FDA Briefing Document

Pharmacy Compounding Advisory Committee (PCAC) Meeting

July 23 - 24, 2026

The attached package contains background information prepared by the Food and Drug Administration (FDA or Agency) for the panel members of the Pharmacy Compounding Advisory Committee (advisory committee). We are bringing certain compounding issues to this advisory committee to obtain the advisory committee's advice. The background package may not include all issues relevant to the final committee recommendation and instead is intended to focus on issues identified by the Agency for discussion by the advisory committee. The FDA will not issue a final determination on the issues at hand until input from the advisory committee process has been considered and all reviews have been finalized. The final determination may be affected by issues not discussed at the advisory committee meeting.

TB-500-Related Bulk Drug Substances (TB-500 (Free Base) and TB-500 acetate)

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FDA Evaluation of TB-500-Related Bulk Drug Substances (TB-500 (Free Base) and TB-500 acetate)

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TO: Pharmacy Compounding Advisory Committee

SUBJECT: Evaluation of TB-500-related Bulk Drug Substances for Inclusion on the 503A Bulk Drug Substances List

U.S. Food & Drug Administration
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List of Abbreviations

Abbreviation	Term
AE	adverse event
API	active pharmaceutical ingredient
β4	beta-4
BDS	bulk drug substance
BET	bacterial endotoxins test
CAERS	CFSAN Adverse Event Reporting System
CAS	Chemical Abstract Service
CDC	Centers for Disease Control and Prevention
CFSAN	Center for Food Safety and Nutrition
CoA	Certificate of Analysis
CQA	critical quality attribute
DIPEA	diisopropylethylamine
FAERS	FDA Adverse Event Reporting System
FD&C Act	Federal Food, Drug, and Cosmetic Act
Fmoc	fluorenylmethoxycarbonyl
GRAS	Generally Recognized as Safe
HFCS	Human Foods Complaint System
HFP	Human Foods Program
HPLC	high-performance liquid chromatography
IM	intramuscular
INN	International Nonproprietary Name
IV	intravenous
LCMS	liquid chromatography-mass spectrometry
MTBE	methyl tert-butyl ether
NF	National Formulary
NIH	National Institutes of Health
OSE	Office of Surveillance and Epidemiology
OTC	over-the-counter
ROA	route of administration
SC	subcutaneous
SPPS	Solid Phase Peptide Synthesis
USP	United States Pharmacopeia
WADA	World Antidoping Agency

I. INTRODUCTION

The Food and Drug Administration (FDA, the Agency, or we) received a nomination for TB-500-related bulk drug substances (BDSs) for inclusion on the list of BDSs that can be used in compounding under section 503A of the Federal Food, Drug, and Cosmetic Act (FD&C Act).[1] The nominator of TB-500-related BDSs provided inconsistent information in the nomination package regarding the specific BDS proposed. Specifically, it is unclear whether the nomination was for TB-500 (free base) or TB-500 acetate. TB-500 (free base) and TB-500 acetate are different active pharmaceutical ingredients (APIs) and hence are considered different BDSs. Please see additional information in section II.A. The nomination was withdrawn[2] and FDA is evaluating the substances at its discretion.

TB-500 (free base) and TB-500 acetate are reported to be peptides consisting of seven amino acids. They are proposed for the intramuscular (IM) and subcutaneous (SC) routes of administration (ROAs). FDA has decided to evaluate both TB-500 (free base) and TB-500 acetate on its own initiative because it is unclear which substance the nominator intended to nominate.

TB-500-related BDSs were evaluated for the following use: wound healing.[3,4] The product proposed in the nomination is a 3 mg/mL lyophilized powder for SC and IM injection.

There is no applicable United States Pharmacopeia (USP) or National Formulary (NF) drug substance monograph for TB-500 (free base) or its acetate form, and neither is a component of an FDA-approved drug.

We have evaluated publicly available data on the physicochemical characteristics, historical use, effectiveness, and safety in compounding of these substances. For the reasons discussed below, we believe the evaluation criteria ***weigh against*** placing TB-500 (free base) or TB-500 acetate on

[1] The nomination submitted by Wells Pharmacy Network (Document ID: FDA-2015-N-3534-0304) can be accessed at https://www.regulations.gov/document/FDA-2015-N-3534-0304. This nomination was withdrawn, but because FDA is evaluating TB-500 (free base) and TB-500 acetate on its own initiative, FDA considered information submitted in these nominations as part of this evaluation.

[2] Document ID: FDA-2015-N-3534-0484.

[3] The proposed use for TB-500-related BDSs listed in the nomination, "LKKTETQ (the active site within the protein thymosin β4 responsible for actin binding, cell migration and wound healing)," therefore, we evaluated its use in wound healing.

[4] We have explained that it is necessary to evaluate a nominated BDS in the context of the uses proposed for compounded drug products that include the substance, though we acknowledge that inclusion of a substance on the 503A Bulks List may not be limited to a specific use. See 84 FR 4696, 4701.

the list of BDSs that can be used to compound drug products in accordance with section 503A of the FD&C Act (503A Bulks List).

II. EVALUATION CRITERIA

A. Is the Substance Well-Characterized, Physically and Chemically?[5]

TB-500 (free base) is understood today to be a seven amino acid synthetic fragment of thymosin beta-4 (β4) from amino acids 17 to 23 and an acetyl group at the N-terminal leucine amino acid (Ac-Leu-Lys-Lys-Thr-Glu-Thr-Gln-OH). However, TB-500 is a common name and not a United States Adopted Name (USAN).[6] FDA has encountered multiple salts, and derivatives, including different active moieties, sold commercially under the same common name. Inconsistent naming conventions that do not follow established chemical nomenclature standards (e.g., INN[7], IUPAC[8], USAN) represent a safety risk for patients as they may be dosed with a different BDS than the physician ordered. From a chemical analysis standpoint, inconsistent naming conventions for TB-500-related BDSs also introduce risks because of the inability to determine which BDS a particular reference standard is referencing.

[5] Among the conditions that must be met for a drug compounded using BDSs to be eligible for the exemptions in section 503A of the FD&C Act is that the BDSs are manufactured by an establishment that is registered under section 510 of the FD&C Act and that each BDS is accompanied by a valid CoA. Sections 503A(b)(1)(A)(ii) and (iii). A BDS is deemed to be adulterated if the methods used in, or the facilities or controls used for, its manufacture, processing, packing, or holding do not conform to or are not operated or administered in conformity with current good manufacturing practice. Section 501(a)(2)(B).

[6] United States Adopted Name (USAN) is a unique, nonproprietary name for a drug sold in the United States. The USAN Council, which is sponsored by several organizations, assigns USANs.

[7] International Nonproprietary Names (INN) facilitate the identification of pharmaceutical substances or active pharmaceutical ingredients. Each INN is a unique name that is globally recognized and is public property. A nonproprietary name is also known as a generic name.

[8] The International Union of Pure and Applied Chemistry (IUPAC) is an international federation of National Adhering Organizations working for the advancement of the chemical sciences, especially by developing nomenclature and terminology.

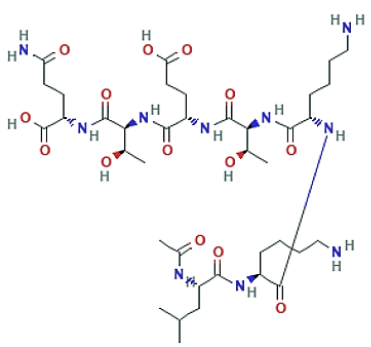
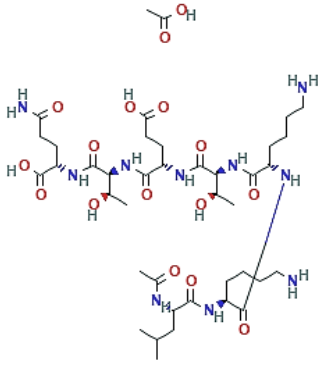
A BDS or API[9] used in a drug product may be a free base (i.e., the native molecule) or a salt or an ester of the free base, all of which share the same active moiety.[10] Different active moieties are not interchangeable because they can have different safety and efficacy profiles. Similarly, a free base or the various salts or ester of an active moiety are distinct chemical entities, each with a different chemical structure and unique physical/chemical, or pharmacokinetic/pharmacodynamic characteristics. As a result, each may offer distinct properties (e.g., different solubilities, permeability, melting points, stability, or flow characteristics) and may also have different safety and/or efficacy profiles. All distinct active moieties, as well as free bases, salts, or esters of any given active moiety, are distinct BDSs for these reasons.

Table 1 below summarizes available identifying information obtained from the public domain for each BDS.

[9] The terms BDS and API are used interchangeably in the compounding context. See 21 CFR 207.3 ("*Bulk drug substance*, as referenced in sections 503A(b)(1)(A) and 503B(a)(2) of the Federal Food, Drug, and Cosmetic Act, previously defined in § 207.3(a)(4), means the same as "active pharmaceutical ingredient" as defined in § 207.1."). An API is defined in FDA regulations at 21 CFR 207.1, which states "*Active pharmaceutical ingredient* means any substance that is intended for incorporation into a finished drug product and is intended to furnish pharmacological activity or other direct effect in the diagnosis, cure, mitigation, treatment, or prevention of disease, or to affect the structure or any function of the body. Active pharmaceutical ingredient does not include intermediates used in the synthesis of the substance."

[10] "*Active moiety* is the molecule or ion, excluding those appended portions of the molecule that cause the drug to be an ester, salt (including a salt with hydrogen or coordination bonds), or other noncovalent derivative (such as a complex, chelate, or clathrate) of the molecule, responsible for the physiological or pharmacological action of the drug substance." 21 CFR 314.3.

Table 1. Summary of Basic Information on TB-500 (Free Base) and TB-500 Acetate

Characteristic	TB-500 (Free Base)	TB-500 Acetate
UNII Code	QHK6Z47GTG	Not available
*** Chemical Abstract Service (CAS) Number**	885340-08-9	Not available
Molecular Formula/ Molecular Weight (g/mol)	$C_{38}H_{68}N_{10}O_{14}$/889.01	$C_{38}H_{68}N_{10}O_{14}\cdot CH_3COOH$/949.1
Chemical Structure		
Supplier[11]	Yes	Yes
Active Moiety	TB-500 (free base)	TB-500 (free base)

*On several suppliers' websites, the CAS number for TB-500 (free base) (885340-08-9) is used for TB-500 Acetate.
Abbreviations: CAS, Chemical Abstract Service; UNII, Unique Ingredient Identifier

One nomination was submitted, which, as discussed above, was later withdrawn. The nominator provided inconsistent information about the TB-500 BDS in its nomination package.

