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ANALYTICAL METHOD VALIDATION AND IMPURITY PROFILING OF TIRZEPATIDE: A CRITICAL REVIEW FROM A REGULATORY QUALITY ASSURANCE PERSPECTIVE

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ABSTRACT

Background: Tirzepatide, a dual glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) receptor agonist, represents a significant advancement in the management of type 2 diabetes and obesity. However, its complex peptide structure necessitates robust analytical strategies to ensure quality, safety, and efficacy. **Objective:** The review aims to critically evaluate analytical method validation and impurity profiling approaches for tirzepatide from a regulatory quality assurance (QA) perspective. **Methods:** A comprehensive assessment of reported analytical techniques, including RP-HPLC, LC-MS/MS, and SEC-HPLC, is presented, with emphasis on their applicability in method validation and impurity characterisation. **Results and Discussion:** Key validation parameters such as accuracy, precision, linearity, and sensitivity are discussed in alignment with ICH Q2 guidelines. The role of stability-indicating methods and forced degradation studies in identifying critical degradants, including deamidation and oxidation products, is highlighted. Additionally, the importance of data integrity (ALCOA+) and risk-based approaches in ensuring regulatory compliance is emphasized. **Conclusion:** Robust analytical methodologies, supported by regulatory compliance and lifecycle management, are essential for maintaining the quality of tirzepatide. This review provides a concise framework to address analytical challenges associated with

peptide therapeutics and supports the development of reliable QA strategies.

KEYWORDS: Tirzepatide, Analytical Method Validation, Impurity Profiling, Regulatory Quality Assurance, ICH Guidelines, Stability-Indicating Methods.

1. INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from defects in insulin secretion, insulin action, or both.[1] Type 2 Diabetes Mellitus (T2DM) accounts for approximately 90% of all diabetes cases worldwide and is strongly associated with insulin resistance and obesity.[4,11] The management of T2DM has evolved significantly over time, with an increasing focus on therapies that not only regulate blood glucose levels but also provide additional cardiovascular and weight-loss benefits.[11,14]

Tirzepatide is a novel, first-in-class, once-weekly subcutaneous injectable therapy that acts as a dual agonist of both glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) receptors.[7,11,16] Through the simultaneous activation of these two incretin pathways - commonly described as “twincretin” therapy - tirzepatide offers superior control along with significant reductions in body weight when compared to selective GLP-1 receptor agonists such as semaglutide.[9,12,19]

From a quality assurance (QA) and analytical perspective, tirzepatide is a structurally complex molecule. It is a synthetic 39-amino acid linear peptide containing a C20 fatty diacid moiety attached via a linker at the Lys20 position.[16,18,20] This intricate chemical structure makes the molecule highly sensitive to environmental factors such as pH, temperature, and light, which may lead to various degradation pathways, including oxidation, deamidation, and the formation of immunogenic aggregates.[3,26,29,33]

Consequently, the development of robust, stability-indicating analytical methods is a regulatory requirement in the pharmaceutical industry. According to the ICH Q2(R1) guidelines, analytical procedures must be validated to provide documented evidence of their suitability for intended use, ensuring the drug's safety, identity, and purity.[2] This review provides a comprehensive overview of reported analytical techniques such as RP-HPLC, LC-MS/MS, and SEC-HPLC used for the estimation of tirzepatide, with a focus on critical validation parameters including accuracy, precision, and linearity, in accordance with stringent regulatory quality assurance standards.[6,10,47,48,54]

2. DRUG PROFILE AND PHARMACOLOGY

Tirzepatide is a novel therapeutic agent that represents a significant advancement in the treatment of metabolic disorders.[7,11] Understanding its chemical and pharmacological profile is essential for developing accurate quality assurance (QA) protocols.[2,16]

Table 1: Comprehensive Physicochemical and Pharmacological Profile of Tirzepatide.[50]

