2.3.S. DRUG SUBSTANCE

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INTRODUCTION

The drug substance used in the manufacture of <TERIPARATIDE 20µg/80µL solution for injection in pre-filled pen> is Teriparatide.

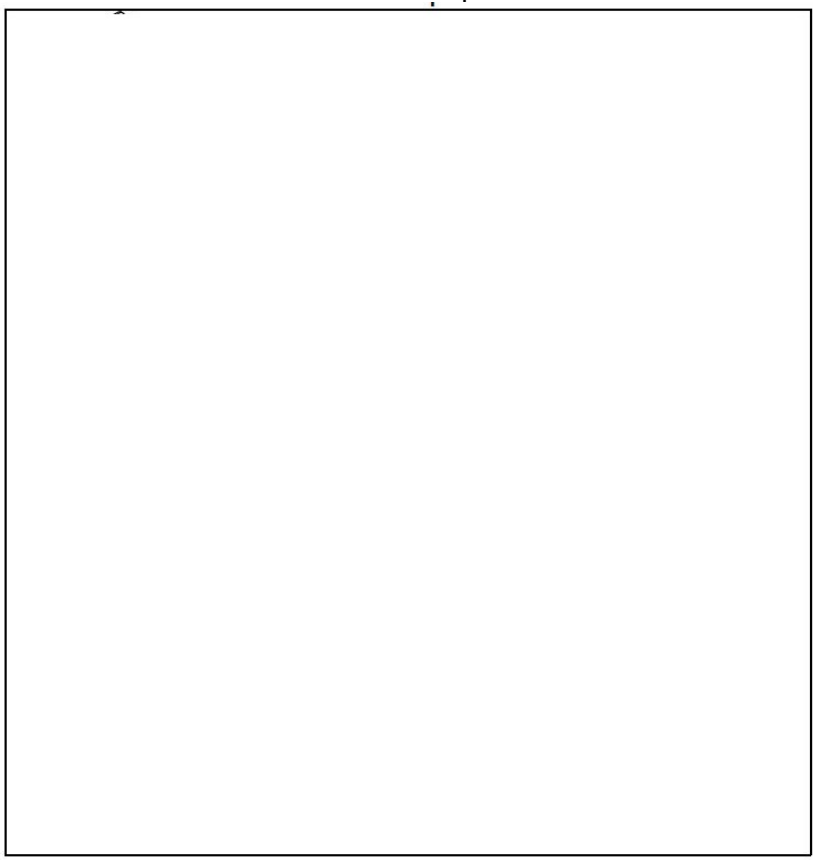
Teriparatide used in the drug product manufacture is supplied by and it is manufactured and controlled according to current GMP relative to active substances. The relevant quality characteristics of the substance are well defined and controlled. The manufacturer holds a Drug Master File for Teriparatide. Open Part of the DMF is included in Section 3.2.S-BCN. An overview of the information included in the Open Part of the DMF is provided in the following pages.

2.3.S.1 GENERAL INFORMATION

Teriparatide, also known as a Parathyroid hormone (1-34) human, is a white or off-white amorphous powder which is freely soluble in water. It is a polypeptide that consists on the 1- 34 amino-acid fragment of human The structural formula of the substance is shown in FIGURE 1.

Teriparatide (molecular formula (as free base)) is a polypeptide with an average molecular weight of 4117, 72 u.m.a.