1. The nominated BDS is TB-500 (free base) while the BDS in the accompanying Certificate of Analysis (CoA) is TB-500 acetate.
2. In the CoA, the CAS number, molecular formula, and molecular weight were for TB-500 acetate, not for TB-500 (free base).
3. The molecular formula provided in the nomination package does not match with either TB-500 (free base) or TB-500 acetate.
4. The "AKA CAS #" provided in the nomination package (AKA CAS #: 476014-70-7) does not match with either TB-500 (free base) or TB-500 acetate.

Due to these inconsistencies in the nomination package, the nominated BDS is unclear. All chemistry related information about the BDSs provided by the nominator is summarized in Table 2.

[11] The existence of a supplier of BDS may be relevant to FDA's characterization analysis because it indicates that consistent production of the BDS according to a standard may be possible. BDSs with suppliers are also frequently accompanied by CoAs associated with their production, which can help FDA to identify and characterize BDSs.

Table 2. Summary of Information Submitted in the Withdrawn Nomination

Nominator	**1**
Nominated BDS	TB-500 (free base)
BDS per UNII code	QHK6Z47GTG *(matches TB-500 free base)*
CoA	CoA provided for TB-500 Acetate
CAS No.	885340-08-9 *(matches TB-500 free base) ("AKA CAS # 476014-70-7" provided in the nomination package matches neither TB-500 free base nor TB-500 acetate)*
Molecular Formula	$C_{38}H_{66}N_{10}O_{14}$ *(provided in the nomination package) (matches neither TB-500 free base nor TB-500 acetate) (Molecular Formula provided in the CoA is not legible)*
Molecular Weight	889.01 *(provided in the CoA) (matches TB-500 free base)*
Chemical Name	N-acetyl-L-leucyl-L-lysyl-L-lysyl-L-threonyl-L-alpha-glutamyl-L-threonyl-L-glutamine *(matches TB-500 free base)*
Proposed Products	Subcutaneous/Intramuscular Injectable, 3 mg/mL

Italics in the table above represents the information identified by FDA.
Abbreviations: BDS, bulk drug substance; CAS, Chemical Abstract Service; CoA, Certificate of Analysis; UNII, Unique Ingredient Identifier

FDA is choosing to concurrently evaluate both BDSs, TB-500 (free base) and TB-500 acetate, in this section under two different sub-sections (II.A.1 and II.A.2) and will provide a separate conclusion for each BDS.

The nominator has proposed to compound this BDS into the following dosage form:

- Subcutaneous/Intramuscular Injectable

For an injection product, critical quality attributes (CQAs) including sterility, bacterial endotoxins test (BET) and foreign particulates are critical safety factors. For this reason, bioburden load (i.e., microbial enumeration test) and BET are critical for the BDSs to be used in compounding injections. Evaluation of the solubility of the BDS is also critical to ensure that no BDS precipitates are formed in the compounded drug product.

We reviewed physical and chemical characterization-related information provided by the nominator and performed a literature search for additional information on TB-500 (free base) and its acetate form. Databases searched for information included SciFinder, Analytical Profiles of Drug Substances, PubMed, the European Pharmacopoeia, and the USP-NF.

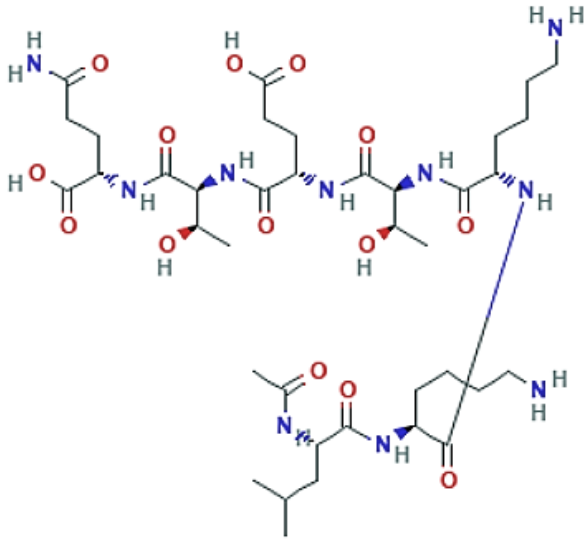
1. TB-500 (Free Base)

TB-500 (free base) is reported to be a seven amino acid synthetic fragment of thymosin β4 from amino acids 17 to 23 and an acetyl group at the N-terminal leucine amino acid (Ac-Leu-Lys-Lys-Thr-Glu-Thr-Gln-OH). Figure 1 shows the structure of TB-500 (free base). The molecular formula of TB-500 (free base) is $C_{38}H_{68}N_{10}O_{14}$ and its molecular weight is 889.01 g/mol.[12] The

[12] https://pubchem.ncbi.nlm.nih.gov/compound/62707662. Accessed 04/04/25.

melting point of TB-500 (free base) is 152-156°C.[13] There is no CoA for TB-500 (free base) in the nomination.

Figure 1. Chemical Structure of TB-500 (Free Base)[14]



a. Stability of the API and Likely Dosage Forms

As reported in the Product Data Sheet,[15] TB-500 (free base) should be stored in a sealed container away from moisture and light under nitrogen atmosphere at temperatures -80°C for 2 years and -20°C for a year in powder form. In solvent, TB-500 (free base) should be stored in a sealed container away from moisture and light under nitrogen atmosphere at temperatures -80°C for 6 months and -20°C for one month.

FDA notes that peptides such as TB-500 (free base) can be extremely sensitive to product formulation, process, and environmental conditions (e.g., pH, heat (temperature), concentration, in-process related impurities, excipients etc.), which may lead to the aggregation and degradation of peptides. This could result in loss of their biological activity (Zapadka et al. 2017). Multiple analytical methods may be needed to detect various aggregates, including size exclusion chromatography or field flow fractionation. Hence, peptides may require more and/or specific analytical in-process and finished product testing for impurities than what is required for small molecules. Uncontrolled manufacturing processes as well as impurities may increase the risk of product aggregation. Product formulation is critical to the quality and stability of peptide drug products, as it is necessary to maintain the peptide molecules in their native state (in the

[13] https://www.echemi.com/produce/pr23030728225-tb500-885340-08-9.html. Accessed 04/04/25.

[14] Unii-qhk6Z47gtg | C38H68N10O14 | CID 62707662 - PubChem. Accessed 07/02/25.

[15] https://file.medchemexpress.com/batch_PDF/HY-P0170/TB500-DataSheet-MedChemExpress.pdf. Accessed 04/04/25.

formulation) to the extent possible. Significant amounts of aggregates can form in formulated products, especially during storage or when exposed to stress conditions.

b. Probable Routes of Bulk Drug Substance Synthesis

TB-500 (free base) is a synthetic peptide fragment of a naturally occurring protein, Thymosin β4. TB-500 (free base) can be synthesized by utilizing 9-fluorenylmethoxycarbonyl (Fmoc) - Solid Phase Peptide Synthesis (SPPS) technique (Fields and Noble 1990). In the first step of the synthesis of TB-500 (free base) (Esposito et al. 2012), C-terminal amino acid, FmocGln(Trt)OH,[16] is coupled with WANG-resin (*p*-Alkoxy-benzyl alcohol polymer-bound) with 0.63 mmol/g loading.[17] General amide coupling methods using 3 equivalents of the preferred amino acid, 3 equivalents of HBTU (O-(Benzotriazol-1-yl)-N,N,N′,N′-tetramethyluronium hexafluorophosphate) as an activator, and 6 equivalents of N,N-Diisopropylethylamine (DIPEA) as a base in a solvent such as DMF (N,N-Dimethylformamide) are used for subsequent peptide couplings. After all the coupling including the coupling to WANG-resin, the Fmoc protecting group is removed using 20% (v/v) piperidine in DMF solvent. The N-terminal amino acid (Leucine) is acetylated in the presence of 1M acetic anhydride in pyridine to provide acylated resin linked heptapeptide. Finally, the resin is cleaved using Reagent K[18] and precipitated in Methyl tert-butyl ether (MTBE).

c. Likely Impurities[19]

Generally speaking, peptide-related impurities and peptide synthesis process-related impurities contribute to and are considered in understanding the impurity profile for all peptides, including TB-500 (free base). For most synthetic peptides, solid-phase peptide synthesis method has been widely used by industry for peptide synthesis. The solid phase synthesis of peptides may lead to potential peptide-related impurities due to incomplete coupling reactions, truncations, or side reactions. These peptide-related impurities are typically similar in structure to the target peptide and may be difficult to identify and quantify without sophisticated analytical methods. Additional potential common impurities may be derived from impurities in the protected amino acid starting materials (e.g., isomeric impurities and free amino acids) and other species that may carry over into the drug substance. In addition, residual solvents, coupling reagents, activators,

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[16] https://www.sigmaaldrich.com/US/en/product/aldrich/47674. Accessed 04/04/25.

[17] https://www.sigmaaldrich.com/US/en/product/aldrich/13618. Accessed 04/04/25.

[18] https://www.sigmaaldrich.com/US/en/technical-documents/technical-article/chemistry-and-synthesis/peptide-synthesis/fmoc-cleavage-deprotection. Accessed 04/04/25.

[19] This evaluation contains a non-exhaustive list of potential impurities in the BDS and does not address fully the potential safety concerns associated with those impurities. The compounder should use the information about the impurities identified in the CoA accompanying the BDS to evaluate any potential safety and quality issues associated with impurities in a drug product compounded using that BDS taking into account the amount of the impurity, dose, route of administration, and chronicity of dosing. Available nonclinical toxicity data for likely impurities of concern (e.g., nitrosamines, potential mutagenic substances, and potential teratogenic substances) in the nominated BDS are discussed in the Nonclinical Assessment at Section D.I. as part of the safety assessment of the substance.

