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ANALYTICAL TECHNIQUES FOR TIRZEPATIDE: A REVIEW ON CURRENT HPLC AND FLUORIMETRY METHODS WITH FUTURE UV SPECTROSCOPY PROSPECTS

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ABSTRACT:

This review focuses on the analytical techniques employed for the analysis of Tirzepatide, a recently developed peptide drug. Despite the limited availability of analytical methods, High-Performance Liquid Chromatography (HPLC) and fluorimetry has emerged as the primary technique for the quantification and purity assessment of Tirzepatide. The review delves into the specifics of the existing HPLC method, highlighting its parameters, such as mobile phase composition, detection wavelength, and column type. It also discusses the method's validation, covering aspects like linearity, sensitivity, accuracy, and precision. The review identifies the need for additional analytical methodologies to address limitations in their current HPLC approach, such as resolving potential impurities and degradation products. Furthermore, the potential for developing complementary techniques, including mass spectrometry and spectroscopic methods, is considered to enhance the comprehensive analysis of Tirzepatide, this review aims to provide a foundation for future research in the analytical characterization of Tirzepatide, guiding the development of more robust and versatile analytical protocols.

1. INTRODUCTION[1-12]

1.1 Introduction of Hyperglycemia and obesity[1-3]

Type 2 diabetes mellitus (T2DM) is a chronic progressive disease associated with microvascular and macrovascular complications and increased cardiovascular mortality. Chronic hyperglycaemia leads to glucose toxicity. which in turn causes pancreatic beta cell dysfunction and exacerbates insulin deficiency in the late phase. The ultimate result of this is a viscous cycle of hyperglycaemia leading to a worsening metabolic state. One of the main modifiable risk factors for diabetes is obesity. The parallel rise in the prevalence of obesity and T2DM (known as 'diabesity') is a global health challenge. Obesity is strongly associated with insulin resistance, one of the key features in pathogenesis of T2DM and one of the barriers for achieving good glycaemic control. Management of T2DM, therefore, includes dietary intervention and lifestyle modification to promote weight loss along with pharmacotherapy to combat hyperglycaemia and optimise metabolic parameters such as blood pressure and lipids, and metabolic surgery in some cases.[1]

Glucagon is a potent hyperglycemic hormone that acts almost exclusively on the liver to increase hepatic glucose production within minutes. Ingestion of carbohydrate elicits a prompt rise in insulin concentration and a decrease in glucagon concentration. The increase in insulin concentrations, which occurs before the rise in arterial glucose concentrations, is thought to be mediated largely via hormonal signals arising in the gastrointestinal tract (incretion effect) [2].

Beta cells that produce insulin, present in the pancreas, play a significant role in the origination and development of T2D by controlling the endocrine system for glucose metabolism and glycemia. In T2D, these cells become inoperative to compensate for insulin resistance, resulting in an insulin-deficient condition called hyperglycemia. Hyperglycemia can result in glucose toxicity that further deteriorates the function of beta cells of the pancreas, giving rise to a deficiency of insulin in the body.

Obesity is defined by the World Health Organization (WHO) as an "abnormal or excessive fat accumulation that may impair health". It is defined by body mass index (BMI), weight in kilograms divided by the square of height in meters, in adults over 30 kg /m2 . It should be highlighted that obesity is related to an increased risk of other serious conditions and diseases such as diabetes, hypertension, cardiovascular disease, cancer, asthma, hypercholesterolemia, and so on.[3]

1.1.1 Classification of Diabetes mellitus (5)

Types of diabetes

Type 1 Diabetes	•Effects children •Caused due to deficiency of insulin •Genetic variations & auto-immune response areleading cause
Type 2 Diabetes	•Effects adults •Caused due to insulin resistance by body •Obesity, inactive lifestyle, hereditary are leadingcause
Gestational Diabetes	•Occurs during pregnancy •Excessive weight gain, genetic history of diabetes
MODY (maturity-onset diabetes of youth)	•Very rare •Caused in adults below 25 years of age •Caused due to genetic variation

Causes

The effect caused by these insulinotropic peptides is called the 'incretin effect'. This effect is characterized by elevation of glucose secretory response by oral glucose administration in comparison to intravenous administration even though they have similar plasma concentrations. The name is given because it is believed to occur due to nutrients triggering the release of incretin hormones and acting upon pancreatic beta cells in an insulinotropic manner.

