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Research

A Simple Stability-Indicating UPLC Method for quantification of Tirzepatide in Bulk drug and Pharmaceutical Formulations

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Abstract

Analytical method development and validation are the continuous and inter-dependent task associated with the research and development, quality control and quality assurance departments. Analytical procedures play a critical role in equivalence and risk assessment, management. It helps in establishment of product-specific acceptance criteria and stability of results. In view of this the present work is intended to develop an efficient and simple UPLC method for the determination of Tirzepatide in bulk and was applied on marketed Tirzepatide products. The method was linear at the concentration range of 5–17.5 µg/ml for Tirzepatide. The method has been validated according to ICH guidelines with respect to system suitability, specificity, precision, accuracy and robustness. LOD and LOQ were found to be 0.01 µg/mL and 0.102 µg/mL for Tirzepatide. Results confirmed that the method was sensitive and can be useful for the detection and analysis of drugs at very lowest concentrations. The method can effectively separate the forced degradation products formed during the stress study confirms the stability indicating nature of the method. Hence it the method reported in the present study was used for the separation and quantification of Tirzepatide in bulk drug, formulations as well as stability testing.

Keywords: Tirzepatide, UPLC method, ICH guidelines, system suitability, specificity, precision, accuracy and robustness etc.

INTRODUCTION

Pharmaceutical analysis is basically concerned with the determination of the chemical composition of matter however, identification of substance, the elucidation of its structure and quantitative analysis of its composition are the aspects covered by modern analytical techniques. Analytical chemistry may be derived as the science and art of determining the composition of material in terms of the elements of compounds contained. By means of analytical techniques both qualitative analysis (the presence or absence of one or more elements) and quantitative analysis (how much amount is present) can be done [1].

The qualitative and quantitative analysis can be done by various analytical methods: various analytical techniques can be revised and some of them give accurate result, example, and spectrophotometric method. Modern analytical techniques employ a range of techniques that vary from simple qualitative chemical test to the use of most sophisticated and expensive computer controlled instruments. Analytical instrumentation plays an important role in the production and evaluation of new products. This instrumentation provides the lower limit of detection required to assure the safety.

Analytical technique is a fundamental scientific phenomenon that has proved useful for providing information on the composition of substance. Analytical method is a specific application of a technique to solve an analytical problem. Analytical chemistry involves the application of range of techniques and methodologies to obtain and access quantitative and structural information on the nature of matter [2,3].

In Pharmaceutical industry the chemical composition of raw material, intermediates and finished products quality and need to be monitored to ensure satisfactory consistency. A drug may be defined as a substance meant for diagnosis, cure, mitigation, prevention or altering any structure or any function of the body or animals. The quality of a drug is the degree of possession of all those characteristics designed and manufactured into it, which contributes in the performance of the intended function, when the drug is used as directed.

METHODS

Preparation of standard drug solution

Preparation of standard stock solution was the primary step prior to experimental work. A standard stock solution of 1000 µg/mL was prepared by weighing accurately 10mg of the standard drug Tirzepatide and was taken in a 10 mL volumetric flask having little amount of Methanol. Dissolve the drug in the solvent and make up to the mark. Then it was filtered through 0.45µ filter paper to remove un-dissolved particles or any solid substances. The solution was preserved safely and used when required.

Table 1: preparation of standard dilutions for Tirzepatide

S.No	Concentration of Stock	Volume taken	Diluent added	Volume prepared	Final concentration
1	100 µg/mL	0.5 mL	9.5 mL	10 mL	5 µg/mL
2	100 µg/mL	0.75 mL	9.25 mL	10 mL	7.5 µg/mL
3	100 µg/mL	1.0 mL	9.0 mL	10 mL	10 µg/mL
4	100 µg/mL	1.25 mL	8.75 mL	10 mL	12.5 µg/mL
5	100 µg/mL	1.5 mL	8.5 mL	10 mL	15 µg/mL
6	100 µg/mL	1.75 mL	8.25 mL	10 mL	17.5 µg/mL

Preparation of formulation solution

An exactly pipetted one mL of solution was taken in a 10 mL flask having 5 mL of methanol. It was kept on a sonicator which was kept in ice bath for 30 min. Then it was kept in a bench top till it reach to normal temperature and flask volume was bring up to mark with same solvent. Finally, the solution was centrifuged up to 5 min at 4000 RPM. Then the solution diluted such that the resultant solution contains 10 µg/mL of Tirzepatide.

UPLC Method Development

Selection of detector wavelength

The appropriate detector wavelength was selected based on the maximum absorption wavelength through detector.

Selection of stationary phase

Since the Tirzepatide is a Polar drug, a non-polar C18 column was selected for the separation of the drug. Different columns of different companies, manufactures and configurations were tested.