Parameter	Technical Description & Specification
Chemical formula	$C_{225}H_{348}N_{48}O_{68}$
Drug Category	First-in-class Dual GIP and GLP-1 Receptor Agonist (antidiabetic)
IUPAC Name	synthetic 39-amino acid linear peptide with a C20 fatty diacid moiety attached to lysine at position 20
CAS Number	2023788-19-2
Regulatory Status	FDA (2022), EMA (2022), CDSCO India (2024) Approved
Molecular Weight	4813.53 g/mol (Highly complex synthetic peptide)
Appearance	White to off-white lyophilized solid / sterile clear solution
Isoelectric Point (PI)	~4.5 – 5.2 (Critical for mobile phase selection in HPLC)
Solubility	Highly soluble in water and 0.9% sodium chloride injection
Pharmacokinetics	Half-life: ~5 days. Bioavailability: ~ 80% (subcutaneous)
Metabolism	Proteolytic cleavage of the peptide backbone and beta-oxidation of the fatty acid side chain
Brand Name	Mounjaro® (for T2DM), Zepbound® (for obesity)
Dosage Form	Pre-filled single-dose pens (injections)
Route of Administration	Subcutaneous (once weekly)
Storage	Refrigerated at 2ºC to 8ºC. Protect from light and freezing

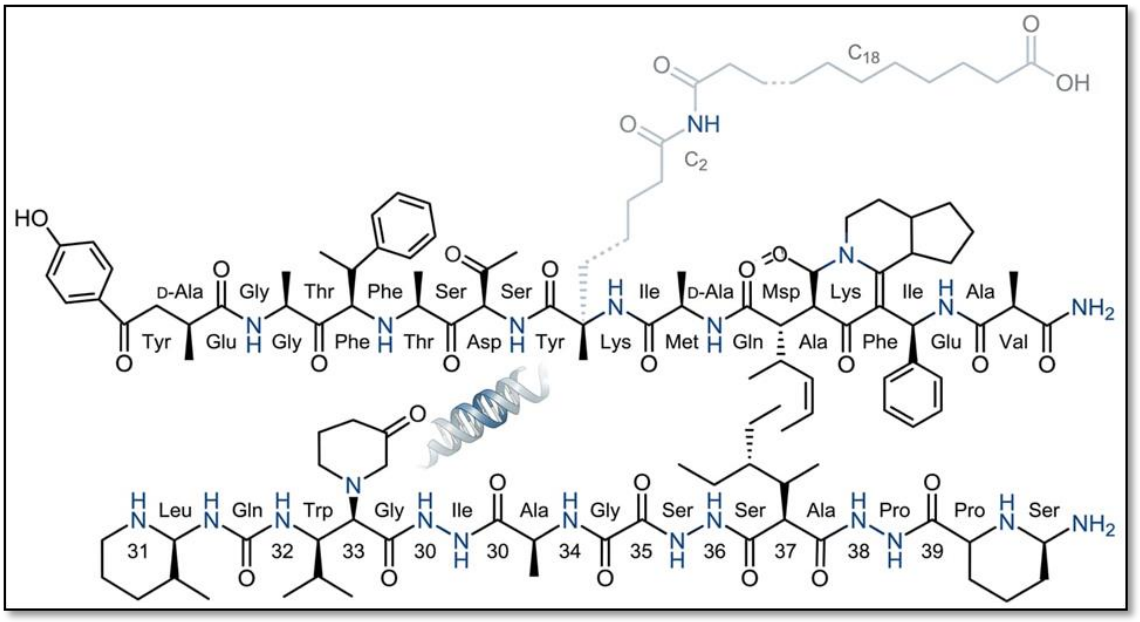

Figure 1: Chemical structure of Tirzepatide.