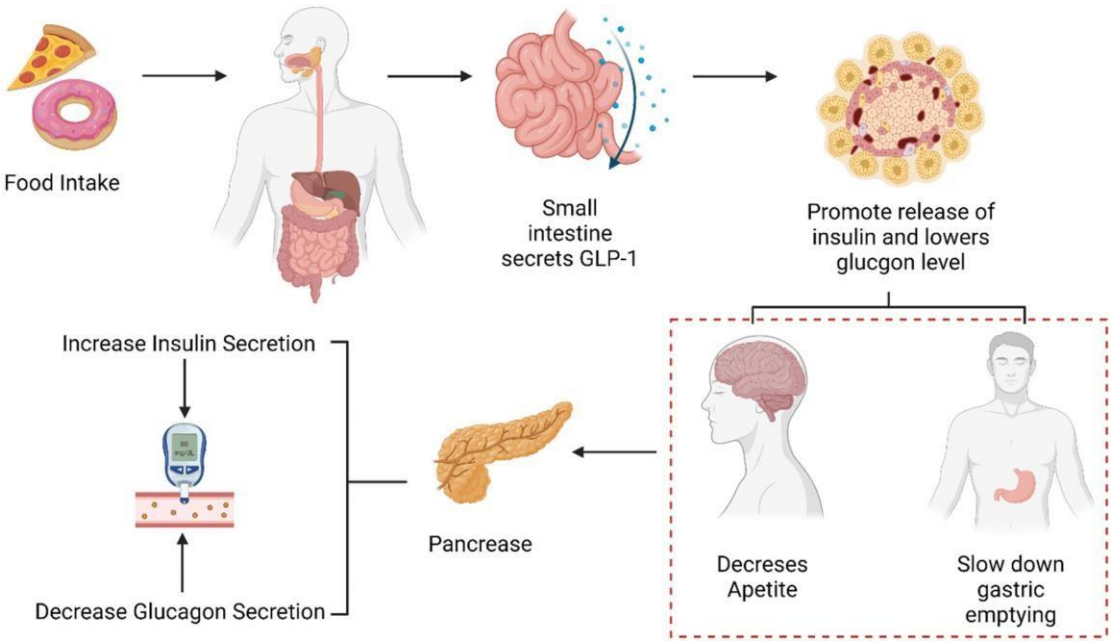


Figure 1.Role of GLP-1 in glucose metabolism

GLP-1 and GIP are peptide hormones that are secreted with the benefit of utilizing cells from intestines called enteroendocrine cells in reaction to the consumption of nutrients, and they have an important function in postprandial metabolism. With the identification of glucose equilibrium, their most favorable condition, the incretin effect, begins to improve the glucose-stimulated release of insulin from the pancreas. GIP is the essential incretin hormone accounting for this effect, although when administered together, they exhibit a synergetic effect.[3]

One of the most common high-risk areas for medication errors and preventable adverse drug events (ADEs) appears to be the use of insulin and the overall management of diabetes mellitus in acute care. The 2001 report of Medmarx, a national database for hospital medication error reporting, identified insulin as the medication most frequently involved in harmful medication errors, especially errors in dosage, errors of omission, and prescribing errors. The most common cause contributing to hyperglycemia was the lack of ownership of diabetes management when patients were admitted for reasons other than diabetes (e.g., the physician indicated that treating the diabetes was not his or her responsibility).[4]

1.1.2 Hyperglycemic drugs :

A broad range of commonly prescribed medications can lead to hyperglycemia or new onset diabetes (NOD). The true incidence and prevalence of drug induced diabetes is not known due to short duration of diabetes and lack of systematic ascertainment of both diabetes and its long-term complications. Diabetes is diagnosed by fasting blood glucose≥ 126 mg/dl, random or 2 h post prandial blood glucose

≥200 mg/dl or a hemoglobin A1C ≥ 6.5% (HbA1c). This paper reviews the recent literature on drug induced hyperglycemia and NOD.[6]

The number of individuals diagnosed with type 2 diabetes mellitus, which is caused by insulin resistance and/or abnormal insulin secretion, is increasing worldwide , creating a strong demand for the development of more effective anti-diabetic drugs. Blood glucose levels are regulated by hormones such as insulin that regulate glucose uptake and metabolism in tissues throughout the body. Evaluation of the effects of anti-diabetic drugs thus requires the use of an animal model. The use of mammalian animals to screen for anti-diabetic drugs, however, is not only very expensive from an animal husbandry perspective, but also presents ethical problems in terms of animal welfare.[7]

Drug induced diabetes is clinically important and underreported in clinical studies. Risks for developing drug induced diabetes include dose and duration of treatment and usual risk factors like age, family history of diabetes and BMI. The severity of hyperglycemia is variable and marked hyperglycemia may be a feature of therapy with glucocorticoids, somatostatin analogues, androgen deprivation, antipsychotics, interferon, older anti-retroviral agents and mTOR inhibitors. The distal glucoregulatory pathways (insulin secretion and insulin action) are impaired in diabetes and the proximal pathways are poorly understood, likely drug-specific, and this is a hindrance to the development of targeted treatment. Different drugs within a class may differ in diabetogenic potential as with statins and antipsychotics. Drug induced diabetes is usually type 2 and typically resolves upon drug discontinuation. An exception to this fact is Interferon which is associated with type 1 diabetes. The true incidence of drug induced diabetes and its relationship to long-term diabetic micro and macrovascular complications is unknown which contributes to clinical uncertainty.[4]

1.1.2.1 Classification of hyperglycemic drugs[1]	
Enhance insulinsecretion	K(atp) channel blockers : Sulfonylureas Ex. Tolbutamide, glibenclamide, glipizide, gliclazide, glimepiride
	K(atp) channel blockers : Phenylalanine analogues Ex. Repaglinide, nateglinide
	Dipeptidyl peptidase-4 inhibitors Ex. Sitagliptin, vildagliptin, saxagliptin
Overcome insulinresistance	Biguanide Ex. Metformin
	Thiazolidinedione Ex. Pioglitazone
Miscellaneousdrugs	β-adrenergic blockers Ex. Propranolol,Metoprolol,Atenolol
	Dopamine d2 agonist Ex. Bromocriptine
Retard carbohydrateabsorption	Alfa- Glucosidase inhibitors Ex. Acarbose, Miglitol, Volgibose

1.2 Introduction of Tirzepatide[7]

In early 2016, Eli Lilly and Company (Indianapolis, IN, USA) first applied a method of glycemic control using tirzepatide. On 14 May 2022, Eli Lilly unlocked one more achievement by receiving US FDA approval for the highly anticipated anti-diabetic drug Mounjaro® (tirzepatide). Tirzepatide is a peptide molecule that is produced synthetically that acts on both GIP and GLP-1 receptors as a receptor agonist. Due to this unique dual activity property, it is also referred to as 'twincretin'. Subcutaneous administration once a week is adequate, as it possesses a half-life of about 5 days.[7]

