The Significance of Monitoring Impurities in GLP-1 drugs

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Glucagon-like peptide-1 (GLP-1) receptor agonists, such as liraglutide, semaglutide, exenatide, and tirzepatide, have transformed the treatment landscape for type 2 diabetes and obesity. By stimulating insulin secretion, suppressing glucagon release, and slowing gastric emptying, these peptide therapeutics provide powerful metabolic control resulting in beneficial weight loss. However, the nature of their peptide structure makes them highly susceptible to chemical and physical degradation. Impurities - whether arising from synthesis, formulation, storage, or degradation pathways - pose significant risks. Identifying and controlling these impurities is therefore a cornerstone of pharmaceutical development and quality assurance.

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Why do impurities matter?

In the context of analytical chemistry, an impurity is any substance present in a chemical sample that is not the intended compound, in this case the Active Pharmaceutical Ingredient (API). For therapeutic peptides like GLP-1s, impurities such as truncated sequences, misfolded aggregates or process impurities (e.g., oxidised or reduced forms, deamidation, phosphorylation, and acylation) can trigger immune responses, toxicity or increased organ stress, effecting consumer safety.

These same impurities can alter the pharmacokinetics of the drug, changing the absorption, distribution, metabolism and elimination profiles, thereby reducing therapeutic efficacy. Degradation products resulting from storage, shipment or other environmental exposures can significantly reduce the potency of the drug product, and the dose reliability, compromising treatment outcomes.

For these, and many other reasons, there are significant regulatory compliance requirements from various regulatory agencies (e.g. ICH, EMA, European Pharmacopeia, USP, etc) related to impurity profiling, identification and quantification, and validation to ensure the risks that impurities cause are evaluated, monitored and controlled.

Types of Impurities

Impurities in GLP-1 drugs can be classified according to their origin within the production and lifecycle process. Regarding the GLP-1 Drug Substance, or API, the most common source of impurities is the manufacturing process. Each GLP-1 manufacturer has their own, unique manufacturing process that produces their own, unique process impurities. Whether it be a recombinant process, which uses a genetically engineered organism to produce the GLP-1 peptide, or a synthetic process which assembles the amino acids in the desired sequence, all processes have the potential to create a myriad of byproducts, many of which are structurally similar to the target GLP-1 peptide, often referred to as related substances. These impurities often include D-amino acid isomers, truncated sequences, oxidised or reduced forms and others arising from deamidation, phosphorylation, and acylation. Often, these process impurities have to be monitored throughout the manufacturing process, and in some cases there are final purification steps taken to remove them from the API Final Form.

The next source for impurities is the drug formulation- where the API is combined with non-active substances, called excipients, to produce a final, safe and effective drug product. Excipients can affect pH, buffer composition, and stability of the drug product, potentially leading to degradation. In addition, most GLP-1 drug products are injectable medications, complicating the formulation and emphasising stability requirements. One example of this increased focus is aggregation- the formation of multi-peptide molecules that form through non-covalent interaction. Aggregation can affect immunogenicity of the drug, as they can be recognised as antigens leading to adverse immune reactions.

The stability of the final form drug product is one of the most important sources of impurities to be monitored. The storage, shipping and handling of injectable GLP-1 drug products create ample opportunities for the creation of degradation impurities, which could affect the dosage, toxicity and overall safety of these therapeutics. As part of regulatory requirements, the many degradation pathways are monitored. The drug product is often subjected to a series of stress tests, which included acidic degradation (e.g., 0.1 mL of 1N Hydrochloric Acid at room temperature for 5 hours), basic degradation (e.g., 0.1 mL of 0.1N Sodium Hydroxide at room temperature for 4 hours), oxidative degradation (e.g., 0.1 mL of 1% Hydrogen Peroxide at room temperature for 1 hour), and thermal degradation (e.g., 80°C water bath for 15 hours) in order to identify and monitor the possible degradation impurities throughout the drug's life cycle.

Separation and Identification

Throughout the development, manufacturing and Quality Assurance/Quality Control of GLP-1 treatments, several techniques are used to separate, identify and monitor the various impurities from the various sources. One of the more popular techniques deployed is High Performance Liquid Chromatography (HPLC) due to the specificity required to separate these very closely related compounds. Combining different HPLC modes with different detection systems creates ample opportunity for high resolution, sensitivity, selectivity and reproducibility in separating these complex peptide mixtures.

Reversed Phase Chromatography is the most common technique used in the analysis and purification of GLP-1s, due to its high efficiency and selectivity for the closely related chemical properties of the peptide impurities, and its compatibility with many valuable detectors. For example, process impurities and degradation products are analysed in separate reversed phase methods, due to their unique profiles and place within the process, however both methods are often similar in approach. In addition, these impurities are so closely related, often differing by small differences in amino acid sequences, Ultra-High-Performance Chromatography (UHPLC) is often deployed to maximise chromatographic efficiency, enhancing the resolution between the API and impurities. Another important factor in achieving effective separation of these impurities is careful selection of the stationary phase of the chromatographic media. Enhanced retention and selectivity are necessary to distinguish between the numerous peptide impurities that closely resemble one another hydrophobically, in polarity, and often structurally. Analytically, highly efficient reversed phase separations are critical in many analytical methodologies which separate and monitor the myriad of impurities mentioned. In addition, many GLP-1s are purified in a final step, removing challenging, low level impurities by utilising large scale reversed phase chromatographic processes, often at multi-kilogram scale, to achieve the >98-99% purity needed for therapeutic use.

Size-Exclusion Chromatography is a mode of chromatography commonly utilised in monitoring impurities in GLP-1 drugs, specifically to monitor peptide aggregation in the manufacturing process and in storage and stability of final drug product forms.

Ion-exchange chromatography (IEX) is used in GLP-1 impurity analysis and purification by exploiting charge differences between the target peptide and impurities. It allows selective binding and elution of different charges species, complimenting Reversed Phase analysis.

All of these separation techniques, RP-HPLC, SEC, IEX and the UHPLC forms of each, can be combined with different detection techniques, each with their own benefits towards identification and sensitivity.

Due to their structural complexity and impurity diversity of GLP-1 impurities, multiple techniques are often needed to properly detect and characterise the various analytes.

Ultraviolet Detection (UV) is the most common detection for most of the mentioned analysis due to its universality, quantitative accuracy, cost effectiveness and relative ease of use.
Where UV lacks selectivity and specificity, Mass Spectrometry (MS) can be used with most reversed phase, and SEC methods to provide information related to the molecular weight, fragmentation and structure of the analytes that can aid significantly in identification.

Multi-angle light scattering (MALS) is commonly used in the SEC aggregate analysis to also aid in determining molecular weight of the higher order structures. In combination, these and other detection techniques are used to characterise, and accurately quantity the myriad of impurities possible in the complexity of GLP-1 drugs.

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Conclusion

The significance of separating, identifying and monitoring impurities in GLP-1 drugs cannot be overstated. These therapeutics are at the forefront of managing diabetes and obesity, but their complexity makes them vulnerable to degradation and impurity formation. Patient safety, therapeutic efficacy, and regulatory compliance all hinge on robust impurity profiling. Advances in analytical science are enabling more precise detection and characterisation, ensuring that GLP-1 drugs remain safe, effective, and reliable. As the market for these therapies expands, impurity identification will remain a defining factor in their success.