6 mm, 3µm) with a mobile phase of NAH_2PO_4 buffer (pH 4) and methanol under gradient elution. System suitability parameters, including resolution (>2), tailing factor (<2), and theoretical plate count (>2000), confirmed the method's efficiency and reliability. Linearity was demonstrated across concentration ranges of 40–200 µg/mL for Liraglutide and 4–20µg/mL for impurities, with correlation coefficients (R^2>0.999) indicating excellent linearity. The method exhibited high precision (%RSD <2%) and accuracy (recovery 98–102%) across multiple concentration levels. Sensitivity was confirmed with acceptable limits of detection and quantification. Robustness was validated through deliberate variations in flow rate and mobile phase composition, showing minimal impact on performance. Forced degradation studies under various stress conditions verified the method's stability-indicating capability, effectively separating and quantifying degradation products. This validated RP-HPLC method is suitable for routine quality control and stability testing of Liraglutide formulations, ensuring reliable detection of impurities and degradation products.
Keywords: RP-HPLC, Liraglutide, Des-acetyl Liraglutide, Glycosylated Liraglutide, method validation, impurities, gradient elution, system suitability, precision, accuracy

Introduction

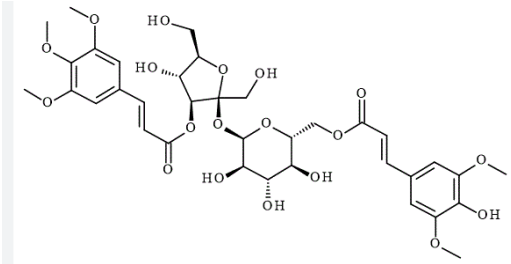

Fig.1: Liraglutide

Table.1: Drug Profile

Property	Information
Molecular Formula	C172H265N43O51
Molecular Weight	3751.2 g/mol
IUPAC Name	(IUPAC name is complex and lengthy, typically not used in common references)
Chem Spider ID	34980716
Polar Surface Area	202.0 Å²
LogP (Octanol/Water)	5.63
Generic Name	Liraglutide
Brand Names	Victoza, Saxenda
Drug Category	GLP-1 receptor agonist
Indications	Type 2 diabetes, chronic obesity
Pharmacology	Mimics GLP-1, increases insulin secretion, decreases glucagon secretion, slows gastric emptying
Potency	Effective at low doses
Tolerability	Generally well-tolerated; common side effects include nausea, vomiting, diarrhea
Contraindications	Personal or family history of medullary thyroid carcinoma, multiple endocrine neoplasia syndrome type 2
Adverse Effects	Nausea, vomiting, diarrhea, pancreatitis, hypoglycemia (when used with insulin or sulfonylureas)
Availability	Prescription only

Materials and Methods

Wave length selection:
UV spectrum of 10 µg / ml Liraglutide in diluent (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength selected as 228nm. At this wavelength both the drugs show good absorbance.

Preparation of mobile phase:

Preparation of Mobile Phase A (NAH2PO4 PH4):
Weigh and transfer accurately 6.4g of NAH2PO4 in 1000ml water and mix. Adjust the PH to 4.Filter the solution through 0.45µm membrane filter paper and degas it.

Preparation of mobile phase B (MEOH):

Diluent Preparation: MEOH: NAH_2PO_4.

Standard Solution Preparation:
Accurately weigh and transfer 20 mg working standard into a 20 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).Further pipette 1.2ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Impurity Solution Preparation:
Accurately weigh and transfer the equivalent weight of 2mg of Impurity -A and 2mg of Impurity-B into a 20 ml clean dry

volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 1.2 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure: Inject 20µL of the standard, sample into the chromatographic system and measure the areas for peaks and identify the impurities known and unknown.

System suitability:

Tailing factor for the peaks in Standard solution should not be more than 2.0. Theoretical plates for the peaks in Standard solution should not be less than 2000. Resolution for the peaks in standard solution should not be less than 2.