2.1 Mechanism of Action (MOA)

Tirzepatide acts through a unique dual agonist mechanism involving two primary incretin receptors, namely the glucose-dependent insulinotropic polypeptide (GIP) receptor and the glucagon-like peptide-1 (GLP-1) receptor.[7,11] Unlike conventional therapies that target a single receptor, tirzepatide simultaneously activates both pathways. Binding to GIP receptors enhances both the first and second phases of insulin secretion and improves metabolic flexibility.[7,16] In addition, activation of GLP-1 receptors results in suppression of glucagon secretion, thereby reducing hepatic glucose production, and also delays gastric emptying.[11,16] The combined action of these mechanisms produces a synergistic effect at the central level, leading to increased satiety and reduced food intake, which significantly contributes to its enhanced efficacy in weight management.[9,14]

2.2 Therapeutic Indications[3,8,13]

- **Type 2 Diabetes Mellitus (T2DM):** Used as an adjunct to diet and exercise to improve glycemic control in adults.
- **Chronic Weight Management:** Indicated for adults with obesity (BMI $\geq$ 30 kg/m^2) or overweight individuals (BMI $\geq$ 25 kg/m^2) with at least one weight-related comorbidity, such as hypertension or dyslipidemia.

2.3 Advantages

- **Superior HbA1c Reduction:** Demonstrates a significantly greater reduction in blood sugar levels compared to agents such as semaglutide and insulin glargine.[9,19]
- **Significant Weight Loss:** Clinical studies, particularly the SURPASS trials, have reported weight reduction of up to 20-22% of total body weight.[9,12]
- **Once-Weekly Dosing:** The long half-life allows for once-weekly administration, significantly improving patient compliance and reducing injection-related anxiety.[3]
- **Cardio-Metabolic Benefits:** Improves lipid profiles and reduces blood pressure, thereby contributing to overall cardiovascular risk reduction.[14]

2.4 Adverse Effects and Contraindications

- **Gastrointestinal:** Nausea, diarrhoea, and vomiting are commonly observed, particularly during the initial dose-escalation phase.[3,37]
- **Pancreatitis:** Rare cases have been reported; therefore, careful monitoring is recommended during therapy.[3,30]

- **Boxed Warning:** Contraindicated in patients with a personal or family history of medullary thyroid carcinoma (MTC) or multiple endocrine neoplasia syndrome type 2 (MEN 2).[3,17]
- **Immunogenicity:** Since tirzepatide is a synthetic peptide, there is a risk of anti-drug antibody (ADA) formation, which requires monitoring during long-term therapy.[29]

3. ANALYTICAL METHODS FOR THE ESTIMATION OF TIRZEPATIDE

Tirzepatide is a synthetic peptide composed of 39 amino acids, with a C20 fatty acid diacid moiety attached via a linker. Due to its high molecular weight (approximately 4813 Da) and structural complexity, the development of selective, sensitive, and robust analytical methods remains a significant challenge in pharmaceutical quality assurance (QA).[1,4]

3.1 High-Performance Liquid Chromatography (RP-HPLC)

Reverse-phase HPLC is widely accepted as a primary analytical technique for the routine analysis and purity assessment of tirzepatide. From a regulatory QA perspective, gradient elution is commonly employed to achieve adequate resolution between tirzepatide and its closely related impurities, including deamidated and oxidized variants.[6,10]

- **Column Selection:** A C_{18} stationary phase (e.g., 250×4.6 mm, 5 µm) with a pore size of approximately 300 Å is typically utilized to facilitate peptide penetration and accommodate hydrophobic interactions associated with the fatty acid side chain.[8,21]
- **Mobile Phase Chemistry:** The inclusion of 0.1% trifluoroacetic acid (TFA) or formic acid serves as an ion-pairing agent, reducing peak tailing and improving peak symmetry by masking residual silanol interactions on the silica surface.[6,9,22]
- **Detector wavelength:** Detection at 214 nm is commonly used for peptide bond absorption, whereas 280 nm is preferred for monitoring aromatic amino acid residues.[23]

3.2 Hyphenated Techniques (LC-MS/MS)

For comprehensive impurity profiling, RP-HPLC alone may be insufficient. Hyphenated techniques such as liquid chromatography-tandem mass spectrometry (LC-MS/MS) are employed for the structural elucidation of unknown degradants and impurities.[10]

