

HPLC Method for Simultaneous Estimation of Tirzepatide Using Analytical QbD

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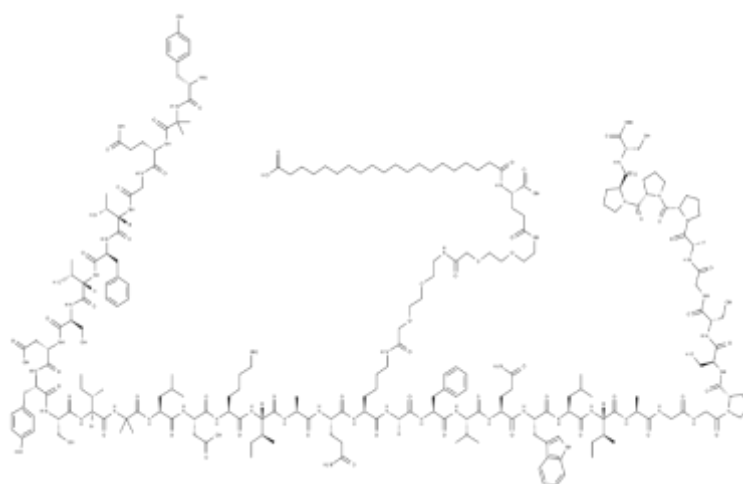
Abstract:

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 ($R_s > 2.0$), 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.

INTRODUCTION

Obesity and diabetes remain among the most important chronic metabolic disorders affecting public health worldwide. According to the World Health Organization, obesity prevalence has risen markedly over recent decades and, by 2022, one in eight people globally were living with obesity. The International Diabetes Federation likewise reports that hundreds of millions of adults currently live with diabetes, and the global burden continues to increase toward 2050. These twin disorders create a strong need for therapies that can address both glycemic control and body-weight management while remaining suitable for long-term use. The modern therapeutic shift toward incretin-based medicines has changed the way these conditions are treated. Drugs acting on GLP-1 receptor pathways have already demonstrated major clinical value, but the development of dual agonists has extended this paradigm further by acting through complementary biological mechanisms. Tirzepatide represents one of the most significant outcomes of this transition, and its growing therapeutic adoption means that laboratories must now establish robust quality-control methods for assay, release testing, stability evaluation, and ongoing product surveillance.



Tirzepatide

MATERIALS AND METHODS

Pure tirzepatide drug was purchased from Spectrum labs in Divya Enterprises, Chh.Sambhajinagar. The chemicals and reagents that were utilized in this investigation were all purchased from Aventar chemicals Pvt Ltd. in India. In order to produce the solutions, borosilicate glassware of the highest grade was utilized.

Material / reagent	Purpose
Tirzepatide API / reference standard	Calibration, system suitability, and validation
Marketed tirzepatide injection	Assay and sample analysis
Hydrochloric acid	Acid stress / pH adjustment
Sodium hydroxide	Alkaline stress / pH adjustment
Hydrogen peroxide	Oxidative stress
Membrane filters	Sample and mobile-phase filtration

RESULT AND DISCUSSION

1.1 Calibration Curve of Tirzepatide

Concentration	Absorbance
10	43.98
20	55.09
30	73.02
40	79.44
50	82.54
60	88.34
70	92.05