Calculation: (For Liraglutide)

$$\%\ Assay = \frac{AT}{AS} * \frac{WS}{DS} * \frac{DT}{WT} * \frac{Average\ weight}{Label\ Claim} * \frac{P}{100} * 100$$

Where:

AT = average area counts of sample preparation.
AS = average area counts of standard preparation.
WS = Weight of working standard taken in mg.
P = Percentage purity of working standard
LC= Label Claim mg/ml.

Results and Discussion

Optimized Chromatographic Conditions

Instrument used : High performance liquid chromatography equipped with Auto Sampler and PDA

Temperature	:	25^0C
Mode	:	Gradient
Column	:	Inertsil C18, (250×4.6mm, 3µm)
Buffer	:	NAH2PO4 PH4
Mobile phase	:	NAH2PO4 PH4: MEOH
Diluent	:	Water: MEOH: 1N NaoH
Flow rate	:	1.0 ml per min
Wavelength	:	246 nm
Injection volume	:	10 µl
Run time	:	25 min.

Standard Solution Preparation

Weigh and transfer accurately 20mg of Liraglutide and in 20ml distilled Methanol and Spike 2mg of Impurity A-Des aceyl Liraglutide and 2mg of Impurity B-Glycosylated Liraglutide ,sonicate for 5 min and Filter the solution through 0.45µm membrane filter.

Procedure: Inject 20µl of the standard solution and observe the system suitability parameters are passed.

