21 July 2016
EMA/536977/2016
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Inhixa

International non-proprietary name: enoxaparin sodium

Procedure No. EMEA/H/C/004264/0000

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

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Administrative information

Name of the medicinal product:	Inhixa
Applicant:	Techdow Europe AB Banergatan 36 752 37 UPPSALA SWEDEN
Active substance:	Enoxaparin sodium
International Non-proprietary Name:	Enoxaparin sodium
Pharmaco-therapeutic group (ATC Code):	antithrombotic agents, heparin group (B01AB05)
Therapeutic indication(s):	-Prophylaxis of venous thromboembolism, particularly in patients undergoing orthopaedic, general or oncological surgery. -Prophylaxis of venous thromboembolism in patients bedridden due to acute illnesses including acute heart failure, acute respiratory failure, severe infections, as well as exacerbation of rheumatic diseases causing immobilisation of the patient (applies to strengths of 40 mg/0.4 mL). -Treatment of deep vein thrombosis (DVT), complicated or uncomplicated by pulmonary embolism. -Treatment of unstable angina and non-Q-wave myocardial infarction, in combination with acetylsalicylic acid (ASA). -Treatment of acute ST-segment elevation myocardial infarction (STEMI) including patients who will be treated conservatively or who will later undergo percutaneous coronary angioplasty (applies to strengths of 60 mg/0.6 mL, 80 mg/0.8 mL, and 100 mg/1 mL).

	-Blood clot prevention in the extracorporeal circulation during haemodialysis.
Pharmaceutical form(s):	Solution for injection
Strength(s):	2,000 IU (20 mg) in 0.2 mL, 4,000 IU (40 mg) in 0.4 mL, 6,000 IU (60 mg) in 0.6 mL, 8,000 IU (80 mg) in 0.8 mL and 10,000 IU (100 mg) in 1 mL
Route(s) of administration:	Intraarterial use, Intravenous use, Subcutaneous use
Packaging:	pre-filled syringe
Package size(s):	10 pre-filled syringes and 2 pre-filled syringes

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List of abbreviations

AISI	American Iron and Steel Institute
Al	Aluminium
anti-FIIa	Factor IIa inhibition
anti-FXa	Factor Xa inhibition
aPTT	Activated Partial Thromboplastin Time
AT	Antithrombin
AU	Absorbance
BE	Bacterial Endotoxins
BP	British Pharmacopoeia
cfu	Colony-forming unit
CPPs	Critical process parameter
CQA	Critical quality attribute
CS	Chondroitin Sulfate
Ct	Threshold cycle
CV	coefficient of variation
Dp	Degree of polymerisation
DLS	Dynamic light scattering
DS	Dermatan Sulfate
ETO	Ethylene Oxide
EU	Endotoxin Unit
FAM probe	6-carboxyfluorescein probe
FMEA	Failure Mode Effectiveness Analysis
FTIR	Fourier transform infrared spectroscopy
GC-MS	Gas chromatography–mass spectrometry
GPC	Gel Permeation Chromatography
HPA	Hydroxy propyl acrylate

HPLC	High Performance Liquid Chromatography
HSQC	1H-13C-heteronuclear single quantum coherence
ICP-OES	Inductively coupled plasma optical emission spectrometry
IPC	In-Process Control
IV	Intrinsic Velocity
LC	Liquid chromatography
LMWH	Low Molecular Weight Heparin
LOD	Limit of Detection
MP	P-average molecular mass
MS	Mass Spectrometry
MVD	Maximum valid dilution
MW	Molecular weight
MWCO	Molecular weight cut-off
NIST	National Institute of Standards and Technology
NMR	Nuclear Magnetic Resonance
OSCS	Over-Sulfated Chondroitin Sulfate
PCS	Photon correlation spectroscopy
Ph. Eur.	European Pharmacopoeia
PVDF	Polyvinylidene fluoride
qPCR	Quantitative Polymerase Chain Reaction
Rh	Hydrodynamic radius
RPIP	Reverse Phase Ion-pair
RPN	Risk priorization number
SAL	Sterility assurance level
SEC	Size Exclusion chromatography
TEGDA	Triethylene glycol diacetate
TFPI	Tissue factor pathway inhibitor
TxR probe	Texas red probe
TSE	Transmissible Spongiform Encephalopathy
UFG	Unfractionated heparin
ULC	Ultralarge complexes

UPLC	Ultra Performance Liquid Chromatography
US EPA	United Stated Environmental Protection Agency
WFI	Water for injections
Zn	Zinc

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Techdow Europe AB submitted on 27 May 2015 an application for marketing authorisation to the European Medicines Agency (EMA) for Inhixa, through the centralised procedure under Article 3 (2) (b) of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 27 June 2013. The eligibility to the centralised procedure under Article 3(2)(b) of Regulation (EC) No 726/2004 was based on demonstration of interest of patients at Community level.

The applicant applied for the following indications:

-Prophylaxis of venous thromboembolism, particularly in patients undergoing orthopaedic, general or oncological surgery.

-Prophylaxis of venous thromboembolism in patients bedridden due to acute illnesses including acute heart failure, acute respiratory failure, severe infections, as well as exacerbation of rheumatic diseases causing immobilisation of the patient (applies to doses of 40 mg/0.4 mL).

-Treatment of deep vein thrombosis (DVT), complicated or uncomplicated by pulmonary embolism.

-Treatment of unstable angina and non-Q-wave myocardial infarction, in combination with acetylsalicylic acid (ASA).

-Treatment of acute ST-segment elevation myocardial infarction (STEMI) including patients who will be treated conservatively or who will later undergo percutaneous coronary angioplasty (applies to doses of 60 mg/0.6 mL, 80 mg/0.8 mL, and 100 mg/1 mL).

-Blood clot prevention in the extracorporeal circulation during haemodialysis.

The legal basis for this application refers to:

Article 10(4) of Directive 2001/83/EC – relating to applications for a biosimilar medicinal products.

The application submitted is composed of administrative information, complete quality data, appropriate non-clinical and clinical data for a similar biological medicinal product.

Information on Paediatric requirements

Not applicable

Information relating to orphan market exclusivity

Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did not submit a critical report addressing the possible similarity with authorised orphan medicinal products because there is no authorised orphan medicinal product for a condition related to the proposed indication.

Scientific Advice

The applicant received Scientific Advice from the CHMP on 24 May 2012. The Scientific Advice pertained to quality, non-clinical and clinical aspects of the dossier.

1.2. Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

Rapporteur: Andrea Laslop Co-Rapporteur: Patrick Salmon

CHMP Peer reviewer(s): Martina Weise

- The application was received by the EMA on 27 May 2015.
- The procedure started on 25 June 2015.
- The Rapporteur's first Assessment Report was circulated to all CHMP members on 12 June 2015 (Annex 1). The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on 12 June 2015 (Annex 2). The PRAC Rapporteur's first Assessment Report was circulated to all PRAC members on 26 June 2015 (Annex 3)
- During the meeting on 9 July 2015, the PRAC agreed on the PRAC Assessment Overview and Advice to CHMP. (Annex 4)
- During the meeting on 23 July 2015, the CHMP agreed on the consolidated List of Questions to be sent to the applicant. The final consolidated List of Questions was sent to the applicant on 24 July 2015 (Annex 5).
- The applicant submitted the responses to the CHMP consolidated List of Questions on 25 February 2016.
- The following GMP inspection(s) were requested by the CHMP and their outcome taken into consideration as part of the Quality/Safety/Efficacy assessment of the product:
  - A GMP inspection at one finished product manufacturing site in Taiwan between 20th and 23rd July 2015. The outcome of the inspection carried out was issued on 10 December 2015.
  - A GMP inspection at one active substance manufacturing site in China between 26th and 29th October 2015. The outcome of the inspection carried out was issued on 8th March 2016.
  - GMP inspections at one finished product manufacturing site in China between 26th and 29th October 2015 and 5th and 7th April 2016. The outcome of the inspections carried out was issued on 7th June 2016.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Questions to all CHMP members on 4 April 2016 (Annex 6).
  - During the PRAC meeting on 14 April 2016, the PRAC agreed on the PRAC Assessment Overview and Advice to CHMP. (Annex 7).
- During the CHMP meeting on 28 April 2016, the CHMP agreed on a list of outstanding issues to be addressed in writing and/or in an oral explanation by the applicant (Annex 8).
- The applicant submitted the responses to the CHMP List of Outstanding Issues on 17 June 2016.
- During the meeting on 18-21 July 2016, the CHMP, in the light of the overall data submitted and the

scientific discussion within the Committee, issued a positive opinion for granting a marketing authorisation to Inhixa on 21 July 2016.

2. Scientific discussion

2.1. Problem statement

The present marketing authorisation application is for medicinal product containing enoxaparin sodium and claiming biosimilarity to Clexane (Sanofi Aventis, Surrey, United Kingdom [UK]), used for the data exclusivity/market protection purpose and authorised pursuant to Article 10(4) of Directive 2001/83/EC, as amended. The Clexane authorised in Poland and belonging to the same Global Market Authorisation as the UK product is the reference medicinal product (RMP), to which comparability studies have been performed. As stated in the *EMA Guideline on similar biological medicinal products (CHMP/437/04 Rev 1)*, a single reference medicinal product should be used throughout the comparability program for quality, safety and efficacy studies during the development of a similar biological medicinal product in order to allow the generation of coherent data and conclusions. Therefore the batches used for the comparability exercise should belong to the medicinal product, i.e. Clexane authorised in Poland.