- **Mass Range:** A scan range of 500–5000 m/z is typically required to detect the multiple charged ion species of tirzepatide.[3,10,31]
- **QA Utility:** LC-MS/MS plays a critical role in identifying degradation products, including deamidated species and process-related impurities generated during solid-phase

peptide synthesis (SPPS), thereby ensuring regulatory compliance and product quality.[12,35,42]

3.3 Size Exclusion Chromatography (SEC-HPLC)

Peptide-based therapeutics are susceptible to physical instability, particularly aggregation. SEC-HPLC is utilized to detect high-molecular-weight aggregates that may pose a risk of immunogenicity in patients.[15,29,30]

Aggregation is considered a critical quality attribute (CQA), and its monitoring is essential during stability studies to ensure compliance with ICH Q1A(R2) guidelines and maintain product safety and efficacy.[1,15]

Table 2: Summary of Reported Analytical Methods for Tirzepatide.

Drug	Analytical Method		Analytical Conditions	Ref.
TIR	UV-Vis		Solvent: Methanol: Water (80:20); Detection: 214nm.	[5,41]
TIR	RP-HPLC		Column: C_{18} (250 × 4.6 mm, 5 µm) Mobile Phase: 0.1% TFA in Water: Acetonitrile (gradient) Detection: 214 nm Column temperature: 25-30ºC Flowrate: 1.0 mL/min Retention time: 14.5 min Run time: 30 min	[6,10,23,27,50]
TIR	LC-MS/MS		Column: Waters BEH C_{18} (1.7 µm) Mobile Phase: Ammonium Acetate: ACN. Column temperature: 50ºC Mass range: 500-5000 m/z	[21,25,31,35]
TIR	SEC-HPLC		Column: TSKgel G2000SWxl; Mobile Phase: Phosphate Buffer (pH 6.8). Flow rate: 0.5 mL/min Detection: 214 nm	[15,29,30]
TIR	Stability-indicating RP-HPLC method		Stationary phase: Agilent Eclipse XDB C18 (150 × 4.6 mm, 3.5µm) Mobile Phase: acetonitrile-0.1% triethylamine buffer pH 2.5 (30:70 v/v) Detection: 234nm Run Time: 5 min	[51]
	Stress Conditions	% Degraded		
	Acid	4.9		
	Alkali	9.3		
	Peroxide	11.6		
	Reduction	12.6		
	Thermal	2.3		
	Photolytic	2.7		
TIR	Stability-indicating LC-		Column: C18 RP-COLUMN (100 × 2.1	[52]

<table>
<tr><td rowspan="8"></td><td colspan="2">MS/MS method</td><td rowspan="8">mm, 1.7 µm)
Mobile Phase A: 0.1% formic acid in water
Mobile Phase B: 0.1% formic acid in acetonitrile
Flow rate: 0.3 mL/min</td><td rowspan="8"></td></tr>
<tr><td>Stress Conditions</td><td>% Degraded</td></tr>
<tr><td>Acid</td><td>5.2</td></tr>
<tr><td>Alkali</td><td>9.3</td></tr>
<tr><td>Oxidative</td><td>11.6</td></tr>
<tr><td>Thermal</td><td>2.5</td></tr>
<tr><td>Photolytic</td><td>2.7</td></tr>
<tr><td rowspan="7">TIR</td><td colspan="2">Stability-indicating SEC-HPLC method</td><td rowspan="7">Column: TSKgel G2000SWxl (300×7.8mm, 5µm)
Mobile Phase: 0.1 M phosphate buffer (pH 7.0)
Flow rate: 0.5 mL/min
Detection: 214nm
Run Time: 30 min</td><td rowspan="7">[53]</td></tr>
<tr><td>Stress Conditions</td><td>% Degraded</td></tr>
<tr><td>Acid</td><td>4.5</td></tr>
<tr><td>Alkali</td><td>3.2</td></tr>
<tr><td>Oxidation</td><td>2.1</td></tr>
</table>

4. ANALYTICAL METHOD VALIDATION

Method validation is the process of establishing documented evidence that an analytical procedure is suitable for its intended purpose. As per ICH Q2(R2) guidelines, key validation parameters are essential to ensure regulatory compliance and reliability in the analysis of Tirzepatide.[2,38]

