International Research Journal of Pharmacy and Medical Sciences
ISSN (Online): 2581-3277

Development and Validation of a Stability-Indicating RP-HPLC Method for Quantitative Determination of Tirzepatide in Bulk Drug and Injectable Dosage Form

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***Abstract*—Background**: *Tirzepatide is a novel dual glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) receptor agonist approved for the treatment of type 2 diabetes mellitus and obesity. Due to its increasing clinical significance and growing pharmaceutical utilization, the development of a reliable analytical method for its routine quality control and stability assessment is essential.* **Objective**: *The present study aimed to develop and validate a simple, accurate, precise, and stability-indicating reverse-phase high-performance liquid chromatographic (RP-HPLC) method for the quantitative estimation of tirzepatide in bulk drug and injectable dosage form.* **Methods**: *Chromatographic separation was achieved on a Phenomenex Kinetex XB-C8 column (150 × 4.6 mm, 5 µm) using a mobile phase consisting of 25 mM phosphate buffer (pH 5.0) and acetonitrile (58:42, v/v) at a flow rate of 1.0 mL/min. Detection was carried out at 220 nm using a diode-array detector. The method was validated according to ICH Q2(R1) guidelines for specificity, linearity, precision, accuracy, robustness, limit of detection (LOD), limit of quantification (LOQ), and forced degradation studies.* **Results**: *The developed method exhibited satisfactory chromatographic performance with a well-resolved tirzepatide peak and acceptable system suitability parameters. The method demonstrated excellent linearity within the concentration range studied, with correlation coefficients exceeding 0.999. Accuracy and precision results were within acceptable limits, while robustness studies confirmed the reliability of the method under small deliberate variations. Forced degradation studies under acidic, alkaline, oxidative, thermal, and photolytic conditions established the stability-indicating nature of the method.* **Conclusion**: *The validated RP-HPLC method was found to be simple, economical, sensitive, precise, accurate, and stability-indicating, making it suitable for routine quality control and stability studies of tirzepatide in pharmaceutical formulations.*

***Keywords*—** *Tirzepatide, RP-HPLC, Method Validation, Stability-Indicating Method, Forced Degradation, ICH Q2(R1), Injectable Formulation.*

I. INTRODUCTION

Type 2 diabetes mellitus (T2DM) is one of the most prevalent chronic metabolic disorders worldwide and continues to represent a major healthcare challenge due to its association with cardiovascular disease, nephropathy, neuropathy, retinopathy, and increased mortality. The global incidence of diabetes has increased substantially over the last few decades, primarily due to sedentary lifestyles, unhealthy dietary habits, obesity, and population aging. Despite significant advances in therapeutic approaches, achieving optimal glycemic control while minimizing adverse effects remains a major clinical concern.

Traditional antidiabetic agents such as metformin, sulfonylureas, thiazolidinediones, and insulin have demonstrated effectiveness in controlling blood glucose levels. However, limitations including hypoglycemia, weight gain, gastrointestinal disturbances, and reduced long-term efficacy have encouraged the development of newer therapeutic alternatives. Incretin-based therapies have emerged as a promising strategy due to their ability to improve glucose regulation while promoting weight reduction and reducing cardiovascular risk.

Tirzepatide is a first-in-class dual agonist of glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) receptors. Structurally, it is a synthetic 39-amino acid peptide conjugated to a C20 fatty diacid moiety, which enhances albumin binding and prolongs systemic circulation, allowing convenient once-weekly administration. Tirzepatide was approved by regulatory authorities for the treatment of type 2 diabetes mellitus and subsequently for chronic weight management in obese and overweight patients. Its dual receptor agonism provides superior glycemic control and body weight reduction compared with selective GLP-1 receptor agonists.

Pharmacologically, tirzepatide stimulates glucose-dependent insulin secretion, suppresses glucagon release, delays gastric emptying, reduces appetite, and improves insulin sensitivity. Clinical studies have demonstrated significant reductions in glycated hemoglobin (HbA1c), fasting plasma glucose levels, and body weight, making tirzepatide one of the most promising therapeutic agents in metabolic disease management.

The increasing clinical use of tirzepatide has generated a need for reliable analytical methods capable of accurately quantifying the drug in bulk materials and pharmaceutical dosage forms. Analytical methods used for routine quality control should provide adequate sensitivity, specificity, precision, accuracy, and robustness. Furthermore, stability-indicating methods are essential to ensure the quality, safety, and efficacy of pharmaceutical products throughout their shelf life by detecting potential degradation products formed under stress conditions.

High-performance liquid chromatography (HPLC) remains one of the most widely used analytical techniques in pharmaceutical analysis because of its high sensitivity,

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selectivity, reproducibility, and suitability for complex formulations. Several chromatographic methods have been reported for the determination of tirzepatide, including RP-HPLC, UPLC, and LC-MS/MS techniques. However, many of these methods require sophisticated instrumentation, lengthy analysis times, gradient elution systems, or complex sample preparation procedures, limiting their applicability for routine quality control laboratories.

Therefore, the present investigation was undertaken to develop and validate a simple, rapid, economical, and stability-indicating RP-HPLC method for the quantitative estimation of tirzepatide in bulk drug and injectable dosage forms in accordance with International Council for Harmonisation (ICH) Q2(R1) guidelines. The developed method was further evaluated through forced degradation studies to establish its suitability for routine pharmaceutical analysis and stability assessment.

II. Materials and Methods

2.1 Materials

Tirzepatide reference standard was obtained from Aadhaar Life Sciences Pvt. Ltd., Solapur, India. HPLC-grade acetonitrile was procured from Thomas Fisher Scientific India Pvt. Ltd. Potassium dihydrogen phosphate and HPLC-grade water were obtained from Merck Specialities Pvt. Ltd., Mumbai, India. All reagents and solvents used throughout the study were of analytical or HPLC grade.

2.2 Instrumentation

Chromatographic analysis was carried out using an Agilent 1260 Infinity II HPLC system equipped with a quaternary pump, autosampler, diode-array detector (DAD), column oven, and OpenLab EZChrom software. Separation was achieved using a Phenomenex Kinetex XB-C8 column (150 mm × 4.6 mm, 5 µm particle size).

2.3 Chromatographic Conditions

Chromatographic separation was performed using a mobile phase consisting of 25 mM phosphate buffer (pH 5.0) and acetonitrile in the ratio of 58:42 (v/v). The mobile phase was filtered through a 0.45 µm membrane filter and degassed by ultrasonication prior to use. The flow rate was maintained at 1.0 mL/min, the column temperature was set at 30°C, and the injection volume was 10 µL. Detection was carried out at 220 nm with a total run time of 10 min.

2.4 Preparation of Standard Solution

A stock solution of tirzepatide was prepared by accurately weighing 5 mg of the drug and transferring it into a 100 mL volumetric flask. The drug was dissolved in diluent consisting of 0.1% phosphoric acid and acetonitrile (50:50, v/v), and the volume was adjusted to obtain a final concentration of 50 µg/mL. Further dilution was performed to obtain a working standard concentration of 5 µg/mL.

2.5 Sample Preparation

Commercial tirzepatide injection equivalent to 5 mg/0.5 mL was used for assay determination. The contents were diluted appropriately using the diluent and filtered through a 0.45 µm membrane filter before chromatographic analysis. Final sample concentration was adjusted to 5 µg/mL.

2.6 Method Validation

The developed RP-HPLC method was validated according to ICH Q2(R1) guidelines for specificity, system suitability, linearity, accuracy, precision, robustness, limit of detection, and limit of quantification.