Structure and Activity

Tirzepatide is a synthetic linear peptide molecule containing 39 amino acids. Residues derive from GLP-1, GIP and semaglutide, and a few residues are unique. More specifically, the structure is based on the native GIP sequence and includes C20 fatty diacid moiety (eicosanedioic acid) linked via hydrophilic linkers (γ-Glu-2xAdo, gamma glutamate and bis-

aminodiethoxyacetyl) connected to lysine residue at C20 position . The peptide sequence of tirzepatide contains two non-coded amino acid residues (Aib, α- amino isobutyric acid) at position 2 and 13, which are responsible for its long half-life and high affinity to albumin . The C-terminus of the peptide is amidated . The molecular formula of tirzepatide is C225H348N48O68 and the molecular weight is 4813.45. Tirzepatide is the first agent that functions as a dual agonist for the two main human GLP-1 and GIP incretins, a promising drug against both T2D and obesity. It has impressive glycemic efficacy. Moreover, it is the first effective drug to have demonstrated notable body weight loss in a phase 3 study in patients with T2D[(7)]

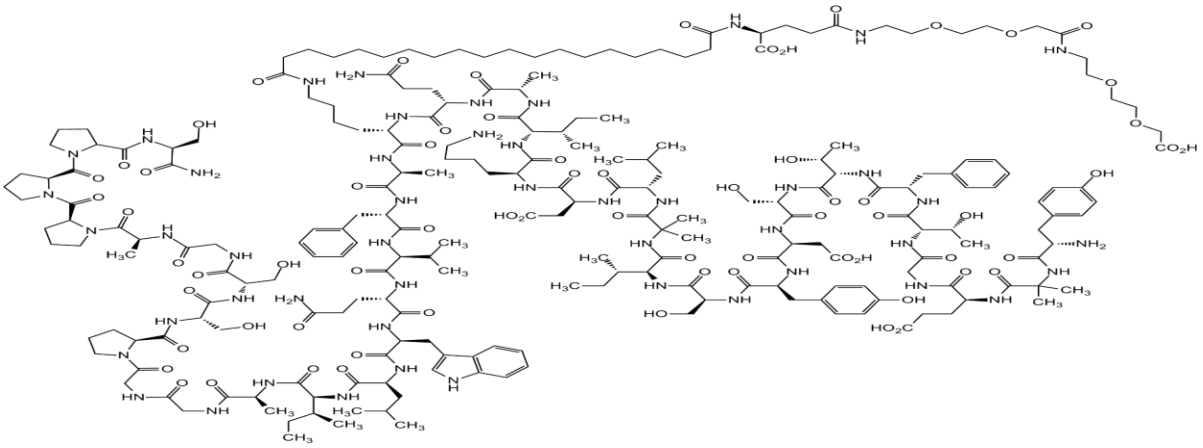


Figure 1. structure of Tirzepatide

1.3 Introduction to analytical method[(8-10)]

Analytical technique is a method used to determine achemical or physical property of a chemical substance, chemical element, or mixture. There is a wide variety of techniques used for analysis, from simple weighing to advanced techniques using highly specialized instrumentation.

Spectrometer can determine chemical composition through its measure of spectrums. The common spectrometer used in analytical chemistry is Mass spectrometry. In a mass spectrometer, a small amount of sample is ionized and converted to gaseous ions, where they are separated and analyzed according to their mass-to-charge ratios.

NMR Spectroscopy involves exciting a NMR-active sample and then measuring the effects of this magnetic excitation. From this, the bonds present in a sample can be determined.

Electroanalytical methods utilize the potential or current of a electrochemical cell. Thethreemain sections of this type of analysis are potentiometry, coulometry and voltammetry. Potentiometry measures the cell's potential, coulometry measures the cell's current, and voltammetry measures the change in current when cell potential changes.

Chromatography separates the analyte from the rest of the sample so that it may be measured without interference from other compounds. There are different types of chromatography that differ from the media they use to separate the analyte and the sample. In Thin-layer chromatography, the analyte mixture moves up and separates along the coated sheet under the volatile mobile phase. In Gas chromatography, gas separates the volatile analytes. A common method for chromatography using liquid as a mobile phase is High-performance liquid chromatography.[8]

1.3.1 Introduction of UV Spectroscopy

UV-VIS spectroscopy involves the study of interaction of electromagnetic radiation with matter. The energy is either absorbed or emitted by matter in distinct amount called quanta. It is one of the earliest instrumental techniques for the analysis and determination of micro and semi micro quantities of analyte in a sample which is based on atomic and molecular spectroscopy. It is used for analysis of different types of solvents and substances such as inorganic, organic, and biomolecules and is preferred mostly for its simplicity, versatility, accuracy, speed and cost effectiveness and maintenance is easy. It is used to determine the identity, strength, quality, and purity of several compounds. It is a measuring device which is used for quantitative analysis generally used for chemical substance by determining amount of light that is partially absorbed by analyte present in the solution. It can be classified according to the spectral region, such as UV (From 190nm to 380nm), VIS (From 380nm to 750nm), and near infrared (From 800nm to 2500nm).

Figure 1.2 UV spectrophphotometer

1.3.1.1 Principle of UV Spectrophotometer

UV/VISIBLE Spectroscopy involves the study of how a sample responds to light. When a monochromatic beam of light passes through sample, certain amount of light may be absorbedand the remaining is transmitted through the sample. It is a technique based on the measurement on depletion of electromagnetic radiation by absorbing substance. The depletion electromagnetic radiation has resulted from the reflection, scattering, absorption, or interference.