Method Development trails

Trail 1:

S. No	Parameter	Condition
1	MP	Methanol and acetonitrile in 70:30 (v/v)
2	Wavelength	245 nm
3	Stationary Phase	Acquity UPLC BEH C18 (50mm × 2.1 mm; 1.7µm)

4	Flow Rate	0.3 mL/min

Trail 2:

S. No	Parameter	Condition
1	MP	Methanol and acetonitrile in 20:80 (v/v)
2	Wavelength	245 nm
3	Stationary Phase	Acquity UPLC BEH C18 (50mm × 2.1 mm; 1.7µm)
4	Flow Rate	0.3 mL/min

Trail 3:

S. No	Parameter	Condition
1	MP	Methanol and water in 50:50 (v/v)
2	Wavelength	245 nm
3	Stationary Phase	Acquity UPLC BEH C18 (50mm × 2.1 mm; 1.7µm)
4	pH of MP	5.5
5	Flow Rate	0.3 mL/min

Trail 4:

S. No	Parameter	Condition
1	MP	methanol: acetonitrile: water 30:20:50 (v/v)
2	Wavelength	245 nm
3	Stationary Phase	Acquity UPLC BEH C18 (50mm × 2.1 mm; 1.7µm)
4	pH of MP	5.6
5	Flow Rate	0.3 mL/min

Trail 5:

S. No	Parameter	Results
1	MP	Methanol: acetonitrile: 0.1 % sodium dihydrogen orthophosphate in 50:30:20 (v/v)
2	Wavelength	245 nm
3	Stationary Phase	Acquity UPLC BEH C18 (50mm × 2.1 mm; 1.7µm)
4	pH of MP	5.9
5	Flow Rate	0.3 mL/min
6	Pump Mode	Isocratic

Analytical Method Validation

UPLC analytical method validation is a systematic and scientific approach to establishing the reliability, accuracy, and suitability of an analytical method for its intended use. It is an integral part of ensuring the quality and safety of pharmaceutical products and other analytical applications.

The method was validated with respect to linearity, accuracy, precision, repeatability, selectivity, and specificity, according to the ICH guidelines. Validation studies were carried out by replicate injections of the sample and standard solutions into the chromatograph.

Specificity

Specificity of the method was checked by injecting the solution into the chromatograph. Specificity of the method was assessed by comparing the chromatogram of Tirzepatide (standard), blank and sample solutions to those obtained for tablet solutions. Retention time of the Tirzepatide in standard solution, and in the sample, solution was compared to determine the specificity of the method.

System suitability

The system suitability was determined by making six replicate injections of the standard solution and analyzing Tirzepatide for its peak area, peak USP tailing factor, and number of theoretical plates. The proposed accepted criteria are not more than 2% for RSD%, not less than 2 for resolution, not more than 2 for USP tailing factor, and not less than 2000 for the number of theoretical plates.

Sensitivity of the method

The limit of detection (LOD) and limit of quantitation (LOQ) were defined as the lowest concentration of analyte in a sample that can be detected and quantified. The standard solutions of Tirzepatide for LOD and LOQ were prepared by diluting them with suitable solvent. The LOD and LOQ were determined by the signalto-noise (S/N) ratio for each compound through analyzing a series of diluted solutions until the S/N ratio yield 3 for LOD and 10 for LOQ, respectively.

Linearity and Range

The calibration curve in the developed method was constructed from LOQ concentration. Tirzepatide standard stock solution of 100 mg/mL was used for preparation of subsequent aliquots. Various aliquots were prepared by serial dilution as given in table 5.2. Sample solution was loaded and 20 µL was injected into column. All measurements were repeated for each concentration. The calibration curve of the area under curve versus concentration were recorded. Form the calibration curve, correlation and regression values were calculated for Tirzepatide.

Precision

The precision studies were carried out by estimating response of Tirzepatide six times at a standard concentration of 10 µg/mL and results are reported in terms of %RSD. The intra-day and inter-day precision studies were carried out by estimating the corresponding responses six times on same day for intraday and interday for three different days and it was expressed as the percentage relative standard deviation (%RSD) which was calculated as per the following expression

%RSD = (standard deviation / mean) x 100.

Accuracy/ Recovery

Accuracy of method was observed by recovery result from two placebos preparations accurately spiked with different concentration of Tirzepatide. Recovery assessment was obtained by using standard addition technique which was by adding known quantities of pure standards at three different levels in 50%, 100% and 150% to the pre analyzed sample formulation. From the amount of drug found, amount of drug recovered and percentage recovery were calculated by using the formula.

%RSD = (standard deviation / mean) x 100.

