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Impurity Analysis & Quality Control for Solid-Phase Synthesis of Hypoglycemic Peptide Drugs

1.Research Background

GLP-1 based hypoglycemic peptides have become first-line clinical medicines for type 2 diabetes treatment, with semaglutide, liraglutide and exenatide dominating the global diabetes drug market due to outstanding effects on blood glucose reduction, weight loss and cardiovascular protection. With soaring market demand worldwide, solid-phase peptide synthesis (SPPS) has evolved into the dominant industrial production route for such peptide APIs, thanks to its convenient operation, high automation and scalable production advantages compared with liquid-phase synthesis and microbial fermentation. Nevertheless, SPPS still faces prominent quality challenges triggered by diversified related impurities generated during amino acid coupling, Fmoc deprotection, resin cleavage and finished-product storage. Various byproducts including deletion peptides, racemic isomers and high-molecular aggregates may alter pharmacological activity, raise immunogenic risk and cause adverse clinical reactions. Therefore, systematic investigation of impurity formation rules is essential

for stable industrial manufacturing of high-quality hypoglycemic peptide products.

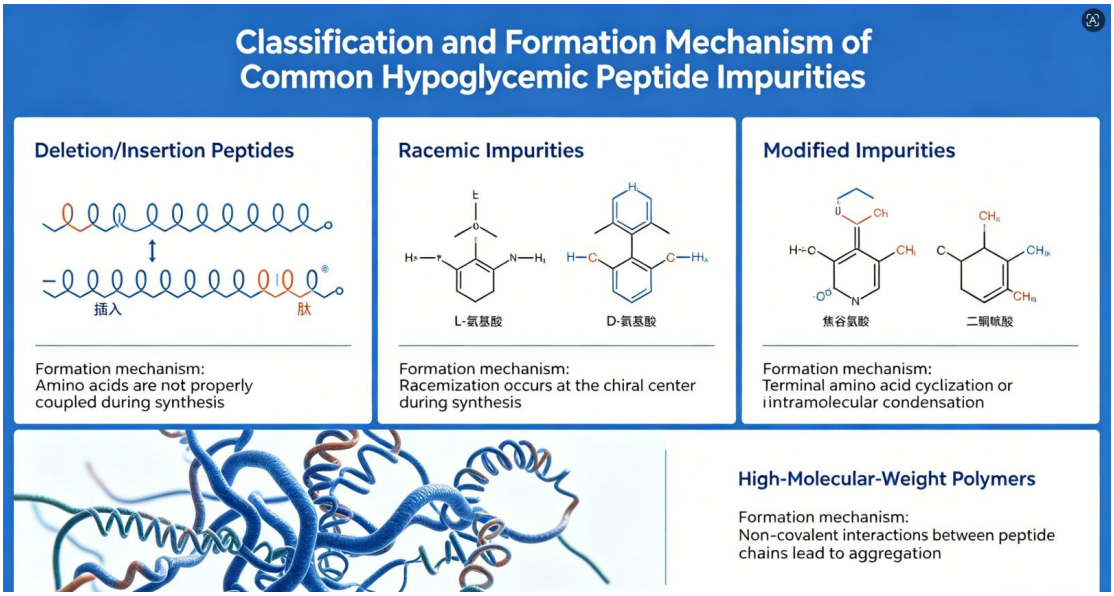
2.Research Purpose and Significance

This paper systematically reviews the origin, formation mechanism and classification of impurities generated throughout the solid-phase synthesis workflow of mainstream GLP-1 hypoglycemic peptides. It sorts out characteristic side reactions of typical amino acid residues and summarizes feasible process optimization strategies for impurity suppression. The summarized research data can serve as practical technical reference for pharmaceutical enterprises to upgrade synthesis parameters, improve purification procedures and establish standardized impurity limit specifications. Meanwhile, the research provides theoretical guidance for reducing production costs while complying with global pharmacopoeia quality requirements, accelerating the industrial upgrading of domestic hypoglycemic peptide preparations.

3.Research Content

Impurity formation in SPPS is mainly driven by incomplete coupling, side-chain chemical transformation and long-term storage degradation. Different amino acid residues show unique side reaction tendencies: Glutamine and asparagine tend to cyclize into pyroglutamate impurities under acidic or alkaline conditions; serine, threonine and tyrosine are prone to β-elimination degradation in alkaline environment; cysteine easily

undergoes oxidation and intermolecular dimerization; proline generates diketopiperazine by-products; tryptophan and histidine suffer from racemization and alkylation; hydrophobic residues like valine, isoleucine and alanine form difficult sequences which lead to incomplete amino acid attachment.



According to structural features, related substances are divided into four core categories: First, deletion (Des) and insertion (Endo) impurities originating from incomplete Fmoc removal or insufficient amino acid activation; Second, D-configured racemic impurities resulting from chiral center inversion during coupling, which severely change peptide bioactivity; Third, modified impurities including acetylation, amidation and side alkylation derivatives induced by residual protecting groups; Fourth, high-molecular-weight polymers (HMWP) formed via intramolecular hydrophobic stacking, which are closely linked to elevated drug immunogenicity.

Proper selection of condensation reagents effectively cuts down impurity generation. The combination of DIC-HOBt or PyBOP-HOBt can remarkably inhibit asparagine dehydration and amino acid racemization. In addition, raw material purity, cleavage cocktail formulation, pH control and finished-product storage temperature all exert notable influences on final impurity profile. Practical comparison between synthetic and biotechnologically-produced liraglutide reveals obvious differences in impurity composition and immunogenic performance, while semaglutide presents lower inherent immunogenic risk under optimized manufacturing processes.

4.Conclusion & Outlook

Impurity generation of hypoglycemic peptides is affected by amino acid property, condensation system, reaction pH and post-processing conditions simultaneously, and rational optimization of raw material screening, coupling procedure and cleavage condition can efficiently reduce total impurity level. Looking ahead, further research will focus on developing novel low-toxicity anti-racemization coupling reagents, optimizing synthetic routes for difficult peptide sequences and innovating high-efficiency chromatographic purification technologies. Continuous technological iteration will further simplify impurity spectrum, enhance API purity and medication safety, facilitate large-scale high-purity production of GLP-1 peptides and promote the popularization of safe, cost-

effective hypoglycemic peptide drugs in global clinical application.