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TIRZEPATIDE – AN OVERVIEW ON ITS ANALYTICAL TECHNIQUES

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ABSTRACT

Tripeptide has emerged as a promising therapeutic agent for the management of type 2 diabetes and obesity, functioning as a novel dual agonist of the glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) receptors. Due to its structural complexity as a peptide-based biologic, robust and precise analytical methodologies are crucial for its characterization, quantification, and stability evaluation. This review highlights the key analytical techniques employed in the development and clinical application of Tripeptide, with a focus on Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) and Ultra Performance Liquid Chromatography (UPLC). In addition, it discusses current challenges and recent advancements in the validation of these analytical methods to ensure reliability and regulatory compliance.

KEYWORDS: Tirzepatide, T2DM, Analytical techniques, RP-HPLC, UPLC, Stability studies.

1. INTRODUCTION

The pharmaceutical field is expanding at an incredible pace as new drugs and new analytical techniques are being developed day-to-day. This leads to the development of various optimized analytical techniques that are being accepted world-wide. Analytical technique is a fundamental scientific phenomenon that has proved useful for providing information on the composition of substance.[1]

A wide range of analytical techniques can be employed for both qualitative and quantitative analysis. For instance, the spectrophotometric method is one of the methods that can be used to acquire accurate results. From basic qualitative chemical testing to the employment of the most advanced and costly computer-controlled instruments, modern analytical techniques use a variety of methods. The development and assessment of new products heavily relies on analytical equipment. The lower limits of detection needed to ensure safety are provided by this equipment.[2]

1.2 DIABETES AND ITS COMPLICATIONS

In affluent countries, chronic illnesses such as diabetes and obesity exhibit elevated mortality rates and contribute significantly to global morbidity. These two conditions are often described as the most consequential epidemics of the 21st century. They are complex health issues influenced by a multifaceted interplay of genetic, environmental, behavioral, socioeconomic, and epigenetic factors.[3, 4] Chronic hyperglycemia—a hallmark of diabetes—can arise from inadequate insulin secretion, impaired insulin action, or a combination of both. Insulin, a crucial anabolic hormone, plays a key role in the regulation of protein, lipid, and carbohydrate metabolism.

1.3 CLASSIFICATION OF DIABETES MELLITUS

Diabetes mellitus is commonly classified into several major types. Type 1 diabetes (T1D) is primarily an autoimmune condition that results in the destruction of pancreatic β-cells, leading to absolute insulin deficiency. Subtypes of T1D include *idiopathic type 1 diabetes*, which lacks clear autoimmune markers or identifiable causes, and *fulminant type 1 diabetes*, a rapidly progressing form predominantly seen in East Asian populations, marked by sudden onset and near-complete β-cell destruction at diagnosis. Type 2 diabetes (T2D), the most prevalent form, arises due to insulin resistance combined with a relative insulin deficiency and is closely associated with obesity, physical inactivity, and genetic predisposition. Gestational diabetes mellitus (GDM) is another form that manifests as glucose intolerance during pregnancy, posing health risks for both the mother and child and increasing the long-term risk of type 2 diabetes in affected women. Additionally, hybrid forms of diabetes exhibit overlapping features of both type 1 and type 2 diabetes. These include *slowly evolving immune-mediated diabetes*, also known as latent autoimmune diabetes in adults (LADA), which progresses gradually and involves autoimmunity, and *ketosis-prone type 2 diabetes*, characterized by episodic ketoacidosis in the

absence of autoantibodies, often accompanied by insulin resistance. Understanding these varied forms is essential for accurate diagnosis, management, and prevention strategies tailored to individual patient profiles.

1.4 COMPLICATIONS ASSOCIATED WITH DM

Type 2 diabetes and obesity are not only metabolic disorders but also key contributors to the development of various chronic complications that significantly impact overall morbidity and mortality. Among these, **liver cirrhosis**, particularly non-alcoholic steatohepatitis (NASH)-related cirrhosis, has emerged as a growing concern due to the strong association between insulin resistance and hepatic fat accumulation. **Cardiovascular disease (CVD)** remains the leading cause of death among individuals with type 2 diabetes, driven by a combination of hyperglycemia, dyslipidemia, hypertension, and chronic inflammation. Additionally, **diabetic kidney disease (DKD)**, also known as diabetic nephropathy, is a major microvascular complication and a leading cause of end-stage renal disease worldwide. **Diabetic retinopathy**, another significant microvascular complication, is a leading cause of vision impairment and blindness among working-age adults. These interrelated conditions underscore the systemic nature of diabetes and highlight the importance of early detection, comprehensive management, and targeted therapies to mitigate long-term complications.

