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Review Article

Review on QBD Approaches to HPLC Method Development and Validation on Tirzepatide

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A B S T R A C T

Tirzepatide, a novel dual GIP and GLP-1 receptor agonist, requires highly sensitive and robust analytical methods for quality control. This study describes a systematic Quality by Design (QbD) approach for the development and validation of a stability-indicating RP-HPLC method. The Analytical Target Profile (ATP) was defined to ensure a short runtime with high resolution. Critical Quality Attributes (CQAs), including the tailing factor (T \leq 1.5) and resolution (Rs > 2.0), were monitored against Critical Method Parameters (CMPs) such as mobile phase composition (Acetonitrile percentage), flow rate, and buffer pH. Using a Central Composite Design (CCD), the design space was established to identify the Method Operable Design Region (MODR). Forced degradation studies under acidic, alkaline, and oxidative conditions confirmed the method's stability-indicating nature, as degradants were well-resolved from the Tirzepatide peak. The results indicate that the proposed QbD-based method is cost-effective, robust, and highly suitable for routine pharmaceutical analysis.
Keywords: Tirzepatide, HPLC, QbD, Method development.

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INTRODUCTION

Type 2 Diabetes (T2DM) is a complex condition where the body struggles to manage blood sugar because the insulin-producing cells (β-cells) wear out and the body's tissues become "resistant" to insulin. Over time, this can lead to serious damage to both small and large blood vessels.While we have many existing drugs—like Metformin or SGLT2 inhibitors—most people eventually need a combination of medicines to stay healthy. Recently, incretin-based therapies (hormone mimics) have become the "gold standard" because they lower blood sugar effectively without causing weight gain or dangerous drops in blood sugar (hypoglycemia). Tirzepatide is a "first-of-its-kind" medication because it targets two different hormone receptors at once: GIP and GLP-1.By activating both, it creates a "synergy" that helps the body in four main way, Boosts Insulin: It tells the pancreas to release insulin, but only when blood sugar is actually high, Cuts Glucagon: It lowers the hormone that tells your liver to dump extra sugar into the blood , Slows Digestion: It keeps food in the stomach longer, so sugar enters the bloodstream more slowly, Weight Loss: It acts on the brain to reduce appetite, leading to significant weight reduction[1]

Administration and uses

1. **Method:** Administered via subcutaneous injection.
2. **Location:** Injection into the abdomen, thigh or upper arm.
3. **Frequency:** Once every 7days at any time of day, with or without.
4. **Dosage:** Start at a lower dosage and increased by doctor to maintain level of 5mg, 10 mg, 15 weekly.

Mechanism of action:

Tirzepatide is a synthetic polypeptide dual agonist for GLP-1 and GIP. Tirzepatide, "twincretin," exhibits distinct characteristics from GLP-1 receptor agonists. The medication comprises 39 amino acids and is an analog of the gastric inhibitory polypeptide. Functionally, tirzepatide stimulates insulin release from the pancreas and reduces hyperglycemia. In addition, tirzepatide also increases the levels of adiponectin.[2]

