

Guidance for Industry

for the Submission of Chemistry, Manufacturing, and Controls Information for Synthetic Peptide Substances

**Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)**

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TABLE OF CONTENTS

I.	INTRODUCTION	1
II.	DESCRIPTION AND CHARACTERIZATION	2
	A. Description	2
	B. Characterization/Proof of Structure	2
III.	SYNTHESIS/METHOD OF MANUFACTURE	4
	A. Starting Materials	4
	1. Amino Acids and Derivatives	4
	2. Resins Used for Peptide Synthesis	4
	3. Chemical Reagents and Solvents	5
	B. Flow Chart of Synthesis	5
	1. Solution-Phase Synthesis	5
	2. Solid-Phase Synthesis	5
	C. Detailed Description of Synthesis	5
	1. Solution-Phase Synthesis	5
	2. Solid-Phase Synthesis	6
	3. Modification of the Completed Peptide	7
	D. Purification of the Peptide	7
	1. Purification Strategy	7
	2. Description of the Purification Process	7
	3. Drying of Purified Drug Substance	8
IV.	PROCESS CONTROLS	8

A.	Reaction Completion	8
1.	Solution-Phase Synthesis	8
2.	Solid Phase Synthesis	8
3.	Disulfide Linkage	8
B.	Intermediate Specifications and Tests	9
C.	Column Performance	9
D.	Removal of Solvents and Reagents	9
V.	REFERENCE STANDARD	9
VI.	SPECIFICATIONS/ANALYTICAL METHODS	11
VII.	CONTAINER-CLOSURE SYSTEM	11
VIII.	STABILITY	12

GUIDANCE FOR INDUSTRY¹
FOR THE SUBMISSION OF
CHEMISTRY, MANUFACTURING, AND CONTROLS
INFORMATION
FOR SYNTHETIC PEPTIDE DRUG SUBSTANCES

I. INTRODUCTION

Interest in peptides as potential drug candidates has increased since the early 1970s with the development of solid-phase peptide synthesis and high performance liquid chromatography (HPLC) for the purification and analysis of peptides. Recent advances in nuclear magnetic resonance (NMR) spectroscopy, mass spectroscopy (MS), and synthetic methods have enabled investigators to create compounds with altered pharmacological profiles compared with naturally occurring peptides. These modified peptides may have increased receptor binding affinity, improved selectivity for receptors, antagonist properties, increased resistance to enzymatic degradation, or improved pharmacokinetic profiles. As a result of these advances, many peptide analogs may become useful therapeutic agents.

This guidance deals only with chemically synthesized peptide drug substances, focusing on those issues which are unique to these compounds. Because there are no issues concerning drug products made from synthetic peptides which are different from the issues in the "Guideline for Submitting Documentation for the Manufacture of and Controls for Drug Products," drug products will not be discussed.

¹This guidance has been prepared by the Biotechnology Technical Committee of the Chemistry Manufacturing Controls Coordinating Committee (CMC CC) of the Center for Drug Evaluation and Research (CDER), with input from the Center for Biologics Evaluation and Research (CBER), at the Food and Drug Administration. Although this guidance does not create or confer any rights for or on any person and does not operate to bind FDA or the industry, it does represent the agency's current thinking on synthetic peptide substances. For additional copies of this guidance, contact the Division of Communications Management, HFD-210, CDER, FDA, 5600 Fishers Lane, Rockville, MD 20857 (Phone: 301-594-1012) or the Division of Congressional and Public Affairs (HFM-44), CBER, FDA, 1401 Rockville Pike, Rockville, MD 20852-1448. Send one self-addressed adhesive label to assist the offices in processing your request. An electronic version of this guidance is also available via Internet the World Wide Web (WWW) (connect to the FDA Home Page at WWW.FDA.GOV/CDER and go to the "Regulatory Guidance" section).

Multiple Antigen Peptides (MAPs) intended to be used as immunogens may require additional guidance. Manufacturers should contact CBER regarding these types of products.

To comply with section 505(b) of the Food, Drug, and Cosmetic Act, 21 U.S.C. § 355(b), certain information pertaining to the method of preparation of the drug substance and the control testing used to monitor its identity, strength, quality, and purity should be provided in all submissions to the Centers. Certain biological characteristics, such as potency, immunogenicity, or antigenicity, may also be necessary. This information is pertinent to investigational new drug applications (IND), new drug applications (NDA), abbreviated new drug applications (ANDA), product licensing applications (PLA), drug master files (DMF), and biological master files (BMF).

For general information on chemistry, manufacturing and controls of the drug substance, refer to the "Guideline for Submitting Supporting Documentation in Drug Applications for the Manufacture of Drug Substances" (Drug Substance Guideline). The present guide only covers synthetic peptides manufactured by either solution-phase methods or solid-phase methods. The depth and extent of documentation necessary to support the application will depend on the type of submission.