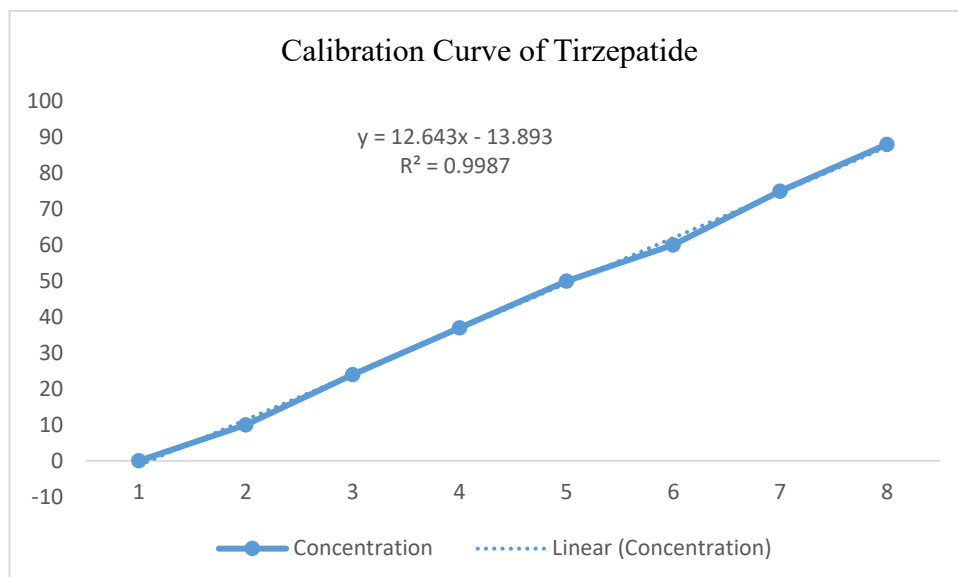


Figure 1 Calibration Curve of Tirzepatide at 234nm.

Optimized Method

The process was optimized by doing several trials from the CCD where various solvents and ratios of organic solvent system, column temperature and flow rates were examined to optimize the parameters with satisfactory system suitability.

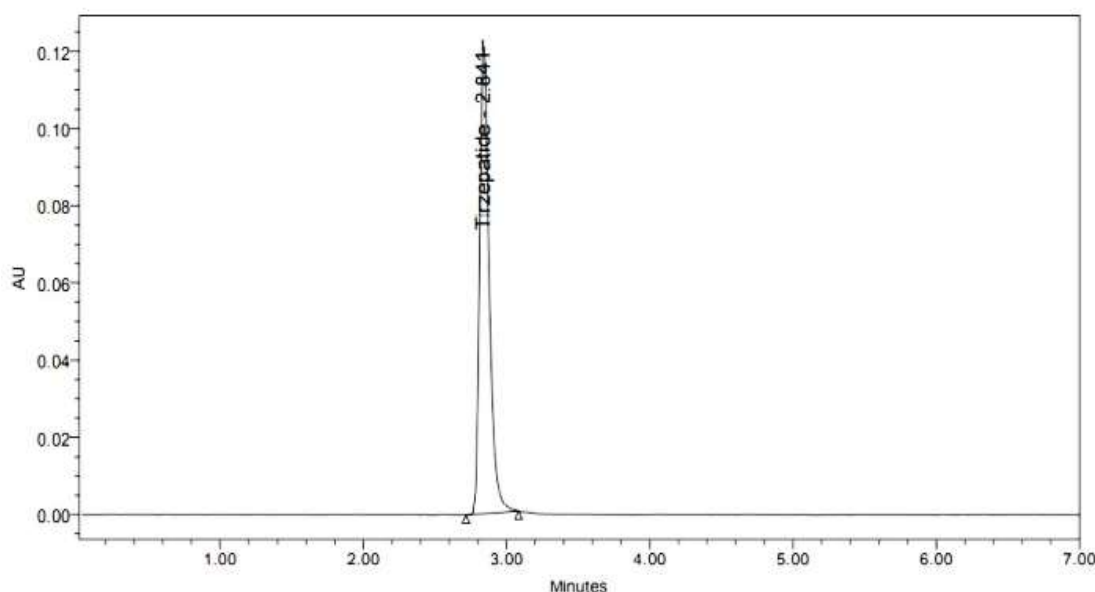


Figure 2. Chromatogram of optimized method

Linearity and Range Linearity was performed by preparing mixed standard solutions of Tirzepatide at different concentration levels including working concentration mentioned in experimental condition i.e. 10 µg/ml. Twenty micro liters of each concentration was injected in duplicate into the UPLC system.