FIGURE 1: Chemical structure of Teriparatide



3.2.S.2. MANUFACTURE

Manufacturer

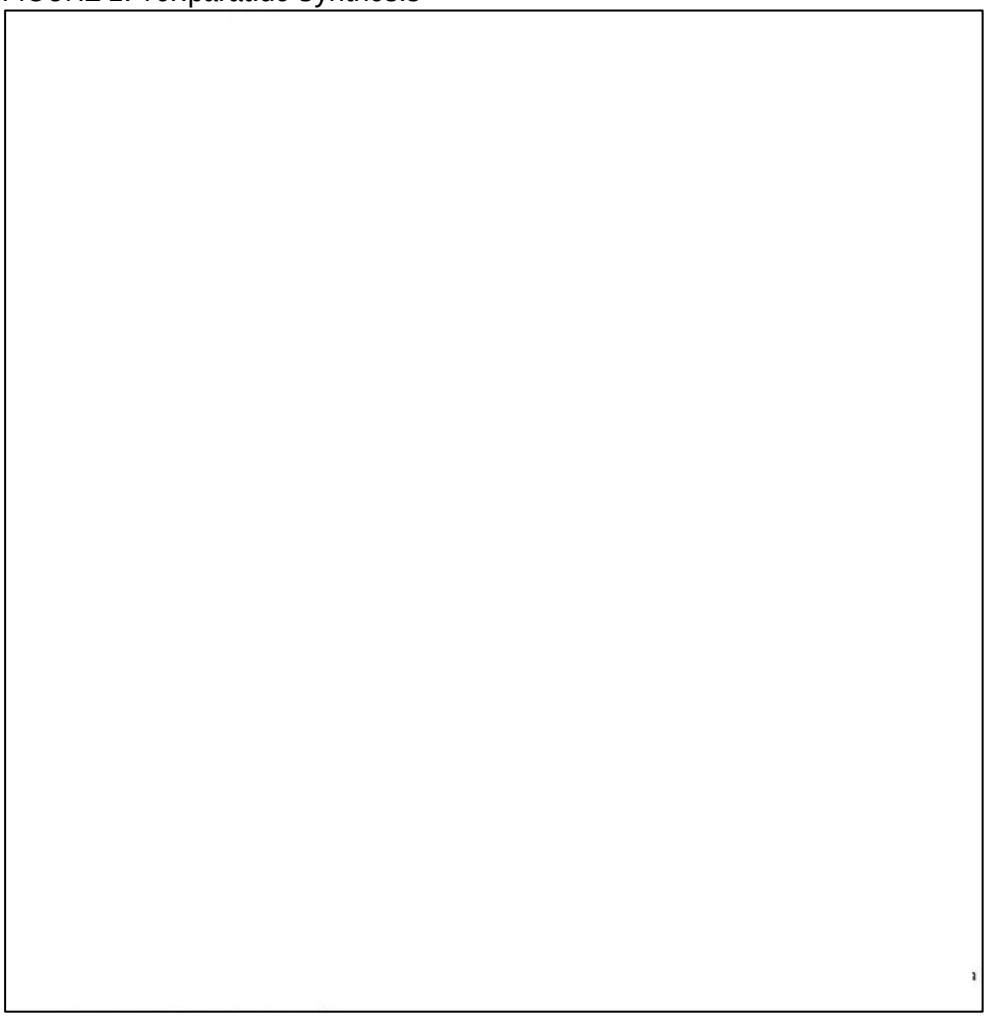
Functions: manufacture of drug substance

Laboratorios Echevarne

Manufacturing Process

Teriparatide is prepared by linear stepwise solid phase peptide methodology with Fmoc/tBu protection strategy. Linear stepwise solid phase peptide synthesis lies in the principle of total coupling of a temporally α-N protected aminoacid on a derivatized polymeric support. After the coupling of the last amino acid the complete protected sequence on the resin is obtained. A subsequent acidolitic treatment which cleaves the peptide-resin bond and removes the side-chain protecting groups, yields the final Teriparatide crude that will be purified. After purification the product is submitted to an ion exchange process to obtain the final product in the acetate form. The process is schematized in FIGURE *2*.

FIGURE 2: Teriparatide Synthesis



Control of Materials

Depending on the nature of the product the frequency of the identity test and/or the purity test is different. For those materials which are an integral part of the backbone of the molecule such as amino acids and AcOH all containers received are sampled and analyzed individually for identification test and purity. For amino acids, a mass (ES-MS) identification, a purity HPLC analysis and an enantiomeric purity HPLC analysis are performed. All the others raw materials (solvents, scavengers, etc.) will be sampled depending on the nature, amount and frequency of reception, as detailed in Section 3.2.S.2.3 of the Open Part of the DMF.