There are three types of ground state orbitals (Lower energy) may be involved:

i. σ (bonding) molecular orbital

ii. π (bonding) molecular orbital

iii. n (bonding) atomic orbital

The electronic transition occurring by absorption of ultraviolet and visible light are as follows:

i. σ to σ*

ii. π to π*

iii. n to σ*

iv. n to π^*

1.3.1.2 Instrumentation of UV Spectrophotometer

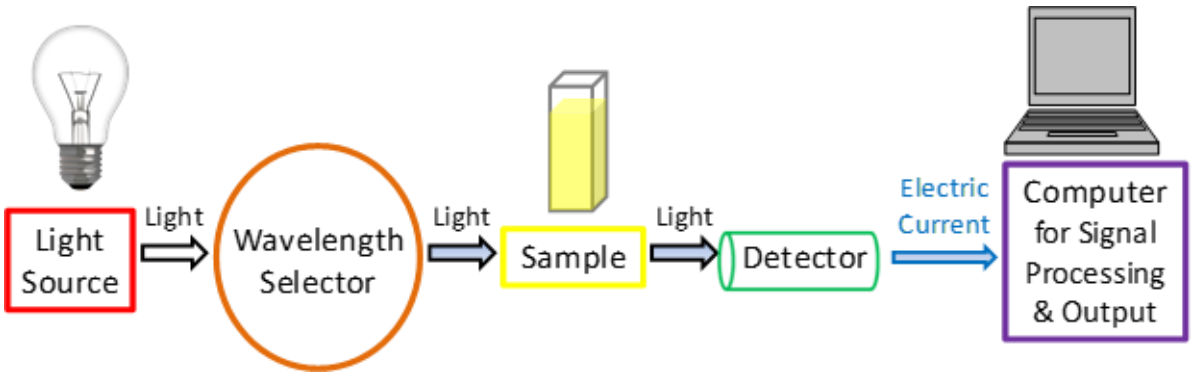


Figure 1.3 Instrumenttion of Uv Spectrphotometer

A. Light Source

Hydrogen (or) deuterium lamps, Xenon discharge lamps, Mercury arcs are most frequently used radiation sources. By passing the electrons through gas, collision occurs between gas molecules and electrons resulting in electronic, vibrational, and rotational excitation in the gas molecules.

A. Lasers

B. Led lamp

C. Deuterium lamps

D. Tungsten lamp

E. Xenon discharge lamp

B. Filter

A filter is a device which transmit radiation of some wavelength but absorbs wholly or partially other wavelengths. Among the several devices that produce limited bands of radiation, filters are the simplest and least expensive and the filters are of two types: 1. Absorption filters 2. Interference filters

B. Monochromator

It can be achieved by filtering out the undesirable wavelength from radiation source. The monochromator is used to convert the polychromatic light into desirable monochromatic light. The main function of the monochromator is to disperse the beam of light obtained from the primary source into its components.

Through the entrance slit, polychromatic light is entered the monochromator and followed by collimation, beam is directed at an angle towards the dispersion component.

It includes the following types: 1. Prism monochromator 2. Grating monochromator

C. Sample and Reference

The cuvette must be constructed with quartz, if the work must be done in the UV region and for Visible region, they are made up of color corrected fused glass. The cuvette thickness depends upon whether the absorption is strong or weak. It may be either rectangular or cylindrical or cylindrical with flat ends.

D. Detector

When the transmitted radiation strikes the detector, it allows the detector in determining the amount of radiation absorbed by the sample. The detector is placed in a position such that they receive the resulting monochromatic radiation after passed through sample and reference substance which is stored in quartz cuvettes. Detectors are usually responding with high sensitivity with low noise having a linear response range, fast response, and low consumption of sample. Converting the light source into electrical signal is the main function of the detector. Detectors are instrument that are used for measurement and to convert transmitted or reflected light from the sample to signal.(9)

1.3.2 Introduction of HPLC (10)

High Performance Liquid Chromatography (HPLC) was developed in the early 1960's. Today it has grown into an essential tool for the modern analytical laboratory, and it has replaced gas chromatography (GC) for a variety of analyses. HPLC is a technique that is usually covered in undergraduate courses devoted to instrumental analytical methods. In its applications to food analysis, the technique has gained increased acceptance mainly because it met two basic factors, namely: i) the need for a wide range of rapid analyses for nutrients; and ii) the need for methods that can be easily automated. Despite those notable advantages, the integration of

HPLC in the food laboratory has been slow compared to other areas like pharmaceutical chemistry and forensic toxicology.

1.3.2.1 Basic Principles

HPLC is a form of liquid chromatography, where separation (or partition) occurs between a mobile phase (the solvent) and a stationary phase (the column packing). It is the ability with which the sample constituents will distribute themselves between the two phases that will affect the separation.

THE MOBILE PHASE- The solvent

The choice of the solvent will depend on the nature of the operation mode, i.e., isocratic or gradient elution (and, of course, on the solubility of the sample in the chosen elution medium). The polarity for such an elution medium can, therefore, vary from buffered aqueous solutions to hydrocarbons. The chosen medium (including water) needs to always be very pure. HPLC grade solvents are commercially available and should always be used. The choice of a gradient eluent is always done by trial and error, usually starting with a single solvent and increasing the concentration of the second mobile phase component by usually using an initial mixing rate of 2% per minute to achieve the desired conditions in the mobile phase.

