



A REVIEW ON METHOD DEVELOPMENT AND VALIDATION OF SELECTED ANTI HYPERTENSIVE DRUG TIRZEPATIDE BY USING DIFFERENT ANALYTICAL TECHNIQUES

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ABSTRACT

Tirzepatide is a novel dual glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) receptor agonist used primarily for the management of type 2 diabetes mellitus and obesity. It mimics the actions of GIP and GLP-1, thereby enhancing glucose-dependent insulin secretion and reducing glucagon secretion. Tirzepatide also delays gastric emptying and promotes satiety, contributing to improved glycemic control and body-weight reduction. Analytical method development is essential for the accurate identification, quantification, purity assessment, and quality control of tirzepatide in pharmaceutical formulations. Various analytical techniques, including UV-visible spectroscopy, HPLC, RP-HPLC, HPTLC, LC-MS, and other chromatographic methods, can be employed for its estimation. Method validation is performed to ensure the reliability and consistency of the developed analytical procedures. Important validation parameters include accuracy, precision, specificity, linearity, robustness, range, limit of detection, and limit of quantification. This review focuses on different analytical methods reported for the estimation of tirzepatide and compares their analytical performance and applicability. The review also highlights the importance of validated analytical methods for routine quality control and pharmaceutical analysis of tirzepatide.

KEYWORDS: Tirzepatide, GIP receptor, Dual agonist, Analytical method development, Method validation, RP-HPLC, LC-MS, Pharmaceutical analysis.

INTRODUCTION

Analytical method development

Analytical method development is the process of designing a reliable procedure for identifying and quantifying an analyte in a sample. It involves selecting a suitable analytical technique based on the properties of the analyte and sample matrix. Various conditions such as mobile phase, pH, flow rate, temperature, and

detection wavelength are optimized. The developed method is evaluated for parameters such as accuracy, precision, specificity, linearity, and robustness. Finally, the method is validated and documented to ensure it is suitable for its intended purpose.

Criteria for the development of new analytical method

The development of a new analytical method should be based on the purpose of analysis, nature of the analyte, sample matrix, and required sensitivity. The method should provide good specificity, accuracy, precision, linearity, and sensitivity. Suitable analytical techniques and experimental conditions should be selected according to the physical and chemical properties of the analyte.

A good analytical method should be simple, rapid, economical, reproducible, and robust for routine use. It should require minimum sample preparation and provide reliable results under normal laboratory conditions. Finally, the method should be validated to demonstrate that it is suitable and reliable for its intended purpose.

Steps for analytical method development

The procedure for analytical method development follows a set of steps as below:

Purpose of Analytical Method

Development In the pharmaceutical industry, analytical method development gives important information on the potency of a drug, the drugs' bioavailability, the drugs' stability and also its effects. In the very first step, the purpose of conducting any Analytical Method Development is established.

Highlighting of Steps

In the second step of Analytical Method Development, the steps involved in the development are recorded in a laboratory book.

Characterization of the Analyte

In this step, both the biological and chemical properties in addition to the physical properties of the analyte are collected. After that, the analyte is obtained and stored according to its specific requirements. The methods for analysis are then recorded with an example being the chromatography technique which employs different methods such as the High-Performance Liquid Chromatography.

Definition of Requirements

Requirements for the method development of the analysis are done and recorded. All the materials, reagents and instruments are procured those are required for the analysis of the sample.

Review of Literature and Previous Methods

All literature information related to the specific analyte e.g. a specific drug is assessed for any biological, chemical and chemical properties regarding the analyte. Reference is then made from journals, books and any other publications.

Choosing an Analytical Method

From the information obtained from the literature during the literature review, a specific methodology is modified to cater for accurate output and also because methods change with the requirements of the analyte. If there are no previous methods in the literature being reviewed regarding the analyte, the procedure goes on uninterrupted.

Setting up of Instruments

Appropriate instruments for the analytical method development are set up in the laboratory by each of the instruments standard operating procedures. Standard Operating Procedures usually abbreviated as SOP's are a set of instructions or steps to aid in performing a specific procedure in a laboratory set up. They are usually universal and standardized for ease of use in any laboratory set up.

