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Degradation Studies and Method Development and Validation of Lanadelumab Using UV Detector in RP-HPLC

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Abstract The RP-HPLC methodology was used to establish a straightforward, accurate, and precise method for estimating Lanadelumab. The following chromatographic conditions were used: 5. Mobile phase: 0.1% OPA buffer: Acetonitrile in a ratio of 65:35; 5. Stationary phase: Agilent C18 250 x 4.6 mm; 5. Detection wavelength: 228.0 nm; column temperature: 30 °C; diluent: 50:50 acetonitrile: water; retention time: 2.280 min. As the most efficient approach, conditions were finalized. The standard was injected six times to study the system appropriateness characteristics, and the results fell well within the acceptable range. An analysis of linearity was conducted at 25% to 150% levels, and the R2 score was 0.999. Standard precision was determined to be 0.8, whereas repeatable precision was found to be 0.5. 0.08µg/ml is the LOD, while 0.24µg/ml is the LOQ. The assay of the marketed formulation was conducted using the described method, and 100.14% was found.

Keywords HPLC, Lanadelumab

1. Introduction

Pharmaceutical analysis, a core aspect of pharmacy, focuses on drug identification and quantification using analytical chemistry. This field includes qualitative (identifying compounds) and quantitative (measuring amounts) analysis. Techniques like spectrophotometry and chromatography are essential in drug analysis. This plays a vital role in drug development, quality control, forensics, and environmental monitoring.

Introduction to HPLC

It is a precise and reliable method used for separating and analyzing components in a mixture. It offers high efficiency, requires small sample volumes, and is adaptable to various types of analyses.

Method Development in HPLC

Effective method development considers:

• Mobile Phase: Polarity affects retention; usually starts with isocratic runs, followed by gradient runs.

• Detector: UV-visible detectors are widely used.

• Column Parameters: Length, diameter, and stationary phase (e.g., C8 or C18) affect resolution and

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retention.

• Gradient Programming: Fast gradients help identify optimal separation conditions.

• pH and Buffers: pH affects analyte ionization and retention. Buffers improve reproducibility and peak shape.

Method Validation Parameters (as per ICH Guidelines)

1. Specificity – Ability to identify the analyte without interference.

2. Linearity – Proportionality between concentration and response.

3. Range – Interval where accuracy, precision, and linearity are validated.

4. Accuracy – Close of the measured value to the true value.

5. Precision – Repeatability and reproducibility of results.

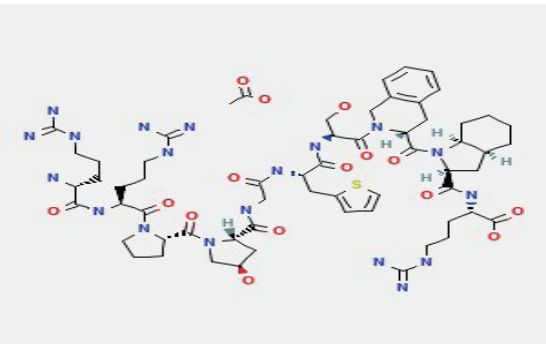
6. Detection Limit (LOD) – Lowest detectable amount.

7. Quantitation Limit (LOQ) – Lowest quantifiable amount.

8. Robustness – Reliability under slight variations in method conditions.

9. System Suitability Testing (SST) – Ensures system performance using parameters like RT, Theoretical Plates (N), Resolution, Tailing Factor & %RSD.

2. Drug Profile

Drug Name	Lanadelumab
Structure	
CAS Number	1426055-14-2
Molecular weight	Average: 146000.0 Da
Protein chemical Formula	C6468H10016N1728O2012S47
Appearance	Powder
Physical	Solid
Solubility	Water Solubility (50mg/mL)
Melting Point (oC)	80-90
Boiling Point (oC)	60-70
Brand Name	Takhzyro
Indication	Lanadelumab is to prevent attacks in adult and paediatric patients aged 2 years and older with hereditary angioedema
Mechanism of action	Lanadelumab is a plasma kallikrein inhibitor
Half Life	2 weeks
Dosage Form	Subcutaneous Injection
Dose	Adult – 30mg per 2 weeks Paediatric – Based on weight

3. Literature Review

Tachdjian et al. (2025) conducted a study titled "Effective long-term prophylaxis with lanadelumab in adolescents with hereditary angioedema: EMPOWER/ENABLE" in the journal Paediatric Allergy and Immunology. This tells that pooled data from two Phase 4 studies (EMPOWER and ENABLE) demonstrated significant reductions in monthly HAE attack rates in both new and established users. New

users showed a reduction from a mean of 3.8 to 0.65 attacks/month. The drug was well tolerated, with most adverse events being mild or moderate. The study supports lanadelumab as an effective and safe long-term prophylactic option in real-world adolescent populations with HAE.

