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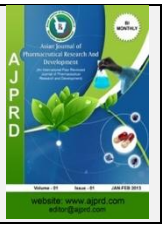

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Review Article

Review on Analytical Method Validation on Tirzepatide:-

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A B S T R A C T

Background: - Type 2 diabetes mellitus (T2DM), obesity, cardiovascular diseases in T2DM, heart failure, non- alcoholic steato hepatitis, obstructive sleep apnea, and obesity-related mortality and morbidity are among the conditions for which Eli Lilly is developing Tirzepatide. As a supplement to diet and exercise, Tirzepatidewas initially approved in the USAin May2022 to help adults with type 2 diabetes improve their glycaemic control. Tirzepatide is a novel antidiabetic medication, single-molecule agonistoftheglucose-dependentinsulinotropicpolypeptideandglucagon-like peptide-1 receptors. It is marketed under the brand names MOUNJARO and ZEPBOUND.

Method:-Techniques such as Reversed-phase High-performance liquid chromatography (RP-HPLC), High-performance liquid chromatography (HPLC), Ultraviolet spectroscopy (UV spectroscopy), Fluorescence spectroscopy, and others have been used for analysis.

Conclusion: - An HPLC Method Validated using a Quality-by-Design (QBD) approach concludes that the method is linear, accurate, precise, and robust for the Quality control of tirzepatide in bulk and dosage forms and LC-MS bioanalytical method for quantifying tirzepatide in human plasma is typically concluded to be specific, sensitive, and accurate for pharmacokinetic studies.

Keywords: Tirzepatide, Type 2 diabetes mellitus (T2DM), Non- alcoholic steatohepatitis (NASH), Obstructive sleep apnea (OSA).

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INTRODUCTION:

Tirzepatide analogue that functions as a dual agonist for the glucagon-like peptide-1(GLP-1) and glucose-dependent insulin otropic polypeptide (GIP) receptors. This dual mechanism yields enhanced insulin secretion, improved insulin sensitivity, and appetite suppression. Tirzepatide is used in the treatment of type 2 diabetes and obesity. According to the International Diabetes Federation (IDF), as of 2024, approximately 89.8 million people aged 20-79 have diabetes in India. According to the International Diabetes Federation (IDF), as of 2024, approximately 89.8 million people aged 20-79 have diabetes in India. Also, the World Health Organization (WHO) notes that in India, there are approximately 77 million people over 18 with type 2 diabetes, with a growing number of pre-diabetic individuals. Data suggest that over 43% of diabetic individuals are aged 60 and above, while the 39-58 age group represents about 37.5%. Men show a slightly higher prevalence (~6.9%) compared to women. Tirzepatide is a promising, highly effective treatment for obesity, demonstrating significant, dose-dependent weight loss and improvements in obesity-related conditions like sleep apnea and Diabetes. Obesity has risen globally, affecting16% of adults in 2022(about one in eight people), with ~880 million adults living with obesity worldwide. Prevalence the rates are 22.9 % in men and 24.0% in women. Although Tirzepatide itself is FDA approved,

Tirzepatide formulations are compounded and experimental, with limited scientific validation. Developed by Eli Lilly and Company, Tirzepatide was approved for the treatment of diabetes. In the US, marketed as **Mounjaro** for type 2 diabetes (T2D) and **Zepbound** for weight management, it also offers benefits such as reduced fat mass and improved cardio metabolic markers.Common side effects: Gastrointestinal symptoms, including nausea, diarrhoea, and vomiting, are not suitable for everyone. It can be injected into the thigh, abdomen, or upper arm and can be administered with or without meals at any time of day. Tirzepatide is administered via subcutaneous injections (once a week).