Venous thromboembolism (VTE), which encompasses deep vein thrombosis (DVT) and pulmonary embolism (PE), is responsible for the death of more than half a million people in Europe each year and is the third leading cause of death from cardiovascular causes only ahead of myocardial infarction and stroke (Gomez-Outes et al 2012). VTE is a common and potentially avoidable cause of morbidity and mortality in patients hospitalised for acute medical illness. Despite significant advances in the prevention and treatment of VTE, PE is a common preventable cause of hospital death; without appropriate prophylaxis, 1 in 20 hospitalised medical patients may suffer a fatal PE. Without improvement in the use of VTE prophylaxis in medical patients, unexpected PE will remain a serious problem (Albertsen et al 2012). Worldwide, coronary artery disease (CAD) is the single most frequent cause of death. Over seven million people every year die from CAD, accounting for 12.8% of all deaths. Every sixth man and every seventh woman in Europe will die from myocardial infarction (Steg et al 2012). Antithrombotic therapy has become the standard of care in the treatment of acute coronary syndromes (ACS) (Christy 2008).

Various pharmacologic agents are used alone or in combination to treat or prevent thromboembolic disease, both arterial and venous. In general, treatment of arterial thromboembolic disease relies on both antiplatelets and anticoagulants, while prevention and treatment of venous thromboembolic (VTE) disease primarily involves anticoagulants (Iqbal and Cohen 2011). The therapeutic arsenal of anticoagulants available for prophylaxis against venous thromboembolism is mainly composed of parenteral agents, such as low molecular weight heparins (Gomez-Outes et al 2012). Native heparin is a glycosaminoglycan generally obtained from porcine or bovine sources. The various low molecular weight heparins are prepared from heparin by depolymerisation in a variety of processes; as a consequence, although the products of depolymerisation are of similar molecular weight to the low molecular weight constituents of unfractionated heparin, they exhibit different physicochemical and biochemical properties. Thus, the low molecular weight heparins are pharmacologically distinct entities (Buckley and Sorkin 1992). For many years, unfractionated heparin (UFH) was the anticoagulant of choice in indications such as the prevention and treatment of venous thromboembolism. One of the main biophysical limitations of UFH is that, as a result of its large molecular size, it is unable to inactivate surface-bound factor IIa or factor Xa. This may explain the limited efficacy of UFH in unstable angina and coronary thrombolysis. UFH is also limited by its adverse effects. Like all anticoagulants, it can cause unwanted

bleeding. The limitations of UFH have led to the development of low molecular weight heparins (Fareed et al 2003). Interest in potential antithrombotic applications of low molecular weight heparins was first aroused by the observations that low molecular weight fractions of heparin had relatively less ability than unfractionated heparin to prolong the activated partial thromboplastin time (APTT), while retaining antifactor Xa activity. The low molecular weight heparins were considered to represent the possibility of separation of the antithrombotic and haemorrhagic profiles of unfractionated heparin (Buckley and Sorkin 1992).

Enoxaparin, a low molecular weight heparin (LMWH), is one of the most widely used amongst its class (Iqbal and Cohen 2011). Now-a-days, enoxaparin has become the treatment of choice for various thromboembolic diseases (Ingle and Agarwal 2014). The use of enoxaparin has been extensively reviewed (Beguin and Hemker 1990, Fareed et al 1990 and 2003, Buckley and Sorkin 1992, Noble et al 1995, Noble and Spencer 1998, Warner and Perry 2001, Ibbotson and Goa 2002, Turpie and Mason 2002, Siddiqui and Wagstaff 2005, Carter et al 2008, Meneveau 2009, Iqbal and Cohen 2011, Ingle and Agarwal 2014). Enoxaparin sodium is the sodium salt of a LMWH that is obtained by alkaline depolymerisation of the benzyl ester derivative of heparin from porcine intestinal mucosa. Enoxaparin sodium is an antithrombotic agent (ATC code. B01A B05) characterized by a higher ratio of antithrombotic activity to anticoagulant activity compared to unfractionated heparin. Specifically, enoxaparin sodium as the sodium salt of depolymerized heparin, is subject to characteristic structural modifications from the effects of the depolymerization reaction. Characteristic structures such as partial transformation of glucosamines into mannosamines, formation of 1,6-anydro derivatives and 2,3 epoxide residues with subsequent conversion to galacturonic acid and the change in the proportion of the sulfated and non-sulfated residues are some of the changes that constitute enoxaparin, a structurally difficult to characterize molecule. As such no single technique or parameter is adequate to fully characterize the enoxaparin sodium molecule as part of a biosimilarity exercise against the reference. Therefore, a number of comparisons between test and reference products have been carried out. These biosimilarity comparisons have involved analysis and comparison of a broad range of physico-chemical properties which were also combined with Ph. Eur. based parameters and tests such as UV spectral analysis, free sulphate/carboxylate ratio, sodium and nitrogen contents of the oligosaccharide chains. Furthermore, as the depolymerization reaction can also affect the length of produced Enoxaparin chains, a number of analyses that determine the molecular weight of the Enoxaparin chain have also been carried out. In addition to Ph. Eur. established methodologies for Molecular weight (Mw) measurements, a number of additional Mw parameters such as mean molecular weight (Mn), weight-average mean molecular weight (Mw), and degree of polydispersity (Mw/Mn) were also determined. Additional Mw determinations have been carried out using HP-SEC with Triple Detector Arrays (HP-SEC/TDA). Furthermore, a number of NMR based techniques were used to determine various saccharides that form an integral part of the disaccharide building blocks in Enoxaparin chain including assessment of residues at both reducing and non-reducing ends with the aim of producing quantitative assessments of various chain disaccharides. NMR determined parameters include monosaccharide composition, sulfation patterns and configuration of linkages between glucosamine and uronic acid residues. Comparisons of various saccharides following enzymatic digestions with Heparinases and nitrous acid have also been performed using liquid based chromatography techniques with a strong anion mode of separation (HPLC-SAX) combined with ion-pair based chromatography and Mass Spectrometry detection (RPIP-UPLC-ESI-MS). Equivalence of the Heparin intermediate and its respective mode of depolymerisation was also carried out. In addition, comparative assessment of Antithrombin III (AT-III) affinity chromatography derived High Affinity (HA) and Low affinity (NA) oligosaccaharide fractions was also performed as Enoxaparin's anti-coagulant activity is attributed to these HA fractions which contain the Anti-thrombin binding sequence (AT-bs). This sequence is a pentasaccharide represented as AGA*IA, (asterisk denotes a rare 3-O-sulfated GlcNSO3). Isolated HA fractions isolated from both Enoxaparin sodium and reference Clexane have also been compared in order to assess the degree of similarity in overall HA and NA fractions extracted, as well as to compare the content of various disaccharides contained in the HA fraction such

as the saccharides in the above pentasaccharide sequence AGA*IA. Other comparisons of saccharide content in HA fractions have been carried out such as the comparison of decasaccharides. A comparative in depth comparison of tetrasaccharides, as well as chain mapping of isolated hexasaccharides, octasaccharides and decasaccharides from both enoxaparin sodium and Clexane have also been carried out with an IPRP-HPLC/UV/ESI-IT-MS based method. Quantification of impurity profiles was also part of the biosimilarity exercise. Impurity profile comparisons have included measurements of galacturonic acid, epoxide and chloride content, while levels of nucleotide and protein impurities have also been compared and assessed in both test and reference formulations. A number of in-vitro tests that assess inhibition of coagulation factors Xa (Anti-FXa) and IIa (Anti-IIa), and clotting tests such as activated partial thromboplastin time (aPTT) and Heptest have also been compared between the test enoxaparin sodium injection and Clexane formulations. Biosimilarity comparisons of test and reference enoxaparin formulation have also examined the effect of sample shelf-life. In this case aged test and reference enoxaparin samples have been compared using a number of shelf-life acceptance quality attributes but also in terms of Mw, NMR determined disaccharide composition and free sulphate content. Results from test and reference products during shelf-life studies have shown that the test formulation not only retains all its acceptance shelf-life specifications but also possess a shelf-life behaviour similar to that of the reference product.

Test and RMP formulations have also been compared *in vivo* in a PD endpoint study for anti-FXa, anti-FIIa (TFPI was carried out for information purposes, as the AUC(0-t) and Cmax of anti-Xa and anti-IIa activities as primary parameters for enoxaparin evaluation were used to assess bioequivalence).

The biosimilar formulation of enoxaparin sodium is available as a solution for injection in prefilled syringes of 2,000 IU (20 mg) in 0.2 mL, 4,000 IU (40 mg) in 0.4 mL, 6,000 IU (60 mg) in 0.6 mL, 8,000 IU (80 mg) in 0.8 mL and 10,000 IU (100 mg) in 1 mL.

It is indicated for adults for:

- Prophylaxis of venous thromboembolism, particularly in patients undergoing orthopaedic, general or oncological surgery.

- Prophylaxis of venous thromboembolism in patients bedridden due to acute illnesses including acute heart failure, acute respiratory failure, severe infections, as well as exacerbation of rheumatic diseases causing immobilisation of the patient (applies to strengths of 40 mg/0.4 mL).

- Treatment of deep vein thrombosis (DVT), complicated or uncomplicated by pulmonary embolism.