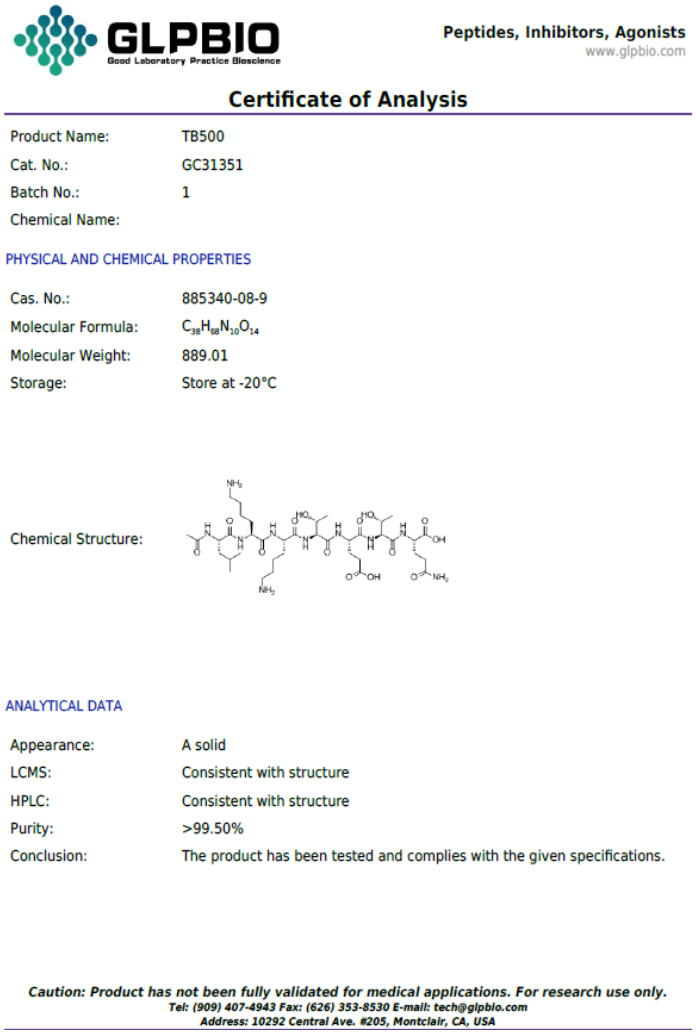
catalysts, and scavengers may exist as SPPS process-related impurities. Drug substance and its proposed product-related impurities may also include peptide-related aggregates.

No CoA for TB-500 (free base) was submitted in the nomination package. We conducted a literature search, and a representative CoA is shown below (Figure 2).[20] This CoA includes only appearance, identity by Liquid Chromatography-Mass Spectrometry (LCMS) and high-performance liquid chromatography (HPLC) and peptide purity. However, assay, impurities, bacterial endotoxins, and aggregates are not tested or controlled for TB-500 (free base).

Since there is a lack of information regarding potential impurities that can be present in TB-500 (free base) and a lack of information on the potential of peptide aggregation, we cannot rule out the potential for immunogenicity associated with these impurities and peptide-related aggregates.

[20] https://www.glpbio.com/tb500.html. Accessed 04/04/25.

Figure 2. Example of CoA for TB-500 (Free Base)



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Certificate of Analysis

Product Name:	TB500
Cat. No.:	GC31351
Batch No.:	1
Chemical Name:	

PHYSICAL AND CHEMICAL PROPERTIES

Cas. No.:	885340-08-9
Molecular Formula:	$C_{38}H_{68}N_{10}O_{14}$
Molecular Weight:	889.01
Storage:	Store at -20°C

Chemical Structure:

ANALYTICAL DATA

Appearance:	A solid
LCMS:	Consistent with structure
HPLC:	Consistent with structure
Purity:	>99.50%
Conclusion:	The product has been tested and complies with the given specifications.

Caution: Product has not been fully validated for medical applications. For research use only.
Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com
Address: 10292 Central Ave. #205, Montclair, CA, USA

d. Physicochemical Characteristics Pertinent to Product Performance, Such as Particle Size and Polymorphism

TB-500 (free base) is reported as a white to off-white solid,[21] which is soluble in water with a solubility of 50 mg in 1 mL water.[22] Because the BDS is soluble in water and would be solubilized prior to administration, particle size and polymorphism are not considered CQAs that affect performance for the proposed injection dosage form.

[21] TB500 | Thymosin β 4 Synthetic Molecule | MedChemExpress. Accessed 04/04/25.

[22] TB500 | Cas# 885340-08-9 - GlpBio. Accessed 04/04/25.

e. Any Other Information About the Substance That May Be Relevant, Such as Whether the Bulk Drug Substance is Poorly Characterized or Difficult to Characterize

No CoA for TB-500 (free base) was submitted in the nomination package. The CoA of TB-500 (free base) from the public domain does not control Bacterial Endotoxin levels in addition to Impurities/Aggregates. No additional relevant information regarding the physical and chemical characterization of TB-500 (free base) is identified from the public domain.

Conclusions: TB-500 (free base) is reported to be a peptide containing seven amino acids with an acetyl group at the N-terminal Leucine (Ac-Leu-Lys-Lys-Thr-Glu-Thr-Gln-OH). TB-500 (free base) is reported to be stable below -20°C under reported storage conditions (sealed storage, away from moisture and light, under nitrogen).

TB-500 (free base) is not physically and chemically well characterized based on (1) inconsistent naming conventions that do not follow established chemical nomenclature standards (e.g., INN, USAN, IUPAC), and (2) certain critical characterization data specific to TB-500 (free base), such as potential peptide impurities, were not found in publicly available scientific literature and CoA. Specific tests for TB-500 (free base) are not available in the public domain or in the publicly available CoA, such as tests for impurities, aggregates, microbiological quality, and bacterial endotoxin. As discussed in the Section II.D.2.d, FDA is concerned about the potential for immunogenicity of TB-500 (free base) when formulated in an injectable dosage form for SC/IM administrations due to the potential for aggregation as well as potential peptide-related impurities, as discussed in the impurities section. Injectable ROAs may present a particular risk for immunogenicity. We also note that the stability, pharmacological activity, and immunogenic properties of peptides are highly sensitive to the manufacturing process and quality attributes of the compounded/finished drug product.

2. TB-500 Acetate

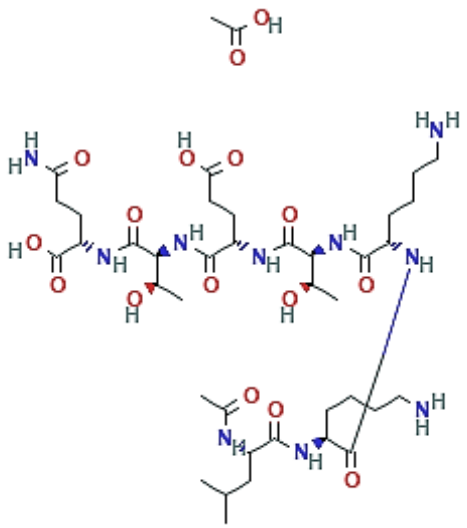
TB-500 acetate is the acetate salt form of TB-500 (free base). Figure 3 illustrates the chemical structure of TB-500 acetate. TB-500 acetate is reported as a white to off-white powder[23] and the molecular formula and molecular weight are $C_{38}H_{68}N_{10}O_{14}\cdot CH_3COOH$ and 949.1 g/mol, respectively.[24]

The nominator provided a CoA of TB-500 acetate from Darmerica with the nomination package. It included the test results for appearance, solubility, amino acid composition, water, acetate content, impurities by HPLC (% total impurities: ≤2.0%; % largest single impurity: ≤1.0%), and purity. Testing results are not provided for Identification, Assay, Aggregates, and Bacterial Endotoxin levels.

[23] https://www.guidechem.com/cas/77591-33-4.html. Accessed 04/11/26.

[24] https://pubchem.ncbi.nlm.nih.gov/compound/TB500-acetate. Accessed 04/04/25.

Figure 3. Chemical Structure of TB-500 Acetate[25]



a. Stability of the API and Likely Dosage Forms

As recommended in the CoA from Darmerica submitted by the nominator, the recommended storage temperature for TB-500 acetate is 2-8°C in a refrigerator or a freezer when stored in a sealed container. Based on the information from the public domain, the recommended storage temperature for TB-500 acetate is < -15°C and protect from light and keep in a tightly closed container.[26]

FDA notes that peptides such as TB-500 acetate can be extremely sensitive to product formulation, process, and environmental conditions (e.g., pH, heat (temperature), concentration, in-process related impurities, excipients, etc.), which may lead to the aggregation and degradation of peptides. This could result in loss of their biological activity. Multiple analytical methods may be needed to detect various aggregates, including size exclusion chromatography or field flow fractionation. Hence, peptides may require more and/or specific analytical in-process and finished product testing for impurities than what is required for small molecules. Uncontrolled manufacturing processes as well as impurities may increase the risk of product aggregation. Product formulation is critical to the quality and stability of peptide drug products, as it is necessary to maintain the peptide molecules in their native state (in the formulation) to the extent possible. Significant amounts of aggregates can form in formulated products, especially during storage or when exposed to stress conditions.

[25] https://pubchem.ncbi.nlm.nih.gov/compound/TB500-acetate. Accessed 04/04/25.

[26] www.biosynth.com/Files/MSDS/FT/18/MSDS_FT183581_5000_EN.pdf. Accessed 04/04/25.

b. Probable Routes of Bulk Drug Substance Synthesis

Synthesis of TB-500 acetate is not reported in the literature. Synthetic methods described in Section A.1.b for TB-500 (free base) are applicable for TB-500 acetate. General method for the synthesis of acetate salt can be utilized for the synthesis of TB-500 acetate (Isidro-Llobet et al. 2019).