III. Results and Discussion

3.1 Method Development and Optimization

The primary objective of method development was to establish a simple, rapid, precise, and stability-indicating RP-HPLC method for the determination of tirzepatide in bulk drug and injectable dosage form. Considering the peptide nature of tirzepatide, several chromatographic trials were performed by varying the composition of the mobile phase, flow rate, pH, detection wavelength, and stationary phase to obtain an optimum chromatographic response.

Initially, chromatographic separation was attempted using orthophosphoric acid-acetonitrile mixtures at different ratios. However, early trials resulted in either no peak elution, broad peak formation, excessive retention, or poor peak symmetry. Optimization studies revealed that the use of a phosphate buffer system significantly improved peak shape and chromatographic performance. Among the investigated mobile phase compositions, a mixture of 25 mM phosphate buffer (pH 5.0) and acetonitrile in the ratio of 58:42 (v/v) produced a sharp, symmetrical, and well-resolved tirzepatide peak.

Selection of an appropriate stationary phase was crucial because of the high molecular weight and peptide nature of tirzepatide. The Phenomenex Kinetex XB-C8 column (150 × 4.6 mm, 5 µm) provided superior chromatographic performance compared with other investigated conditions, resulting in improved peak symmetry, enhanced theoretical plate count, and acceptable retention characteristics.

The detection wavelength was selected following UV spectral scanning between 190 and 400 nm using a PDA detector. Tirzepatide exhibited significant absorbance at 220 nm, which provided adequate sensitivity and reproducibility for quantitative analysis.

The optimized chromatographic conditions produced a symmetrical tirzepatide peak with satisfactory system suitability characteristics and acceptable run time, making the method suitable for routine quality control applications.

Several chromatographic trials were performed to achieve satisfactory separation, peak symmetry, retention characteristics, and system suitability parameters for tirzepatide. Initial trials using orthophosphoric acid-acetonitrile combinations resulted in poor peak shape, broad peaks, or inadequate retention. Optimization studies demonstrated that a phosphate buffer system provided superior chromatographic performance. The final optimized chromatographic conditions consisted of 25 mM phosphate buffer (pH 5.0): acetonitrile (58:42, v/v) delivered through a Phenomenex Kinetex XB-C8 column (150 × 4.6 mm, 5 µm) at a flow rate of 1.0 mL/min with UV detection at 220 nm.

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Under these conditions, tirzepatide eluted at approximately 2.97 min with excellent peak symmetry and theoretical plate count.

3.2 Specificity

The specificity of the developed RP-HPLC method was evaluated by comparing chromatograms obtained from blank, standard, and pharmaceutical formulation samples. No interfering peaks were observed at the retention time of tirzepatide in the blank chromatogram. The retention time of tirzepatide in the standard and drug product chromatograms was approximately 2.97–2.98 min, indicating excellent specificity of the developed method. Furthermore, peak purity analysis confirmed the absence of co-eluting components.

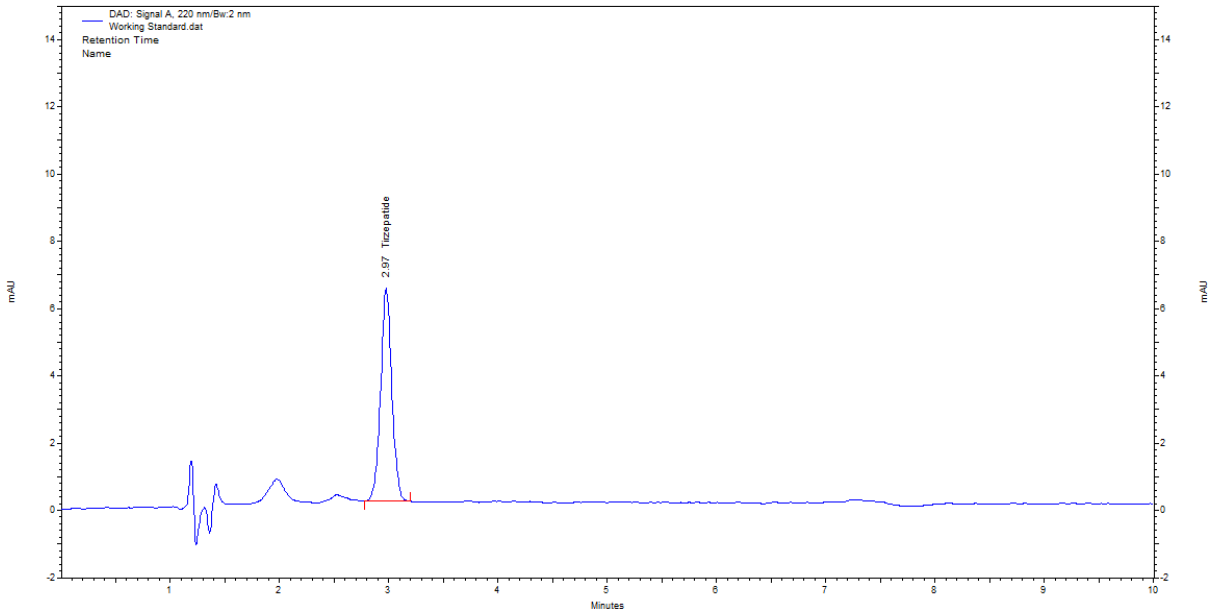

Figure 1: Optimized RP-HPLC Chromatogram of Tirzepatide (Working Standard)

