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Peptides: Best Practices On Regulatory & Control Strategies, Analytical Methods, & More

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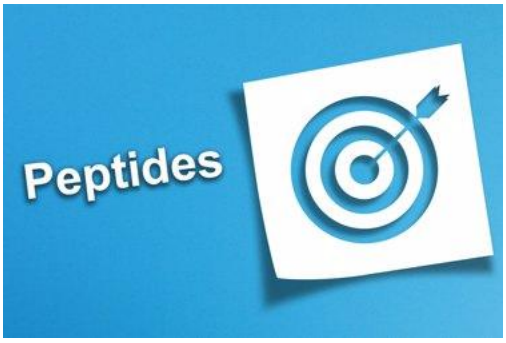

Industry, regulatory, and academic experts gathered at the United States Pharmacopeial Convention's (USP's) annual Peptides & Oligonucleotides Workshop virtually March 1-5, 2021, to share best practices on chemistry, manufacturing, and controls (CMC) for these rapidly growing classes of therapeutics. USP and the BIO1 Peptides & Oligonucleotides Expert Committee use these workshops to help inform development of new standards that can help support and ensure quality of these therapeutics. In response to recent manufacturing and analytical advances, the 2021 workshop steering committee assembled a program that covered regulatory and control strategies, innovative analytical approaches, raw material characterization, and formulation and delivery advances across both peptide and oligonucleotide therapeutic classes. This summary shares highlights from the talks related to control of commercialized therapeutic peptides as well as for those in development.

Regulatory Considerations For Peptides: Impurities And Immunogenicity

Regulators and regulatory experts reviewed current requirements and considerations for peptides. In the United States, when peptides (40 amino acids or less), produced either synthetically or recombinantly, are regulated as drugs, they fall outside the scope of guidance documents written for traditional small molecule drugs as well as for biologics. Among many issues, this can make it particularly challenging for new manufacturers to determine appropriate impurity limits for peptides. Dr. René Thürmer from the German Federal Institute for Drugs and Medical Devices (BfArM) compared peptide and oligonucleotide regulatory considerations. In Europe, the European Pharmacopeia's monograph *Substances for Pharmaceutical Use* contains reporting, identification, and qualification thresholds for impurities in synthetic peptides, but this is not globally followed and does not apply to genotoxic impurities. He noted that, while distinct impurity peaks for peptides are typically observed, orthogonal purity methods are recommended to minimize the risk of undetected impurities coeluting with the main peak or with each other. To this end, ultra-performance liquid chromatography (UPLC) is becoming the state-of-the-art method for impurities and for content. Other trends include the use of tandem mass spectrometry (MS/MS) for complete sequencing of the peptide and spectroscopic methods for longer peptides with higher order structures.

Patients can sometimes develop an immune response when therapeutic proteins are delivered. The U.S. FDA's guidance, *ANDAs for Certain Highly Purified Synthetic Peptide Drug Products that Refer to Listed Drugs of rDNA Origin,*[1] that was just finalized in May 2021 stressed that this is important to consider for synthetic peptides as well, even though the guidance is specific to only five peptides and their related impurities. Anti-drug antibodies can impact product safety and efficacy through several mechanisms, including:

- neutralizing the peptide by blocking its binding to target,
- breaking tolerance and leading to autoimmune responses to endogenous proteins and peptides, or by
- impacting its pharmacokinetic/pharmacodynamic profile of the peptide.[2]

While immunogenicity remains an ongoing challenge that usually leads to post-marketing clinical commitments for therapeutic proteins, advances across in silico, in vitro, and preclinical in vivo methods are helping to provide manufacturers and regulators with tools that may inform immunogenicity risk assessments of generic peptides and their impurities in the absence of additional clinical studies.[3],[4] Dr. Jeff Jiang of the Sweetwood Group gave an overview of comparative studies for complex synthetic peptide products that included points to consider related to the 2017 draft guidance (which was still effective at the time of the workshop) and the latest tools to assess potential peptide immunogenicity. However, the workshop discussion demonstrated that this is an ongoing challenge for industry to identify minor impurities and produce these impurities in sufficient, relatively pure quantities to test for their immunogenicity even in a laboratory environment.

The final guidance did not change significantly regarding expectations for new impurities. If a new impurity (not present in the reference listed drug is present at <0.1% of the drug substance, then the ANDA path is usually suitable. For any new impurity between ≥0.1% and ≤0.5% of the drug substance, they must each be identified, and data should be provided to justify that the impurity(s) would

not modify the drug's physicochemical properties, biological activity, or risk of immunogenicity in order to follow the ANDA path. For impurities >0.5%, the ANDA path may not be suitable, but manufacturers are encouraged to discuss their findings with the FDA to determine a suitable path.