- Treatment of unstable angina and non Q wave myocardial infarction, in combination with acetylsalicylic acid (ASA).

- Treatment of acute ST segment elevation myocardial infarction (STEMI) including patients who will be treated conservatively or who will later undergo percutaneous coronary angioplasty (applies to strengths of 60 mg/0.6 mL, 80 mg/0.8 mL, and 100 mg/1 mL).

- Blood clot prevention in the extracorporeal circulation during haemodialysis.

Type of Application and aspects on development

The current application for marketing authorisation has been submitted as similar biological medicinal product pursuant to Article 10(4) of Directive 2001/83/EC, as amended.

The chosen RMP, Clexane 100mg/ml solution for injection has been licensed for more than 10 years on the basis of a complete dossier. It has first been authorised by Aventis Pharma Limited in 1990 in UK.

The indications applied for are identical to those approved for the reference Clexane.

In support of their application, the applicant has submitted one PK/PD study. In this study which was a randomised, open-label, single-dose, 2-way cross-over comparative PK/PD study of biosimilar enoxaparin sodium 40 vs. reference medicinal product Clexane after subcutaneous administration in healthy volunteers. In this study surrogate markers such as anti-Xa, anti-IIa and TFPI activity in plasma were investigated for the test and reference product.

The development programme of Inhixa has specifically considered the EU guidelines for similar biological medicinal products including specific guidelines for LMWH (see list below).

Guideline	Document Reference
CHMP Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: quality issues (revision 1)	(EMA/CHMP/BWP/247713/2012)
CHMP Guideline on Similar Biological Medicinal Products containing Biotechnology-Derived Proteins as Active Substance: Non-Clinical and Clinical Issues	(EMEA/CHMP/42832/05)
Guideline on Non-Clinical and Clinical Development of Similar Biological Medicinal Products containing Low-Molecular-Weight-Heparins	(EMEA/CHMP/BMWP/118264/2007)
Concept paper on the revision of the guideline on nonclinical and clinical development of similar biological medicinal products containing low molecular-weight heparins	EMA/CHMP/BMWP/522386/2011

The applicant received scientific advice (SA) from the CHMP with respect to quality, pre-clinical, clinical and regulatory aspects of the product on three occasions: in 2012, 2013 and 2014. For quality questions, the applicant requested advice on the analytical package used to demonstrate biosimilarity molecular weight distribution, chain mapping, 1H-NMR, 13C-NMR, disaccharide building block analysis, fragment mapping, chain mapping. In the final advice letters, the CHMP agreed with much of the applicant's proposed approach. Additional analyses were also advised to ensure a greater understanding of the complex structure of heparin. This included analysis of tetrasaccharides, hexasaccharides, octasaccharides by mass spectrometry, binding to antithrombin III and the use of heparinases II and III in addition to heparinase I. In general, the applicant has included the additional tests recommended by the SA.

The applicant has also widely followed the recommendations given in previous SA procedures, on preclinical and clinical level e.g. points proposed about the design of a to be performed PK/PD study, choice of endpoints etc.

were found to be acceptable by CHMP. However certain issues, especially pertaining to immunogenicity testing have not been addressed in detail in the procedures in question.

2.2. Quality aspects

2.2.1. Introduction

The present Marketing Authorisation Application is related to a product claiming biosimilarity to Clexane (Sanofi Aventis, Surrey, UK) syringes and seeking authorisation pursuant to Article 10(4) of Directive 2001/83/EC, as amended.

Enoxaparin sodium is the sodium salt of a low-molecular-mass heparin that is obtained by alkaline depolymerisation of the benzyl ester derivative of heparin from porcine intestinal mucosa. Enoxaparin sodium is an antithrombotic agent (ATC code B01AB05) characterized by a higher ratio of antithrombotic activity to anticoagulant activity compared to unfractionated heparin.

Specifically, Enoxaparin sodium as the sodium salt of depolymerized Heparin is subject to characteristic structural modifications from the effects of the depolymerization reaction. Characteristic structures such as partial transformation of glucosamines into mannosamines, formation of 1,6-anydro derivatives and 2,3 epoxide residues with subsequent conversion to galacturonic acid and the change in the proportion of the sulfated and non-sulfated residues are some of the changes that constitute Enoxaparin a structurally difficult to characterize molecule. As such no single technique or parameter is adequate to fully characterize the Enoxaparin molecule as part of a biosimilarity exercise against the reference.

The finished product enoxaparin sodium solution for injection is presented in pre-filled syringes of 2,000 IU (20 mg) in 0.2 mL; 4,000 IU (40 mg) in 0.4 mL; 6,000 IU (60 mg) in 0.6 mL; 8,000 IU (80 mg) in 0.8 mL and 10,000 IU (100 mg) in 1 mL.

2.2.1. Active Substance

General information

Heparin Sodium is the sodium salt of sulfated glycosaminoglycans present as a mixture of heterogeneous molecules varying in molecular weights that retains a combination of activities against different factors of the blood clotting cascade. It is composed of polymers of alternating derivatives of α- D-glucosamido (N-sulfated, O-sulfated, or N-acetylated) and O-sulfated uronic acid (α-L-iduronic acid or β-D-glucuronic acid).

Enoxaparin Sodium is manufactured from heparin sodium obtained exclusively from porcine intestinal mucosa. It consists of a complex set of oligosaccharides that have not yet been completely characterized. The majority of the components have a 4-enopyranose uronate structure at the non-reducing end of their chain. About 20% of the materials contain a 1,6-anhydro derivative structure on the reducing end of the chain, the range being between 15% and 25%.

Manufacture, process controls and characterisation

Description of manufacturing process and process controls

Heparin Sodium (Intermediate)

Heparin sodium is obtained from the intestinal mucosa of pigs (starting material). The manufacture of crude heparin sodium (which is an intermediate in the manufacture of enoxaparin sodium active substance) is adequately described and in accordance with EMA/CHMP/BWP/429241/2013 pooled porcine intestinal mucosa is defined as the starting material. Suppliers of the crude heparin have been registered in the dossier. The QP declaration confirms that appropriate levels of GMP are in place from introduction of the starting material into the manufacturing process. Sufficient information regarding the starting material pooled porcine intestinal mucosa has been provided. Specification/quality criteria for porcine intestines have been included in the dossier.

A detailed narrative description of the manufacture has been provided. CQAs and CPPs have been adequately summarized and the process can be regarded as being stable. Tests and acceptance criteria performed at the critical steps have been provided. The in-process controls are sufficiently described for the intermediate heparin sodium. A qualitative PCR method is included in the crude heparin specification for the purpose of species determination.

The current heparin sodium manufacturing process has been appropriately validated to assure that the intermediate can meet the specifications. Proposed specifications are in accordance with the Ph. Eur. monograph 08/2013:0333 for Heparin Sodium and are generally considered acceptable.

Enoxaparin Sodium (Active Substance)

Enoxaparin Sodium is manufactured at Shenzhen Techdow Pharmaceutical Co., Ltd, Shenzhen City, China and has a valid GMP certificate.

To manufacture Enoxaparin Sodium active substance, the intermediate heparin sodium is salified, esterified and then depolymerized to obtain enoxaparin sodium crude. The crude enoxaparin sodium is then purified by multiple steps and lyophilized to obtain the final active substance. The specifications for materials used at Shenzhen Techdow for manufacture of Enoxaparin Sodium are, in general, acceptable. Process validation data has been presented. All analytical results for validation batches complied with release specifications.

The manufacturing process has been presented and the CQAs have been discussed in detail and have been justified. Process validation data show that all CQAs have been followed. The control strategy for the active substance manufacturing process is acceptable.

Characterisation

Heparin Sodium (Intermediate)

The characterisation profile to confirm the structure and other characteristics includes a range of methods such as IR and NMR. The Applicant has updated 3.2.S.3.1 Elucidation of Structure and Other Characteristics-Heparin to include two additional characterisation tests to further characterise heparin sodium. Characterisation has been performed between heparin sodium and the EP identification reference standard, for the purpose of identifying consistency in molecular structures.

Enoxaparin Sodium (Active Substance)

The characterisation profile to confirm the structure and other characteristics with a number of techniques such as IR and NMR used to quantitatively study various structural chain parameters including amines, uronic acids, the linkage region, 1,6-anhydro component, total glucuronic acid and total iduronic acid has been provided. The characterisation study is considered adequate.

The discussion on possible impurities (including reagents, residual solvents and heavy metals) is sufficient. Data showing the impurity profile in several batches of enoxaparin sodium were also provided showing that the impurity profile is constant and well controlled in the final API. Additionally, results are shown that demonstrate that elemental impurities conform to Ph. Eur. (5.20) and ICH Q3D requirements. Moreover, it was demonstrated that the impurity profile of the API is comparable to or lower than the originator.

Specification

The specification of enoxaparin sodium is established based upon current Ph. Eur. monograph 04/2014:0828 and the majority of analytical methods are adopted from the current Ph. Eur. monograph. Limits for the residual solvents were based upon the requirements of ICH. Additional tests and limits for various parameters including residual solvents and microbial bioburden (acceptance criteria for microbiological quality of non-sterile substances for pharmaceutical use are followed) are part of the specification. The Applicant has identified a number of potential impurities and presented and justified a control strategy for them. The Applicant has adequately defined what impurities are to be controlled in the active substance release specification and justified not testing of other named impurities.

Analytical methods

Method descriptions are in general acceptable and sufficient detail has been provided.