Optimization of the Method

In carrying out the optimization of the analytical method, parameters are changed individually depending on the arising interests. Optimization of an analytical method is done in reference to a systematic and procedural plan while making sure to critically follow all the documented steps.

Analytical Figures of Merit Documentation

Documentation of the analytical figures of merit decided upon is done. These analytical figures of merit include quantification limits, detection limits, analysis time frame, operational costs and sample preparation.

Development Method Evaluation

The resultant product of analysis should give a desirable result as expected in the identification of the analyte.

Sample Estimation, Quantitative Demonstration and Analysis of Samples

Estimation of an analyte with an example being a drug in a matrix sample containing the analyte is done here.

Analytical method validation

It establishes documented evidence that the procedure adapted for a test is fit for the intended purpose in terms of quality, reliability, and consistency of results.

Parameters to be checked for method validation**1. Selectivity/specificity**

Specificity is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present. The terms selectivity and specificity are often used interchangeably. According to ICH the term specific generally refers to a method that produces a response for a single analyte only while the term selectivity refers to a method that provides responses for a number of chemical entities that may or may not be distinguished from each other.

2. Precision

ICH defines the precision of an analytical procedure as the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision is the measure of how close the data values are to each other for several measurements under the same analytical conditions.

3. Accuracy

The accuracy of an analytical method is the degree of agreement of test results generated by the method to the true value.

Accuracy is measured by spiking the sample matrix of interest with a known concentration of analyte standard and analysing the sample using the “method being validated.” The procedure and calculation for Accuracy (as% recovery) will be varied from matrix to matrix and it will be given in respective study plan or amendment to the study plan.

4. Linearity

The linearity of an analytical method is its capability to elicit check consequences which might be at once, or with the aid of well described mathematical adjustments, proportional to the concentration of analytes in within a given range.

Linearity is determined by injecting a series of standards of stock solution/diluted stock solution using the solvent/mobile phase, at a minimum of five different concentrations in the range of 50–150% of the expected working range. The linearity graph will be plotted manually/using Microsoft Excel or software of the computer (Concentration vs. Peak Area Response) and which will be attached to respective study files.

5. Range

The range of an analytical method is the interval between the upper and lower levels that have been demonstrated to be determined with precision, accuracy and linearity using the set method. This range will be the concentration range in which the Linearity test is done.

6. Limit of detection and limit of quantitation

The limits of detection (LOD) and quantification (LOQ) are defined as the lowest concentration of the analyte that can be reliably detected and quantified, respectively. Usually, the LOD and LOQ refer to the limits associated with 95% probability of obtaining a correct result Limit of detection.

Different Analytical techniques

Liquid chromatography-mass spectrometry (LC–MS)

LC–MS stands for Liquid Chromatography–Mass Spectrometry and is an advanced instrument used to analyze different substances present in a sample. In this technique, liquid chromatography first separates the compounds, and mass spectrometry then detects and identifies them according to their mass-to-charge ratio. It is capable of detecting even very small amounts of substances with high accuracy. LC–MS is known for its good sensitivity, selectivity, and reliability. It is commonly used in pharmaceutical research and drug analysis. It can also be used to detect impurities, metabolites, and other chemical compounds. The technique is useful for both identification and measurement of substances. It is widely applied in pharmaceutical, biological, clinical, environmental, and food laboratories. LC–MS is particularly useful for analyzing complex samples containing many different compounds. Therefore, it is an important and widely used technique in modern analytical science.