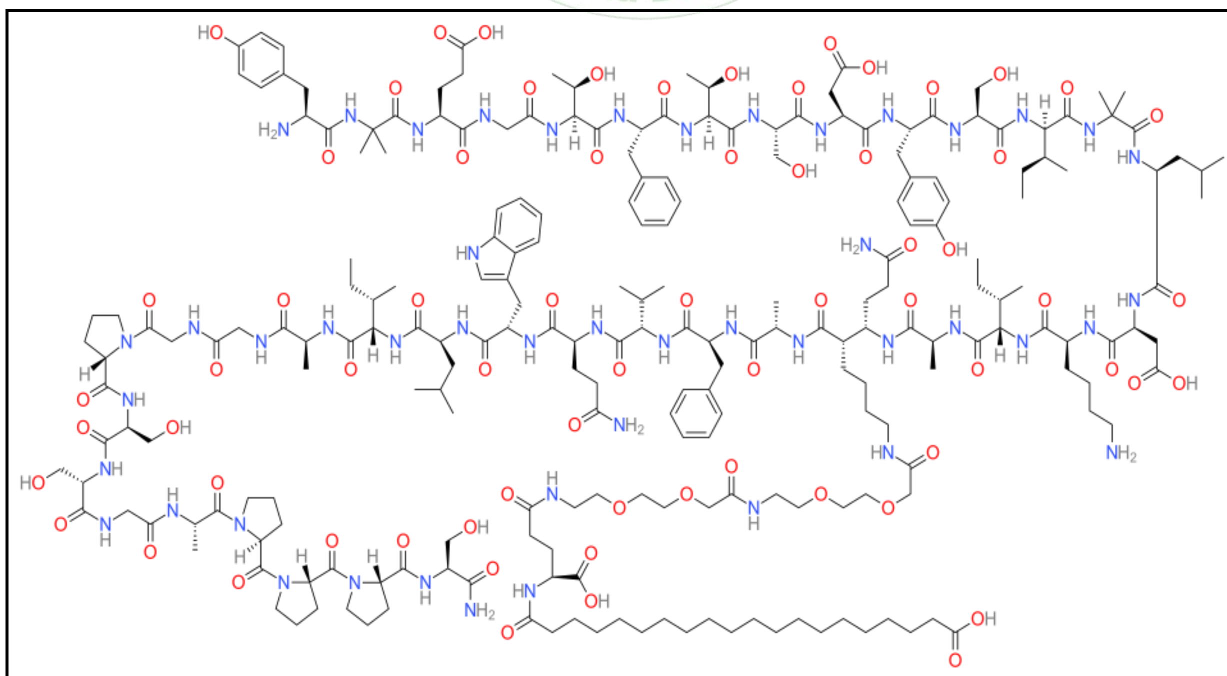
Pharmacokinetics:

- Absorption: bioavailability of tirzepatide 80%
- Distribution: volume of distribution is 10.3 L
- Metabolism: the tirzepatide are metabolise in various amino acid in various tissue, invluding liver.
- Elimination: half- life is 5day.[3]

DRUG PROFILE

Drug name	Tirzepate
Synonyme	Mounjaro
IUPAC name	25)-2-[[20[[|(5S)-6-{(2S,3S)-1-[(2S)-1-[(2S5-amino-1-25)-6-amino-1-[(25)-1-[(25)-1-(28)-1-II(25)-5-amino-1-[(25)-1-[(25)-1-[](25,35)-1-[(2S)12 [2-[(25) 2 (2S)-1-[(2S) 1 2 (25)(2S)-2-(25)-2-(25)-2-|(25)-1-amino-3-hydroxy-1-axopropan-2-ylcarbamoylpyrrolidine-1-carbonylpyrrolidine-1 carbonylpyrrolidin-1-yl]-1-ox opropon 2ylamino) 2 oxoctnylamino) 3- hydroxy-1-oxopropan-2-yllarminol-3-hydroxy-1-oxopopan-2-ylcarbamoylpyrrolidin-1-yl-2-axoethyl amino-2-oxcethyl)amino]-1-cxopropan-2-ylam ino] 3 methyl 1 oxopentan 2 ylamino) 4 methy 1-1-cxopentan-2-ylamino)-3-(1H-intal-3-yl)-1-0xopropan-2-ylamino)-1,5-dioxopentan-2-yllamınol-3-methyl-1-cxobutan-2-yl)amino]-1-oxo-3-phenylpropan-2-ylamino)-1-oxopropan-2-yllarninoj-1-oxohexan-2-ylamino)-1,5-dioxopentan-2-ylamino)-1-oxopropan-2-yl)amino)-3methyl-1- oxopentan-2-ylamino)-5-[[(2S)-2-[[(2S)-2-[2-[(2 S,3S)-2-(23)-2-(25)-2-[](2S)-2-[(25)-2-(25,3R)-2-(25)-2-(25.3R)-2-([2-[(25)-2-12-28)-2-amino-3-hydroxyphenyl)propancyl]amino)-2-methylpropanoylamino)-4-carboxybutanoyla manojacetylamino]-3-hydroxybutanoylarminol-3-phenylpropanoy(amino]-3-hydroxybutanoyamino-3-hydroxypopanoўjamino-3-carboxypropanoylamino)3-(4 hydroxyphenyl)propancyamino]-3-hydroxypropanoy(amirol-3-methylpentancyl]amino]-2-methylpropanoyl)amino]-4methylpentancyfjamino]-3-carboxypropanicyl]amino)-6-oxohexylamino)-20-oxoicosancyllamino-5-12-12-12-12-(carboxymethoxy)ethoxylethylamino)noethoxylethoxyjethylamino)-5-opentanoic acid
Chemical formula	$C_{225}H_{348}N_{48}O_{68}$
Molecular weight	4813.527 mg/mol
Solubility	Water
P^H	Slightly acidic to neutral