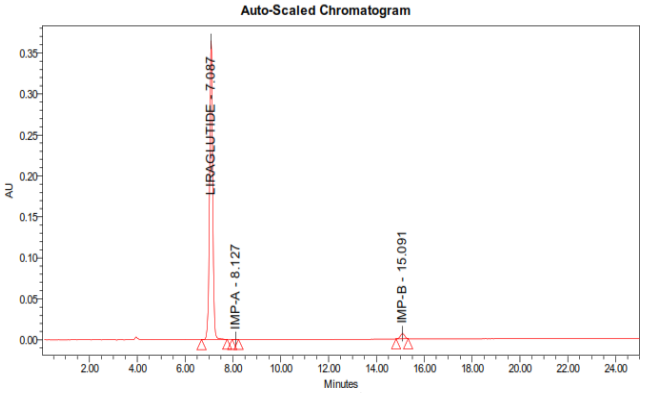

Fig.2: Chromatogram for system suitability

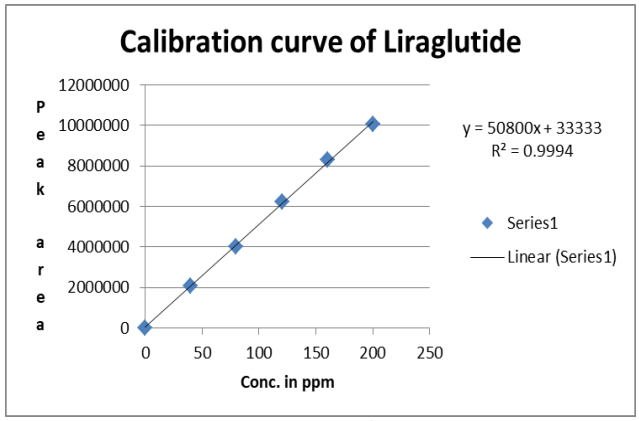

Fig.3: Calibration graph for Liraglutide

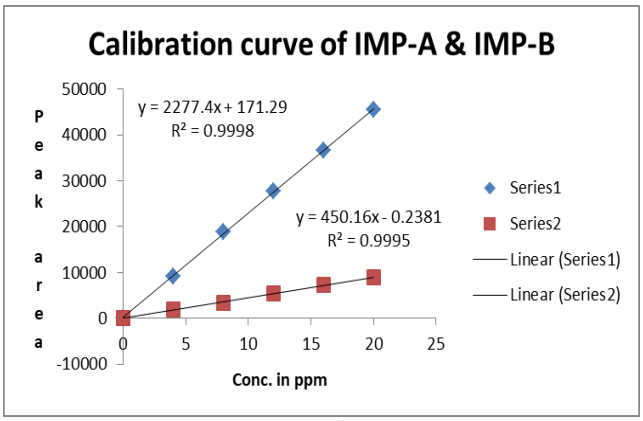

Fig.4: Calibration graph for Impurity A and B

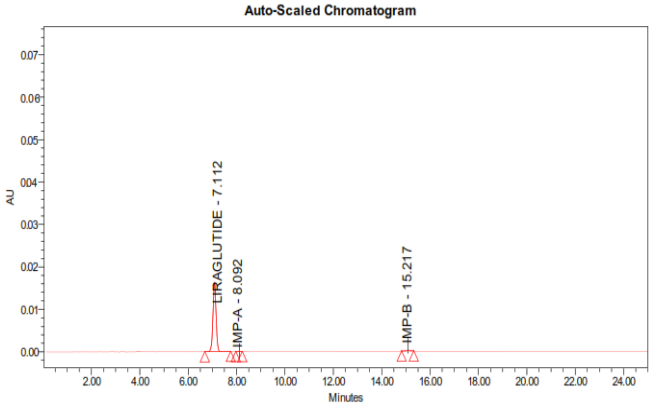

Fig.5: Chromatogram of Liraglutide showing LOD

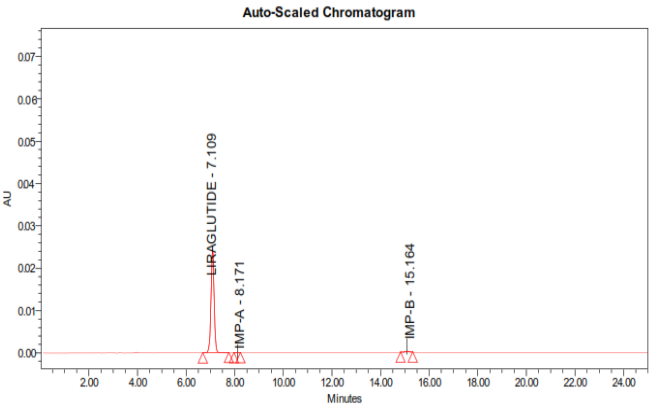

Fig.6: Chromatogram of Liraglutide showing LOQ

Table.2: Results of system suitability parameters

S.No	Name(STD)	RT(min)	Area (µV sec)	Height (µV)	Resolution	USP tailing	USP plate count
1	Liraglutide	7.087	6217451	656136		1.32	8263
2	Impurity A	8.127	27415	6080	3.60	1.21	3296
3	Impurity B	15.091	5417	2052	6.32	1.02	4245

Table.3: Analytical performance parameters of Liraglutide, Impurity A and B

Parameters	Liraglutide	Impurity A	Impurity B
Slope (m)	50800	2277.4	450.16
Intercept (c)	33333	171.29	0.2381
Correlation coefficient (R^2)	0.9994	0.9998	0.9995

Table 4: Results of Intermediate precision for Liraglutide

Injection	Areas	Areas	Areas
Injection-1	6321458	28544	5563
Injection-2	6395478	28554	5547
Injection-3	6321587	28631	5489
Injection-4	6399851	28641	5412
Injection-5	6320145	27999	5533
Injection-6	6320147	28514	5537
Average	6346444	28481	5514
Standard Deviation	39703.86	241.17	55.511
%RSD	0.6	0.8	1.0

Table 5: Accuracy (recovery) data for Liraglutide

%Concentration Liraglutide (at specification Level)	Area*	Amount Added (mg)	Amount Found(mg)	% Recovery	Mean Recovery
50%	3088741	10	9.92	99.4	99.0
100%	6185471	20	19.86	99.3	
150%	9215741	30	29.59	98.6	