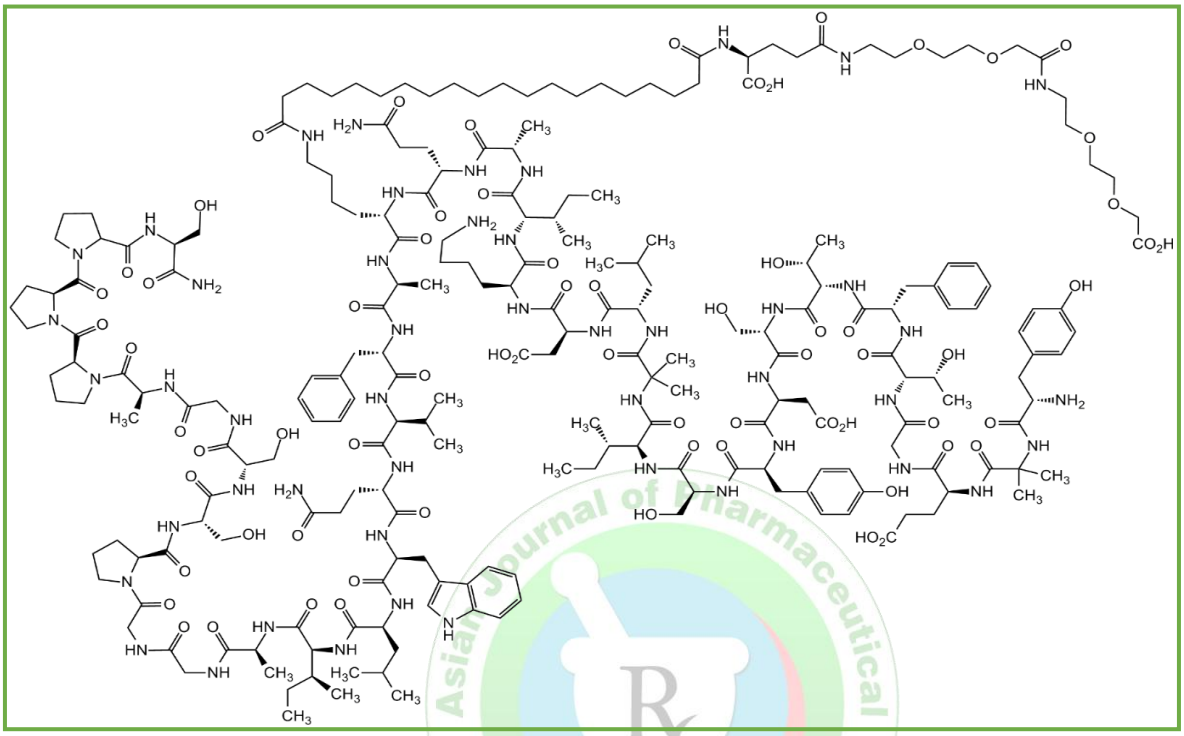

Figure 1: Structure of Tirzepatide

Tirzepatide is long half-life and strong affinity for albumin are attributable to two non-coded amino acid residues (Aib, α-amino isobutyric acid)at positions 2 and 13 in its peptide 13initspeptidesequence. Tirzepatide molecular weight is 4813.45, and its chemical formula is $\mathbf{C_{225}H_{348}N_{48}O_{68}}$.

Additional uses:

1. Obesity and Weight management
2. Cardiovascular risk reduction
3. Non- alcoholic
4. Steatohepatitis (NASH) /fatty liver
5. Disease Obstructive Sleep Apnea (OSA)
6. Polycystic Ovary Syndrome (PCOS)-investigation.

Drug Profile:

Parameter	**Detail**
Drug Name	Tirzepatide
Class	An Antidiabetic peptide drug and an obesity-reducing drug
Pharmacokinetics	**Absorption:** -80 % absorbed following subcutaneous administration. **Distribution:** -Minimally distributed to tissue. **Proteinbinding:** -99% **Metabolism:** -Metabolized by proteolytic cleavage, beta oxidation and amide hydrolysis. **Excretion:** -excreted in urine and faeces, with tiny amounts being eliminated as unchanged drug.

Pharmacological action	Act as a GIP and GLP-1 receptor agonist; increase insulin secretion and reduce glucagon secretion, both in dependent manners—also, slow gastric emptying time. Also helps suppress appetite, leading to a decrease in caloric intake.
Dosage form	Subcutaneous injection (peptide formulation).
Dose	Once a week
Half life	5 days
Therapeutic indication	Treatment of Type 2 Diabetes Mellitus (T2DM) and chronic weight management.
Advantages	Better energy balance, Weight loss potential, Dual/triple incretin pathway activation.
Adverse effect	**CV: -** Increase heart rate, Hypotension **Skin: -** Hair loss **Endo: -** Hypoglycemia **GI** Constipation, nausea, diarrhea, increased amylase & lipase **Metabolic: -**Decreasedappetite
Contraindication	**Hypersensitivity.** Type 1 diabetes. Severe gastro paresis. Personalorfamilyhistoryofmedullarythyroid carcinoma.