1.3.2.2 Instrumentation of High-Performance Liquid Chromatography (HPLC)

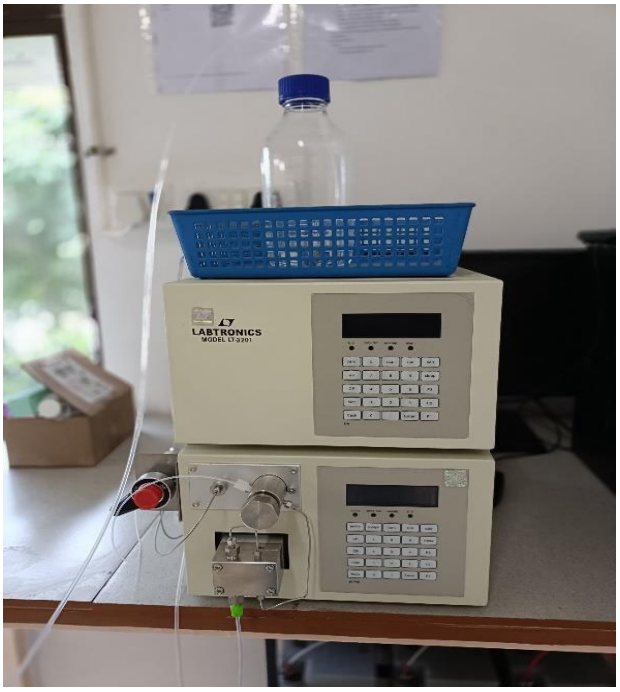


Figure 1.5Instrumentation of HPLC

The Pump

The pumps are electronically controlled to regulate pressure and flow rates within narrow limits. A programmer is normally used to regulate the delivery of a solvent mixture of fixed (isocratic) or changing composition (gradient). It is important to maintain the pumping system so that the solvent flow is constant during the analysis. A change in solvent flow will influence the retention times as noted earlier, and therefore induce errors in the identification of the sample. Reproducibility in chromatograms of standards and samples is essential. Modern analytical HPLC pumps are capable of pumping flow rates as low as 1 or 10 pL/min up to 5 or 10 mL/min. Since the efficiency in separation increases as the flow decreases, flow rates are generally maintained low.

Injector

A few methods are used to inject the sample onto the column, the simplest one being a direct injection with a micro-syringe. But most systems will use sampling devices. The most common way of injecting the sample on the column is an injection valve in which the sample is injected into a holding loop. Loops are designed to inject a specific volume onto the column, usually of the order of 10 to 20 pL for an analytical column.

The Stationary Phase - The column

are the selection of the separation mode and the appropriate column packing. The columns most encountered have an internal diameter (i.d.) of 4.5 to 5 mm and are 10 to 25 cm in length; they are packed with stationary phases having 5 to 10 Tm in diameter. Usually made of stainless steel, the compressing end fittings of the columns are of various designs. Column packing is very important for chromatography resolution.

Detector

The detector can be considered as the "soul" of a HPLC system. Connected to the outlet end of the column, its role is to monitor the column effluent in real time. Detectors can be the most sophisticated and expensive component of the system. Classification of detectors is of two sorts, selective detectors which give different responses depending on the molecular structure of the sample under analysis, or universal detectors, for whom the response is similar for most compounds.

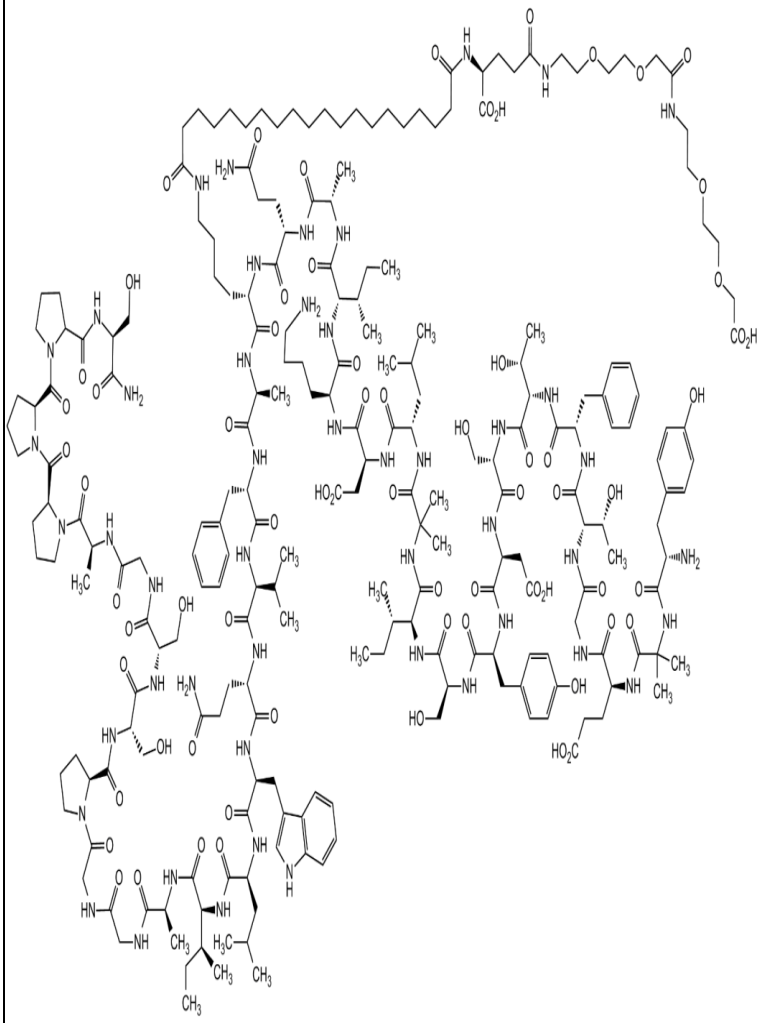
1.3.2.3 Applications of High-Performance Liquid Chromatography (HPLC)

- A noteworthy feature of HPLC is that it is often suitable for organic compounds that are too unstable or insufficiently volatile to be amenable to gas chromatography analysis without prior derivatisation.
- It is suited for the separation of a wide range of chemicals, including pharmaceuticals, foods, heavy industrials and biochemicals.
- The number of published HPLC analytical methods these days is so large that a literature search is likely to provide a set of chromatographic conditions for almost any type of compounds.
- Alteration of these conditions to suit the matrix involved might be the only required step for the food scientist to perform a HPLC analysis of a new compound.
- In food science, HPLC has been applied to several categories of substances carbohydrates, lipids, vitamins, additives, synthetic colourings, natural pigments, contaminants (degradation products, pesticides, or naturally occurring substances) as well as amino acids and others

1.4 Drug Profile for Tirzepatide: (11)

Table 1.2 Drug profile of Tirzepetide

INTRODUCTION	
Name	Tirzepetide
Officialin	Tirzepetide is not official in anyof thepharmacopoeia(i.e.USP,EP,BPandIP).
Description	Tirzepatide is currently utilized as a second-line diabetes medication akin to GLP-1 drugs, such as semaglutide, and is administered once weekly via subcutaneous injection with incremental dosage adjustments.