Ruggedness

Two laboratory analysts carried out the precision of Tirzepatide at a standard concentration of 10 µg/mL was prepared by different analysts in the laboratory conditions, the prepared solution were analyzed in the optimized conditions. Peak area that obtained was used for the determination of ruggedness of the method. Ruggedness was expressed in terms of %RSD which must be less than 2.

Robustness

Robustness of the proposed method included six deliberate variations to some chromatographic parameters. The modifications include different mobile phase ratios and different detector wavelengths and different percentage in the mobile phase (in the range of ± 5 of the nominal value and the normal %). The % change in each of the changed condition was calculated.

METHODOLOGY

Forced degradation study

A forced degradation study is conducted to understand the stability characteristics and potential degradation pathways of a pharmaceutical product under stress conditions. This study helps in identifying and characterizing degradation products, which, in turn, aids in developing robust formulations and ensuring the product's quality and safety.

Acid Hydrolysis: 50 mg of drugs were mixed with 50 ml of 0.1N HCl solution. The solution was neutralized and diluted up to standard concentration (10 µg/mL) and was analyzed in the developed method condition

Base Hydrolysis: 50 mg of drugs were mixed with 50 ml of 0.1N NaOH solution. The solution was neutralized and diluted up to standard concentration i.e 10 µg/mL and was analyzed in the developed method condition

Oxidative Degradation: 0 mg of drugs were with 50 ml of 3% Peroxide solution. The solution was neutralized and diluted up to standard concentration (10 µg/mL) and was analyzed in the developed method condition

Photolytic Degradation: 50 mg of drug sample was kept in UV light [254 nm]. After the selected time of light expose, the drug solution was prepared and was analyzed

Thermal Degradation: 50 mg of drug sample was kept in oven at 60^0C. After the selected time of light expose, the drug solution was prepared and was analyzed

Formulation analysis

This proposed method was applied to the determination of Tirzepatide in commercially in injection form. The sample solution at a concentration of 10 µg/mL of Tirzepatide was analyzed in the optimized conditions. Peak area of the resultant chromatogram was used for the estimation of assay using label clime recovery method. The % assay was calculated for Tirzepatide using the standard calibration values.

UPLC Method Development

The present work aimed to develop a simple and accurate UPLC method for the quantification of Tirzepatide in pharmaceutical formulations. The method development was initiated with the determination of suitable detector wavelength for the detection of Tirzepatide using UV detector in UPLC. Hence the maximum absorption wavelength was determined using UV detector in UPLC.

For developing the method, a systematic study of the effect of various factors was undertaken by varying one parameter at a time and keeping all other conditions constant. Method development consists of selecting the appropriate wavelength by spectrophotometer and choice of stationary and mobile phases.

RESULTS AND DISCUSSIONS

Trail 1:

Under these conditions, no proper identification was observed. The chromatogram shows peaks corresponding to Tirzepatide, but there was no clear baseline observed. Therefore, the conditions were not suitable for the separation and analysis of Tirzepatide. The chromatographic results suggest that the mobile phase used in this trial was not suitable for the resolution of analytes. Consequently, further study was conducted with a change in the mobile phase while keeping the same column. The chromatogram obtained in this condition was given figure 1.

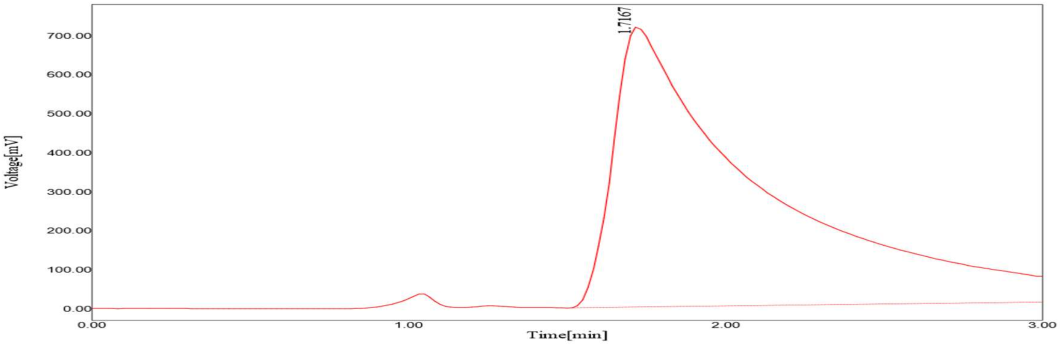


Fig 1: Chromatogram observed for Tirzepatide in trail 1

Trail 2:

Under these conditions, a broad peak with very poor peak area intensity and peak area response was noticed. The baseline of the chromatogram was disturbed, with no additional detections observed. The chromatographic results suggest that the mobile phase used in this trial was not suitable for the resolution of analytes. Consequently, further study was conducted with a change in the column while keeping the same mobile phase. The chromatogram obtained in this condition was given figure 2.