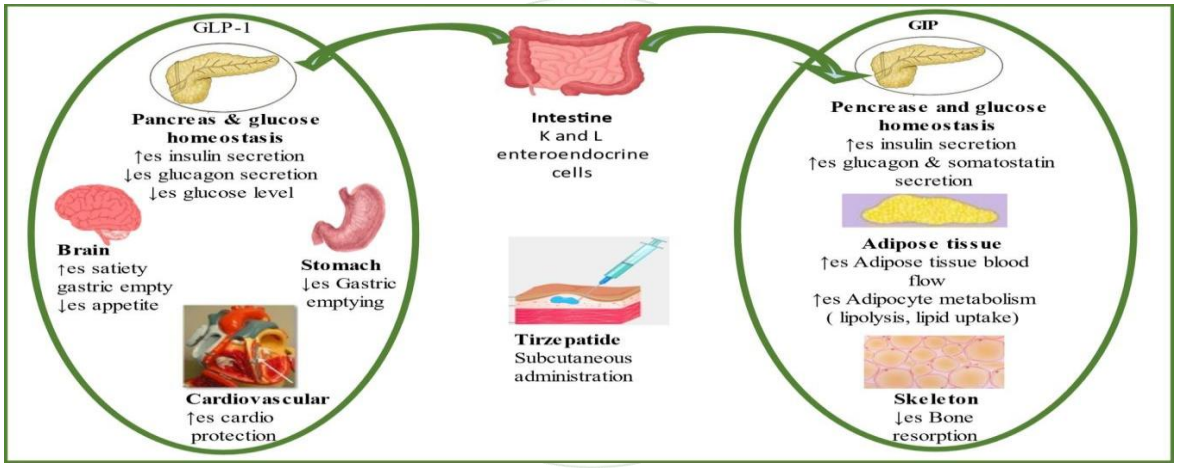

Figure 1: Mechanism of action of Tirzepatide

Pharmaceutical Dosage form:

Table1: Pharmaceutical Dosage form

Sr. No.	Brand name	Manufacturer	Dose
1.	Mounjaro injection	Jishnu Pharmaceutical Pvt. Ltd	2,5mg
2.	Zepbound injection	Kredence Multi Trading Limited	2.5mg
3.	Mounjaro injection	Softex Industrial Private Limited	5mg
4.	Zepbound injection	Aayuexport	15mg
5.	Mounjaro injection	Jishnu Pharmaceutical Pvt. Ltd	7.5mg
6.	Zepbound injection	Meditune Healthcare Private Limited	10mg
7.	Mounjaro injection	Hetero Healthcare Limited	12.5mg

Figure 2: Zepbound

Figure 3: Mounjaro

Analytical Method Validation:

S r No.	Drugs	Analytical method	Description	Ref. No.
1.	Tirzepatide	RP-HPLC	**Mobile phase:**-0.01N KH_2PO_4 :Acetonitrile41:59(%v/v) **Column:**-C18(150nm*4.6mm*5µm) **Flowrate:** -1.0ml/min **Columntemperature:**-25-30˚C **Wavelength:**-250nm **Runtime:**-20-30min	4
2.	Tirzepatide	HPLC	**Mobile phase:** - (A): Water (B): Acetonitrile **Gradient:**-5% Bincreaseto 95%B within 1.3min, 95% B for 1.7min **Column:**- Primesep B (3.2 ×50mm*5µm) **Flowrate:**-0.5ml/min **Wavelength:**-280nm	5
3.	Tirzepatide	LC/MSAnalysis	**Mobile phase:- (**A) water:0.1 % formic acid (B) Acetonitrile:0.1% formic Acid **Column:**- Agilent Phoroshell120EC- C18 (4.6*50mm,4-micron) **Column temperature:** - 50˚C **Wavelength:**-214nm	3
4.	Tirzepatide	UVSpectroscopy	**Wavelength:**-214nm **RetentionTime:**-7.600min	5
5.	Tirzepatide	Fluorescence spectroscopy	**Solvent:**-ethanol **Wavelength:**-native fluorescence 294.8 and 225nm	7

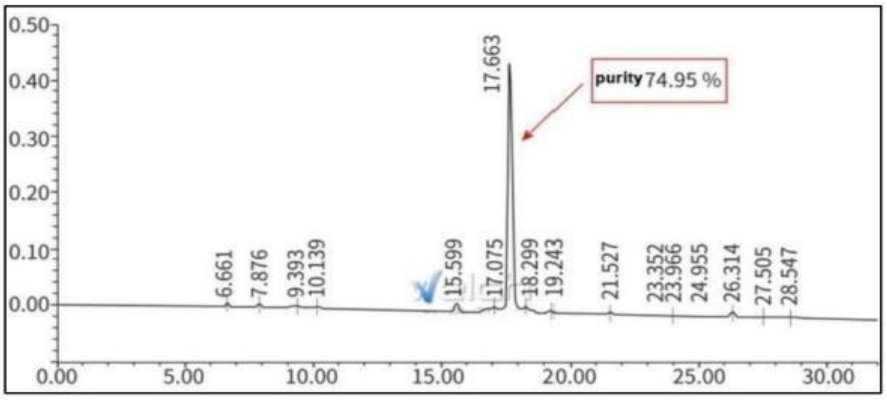

Figure: Chromatogram of Tirzepatidein human plasma with RP-HPLC Method

CONCLUSION:

The presented review on the analytical method validation of various methods reported on Tirzepatide. Some introduction on Tirzepatideis a novel dual GIP and GLP-1 receptor agonist that offers effective control of Type 2 Diabetes Mellitus and obesity by improving insulin secretion, reducing glucagon, and promoting weight loss.Validated analytical methods like HPLC, LC/MS, UV, and Fluoroscene ensure its accurate evolution.These techniques are appropriate for supporting important pharmacokinetic and bio analytical studies as well as for quality control during drug

standardization.These technique proven ability to indicate stability further guarantee's that Tirzepatide can be accurately measured even when its breakdown products are present.Validated and economical techniques are available to support this significant therapeutic agent's continued research, quality control, and clinical use.

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