Reference materials

Information regarding the reference standards used is sufficient. With respect to method validation, sufficient validation data for methods have been provided.

Batch analysis

Batch data has been provided for validation batches of enoxaparin sodium.

Stability

Heparin Sodium (Intermediate)

The Applicant has performed a long-term study and an accelerated stability study. Based on the provided batch data the proposed shelf-life for heparin sodium was acceptable.

Enoxaparin Sodium (Active Substance)

According to the stability protocol, long term studies were in line with the requirements of ICH Q5C. It was also shown that during storage of enoxaparin sodium API, related impurity content is stable, and the results meet the Pharmacopoeial statutory requirements. On the basis of the data provided the proposed shelf-life for Enoxaparin Sodium was considered acceptable.

2.2.2. Finished Medicinal Product

Description of the product and pharmaceutical development

Inhixa is a solution for injection containing Enoxaparin sodium as API. It is a clear, colourless or pale yellow solution. The finished product and its composition have been sufficiently described.

The product is supplied in pre-filled syringes (PFS) and consists of a clear type I borosilicate glass syringe barrel with stainless steel needle and needle shield, and a chlorobutyl rubber, installed with a plunger rod.

A detailed discussion on compatibility of the active substance and the only excipient (water for injections), based on the results of stress condition, long-term, intermediate and accelerated stability studies has been provided. The formulation is identical to the reference product Clexane. Comparability of enoxaparin sodium solution for injection with the reference product Clexane has been shown with a comparative biosimilarity approach. No overage is proposed. Physicochemical and biological parameters relevant for finished product performance are addressed.

Pharmaceutical Development

The manufacturing process development has been sufficiently discussed including compatibility of the finished product solution and manufacturing equipment and assessment of potential leachables from the sterilization filter. Nevertheless, the Applicant is recommended to perform a study for non-volatile leachables of transfer hoses as a post authorisation-measure. The Applicant already committed to do so.

Detailed information on the container closure system (pre-filled syringe) development has been provided. Packaging materials are described. For primary packaging materials, coming into contact with finished product solution, compliance with the respective Ph. Eur. requirements is confirmed. Syringeability and container closure integrity are sufficiently demonstrated. Potential extractables and leachables of the primary packaging material were thoroughly investigated and found to be acceptable. Dosage accuracy of final pre-filled syringes is sufficiently addressed.

The finished product is a sterile preparation. Sterilization is done by aseptic filtration, which has been sufficiently justified. Compatibility studies of finished product and proposed diluents have been performed. Compatibility of the solution for infusion with infusion administration devices and kits has been sufficiently demonstrated.

Manufacture of the product and process controls

EU GMP certificates were provided for all sites involved in manufacturing, testing and batch release of the finished product. Detailed information on the finished product manufacturing process as performed at both proposed finished product manufacturing sites has been provided.

Manufacture of the finished product, involves weighing and dissolution of enoxaparin sodium in water for injections, aseptic filtration and filling of syringes. Post approval commitment for harmonization of microbiological enumeration tests at the two manufacturing sites was recommended by CHMP. The Applicant has committed to do so.

Based on the provided information so far, the proposed manufacturing process as performed at all manufacturing sites is considered to be acceptable and to be comparable. Appropriate IPCs have been identified and the process by which these were assigned as CQAs and CPPs has been described. Detailed information on process validation has been provided.

Appropriate production scale process validation data have been presented for both proposed finished product manufacturing sites. Bacterial challenge test results indicate suitability of the proposed sterilization filter for its intended use. Sites and methods of sterilization of primary packaging material are indicated and sufficient validation data has been provided. Syringe barrels are sterilized with Ethylene oxide. Ethylene oxide residuals are included in the finished product manufacturer's in-house specification for syringe barrels. However, the CHMP recommended the Applicant to validate the analytical method as a post-authorisation measure. The Applicant has committed to do so.

Product specification

A release specification has been provided for Enoxaparin sodium injection. The Applicant has justified the proposed specification limits as complying with BP (2015) requirements for Enoxaparin Sodium Injection as well as with regard to data obtained from Enoxaparin Sodium Injection from lots of different manufacturing sites, data from stability studies, relevant bio-batch data and Clexane batches. The established specification limits (taking the methodological variability into account) are acceptable. Free sulfate content is included, additionally to BP requirements for Enoxaparin Sodium Injection. Additional parameters relevant with regard to the proposed dosage form, which were not addressed in above described specific pharmacopoeial monographs, are included in the release specification. Functionality of the drug delivery system by means of piston break-free force and extrusion force is sufficiently addressed. Container closure integrity testing is included in the release specification by means of methylene blue testing.

Batch analysis has been conducted on validation batches from all manufacturing sites. There are no apparent differences between batches from different syringe suppliers. Moreover, batches manufactured at both sites are of comparable quality. Certificates of analysis for the biobatch used were also provided.

The proposed shelf-life specification is identical to the release specification. Analytical test methods for control of finished product have been adequately described. The methods have been validated.

Stability of the product

Formal stability studies have been provided for batches from both proposed finished product manufacturers. The packaging of the selected batches is the same as that proposed for marketing and packaging material from both suppliers. The proposed stability protocols are in compliance with relevant guidance recommendations regarding storage conditions and test intervals. Long-term conditions stability results for the proposed shelf life comply with the proposed shelf-life specification. Almost no difference in finished product stability is seen for batches of different finished product manufacturers or packaging materials from different suppliers.

The provided stability data are sufficient to justify the proposed shelf-life under long term storage conditions of 24 months when stored below 25°C.

Adventitious agents

The TSE declarations provided are acceptable. Since the active substance is obtained from the porcine intestinal mucosa, specific viral assays with appropriate model viruses have been adequately described and validated showing that the process is capable to effectively inactivate virus.

A discussion of the differences in process parameters and their potential impact on the validity of the viral validation studies has been provided. Parameters applied represent worst case scenario in terms of viral reduction. It has also been confirmed that the mucosa is sourced from pigs veterinary certified as healthy and fit for human consumption; representative veterinary certificates have been provided.

Comparability exercise (Biosimilarity)

The pharmaceutical form of the product enoxaparin sodium injection is identical to the pharmaceutical form of the reference product Clexane, which are ready-to-use pre-filled syringes. The establishment of the formulation has been based on the listed product information of the reference product Clexane. The active substance and water for injections make up the formulation.

An extensive comparison strategy has been carried out in order to demonstrate the biosimilarity of the test Enoxaparin sodium injection and the reference product Clexane. Biosimilarity comparisons have involved broad physico-chemical properties, oligosaccharide chain characterisation, and molecular weight determinations which show Enoxaparin Sodium to be within the reference range of Clexane. Assessment of various monosaccharide building blocks in the enoxaparin chain was also carried out with a range of orthogonal techniques.

Data from both manufacturing sites demonstrated the products to be comparable i.e. within the min-max range of the reference product.

A side-by-side comparison of results was presented and overall demonstrates good comparability of the manufactured batches including individual results for anti-factor IIa and Xa which appear to be consistent across all the batches.

Chain distribution was analysed using UPLC and mass spectrometry based techniques. Saccharides were separated based on the degree of polymerisation and mass spectrometry was used to identify each peak. For all peaks the %content of Enoxaparin Sodium was within the min/max range of the reference product.

Disaccharide building blocks were compared following enzymatic digestion with heparinises I, II and III followed by HPLC analysis. The content of individual disaccharides including 1,6 anhydro derivatives was within the min max range of the reference product.

Some differences in the content of link region (LR) were found between Inhixa and the reference product. The Applicant provided justification that the LR region is a structural feature of Enoxaparin which has no known pharmacological role that directly or indirectly affects either Heparin or Enoxaparin molecules. Furthermore, an assessment of LR levels in Inhixa and various other marketed Heparin products supports the conclusion that there are no safety concerns for the slightly increased LR levels found in Inhixa.

Heparinase-specific fragment maps were generated by digesting with single heparinases. Results were in general comparable while any differences been adequately explained and justified by the Applicant.

Comparative oligosaccharide analysis was conducted by isolating various oligosaccharide fractions and comparing the content of glucosamine and uronic acid monosaccharides using NMR. The % content of Inhixa

monoscaccharides in the various factions were close to the reference range. Markers of the active pentasacchride sequence (responsible for binding to anti-thrombin III) were found to be within the reference range. Oligosaccharide chain mapping was also carried out by HPLC-MS analysis.

Affinity chromatography was used for comparative assessment of antithrombin-III binding in oligosaccharide fractions. Results showed the isolated fractions for Inhixa were within the reference range. Structure characterisation of fractions with NMR and LC based techniques showed the products to be similar. Further analysis of the antithrombin-III binding decasaccharide fraction was also carried out with NMR, HPLC and MS based techniques which showed both products to be similar.

A number of *in-vitro* tests that assess inhibition of coagulation factors Xa (anti-FXa) and IIa (anti-FIIa), and clotting tests such as activated partial thromboplastin time (aPTT) and HEPTEST have also been compared between the test Enoxaparin sodium injection and Clexane formulations. The aPTT and HEPTEST assay results were compared using an equivalency test. The data in general show the test results to be comparable.

Multivariate statistical analysis results show that for all characteristics all test batches lie within the confidence boundary built by data from the reference. Even though, the statistical analysis was considered supportive, the quality assessment of the individual components is still of utmost relevance.