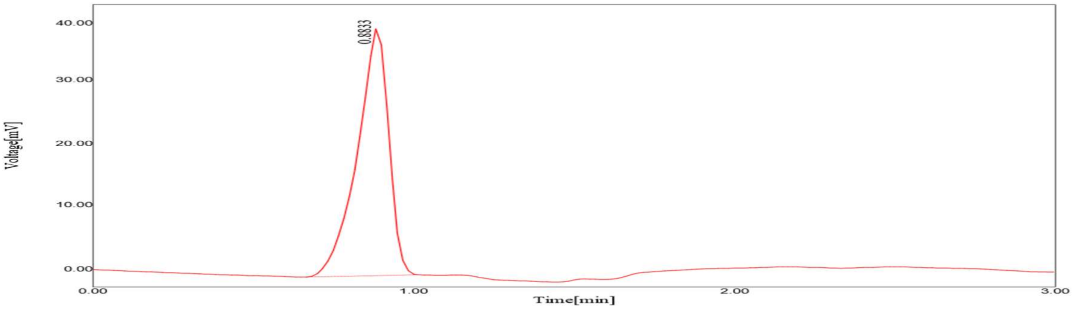


Fig 2: Chromatogram observed for Tirzepatide in trail 2

Trail 3:

Under these conditions, one peak corresponding to Tirzepatide was observed. However, the peak was broad and exhibited a high tail factor, indicating significant tailing. Despite this, the peak intensity and peak area response were enhanced compared to previous trials, showing improved detection of the analyte. The baseline of the chromatogram was still disturbed, but these disturbances were significantly reduced compared to earlier conditions. The chromatographic results suggest that the column used in this trial was suitable for the resolution of analytes. Therefore, further study was conducted using the same column while varying the mobile phase composition to optimize the separation and analysis conditions. The chromatogram obtained in this condition was given figure 3.

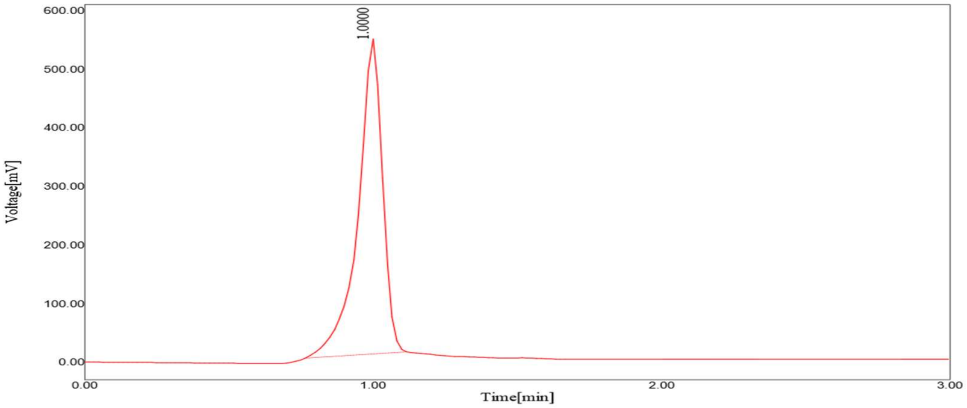


Fig 3: Chromatogram observed for Tirzepatide in trail 3

Trail 4:

In these conditions, Tirzepatide's peak was clearly identified in the chromatogram, and the baseline was observed to be clear with additional detections noted. However, there was a significant decrease in the peak area response compared to previous experimental settings. This decrease in peak intensity suggests that the presence of water in the mobile phase affected the elution behavior, particularly for Tirzepatide. Water in the mobile phase likely caused retention or altered the chromatographic behavior of Tirzepatide, leading to less efficient separation and detection. Consequently, it was concluded that water was not suitable for achieving optimal resolution of Tirzepatide under these chromatographic conditions. As a result, a subsequent trial was proposed with adjustments to the mobile phase composition, specifically without the inclusion of water, to improve the separation and analytical performance for Tirzepatide. Figure 5.4 shows the chromatogram noticed in trail 4 conditions during the optimization of method for quantification of Tirzepatide.