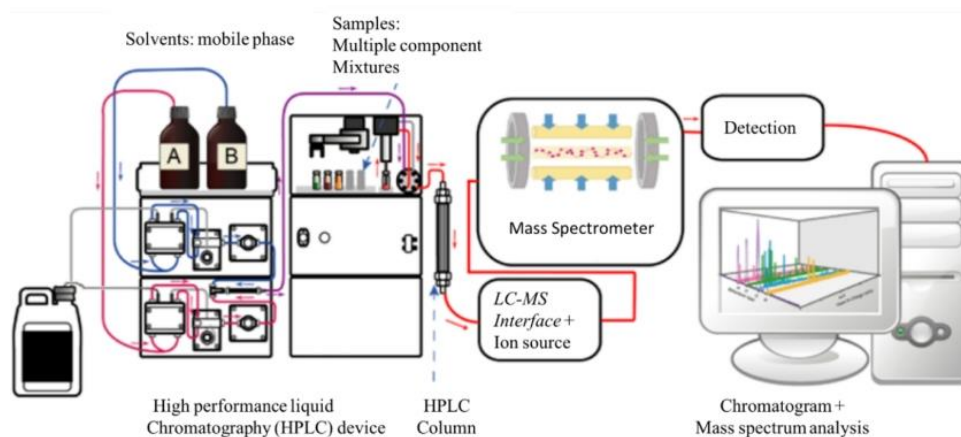


Fig.1: LC-MS Instrumentation.

Ultra Performance Liquid Chromatography

UPLC stands for Ultra Performance Liquid Chromatography, a technique used in liquid chromatography analysis. It's an improvement on HPLC (high-performance liquid chromatography) and offers better resolution and sensitivity, shorter run times, and the ability to analyse more samples in less time. Ultra-performance liquid chromatography (UPLC), developed in 2004 by Waters, is a modern liquid chromatographic system, which is simply the modification of HPLC.

The smaller particle size of sorbent (diameter less than 2 μm) in UPLC offers better resolution, speed, and sensitivity compared with HPLC. The basic principle of UPLC for the separation of components in a matrix is

same as HPLC, the main difference is in the particle size of sorbent of the column, which is less than 2 μm . The small particles in UPLC require a high pressure (6000 psi) to work with. A significant decrease in the particle size of stationary phase below 2.5 μm provides a remarkable increase in the effectiveness of the column, which does not reduce on increasing the linear speed or rate of flow. The use of speed, particles with a small radius, and maximum number of resolvable peaks (peak capacity) comprehends the efficiency together with resolution. UPLC cuts the mobile phase volume consumption by at least 80% compared to HPLC with a very short runtime (even less than 1 min).

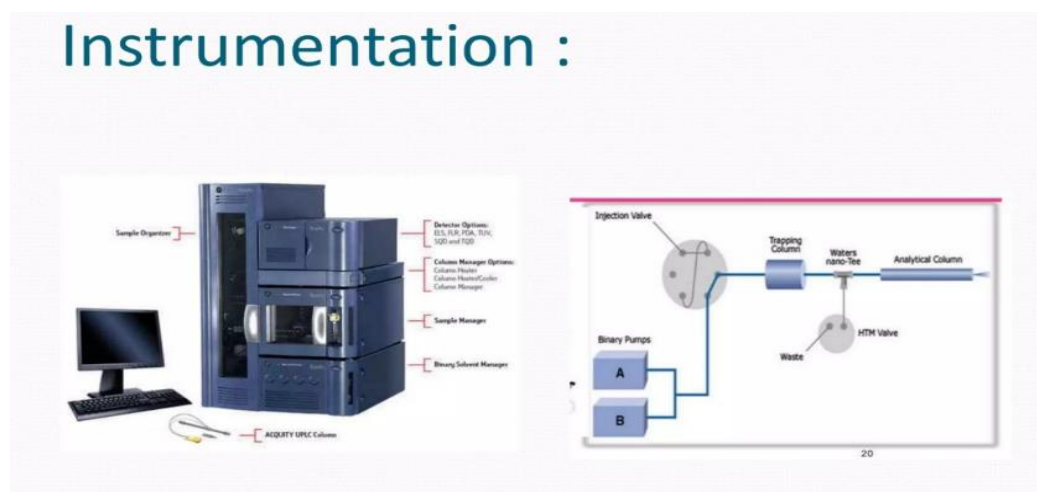


Fig. 2: UPLC Instrumentation.

High-Performance Liquid Chromatography

HPLC, or High-Performance Liquid Chromatography, is a widely used analytical technique for separating, identifying, and quantifying different components present in a mixture. In HPLC, a liquid mobile phase carries the sample through a column packed with a stationary phase. Different compounds move through the column at different rates and are separated based on their chemical properties. The separated compounds are detected using a suitable detector, such as a UV detector.