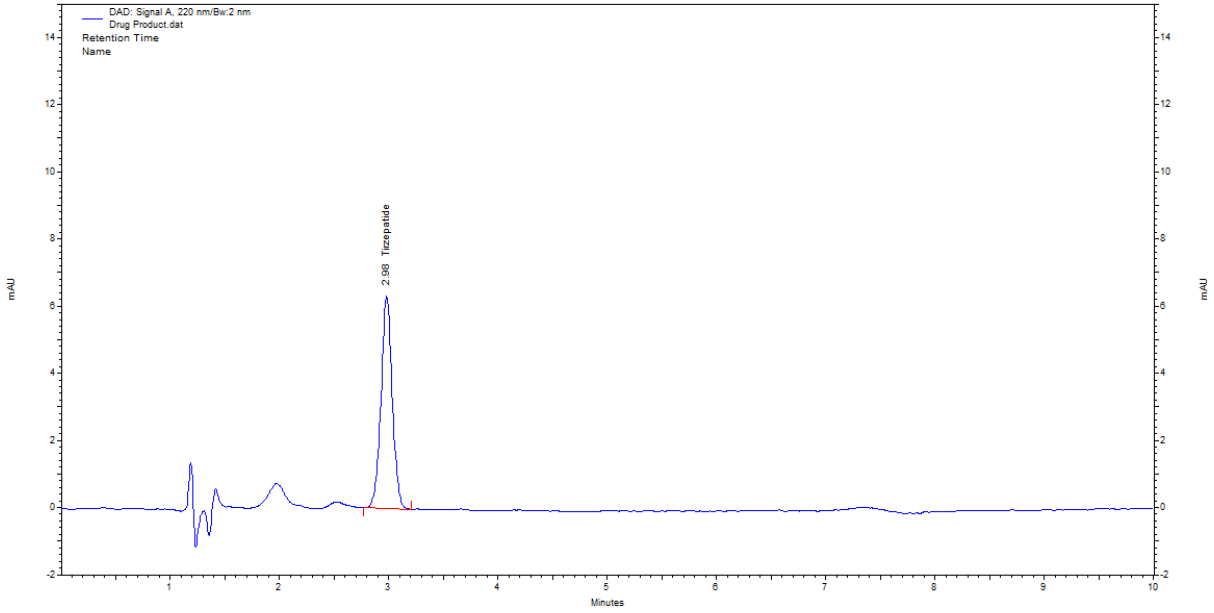

Figure 2: Drug Product Chromatogram of Tirzepatide Injection

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International Research Journal of Pharmacy and Medical Sciences
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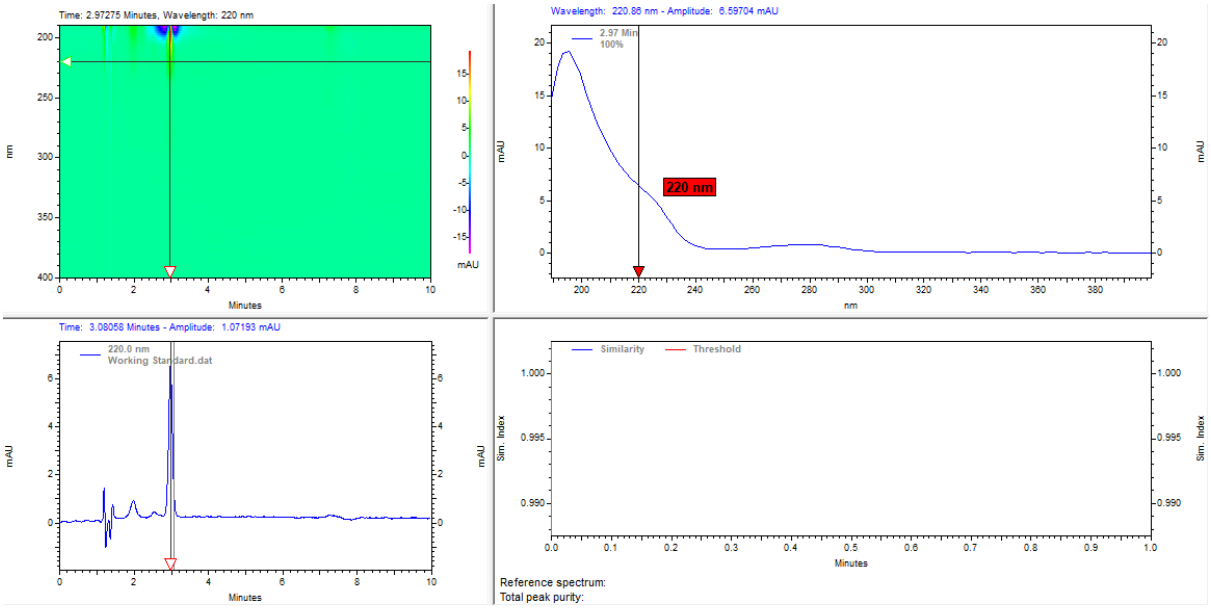

Figure 3:DAD Spectrum Used for Wavelength Selection

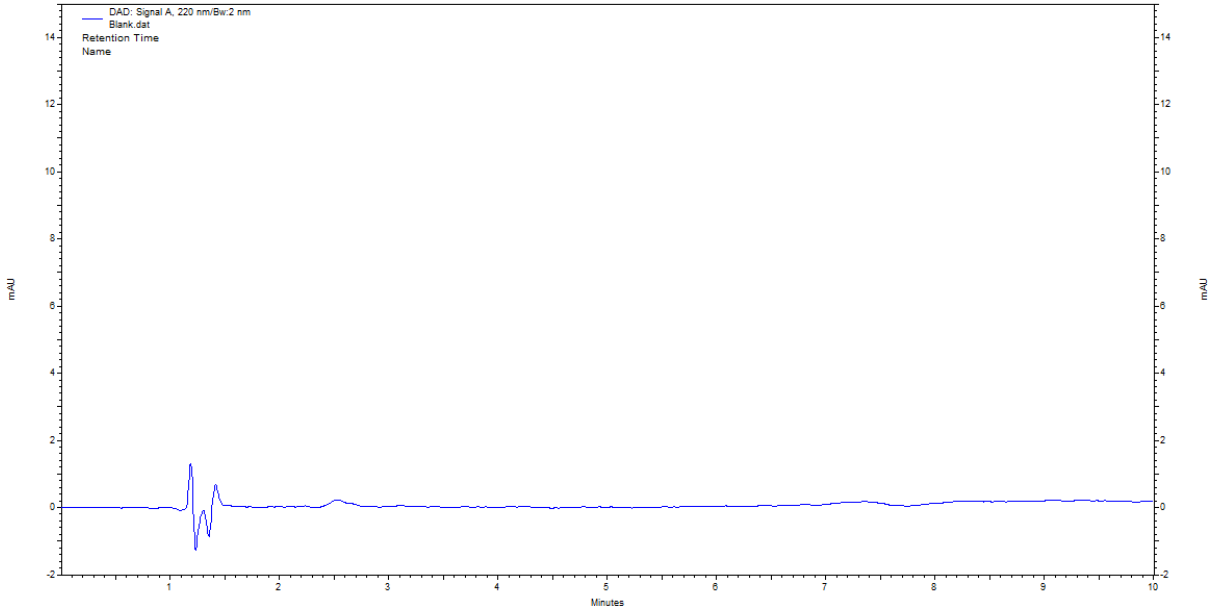

Figure 4: Blank Chromatogram

3.3 System Suitability and Repeatability

System suitability studies were performed using six replicate injections of a standard solution containing tirzepatide at the target assay concentration. The retention time remained constant at 2.97 min, while peak asymmetry values were close to unity, indicating symmetrical peak shape. Theoretical plate counts exceeded 42,000, demonstrating excellent column efficiency. The percentage relative standard deviation (%RSD) for peak area was only 0.20%, which is well within the acceptance limit of less than 2%. These results confirmed the suitability and reproducibility of the chromatographic system.

TABLE 1. System Suitability and Repeatability Results

Injection	Peak Area	RT (min)	Theoretical Plates	Tailing Factor	Peak Purity
Rep 1	94416	2.97	42387	1.04	1.00
Rep 2	94785	2.97	42184	0.99	1.00
Rep 3	94284	2.97	42779	0.97	1.00
Rep 4	94331	2.97	42371	1.00	1.00
Rep 5	94584	2.97	42339	1.01	1.00
Rep 6	94381	2.97	42145	1.05	1.00
Mean	94464	2.97	42368	1.01	1.00
SD	188.00	0.00	—	—	—
%RSD	0.20	0.00	—	—	—

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3.4 Linearity and Range

Linearity was established over the concentration range of 4–6 µg/mL, corresponding to 80–120% of the target assay concentration. The calibration plot demonstrated a strong linear relationship between concentration and peak area response. The regression equation obtained was:

$$y = 18781.8x + 386.8$$

with a correlation coefficient ($R^2 = 0.9998$), indicating excellent linearity across the studied range. The negligible intercept and high slope further confirmed the reliability of the method for quantitative analysis.