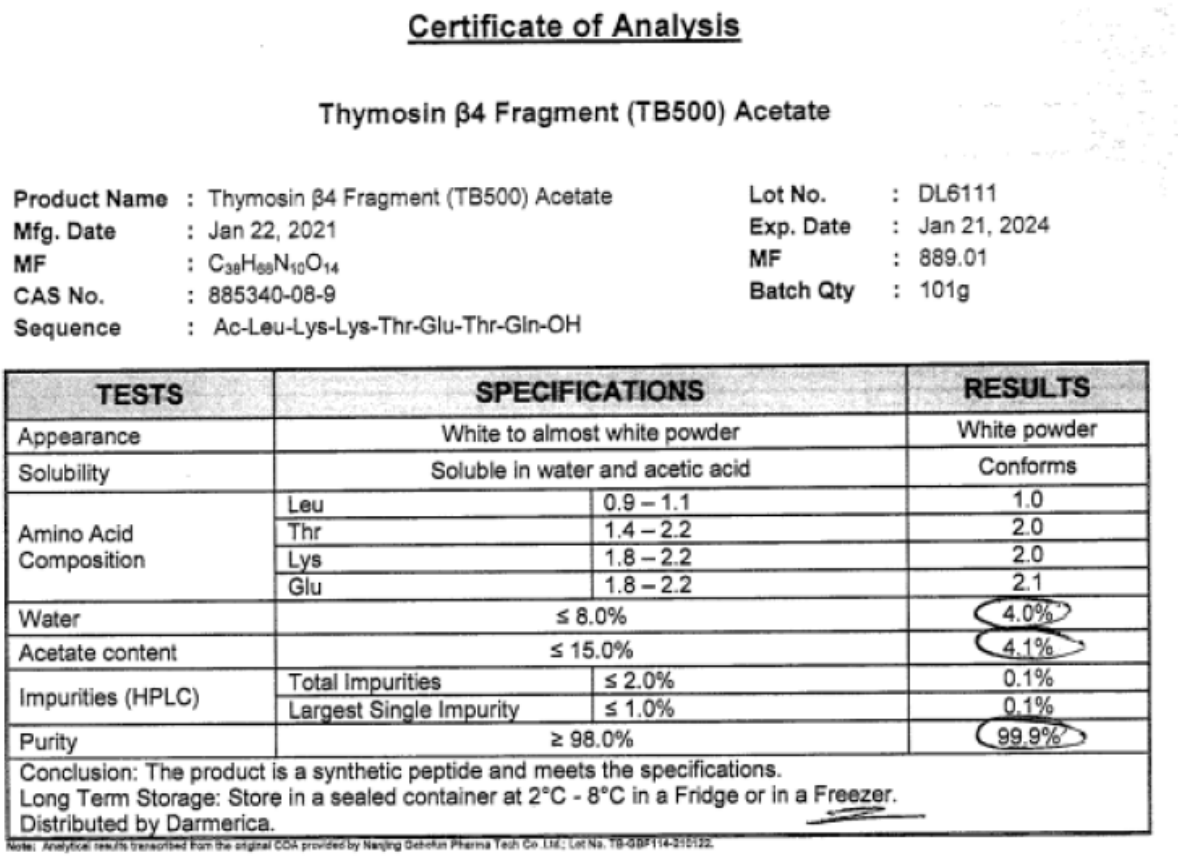
c. Likely Impurities[27]

Generally speaking, peptide-related impurities and peptide synthesis process-related impurities contribute to and are considered in understanding the impurity profile for all peptides, including TB-500 acetate. For most synthetic peptides, solid-phase peptide synthesis method has been widely used by industry for peptide synthesis. The solid phase synthesis of peptides may lead to potential peptide-related impurities due to incomplete coupling reactions, truncations, or side reactions. These peptide-related impurities are typically similar in structure to the target peptide and may be difficult to identify and quantify without sophisticated analytical methods. Additional potential common impurities may be derived from impurities in the protected amino acid starting materials (e.g., isomeric impurities and free amino acids) and other species that may carry over into the drug substance. In addition, residual solvents, coupling reagents, activators, catalysts, and scavengers may exist as SPPS process-related impurities. Drug substance and its proposed product-related impurities may also include peptide-related aggregates.

The CoA from Darmerica, submitted by the nominator, for TB-500 acetate controls 'Largest Single Impurity' at a specification limit of ≤ 1.0% and 'Total Impurities' at ≤ 2.0% (see below; Figure 4).

[27] This evaluation contains a non-exhaustive list of potential impurities in the BDS and does not address fully the potential safety concerns associated with those impurities. The compounder should use the information about the impurities identified in the CoA accompanying the BDS to evaluate any potential safety and quality issues associated with impurities in a drug product compounded using that BDS taking into account the amount of the impurity, dose, route of administration, and chronicity of dosing Available nonclinical toxicity data for likely impurities of concern (e.g., nitrosamines, potential mutagenic substances, and potential teratogenic substances) in the nominated BDS are discussed in the Nonclinical Assessment at Section II.D.1. as part of the safety assessment of the substance.

Figure 4. Example of CoA for TB-500 Acetate



Certificate of Analysis

Thymosin β4 Fragment (TB500) Acetate

Product Name	: Thymosin β4 Fragment (TB500) Acetate	**Lot No.**	: DL6111
Mfg. Date	: Jan 22, 2021	**Exp. Date**	: Jan 21, 2024
MF	: $C_{38}H_{68}N_{10}O_{14}$	**MF**	: 889.01
CAS No.	: 885340-08-9	**Batch Qty**	: 101g
Sequence	: Ac-Leu-Lys-Lys-Thr-Glu-Thr-Gln-OH		

TESTS	SPECIFICATIONS		RESULTS
Appearance	White to almost white powder		White powder
Solubility	Soluble in water and acetic acid		Conforms
Amino Acid Composition	Leu	0.9 – 1.1	1.0
	Thr	1.4 – 2.2	2.0
	Lys	1.8 – 2.2	2.0
	Glu	1.8 – 2.2	2.1
Water	≤ 8.0%		4.0%
Acetate content	≤ 15.0%		4.1%
Impurities (HPLC)	Total Impurities	≤ 2.0%	0.1%
	Largest Single Impurity	≤ 1.0%	0.1%
Purity	≥ 98.0%		99.9%
Conclusion: The product is a synthetic peptide and meets the specifications. Long Term Storage: Store in a sealed container at 2°C - 8°C in a Fridge or in a Freezer. Distributed by Darmerica.			

Note: Analytical results transcribed from the original COA provided by Nanjing Gotekin Pharma Tech Co.,Ltd.; Lot No. TB-GBF114-210122.

Because of the lack of information regarding the nature of individual impurities that can be present at up to 1.0% level and a lack of information on the potential of peptide aggregation, there is a concern about the potential immunogenicity associated with these impurities and peptide-related aggregates of TB-500 acetate.

d. Physicochemical Characteristics Pertinent to Product Performance, Such as Particle Size and Polymorphism

TB-500 acetate is reported as a white to off-white powder.[28] Though the specific solubility data of TB-500 acetate (mg/mL) is not available in the nomination package or in the public domain, the CoA from Darmerica submitted with the nomination package indicated that TB-500 acetate is soluble in water. For subcutaneous/intramuscular injectable dosage forms, particle size and polymorphism of the BDS are not critical to the performance of the final drug product as it is in liquid form prior to the administration.

[28] https://www.guidechem.com/cas/77591-33-4.html. Accessed 04/11/26.

e. Any Other Information About the Substance That May Be Relevant, Such as Whether the Bulk Drug Substance is Poorly Characterized or Difficult to Characterize

The CoA from Darmerica submitted with the nomination package for TB-500 acetate does not provide information about Identification, Assay, Aggregates, and Bacterial Endotoxin levels. Also, no additional relevant information regarding the physical and chemical characterization of TB-500 acetate is identified from the public domain.

Conclusions: TB-500 acetate is the acetate salt form of TB-500 (free base). TB-500 acetate is reported to be stable below -15°C under reported storage conditions (protected from light and kept in a tightly closed container).

TB-500 acetate is not physically and chemically well characterized based on (1) inconsistent naming conventions that do not follow established chemical nomenclature standards (e.g., INN, USAN, IUPAC), and (2) certain critical characterization data specific to TB-500 acetate, such as potential peptide impurities, were not found in publicly available scientific literature or the provided CoA. Specific tests for TB-500 acetate are not available in the public domain or in the submitted CoA, such as tests for impurities, aggregates, microbial test, and bacterial endotoxin test. As discussed in the Section II.D.2.d below, FDA is concerned about the potential for immunogenicity of TB-500 acetate when formulated in an injectable dosage form for SC/IM administrations due to the potential for aggregation as well as potential peptide-related impurities, as discussed in the impurities section. Injectable ROAs may present a particular risk for immunogenicity. We also note that the stability, pharmacological activity, and immunogenic properties of peptides are highly sensitive to the manufacturing process and quality attributes of the compounded/finished drug product.

B. Has the Substance Been Used Historically in Compounding?

This evaluation focuses on TB-500 (free base) and TB-500 acetate for SC and IM injection and their use in wound healing; however, FDA searched generally for information on the historical use of TB-500-related BDSs in compounding. Databases searched for information on TB-500-related BDSs for this evaluation included PubMed, Embase, GlobalEdge.com database for pharmaceutical regulatory agencies, NatMed Pro database, USP-NF,[29] FDA Adverse Event Reporting System (FAERS) public dashboard,[30] Google, Compounding Today[31], European Pharmacopeia,[32] Japanese Pharmacopeia,[33] and the Outsourcing Facility Product Reporting

[29] Information available at https://www.uspnf.com/ (subscription required). Accessed 02/15/24.

[30] Information available at https://fis.fda.gov/sense/app/95239e26-e0be-42d9-a960-9a5f7f1c25ee/sheet/7a47a261-d58b-4203-a8aa-6d3021737452/state/analysis. Accessed 02/15/24.

[31] Information available at https://compoundingtoday.com (subscription required). Accessed 02/15/24.

[32] Information available at https://pheur.edqm.eu/home (subscription required). Accessed 02/15/24.

[33] Information available at https://www.pmda.go.jp/english/rs-sb-std/standards-development/jp/0029.html. Accessed 02/15/24.

Database.[34] It is often unclear whether the TB-500 discussed in this section is the salt formulation or the free base and whether it was compounded or not. Therefore, FDA will consider the information discussed in this section in its evaluation of both the free base and salt form as appropriate.

1. Length of Time the Substance Has Been Used in Compounding

The nominator stated that TB-500 has been used to compound drug products but did not provide any additional information regarding the historical use of TB-500 in compounding. The nominators submitted three articles (Philp et al. 2003; Shah et al. 2018; Sosne et al. 2010) but none of the articles discussed the use of a compounded formulation of TB-500.

Using available data, the extent to which TB-500-related BDSs have been used in compounding is unclear. After conducting a literature search, TB-500 appears to have first been synthesized from thymosin β4 in 2003 (Philp et al. 2003). A veterinary preparation of TB-500 appeared in 2011 and was marketed to boost performance in equine and greyhound sports (Ho et al. 2012). No studies were found in which TB-500 was used as a compounded drug product and no outsourcing facilities have reported compounding drug products containing TB-500 to FDA.

2. The Medical Condition(s) It Has Been Used to Treat

After conducting a literature search, no articles were found in which TB-500 was administered to humans.