4.1 Linearity and Range

Linearity refers to the ability of an analytical method to obtain test results that are directly proportional to the concentration of the analyte within a given range. For tirzepatide, linearity is typically established over a concentration range of 5–50 µg/mL or 80–120% of the target concentration. A correlation coefficient (r^2) greater than 0.999 is generally expected to demonstrate method suitability and regulatory acceptability.[2,41]

4.2 Accuracy (Recovery Studies)

Accuracy is evaluated through recovery studies by spiking known quantities of tirzepatide into the formulation matrix. Regulatory expectations typically require mean recovery within the range of 98.0% to 102.0%, confirming that excipients do not interfere with quantification and that the method is reliable for its intended use.[2,33]

4.3 Precision

Precision reflects the degree of agreement among individual test results when the method is applied repeatedly. It is assessed at multiple levels:

- **Repeatability (Intra-day):** Evaluation under the same operating conditions over a short time interval.

- **Intermediate Precision (Inter-day):** assessment across different days, analysts, or instruments.

A % relative standard deviation (%RSD) of less than 2.0% is typically considered acceptable, indicating good method consistency and reproducibility.[2,38]

4.4 Limit of Detection (LOD) and Limit of Quantitation (LOQ)

For impurity profiling, LOD and LOQ are the most critical parameters to the ensure sensitivity of the analytical method.

- **LOD:** The lowest concentration of an analyte that can be reliably detected but not necessarily quantified.
- **LOQ:** The lowest amount that can be quantified with acceptable accuracy and precision.

Given the high potency and complexity of tirzepatide, analytical methods must demonstrate sufficiently LOQ values (typically in the nanogram range) to enable detection and quantification of trace-level impurities in compliance with regulatory requirements.[2,34]

4.5 Robustness and System Suitability

Robustness evaluates the ability of an analytical method to remain unaffected by small, deliberate variations in method parameters, such as changes in flow rate (± 0.1 mL/min) or mobile phase pH (± 0.2). This parameter ensures the reliability of the method under normal usage conditions and is a critical component of method validation as per regulatory expectations.[2,38] System suitability testing is performed prior to analysis to verify that the analytical system is functioning properly. Key parameters such as tailing factor (≤ 2.0) and theoretical plates (≥ 2000) must meet predefined acceptance criteria to ensure system performance and data integrity.[23,44]

Table 3: Typical Validation Parameters for Tirzepatide (Summary of Literature).

Validation Parameter	Acceptance Criteria (ICH Q2)	Reported Results for Tirzepatide	Regulatory Significance
Linearity (r2)	≥ 0.999	0.9995-0.9999	Demonstrates proportional response.
Accuracy (% Recovery)	98%-102%	99.2-100.8%	Confirms accuracy of quantification
Precision (%RSD)	≤ 2.0%	0.4%-1.2%	Ensures method reproducibility
LOD	Signal-to-Noise ≥ 3:1	0.05-0.15 µg/mL	Enables detection of trace impurities
LOQ	Signal-to-Noise ≥ 10:1	0.15-0.45 µg/mL	Define minimum quantifiable limit.

Robustness	No significant variation	Complies under small changes	Ensures method reliability

5. IMPURITY PROFILING OF TIRZEPATIDE

Impurity profiling is a regulatory requirement to ensure the safety and efficacy of peptide-based therapeutics. According to ICH Q3A(R2) and Q3B(R2), impurities in tirzepatide must be identified, characterized, and quantified. These impurities are generally classified into two categories: process-related and product-related (degradants).[33,34]

5.1 Process-Related Impurities

These impurities arise during the solid-phase peptide synthesis (SPPS) of tirzepatide.