TABLE 2. Linearity Data for Tirzepatide

% Level	Concentration (µg/mL)	Peak Area
80	4.0	75509
90	4.5	84956
100	5.0	94416
110	5.5	103313
120	6.0	113285

Regression Equation: y = 18781.8x + 386.8
Correlation Coefficient (R^2): 0.9998

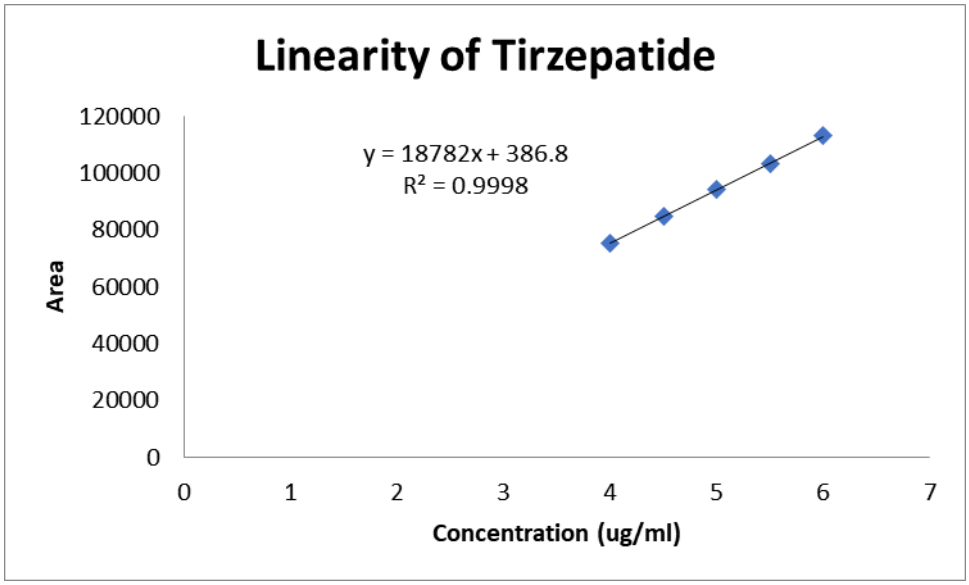

Figure 5: Linearity at 80% concentration

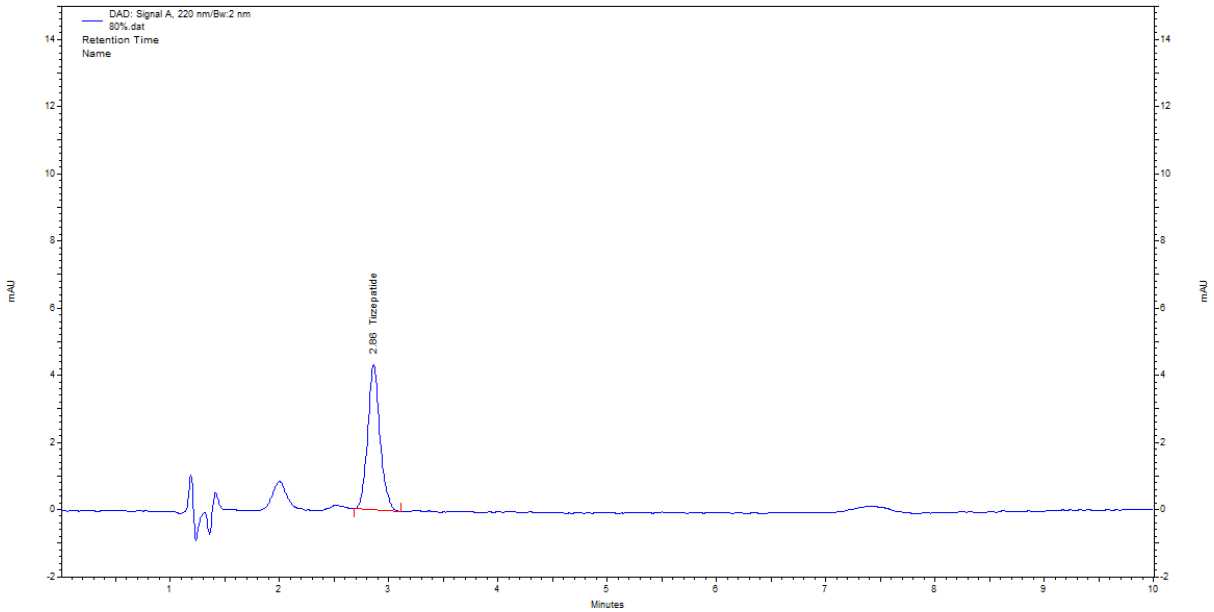

Figure 6: Linearity at 80% concentration

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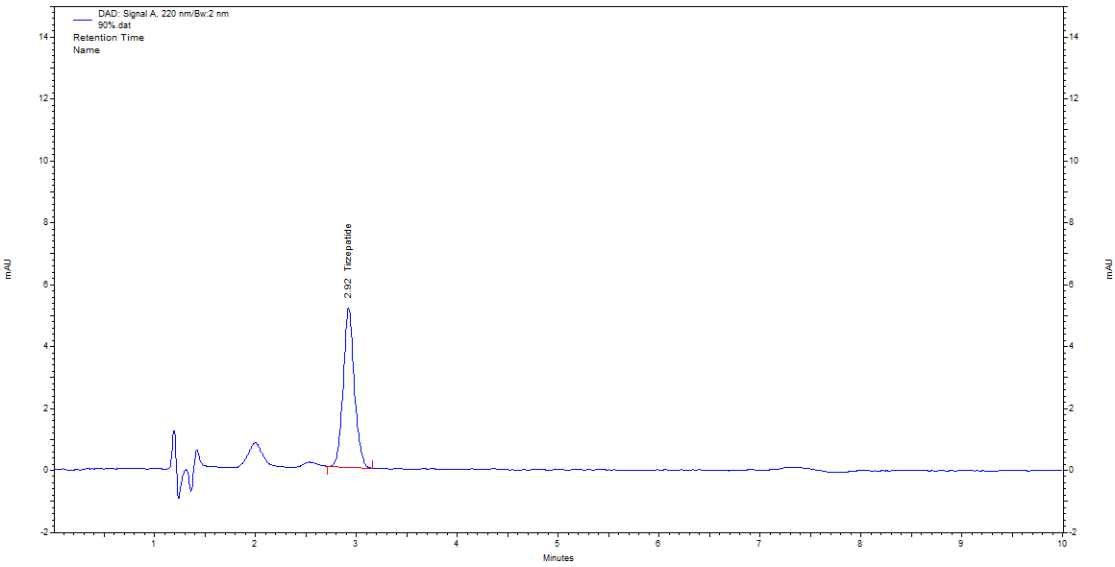