TABLE *1* describes all reagents and solvents used for this synthesis along with the suppliers of the starting materials.

TABLE 1 .-. Solvents ad reagents used during Teriparatide Synthesis

Reagent Type	Reagent	Suppliers

Table 2 Solvents and reagents used during the synthesis of Teriparatide

Specifications of raw materials are detailed in Section 3.2.S.2.3 of the DMF Open Part.

does not use within the process any material coming from animal or human origin. The quality policy assures that no material is accepted in the warehouse without a certificate of origin stating that the product is not of human or animal origin.

3.2.S.3 CHARACTERISATION

Elucidation of structure and other characteristics

The confirmation of the chemical structure of the active substance is based on the results obtained from the elemental analysis, amino acid analysis and from the results obtained from the spectroscopic techniques such as I.R., H-HMR (1D and 2D 2D NOESY and TOCSY), UV MS-MS sequencing, Mass spectrometry.

C-NMR is not performed because peptides are molecules with a large amount of Carbons and overlapping of the signals may occur. Furthermore, this technique is not able to give us information on the sequence of the peptide, so the information that could be obtained from this analysis is not useful for the characterization of the product. X-Ray Diffraction is not performed because our product is not a crystal, it is an amorphous powder and therefore this technique can not be used.

Full details on the characterization results are included in Section 3.2.S.3.1.

Impurities

Full details on the characterization of impurities potentially to be present in the drug substance are included in Section 3.2.S.3.2.

TABLE 2 lists potential impurities that can be found in the drug substance. The impurities present at levels higher than 0.1% in two batches produced are listed in TABLE 3.

TABLE 2 .-. Teriparatide identified potential impurities

Tentative Identification [Abbreviated name]	Relative Rt(HPLC)	Origin

TABLE 3 .-. Impurities in the drug product

Batch	rrt	Abbreviated name	Area%

Residual Solvents

The presence of the following residual solvents was analyz ed and validated. None of these solvents are used in the final step of the manufacturing process:

- Dimethylformamide (DMF)
- Dichloromethane (DCM)
- Acetonitrile
- Methanol
- Diethyl ether

Three consecutive batches were analyzed, using a validated GC method, in order to validate the absence of residual solvents in the product.

The results obtained (see TABLE 4), taking into consideration the maximum daily dose of Teriparatide (0.02 mg), show that the maximum daily exposure of the patient to these solvents is, at least, 5 orders of magnitude below the 10% of the permitted daily exposure for all the solvents.

TABLE 4 – Daily exposure residual solvents

Solvent	PDE (ICH) (µg/day)	10%PDE (µg/day)	PTH0901 (µg/day)	PTH0902 (µg/day)	PTH0903 (µg/day)

According to the guidelines CPMP/ICH/283/95 (Impurities: Residual Solvents) and its annex 1 (Specifications for Class 1 and Class 2 residual solvents in active substances), the analysis for residual solvents is not necessary to be performed as routine analysis for Teriparatide since the daily exposure of the patient to any solvent is not more than the 10% of the Permitted Daily Exposure (PDE) established in the ICH guidelines CPMP/ICH/283/95 (Impurities: Residual Solvents).

Therefore, the absence of residual solvents in Teriparatide is considered validated.

Elemental impurities

No metals or catalysts are used in the manufacturing process of Teriparatide. Full details on the elemental impurities to be present in Teriparatide are included in Section 3.2.S.3.2.

TABLE 5 shows the results obtained for the three different Teriparatide batches analyzed. The results are given as ppm (µg/g) and as daily dose (µg/day) of each elemental impurity in each batch.

