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Advances in Peptide Chemical Synthesis Technology and Current Status of Peptide Quantification Development

Peptides are bioactive molecules composed of amino acids linked by amide bonds, usually less than 10 kDa, which play a key role in physiological processes such as immunomodulation, hormone regulation, antioxidant, and anti-cancer, and have been widely used in biomedicine, food, cosmetics and new materials, with important economic and application value. As the peptide product market continues to expand, its efficient synthesis technology and standardized quality control have become the focus of the industry. This paper systematically sorts out the advantages and disadvantages of the mainstream technology of peptide chemical synthesis, and reviews the development status, application scenarios and core challenges in the field of peptide metrology, so as to provide reference for peptide product research and development and quality control.

The preparation methods of peptides mainly include natural extraction, enzymatic hydrolysis, fermentation, genetic recombination and chemical synthesis. Among them, chemical synthesis has become the mainstream preparation method of peptide drugs due to its advantages such as short

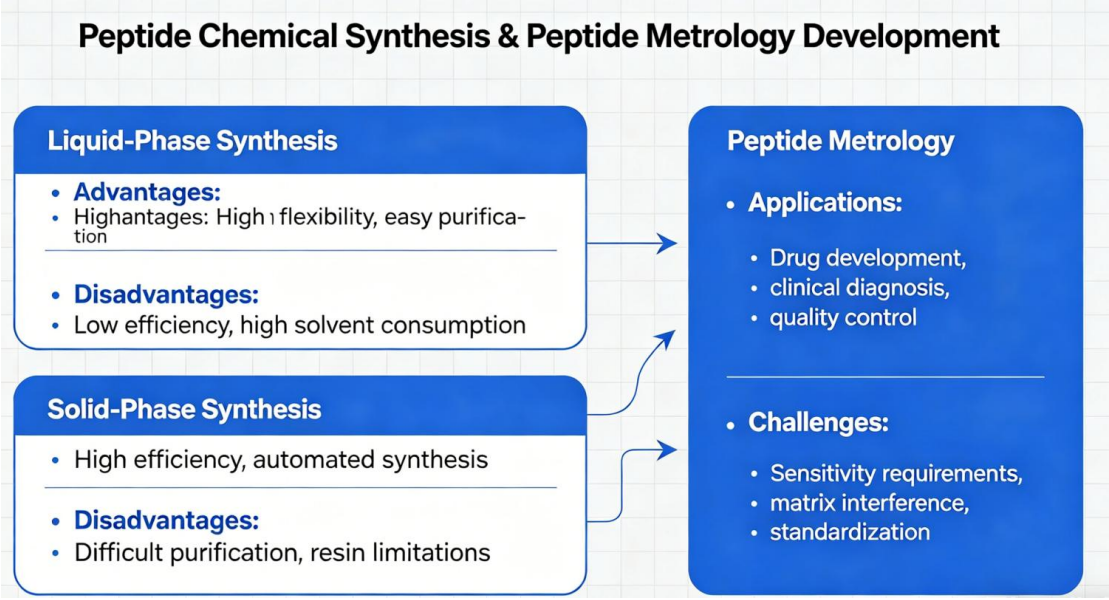
research and development cycle, flexible operation, and easy automation, and more than 90% of peptide drugs on the market are produced using this technology. Chemical synthesis is mainly divided into two routes: liquid phase synthesis and solid phase synthesis.

Liquid phase synthesis is the earliest developed peptide synthesis method, which is carried out in homogeneous solution, and it is necessary to selectively protect and activate the amino groups, carboxyl groups and side chain active groups of amino acids. This method has the advantages of mild reaction conditions, low cost, separable and purified intermediates, and easy large-scale production, and is suitable for the synthesis of short-chain peptides and cyclic peptides. However, the automation of liquid phase synthesis is low, and each step of the reaction needs to be purified, which is cumbersome, long cycle, and has a large product loss, which limits its application in the preparation of long-chain peptides.

Solid-phase peptide synthesis (SPPS), established in 1963, revolutionized peptide preparation and is now the technology of choice in laboratories and industry. The core principle is to fix the first amino acid on the insoluble resin carrier, gradually extend the peptide chain through the cycle of "coupling-washing-deprotection-neutralization", and finally cleave the peptide from the resin and remove the side chain protection group to obtain the product. Solid-phase synthesis is easy to operate, over-feed ensures complete reactions, and is easy to automate, allowing for efficient synthesis

of peptides up to 50 amino acids. However, there are still obvious shortcomings in this method, such as the large use of toxic solvents such as DCM and DMF, high reagent consumption, high cost, and outstanding environmental pressure.

As peptide synthesis technology continues to mature, accurate measurement and standardized control of product quality are becoming increasingly important, and peptide/protein measurement has become a key area to support the development of industry norms. Biometrics aims to achieve traceability and reproducibility of measurement results, and ensures the accuracy and consistency of peptide content, purity, activity and structure determination by establishing standard methods, reference materials and measurement value transfer systems. At present, quantitative technologies such as isotope dilution mass spectrometry and mass balance method are becoming more and more perfect, and more than ten peptide reference materials such as insulin, oxytocin, angiotensin, and natriuretic peptides have been successfully developed, and calibration specifications for relevant measurement equipment have been gradually released to provide metrological support for clinical testing, biomedicine, food safety and other scenarios.



Peptide quantification has achieved significant applications in clinical diagnostics, toxin detection, food nutrition analysis, vaccine evaluation, and other fields. In clinical practice, precise quantification of peptide biomarkers such as insulin, C-peptide, and natriuretic peptides can provide reliable evidence for disease diagnosis; in the food field, traceable measurement of nutritional components can ensure the accuracy of ingredient labeling; vaccine and toxin detection rely on standard substances to achieve comparable and reliable results. However, peptide quantification still faces many challenges: some peptides undergo complex post-translational modifications, making SI (International System of Units) traceability difficult; the variety of standard substances is insufficient, especially for cyclic peptides, long-chain peptides, and modified peptides; the standardization of measurement methods is not high, and data consistency across different platforms needs improvement.

In summary, chemical synthesis of peptides has formed a complementary technical system of liquid-phase and solid-phase synthesis, with solid-phase synthesis dominating due to its efficiency and automation advantages. As a core means of quality assurance, peptide quantification has made important progress in standard substances, traceability systems, and measurement methods, but basic research and technological innovation still need to be strengthened. In the future, with continuous breakthroughs in green synthesis, continuous-flow synthesis, and high-precision quantification, the peptide industry will be further promoted toward high-efficiency, green, and standardized high-quality development.

Reference： Zhao, Y., Zhao, H. B., Leng, X. J., Zhao, X. N., & Wang, B. Y. Advances in Peptide Chemical Synthesis Techniques and the Current Status of Peptide Metrology[J]. Metrology Science and Technology, 2024, 68(12): 39-44.