Figure 7: Linearity at 90% concentration

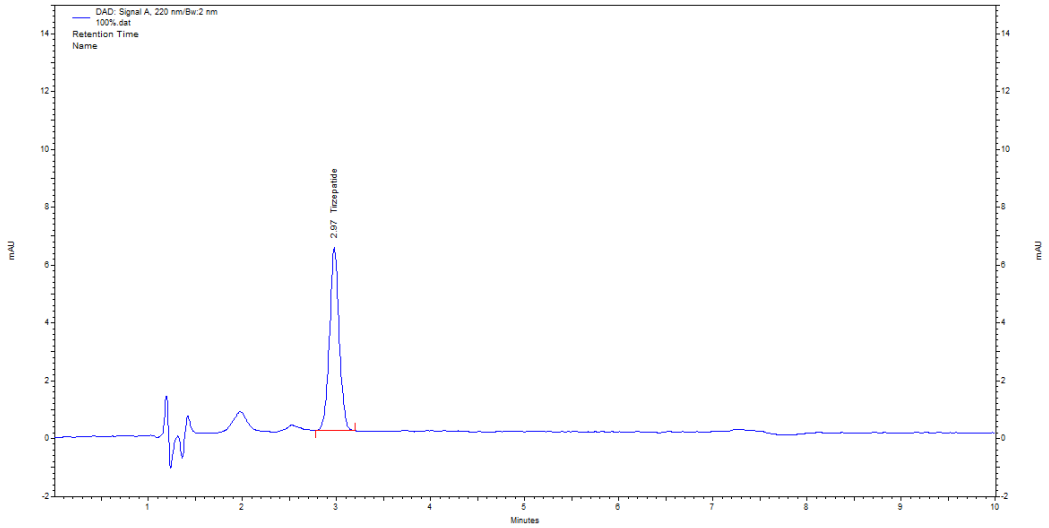

Figure 8: Linearity at 100% concentration

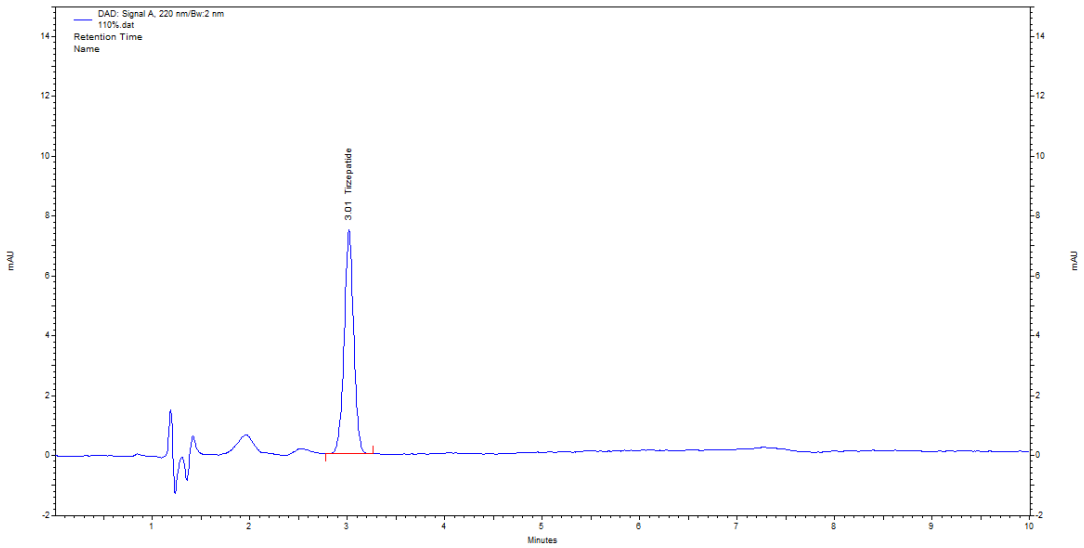

Figure 9: Linearity at 110% concentration

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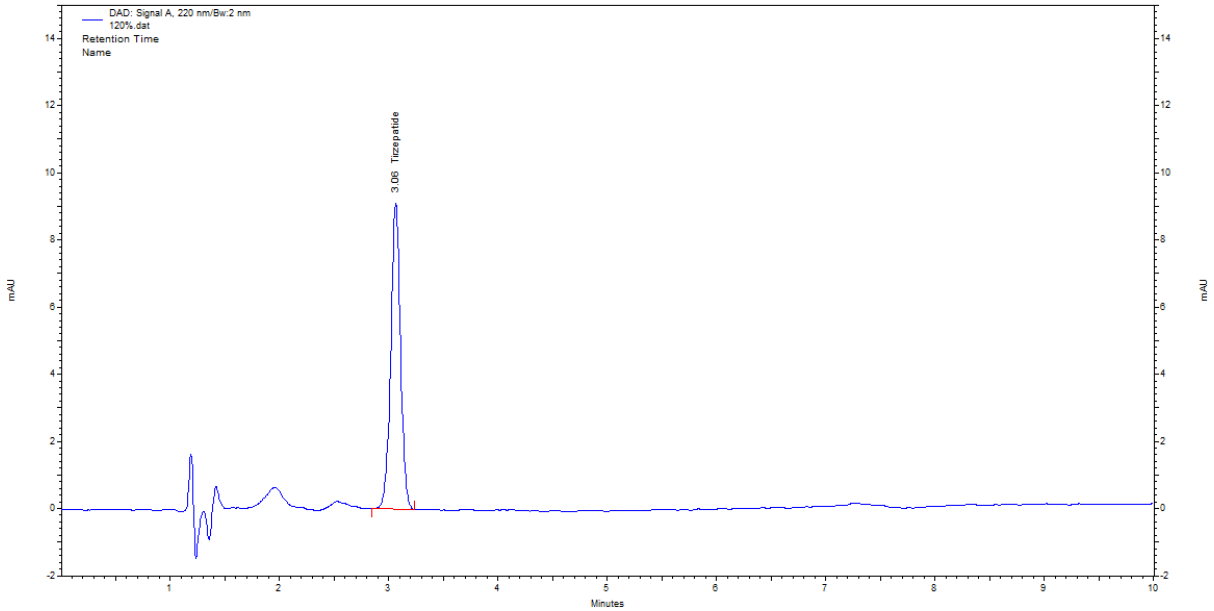

Figure 10: Linearity at 120% concentration

3.5 Accuracy

Accuracy was evaluated by recovery studies using the standard addition technique at 80%, 100%, and 120% concentration levels. The recovery values ranged between 99.81% and 100.34%, demonstrating excellent accuracy of the developed analytical method. The %RSD values remained below 0.30%, indicating high reproducibility of recovery measurements.