Structure	
ChemicalFormula	**C225H348N48O68**
Mol.Weight	**4813.527**gm/mol
	20-[[(1*R*)-4-[2-[2-[2-[2-[2-[2-[[(5*S*)-5-[[(2*S*)-5-amino-2-[[(2*S*)-2-

IUPACName	[[(2*S*,3*S*)-2-[[(2*S*)-6-amino-2-[[(2*S*)-2-[[(2*S*)-2-[[2-[[(2*S*,3*S*)-2-[[(2*S*)-2-[[(2*S*)-2-[[(2*S*)-2-[[(2*S*)-2-[[(2*S*,3*R*)-2-[[(2*S*)-2-[[(2*S*,3*R*)-2-[[2-[[(2*S*)-2-[[2-[[(2*S*)-2-amino-3-(4-hydroxyphenyl)propanoyl]amino]-2-methylpropanoyl]amino]-4-carboxybutanoyl]amino]acetyl]amino]-3-hydroxybutanoyl]amino]-3-phenylpropanoyl]amino]-3-hydroxybutanoyl]amino]-3-hydroxypropanoyl]amino]-3-carboxypropanoyl]amino]-3-(4-hydroxyphenyl)propanoyl]amino]-3-hydroxypropanoyl]amino]-3-methylpentanoyl]amino]-2-methylpropanoyl]amino]-4-methylpentanoyl]amino]-3-carboxypropanoyl]amino]hexanoyl]amino]-3-methylpentanoyl]amino]propanoyl]amino]-5-oxopentanoyl]amino]-6-[[(2*S*)-1-[[(2*S*)-1-[[(2*S*)-1-[[(2*S*)-5-amino-1-[[(2*S*)-1-[[(2*S*)-1-[[(2*S*,3*S*)-1-[[(2*S*)-1-[[2-[[2-[(2*S*)-2-[[(2*S*)-1-[[(2*S*)-1-[[2-[[(2*S*)-1-[(2*S*)-2-[(2*S*)-2-[(2*S*)-2-[[(2*S*)-1-amino-3-hydroxy-1-oxopropan-2-yl]carbamoyl]pyrrolidine-1-carbonyl]pyrrolidine-1-carbonyl]pyrrolidin-1-yl]-1-oxopropan-2-yl]amino]-2-oxoethyl]amino]-3-hydroxy-1-oxopropan-2-yl]amino]-3-hydroxy-1-oxopropan-2-yl]carbamoyl]pyrrolidin-1-yl]-2-oxoethyl]amino]-2-oxoethyl]amino]-1-oxopropan-2-yl]amino]-3-methyl-1-oxopentan-2-yl]amino]-4-methyl-1-oxopentan-2-yl]amino]-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino]-1,5-dioxopentan-2-yl]amino]-3-methyl-1-oxobutan-2-yl]amino]-1-oxo-3-phenylpropan-2-yl]amino]-1-oxopropan-2-yl]amino]-6-oxohexyl]amino]-2-oxoethoxy]ethoxy]ethylamino]-2-oxoethoxy]ethoxy]ethylamino]-1-carboxy-4-oxobutyl]amino]-20-oxoicosanoic acid
Categories	Glucose dependent insulinotropic poplypeptide (GIP) receptor and Glucagon like peptide 1 (GLP-1) receptor agonist
Solubility	Water solubility (<1mg/ml>)

CDSCO Approved Date	19/01/2024
PHARMACOLOGY	
Classes	Antidiabetic
Mechanismofaction	Tirzepatide activation of GIP receptors augments insulin sensitivity and secretion and thereby helps reinforce the mechanisms regulating bloodglucose levels
PROPERTIES	
State	Solid
CAS NO.	2023788-19-2
Nature	Ph 6.5-7.5 (Neutral)

1.2Analytical Method Validation(12)

1. ANALYTICAL PROCEDURE

The analytical procedure refers to the way of performing the analysis. It should describe in detail the steps necessary to perform each analytical test. This may include but is not limited to the sample, the reference standard and the reagents preparations, use of the apparatus, generation of the calibration curve, use of the formulae for the calculation, etc.

2. SPECIFICITY

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically, these might include impurities, degradants, matrix, etc. Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedure(s). This definition has the following implications Identification to ensure the identity of an analyte. Purity Tests: to ensure that all the analytical procedures performed allow an accurate statementof the content of impurities of an analyte, i.e. related substances test, heavy metals, residual solvents content, etc. Assay (content or potency) to provide an

exact result which allows an accurate statement on the content or potency of the analyte in a sample.

3. ACCURACY

The accuracy of an analytical procedure expresses the closeness of agreement between the value whichis accepted either as a conventional true value or an accepted reference value and the value found. Thisis sometimes termed trueness.

4. PRECISION

The precision of an analytical procedure expresses 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 may be considered at three levels repeatability, intermediate precision and reproducibility. Precision should be investigated using homogeneous, authentic samples. However, if it is not possible to obtain a homogeneous sample it may be investigated using artificially prepared samples or a sample solution. The precision of an analytical procedure is usually expressed asthe variance, standard deviation or coefficient of variation of a series of measurements.

5. DETECTION LIMIT

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

6. QUANTITATION LIMIT

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a samplewhich can be quantitatively determined with suitable precision and accuracy. The quantitation limit is aparameter of quantitative assays for low levels of compounds in sample matrices and is used particularlyfor the determination of impurities and/or degradation products.

7. LINEARITY

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

8. RANGE

The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstratedthat the analytical procedure has a suitable level of precision, accuracy and linearity.

9. ROBUSTNESS

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normalusage.