HPLC provides accurate, precise, and reliable results and can analyze very small quantities of substances. It is widely used in pharmaceutical analysis, quality control, clinical research, food analysis, and environmental testing. HPLC is commonly used to determine the purity, assay, and stability of drugs. It can also be used for the detection of impurities and degradation products. Because of its high sensitivity and versatility, HPLC is an important technique in modern analytical laboratories.

High Performance Liquid Chromatography (HPLC)

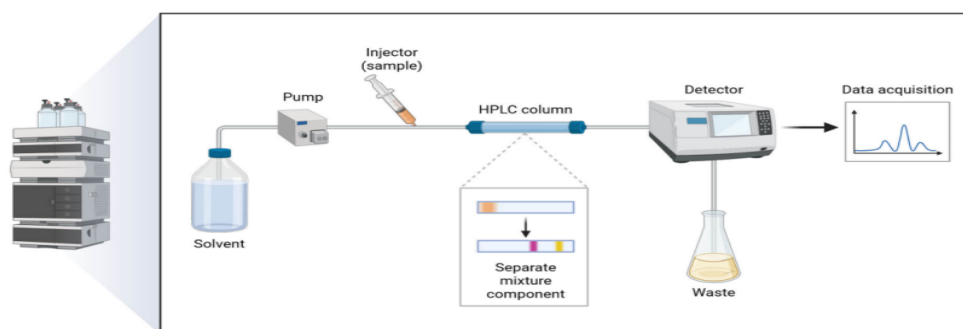


Fig. 3: HPLC Instrumentation.

Gas chromatography

Gas Chromatography (GC) is an analytical technique used to separate, identify, and quantify compounds that can be converted into a gas or vapor. In GC, the sample is vaporized and carried through a column by an inert carrier gas such as helium or nitrogen. Different compounds move through the column at different rates and are separated based on their properties and interaction with the stationary phase. A detector then

detects the separated compounds and produces a chromatogram. GC provides accurate, sensitive, and reproducible results. It is commonly used for analyzing volatile and thermally stable substances. GC is widely applied in pharmaceutical, environmental, food, forensic, and chemical analysis. It is useful for identifying impurities, solvents, and other volatile compounds.

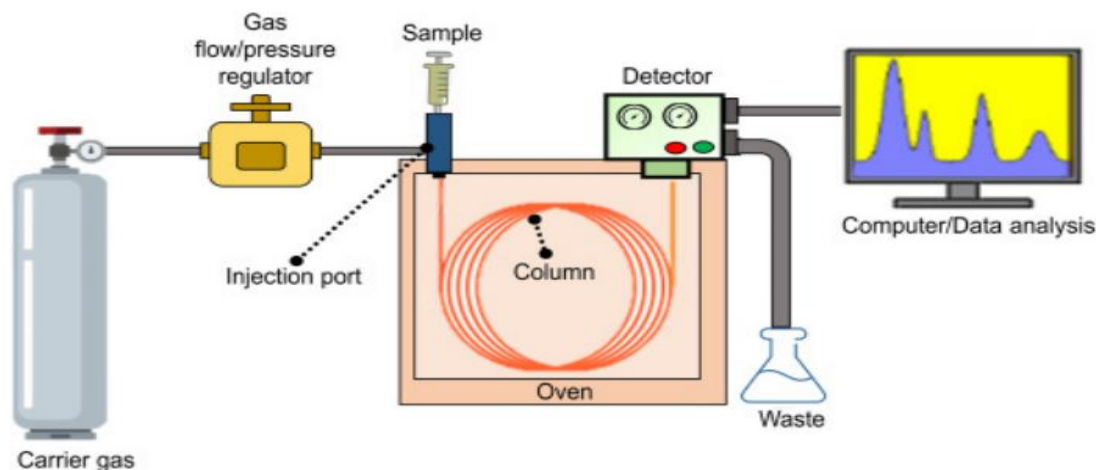


Fig. 4: Gas Chromatography Instrumentation.