Structure



QUALITY BY DESING

Predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management". It implies that product and process performance characteristics need to be scientifically designed to tuinll the specific objectives. Analogous in process QbD, the outcome of QbD is a well understand, it for purpose, and robust method that consistently deliver the intended performance throughout its lifecycle, The bread knowledge obtained from this process is used to establish a method operable design (MODR), a multidimensional space based on the method factors and setting that provides suitable method performance.[4]

Method development by QbD approach

Step 1: Defining method intent

Since pharmaceutical QbD is a systematic, scientific, holistic, menace-based, and practical approach that begins with predefined objectives and lays emphasis on product and process understanding and control so the goals of HPLC method development have to be clearly defined. The eventual goal of the analytical method is to separate and quantify the main compound.[4]

Step 2: Performing experimental design

Experimental design can be efficiently used for rapid and systematic method optimization. A systematic experimental design is considered necessary to aid in obtaining profound method understanding and performing optimization. forms a chromatographic database that will help out with method understanding, optimization, and selection. In addition, it can be used to evaluate and implement the change of the method, should it It be needed in the future, for example, should the chromatographic column used no longer be commercially available, or impurity is no longer relevant.

Step 3: Evaluation of experimental results and selection of final method conditions

The conditions for the method need to be evaluated using the three-tiered approach. At first, the conditions should be evaluated for peak symmetry, peaks fronting and peaks tailing. Later these conditions should be further evaluated by using more stringent criteria, such as the tailing factor should be less than 1.5, etc.

Step 4: Performing risk assessment with robustness and ruggedness evaluation[5]

Once the final method is selected against method attributes, it is highly likely that the selected method is reliable and will remain operational over the lifetime of the product. The fourth step of method development is mainly for the method verification and finalization and the evaluation of method robustness and ruggedness to be carried out.

QbD for various analytical methods which include,

Chromatographic techniques like HPLC (For stability studies, method development, and determination of impurities in pharmaceuticals).[6]

- Hyphenated techniques like LC-MS.
- Advanced techniques like mass spectroscopy, UHPLC, and capillary electrophoresis.
- Karl Fischer titration for determination of moisture content.
- Vibrational spectroscopy for identification and quantification of compounds e.g., UV method.
- Analysis of genotoxic impurity.
- Dissolution studies.
- Biopharmaceutical processes.
- Benefit of QbD study
- Scientific understanding of pharmaceutical process and method.
- It provides a space for invention of new techniques by continuous improvement throughout life cycle.
- Critical quality attributes are identified and their effect on final quality of product is analyzed.
- It provides required design space for development.[7]

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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.[7]

Basic principle: 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.[8]

Analytical method validation

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.[9]

1. **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.[10]

2. **Accuracy**
   The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness.
3. **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.[11]
4. **Limit of detection**
   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.
5. **Limit of quantification**
   The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds.
6. **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.
7. **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 normal usa

CONCLUSION

The application of QbD to Tirzepatide HPLC development offers a significant advantage over traditional methods. By identifying Critical Method Parameters early and defining a Method Operable Design Region, the resulting method is not only validated but "future-proofed" against minor laboratory variations. This systematic approach ensures that the purity and potency of Tirzepatide can be monitored with high precision, 13. supporting the safety and efficacy of the drug throughout its commercial life. QbD approach transforms Tirzepatide HPLC analysis from a rigid procedure into a flexible, scientifically sound process. It ensures that the method remains reliable even when moved between different instruments or laboratories, which are essential for the global supply of this critical metabolic medication.

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