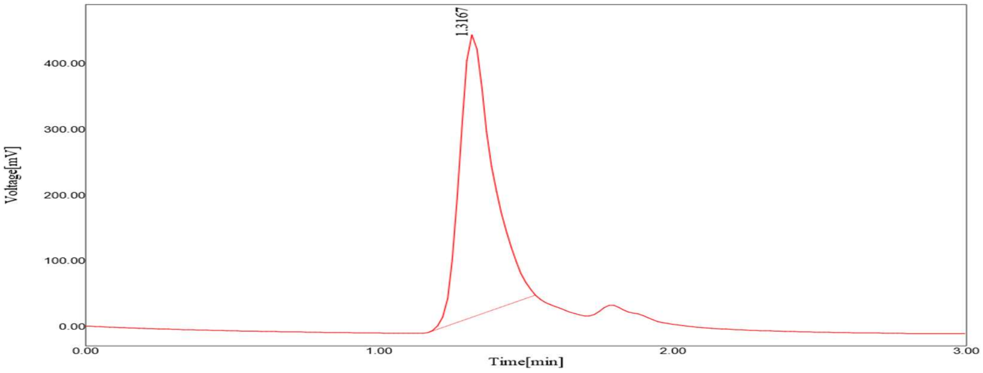


Fig 4: Chromatogram observed for Tirzepatide in trail 4

Trail 5:

In these conditions, the peak corresponding to Tirzepatide was identified, appearing slightly sharper compared to previous trials where broader peaks were observed. The peak area response was significantly higher under these conditions than in previous experiments. However, despite these improvements, the conditions were still deemed unsuitable (Figure 5). Therefore, it is clear that further experimentation or alternative approaches are needed to optimize the separation conditions for Tirzepatide.

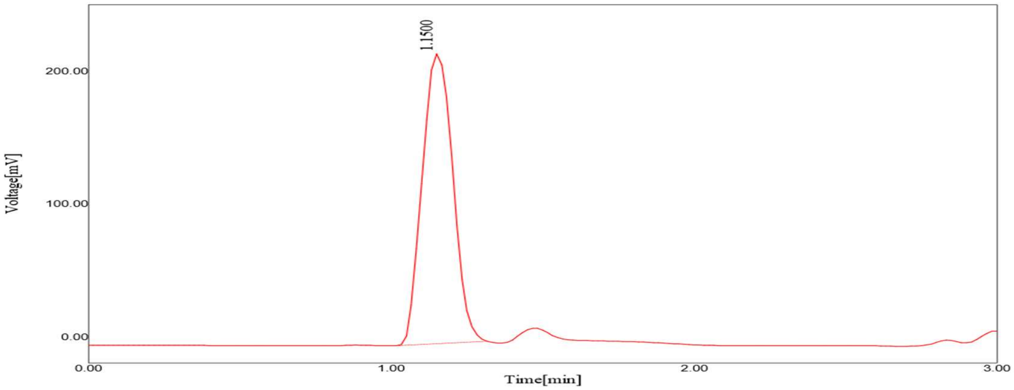


Fig 5: Chromatogram observed for Tirzepatide in trail 5

In the optimized conditions, well resolved, retained and accepted system suitability was observed. The optimized conditions were given in table 2.

Table 2: Optimized chromatographic conditions

S. No	Parameter	Results
1	Mobile phase	Methanol: acetonitrile: 0.1 % sodium dihydrogen orthophosphate in 70:10:20 (v/v)
2	Wavelength	245 nm
3	Stationary Phase	Acquity UPLC BEH C18 (50mm × 2.1 mm; 1.7µm)
4	pH of MP	5.9
5	Flow Rate	0.3 mL/min
6	Pump Mode	Gradient
7	Pump Pressure	9.1±5 MPa
8	Run time	3 min

Method Validation

The proposed method was validated as per ICH guidelines. The parameters studied for validation were specificity, linearity, precision, accuracy, robustness, system suitability, limit of detection, limit of quantification, and stress degradation studies.

Specificity

The specificity of method was performed by comparing the chromatograms of standard (Figure 6) blank (Figure 7) and sample solutions (Figure 8). It was found that there is no interference due to excipients in the formulation and also found good correlation between the retention times of standard and sample. Sharp peak was obtained for Tirzepatide at retention times of 1.0 min. This peak was not detected in the blank solution. Retention times of the drugs in standard solutions, in the mixed standard solutions and in the sample solutions were the same. This result indicated specificity of the method. Furthermore, there was no interference from the excipients present in the tablets; thus, the method was considered specific and selectivity.