Magerl et al. (2024) in their work titled "Real-World Effectiveness of Lanadelumab in Hereditary Angioedema: Multicountry INTEGRATED Observational Study" In Practice. This is about assess the real-world effectiveness of lanadelumab in a multicountry cohort of 198 patient aged 12 years and older with HAE types I and II, across Germany, France, Austria, and Greece. This tells that lanadelumab significantly improved the attack-free rate (AFR), rising from 0% before treatment to 54.4% at 12 months and even higher beyond two years. Physicians often increased the dosing interval from every 2 weeks to 4 weeks or more once patients became attack-free. The results highlight lanadelumab's sustained efficacy and support dose individualization based on clinical response. This study strengthens the evidence for lanadelumab's long-term benefits and flexible dosing in routine clinical settings.

Buttgereit et al. (2024) published the article "The real-life experience goes on: update after 4 years on the first cohort treated with lanadelumab at our center" in the journal Frontiers in Immunology. Over time, more than 70% became attack-free, and all patients reported well-controlled disease and improved quality of life. Most did not require additional short-term prophylaxis even before invasive procedures. The findings confirm lanadelumab's effectiveness and safety in the long term, while also questioning the necessity of short-term prophylaxis in stable patients.

4. Materials and Methods

Materials

Lanadelumab (API) received from spectrum labs.

Chemical and Reagents

Acetonitrile, water, Methanol.

Potassium di hydrogen orthophosphate(PDHO), orthophosphoric acid (analytical reagent grade).

Equipment and Instruments

The analysis was carried out on a Shimadzu HPLC with UV detector. Other instruments used include Shimadzu UV-Visible Spectrophotometer, Infradigi Analytical Balance, Systronics pH meter, Lab Man Ultrasonicator, and Hot Air Oven.

Methods

Diluent

Acetonitrile and Water taken in the ratio of 50:50

Preparation of buffer

0.1%Ortho Phosphoric Acid Buffer: 1ml of Perchloric acid was diluted to 1000ml with HPLC grade water.

Preparation of standard solution: A 50 ml volumetric flask containing 30 mg of precisely weighed Lanadelumab was filled with 3/4 diluent, and the flasks were sonicated for 10 minutes. The flask was labeled "Standard stock solution" and filled with diluents. 600 µg of Lanadelumab per milliliter.

Standard working solution preparation involved pipetting 1 ml of the stock solution into a 10 ml volumetric flask and adding diluent to make it up. (Lanadelumab 60µg/ml).

Sample stock solution preparation involved taking a vail of Takhzyro injection and transferring it into a 100 ml volumetric flask. Then, adding 50 ml of diluents and sonicating it for 25 minutes. The volume was then adjusted with diluent and filtered using HPLC filters. Lanadelumab at 1500 µg/ml.

Preparation of Sample working solution: A 10 ml volumetric flask was filled with 0.4 ml of the filtered sample stock solution, which was then diluted. 60 µg of Lanadelumab per milliliter.

5. Results and Discussion

Optimize method:

Mobile phase: ACN: 0.1% Ortho phosphoric acid (65:35 v/v)

Flow rate: 1.0 ml/min

Column: Agilent C18 (250* 4.6mm 5.0mm).

Detector wave length: 228nm

Column temperature: 30°C

Injection volume: 10.00 mL

Run time: 5.0 minutes

Diluent: Water: Acetonitrile (50:50 v/v)