TB-500 is marketed online for use in a variety of conditions including promoting tissue repair and regeneration; healing damage to tendons, muscles, ligaments, and bones; wound healing, promoting angiogenesis; reducing inflammation; increasing muscle growth and muscle tone; improving endurance, stamina, and performance; improving cardiovascular health; promoting hair growth; treatment of blood clots; treatment of chronic diseases and inflammatory conditions; enhancing neurogenesis; reducing scar tissue and adhesions; improving flexibility and mobility; boosting the immune system; dry eye disorder; antioxidant; and increasing collagen.[35,36]

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[34] Information available at https://dps.fda.gov/outsourcingfacility. Accessed 02/15/24.

[35] Several websites state that TB-500 is a synthetic version of thymosin beta-4 and often use the terms interchangeably. However, as noted previously, thymosin beta-4 and TB-500 are not the same substance.

[36] Information available at https://www.transformyou.com/thymosin-beta-4, https://peptidesworld.com/product/tb-500-tb4-frag-17-23-10-mg/, https://www.peptides.org/category/tb-500/, https://limitlesslifenootropics.com/product/tb-500/, https://www.corepeptides.com/peptides/tb-500/ref/7/, https://accelerate-labs.us/products/tb-500-5mg, https://purerawz.co/product/tb-500/, https://theremedyiv.com/everything-you-need-to-know-about-tb-500-peptide/, https://drjennpb.com/product/tb-500-injury-repair-peptide/, https://agerejuvenation.com/tb500/, https://www.frontlinealternative.com/what-you-should-know-about-tb-500, https://atlantamensclinic.com/peptide-therapy/tb-500/, https://biotechpeptides.com/product/tb-500-10mg/, and https://livvnatural.com/no-more-tylenol-or-advil-treat-inflammation-more-naturally-with-the-bpc157-and-tb500-wolverine-protocol/. Accessed 02/15/24.

3. *How Widespread Its Use Has Been*

No outsourcing facilities have reported compounding drug products containing TB-500 to FDA. Two websites were found that state that they obtain TB-500 from a compounding pharmacy, but no details were provided about the pharmacy from which the product is obtained;[37] additionally, no pharmacies were found that market compounded drug products containing TB-500.

One article was found as part of the literature search that analyzed online discussion forums to "identify doping products and online sellers, as well as to evaluate their popularity and their temporal trends regarding the number of users discussing them" (Pineau et al. 2016, 105). Of the 13 forums that were analyzed, the study found that discussions about peptides and growth factors have increased since 2001 and that, when the forums were analyzed based on the "brand name" of peptides and growth factors discussed, TB-500 was one of the two most frequently mentioned substances (Pineau et al. 2016). Additionally, the study identified that, after 2010, TB-500 emerged as a topic of discussion, and the number of discussions has continued to trend upward (Pineau et al. 2016). It is unclear whether the products discussed are compounded products or how the products are obtained. The World Antidoping Agency (WADA) approved a project in 2013 to, among other things, determine the detection limits of the relevant metabolites of TB-500 and implement the metabolites into peptide-screening methods.[38] TB-500 is on the list of prohibited substances under section S2.3 of the WADA.[39]

One website was found that provided details on how to purchase TB-500 as well as reconstitution and administration instructions.[40] Several websites were found offering TB-500 products including a 2 mg, 3 mg, 5 mg, 10 mg, and 15 mg powder for reconstitution; the websites state that the products are intended for research use only.[41] Additionally, several

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[37] Information available at https://drjennpb.com/product/tb-500-injury-repair-peptide/ and https://store.matrixreformed.com/products/e48bbf8e72/4979201000004700010. Accessed 02/15/24.

[38] Information available at https://www.wada-ama.org/en/resources/scientific-research/investigation-vitroex-vivo-tb-500-metabolism-synthesis-relevant. Accessed 02/15/24.

[39] World Anti-Doping Agency Prohibited List available at https://www.wada-ama.org/en/prohibited-list. Accessed 02/15/24.

[40] Information available at https://bengreenfieldlife.com/article/supplements-articles/how-to-use-tb-500/. Accessed 02/15/24.

[41] Information available at https://www.paradigm-peptide.com/product-page/tb-500-5mg https://www.peptides.org/category/tb-500/, https://limitlesslifenootropics.com/product/tb-500/, https://www.corepeptides.com/peptides/tb-500/ref/7/, https://accelerate-labs.us/products/tb-500-5mg, https://purerawz.co/product/tb-500/, https://biotechpeptides.com/product/tb-500-10mg/, and https://livvnatural.com/no-more-tylenol-or-advil-treat-inflammation-more-naturally-with-the-bpc157-and-tb500-wolverine-protocol/. Accessed 02/15/24.

websites were found in which a consultation could be scheduled to discuss use of TB-500; no details were provided on how TB-500 would be obtained.[42]

4. Recognition of the Substance in Other Countries or Foreign Pharmacopeias

TB-500 is not recognized in either the European or Japanese Pharmacopeias. There are no approved products containing TB-500 in Canada, Australia, the United Kingdom, Belgium, Ireland, France, Norway, Germany, Spain, and Italy. Additionally, there are no products containing TB-500 that have been authorized for use in the European Union by the European Medicines Agency.

Conclusions: It is often unclear whether the TB-500 discussed in the sources considered for this section is the salt form or the free base. Using available data, the extent to which TB-500-related BDSs have been used in compounding is unclear. Since 2010, interest in TB-500 has continued to increase, several websites discuss the use of TB-500, and websites were found that sell products containing TB-500. However, it is unclear if these are compounded products; no pharmacies were found that compound products containing TB-500-related BDSs. At the time of this evaluation, currently available data and published literature are too limited for FDA to understand the historical use of TB-500-related BDSs in compounded drug products.

C. Available Evidence of Effectiveness or Lack of Effectiveness of Drug Products Compounded With the Substance

The following databases were consulted for the preparation of this section: PubMed, Embase, ClinicalTrials.gov, DailyMed, Drugs@FDA, relevant professional healthcare organization websites, and various online clinical references and websites such as information from National Institutes of Health (NIH) and Centers for Disease Control and Prevention (CDC).

The references submitted by the nominator do not clearly identify whether the TB-500 form was a salt formulation or the free base, and our search did not identify data, such as clinical studies, on these substances administered in humans. Therefore, throughout this section, the substances will be generally referred to as TB-500 unless otherwise specified as free base or acetate salt.

TB-500-related BDSs were evaluated for use in wound healing. The nomination cited three literature references[43] in support of the nomination; none were studies of TB-500 administered in humans. The nominated use is briefly described below.

Wound Healing: A wound is a disruption of the normal structure and function of the epidermis and associated underlying tissues. Wounds are broadly classified as either acute or chronic and

[42] Information available at https://www.transformyou.com/thymosin-beta-4, https://theremedyiv.com/everything-you-need-to-know-about-tb-500-peptide/, https://agerejuvenation.com/tb500/, https://antiagingnorthwest.com/about/bellingham-anti-aging-treatments/, https://www.frontlinealternative.com/what-you-should-know-about-tb-500, and https://atlantamensclinic.com/peptide-therapy/tb-500/. Accessed 02/15/24.

[43] The nominator cited three references in their nomination; three references are studies on Thymosin β4.

by their clinical presentation. Treatments vary depending on the specific wound type.[44] We note that the nominator has not provided information about what specific type of wound that TB-500 is intended to be used for.

1. *Reports of Trials, Clinical Evidence, and Anecdotal Reports of Effectiveness, or Lack of Effectiveness, of the Bulk Drug Substance*

We reviewed information from the nominator-cited references; the nominator did not provide clinical evidence on the use of TB-500 in wound healing. We performed our own search of published medical literature on additional relevant information on this substance; however, we did not identify data, such as clinical studies, on the use of TB-500 in humans.[45]

The nominator has not provided clinical evidence on the use of TB-500 in wound healing.

2. *Whether the Product Compounded With This Bulk Drug Substance is Intended To Be Used in a Serious or Life-Threatening Disease*

Wounds can be serious or life-threatening.

3. *Therapies That Have Been Used for the Condition Under Consideration*

There are FDA-approved drug products that treat the same medical condition as those proposed for the TB-500 compounded drug product(s).[46] For wound healing, treatments vary depending on the specific wound type.

Conclusion: There is a lack of evidence to evaluate the effectiveness of TB-500 (free base) and TB-500 acetate products for the nominated use of wound healing. The nomination did not include, and FDA did not find any information in the medical literature where, TB-500 was administered to patients to treat any disease or condition including its use in wound healing. Acute and chronic wounds are associated with morbidity and can be serious or life-threatening. In addition, there are currently various FDA-approved therapies with established efficacy for the management of wounds.

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[44] Basic Principles of Wound Healing, available at Basic principles of wound healing - UpToDate. Accessed 08/05/24.

[45] See 80 FR 65765 for information necessary to fully evaluate a substance.

[46] FDA considers the existence of FDA-approved or over-the-counter (OTC) monograph drug products to treat the same condition as that proposed for the nomination relevant to FDA's consideration of the effectiveness criterion, to the extent there may be alternative therapies that have been demonstrated to be effective for certain conditions. See 84 FR 4696.

D. Are There Concerns About the Safety of the Substance for Use in Compounding?

1. Nonclinical Assessment

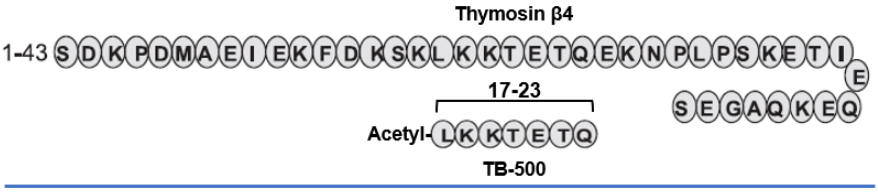
The nominator submitted nonclinical information. Specifically, the nominator submitted one article that reports a nonclinical pharmacological study of TB-500 among other substances (Sosne et al. 2010) and two articles that report nonclinical pharmacological studies of the non-acetylated heptapeptide LKKTETQ (Philp et al. 2003; Shah et al. 2018).

The following databases were consulted in preparation of this section: Drugs@FDA, Embase, European Chemicals Agency, FDA's Generally Recognized as Safe (GRAS) Notice Inventory, Google, Google Scholar, NIH's dietary supplement label database, National Toxicology Program website, Pharmapendium, PubMed, Society of Toxicology, USP, and Web of Science.

a. General Pharmacology of the Drug Substance

TB-500 is a synthetic N-acetylated heptapeptide containing the amino acid sequence 17 through 23 (LKKTETQ) of the full-length (43-amino acid) peptide thymosin β4 (Ho et al. 2012; Sosne et al. 2010). Figure 5 illustrates the amino acid sequence of TB-500, the presumed active moiety of TB-500 (free base) and TB-500 acetate.