TABLE 3. Accuracy Results

Level	Mean Recovery (%)	%RSD
80%	100.03	0.27
100%	100.03	0.27
120%	100.02	0.10

Overall Mean Recovery = 100.03%

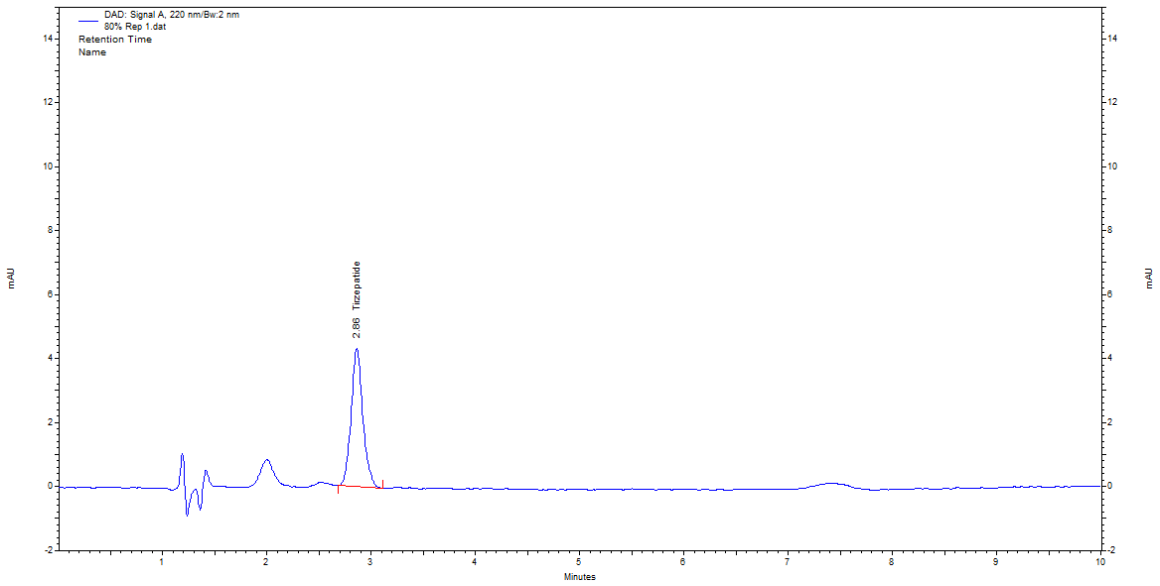

Figure 11: Accuracy 80% Rep 1

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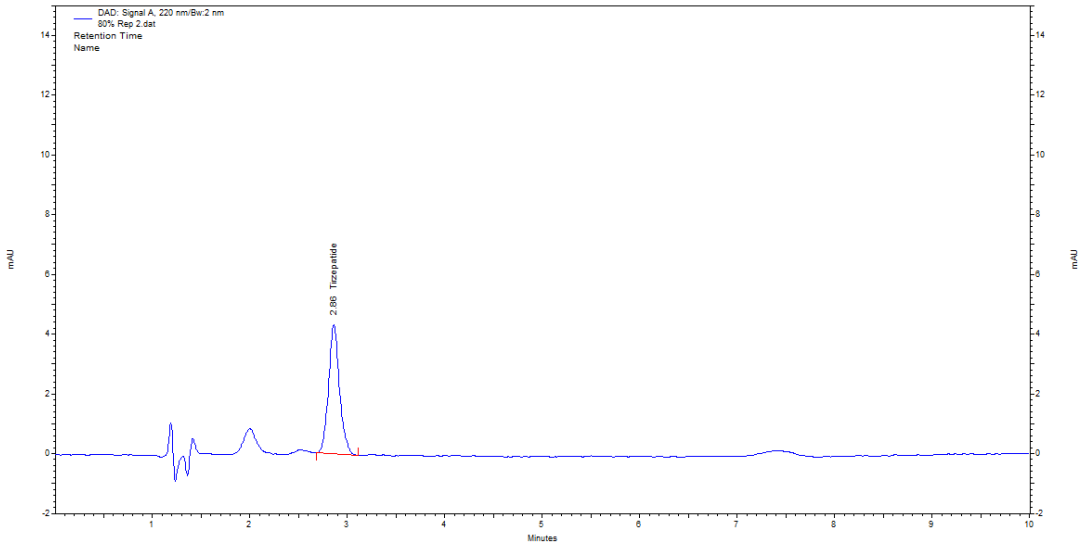

Figure 12: Accuracy 80% Rep 2

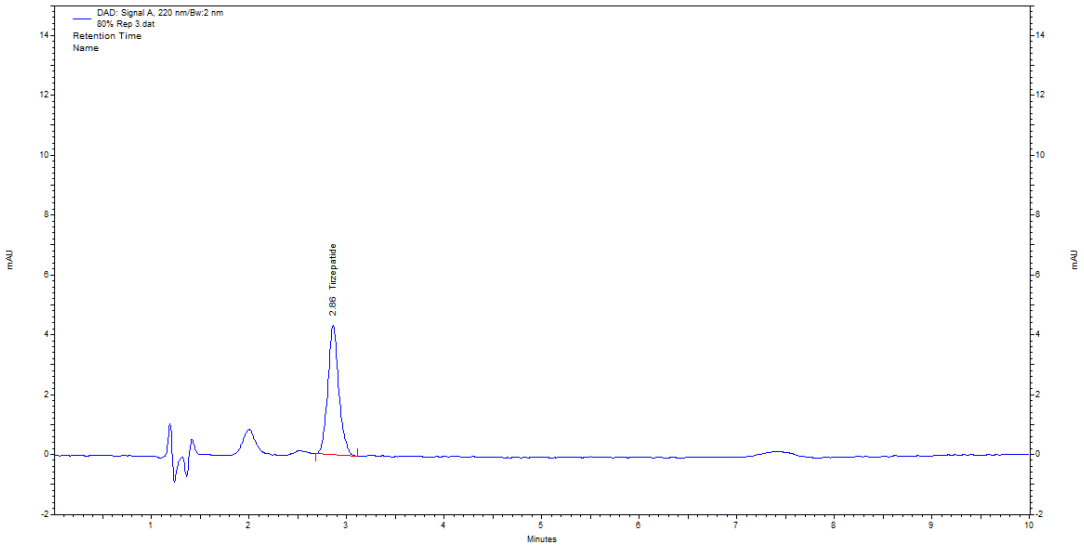

Figure 13: Accuracy 80% Rep 3

3.6 Precision

The precision of the method was assessed through intra-day and inter-day studies. The %RSD values obtained for peak area and assay results were below 1%, indicating excellent method precision and reproducibility. Retention times remained consistent throughout the study period.

TABLE 4. Intermediate Precision Results

Study	%RSD RT	%RSD Peak Area
Intra-day	0.39	0.34
Inter-day	0.75	0.29

3.7 Sensitivity (LOD and LOQ)

The developed method demonstrated excellent analytical sensitivity. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.14 µg/mL and 0.44 µg/mL, respectively. These low values indicate that the method is capable of detecting and quantifying tirzepatide even at trace concentration levels, making it suitable for degradation and stability studies.

TABLE 5. Sensitivity Parameters

Parameter	Value (µg/mL)
LOD	0.14
LOQ	0.44

3.8 Robustness

Robustness studies were conducted by varying column temperature (±2°C) and detection wavelength (±2 nm). The chromatographic performance remained unaffected by these deliberate changes. The %RSD values for assay results remained below 1%, confirming robustness of the analytical procedure.

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TABLE 6. Robustness Study (Temperature Variation)

Temperature	Assay (%)
28°C	100.25
30°C	100.58
32°C	100.38

Mean Assay = 100.40%
%RSD = 0.16

3.9 Forced Degradation Studies

Forced degradation studies demonstrated the stability-indicating capability of the developed RP-HPLC method. Tirzepatide exhibited maximum degradation under alkaline conditions (9.74%), followed by thermal degradation (7.47%). Moderate degradation was observed under oxidative and photolytic conditions, whereas acidic degradation was minimal. Importantly, all degradation products were well resolved from the principal drug peak, confirming the specificity and stability-indicating nature of the method.