1.5 TIRZEPATIDE

A novel molecule with dual agonist activity on both the glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) receptors—termed a "twincretin"—has been developed to leverage the synergistic metabolic benefits of these incretin hormones. **Tirzepatide (LY3298176)** is the first twincretin, a synthetic peptide composed of 39 amino acids based on the native GIP sequence, combining dual agonism at both GIP and GLP-1 receptors to improve glycemic control and promote weight loss.[6–10] Tirzepatide demonstrates an affinity for the GIP receptor equivalent to that of native GIP, while its affinity for the GLP-1 receptor is approximately five times lower than that of native GLP-1.[11,12] Beyond its metabolic effects, tirzepatide also contributes to improvements in cardiovascular risk factors, including reductions in blood pressure, waist circumference, low-density lipoprotein (LDL) cholesterol, and circulating triglycerides.[13–17] The efficacy and safety of tirzepatide have been established in several large-scale clinical trials, reinforcing its potential as a transformative therapy for type 2 diabetes and obesity. (Figure 1).

1.6 ANALYTICAL TECHNIQUES

Drug estimation encompasses a range of chemical and instrumental techniques developed over time to ensure that pharmaceutical products perform their intended therapeutic functions effectively and safely. Throughout the stages of manufacturing, storage, and transportation, pharmaceutical compounds are susceptible to contamination or degradation, which may compromise their safety and efficacy. Therefore, the identification and quantification of both active ingredients and potential impurities are essential. In this context, analytical tools play a vital role in maintaining quality control and regulatory compliance.[18] A variety of techniques are employed in pharmaceutical analysis, including **titrimetric methods**, which are among the earliest approaches, and more advanced **chromatographic techniques** such as thin-layer chromatography (TLC), high-performance thin-layer chromatography (HPTLC), high-performance liquid chromatography (HPLC), and gas chromatography (GC). **Spectroscopic techniques**—including spectrophotometry, near-infrared spectroscopy (NIRS), nuclear magnetic resonance (NMR) spectroscopy, fluorimetry, and phosphorimetry—are widely used for structural elucidation and quantitative analysis. Additionally, **electrochemical methods**, the **kinetic method of analysis**, and **electrophoretic techniques** offer specific applications in trace analysis and biomolecular separations. Modern advancements have introduced **flow injection** and **sequential injection analysis**, as well as **hyphenated techniques**, which integrate multiple analytical methods (e.g., LC-MS, GC-MS) to enhance sensitivity and selectivity. Together, these techniques form a comprehensive toolkit for ensuring drug safety, efficacy, and quality.

2. MATERIALS AND METHODS

2.1 REPORTED ANALYTICAL METHOD OF TIRZEPATIDE USING UPLC[2]

Chandana Mannepalli et al. – A Simple Stability-Indicating UPLC Method for Quantification of Tirzepatide in Bulk Drug and Pharmaceutical Formulations.

Chandana Mannepalli and colleagues developed a stability-indicating Ultra Performance Liquid Chromatography (UPLC) method for the quantification of tirzepatide in both bulk drug and pharmaceutical formulations. The method was validated for accuracy, precision, and stability, providing an efficient and reliable means of quantifying tirzepatide while ensuring its stability under various conditions. The optimized UPLC conditions and their corresponding parameters in **Table 1** were specifically designed to meet the regulatory requirements for drug analysis, ensuring the robustness of the method across different formulations. (Figure 2 & Figure 3).

2.2 REPORTED ANALYTICAL METHOD OF TIRZEPATIDE USING RP-HPLC[19]

VM Goud et al. – QbD Approach for the Analysis of Tirzepatide in Its Bulk and Marketed Formulation by a Stability-Indicating RP-HPLC Method.

VM Goud and colleagues utilized a **Quality by Design (QbD)** approach to develop a stability-indicating **Reverse Phase High-Performance Liquid Chromatography (RP-HPLC)** method for the analysis

of tirzepatide in both bulk drug and marketed formulations. The method was designed to be both precise and robust, ensuring reliable quantification of tirzepatide while evaluating its stability under various stress conditions. The QbD approach focused on optimizing key chromatographic parameters to improve method performance and ensure the reproducibility of results across different batches.

The analytical method was validated according to standard guidelines, including testing for accuracy, precision, specificity, and stability, making it suitable for routine quality control in pharmaceutical manufacturing. **Table 2** outlines the key parameters used in the RP-HPLC method, and **Figure 4** presents the chromatogram obtained using the optimized condition.

3. DISCUSSION

3.1 METHODS ESTABLISHED

For the analysis of Tirzepatide, two primary analytical techniques have been reported: Ultra Performance Liquid Chromatography (UPLC) by Chandana Mannepalli et al. and Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) by VM Goud et al.