Figure 5. Amino Acid Sequences of TB-500 and Thymosin β4 (Adapted from Sosne et al. 2010)

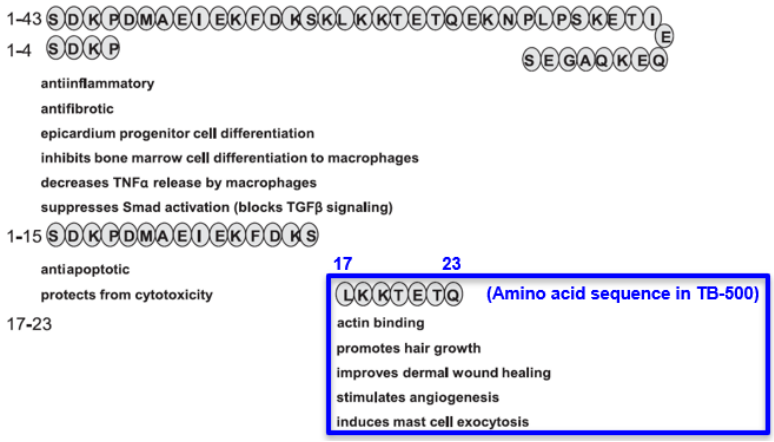


Amino acid sequences of thymosin β4 and TB-500 from the N- to the C-terminal domains. A: alanine; D: aspartic acid; E: glutamic acid; F: phenylalanine; G: glycine; I: isoleucine; K: lysine: L: leucine; M: methionine; N: asparagine; P: proline; Q: glutamine; S: serine; T: threonine. All are natural L-amino acids.

Thymosin β4 is a highly conserved peptide that was originally isolated from calf thymus and proposed to be a thymic hormone capable of stimulating the hypothalamic-pituitary-gonad axis and the immune system (Low et al. 1981; Rebar et al. 1981). In subsequent studies, thymosin β4 was found to be ubiquitously expressed in various cell types and to be secreted in various body fluids, including blood, plasma, saliva, tears, and wound fluid (Kleinman and Sosne 2016; Sosne et al. 2010).

As illustrated in Figure 6, thymosin β4 has several biological activities that have been attributed to specific amino acid sequences within the peptide (Hannappel et al. 1982; Kleinman and Sosne 2016; Shah et al. 2018; Sosne et al. 2010).

Figure 6. Schematic Representation of the Pharmacological Properties of Thymosin β4-Derived Peptides (Adapted from Sosne et al. 2010)



The TB-500 amino acid sequence (LKKTETQ) has the 6-amino acid segment (LKKTET) known as the actin-binding region of thymosin β4 (Mannherz and Hannappel 2009). It has been hypothesized that, by binding to globular G-actin but not filamentous F-actin, LKKTETQ may have the same G-actin buffering capacity of thymosin β4 that allows the flux of the G-actin monomers between the thymosin β4-bound pool and F-actin (Sosne et al. 2010). Sequestration of G-actin by thymosin β4 appears essential to the elongation of actin filaments and maintenance of the actin cytoskeleton that is critical to many cellular functions, including regulation of cell shape, motility, intracellular communication, intracellular transport, and intracellular transduction of extracellular signals (Goldstein et al. 2005). It remains to be determined whether actin binding contributes to the ability of the heptapeptide LKKTETQ to stimulate angiogenesis, induce mast cell degranulation, increase angiogenesis, and promote wound healing (Sosne et al. 2010).

The wound-healing properties of the non-acetylated heptapeptide LKKTETQ were assessed in a pharmacological study conducted in an aged mouse model of impaired healing (Philp et al. 2003). In this study, four 3-mm punch wounds were made on the dorsal region of 26-month-old female BALB/cBy mice. On the day of and 48 hours after the wound generation, 50 µl of the following treatments were applied topically to each wound: (i) thymosin β4 [0.1 % in phosphate-buffered saline (PBS) or 0.05% in hydrogel], (ii) non-acetylated heptapeptide LKKTETQ [0.01% in PBS], or (iii) vehicle [hydrogel or PBS]. Histological assessments conducted on day 7 after the wound generation suggested that topical application of either thymosin β4 or the non-acetylated heptapeptide LKKTETQ significantly increased epidermal closure and collagen content at the wound sites in aged mice (Philp et al. 2003). It is unknown whether the apparent wound-healing properties of the non-acetylated heptapeptide LKKTETQ can be generalized to other wound types. In addition, the data should be interpreted with caution in part because the study lacks an assessment of the dose-response relationship for the non-acetylated heptapeptide to improve wound healing.

It is important to note that, because acetylation of peptides and proteins irreversibly alters their charge, hydrophobicity, and size, it can modify their lifespan, folding characteristics, and binding properties (Ree et al. 2018). Therefore, the pharmacological profile of the non-acetylated

heptapeptide LKKTETQ cannot be directly extrapolated to the N-acetylated heptapeptide TB-500. For instance, although wound healing is a pharmacological effect normally associated with the heptapeptide sequence LKKTETQ (reviewed in Sosne et al. 2010), TB-500 (free base) appeared to be devoid of wound-healing properties in an in-vitro study (Rahaman et al. 2024). In this study, cultured confluent fibroblasts with uniform wounds generated by controlled physical scratches were incubated for 8 hours with medium containing TB-500 (free base; 50 µg/mL) or vehicle. Wound closure, which was quantified by subtracting the post-incubation from the pre-incubation wound area, was not significantly different between cultures incubated with TB-500 (free base) and vehicle (Rahaman et al. 2024). The study lacks a concentration-response relationship analysis. However, under the same experimental conditions, the TB-500 metabolite N-acetylated LKKTE (50 µg/mL) caused a small, but significant wound closure (Rahaman et al. 2024). It remains unknown whether TB-500 (free base) and TB-500 acetate could have wound-healing properties in vivo and, if so, whether their pharmacological activity depends on the conversion of the TB-500 moiety to a pharmacologically active metabolite.

b. Pharmacokinetics/Toxicokinetics

According to an in-vivo pharmacokinetic study conducted in thoroughbred geldings treated with TB-500 (free base; 10 mg, SC), plasma concentrations of TB-500 peaked at 0.05 and 0.08 ng/mL between 60 and 120 minutes after the treatment and declined subsequently becoming unquantifiable between 6 and 10 hours after the treatment (Ho et al. 2012). In this study, experiments were not conducted with TB-500 (free base) delivered via the intravenous (IV) ROA, precluding the establishment of the absolute bioavailability of TB-500 (free base) via the SC ROA.

The major metabolites of TB-500 identified in an in-vitro experiment in which homogenized equine liver was incubated with TB-500 (free base) at 37°C for 1 hour included N-acetylated LKKTET (M1), N-acetylated LKKTE (M2), N-acetylated LKKT (M3), N-acetylated LKK (M4), and N-acetylated LK (M5). These metabolites were generated from a sequential loss of amino acid residues from the C-terminal domain of TB-500. Additional metabolites were generated by enzymatic stereoisomerization of the parent peptide and the primary metabolites M1 and M2. Likely due to non-enzymatic hydrolysis of the peptide bonds, traces of metabolites M1, M2, M3, M4, and M5 could also be the detected in the absence of homogenized liver (Ho et al. 2012).

C-terminus-truncated metabolites of TB-500 are also generated in vivo, because they were detected in plasma and urine samples collected from TB-500 (free base)-treated horses. Specifically, a plasma sample drawn from horses at approximately 2 hours after their SC treatment with TB-500 (free base; 10 mg) contained the parent peptide, its stereoisomer, and the C-terminus-truncated metabolites M2 and M5. A urine sample collected from the same horses at approximately 6 hours after their SC treatment with TB-500 (free base; 10 mg) contained the parent peptide in addition to its stereoisomer and the M1, M2, M4, and M5 (Ho et al. 2012).

The in-vivo metabolic profile of TB-500 (free base) in horses resembles that observed in rats treated with the peptide. Specifically, the main metabolites identified and quantified in the urine of young adult (6-week-old) Sprague-Dawley male rats that received an intraperitoneal (IP) injection of TB-500 (free base; 50 mg/kg) included the N-acetylated metabolites M2, M4, and

M5. Traces of N-acetylated lysine (M6), a metabolite not detected in the urine of TB-500-treated horses, were detected in the urine of TB-500-treated rats (Rahaman et al. 2024).

Rahaman and collaborators conducted in-vitro experiments in which TB-500 (free base) was incubated with human kidney microsomes, rat liver microsomes, cytosol pooled from human liver, S9 pooled from human liver, and human serum at 37°C for 22 hours. In each of the enzymatic systems, the primary TB-500 metabolites were the C-terminal-truncated M2, M4, M5, and M6 peptides. The metabolites M2 and M4 were disproportionally generated in human serum and human kidney microsomes, respectively, compared to rat liver microsomes incubated with TB-500 for 22 hours (Rahaman et al. 2024).

The metabolic profile of TB-500 was further assessed in human kidney microsomes, S9 pooled from human liver, human liver microsomes, and human serum incubated with TB-500 (free base) for a short time – 2 hours (Zvereva et al. 2016). In line with the findings discussed above, Zvereva and colleagues reported that: (i) the primary metabolites of TB-500 were the C-truncated metabolites M2, M3, M4, and M5, (ii) the proportion of the metabolites varied with the enzymatic system assayed; for instance, M2 and M4 were the predominant metabolites in TB-500-incubated human serum and M5 was the prevalent metabolite in TB-500-incubated human liver microsomes, and (iii) TB-500 was not metabolically deamidated in vitro (Zvereva et al. 2016).

At the time of this evaluation, the nominator did not submit, and FDA did not identify pharmacokinetic studies of TB-500 (free base) or TB-500 acetate delivered via parenteral ROAs other than the SC and IP described above.

c. Acute Toxicity[47]

At the time of this evaluation, the nominator did not submit, and FDA did not identify acute toxicity studies of TB-500 (free base) or TB-500 acetate.

d. Repeat-Dose Toxicity[48]

At the time of this evaluation, the nominator did not submit, and FDA did not identify repeat-dose toxicity studies of TB-500 (free base) or TB-500 acetate.