UV Spectroscopy

Spectroscopy is the measurement and interpretation of electromagnetic radiation absorbed or emitted when the molecules or atoms or ions of a sample move from one energy state to another energy state. UV spectroscopy is a type of absorption spectroscopy in which light of the ultra-violet region (200-400 nm) is absorbed by the molecule which results in the excitation of the electrons from the ground state to a higher energy state.

UV Vis spectroscopy is a scientific technique used to measure the amount of light that is absorbed or transmitted by a sample at different wavelengths of ultraviolet (UV) and visible (Vis) light.

The process involves shining a beam of UV Vis light through the sample and measuring the amount of light that passes through it. By analysing the pattern of absorption and transmission of light, scientists can identify and quantify the components of the sample.

A unique relationship exists between the substance and its UV Vis spectrum when a substance absorbs the maximum light at a specific wavelength. This relationship can be used for:

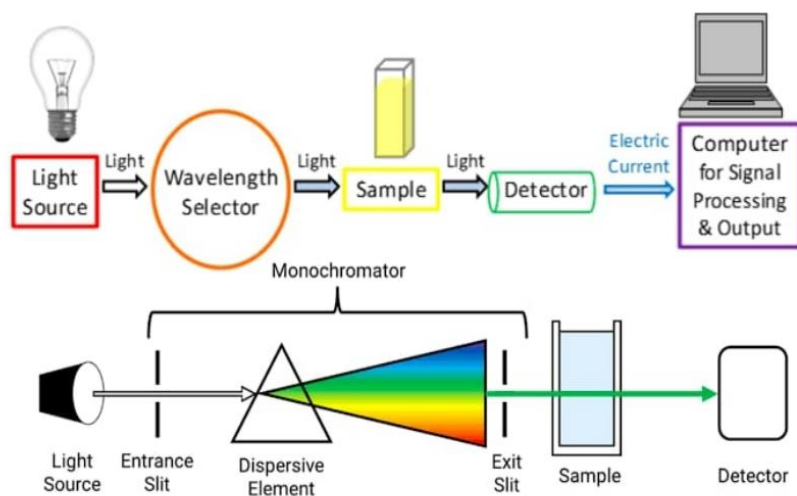
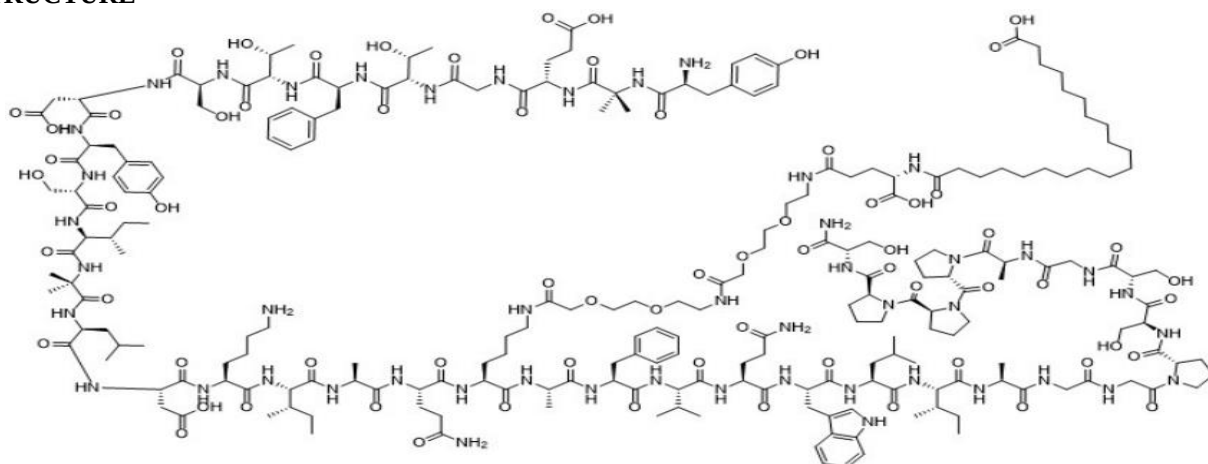


Fig. 5: UV Spectrophotometer Instrumentation.