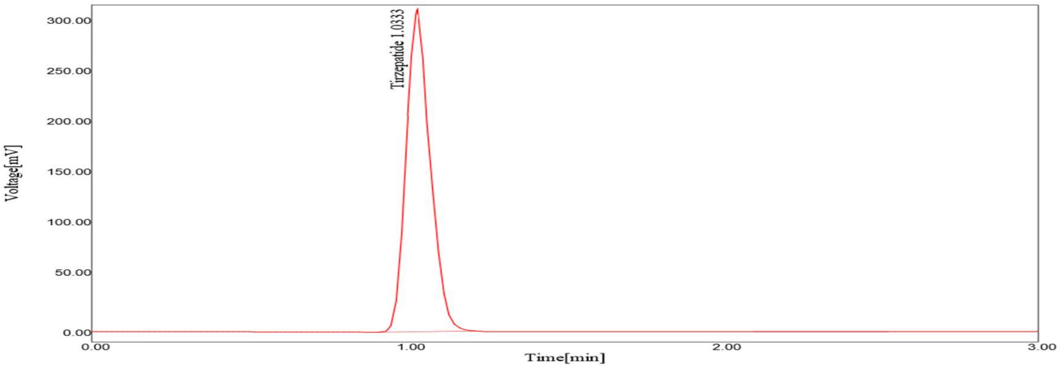


Fig 6: Chromatogram of Standard in the optimized conditions

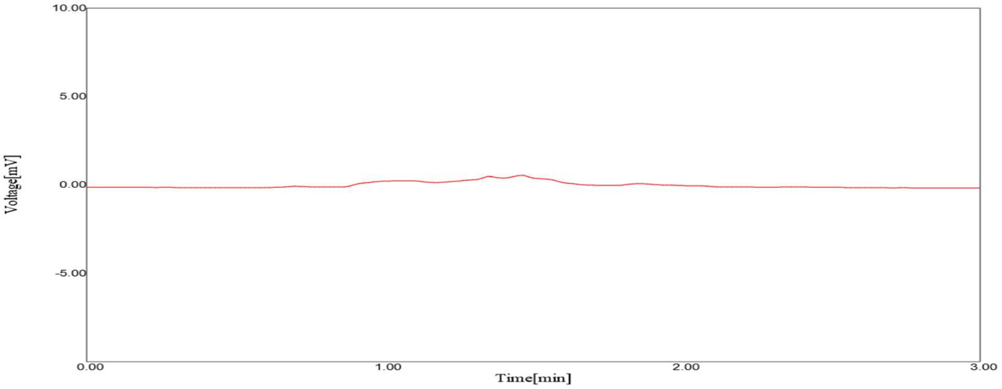


Fig 7: Chromatogram of Blank in the optimized conditions

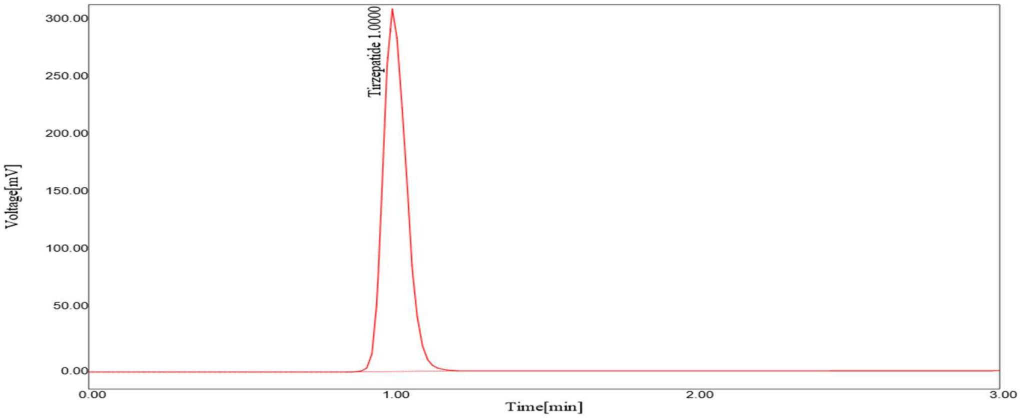


Fig 8: Chromatogram of Sample in the optimized conditions

System suitability

In the optimized conditions, peak tailing factors of was found to be 0.96 for Tirzepatide whereas the number of theoretical plates was found to be 4908. This method met the accepted requirements. Table 3 shows the results of the system suitability results of the proposed method.

Table 3: system suitability results of Tirzepatide

S. No	Parameter	Results
1	Api Concentration	10 µg/mL

2	RT	1.0 min
3	Resolution	Not applicable
4	Area	267892.2
5	Theoretical Plates	4908
6	Tailing Factor	0.96

Linearity and Range

Linearity was performed by preparing mixed standard solutions of Tirzepatide at different concentration levels including working concentration mentioned in experimental condition i.e.10 µg/ml. Twenty micro liters of each concentration was injected in duplicate into the UPLC system. The response was read at 245 nm and the corresponding chromatograms were recorded. From these chromatograms, the mean peak areas were calculated and linearity plots of concentration over the mean peak areas were constructed individually. Linearity was observed in the concentration range of 5-17.5 µg/ml for Tirzepatide with Linear regression equation of $y = 26756x - 5393.2$ ($R^2 = 0.9993$). The linearity results were given in table 4, linearity graph was given in figure 9 and linearity chromatograms were given in figure 10.