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[47] Acute toxicity refers to adverse effects observed following administration of a single dose of a substance, or multiple doses given within a short period (approximately 24 hours). For more information on general approaches for acute toxicity studies, please refer to FDA's guidance for industry *M3(R2) Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals* (January 2010), available at https://www.fda.gov/media/71542/download.

[48] Repeat-dose toxicity studies consist of in-vivo animal studies that seek to evaluate the toxicity of the test substance when it is repetitively administered daily for an extended period. For more information on general approaches for repeat-dose toxicity studies, please refer to FDA's guidance for industry *M3(R2) Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals* (January 2010), available at https://www.fda.gov/media/71542/download.

e. Genotoxicity[49]

At the time of this evaluation, the nominator did not submit, and FDA did not identify genotoxicity studies of TB-500 (free base) or TB-500 acetate.

f. Developmental and Reproductive Toxicity[50]

At the time of this evaluation, the nominator did not submit, and FDA did not identify nonclinical developmental and reproductive studies of TB-500 (free base) or TB-500 acetate.

g. Carcinogenicity[51]

At the time of this evaluation, the nominator did not submit, and FDA did not identify nonclinical carcinogenicity studies of TB-500 (free base) or TB-500 acetate.

Conclusions: At the time of this evaluation, the nominator did not submit, and FDA did not identify nonclinical in-vivo pharmacological studies assessing the potential for TB-500 (free base) or TB-500 acetate to promote wound healing. Instead, FDA identified an in-vitro study reporting that TB-500 (free base; at the concentration of 50 µg/mL) did not induce wound healing in confluent cultures of fibroblasts with scratch-generated wounds. FDA also identified nonclinical pharmacokinetic studies indicating that TB-500 (free base) delivered via the SC and IP ROAs reaches the systemic circulation and is metabolically hydrolyzed into smaller peptides. The nominator did not submit, and FDA did not identify nonclinical PK studies in which animals received TB-500 (free base) or TB-500 acetate via the nominated IM ROA. While the TB-500 metabolite N-acetylated LKKTE (50 µg/mL) appeared to have wound-healing properties in vitro,

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[49] The genotoxicity assessment battery usually consists of a gene mutagenicity assay (for single dose trials) and a variety of clastogenicity/genotoxicity assays. To support multiple dose administration in humans, additional genotoxicity testing assessment is usually conducted to detect chromosomal damage in mammalian systems. For more information on general approaches for genotoxicity studies, please refer to FDA's guidance for industry *S2(R1) Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use* (June 2012), available at https://www.fda.gov/media/71980/download.

[50] Developmental and reproductive toxicity studies are usually designed to assess the potential adverse effects of a substance within a complete reproductive cycle, from conception to reproductive capacity in subsequent generations, and to identify the potential effects of a substance on pre-, peri-, and postnatal development. Developmental toxicity or teratogenicity refers to adverse effects (can include embryo-fetal mortality, structural abnormalities, functional impairment, or alterations to growth) and can occur in pups either as a result of the exposure of their parents to the substance, prior to the pups' birth, or by direct exposure of the pups to the substance after birth. For more information on general approaches for reproductive and developmental toxicity studies, please refer to FDA's guidance for industry *S5(R3) Detection of Reproductive and Developmental Toxicity for Human Pharmaceuticals* (May 2021), available at https://www.fda.gov/media/148475/download.

[51] Studies that assess cancer risk in animals are used as predictive tools to evaluate the potential for drugs to cause tumors when used by humans on a chronic basis. Carcinogenicity studies are conducted if the clinical use is expected to be continuous for a minimum of 6 months of life, or if intermittent clinical use is expected to total 6 months or more of life. For more information on general approaches for carcinogenicity studies, please refer to FDA's guidance for industry *S1B Testing for Carcinogenicity of Pharmaceuticals* (July 1997), available at https://www.fda.gov/media/71935/download.

the pharmacological profile of the other TB-500 metabolites is unknown. At the time of this evaluation, the nominator did not submit, and FDA did not identify nonclinical toxicity studies of TB-500 (free base) or TB-500 acetate. In conclusion, nonclinical pharmacological evidence is currently lacking to support the potential for TB-500 to promote wound healing, and nonclinical toxicological studies are not available to inform safety considerations for potential clinical uses of TB-500 (free base) or TB-500 acetate.

2. Human Safety

The following databases were consulted in the preparation of this section: PubMed, Embase, FAERS, Human Foods Program (HFP)[52] Human Foods Complaint System (HFCS),[53] ClinicalTrials.gov, professional healthcare organization websites, and various online clinical references and websites. Note that throughout this section, the substance will be generally referred to as TB-500 unless otherwise specified as free base or acetate salt.

The nomination did not include, and FDA did not find information on products containing TB-500 (free base) or TB-500 acetate administered in humans.

a. Pharmacokinetic Data

FDA did not identify clinical studies in humans assessing pharmacokinetics or pharmacodynamics of TB-500 (free base) or TB-500 acetate via any ROA.

b. Reported Adverse Reactions (Case Reports and Anecdotal Cases Assessing Safety)

FAERS

The Office of Surveillance and Epidemiology (OSE) conducted a search of the FAERS database for reports of adverse events (AEs) associated with TB-500 through March 26, 2025. The FAERS search did not retrieve any reports, and the literature search did not identify any cases of AEs. It is important to note that FAERS data have limitations.[54]

HFCS

HFP collects reports of AEs and product complaint reports submitted to FDA for food, dietary supplements, and cosmetics in the HFCS. A search of HFCS was conducted for AEs associated

[52] Formerly Center for Food Safety and Nutrition (CFSAN).

[53] Formerly CFSAN Adverse Event Reporting System (CAERS).

[54] It is important to note that FAERS data have limitations. Reporting is voluntary. In general, there is no certainty that a reported AE was due to the suspect product. Further, FDA does not receive all AE reports that may potentially occur with a product, especially for compounded products. Considering these limitations, FDA cannot make definitive conclusions regarding the safety of TB-500. For additional information, see https://fis.fda.gov/extensions/FPD-FAQ/FPD-FAQ.html#_Toc514144622. Accessed 3/24/25.

with TB-500 for the date range of 1/1/2004 to 3/10/2025 and retrieved two cases about "blended TB-500 and BPC-157"; however, the reports did not include safety assessments.

We did not find case reports in the published medical literature on the use of TB-500 in humans.

c. Clinical Studies Assessing Safety

The nomination did not include, and FDA has not identified, any clinical studies or human exposure data using TB-500 via any ROA. Therefore, potential safety risks associated with the use of TB-500 in humans are unknown.

d. Other Safety Information (e.g., Relevant Safety Information From Other Regulatory Agencies as Appropriate)

Immunogenicity and Aggregation Concerns

FDA has issued guidance regarding immunogenicity assessment for therapeutic protein products.[55] The guidance describes immunogenicity as the propensity of a therapeutic protein product to generate immune responses to itself and to related proteins including endogenous proteins or peptides, or to induce immunologically related adverse clinical events. Although this guidance addresses therapeutic protein products, the concerns about immunogenicity are also relevant to peptides (such as TB-500 (free base) and TB-500 acetate), which can similarly elicit an immunogenic response. This immunogenic response may be enhanced when peptides are given via injectable ROA, such as IV, IM, and SC. In general, intradermal, SC, inhalational ROA are associated with increased immunogenicity compared to IM and IV ROA.

The consequences of triggering an immune response may range from antibody responses with no apparent clinical manifestations to life-threatening and catastrophic reactions. Such outcomes are often unpredictable in patients administered therapeutic protein or peptide products. One possible consequence of the development of an immune response is the development of neutralizing antibody activity that may lead to loss of efficacy or even result in the neutralization of the activity of the endogenous peptide counterpart.

In addition, compared to small molecule APIs, peptides are distinct because they may have an inherent tendency to aggregate. Aggregation refers to the processes through which peptides associate into larger species consisting of multiple peptide chains. Aggregates can be highly ordered or amorphous and the formation can be reversible or irreversible (Zapadka et al. 2017). Peptides with as few as two amino acids have been shown to aggregate (Frederix et al. 2011). Aggregates can impact the pharmacology of the peptide. In addition, aggregation is a risk factor in immunogenicity and for decreased pharmacotherapeutic effect of the drug product due to effects on bioavailability, formation of precipitates, or anti-drug antibody production (Ratanji et al. 2014).

[55] See FDA's guidance for industry *Immunogenicity Assessment for Therapeutic Protein Products* (August 2014), available at https://www.fda.gov/media/85017/download.

TB-500 (free base) and TB-500 acetate are peptides consisting of seven amino acids proposed for the IM and SC ROA. As a peptide that is administered through injectable ROAs (SC and IM), TB-500 may pose a significant risk for immunogenicity, potentially amplified by aggregation as well as potential peptide-related impurities, as discussed above. The nominator did not provide, and FDA did not identify clinical studies assessing immunogenicity nor aggregation of TB-500.

e. Therapies That Have Been Used for the Condition(s) Under Consideration

There are FDA-approved drug products that treat the same medical condition as that proposed for TB-500-related BDSs.[56]

Conclusions: There is a lack of clinical and nonclinical safety information on the use of TB-500 (free base) and TB-500 acetate. FDA is particularly concerned about the absence of human data on drug products containing these substances administered via any ROA. Peptides administered via injectable ROAs, such as the proposed IM and SC routes, may pose a significant risk for immunogenicity, potentially amplified by aggregation as well as potential peptide-related impurities. The nomination did not include, and FDA has not identified, information about TB-500-related substances to suggest that these substances do not present such risks. Therefore, potential safety risks associated with the use of these substances in humans are unknown. There are currently available FDA-approved therapies for the management of wounds.

III. CONCLUSION AND RECOMMENDATION

We have balanced the criteria described in section II above to evaluate TB-500 (free base) and TB-500 acetate for the 503A Bulks List. After considering the information currently available, a balancing of the criteria ***weighs against*** TB-500 (free base) and TB-500 acetate being placed on that list based on the following:

1. Conclusions on the physical and chemical characterization for each TB-500-related BDS, TB-500 (free base) and TB-500 acetate, are included in subsections 1.1 and 1.2, respectively.

   1.1.

   TB-500 (free base) is reported to be a peptide containing seven amino acids with an acetyl group at the N-terminal Leucine (Ac-Leu-Lys-Lys-Thr-Glu-Thr-Gln-OH). TB-500 (free base) is reported to be stable below -20°C under reported storage conditions (sealed storage, away from moisture and light, under nitrogen).