DRUG PROFILE**TIRZEPATIDE****CHEMICAL FORMULA:** C₂₂₅H₃₄₈N₄₈O₆₈**MOLECULAR MASS:** 4810.52**STRUCTURE****Fig. 6: Structure of Tirzepatide.****PHARMACODYNAMICS**

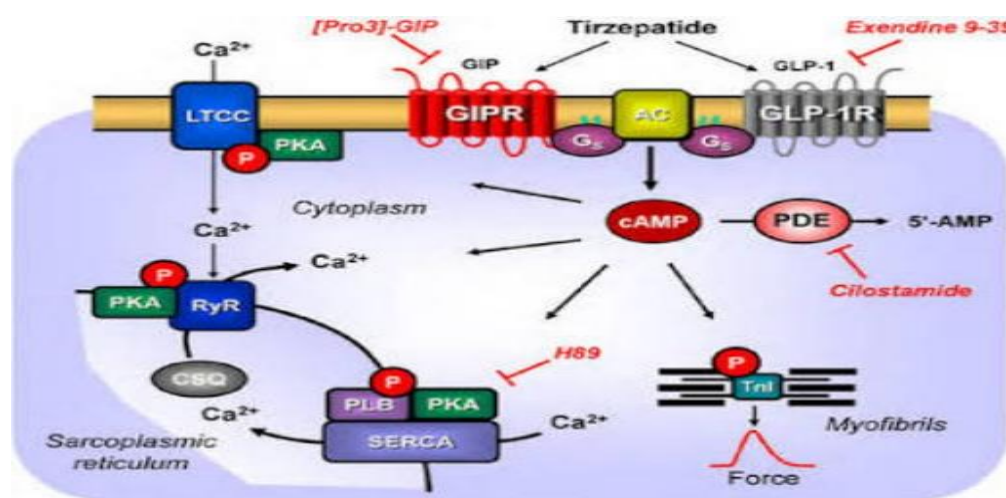
Tirzepatide is administered subcutaneously once weekly, with or without meals. It is a dual GIP and GLP-1 receptor agonist that enhances glucose-dependent insulin secretion, suppresses glucagon secretion, delays gastric emptying, and reduces appetite and food intake. These effects improve glycemic control and promote weight loss.

MECHANISM OF ACTION

Glucagon-like peptide-1 (GLP-1) receptors (GLP-1R) are expressed throughout the body, including pancreatic beta-cells and the gastrointestinal tract. They have been implicated in the pathophysiology of type II diabetes mellitus as GLP-1R signalling is involved in glucose control by enhancing glucose-stimulated insulin secretion, delaying gastric transit, decreasing plasma glucagon levels, and reducing body weight by activating anorexigenic pathways in the brain.

Both glucose-dependent insulinotropic polypeptide (GIP) and GLP-1 are peptide hormones involved in glucose homeostasis: they promote glucose-stimulated insulin secretion from the pancreatic beta-cells.

The exact mechanism of action of tirzepatide has not been fully elucidated; however, dual agonism at GIP and GLP-1R may contribute to the glycemic and weight control effects of the drug. Studies demonstrated that co-administration of GIP and a GLP-1R agonist more significantly increased insulin response and suppressed glucagon secretion compared to separate administration of either hormone alone. Tirzepatide binds to GIP and GLP-1R with high affinity. In vitro, tirzepatide has a comparable GIP receptor binding affinity to native GIP and five times lower GLP-1R affinity than that of native GLP-1.

**Fig 7: Tirzepatide mechanism of action.**

VOLUME OF DISTRIBUTION: The mean apparent volume of distribution of tirzepatide is 9.7 L at steady state in patients with overweight/obesity; about 10.3 L in patients with type 2 diabetes mellitus

PROTEIN BINDING

99% of tirzepatide bound to plasma albumin.

METABOLISM

Tirzepatide is metabolized mainly through proteolytic cleavage of its peptide backbone. Its structure also undergoes β -oxidation of the C20 fatty-diacid side chain and amide hydrolysis. These metabolic processes produce smaller, inactive metabolites. Unchanged tirzepatide is not detected in urine or feces. The resulting metabolites are eliminated primarily through urine and feces.