Quantification of impurity profiles was also part of the biosimilarity exercise. It was demonstrated that the impurity profile of the API is comparable to or lower than the originator.

It is clear that the Applicant has put considerable effort into the analytical testing of both products for the biosimilarity exercise. Taken together, the Applicant has generated a significant amount of data, which supports the Applicant's claim of biosimilarity.

2.2.3. Discussion on chemical, pharmaceutical and biological aspects

Sufficient information regarding the starting material pooled porcine intestinal mucosa has been provided. An extensive biosimilarity study that encompasses comparison of heparin source material and mode of depolymerisation, physico-chemical properties, structural comparisons (similarity of oligosaccharides sequence, similarity of oligosaccharide fragments, similarity of disaccharide building blocks and similarity of affinity components) and *in-vitro* biological (clotting tests such as activated partial thromboplastin time (aPTT) and HEPTEST)) and biochemical activity (inhibition of coagulation factors Xa (anti-FXa) and IIa (anti-FIIa)) has been carried out. Therefore it can be concluded that biosimilarity has been demonstrated between Enoxaparin Sodium and Clexane.

2.2.4. Conclusions on the chemical, pharmaceutical and biological aspects

Information about the active substance and finished product was of acceptable quality. The manufacturing processes are well described and properly controlled both for active substance and finished product. Specification limits and analytical methods are suitable to control the quality of the active substance and the finished product. The stability program is considered satisfactory. The results generated during the stability studies support the proposed shelf-life and storage conditions as defined in the SmPC.

2.2.5. Recommendations for future quality development

In the context of the obligation of the MAHs to take due account of technical and scientific progress, the CHMP recommended some points for further investigation.

2.3. Non-clinical aspects

2.3.1. Introduction

The non-clinical data included in the initial application presented and discussed solely bibliographical data, not reflecting the specific aspects that need to be considered for an abridged marketing authorisation procedure. According to Eudralex Volume 2A (Notice to Applicants, June 2013) for application in accordance with paragraph 4 of Article 10 "the results of appropriate pre-clinical tests or clinical trials relating to these conditions must be provided." Initially it was not reported if pharmaco-toxicological requirements are fulfilled by assaying representative material by non-clinical means. The Non-clinical Overview submitted within the initial application did not justify the approach taken to compare the product candidate with the reference medicinal product (RMP) to detect differences between the similar and the RMP as requested in the *CHMP Guideline on nonclinical and clinical development of similar biological medicinal product containing low-molecular-weight-heparins (EMEA/CHMP/BMWP/118264/2007)*. But in the course of the procedure the applicant provided the revised Non-clinical Overview to properly integrate data and include a discussion of any quality aspects that might have implications on pharmacology of the product candidate, including safety implications of excipients and considerations on immunogenicity.

2.3.2. Pharmacology

The Non-clinical Overview contained in the initial submission relied on published studies on the active substance, enoxaparin sodium. The mechanism of action of enoxaparin sodium has been elucidated *in vitro* and has been attributed to its ability to bind to anti-thrombin III and inhibit the conversion of prothrombin to thrombin, reducing the thrombin-mediated conversion of fibrinogen to fibrin and thus preventing clot formation. This has been supported by the extensive clinical use of enoxaparin sodium for the treatment of various thromboembolic diseases. No new safety pharmacology studies have been performed and the identified issues in the literature review mainly relate to the potential of enoxaparin to modulate the bleeding times in pre-clinical models, however, these effects were dose dependent and occurred at high doses. No studies were reported for effects on central nervous or respiratory systems.

The Non-clinical Overview did not justify the approach taken to compare the product candidate with the RMP to detect differences between the similar and the RMP as requested per *CHMP Guideline on nonclinical and clinical development of similar biological medicinal product containing low-molecular-weight-heparins (EMEA/CHMP/BMWP/118264/2007)*. Therefore the applicant provided during the procedure a revised Non-clinical Overview properly integrating data and including a discussion of any quality aspects that might have implications on pharmacology of the product candidate, including safety implications of excipients and considerations on immunogenicity.

Comparative *in vitro* studies (to assess inhibition of coagulation factors Xa and IIa, and clotting tests such as aPTT and Heptest®) presented and discussed in Module 3 as well as *in vivo* pharmacodynamic (PD) endpoints (anti-FXa, anti-FIIa and TFPI) presented and discussed in Module 5 largely covered the expectations clarified in the frame of previous scientific advices given to the product.

2.3.3. Pharmacokinetics

All pharmacokinetic (PK) data provided by the applicant originate from studies of authorised presentations in healthy volunteers. No non-clinical PK drug interactions were discussed. The applicant has instead examined

clinical PK drug interactions. However, non-clinical PK data are usually not required for biosimilarity assessment (except for toxicokinetics in cases where a toxicology study is necessary).

2.3.4. Toxicology

A broad range of published toxicology data regarding the RMP, enoxaparin sodium (Clexane/Lovenox) was provided and no comparative or stand-alone toxicity studies were performed with Inhixa and the originator, respectively.

Sub-acute and chronic toxicity studies with enoxaparin were conducted in rats, dogs and monkeys. There were no species differences in the toxicity of enoxaparin; in all animals there were changes in hematology values and organ weights, reflecting the physiological adaptation of animals to long term anticoagulant treatment and resulting hemorrhage.

No significant effects were observed on fertility as demonstrated through reproductive performance in rats. No significant toxicity or teratogenicity was seen in any of the embryo-foetal development publications reviewed, although a number of abortions and resorptions in rabbits were noted at 10 mg/kg/day and 30 mg/kg/day dose groups in two publications. No significant postnatal toxicity was seen in rats asides significantly reduced postnatal body weight and body weights gain at 20 mg/kg/day. In sheep, although enoxaparin did not cross the placenta, it did induce the release of an endogenous dermatan sulphate-like substance which altered fetal coagulation. In humans, prophylactic and therapeutic enoxaparin treatment during pregnancy was safe. However, as there were no adequately powered and well-controlled studies in pregnant women and animal studies are not always predictive of human response, enoxaparin should be used during pregnancy only if the physician has established a clear need. This is reflected in the SmPC.

No local tolerance issues or allergic potential was demonstrated following subcutaneous and intradermal injections in beagle dogs and Pirbright white guinea pigs.

Toxicology studies could be not required if the quality comparability investigations of Inhixa and the RMP (addressing physicochemical parameters/analytical characterisation as well as biological/biochemical parameters and similarity in biological activity) yield the expected results and did not leave open unanswered questions. This position was essentially confirmed by the CHMP in the frame of previous scientific advices given for the product candidate (EMA/CHMP/SAWP/691158/2013; Procedure No.: EMEA/H/SA/2281/1/FU/1/2013/III) and is in line with the *EMA Concept paper on the revision of the guideline on nonclinical and clinical development of similar biological medicinal products containing low-molecular-weight heparins" (EMA/CHMP/BMWP/522386/2011)*. Also studies regarding safety pharmacology, and reproduction toxicology, were considered not required in the context of non-clinical testing of a biosimilar containing low LMWH.

As from a pharmaceutical (anti-Xa, anti-IIa, aPTT, Heptest®) and pharmacological (*in vivo* PD endpoints anti-FXa, anti-FIIa and TFPI integrated into clinical study) perspective essential expectations were covered and reflected in the respective quality and clinical assessment reports, the absence of product candidate specific toxicity studies was not of concern from a nonclinical perspective.

Heparin-induced thrombocytopenia (HIT) presents a rare, but serious adverse event associated with LMWHs but the biosimilar claim regarding this aspect was not initially discussed by the applicant. PF4-heparin-complex antibodies are a prerequisite for HIT, with assays discussed as surrogate for platelet-activating antibodies and used to confirm HIT (Warkentin et al. 2004, Greinacher et al. 2010). It is widely accepted that the immunogenic epitopes are located on PF4, with NMR measurements suggesting that

complex antigenicity is accompanied by structural changes of PF4 (Mikhailov et al 1999). Extrapolation from animals to humans is difficult so immunogenicity studies in animals are not encouraged and it is also well recognized that many antibody-positive patients have normal platelet counts.

Given that an abridged approach was chosen for clinical development the CHMP requested adequate characterisation of the risk of immunogenicity for the biosimilar candidate, based on qualitative data of the biosimilar candidate (e.g. chain length and charge density) as well as a reflection on impurities and supportive state-of-the-art comparative nonclinical studies/in vitro techniques such as exploring measurements of conformational changes in PF4 (e.g. Brandt et al. 2014), applying *in vitro* models of human immunity (e.g. Luna et al. 2015) and elaborating on the size and charge of PF4 aggregates and/or comparative measurement of antiheparin/PF4 total or IgG antibodies.

It was initially not clear from the submitted documentation whether the performed bioassays (presented in the pharmaceutical part) were suitable for demonstrating biosimilarity on the non-clinical level. Therefore, the Applicant was asked to compile all bioassays performed in a table, including a short description of methods and outcome, as well as full references information instead of providing exclusively abstracts. The Applicant was also asked to provide full study reports for all bioassays, also containing raw data, from which the experimental procedures, the positive and negative controls used and the number of data points obtained could be derived.

Also a focussed elaboration on the potential immunogenicity issues, with HITT (Heparin induced Thrombocytopenia and Thrombosis) presenting a rare, but serious adverse event associated with LMWHs was requested. The CHMP requested further supportive information during the procedure to adequately characterise the risk of immunogenicity for the biosimilar candidate.