TABLE 5 – Elemental Impurities results

ICH class	Element	Specifications according to ICH Q3D		Batch Results					
				PTH0903/1		PTH1601		PTH1602	
		Permitted Daily Dose (PDE) (mcg/day)	Control threshold: 30% of the daily dose PDE (mcg/day)	ppm (mcg/g)	Daily Dose (mcg/day)	ppm (mcg/g)	Daily Dose (mcg/day)	ppm (mcg/g)	Daily Dose (mcg/day)

The results obtained from the three batches analyzed show that the manufacturing process of Teriparatide yields a product with a really low burden of elemental impurities (in all cases all metals are below the detection limit of the technique). The elemental impurity level in the drug substance is, at least, 5 orders of magnitude below the control threshold (30% of the permitted daily exposure) of each metal. Therefore, there should be no concern due to the presence of elemental impurities in Teriparatide.

It is concluded that no additional controls are required for Teriparatide. This conclusion agrees with the ICH Q3D guideline, where it is stated that if the daily exposure of the patient to any elemental impurity is not more than the control threshold (30% of the PDE), additional controls are not required. Therefore, the absence of an elemental impurities test in the drug substance specifications is justified.

Genotoxic impurities

Related peptides present in the product, are impurities with a chemical structure very similar to Teriparatide. Therefore, its toxicity should be very similar to that of Teriparatide. Teriparatide is not considered to have any structural alert for genotoxicity. Consequently, all the impurities related to Teriparatide would be genotoxically safe.

Fate and purge of reagents

The rationale provided in Section 3.2.S.3.2. supports that it is highly unlikely that the reagents used for the manufacture of the product are present in the purified Teriparatide. Therefore, no safety concerns should be raised.

3.2.S.4 CONTROL OF DRUG SUBSTANCE

The specifications established by the drug substance supplier and by the product manufacturer for the acceptance for the drug substance to be used in drug product manufacture are stated in TABLE 6. Before drug product manufacture, the drug product manufacturer only performs a test for appearance and drug substance identification by HPLC.

The specification contains all relevant attributes for a synthetic peptide. Since there is no monograph on synthetic Teriparatide currently published in any pharmacopoeia, the specifications proposed are based on the Ph Eur and USP current edition monographs on Teriparatide produced by recombinant DNA technology. For that reason, some parameters have been adapted such as acetic acid content, and some other tests have not been included in the specifications because are tests applicable only to products obtained by recombinant technology such as “Identification by peptide mapping” and “Bioidentity”. Additionally, “Chloride content” has not been included in the specifications because the manufacturing process of Teriparatide at yields the product as an acetate. No chlorides are used in the process. Additionally, has established some specifications that are not considered in the EP and USP monographs, such as mass spectrometry identification, amino acid analysis and trifluoroacetic acid content. A comprehensive justification of the specifications proposed is provided in Section 3.2.S.4.5 of the Open part of the DMF.

The methods of analysis used in are fully described in Section 3.2.S.4.2. The Open Part of the DMF (Section 3.2.S.4.3-BCN) include the analytical validation results for the methods of peptide content (HPLC), related substances (HPLC), Acetic Acid and Trifluoroacetic acid content (HPLC), microbial limit and bacterial endotoxins. The results obtained in the validation studies demonstrate that these methods can be routinely applied

for the analysis of Teriparatide at . The method for Identification by HPLC has been successfully transferred from . Analytical transfer results are included in Section 3.2.S.4.3-Recipharm.

TABLE 6 show the results obtained in the analysis of three commercial batches of drug substance. The corresponding certificates of analysis are included in Section 3.2.S.4.4 BCN.

TABLE 6 .-. Teriparatide Drug Substance. Specifications and Analysis Results

TEST	SPECIFICATION	BATCH ANALYSIS RESULTS		
		PTH1601	PTH1602	PTH1801
CHARACTERS				
IDENTIFICATION				
	: : : : :			
TESTS				
)		%	%	%
•	%	%	%	%
•	%	%	%	%
•	%	%	%	%
	%	%	%	%
	%	%	%	%
	%			
•				
•				

* Current Edition; ** Test conducted at before drug product manufacture

3.2.S.5 REFERENCE STANDARDS OR MATERIALS

The primary reference material is obtained directly from the USP (in form of a CRS standard).

establishes a working standard by analyzing the correspondent material against the Teriparatide CRS standard according to the assay method established for drug substance control. Once the peptide content of the working standard has been established it is dosed in vials.