%Concentration Impurity A (at specification Level)	Area*	Amount Added(mg)	Amount Found(mg)	% Recovery	Mean Recovery
50%	13552	1	0.99	98.7	98.8
100%	26987	2	1.96	98.2	
150%	41047	3	2.99	99.6	

%Concentration Impurity B (at specification Level)	Area*	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	2671	1	0.98	98.2	98.5
100%	5368	2	1.98	98.9	
150%	7993	3	2.95	98.2	

Table 6: Results of LOD

Drug name	Baseline noise(µV)	Signal obtained(µV)	S/N ratio	Conc.
Liraglutide	66	192	2.91	0.04µg/ml
Impurity A	66	195	2.95	0.38µg/ml
Impurity B	66	193	2.92	1.13µg/ml

Table 7: Results of LOQ

Drug name	Baseline noise(µV)	nal obtained(µV)	S/N ratio	CONC.
Liraglutide	66	655	9.92	0.12µg/ml
Impurity A	66	656	9.94	1.29 µg/ml
Impurity B	66	658	9.97	3.85µg/ml

Conclusion

RP-HPLC method was successfully developed and validated for the simultaneous estimation of Liraglutide and its related impurities (Impurity A – Des-acetyl Liraglutide and Impurity B–Glycosylated Liraglutide).Among several trials with varying chromatographic conditions, the optimized method utilized an Inertsil C18 column (250×4.6 mm, 3 µm) with a mobile phase consisting of NAH_2PO_4 buffer (pH 4) and methanol, employing a gradient elution. The method demonstrated excellent system suitability parameters including resolution (>2), tailing factor (<2), and plate count (>2000) for all analytes. Linearity was established over the range of 40–200 µg/ml for Liraglutide, and 4–20 µg/ml for impurities, with correlation coefficients (R^2 > 0.999) indicating excellent linearity. Further, the method showed high precision, with %RSD values well below 2%, and high accuracy, with recovery values ranging from 98–102% at all levels (50%, 100%, and 150%). The LOD and LOQ were found to be well within acceptable limits, confirming the method's sensitivity. Robustness was confirmed through deliberate variations in flow rate and mobile phase composition, which did not significantly impact system performance. The forced degradation studies demonstrated that the method is stability-indicating, with all degradation products effectively

separated and quantified. Thus, the developed HPLC method is suitable for routine analysis and quality control of Liraglutide formulations and can reliably detect its degradation products and impurities.

References

[1] Drucker DJ. The role of gut hormones in glucose homeostasis. J Clin Invest. 2007;117(1):24-32.

[2] Knudsen LB, Lau J. The discovery and development of liraglutide and semaglutide. Front Endocrinol (Lausanne). 2019; 10:155.

[3] Frokjaer S, Otzen DE. Protein drug stability: a formulation challenge. Nat Rev Drug Discov. 2005; 4(4): 298-306.

[4] Joubert MK, Deshpande M, Yang J, et al. Use of in vitro assays to assess immunogenicity risk of antibody-based biotherapeutics. PLoS One. 2016;11(8):e0159328.

[5] Khameneh B, Iranshahy M, Soheili V, Fazly Bazzaz BS. Peptides as a novel generation of anti-biofilm agents in medical sciences. Peptides. 2016;78:91-101.

[6] Gilar M, Olivova P, Daly AE, Gebler JC. Two-dimensional separation of peptides using RP-RP-HPLC system with different pH in first and second separation dimensions. J Sep Sci. 2005;28(14):1694–1703.

[7] International Conference on Harmonisation. ICH Q2(R1): Validation of Analytical Procedures: Text and Methodology. ICH Harmonised Tripartite Guideline. 2005.

[8] International Conference on Harmonisation. ICH Q3A(R2): Impurities in New Drug Substances. 2006; ICH Q3B(R2): Impurities in New Drug Products. 2006.

[9] Pedaprolu JN, Bonthu MG, Vatchavai BR, Kamatham SS, Kolli S, Kapuganti ANJ. A new stability-indicating and validated RP-HPLC method for the estimation of liraglutide in bulk and pharmaceutical dosage forms. Eurasian J Anal Chem. 2017;12(2):31–44.