In the UPLC method proposed by Chandana Mannepalli et al., the mobile phase consisted of a mixture of methanol, acetonitrile, and 0.1% sodium dihydrogen orthophosphate in a 70:10:20 (v/v) ratio. The retention time (RT) for Tirzepatide was found to be 3.00 minutes. In comparison, the RP-HPLC method developed by VM Goud et al. used 0.01N KH_2PO_4: Acetonitrile in a 41:59 (% v/v) ratio, resulting in a shorter RT of 2.841 minutes. Although both methods are effective, the UPLC technique shows a delayed RT relative to RP-HPLC.

Given that Tirzepatide is a peptide-based biologic, additional analytical methods may be employed for comprehensive analysis. Techniques such as Mass Spectrometry (MS), Enzyme-Linked Immunosorbent Assays (ELISA), Capillary Electrophoresis (CE), and bio-analytical techniques for Pharmacokinetics (PK) studies provide further insight into the drug's molecular structure, stability, and pharmacokinetic properties.

3.2 STABILITY TESTING OF TIRZEPATIDE

Stability testing is a critical aspect of ensuring the safety, efficacy, and shelf life of Tirzepatide. Methods like High-Performance Liquid Chromatography (HPLC) are commonly employed to monitor the stability of Tirzepatide in various formulations under different environmental conditions, such as temperature, humidity, and light exposure. HPLC-based methods are particularly effective in assessing the degradation and impurity profiles of the drug over time, which are crucial for evaluating its long-term stability.

These stability studies are not only important for maintaining the quality of the product but are also essential for meeting regulatory standards set by organizations like the FDA and EMA. In one study conducted by VM Goud et al., stability testing using RP-HPLC revealed that a 2N NaOH (base hydrolysis) test conducted over 8 hours resulted in a 8.73% degradation of Tirzepatide, which was higher compared to other stability tests. This finding highlights the importance of identifying conditions that can lead to significant degradation, thereby guiding optimal storage and handling practices for the drug.

4. CONCLUSIONS

Tirzepatide has shown promising therapeutic benefits in the management of type 2 diabetes and obesity, highlighting the importance of developing robust and reliable analytical methods to ensure its safety, efficacy, and quality. Current techniques, including RP-HPLC and UPLC, have proven effective and sensitive for detecting and analyzing Tirzepatide at low concentrations. However, no new analytical methods have been reported to date, signaling a need for further development in this area. The introduction of modern analytical techniques, particularly hyphenated methods, would enhance precision, reduce costs, and improve the overall analytical capability for Tirzepatide. While existing techniques are highly regarded within the pharmaceutical industry, ongoing research into innovative methods is essential to overcome challenges related to sensitivity, specificity, and matrix interference. The continued refinement of these analytical methods will be crucial for ensuring that Tirzepatide remains safe, effective, and of the highest quality for clinical use.

Table 1.

S.NO	CONDITIONS	PARAMETER
1.	Stationary phase	Acquity UPLC BEH C18 (50mm × 2.1 mm; 1.7µm)
2.	Mobile phase	Methanol: acetonitrile: 0.1 % sodium dihydrogen orthophosphate in 70:10:20 (v/v)
3.	pH	5.9
4.	Flow rate	0.3 mL/min
5.	Pump mode	Gradient
6.	Pump pressure	9.1±5 MPa
7.	Wavelength	245 nm
8.	Run time	3 min

Table 2.

S.NO	CONDITIONS	PARAMETER
1.	Stationary phase	BDS C18 (150x4.6 mm, 5m)
2.	Mobile phase	0.01N KH2PO4: Acetonitrile in the 41:59 (%v/v)
3.	Flow rate	0.9 mL/min
4.	Column temperature	31°C
5.	Wavelength	250 nm
6.	Run time	2.841 min

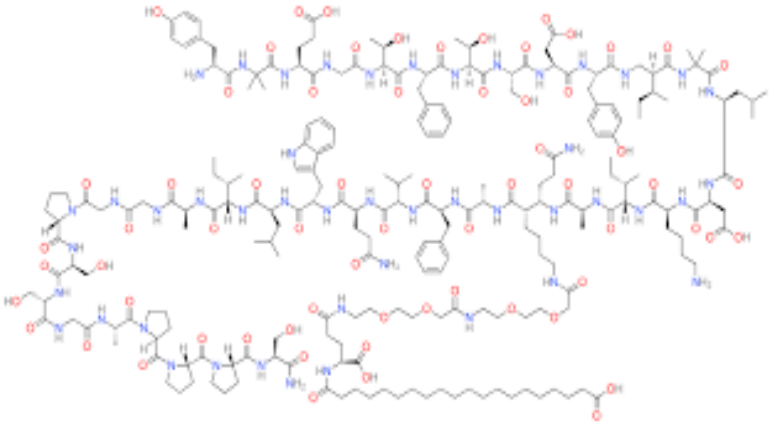


Figure 1: Structure of Tirzepatide.