- **Deletion Sequences:** Missing amino acids during the coupling process.
- **Truncated Peptides:** Shortened chains formed due to incomplete reactions.
- **Residual Solvents and Catalysts:** Trace amounts of piperidine, DMF, or trifluoroacetic acid used during synthesis.[35]
- **Regulatory QA Significance:** These impurities are controlled through stringent in-process control (IPC) to ensure compliance with predefined purity specifications.[20,33]

5.2 Product-Related Impurities (Degradation Products)

Tirzepatide is highly sensitive to environmental stress. The major degradation pathways include:

- **Deamidation:** Conversion of asparagine (Asn) or glutamine (Gln) residues into aspartic acid or glutamic acid, representing the most common degradation pathway in peptides.[20,25]
- **Oxidation:** Primarily affects susceptible amino acid residues under exposure to light, oxygen, or peroxides.[25,26]
- **Aggregation (Physical Impurity):** Formation of dimers, trimers, or higher-order aggregates, which can significantly increase immunogenic risk.[29]

5.3 Characterization of Unknown Impurities

When a new impurity peak appears in the HPLC chromatogram (above the identification threshold of 0.10%), regulatory guidelines require structural elucidation of the impurity.[33, 34]

- **Role of LC-MS/MS:** Tandem mass spectrometry is employed to determine the molecular mass and fragmentation pattern of the impurity, enabling identification of structural modifications in the peptide chain.[31,48]

- **Peak Purity Analysis:** Use of a Photo-Diode Array (PDA) detector helps confirm peak homogeneity and ensures that the main tirzepatide peak is not co-eluting with unidentified impurities.[23,10]

Table 4: Common Impurities and Degradation Products in Tirzepatide.

Types of impurity	Mechanism	Detection Method	Regulatory Limit (ICH)	Ref.
Deamidated Peptide	Hydrolysis at specific pH	RP-HPLC/LC-MS	< 0.15%	[20, 25, 33]
Aggregates	Temperature/Agitation	SEC-HPLC	NMT 1.0%	[26, 29]
Deletion Sequences	Incomplete Synthesis	LC-MS/MS	< 0.10%	[20, 35]
Oxidation Products	Exposure to Light/Oxygen	RP-HPLC	< 0.15%	[25, 26, 34]
Residual Solvents	Manufacturing Process	GC-HS	As per ICH Q3C	[36, 39]

6. REGULATORY QUALITY ASSURANCE (QA) PERSPECTIVE

From a Regulatory Quality Assurance (QA) perspective, the analysis of tirzepatide is not merely about generating data but ensuring the data integrity, safety, and compliance of the therapeutic peptide. QA acts as the bridge between analytical development and final patient safety.[2,38,42]

6.1 Compliance with ICH Guidelines

The regulatory framework for tirzepatide is governed by the International Council for Harmonisation (ICH).

- **ICH Q2(R1):** Governs the validation of every analytical procedure used in the purity and assay testing.[2]
- **ICH Q3A/B:** Sets the thresholds for reporting, identification, and qualification of impurities. For tirzepatide, any impurity above 0.1% must be reported to the regulatory authorities.[34,33]
- **ICH Q1A(R2):** Dictates the stability testing protocols to ensure the drug remains effective throughout its shelf life.[26]

6.2 Data Integrity and Documentation (ALCOA+ Principles)

Regulatory agencies (FDA and EMA) place heavy emphasis on data integrity.

- **Audit Trails:** Every HPLC run must have a secure, time-stamped audit trail that shows who performed the test and if any changes were made to the integration parameters.[32,39]

- **ALCOA+ Concept:** Data must be attributable, legible, contemporaneous, original, and accurate. In Tirzepatide analysis, this prevents "testing into compliance" (repeating tests until a passing result is found).[39,42]

6.3 Specification Setting and Release Testing

QA professionals are responsible for setting the "specifications" (the pass/fail limits).

- **Assay Limits:** Typically, 95.0% to 105.0% of the labeled amount.[2,41]
- **Impurity Limits:** Total impurities must often be NMT (Not More Than) 1.5%-2.0%, with individual unknown impurities NMT 0.10%.[33,34]
- **Out of Specification (OOS):** Any result outside these limits triggers a formal QA investigation to find the "root cause".[2,38,42]

6.4 Quality Risk Management (QRM)

According to ICH Q9, quality risk management (QRM) involves the systematic identification, assessment, and control of risk associated with the analytical and manufacturing processes. For tirzepatide, a key risk is peptide aggregation, which can impact product quality and patient safety.[29,42]

QA ensures that appropriate analytical techniques such as SEC-HPLC are routinely employed to monitor and control high molecular weight aggregates, thereby mitigating the risk of immunogenicity and potential adverse effects.[29,30,54]

Table 5: Regulatory QA Checklist for Tirzepatide Method Lifecycle.