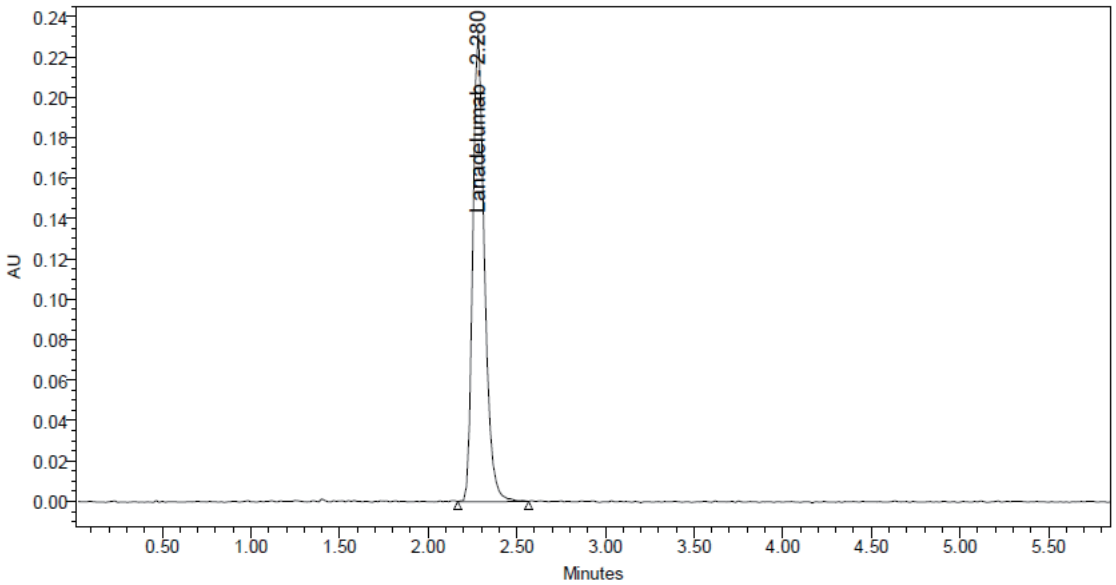


Figure 1: Optimized Chromatogram

System suitability: The method met all system suitability criteria. The %RSD of peak areas was 0.8%, theoretical plates >5000, and tailing factor <1.3

Linearity: The method exhibited linearity in the concentration range of 15–90 µg/mL with R2 = 0.999. Calibration curve showed excellent correlation.

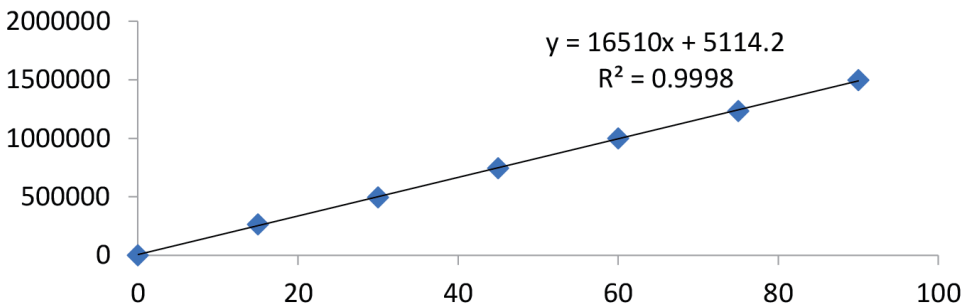


Figure 2: Calibration Curve of Linearity

Precision: Repeatability and intermediate precision were evaluated by six replicate injections. The %RSD values for area were <0.5%, confirming method precision.

Accuracy: Recovery studies at 50%, 100%, and 150% levels yielded recovery within 98% to 102%, validating method accuracy.

Table 1: Recovery data for accuracy studies

Level	Added amount (Mg/ml)	Result (mg/ml)	% Recovery
50%	30	29.78	
100%	60	60.19	99.1%
150%	90	89.44	

LOD and LOQ: It was found to be 0.08 µg/mL and 0.24 µg/mL respectively.

Robustness: Method robustness was confirmed under deliberate variations of flow rate (0.7 - 0.9 mL/min), mobile phase ratio, and temperature (25°C and 35°C).

Table 2: Robustness data under varied conditions

Parameter	Condition	Rt (min)	Area	Tailing factor	Theoretical plates
Flow rate Minus	0.75mL/min	2.45	1046581	1.23	5681
Flow rate plus	0.9 mL/min	2.12	1035123	1.25	5589
Temperature minus	25°C	2.35	1051265	1.22	5745
Temperature plus	35°C	2.18	1037890	1.24	5602

Assay: The assay of the marketed formulation showed 100.14% of Lanadelumab, indicating the accuracy and applicability of the method.

Degradation Studies: Forced degradation studies were carried out under Acid, Base, Peroxide, Thermal, UV, and water conditions. Significant degradation peaks were observed in acid and peroxide conditions, confirming the method's ability to distinguish degradation products from the analyte.

Table3: Degradation studies

S.No	Degradation Condition	%Purity	Drug degraded
1	Acid	92.13	7.87
2	Alkali	95.12	4.88
3	Oxidation	92.00	8.00
4	Thermal	97.46	2.54
5	UV	98.26	1.74
6	Water	99.11	0.89

6. Discussion

The developed RP-HPLC method for Lanadelumab demonstrated high selectivity, acceptable sensitivity, and consistent reproducibility. Validation studies confirmed its reliability for routine quality control. The method demonstrated good robustness and system suitability across all trials. The stability capability of this method makes it suitable for evaluating the Lanadelumab under various stress conditions. This work adds a valuable method for pharmaceutical analysis, ensuring safety and efficacy of the therapeutic agent.

7. Conclusion

The RP-HPLC method that was developed for Lanadelumab is straightforward, reliable, and accurate. It can be used for routine quantitative analysis of bulk Lanadelumab and has been effectively verified. The technique is particular and stability-indicating.

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Conflict of interest

The authors declare that there is no conflict of interest.

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