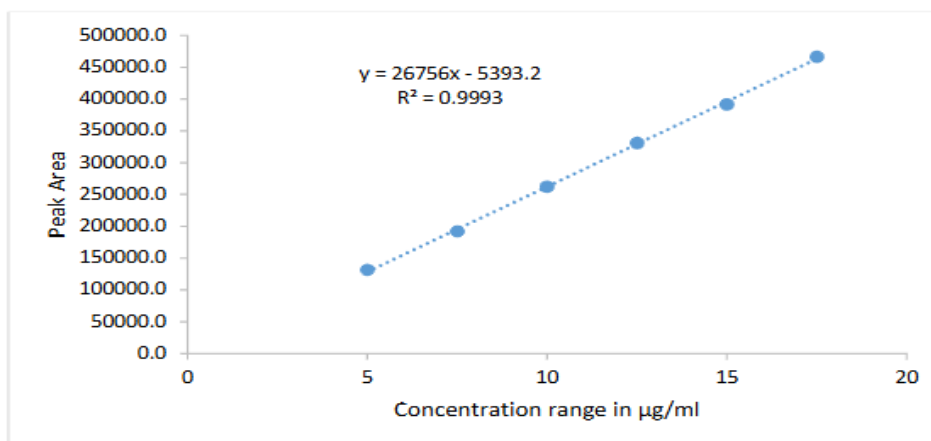


Figure 3 Linearity of Tirzepatide

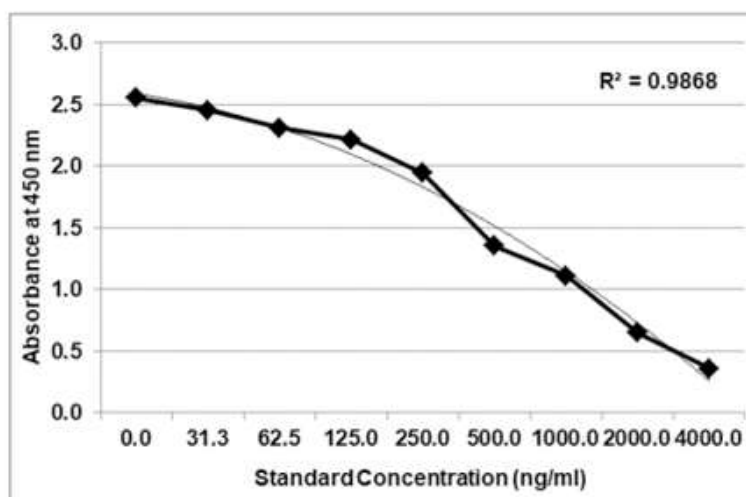


Figure 4 % Recovery outcomes of Tirzepatide

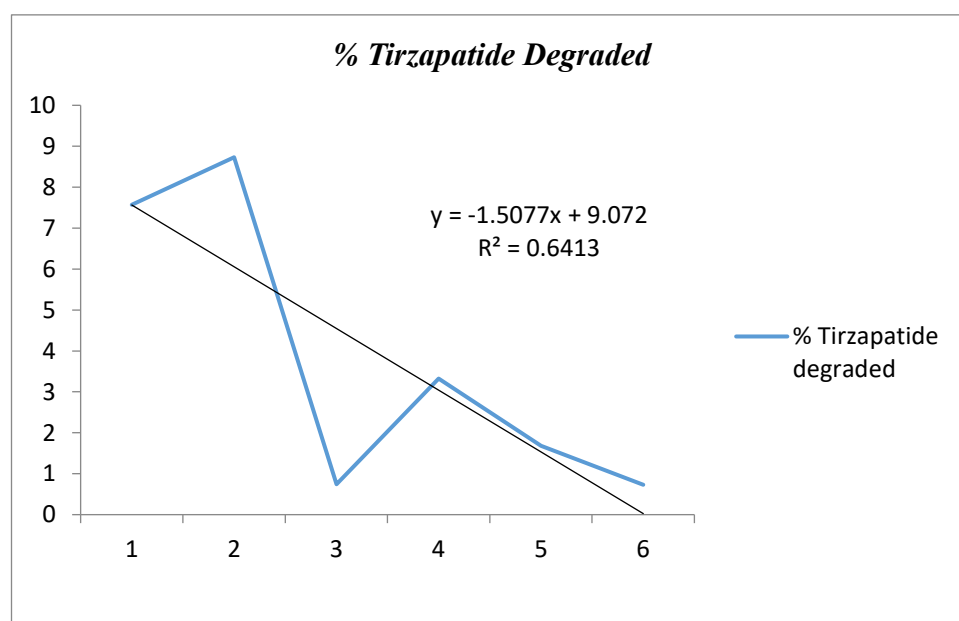


Figure 5 % Tirzepatide Degraded

Stability-indicating assays frequently deploy HPLC owing to its effectiveness in separating numerous components with high sensitivity, specificity, and resolution. The development of a stability-indicating assay method (SIAM) as described by the ICH guidelines necessitates performing forced degradation of the studied drugs under different conditions including oxidation, pH, heat, light, and others, and the separation of the studied drug from its degradates is obligatory.

SUMMARY & CONCLUSION

The evaluation of robustness should be considered during the development phase and depends on the type of procedure under study. It should show the reliability of an analysis with respect to deliberate variations in method parameters.” More generally, a method should be rugged, i.e. insensitive to factors external to the method, such as where it is run and by whom. AQBd approach to method development facilitates achieving these goals

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