TABLE 7. Forced Degradation Results

Stress Condition	% Assay	% Degradation
Acid Hydrolysis	99.63	0.37
Alkali Hydrolysis	90.26	9.74
Oxidative Degradation	97.05	2.95
Thermal Degradation	92.53	7.47
Photolytic Degradation	97.54	2.46

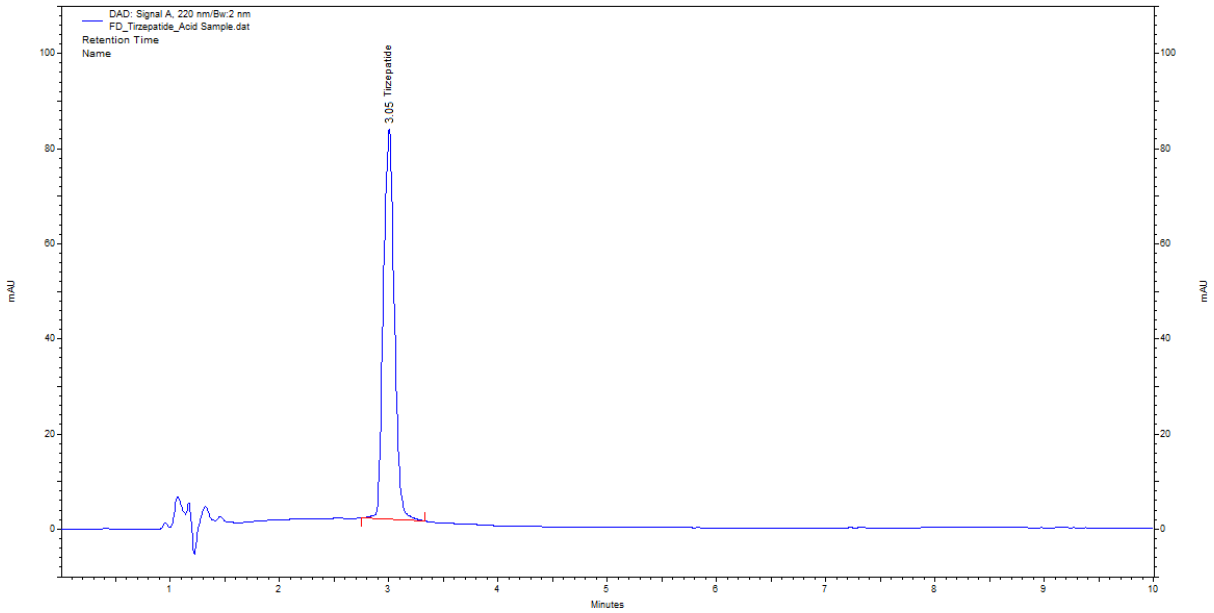

Figure 14: Acid Degradation Chromatogram

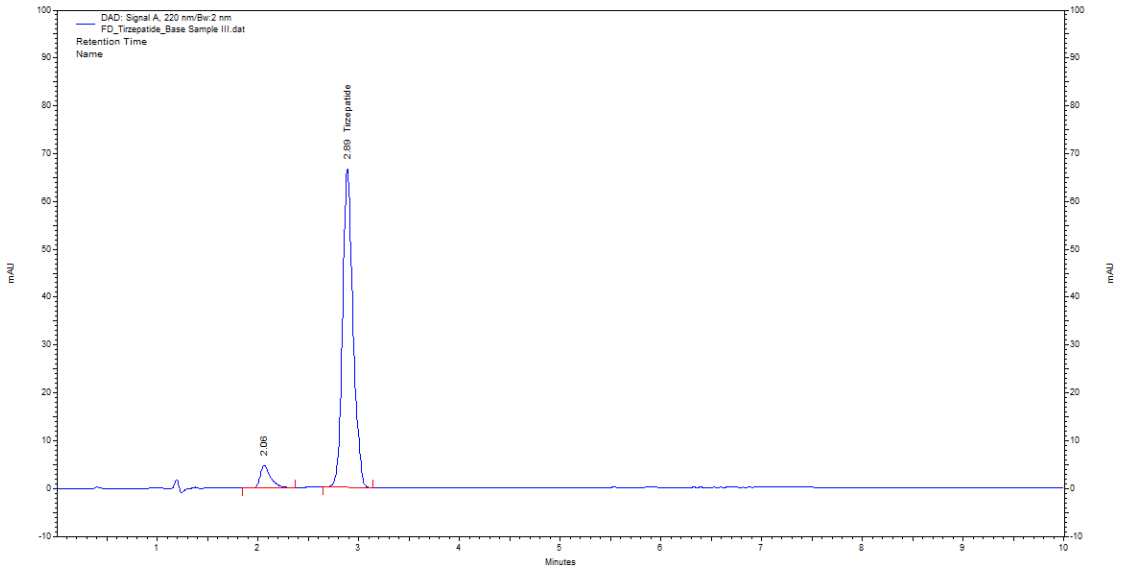

Figure 15: Base Degradation Chromatogram

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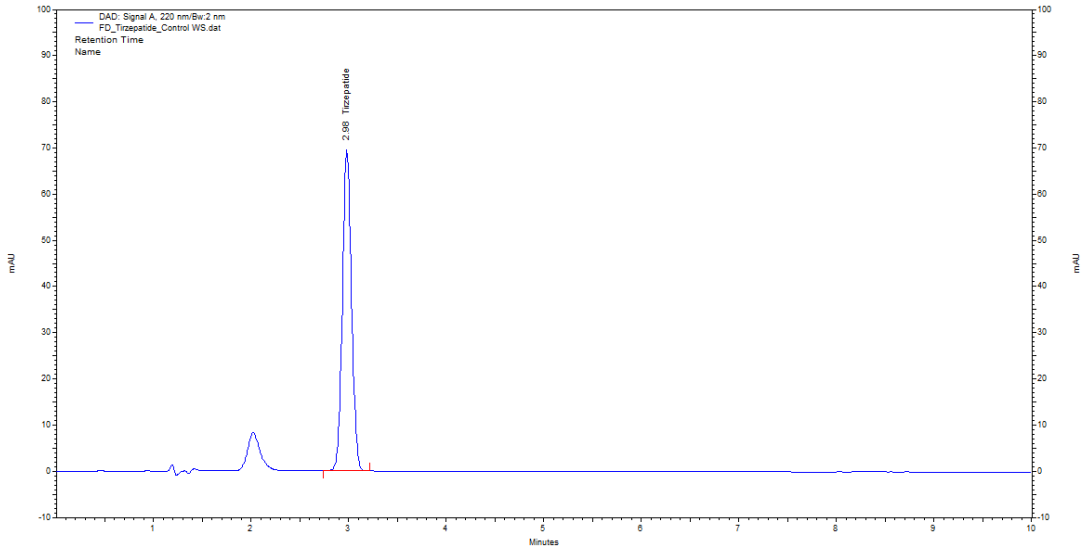