With responses the applicant submitted PF4-LMWH and PF4 analysis by SEC, In-vitro PBMC (peripheral blood mononuclear cell) model and the results of measuring serotonin release as detected after adding PF4-enoxaparin complexes.

PF4-LMWH and PF4 analysis by SEC

Regarding the concern for HIT and its published evidence for a link with platelet-activating IgG antibodies recognizing ultralarge complexes (ULC) composed of PF4 bound to heparin the applicant tried to characterize PF4-enoxaparin complexes formed by the reference medicinal product or the biosimilar candidate by SEC. Report “GLP(2015)00-032” had not entirely meaningful structure, not accurately explained methodology and missing references to source literature. Besides that, also the ultimate scope of providing scientific evidence for the absence of clinical safety concerns was not fulfilled:

PF4-LMWH and PF4 analysis by SEC was developed and optimized on the basis of method as published by Rauova et al. 2005 and Luna et al. 2015 to check for ULCs after incubation of enoxaparin (three reference and three biosimilar candidate batches, measurement in duplicates) with PF4 at 37°C for 60 minutes. In line with the papers, the tested molecular mass of the complex is above 660 kDa, but depolymerized at high salt concentration (0.75 M NaCl). Luna et al. 2015 also used low and high salt (0.15 and 0.75 M NaCl containing 1% glycine) buffer, but used a pH 7.5 buffered solution which was not reported with GLP(2015)00-032 - but the absence of pH stabilization presents a risk for generating less robust data. Luna et al. 2015 also presented the differences in stability of ULCs, yielding the detection of tetrameric PF4 with a branded enoxaparin at high salt levels, which was not the case for other enoxaparins. Also other differences, like poor dissociation under high salt conditions were observed for the non-originator compound.

Neither method optimization nor the applicants' discussion touched this aspect. The absence of relevant control experiments was noted, as it was not shown that under the selected experimental conditions heparin chain length (or at least UFH) is able to influence generation and/ or stability of ULCs.

The applicant showed that the results (of the biosimilar candidate) are (with 0.76-0.264 mAU*min) in the range of the originator product (0.186-0.488), or higher than that – but concluded that the peak area of PF4 is largely the same "*it can be inferred"* that the amount of ULC is in the range of the originator product. A method which allows a clear, direct readout for data definitively needs to be preferred over a method which was not qualified for this inference.

The MWCO of SEC column TSK G3000SWXL is given with 500 kDa, whereas already the paper mentions that ULCs are expected around 670 kDa. Of interest, optimization included a test for the instrument (Thermo Ultimate 3000 vss Waters e2695) – besides optimization of incubation time, PF4-Heparin ratio and other experimental conditions - but did not include the option to select a column with a MWCO appropriate to directly assay for the expected size of the aggregates.

As a column with a suboptimal MWCO has been selected, and even also bigger sizes of aggregates were already detectable by SEC it was questionable, if these results indeed support biosimilarity at all and results may hide bigger aggregates potentially existing for the biosimilar candidate.

In-vitro PBMC (peripheral blood mononuclear cell) model

The applicant studied the immunostimulatory potential (using CD83 – a stringent marker of APC maturation) of three originator and biosimilar candidate lots in an in vitro PBMC model, with PBMCs derived from eight different healthy donors. Immunostimulation was modelled by measuring CD83 expression by flow cytometry as a consequence of APC maturation of monocytes (CD14+) after 3 days of incubation of samples containing Heparin and PF4 at final concentrations of 4.8 and 15.4 µg/mL at 37°C. LPS (50 ng/mL) and R484 (10 µg/mL) presenting positive TLR agonist controls, were used as positive control.

The CHMP noted that the absence of a difference between reference material and biosimilar candidate needs to be interpreted against the background of high variability, a relatively small dynamic range facing also the strong immunostimulatory nature of the positive control sample and cannot be considered discriminatory or indicating regarding TFPI production or clinically relevant safety aspects.

Dose-dependent immunostimulatory potential of PF4 (in absence of LMWH) was shown in the range of 0 and 15.4 µg/mL. As the assay format applied a fixed concentration (15.4 ug/mL) of PF4 the readout rather presents the degree of PF4 consumption leading to a decrease in immunostimulation as assayed by CD83+ cells instead of modelling the inverse correlation of APC activation with decreases in TFPI production.

Finally, in absence of challenging this method for its discriminatory potency by applying additional controls (e.g. PF-4-UFH complexes) even a suggestive link to clinical safety or the context of likely clinical significance was considered not established, but required for product-specific assessment.

Serotonin release as detected after adding PF4-enoxaparin complexes

The applicant described as well in its responses the results of measuring serotonin release as detected after adding PF4-enoxaparin complexes (as prepared for in-vitro PBMC assay) into rich platelet samples isolated from four healthy volunteers against LPS used asa positive control. The applicant presented general information on SRA validation, its value in oncology, theory of electrochemical detection and "principles of quantitative evaluation with internal standard", but no product-specific development or testing data.

As such, SRA data presented with responses were noted, but lacked the presentation of raw data and data evaluation. The applicant also missed to show the sensitivity of the method that SRA values indeed are able to correlate with the content and/or quality of PF4-heparin complexes.

The CHMP concluded that the provided set of bioassays with D121 responses was not able to show its product-specific discriminatory capacity to detect clinically relevant differences. Thus the results provided within these responses – although not indicating differences – did not provide relevant supportive value.

Additional responses provided by the applicant consisted of a series of exploratory, comparative *in vitro* studies trying to complete the initial investigations to diminish immunogenicity concerns by characterizing PF4-enoxaparin complexes formed by the RMP or the biosimilar candidate. The assessment of these responses is provided below.

<u>in-vitro PBMC model</u>

Results of a previously submitted *in vitro* cultured PBMC model (based on CD83 expression after incubation of Enoxaparin/PF4 with human PBMCs, Report K 1560) suffered on high variability, a relatively small dynamic range for the readout and – fundamentally - was not able to show discriminatory potency in absence of additional controls (e.g. PF4-UFH complexes).

A bridging report was submitted, presenting results for CD83 expression of PF4: Heparin complex in the in-vitro PBMC model. Besides the PF4:Heparin samples representing the test article, the examinations of the bridging report contained data for negative control (culture medium), positive control (LPS + R848) and vehicle controls.

The immunostimulatory potential as measured by CD83+% under the test conditions (using human PBMC from two different male donors) showed indeed on average for the PF4:Heparin complex a relatively high value if compared with the LPS/R848 based positive control. But CD83+% for the test article (PF4:Heparin) of human PBMC donor M1 was even higher if compared with the positive control using human PBMCs from the same donor.

This completed the picture provided with the initial report K1560 providing results for PF4:Enoxaparin (test article), positive, vehicle and negative control derived with human PBMC samples from eight different donors.

However, the applicants statement, that "*results from the bridging study also show the ability of the model in establishing an upper immunostimulatory response limit between PF4/Heparin and PF4/Enoxaparin comparisons*" was not convincingly supported by data, due to the high – and overlapping – variability of results and the absence of a head-to-head comparison for PF4/Heparin with PF4/Enoxaparin preparations.

Nevertheless, the inherent limitations of the testing approach together with the efforts undertaken to establish a product-specific strategy were well recognized by the CHMP.

<u>TFPI release from HUVEC cells</u>

This approach was established as a second model, measuring TFPI levels released into the culture medium by ELISA (Evaluation of TFPI release from HUVEC cells), and was not part of previously submitted data. Enoxaparin stimulation from test and reference compounds showed comparable levels, while heparin stimulation induced

higher levels of TFPI release – although the latter may have been driven by a 36 (instead of 24 hour) incubation period.

PF4-Enoxaparin complex supplementary LC characterization

PF4-Enoxaparin Complex Characterization Supplementary LC Report showed robustness for the method using a mobile phase in the pH range 7.3 to 7.7 for the AUC values derived for PF4-Enoxaparin and PF4 applying an 80-120% acceptance criterion (against AUC values derived at pH 7.5). In fact it was shown, that values at pH 7.3 and 7.7 were always below 100% - which sufficiently confirms appropriateness for the selected pH 7.5 of the mobile phase.

As with the previous studies a SEC column of suboptimal MWCO range was selected, 4 different columns (TSKG3000SWXL, TSKG4000SWXL, TSKG6000PWXL, Bio-Rad SEC650) were tested for their capability to detect ultralarge complexes in PF4-UFH complex solution of 1:1/3.4:1/6.8:1/13.6:1/34:1. Although the columns – according to manufacturer's specifications – should in principle be able to cover the relevant MW range no ULCs were detectable, only residual PF4 signal was detectable.

Consequently the applicant conducted control experiments by bypassing the SEC columns, to assay samples derived from different PF4: Enoxaparin and PF4:UFH ratios.

Results indeed showed a trend for a higher ratio and complex size for the PF4/Heparin complex if compared to the PF4/enoxaparin complexes, in absence of differences between test article and reference compound. I.e. it was confirmed, that Heparin consumes higher amounts of PF4 than enoxaparin. PF4:enoxaparin complexes were found also to be more sensitive against increasing salt concentrations if compared to PF4:heparin complexes.

In summary, also this assay showed limitations in analytical capabilities to setup a sensitive methodology to detect formation of PF4:Enoxaparin complexes. But it was acknowledged that size exclusion chromatography based separation may be hampered for highly charged molecules, triggering the need to apply high ionic strength in the mobile phase. But this likely leads to depolymerization of complexes formed by polyanic and highly positive charged structures.