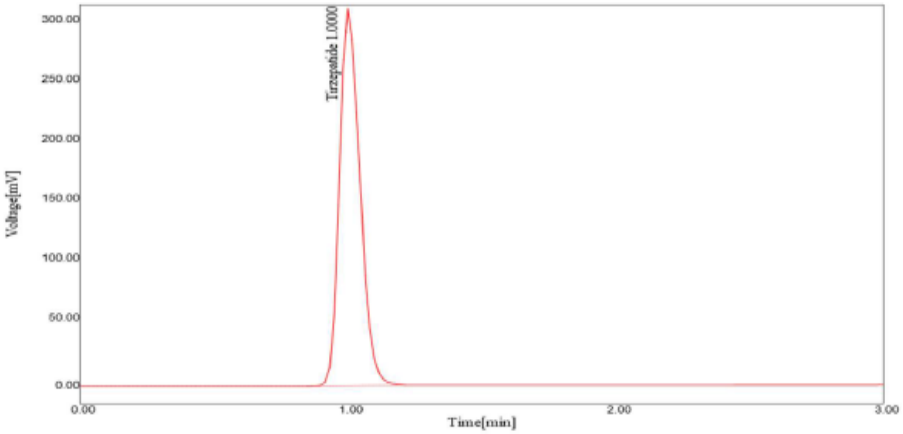


Figure 2: Chromatogram of sample in optimized condition.

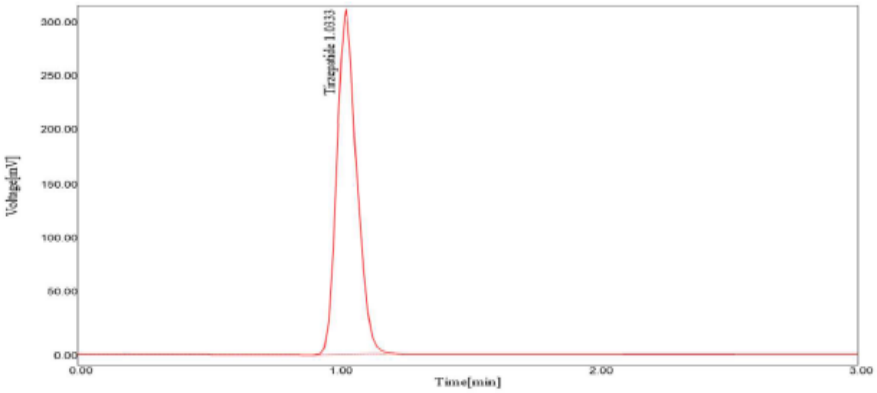
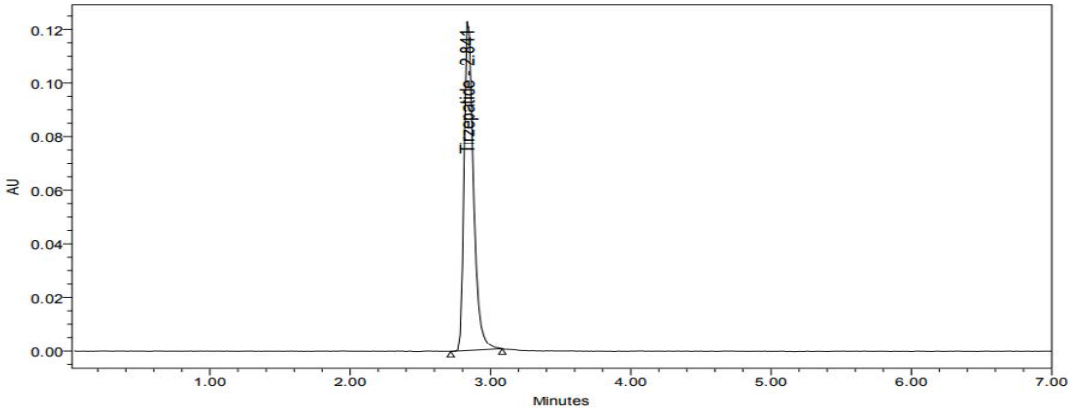


Figure 3: Chromatogram of standard in optimized condition.



	Peak Name	RT	Area	USP Plate Count	USP Tailing	% Area	Purity1 Angle	Purity1 Threshold
1	Tirzepatide	2.841	575086	8829	1.4	100.00	0.298	0.351

Figure 4: Chromatogram of Optimized method of Tirzepatide.

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