In case of shortage of the primary reference standard where no other equivalent international is available, one batch of Teriparatide manufactured and characterized as follows would be taken as the reference standard.

*Peptide content = (100-water content–acetic acid content–trifluoroacetate acid content) * purity*

Elemental analysis would be used as orthogonal technique to confirm the assigned value.
A certificate of analysis of a working standard can be found in Section 3.2.S.5.

3.2.S.6 CONTAINER CLOSURE SYSTEM

Teriparatide is stored in HDPE bottles sealed with HDPE inserts (stoppers) and polypropylene closures. The product is only in contact with HDPE material (body and insert).

The materials used for the manufacture of the containers comply with the European regulations for food contact applications (EC regulations 1935/2004 and 10/2011) and with the FDA criteria for food contact applications 21CFR 177,1520. Therefore, it is compatible with the storage of Active Pharmaceutical Ingredients.

The specifications and method of analysis used for the control of the container closure system are fully described in Section 3.2.S.6.

3.2.S.7 STABILITY

In order to establish the stability profile of the drug substance, long term and accelerated stability studies have been conducted. In addition, stress stability studies have also been conducted.

Standard Stability Program

Storage Conditions and Analysis Schedule

The following stability studies were scheduled:

TABLE 7 – Stability Parameters

STABILITY STUDIES	TIME POINTS OF ANALYSIS							
	0	3	6	9	12	18	24	36
Long-term -20°C±5°C	✓	✓	✓	✓	✓	✓	✓	✓
Accelerated Conditions* 5°C±3°C	✓	✓	✓					

*Only conducted for batch PTH1602

Batches Analyzed

The production batches tested for the stability studies are PTH1601 and PTH1602. Batches were manufactured as described in Section 3.2.S.2.2 of the DMF. The container closure system of the batches is the same as the proposed for marketing (see Section 3.2.S.6).

Analysis and Specification

All stability indicating parameters were tested along the stability studies (see **TABLE 8**). The specifications applied during the studies for the different parameters are the same as those established for drug substance control (as stated in TABLE 6). The methods used for the analysis are the same as those used for the routine analysis of the product. These methods are described in Section 3.2.S.4.2

TABLE 8 – Stability Studies. Analysis Schedule

	long term Temp:-20ºC ± 5ºC								elevated temperature Temp:5ºC ± 3ºC		
	Month 0	Month 3	Month 6	Month 9	Month 12	Month 18	Month 24	Month 36	Month 0	Month 3	Month 6
Appearance	x	x	x	x	x	x	x	x	x	x	x
Peptide Content	x	x	x	x	x	x	x	x	x	x	x
Total of methionyl sulfoxides	x	x	x	x	x	x	x	x	x	x	x
Other individual Impurities	x	x	x	x	x	x	x	x	x	x	x
Total impurities	x	x	x	x	x	x	x	x	x	x	x
Water content	x	x	x	x	x	x	x	x	x	x	x
Acid acetic content	x	x	x	x	x	x	x	x	x	x	x
Microbial Limit	x	‡	‡	‡	‡	‡	‡	x	x	‡	x

(x)-Parameter to be analysed. (‡)- Parameter to be analysed if in that month, some of the parameters that are sistematically analysed (x) are out of specifications.

Results

Results obtained in the stability studies are shown in TABLE 9 and TABLE 10.

TABLE 9 – Stability Results. Batch PTH1601

Batch number.	PTH1601	Manufacturing date	05/2016	Batch size	38.1 g

Temp.	Humidity	Month	Appearance	Peptide content	Imp (Σ ind imp ≥ 0.1%)			Water%	AcOH %	Microbial Limit	
					ind. Imp.*	Total sufoxides methionyl	Total imp. %			TAMC	TYMC
Specifications			White or off white powder	95.0%-105.0%	≤0,5%	≤0,5%	≤2.5%	≤10.0%	≤10.0%	≤10 c.f.u. /100mg	≤10 c.f.u. /100mg
		36									