PF4 complex - secondary structure

Based on published literature (Brandt et al 2014) showing that" *PF4 structural changes resulting in antiparallel β-sheet content >30% make PF4/polyanion complexes antigenic*" the applicant conducted CD spectroscopy examining secondary structure (α-helix, β-sheet, β-turn and random coil) similarity of PF4:Enoxaparin complex formed with test and reference compound.

An increasing amount of enoxaparin was added to a fixed content of PF4 to study its effect on secondary structure of PF4. These effects were compared for test and reference compound. The selected molar ratio of 1.5:1 for PF4:Enoxaparin was based on the maximum impact measured in changes of the secondary structure.

Although slight differences in the content of beta-turn structures were observed if compared to literature a likely explanation was given with the source (and different MW) of PF4. Comparative results indicated no differences between test and reference compound.

PF4 complex-Binding study

A surface plasmon resonance based binding study examined binding of test and reference compound to immobilized PF4, applying blank and heparin as control samples (SPR Binding study report). These new data

showed a comparable PF4 binding for test and reference compound, confirming an expected difference to the affinity of heparin to PF4.

PF4 complex –particle size report

Photon correlation spectroscopy (PCS, also dynamic light scattering DLS) was used to examine particle size of PF4-enoxaparin complexes (PF4/Enoxaparin Complex size report). PCS measures fluctuations in scattered light intensity due to diffusing particles, and could therefore be used to characterize the size of various particles.

Comparative analysis of test material and reference products showed individual values (three batches each) ranging from 1152 to 1597 for the test material and from 1574 to 1650 for the reference material. These results were considered sufficiently overlapping, facing the variability and nature of the testing approach as well as the selection ofd Z-mean particle size as readout.

In summary the CHMP concluded that from a non-clinical perspective relevant assays were conducted and were not able to identify different immunogenic potential for the biosimilar candidate if compared with the RMP. The efforts undertaken by the applicant were acknowledged, although constraints based on the exploratory nature of the testing approaches were experienced. Nevertheless, in addition to the efforts to underline robustness of *in vitro* based human PBMC assays additional examinations based on TFPI release from HUVEC cells, circular dichroism, SPR based PF4 complex-binding studies and PCS based particle size determination were submitted. Also these additional examinations were not able to indicate differences regarding antigenicity between test and reference compounds.

2.3.5. Ecotoxicity / environmental risk assessment

According to Directive 2001/83/EC applicants are required to submit an environmental risk assessment (ERA) also for applications under Art. 10(4) similar biological applications. The applicant submitted a justification for the absence of ERA, referring to the intention to substitute products on the market by the biosimilar medicinal product. The justification included a statement that "*the product does not contain any component which results in additional hazard to the environment*".

The indications for enoxaparin biosimilar are the same as those for the RMP, and therefore the absence of an ERA was considered justified in line with the requirements of *EMA Guideline on the environmental risk assessment of medicinal products for human use (EMEA/CHMP/SWP/4447/00 Corrigendum 2)*.

2.3.6. Discussion on non-clinical aspects

The applicant provided an **exhaustive literature review** regarding non-clinical aspects for enoxaparin sodium. ***In vitro* tests** assessing the inhibition of coagulation factors Xa and IIa, and clotting tests (activated partial thromboplastin time [APTT] and Heptest) and thus examining the comparability of the test enoxaparin sodium injection and RMP formulations were detailed and assessed in the quality section.

Moreover, the applicant performed several non-clinical studies to assess the biosimilarity of the test enoxaparin formulation to that of the reference product. Data of following bioassays were provided: ***in vitro* Xa / IIa assay, Enoxaparin PF4 complex immunogenicity study, the characterization study of PF4-Enoxaparin complex by SEC-HPLC, and the Serotonin Release Assay (SRA)**. However, the provided set of bioassays was not able to show its product-specific discriminatory capacity to detect clinically relevant differences. Besides not providing sufficiently sensitive models to detect differences in immunogenicity, the applicant initially missed to layout a product-specific strategy to link potential differences by establishing limits with clinical safety relevance. Thus the results – although not indicating differences – did not provide relevant supportive value.

Therefore results of **additional, in depth examinations** based on comparative *in vitro* studies were provided and were able to diminish immunogenicity concerns. While further examinations on the validity of the *in vitro* PBMC (peripheral blood mononuclear cell) model, including the supplementary LC studies on PF4-Enoxaparin complex, showed essentially the inherent limitations of the testing approach, the following studies provided additional confidence for the absence of comparative immunogenicity differences: **comparable levels of TFPI released into the culture medium by HUVEC cells, similarity of secondary structural changes of PF4 as measured by CD spectroscopy after addition of test and reference enoxaparin, comparable PF4 binding for test and reference compound based on SPR analysis, and overlapping results of comparative test and reference material analysis by PCS for the PF4/LMWH particle size**.

Regarding toxicology the absence of any *in vivo* studies indeed represent a valid option for biosimilar application. From a pharmaceutical (anti-Xa, anti-IIa, aPTT, Heptest) and pharmacological (*in vivo* PD endpoints anti-FXa, anti-FIIa and TFPI integrated into clinical study) perspective essential expectations for biosimilar applications were considered to be covered. The absence of product candidate specific *in vivo* toxicity studies was not of concern from a nonclinical perspective.

2.3.7. Conclusion on the non-clinical aspects

The CHMP concluded that from the non-clinical perspective relevant assays were conducted and were **not able to identify different immunogenic potential** for the biosimilar candidate when compared to the RMP. The efforts undertaken by the applicant were well recognized, although constraints based on the exploratory nature of the testing approaches were experienced. During the procedure the applicant has provided **additional data further characterizing PF4-enoxaparin complex** indicating a lack of relevant differences between test and the RMP. Additional *in vitro* data were provided as well, however it was acknowledged that its results with respect to immunogenicity have limitations. Taken together the CHMP concluded that the most prominent safety concern associated with LMWHs, HP4 (Heparin Platelet Factor 4) complex binding is most likely similar between the test and the RMP. From this it was inferred that the risk for immunogenicity is most likely also similar. This risk was further addressed in the agreed version of the risk management plan including plans for appropriate pharmacovigilance activities. The CHMP concluded that the submitted non-clinical data support biosimilarity of candidate product to the RMP.

2.4. Clinical aspects

2.4.1. Introduction

GCP

The clinical trials were performed in accordance with GCP as claimed by the applicant.

To support current application the applicant conducted a, randomized, single dose, two-way cross-over, pharmacodynamic (PD) endpoints study of biosimilar enoxaparin sodium 40 as compared to RMP Clexane with quantification of the surrogate factors anti-FXa and anti-FIIa, and tissue factor pathway inhibitor in healthy subjects using subcutaneous administration.

2.4.2. Pharmacokinetics

No new pharmacokinetic (PK) data have been provided. A **comprehensive literature review** has been provided which summarize the PK properties of enoxaparin sodium. Due to the heterogeneity of LMWHs in their physico-chemical characteristics, conventional PK studies could not be performed.

The PK of enoxaparin is based on anti-factor Xa activity, rather than direct detection of the molecular species (Carter et al 2008). The PK properties of enoxaparin have been investigated in healthy volunteers (Frydman et al 1988, Bara et al 1993, Collignon et al 1995), in critically ill patients (Priglinger et al 2003, Haas et al 2005), and in patients presenting with acute coronary syndrome (Aslam et al 2002, Martin et al 2004). Following SC administration, enoxaparin demonstrates high bioavailability; it has been estimated at 91%, compared with 29% for unfractionated heparin (Bara et al 1985, Dawes et al 1986). The maximum plasma anti-Xa activity occurs 1 to 4 hours after injection with peak activities in the order of 0.16 IU/mL and 0.38 IU/mL after doses of 20 mg or 40 mg, respectively. Following a 30 mg IV bolus, immediately followed by 1 mg/kg SC every 12 hours provided initial Amax of 1.16 IU/mL (n=16) and average exposure corresponding to 88% of steady-state levels (Clexane SmPC 2014). The PK of SC enoxaparin was shown to be linear within dose range of 20 to 80 mg (Frydman et al 1988). Following a 40 mg dose, anti-Xa activity may persist in the plasma for 24 hours.

Enoxaparin appears to bind less to plasma proteins than UFH (Young et al 1993). The apparent volume of distribution of enoxaparin in healthy young volunteers has been estimated at between 5.2 and 9.3L (Buckley and Sorkin 1992, Noble et al 1995).

It appears likely that the primary route of elimination of enoxaparin is renal. Enoxaparin seems to possess the relative advantage of dose-independent elimination (Buckley and Sorkin 1992). In addition, desulfation and/or depolymerization of enoxaparin in the liver result in the formation of lower molecular weight species with substantial loss of activity (Siddiqui and Wagstaff 2005) and seem to contribute to elimination.

The terminal half-lives of anti-Xa activity have been shown in healthy volunteers to be approximately 2 and 4 hours (Frydman et al 1988, Collignon et al 1995). In patients with unstable angina and NSTEMI after a single 30mg enoxaparin IV bolus followed by 1.0 or 1.25 mg/kg SC twice a day the elimination half-life 5.0 h (Bruno et al 2003). In general, elimination is characterised by a half-life of 4 to 5 hours.