QA Focus Area	Regulatory Requirement	Analytical Control
Method Validation	ICH Q2(R2) Compliance	Comprehensive validation of accuracy, precision, specificity, LOD, and LOQ
Impurity Control	ICH Q3A/Q3B Guidelines	Identification and quantification of impurities above the reporting threshold (0.10%)
Stability Testing	ICH Q1A(R2) Guidelines	Long-term and accelerated stability studies
Data Integrity	ALCOA+ Principles & 21 CFR Part 11	Use of compliant software with audit trails and secure data handling
Patient Safety	ICH Q9 (QRM)	Monitoring of peptide aggregation using SEC-HPLC to mitigate immunogenicity risk

7. CHALLENGES AND FUTURE PERSPECTIVES

Despite significant advancements in analytical techniques, the characterization of tirzepatide

continues to present unique challenges due to its complex peptide structure and stringent regulatory expectations.[1,38]

7.1 Current Analytical Challenges

- **Complex Matrix Interference:** Analysis of tirzepatide in its final injectable formulation is challenging due to the presence of excipients such as *m-cresol* and glycerol, which may interfere with UV detection and affect method specificity.[6,21]
- **Peptide Adsorption:** Tirzepatide exhibits nonspecific adsorption to surfaces such as glass vials, plastic containers, and chromatographic systems, potentially leading to reduced recovery and underestimation of analyte concentration.[44,12]
- **Distinguishing Related Substances:** The identification and differentiation of structurally similar impurities, including site-specific deamidation variants, require high-resolution analytical techniques such as high-resolution mass spectrometry (HRMS), which may not be readily accessible in routine quality control laboratories.[3,31]

7.2 Future Perspectives

- **Automation and AI in QA:** The integration of artificial intelligence (AI) into chromatographic data systems is expected to enhance automated peak integration and support Out-of-Specification (OOS) investigations, thereby reducing human error and strengthening data integrity in compliance with regulatory expectations.[39,42]
- **Multi-Attribute Method (MAM):** There is a growing shift towards LC-MS/MS-based Multi-Attribute Method, which enables simultaneous identification, quantification, and impurity profiling. This approach aligns with regulatory expectations for lifecycle management and improved analytical efficiency in QA environments.[31,45]
- **Green Analytical Chemistry (GAC):** Future regulatory frameworks are expected to emphasize the adoption of environmentally sustainable analytical methods, including the use of less hazardous solvents and reduced waste generation, in line with green chemistry principles and regulatory sustainability initiatives.[20,43]

8. CONCLUSION

The analytical landscape for tirzepatide represents a complex intersection of advanced analytical techniques and stringent regulatory expectations. This critical review highlights that while RP-HPLC remains the foundational tool for routine assay and purity assessment, advanced hyphenated techniques such as LC-MS/MS and SEC-HPLC are essential for

comprehensive impurity profiling and the detection of peptide aggregates. From a regulatory quality assurance perspective, analytical success extends beyond obtaining acceptable data and integrity maintained throughout the analytical lifecycle. The application of a regulatory framework ensures consistency, traceability, and compliance in analytical practices. As the clinical and commercial use of tirzepatide continues to expand, there is an increasing need for stability-indicating methods capable of addressing evolving degradation pathways and complex impurity profiles. Future advancements in analytical technologies, combined with lifecycle management approaches, will play a critical role in enhancing method performance and regulatory compliance. Ultimately, adherence to ICH guidelines and the implementation of ALCOA+ principles will remain central to ensuring data integrity, thereby safeguarding the quality, safety, and efficacy of tirzepatide for global patient populations.

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