** Different from Methionyl sulfoxides*
n.a.: not analyzed according the protocol.

TABLE 10 – Stability Results. Batch PTH1602

Batch number.	PTH1602	Manufacturing date	05/2016	Batch size	56.0 g

Temp.	Humidity	Month	Appearance	Peptide content	Imp (Σ ind imp ≥ 0.1%)			Water%	AcOH %	Microbial Limit	
					ind. Imp*	Total sufoxides methionyl	Total imp. %			TAMC	TYMC
Specifications			White or off white powder	95.0%-105.0%	≤0,5%	≤0,5%	≤2.5%	≤10.0%	≤10.0%	≤10 c.f.u. /100mg	≤10 c.f.u. /100mg

** Different from Methionyl sulfoxides*
n.a.: not analyzed according the protocol.

Stress Stability Studies

In order to characterize the degradation profile of the molecule and to prove that the methods of analysis are stability indicating, stress stability studies were conducted on batch PTH1601. The stress degradation pathways studied are described below:

a) Photostability testing:

The photostability testing was done according to CPMP/ICH/279/95 guideline. The temperature of the test was 25ºC. The detailed description of the methodology used can be found in **Section 3.2.S.7.1** of the Open Part of the DMF. The results obtained in the studies are shown in the following table.

TABLE 11 – Stress Studies. Photostability

rrt	Reference sample (protected from light)	After photostability
	%Area	%Area

"-" Not detected

The results obtained show that the drug substance is photosensitive and prove that the method is stability indicating.

b) Temperature stress

The temperature stress was conducted at six different temperatures: -20ºC (control study), 5ºC, 25ºC, 40ºC, 60ºC and 80ºC. The samples were kept during three days at the corresponding temperature. The samples were analyzed by HPLC with the related substances method used for batch release. The results obtained are shown in TABLE 12. A marked degradation is observed starting at 60ºC. The results obtained show that the method is stability indicating and that degradation impurities caused by temperature will be detected with the related substances method.

TABLE 12 – Stress Studies. Thermal Stress

rrt	-20°C	5°C	25°C	40°C	60°C	80°C
	%Area	%Area	%Area	%Area	%Area	%Area

*:- Not detected

c) Chemical stress:

The following conditions were assessed. The related substance test established for routine drug substance control was used in the analysis.

- *Alkaline hydrolysis*: A sample was treated with a NaOH solution 0.1N for 15 minutes. After neutralization of the sample 1:1 with HCl solution 0.1N it was analyzed. The results obtained are shown in TABLE 13.
  The results obtained show that the method is stability indicating and that degradation impurities caused by alkaline stress will be detected with the related substances method.

TABLE 13 – Stress Studies. Alkaline Stress

rrt	Reference sample (-20°C)	NaOH 0.1N
	%Area	%Area

"-" Not detected

- *Acidic hydrolysis*: A sample was treated with an HCl solution 3N for 30 minutes. After neutralization of the sample 1:1 with NaOH solution 3N, it was analyzed. The results obtained are shown in TABLE 14.

  The results obtained show that the method is stability indicating and that degradation impurities caused by alkaline stress will be detected with the related substances method.

TABLE 14 – Stress Studies. Acidic Stress

rrt	Reference sample (-20°C)	HCl 3N
	%Area	%Area

"-" Not detected

- *Oxidative stress*: H_2O_2 solution 0.1% for 1 minute. After dilution of the sample 1:1 with water, it was analyzed. The results obtained are shown in TABLE 15.

TABLE 15 – Stress Studies. Oxidative Stress

rrt	Reference sample (-20°C)	H_2O_2 0.1%
	%Area	%Area

"-"Not detected

The results obtained show that the method is stability indicating and that degradation impurities caused by oxidative stress will be detected with the related substances method.

Stability Protocol and Commitment

Following GMP recommendations, at least one batch of drug substance will be entered in stability (under long-term storage conditions) every year. The analysis schedule will be once a year up to three years. The parameters to be analyzed in this study are appearance, peptide content, individual and total impurities, water content, acetic acid content and microbial limit.

LIST OF TABLES

LIST OF FIGURES