Figure 16: Control Standard Chromatogram

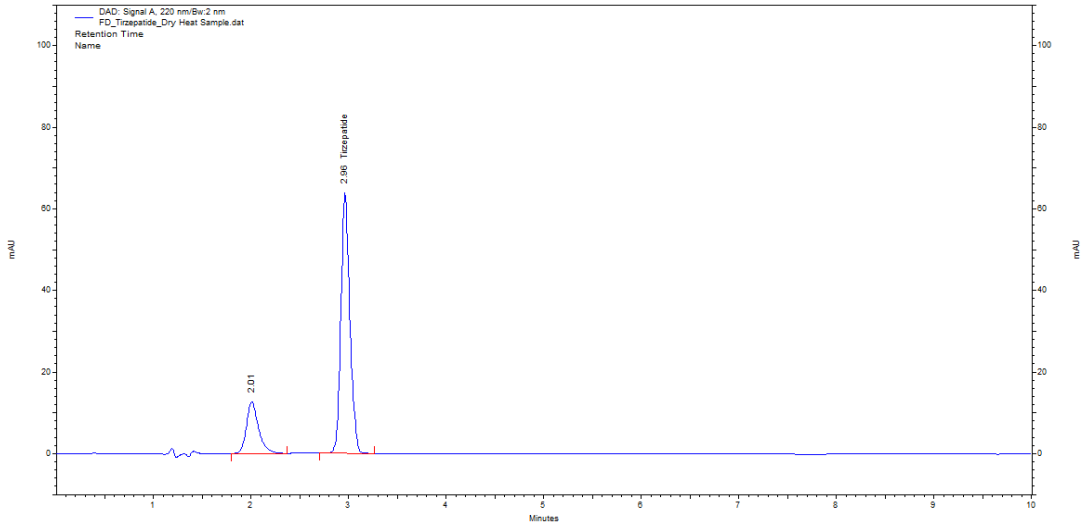

Figure 17: Dry Heat Degradation Chromatogram

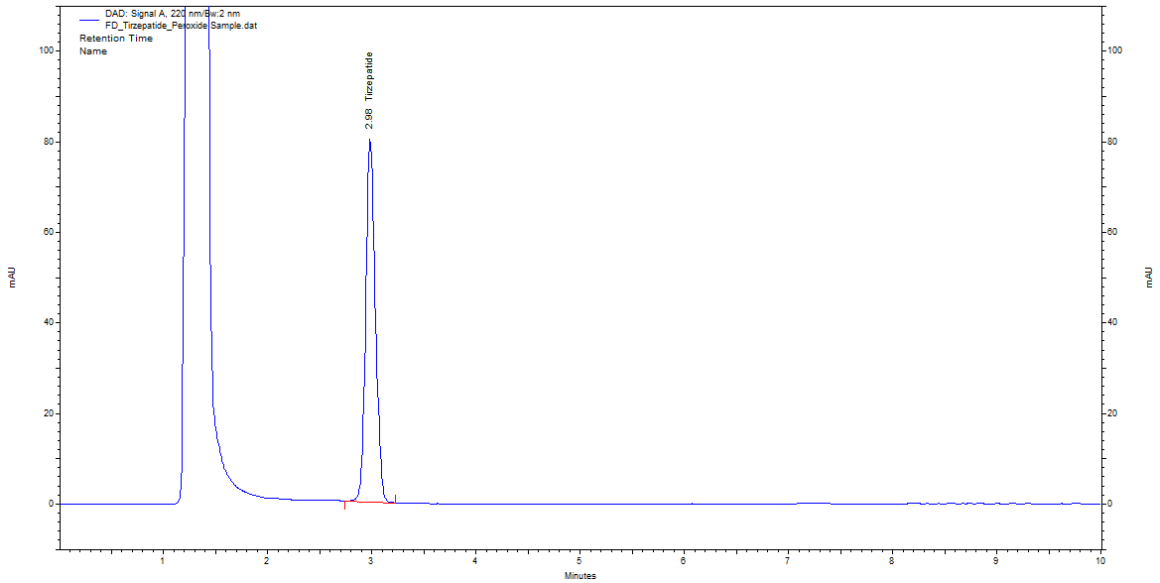

Figure 18: Peroxide Degradation Chromatogram

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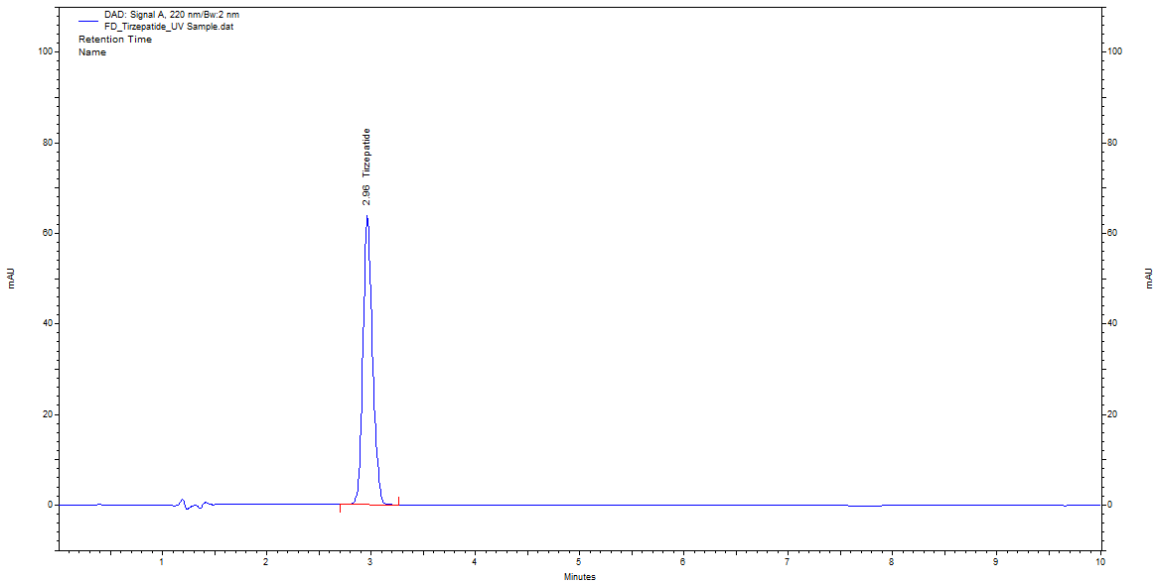

Figure 19: UV Degradation Chromatogram

IV. Discussion

The developed RP-HPLC method demonstrated several advantages over previously reported analytical methods for tirzepatide. Unlike LC-MS/MS and UPLC methods that require sophisticated instrumentation and higher operational costs, the present method utilizes conventional RP-HPLC instrumentation while maintaining excellent analytical performance. The method offers a shorter analysis time, simple sample preparation, satisfactory sensitivity, and compliance with ICH validation requirements.

Furthermore, successful forced degradation studies confirmed the stability-indicating capability of the method, making it suitable for routine quality control, stability testing, and regulatory applications. The overall validation results indicate that the proposed method provides a reliable analytical tool for the quantitative estimation of tirzepatide in pharmaceutical formulations.

V. Conclusion

The present study successfully developed and validated a simple, rapid, accurate, precise, robust, and stability-indicating RP-HPLC method for the quantitative determination of tirzepatide in bulk drug and injectable dosage form. Chromatographic separation was achieved using a Phenomenex Kinetex XB-C8 column with a mobile phase consisting of 25 mM phosphate buffer (pH 5.0) and acetonitrile (58:42, v/v), resulting in satisfactory peak resolution and chromatographic performance.

The developed method was validated according to ICH Q2(R1) guidelines and demonstrated excellent specificity, linearity, accuracy, precision, robustness, and sensitivity. The calibration curve exhibited a strong linear relationship between concentration and peak area within the selected analytical range. Accuracy studies showed satisfactory recovery values, while precision studies indicated excellent repeatability and intermediate precision with percentage relative standard deviation values well within acceptable limits.

Forced degradation studies conducted under acidic, alkaline, oxidative, thermal, and photolytic stress conditions confirmed the stability-indicating nature of the method. The developed chromatographic procedure successfully separated degradation products from the principal tirzepatide peak, demonstrating its suitability for stability testing and routine quality control applications.

Compared with previously reported analytical methods, the proposed RP-HPLC method offers advantages including simplicity, shorter analysis time, economical operation, ease of sample preparation, and utilization of commonly available chromatographic instrumentation. Therefore, the developed method can be effectively employed for routine pharmaceutical quality control, assay determination, stability assessment, and regulatory compliance studies involving tirzepatide formulations.

Acknowledgements

The authors express their sincere gratitude to the management and faculty members of the Department of Pharmaceutical Quality Assurance for providing the necessary laboratory facilities and continuous support throughout the research work. The authors also acknowledge Aadhaar Life Sciences Pvt. Ltd., Solapur, India, for providing the gift sample of Tirzepatide used in the present investigation.

Funding Statement

The authors declare that no external funding was received for conducting this research work.

Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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