II. DESCRIPTION AND CHARACTERIZATION

A. Description

A general description of the synthetic peptide should include such items as USAN, chemical formula, amino acid sequence, pI value (isoelectric pH), solubility, molecular weight and salt form (e.g., acetate or trifluoroacetate). Any modifications, such as esterification or amidation, should be described.

B. Characterization/Proof of Structure

The synthetic peptide should be characterized by, at a minimum, amino acid analysis, MS, and peptide sequencing. Amino acid analysis of the purified peptide provides information on whether the peptide has the correct composition. Mass spectroscopy based on a number of techniques, such as fast atom bombardment, electrospray, plasma desorption, or laser desorption, is frequently used to provide the molecular weight or sequence information. These data can provide

evidence for the authenticity of the peptide and for the absence of impurities that might occur during synthesis.

Edman degradation can be used for the determination of the correct sequence of the synthetic peptide. If the N-terminus of the synthetic peptide is blocked, the N-terminal moiety should be removed by an appropriate method prior to Edman degradation.

If a C-terminal modification, e.g., amidation, arises from a modification of the synthetic resin (see section III.A.2.b), this need not be characterized in the final peptide.

If the peptide contains more than 20 amino acid residues, a peptide map should be provided. The fragments should be isolated and characterized by either MS or peptide sequencing.

When unnatural amino acids (i.e., besides the generally accepted 20 "naturally" occurring amino acids) are used in synthesis, their chromatographic behavior (for amino acid analysis) and that of their derivatives (for peptide sequencing) should be established to verify the presence of these amino acids in the synthetic peptide.

Disulfide bond(s), if present in a peptide, should be determined by reaction with a suitable reducing agent, such as dithiothreitol. The free -SH groups can then be detected by a suitable reagent, such as Ellman's reagent. When a peptide contains two or more disulfide bonds, the correct disulfide linkages should be established.

The chemical structure can be further substantiated by ^1H -NMR and ^{13}C -NMR spectroscopy.

Other test methods used to characterize the drug substance should be fully described (e.g., circular dichroism and fluorescence spectroscopy).

For applications to CBER for *in vitro* diagnostic (IVD) test kits, peptides should be shown to be devoid of blocking groups on the side-chains, or else should be highly characterized regarding the presence of such side-chain blocking groups. Also, it is important to demonstrate the presence of multiple epitopes.

III. SYNTHESIS/METHOD OF MANUFACTURE

A. Starting Materials

1. Amino Acids and Derivatives

If amino acids and derivatives are purchased, the vendor's name and address should be provided. Test results, including the optical rotation, melting point, chemical and chiral purity, and chromatographic behavior using either thin-layer chromatography (TLC) or HPLC, should be provided in a certificate of analysis by the vendor who has demonstrated the capability of supplying materials of consistent quality. If the qualified vendor provides a certificate of analysis, the purchaser may run only an identity test. Otherwise, the purchaser should run the entire set of tests as described above.

When amino acids and derivatives are uncommon, or are synthesized by a novel method, a detailed description of how they are synthesized and characterized should be provided. Their chemical structure should be characterized by NMR, infrared spectroscopy, MS, and optical rotation. Purity should be monitored by TLC and/or HPLC.

2. Resins Used for Peptide Synthesis

When a peptide is synthesized by the solid-phase method, a brief description of the resin and the reason for its choice should be provided. This description should include:

- a. A chemical description of the resin (e.g., hydroxymethyl polystyrene-vinylbenzene copolymer or polyamide);
- b. Any custom functionalization (e.g., phenylacetamido or benzhydrylamine derivatives);
- c. Molar substitution factors;
- d. Swelling factors for the resins in various solvents;
- e. Any instability under identified conditions (e.g., during removal of the peptide from the resin by hydrolysis).

3. Chemical Reagents and Solvents

For information on this topic, refer to the Drug Substance Guideline.

B. Flow Chart of Synthesis

1. Solution-Phase Synthesis

A fragment condensation flow chart should be provided. In each step, solvents, catalysts, and reagents used for synthesis should be given. A brief description of the synthetic strategy should accompany the flow chart.

2. Solid-Phase Synthesis

The flow chart for the solid-phase synthesis should include the initial coupling, a general synthesis cycle [consisting of removal of the N-terminal protecting group, washing, neutralization [if t-butoxycarbonyl (t-Boc) amino acid is used], washing, coupling and washing, and the final cleavage of the peptide from the resin.

Synthesis steps which do not follow the general synthesis cycle should be specified.

C. Detailed Description of Synthesis

1. Solution-Phase Synthesis

Because different coupling methods and deprotection techniques may be employed during peptide synthesis, the procedures used for each step of the synthesis should be described in detail. The process control points should be specified (see Section IV, Process Controls). The sponsor/applicant/holder should refer to the Drug Substance Guideline.