[10] Sharma V, Patel A, Shah P. Establishment of stability-indicating purity method based on the stress degradation behaviour of human GLP-1 analog liraglutide. Indian J Pharm Sci. 2023;85(4):1068–1076.

[11] Shah R, Patel J, Parmar R, Vora S. Simultaneous estimation and impurity profiling of liraglutide using RP-HPLC: A validated stability-indicating method. J Pharm Anal. 2024;14(2):115–124.

[12] TNV Biochem — Liraglutide product page: gives molecular formula ($C_{172}H_{265}N_{43}O_{51}$), molecular weight (~3751.2 Da), mechanism of action, brand names, indications etc. tnvbiochem.com

[13] Chemsrc — Chemical properties and uses of liraglutide: molecular formula, usage in type 2 diabetes and obesity. Chemsrc

[14] ChemicalBook — Detailed chemical properties (solubility, isoelectric point, physical description), molecular weight and formula. amp.chemicalbook.com+1

[15] Sigma Aldrich — Provides assay, drug modifications (acylation, etc.), brand names, and pharmacologic description. MilliporeSigma

[16] NCATS/NIH (Drugs.ncats.io) — Pharmacology, mode of action including GLP 1 receptor agonist activity, plasma half-life (~13 h), stability against degradation etc. Inxight Drugs

[17] PDB 101 / RCSB — Description of structure, modifications (C 16 fatty acid attached via glutamic spacer to Lys 26), approval details (brand names), etc.

[18] Satyanarayana PV, Madhavi AS. Validated RP-HPLC method for the estimation of liraglutide in tablet dosage. Int J Sci Invent Today. 2012;1:17-26.

[19] Pedaprolu JN, Bonthu M, Vatchavai B, Kamatham SS, Kolli S, Kapuganti AN. A new stability-indicating and validated RP-HPLC method for the estimation of liraglutide in bulk and pharmaceutical dosage forms. Eur J Anal Chem. 2017 Jan 1;12(2):31-44.

[20] Barredo-Vacchelli GR, Rodríguez JA, Eloy JA, Camperi SA. A Novel Method for Liraglutide Synthesis and Purification. Peptide Science. 2024:e24351.

[21] Dong S, Gu Y, Wei G, Si D, Liu C. Determination of liraglutide in rat plasma by a selective liquid chromatography-tandem mass spectrometry method: Application to a pharmacokinetics study. Journal of Chromatography B. 2018 Aug 1;1091:29-35.

[22] Giri T, Kukreja D, Sharma N, Shah R. Establishment of Stability-Indicating Purity Method Based on the Stress Degradation Behaviour of Human Glucagon-like Peptide-1 Analog Liraglutide using Reverse Phase-Liquid Chromatography. Indian Journal of Pharmaceutical Sciences. 2023 Jul 1;85(4).

[23] Chavoshi F, Mirjalili SZ, Mohammadi A, Amini M, Somsen GW, Shirangi M. Forced Degradation Products of Liraglutide: A Comparative Study of Similarity Between Originator and Analogue Version by Liquid Chromatography–Mass Spectrometry. International Journal of Peptide Research and Therapeutics. 2024; 30(3): 1-3.

[24] Sudheer, M., Prakash, M. S., Rajashekar, G., Hima Vani, K. V., Ramalingam, P., & Reddy, Y. P. A stability indicating HPLC method with diode array detection for the determination of atorvastatin calcium and fenofibrate in commercial tablets. *Journal of Pharmacy Research*, 2011; *4*(9), 3033-3036.

[25] Oh HS, Choi M, Lee TS, An Y, Park EJ, Kim TH, Shin S, Shin BS. Pharmacokinetics and brain distribution of the therapeutic peptide liraglutide by a novel LC–MS/MS analysis. Journal of Analytical Science and Technology. 2023; 14(1):19.

Source of Support: Nil. Conflicts of Interest: None declared.