   TB-500 (free base) is not physically and chemically well characterized based on (1) inconsistent naming conventions that do not follow established chemical nomenclature standards (e.g., INN, USAN, IUPAC), and (2) certain critical characterization data specific to

[56] FDA considers the existence of FDA-approved or OTC monograph drug products to treat the same condition as that proposed for the nomination relevant to FDA's consideration of the safety criterion, to the extent there may be therapies that have been demonstrated to be safe under the conditions of use set forth in the approved labeling. See 84 FR 4696.

TB-500 (free base), such as potential peptide impurities, were not found in publicly available scientific literature and CoA. Specific tests for TB-500 (free base) are not available in the public domain or in the publicly available CoA, such as tests for impurities, aggregates, microbiological quality, and bacterial endotoxin. As discussed in the Section II.D.2.d, FDA is concerned about the potential for immunogenicity of TB-500 (free base) when formulated in an injectable dosage form for SC/IM administrations due to the potential for aggregation as well as potential peptide-related impurities, as discussed in the impurities section. Injectable routes of administration may present a particular risk for immunogenicity. We also note that the stability, pharmacological activity, and immunogenic properties of peptides are highly sensitive to the manufacturing process and quality attributes of the compounded/finished drug product.

1.2.

TB-500 acetate is the acetate salt form of TB-500 (free base). TB-500 acetate is reported to be stable below -15°C under reported storage conditions (protected from light and kept in a tightly closed container).

TB-500 acetate is not physically and chemically well characterized based on (1) inconsistent naming conventions that do not follow established chemical nomenclature standards (e.g., INN, USAN, IUPAC), and (2) certain critical characterization data specific to TB-500 acetate, such as potential peptide impurities, were not found in publicly available scientific literature or the provided CoA. Specific tests for TB-500 acetate are not available in the public domain or in the submitted CoA, such as tests for impurities, aggregates, microbial test, and bacterial endotoxin test. As discussed in the Section II.D.2.d, FDA is concerned about the potential for immunogenicity of TB-500 acetate when formulated in an injectable dosage form for SC/IM administrations due to the potential for aggregation as well as potential peptide-related impurities, as discussed in the impurities section. Injectable routes of administration may present a particular risk for immunogenicity. We also note that the stability, pharmacological activity, and immunogenic properties of peptides are highly sensitive to the manufacturing process and quality attributes of the compounded/finished drug product.

2. It is often unclear whether the TB-500 discussed in the sources considered for this section is the salt form or the free base. Using available data, the extent to which TB-500-related BDSs have been used in compounding is unclear. Since 2010, interest in thymosin beta-4 has continued to increase, several websites discuss the use of TB-500, and websites were found that sell products containing TB-500. However, it is unclear if these are compounded products; no pharmacies were found that compound products containing TB-500-related BDSs. At the time of this evaluation, currently available data and published literature are too limited for FDA to understand the historical use of TB-500-related BDSs in compounded drug products.

3. There is a lack of evidence to evaluate the effectiveness of TB-500 (free base) and TB-500 acetate products for the nominated use of wound healing. The nomination did not include, and FDA did not find any information in the medical literature where TB-500 was administered to patients to treat any disease or condition including its use in wound healing. Acute and chronic wounds are associated with morbidity and can be serious or life-

threatening. In addition, there are currently various FDA-approved therapies with established efficacy for the management of wounds.

4. At the time of this evaluation, the nominator did not submit, and FDA did not identify nonclinical in-vivo pharmacological studies assessing the potential for TB-500 (free base) or TB-500 acetate to promote wound healing. Instead, FDA identified an in-vitro study reporting that TB-500 (free base; at the concentration of 50 µg/mL) did not induce wound healing in confluent cultures of fibroblasts with scratch-generated wounds. FDA also identified nonclinical pharmacokinetic studies indicating that TB-500 (free base) delivered via the SC and IP ROAs reaches the systemic circulation and is metabolically hydrolyzed into smaller peptides. However, the nominator did not submit, and FDA did not identify nonclinical PK studies in which animals received TB-500 (free base) or TB-500 acetate via the nominated IM route of administration. While the TB-500 metabolite N-acetylated LKKTE (50 µg/mL) appeared to have wound-healing properties in vitro, the pharmacological profile of the other TB-500 metabolites is unknown. At the time of this evaluation, the nominator did not submit, and FDA did not identify nonclinical toxicity studies of TB-500 (free base) or TB-500 acetate. In conclusion, nonclinical pharmacological evidence is currently lacking to support the potential for TB-500 to promote wound healing, and nonclinical toxicological studies are not available to inform safety considerations for potential clinical uses of TB-500 (free base) or TB-500 acetate.

   There is a lack of clinical and nonclinical safety information on the use of TB-500 (free base) and TB-500 acetate. FDA is particularly concerned about the absence of human data on drug products containing these substances administered via any route of administration. Peptides administered via injectable routes of administration, such as the proposed IM and SC routes, may pose a significant risk for immunogenicity, potentially amplified by aggregation as well as potential peptide-related impurities. The nomination did not include, and FDA has not identified, information about TB-500-related bulk drug substances to suggest that these substances do not present such risks. Therefore, potential safety risks associated with the use of these substances in humans are unknown. There are currently available FDA-approved therapies for the management of wounds.

On balance, the physiochemical characterization, information on historical use, lack of any evidence of effectiveness, and safety information for both TB-500 (free base) and TB-500 acetate weigh against their being added to the 503A Bulks List. In particular, FDA's proposal is based on the lack of data related to physiochemical characterization, lack of historical use in compounding, and the non-existing information on the use of these substances administered in humans to make a conclusion on their clinical safety and effectiveness. In addition, peptides administered via the proposed injectable routes of administration, may pose a significant risk for immunogenicity. There are currently FDA-approved therapies for the management of wounds. Accordingly, we propose not adding TB-500 (free base) or TB-500 acetate to the 503A Bulks List.

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TB-500-Related Bulk Drug Substances (TB-500 (Free Base) and TB-500 acetate) Nomination

Company Name	**Wells Pharmacy Network**
Contact Name	**Anthony Campbell, PharmD, BCSCP**
Contact Phone	**352-622-2913**
Contact Email	**ACampbell@wellsrx.com**

503A Bulk Drug Substance Nomination	
What is the name of the nominated ingredient?	**TB-500** **Thymosin Beta-4, Fragment (LKKTETQ)** **Provided as "ABP-7" (Actin Binding Protein)**
Is the ingredient an active ingredient that meets the definition of "bulk drug substance" in 207.3 (a)(4)?	**YES**
Is the ingredient listed in any of the three sections of the Orange Book?	**No**
Were any drug monographs for the ingredient found in the USP or NF monographs?	**No**
What is the chemical name of the substance?	**IUPAC Name:** N-acetyl-L-leucyl-L-lysyl-L-lysyl-L-threonyl-L-alpha-glutamyl-L-threonyl-L-glutamine **IUPAC Condensed:** Ac-Leu-Lys-Lys-Thr-Glu-Thr-Gln-OH **Sequence**: LKKTETQ **InChIKey** ADKDNDYYIZUVCZ-ZQNQAVPYSA-N **CAS#: 885340-08-9** (AKA CAS#: 476014-70-7) $C_{38}H_{66}N_{10}O_{14}$
What is the common name of the substance?	**TB-500 fragment** **TB500** **UNII-QHK6Z47GTG** **Provided as "ABP-7" (Actin Binding Protein)**
Does the substance have a UNII code?	QHK6Z47GTG
What is the chemical grade of the substance?	**Provided by FDA Registered Supplier/COA**
What is the strength, quality, stability, and purity of the ingredient?	**Assay, Description, Solubility, etc.; Example of Certificate of Analysis for this chemical is attached.**

How is the ingredient supplied?	Lyophilized Powder
Is the substance recognized in foreign pharmacopeias or registered in other countries?	No
Has information been submitted about the substance to the USP for consideration of drug monograph development?	No
What dosage form(s) will be compounded using the bulk drug substance?	Subcutaneous/Intramuscular Injectable
What strength(s) will be compounded from the nominated substance?	3mg/mL
What is the anticipated route(s) of administration of the compounded drug product(s)?	Subcutaneous and/or Intramuscular Injection
Are there safety and efficacy data on compounded drugs using the nominated substance?	See Attached Documents
Has the bulk drug substance been used previously to compound drug product(s)?	YES
What is the proposed use for the drug product(s) to be compounded with the nominated substance?	**LKKTETQ (the active site within the protein thymosin β_4 responsible for actin binding, cell migration and wound healing)**
What is the reason for use of a compounded drug product rather than an FDA-approved product?	no FDA-approved product available
Is there any other relevant information?	Added as an Attachment

Darmerica
198 Wilshire Boulevard
Casselberry, FL 32707
www.darmerica.com

Certificate of Analysis

Thymosin β4 Fragment (TB500) Acetate

Product Name : Thymosin β4 Fragment (TB500) Acetate
Mfg. Date : Jan 22, 2021
MF : $C_{38}H_{68}N_{10}O_{14}$
CAS No. : 885340-08-9
Sequence : Ac-Leu-Lys-Lys-Thr-Glu-Thr-Gln-OH

Lot No. : DL6111
Exp. Date : Jan 21, 2024
MF : 889.01
Batch Qty : 101g

TESTS	SPECIFICATIONS		RESULTS
Appearance	White to almost white powder		White powder
Solubility	Soluble in water and acetic acid		Conforms
Amino Acid Composition	Leu	0.9 – 1.1	1.0
	Thr	1.4 – 2.2	2.0
	Lys	1.8 – 2.2	2.0
	Glu	1.8 – 2.2	2.1
Water	≤ 8.0%		4.0%
Acetate content	≤ 15.0%		4.1%
Impurities (HPLC)	Total Impurities	≤ 2.0%	0.1%
	Largest Single Impurity	≤ 1.0%	0.1%
Purity	≥ 98.0%		99.9%
Conclusion: The product is a synthetic peptide and meets the specifications. Long Term Storage: Store in a sealed container at 2°C - 8°C in a Fridge or in a Freezer. Distributed by Darmerica.			

Note: Analytical results transcribed from the original COA provided by Nanjing Gebofun Pharma Tech Co.,Ltd.; Lot No. TB-GBF114-210122.

Based on the review of above information, the lot stands released.

	Name	Title	Signature	Date
Prepared by	Seka Nadima	Quality Assistant	Seka Nadima	06/25/2021
Released by	Harun Kapidzic	Quality Assistant		07/09/2021

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