Table 4: Linearity results

S. No	Concentration in µg/ml	Peak Area
1	5	131323.8
2	7.5	191851.4
3	10	262256.4
4	12.5	330775.7
5	15	391196.2
6	17.5	466234.5

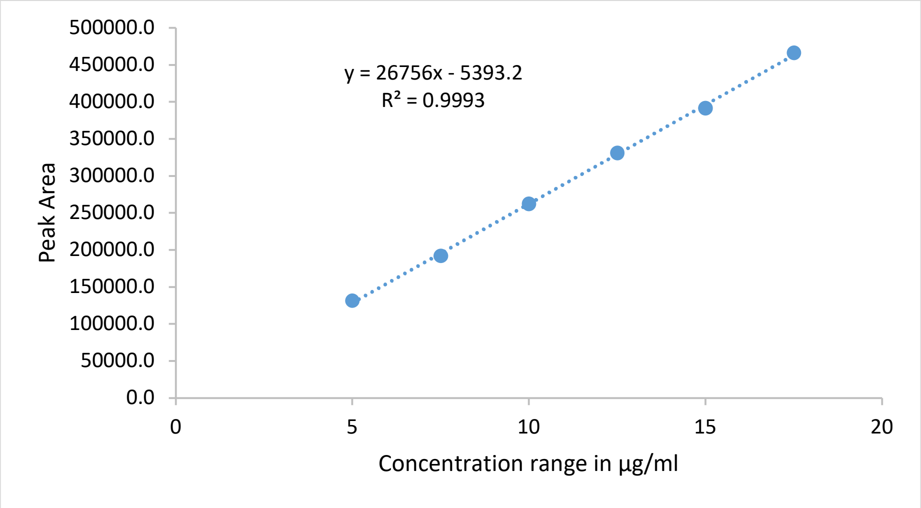
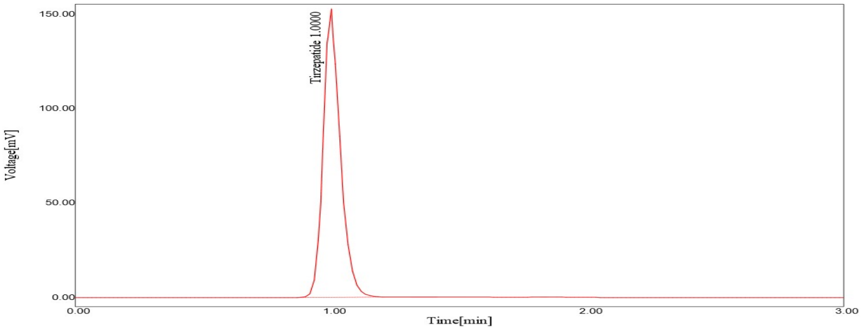
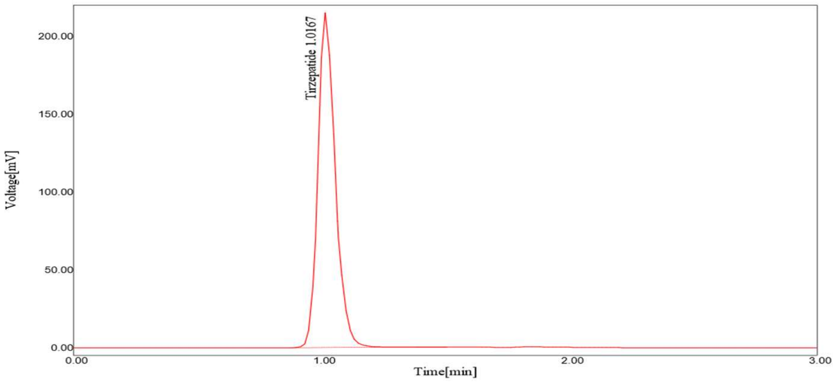