ROUTE OF ELIMINATION

Following administration of radiolabelled tirzepatide, the metabolites are eliminated through both feces and urine. Unchanged tirzepatide is not detected in either feces or urine, indicating that elimination occurs primarily after metabolism. The drug undergoes proteolytic degradation, β -oxidation, and amide hydrolysis before excretion. The resulting metabolites are eliminated via the renal and fecal routes.

HALF-LIFE: The mean effective half-life of tirzepatide.

Table No: 1- Different analytical techniques of Tucatinib.

S.NO	DRUG NAME	ANALYTICAL TECHNIQUE	DESCRIPTION OF TECHNIQUE	AUTHOR & YEAR
1	Tirzepatide	LC-MS/MS	Column: Acquity UPLC Peptide CSH C18 (2.1 x 50 mm 1.7 μ m). Mobile phase: Water+ 0.1%Formic acid& acetonitrile+0.1%Formic acid Flow rate: 0.6 ml/min. Run time: 03min. Linearity :1-1000 μ g/ml Injection volume:10 μ l	Hae-In Choi et al.,(2026)
2	Tirzepatide	HPLC	Column: C18, (250 x 4.6mm,1.8 μ m) Mobile phase: Methanol:0.1N citric acid (50:50 v/v) Flow rate: 0.9 ml/min Linearity: 5-25 μ g/ml LOQ: 0.104 μ g/ml LOD: 0.034 μ gm/ml Injection volume:20 μ l Wavelength: 250nm	Dr. K. Patil et al., (2026)
3	Tirzepatide	RP-HPLC	Column: Phenomenex kinetex XB-C8 (150mm x4.6mm,5 μ m) Flow rate: 1 ml/min. Mobile phase: Phosphate buffer: acetonitrile (58:42 v/v) Linearity :>0.999 μ g/ml Wavelength: 220nm	Safeer Sikander Hawa et al., (2026)
4	Tirzepatide	UPLC	Column: Phenomenex C18(50 x 1.7mm,2 μ m) Mobile phase: Acetonitrile: trifluoroacetic acid buffer (37:7:63 v/v) Flow rate: 0.57 ml/min. LOQ: 2 μ g/ml LOD:0.6 μ gm/ml Linearity:12.5-75 μ g/ml Wavelength: 264nm	Panthagada Sunitha et al., (2026)
5	Tirzepatide	RP-HPLC	Column: Eclipse XDB C18, (150 x4.4mm, 3.5 μ m)	Mendi Tarun et at., (2025)

			Mobile phase: Acetonitrile: triethylamine buffer (30:70 v/v) Flow rate: 1ml/min. LOQ:2.00 µg/ml LOD:0.60 µgm/ml Linearity:25-150 µg/ml Wavelength: 234nm Injection volume:10 µl	
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Table No 2: BRAND NAMES.

S.NO	BRAND NAME	API	MANUFACTURING COMPANY
1	Mounjaro®	Tirzepatide	Eli Lilly and Company
2	Zepbound®	Tirzepatide	Eli Lilly and Company
3	Yurpeak®	Tirzepatide	Cipla Ltd.
4	Lucitirze	Tirzepatide	Lucius
5	Tirzee	Tirzepatide	GetZ Pharma
6	Tizaro	Tirzepatide	Indian Private Ltd
7	Tirzix	Tirzepatide	Beacon Pharmaceuticals

CONCLUSION

Provide a comprehensive overview of analytical methods reported for tirzepatide estimation. Present a systematic comparison of chromatographic, mass-spectrometric, and other applicable analytical techniques. Establish how different method-development parameters influence analytical performance. Summarize the reported validation results for parameters such as linearity, accuracy, precision, specificity, LOD, LOQ, and robustness. Identify the strengths and limitations of currently reported analytical methods. Identify gaps in the existing literature that may provide opportunities for future method development. Provide a useful reference for selecting or developing an appropriate analytical method for the estimation of tirzepatide in pharmaceutical formulations and relevant samples.

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CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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