Starting Material Control Strategies: Vendor Selection And Sourcing Strategy

In addition to process- and product-related impurities, impurities from raw materials and starting materials can adversely impact product quality. Because standard Fmoc-protected amino acid derivatives are considered suitable starting materials, supplier selection criteria for conventional amino acid derivatives focus on reliability, sustainability, control (i.e., vendor quality system), and capacity. In contrast, non-established derivatives can be more challenging, and a specified control strategy and vendor selection criteria based on analytical data and process understanding can inform manufacturers' sourcing strategy.

Dr. Heiko Rinderhagen from Janssen and Dr. Tobias Kapp from Bachem AG presented on how manufacturers can leverage analytical assessments and target specifications to inform their vendor selection strategy for sourcing non-established amino acid derivatives. Impurity profiles across vendors can be assessed and compared via a product-specific use test that covers all stages of the manufacturing process, allowing manufacturers to distinguish critical impurities from non-critical impurities based on their potential to generate active pharmaceutical ingredient impurities that cannot be purged during the manufacturing process.

Strategies In Peptide Synthesis, Formulation, & Delivery

Aside from immunogenicity, therapeutic peptides also need to overcome the challenges of:

- Low oral bioavailability, in vivo instability, short circulating plasma half-life, and low membrane permeability.[5]
- Peptide sequences containing high numbers of hydrophobic residues ("difficult sequences") have low solubility in organic or aqueous solvents and high aggregation potential due to their tendency to form β-sheet or α-helical structures.[6]
- The need to optimize peptide drug binding characteristics.[7]

From nanoparticle encapsulation and carrier molecule conjugation to digital capsules and controlled-release dosage forms, advances in drug delivery technologies are impacting the pharmaceutical industry across therapeutic classes. Meanwhile, there is a need to adopt more efficient, greener manufacturing methods for peptides. On the last day of the workshop, attendees heard from experts in peptide development and drug delivery systems who shared a case study in peptide synthesis and outlined important formulation considerations.

Peptide Synthesis Case Study: Tirzepatide

A hybrid solid-/liquid-phase peptide synthesis (SPPS/LPPS) approach allows for native chemical ligation synthesis, where unprotected peptide fragments are coupled in aqueous media without any epimerization. Drs. Ankur Jalan and Michael Kopach, both from Eli Lilly and Company, reviewed the synthesis of tirzepatide using a hybrid SPPS/LPPS approach and native chemical ligation methodology. This strategy can produce more than 95% conversion of the starting material to the ligation product and has the potential for high yields, no epimerization, and higher purity than traditional convergent hybrid syntheses. Commercial requirements for manufacturing scale-up will likely accelerate advancements in continuous-flow peptide synthesis and further improve efficiency and reduce waste during peptide synthesis.

Delivery System Considerations

Dr. Christopher Rhodes from Drug Delivery Experts provided a high-level overview of peptide formulation approaches and delivery systems. He noted that formulation and delivery system decisions not only require extensive product knowledge, but they also rest on the commercial feasibility and manufacturing scalability because of the higher development cost and risk associated with moving from a traditional vial and syringe delivery system to a more complex formulation or combination device. Regardless of advances in formulation and device technologies, long-acting (once-daily), orally delivered formulations will likely remain the preferred formulation choice of patients and providers, as they are simple and non-invasive, thus improving medication adherence and patient outcomes.

Next Steps

Recordings of the talks are available through USP Education. USP encourages stakeholders to share their expertise and help advance novel and generic therapeutic peptides by collaborating on new standards and tools to ensure peptide quality throughout development and the product life cycle. To provide input on peptide standards, including monographs, general chapters, and Reference Standards, stakeholders are encouraged to contact Dr. Julie Zhang, who is the lead liaison to the USP BIO1 Expert Committee, which is responsible for peptide-related activities.

About The Author

Maura Kibbey, Ph.D., is senior scientific fellow for education and training in USP's Global Biologics department. She leads development of courses, workshops, and forums to engage USP's biologics stakeholders. This role builds on her previous responsibilities directing USP scientists developing compendial standards. Before joining USP, Kibbey worked for several biotechnology and diagnostic companies in the Washington, D.C., area in scientific, management, marketing, and business development roles, as well as performing cancer research at the National Institutes of Health. She has published over 40 peer-reviewed articles and has been an invited speaker or workshop organizer for numerous scientific conferences. You can reach her at mck@usp.org.

[1] https://www.fda.gov/media/107622/download

[2] https://www.fda.gov/media/146112/download

[3] https://www.fda.gov/drugs/news-events-human-drugs/non-clinical-immunogenicity-assessment-generic-peptide-products-development-validation-and-sampling

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