When fragment condensation is part of the synthetic strategy, isolation and purification of the fragments may be important. A description of the purification processes should be provided.

When the final and fully protected peptide synthesized from the solution phase methods is isolated by crystallization, a detailed

description of crystallization procedures should be provided. Disposition of the mother liquor should be described.

2. Solid-Phase Synthesis

When a commercially available synthesizer is used, the sponsor/applicant/holder should provide the model number and the name and address of the manufacturer. Custom designed or modified synthesizers should be described in detail and validated.

For initial coupling, the amount of the first amino acid attached to the resin (millimole substitution) and amount of the resin used for synthesis should be provided.

The following information for each synthesis cycle should be provided:

- a. Volumes and compositions of the solutions used for deprotection, washing, and neutralization;
- b. Amounts of fully protected amino acid derivatives and coupling reagents;
- c. Times used for washing, deprotection, neutralization, coupling, and capping;
- d. The circumstances requiring repeated coupling of individual amino acids should be defined.

The process control points should be specified (see Section IV, Process Controls).

Regarding deprotection and cleavage of the desired peptide from the resin, the sponsor/applicant/holder should provide detailed information on the type of cleavage reaction employed. For example, hydrogen fluoride (HF) is usually employed for t-Boc peptides and a TFA solution for Fmoc peptides. In addition, the amount of scavengers used during cleavage/deprotection should also be stated.

Extraction of the peptide from the resin should be described fully.

3. Modification of the Completed Peptide

Synthesis of the active drug substance often requires modification of the peptide chain. Examples are oxidation, reduction, N-acetylation, amidation, myristoylation, and cyclization. A detailed description of these processes should be provided. In particular, information should be given concerning any oxidation step leading to the formation of specific disulfide linkages.

D. Purification of the Peptide

1. Purification Strategy

An appropriate purification process should be developed to separate the desired peptide from any contaminating materials. These contaminants may include:

- a. diastereomeric (racemized) peptides;²
- b. deletion (incomplete) peptides;
- c. truncated peptides;
- d. reaction by-products;
- e. deamidation peptides;
- f. by-products generated by incomplete deprotection of amino acid side chain protecting groups;
- g. oxidized peptides;
- h. disulfide exchange products;
- i. oligomers and/or aggregates; or
- j. toxic reagents and solvents used in synthesis.

Selection of the route of purification should take into account the size, polarity, and ionic character of the desired peptide and impurities.

2. Description of the Purification Process

A detailed description of the purification of the crude peptide should be provided. When chromatography is used, the applicable conditions should be described. These may include sample load, mobile phase, column material, particle size, column dimensions, gradient programs, detection wavelength, flow rate and column

²Racemization during peptide synthesis is difficult to prevent regardless of what synthetic strategy, solution or solid synthesis, is used, even though reagents (e.g., 1-hydroxybenzotriazole (HOBt)), which are designed to minimize racemization, are added to the reaction mixture.

temperature. The selection criteria for collecting fractions should be specified. Disposition of side fractions should be described. Sample chromatograms for a representative purification run should be provided.

If the peptide is isolated as a salt, the counter-ion used during the final step of the purification should be specified.

3. Drying of Purified Drug Substance

The final preparation may need to be dried to preserve the structure and biological activity. Most peptides are lyophilized but room and high temperature drying may be used if the peptide is not affected by these conditions. The exact conditions (temperature, pressure, rate of change, and duration of each step) should be described.

If a solution of the bulk drug substance is apportioned into individual containers (such as glass vials) prior to lyophilization, the size and specifications for the container-closure should be specified.

IV. PROCESS CONTROLS

A. Reaction Completion

1. Solution-Phase Synthesis

Completion of reaction can be monitored by TLC.

2. Solid Phase Synthesis

Completion of coupling can be monitored by the ninhydrin test.

3. Disulfide Linkage

Completion of disulfide linkages can be monitored by the Ellman test and HPLC. Other suitable procedures may be used.

B. Intermediate Specifications and Tests

Fragments destined for condensation reactions may need to be isolated. Once isolated, these fragments should be characterized. Adequate structural analysis for each fragment should be provided. These analyses may employ tests such as amino acid analysis, amino acid sequencing, MS, optical rotation, and melting point.

For every synthesis cycle, the identity and purity of each fragment should be monitored by TLC and/or HPLC.

C. Column Performance

Validated procedures should be described for performing column regeneration after the purification steps. Criteria should be established for determining column lifetime and evaluating column performance.

D. Removal of Solvents and Reagents

Validation of the removal of solvents and reagents used during synthesis and purification of the peptide should be described.