1.3 Pharmaceutical Dosage Form:

TIRZEPATIDE INJECTION

Table 1.3 Pharmaceutical dosage form

Sr.No	**Brand Name**	**Manufacturer**	**Dose**
1	Mounjaro injection	Jisnu pharmaceutical Pvt. Ltd.	2,5mg
2	Mounjaro injection	Softex industrial private limited	5mg
3	Mounjaro injection	Jisnu pharmaceutical Pvt. Ltd.	7.5mg
4	Mounjaro injection	Hetero healthcare limited	12.5mg
5	Zepbound injection	Kredence multi trading limited	2.5mg
6	Zepbound injection	Aayu export	15mg
7	Zepbound injection	Meditune healthcare private limited	10mg

Figure 1.6 Zepbound 10mg injection

Figure 1.7 mounjaro 12.5mg injection

2. LITERATURE REVIEW(13-19)

These drugs are not official in any pharmacopoeia.

2.1 Reported method of Tirzepatide

Table 1.4 Literature review of Tirzepatide

Sr. No	Drugs	Analytical method	Description	Ref. No.
1	Tirzepatide	Fluorescence spectroscopy	Solvent-ethanol Wavelength- Native fluorescence 294.8 and 303nm and after excited 216 and 225nm	(13)
2	Tirzepatide	RP-HPLC	Mobile phase-:0.01N KH2PO4: Acetonitrile 41:59 (% v/v) Column- C18(250mm*4.6mm*5µm) Flow rate- 0.9 ml/min Wavelength- 250nm	(14)

2.2 Summery of PSAR report: (15-25)

Table 1.5 PSAR report of Tirzepatide

Sr no.	Patent Application Number	Title of patent	Ref.no.
1	WO-2021154593-A1	Therapeutic uses of tirzepatide	15
2	CN110903355A	Preparation method of Tirzepatide	16
3	WO2021154593A1	Therapeutic uses of tirzepatide	17
4	CN115181174A	A preparation method of Tirzepatide	18
5	CN-112661815-A	A kind of purification method of Tirzepatide	19

1. WO-2021154593-A1- The present invention relates to methods for treating, preventing or delaying disorders relating to cognition, such as cognitive decline, cognitive impairment or dementia. The present invention also relates to method for treating, preventing or delaying heart failure, such as heart failure with preserved ejection fraction (HFpEF) or heart failure with reduced ejection fraction (HFrEF).

2. CN110903355A - The invention relates to the technical field of polypeptide drug synthesis and discloses a preparation method of Tirzepatide. The method comprises the following steps: preparing Tirzepatide peptide resin by a solid-phase peptide synthesis method and obtaining Tirzepatide by cracking the Tirzepatide peptide resin; wherein the method for accessing Aib is: 2-6 peptide fragments containing Aib were used. The process of the invention can significantly reduce the generation of related impurities, and at the same time the yield is guaranteed, and the purification is facilitated. In addition, the synthesis of multiple fragments can be carried out simultaneously, and the synthesis cycle is shortened, which is suitable for large-scale industrial production, and is a Tirzepatide preparation method with wide practical value and application prospect.

3. WO2021154593A1 - An embodiment of the invention relates to increasing HDL-C in a patient in need thereof. An embodiment of the invention relates to decreasing high blood pressure. An embodiment relates to a treatment for a patient with refractory type 2 diabetes to provide normal HbA1C glycemia.

4. - CN115181174A The present invention relates to dual incretin peptide mimetic compounds that agonize receptors for both human glucose-dependent insulinotropic

polypeptide (GIP) and glucagon- like peptide-1 (GLP-1) and may be useful for treating type 2 diabetes mellitus (T2D).

5. CN-112661815-A - The invention relates to a method for purifying Tirzepatide, which belongs to the technical field of medicinal chemistry. In the purification method, the crude peptide of Tirzepatide is dissolved in purified water, and the pH is adjusted to 8.0 with ammonia water to be completely dissolved. After obtaining the crude peptide solution, Tirzepatide is obtained by two-step purification. The Tirzepatide prepared by the purification method has high purity, high yield and simple operation, which is beneficial to realize the large-scale preparation of Tirzepatide. The yield of the method of the invention is about 70%, and the obtained Tirzepatide has a purity of more than 99.0% and a single impurity of less than 0.15%.

RATIONALE:

- After conducting patent search & literature survey on analytical method development for Tirzepatide, it was found that only process patent of synthesis, and preparation of formulations are patented.
- Even though, till now there is no analytical method development reported on tirzepatide
- Dosage form containing tirzepatide is also available in market.
- So, I thought to develop the method for estimation of tirzepatide and its impurities.

CONCLUSION:

In conclusion, the current analytical methods for tirzepatide are predominantly limited to High-Performance Liquid Chromatography (HPLC) and fluorimetry. While HPLC provides reliable quantification and purity assessment, and fluorimetry offers sensitive detection of fluorescent derivatives, these techniques alone may not comprehensively address all analytical needs, such as the detailed analysis of impurities and degradation products. This review suggests that future research should explore the application of UV spectroscopy as a future analytical tool. This additional technique would enhance the robustness of quality control measures and facilitate a more thorough characterization of tirzepatide. The exploration of UV spectroscopy, alongside the current methods, promises to provide a more holistic analytical framework, supporting the continued research and development of tirzepatide.