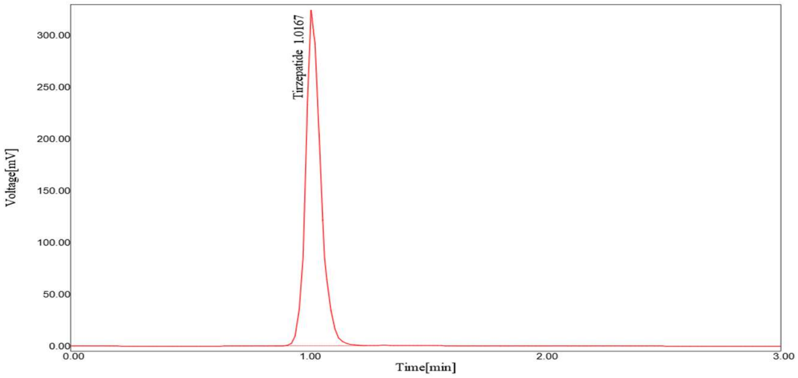
Fig 9: Linearity graph



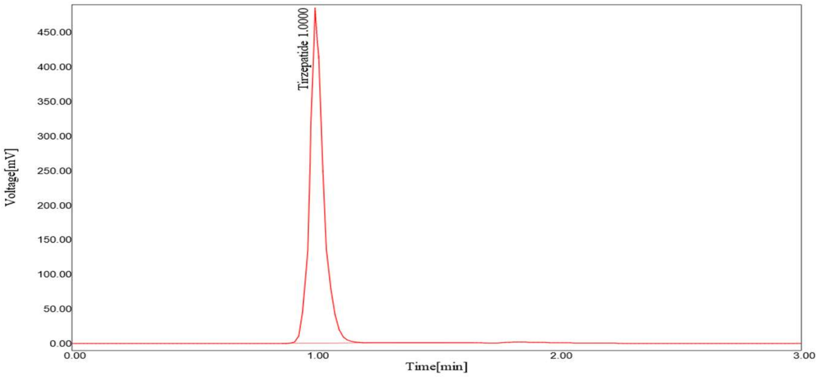
Linearity Level 1: 5 µg/mL chromatogram



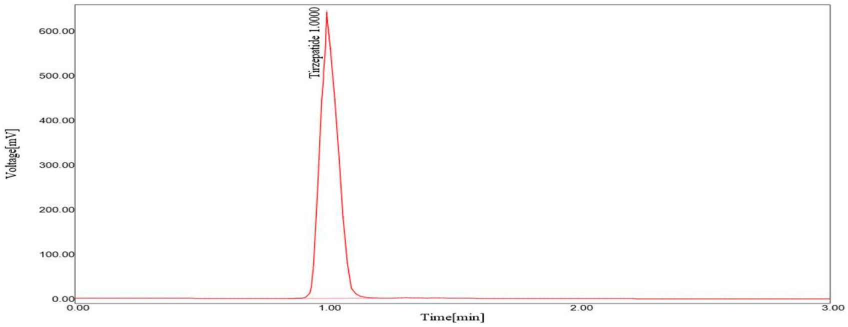
Linearity Level 2: 7.5 µg/mL chromatogram



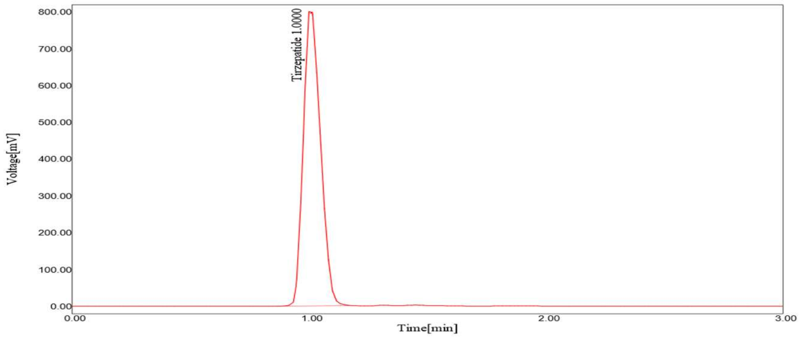
Linearity Level 3: 10 µg/mL chromatogram



Linearity Level 4: 12.5 µg/mL chromatogram



Linearity Level 5: 15 µg/mL chromatogram



Linearity Level 6: 17.5 µg/mL chromatogram

Fig 10: Linearity chromatograms of Tirzepatide in the developed method

CONCLUSION

An efficient and simple UPLC method has been developed and validated for the determination of Tirzepatide in bulk and was applied on marketed Tirzepatide products. The mobile phase used for the chromatographic runs consisted of Methanol: acetonitrile: 0.1 % sodium dihydrogen orthophosphate in 70:10:20 (v/v). The separation was achieved on an Acquity UPLC BEH C18 (50mm × 2.1 mm; 1.7µm) column using isocratic mode. Drug peak was well separated and were detected by a UV detector at 245 nm. The method was linear at the concentration range of 5–17.5 µg/ml for Tirzepatide. The method has been validated according to ICH guidelines with respect to system suitability, specificity, precision, accuracy and robustness. LOD and LOQ were found to be 0.01 µg/mL and 0.102 µg/mL for Tirzepatide. Results confirmed that the method was sensitive and can be useful for the detection and analysis of drugs at very lowest concentrations. The method can effectively separate the forced degradation products formed during the stress study confirms the stability indicating nature of the method. Hence it the method reported in the present study was used for the separation and quantification of Tirzepatide in bulk drug, formulations as well as stability testing.

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