V. REFERENCE STANDARD

A primary reference standard synthesized in the same manner as the drug substance should be established. The preparation of the reference standard should be briefly described. If any modifications of the synthesis and/or additional purification steps are used, detailed information should be provided. Certain elements in the definition of the reference standard, such as the amino acid sequence, need not be provided in the lot release specifications (see section VI, Specifications/Analytical Methods).

A copy of the Certificate of Analysis should be provided. The tests should include: appearance, optical rotation, amino acid analysis, peptide content, counter-ion content, moisture content, peptide purity, residual organic solvents, MS analysis, peptide sequencing, and residual fluoride (if HF is used to cleave the peptide from the resin). Peptide mapping and biological assays may also be required.

When appropriate, the reference standard should be examined for its biological and/or immunologic activities using suitable *in vivo* or *in vitro* tests. With respect to antagonists, biological potency may be measured by using a suitable inhibition assay and a proper reference standard.

The appearance of the solid synthetic peptide should be described. The solubility and pI should be specified.

The optical rotation should be determined. If polarimetry is used, the concentration of the solution and the pathlength should be given.

Amino acid analysis should be performed to provide the amino acid composition and to quantify the amount of peptide present. The results should be consistent with the theoretical values and any discrepancies should be explained.

The amount of counter-ion, such as TFA or acetate, in the peptide can be determined by HPLC.

Determination of the moisture content is essential for the quantitation of the amount of peptide as dry weight. The Karl Fischer method may be used to determine the amount of water in the peptide. For further information, refer to the "Guideline for the Determination of Residual Moisture in Dried Biological Products."

HPLC techniques that are useful for measuring the purity of peptides include reversed-phase, ion-exchange, and hydrophobic interaction. Capillary zone electrophoresis (CZE) has also been used for this purpose. Size-exclusion chromatography and polyacrylamide gel electrophoresis (PAGE) under non-dissociating conditions may be useful in characterizing aggregates and oligomers.

Residual amounts of organic solvents and reagents which have been used during the synthesis and preparation of the peptide should be identified and quantitated. If HF is used during cleavage of the peptide from the resin, residual fluoride should be determined.

The primary amino acid sequence should be determined. Suitable methods include Edman degradation or MS. If the peptide backbone has been modified in such a way as to preclude sequencing by Edman degradation, alternative methods may be used.

The entire covalent structure of the peptide drug should be determined. Included in the covalent structure are the molecular weight, disulfide bonding, peptide cyclization, and any modifications of the peptide backbone, side chains, or terminal groups.

The structure of any unnatural amino acids (see Section II.B., Characterization) used in the preparation of the peptide should be confirmed. MS is the method of choice for structure determination.

If any D-amino acids are used during synthesis, a suitable method should be provided to demonstrate the retention of the original configuration of the amino acid in the peptide.

If the peptide contains more than 20 amino acid residues, a peptide map, including the structural characterization of each peptide fragment, should be provided.

When a working reference standard is employed, appropriate abbreviated characterization should be provided.

VI. SPECIFICATIONS/ANALYTICAL METHODS

The regulatory specifications and tests should be provided. As stated in Section V, Reference Standard, the lot release specifications may omit some of the tests used for the Primary Reference Standard, but should be sufficient to assure the identity, purity, strength and/or potency of the peptide and to demonstrate lot-to-lot consistency. See Drug Substance Guideline.

Specifications should be set for residual reagents and organic solvents. See Drug Substance Guideline.

Validation of the analytical systems should demonstrate the ability to separate the desired peptide from peptide by-products resulting from synthesis (e.g., incomplete peptides), purification (e.g., column leachables, solvents), degradation (e.g., disulfide exchange or oxidation), or aggregation. The degree of purity of the peptide and the amount of impurities present will depend on the product itself and its intended use, and should be discussed on a case-by-case basis.

VII. CONTAINER-CLOSURE SYSTEM

Refer to Drug Substance Guideline. If the container-closure is coated (e.g., siliconized), this process should be described. For IVDs where peptides are bound to the immediate container (e.g., to a microtiter well), information regarding the amount of peptide bound and its efficacy in binding to corresponding antibodies should be provided.

VIII. STABILITY

The "Guideline for Submitting Documentation for the Stability of Human Drugs and Biologics" provides broad guidance in designing stability studies for drug substances. Some of the general concepts are applicable to synthetic peptides. In general, the lyophilized powder of the synthetic peptide is relatively stable during storage at -20°C under dry conditions and protected from light. When a peptide is kept in solution or under high humidity, degradation and/or aggregation will occur more rapidly than when the peptide is kept dry. For example, methionine, tryptophan, and cysteine will be oxidized; asparagine and glutamine will be deamidated; the peptide backbone may be hydrolyzed. Attention should also be given to potential leaching of any coating materials from the container-closure into the drug substance.

A measurement of biological activity may need to be included in the stability program, but preservation of biological activity is not a guarantee of chemical integrity.

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