REFERENCES:

1. Min, T., & Bain, S. C. (2021). The role of tirzepatide, dual GIP and GLP-1 receptor agonist, in the management of type 2 diabetes: the SURPASS clinical trials. Diabetes Therapy, 12(1), 143-157.
2. Giugliano, D., Ceriello, A., & Esposito, K. (2008). Glucose metabolism and hyperglycemia. The American journal of clinical nutrition, 87(1), 217S-222S.
3. Chavda, V. P., Ajabiya, J., Teli, D., Bojarska, J., & Apostolopoulos, V. (2022). Tirzepatide, a new era of dual-targeted treatment for diabetes and obesity: a mini-review. Molecules, 27(13), 4315.
4. Smith, W. D., Winterstein, A. G., Johns, T., Rosenberg, E., & Sauer, B. C. (2005). Causes of hyperglycemia and hypoglycemia in adult inpatients. American Journal of Health-System Pharmacy, 62(7), 714-719.
5. Dave J, Tirgar P, Patel B. A Pharmacological Analysis of Sodium-Glucose Cotransporter-2 Inhibitors for the Treatment of Diabetes and the Complications Associated. Research Journal of Pharmacy and Technology. 2024 Aug 1;17(8):3625-32. DOI: 10.52711/0974-360X.2024.00566
6. Jain, V., Patel, R. K., Kapadia, Z., Galiveeti, S., Banerji, M., & Hope, L. (2017). Drugs and hyperglycemia: A practical guide. Maturitas, 104, 80-83.
7. Matsumoto, Y., Sumiya, E., Sugita, T., & Sekimizu, K. (2011). An invertebrate hyperglycemic model for the identification of anti-diabetic drugs. PloS one, 6(3), e18292.
8. Patel BD, Mishra A, Chaudhary A. RP-HPLC Method Development and Validation for Simultaneous Estimation of Pyrantel Pamoate, Praziquantel, Febantel in Tablet. Research Journal of Pharmacy and Technology. 2022;15(8):3535-9. DOI: 10.52711/0974-360X.2022.00593.
9. Sudharshan, N., & Swetha, V. (2023). UV-VISIBLE SPECTROSCOPY: A COMPREHENSIVE REVIEW ON INSTRUMENTATION.
10. Patel BD, Nayak TA, Hussain SA. Development of RP-HPLC Method of Tizanidine HCL with Some Validation Parameter. Chinese Journal of Applied Physiology. 2024 Jul 9:e20240007.
11. National Center for Biotechnology Information (2024). PubChem Compound Summary for CID 163285897, CID 163285897. Retrieved July 25, 2024, from https://pubchem.ncbi.nlm.nih.gov/compound/163285897..

12. International Conference on Harmonization (ICH) of technical requirements for the registration of pharmaceuticals for human use, Validation of analytical procedures: text and methodology, Q2 (R1), Geneva 2005.
13. Mansour, N. M., El-Masry, A. A., El-Sherbiny, D. T., & Moustafa, M. A. (2024). White analytical insight for sensitive fluorescent determination of semaglutide and tirzepatide in pharmaceuticals and biological matrices. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 313, 124159.
14. Goud VM, Sirisha P, Rachana KN, Godela R, Prasad BD. QbD Approach for Analysis of Tirzepatidein its Bulk and Marketed Formulation by Stability Indicating RP-HPLC. International Journal of Pharmaceutical QualityAssurance. 2023;14(2):404-409.
15. Matthew Paul Coghlan Axel Haupt Masahiro Murakami Jeffrey Scott RIESMEYER. Therapeutic uses of tirzepatide. 2021. https://patents.google.com/patent/WO2021154593A1/en
16. Guo DewenZeng DezhiMoon Young-Kyun. Preparation method of Tirzepatide. 2020. https://patents.google.com/patent/CN110903355A/en
17. Matthew Paul Coghlan Axel Haupt Masahiro Murakami Jeffrey Scott RIESMEYER. 2021. Therapeutic uses of tirzepatide . https://patents.google.com/patent/WO2021154593A1/en
18. Patel BD, Vekaria HJ. Central Composite Design Expert-Supported RP-HPLC Optimization and Quantitative Evaluation of Efonidipine Hydrochloride Ethanolate & Chlorthalidone in Tablet. Journal of Chromatographic Science, 1 (2023) 8. DOI: https://doi.org/10.1093/chromsci/bmad077.
19. B.D.Patel, S.G.Rathi, A.M.Suthar, P.V.Dobariya. RSM-CCD coupled RP-HPLC optimization and quantitative evaluation of antihypertensive drugs in tablet. Azerbaijan chemical journal. 2024, 2, 26-35. DOI: doi.org/10.32737/0005-2531-2024-2-26-35.
20. Patel BD and Vekaria HJ. Quality by Design based method development and its validation for simultaneous estimation of Montelukast sodium and Bilastine in tablet dosage form. International Journal of Biology, Pharmacy and Allied Sciences. 11 (2022) (11) 5506-5516. DOI: https://doi.org/10.31032/IJBPAS/2022/11.11.6608.
21. Hu PengZhang LixiangWu LifenWu FengGu Zuli. 2022. A preparation method of Tirzepatide. https://patents.google.com/patent/CN115181174A/zh
22. Guo DewenZeng DezhiMoon Young-Kyun. A kind of purification method of Tirzepatide . 2019. https://patents.google.com/patent/CN110903355A/en

23. Patel DM, Chaudhary AB and Patel BD. Development and validation of RP-HPLC method for simultaneous estimation Beclomethasone Dipropionate, Phenylephrine Hydrochloride and Lignocaine Hydrochloride in cream. World Journal of Pharmacy and Pharmaceutical Sciences. 7 (2018) (5): 829-841. DOI: 10.47583/ijpsrr.2021.v69i02.019.
24. Patel B., Vekaria H. 23 Factorial design for optimization of HPLC-PDA method for the simultaneous estimation of Efonidipine Hydrochloride Ethanolate and Telmisartan in Tablet Dosage Form. Journal for Basic Sciences. 22 (2022) (12): 1383-1392. DOI:10.37896/JBSV22.12/1673
25. Patel BD, Suthar SP, Mansuri AM, Joshi VD. A Comparative Analysis of Generic Drug Assess-ment and Regulatory Approval in the USA, Europe and India. Zhongguo ying yong sheng li xue za zhi= Zhongguo yingyong shenglixue zazhi= Chinese journal of applied physiology. 2024 Oct 10;40:e20240029.