In patients with ACS that received a full complement of antiischaemic therapy, aspirin, and enoxaparin (30 mg IV, followed by weight-adjusted doses of either 1 mg/kg or 1.25 mg/kg SC every 12 hours) creatinine clearance emerged as the most influential factor on apparent clearance, area under the curve, and anti-Xa activity among a wide range of clinical and laboratory covariates (Becker et al 2002). Similarly, in hospitalised acutely ill elderly patients receiving prophylactic dosages of enoxaparin (4000IU daily for up to 10 days) only CLcr <30 mL/min and bodyweight <50kg were associated with significantly higher anti-Xamax values (Mahe et al 2007). After repeated SC enoxaparin 40mg once-daily doses, anti-Xa clearance decreases with decline in renal function (Sanderink et al 2002a, Hulot et al 2004), with the clearance being 39% lower in patients with severe renal impairment than in healthy volunteers (P=0.0001) (Sanderink et al 2002a), necessitating a reduction in enoxaparin dosage in patients with severe renal impairment (creatinine clearance <30 mL/min) (Siddiqui and Wagstaff 2005).

After IV infusion, total body clearance and volume of distribution at steady state were higher in obese volunteers than in non-obese volunteers, but when adjusted for weight, these values were about 10% lower in obese volunteers. There appears to be no need to modify the recommended dose for obese volunteers (Sanderink 2002b).

An investigation of anti-Xa and anti-thrombin activities in pregnant women and their fetuses at 23 weeks' gestation, following SC administration of enoxaparin to the women, indicated that the drug does not cross the placenta to any significant extent (Forestier et al 1984). Both enoxaparin clearance and volume of distribution were shown to increase during pregnancy (Patel et al 2013). Maintenance of the same doses throughout pregnancy was found to result in a progressive reduction in mean and peak anti-Xa activities (Lebaudy et al 2008). No PK interactions were observed between enoxaparin and thrombolytics when administered concomitantly.

2.4.3. Pharmacodynamics

2.4.3.1. Introduction

While thrombin is the primary focus of action of a heparin anticoagulant, effects on thrombin generation may also be involved. Heparin also acts via inhibition of factor IXa (Beguin et al 1991) and inhibition of the thrombin-mediated feedback activation of factors V and VIII (Beguin et al 1988). Enoxaparin produces dose-dependent inhibition of thrombin but also inhibits prothrombinase activity in the common pathway (Buckley and Sorkin 1992). Two major mechanisms of action for enoxaparin are apparent from studies: direct inhibition of thrombin by binding and enhancement of AT III, and inhibition of prothrombinase-mediated thrombin generation (Buckley and Sorkin 1992, Ofosu et al 1992a and b, Bara et al 1993, Bendetowicz et al 1994a). The major anticoagulant effect of enoxaparin, like that of unfractionated heparin (UFH), is mediated via a conformational interaction with antithrombin III which stimulates the latter molecule to inactivate procoagulatory serine proteases such as factors IIa (thrombin), IXa and Xa. Consequently, enoxaparin indirectly inhibits the conversion of prothrombin to thrombin mediated by the prothrombinase complex (factor Va/factor Xa) and reduces the thrombin-mediated conversion of soluble fibrinogen to insoluble fibrin, thus preventing clot formation (Noble and Spencer 1998).

While the interaction of enoxaparin with the coagulation cascade is complex, inhibition of factors Xa (which converts prothrombin to thrombin) and IIa (which converts fibrinogen to fibrin) are central to its action (Noble et al 1995).

Enoxaparin has a higher ratio of anti-factor Xa to IIa activity (variously estimated between 3.6 : 1 and 14 : 1) than UFH (1 : 1) (Padilla et al 1992, Bara et al 1993, Noble et al 1995, Hirsh et al 2001a, Siddiqui and Wagstaff 2005). In platelet depleted plasma of healthy volunteers, who received one subcutaneous injection of either enoxaparin at 40 mg or 1mg/ kg, or unfractionated heparin at 5,000 IU at one week intervals, higher anti-Xa activity relative to anti-IIa was reported for enoxaparin (1,200 IU) (Bara et al 1993).

The activity of enoxaparin in anti-thrombin assays is relatively less than its anti-factor Xa activity; for equivalent anti-factor Xa activity, enoxaparin has a much lesser effect (up to 5 times less) than unfractionated heparin on thrombin (Vinazzer and Woler 1985, Walenga et al 1985).

Enoxaparin has also been shown to cause the release of tissue factor pathway inhibitor (TFPI) (Fareed et al 2003). Although enoxaparin and UFH produce similar increases in free TFPI concentrations following single-dose administration, total TFPI activity is partially depleted after continuous, multiple-dose, subcutaneous administration of UFH, but not of enoxaparin (Bara et al 1993, Fareed et al 2003).

Intravenous enoxaparin at doses of 25-125 mg administered to human volunteers strongly inhibited fibrinopeptide A generation for as long as 24 hours after administration. The sustained inhibitory effect of enoxaparin on fibrinopeptide A generation is thought to be due to the release of endogenous mediators such as TFPI and glycosaminoglycans (Fareed et al 1986).

Enoxaparin has been shown to inhibit the generation of factor VIIa (Gerotziafas et al 1998 and 2003). Enoxaparin has been shown to have an effect on the release of von Willebrand factor (vWF) which mediates platelet adhesion to exposed subendothelium (Fareed et al 2003).

It is recommended that agents which affect haemostasis should be discontinued prior to enoxaparin therapy unless their use is essential, such as: systemic salicylates, acetylsalicylic acid, NSAIDs including ketorolac, dextran, and clopidogrel, systemic glucocorticoids, thrombolytics and anticoagulants. If the combination cannot be avoided, enoxaparin should be used with careful clinical and laboratory monitoring (Clexane SmPC 2014).

2.4.3.2. Mechanism of action

Enoxaparin, a LMWH, is one of the most widely used amongst its class (Iqbal and Cohen 2011). Now-a-days, enoxaparin has become the treatment of choice for various thromboembolic diseases (Ingle and Agarwal 2014). Two major mechanisms of action for enoxaparin are apparent from studies: direct inhibition of thrombin by binding and enhancement of AT III, and inhibition of prothrombinase-mediated thrombin generation (Buckley and Sorkin 1992, Ofosu et al 1992a and b, Bara et al 1993, Bendetowicz et al 1994a).

2.4.3.3. Primary pharmacology

The major anticoagulant effect of enoxaparin, like that of UFH, is mediated via a conformational interaction with antithrombin III which stimulates the latter molecule to inactivate pro-coagulatory serine proteases such as factors IIa (thrombin), IXa and Xa. Consequently, enoxaparin indirectly inhibits the conversion of prothrombin to thrombin mediated by the prothrombinase complex (factor Va/factor Xa) and reduces the thrombin-mediated conversion of soluble fibrinogen to insoluble fibrin, thus preventing clot formation (Noble and Spencer 1998).

Both UFH and enoxaparin lead to the release of tissue factor pathway inhibitor (TFPI), which has inhibitory effects on the coagulation cascade. This may partially be responsible for the anticoagulant effect (Fareed et al 2003). Increases in the concentration of free TFPI are similar after single doses of UFH or enoxaparin (Bara et al 1993), but the drugs produce different effects after multiple doses (Fareed et al 2003). After five days of administration of UFH, total TFPI activity is partially depleted following repeated and continuous subcutaneous injection of UFH, but not following subcutaneous injection of enoxaparin (Hansen et al 1998, Bendz et al 1999). The difference in depletion of TFPI by UFH and LMWH may be a further explanation of why the anticoagulant effect of enoxaparin is more predictable than that of UFH (Fareed et al 2003).

Major pharmacological characteristics of the LMWH´s compared to UFH (unfractioned heparin):

- High bioavailability (91%, after subcutaneous injection)
- Minimal affinity for binding to plasma, platelet and vessel wall proteins
- Little or no binding to endothelial cells or macrophage receptors
- Higher ratio of anti-factor Xa to anti-factor IIa activity (2 : 1 to 4 : 1 compared with 1 : 1 of UFH)
- More predictable and more durable anticoagulant response
- Sustained biological activity allows once- or twice-daily administration compared with 3-times-daily administration for UFH
- Less effect on platelet function
- Not inhibited by platelet factor 4

- Inhibition of factor Xa on platelet surfaces as well as in the circulation (Noble and Spencer 1998)

2.4.3.4. Secondary pharmacology

N/A

2.4.3.5. Relationship between plasma concentration and effect

No dose-response study has been performed by the applicant. This was considered acceptable.

2.4.3.6. Pharmacodynamic interactions with other medicinal products or substances

No interaction studies have been performed by the applicant and none were required in an authorisation procedure for similar biological medicinal product.

2.4.3.7. Comparative PK/PD Trial: Study No 411/13 (EudraCT No.: 2013-002864-21)

The applicant submitted a single comparative pharmacodynamic (PD) bioequivalence study comparing Enoxaparin sodium 40 mg/0.4 ml solution for injection to Clexane 40 mg/0.4 ml solution for injection via subcutaneous administration in 20 healthy volunteers to support the demonstration of bioequivalence of Enoxaparin Inhixa to the reference medicinal product Clexane.

Study period: 11 October 20013- 21 October 2013

Date Report: 26 June 2014

Methods

Study design

The study was open label, randomized, single dose, two-treatment, two-period, two-sequence, crossover, comparative bioavailability and pharmacodynamic bioequivalence study of Enoxaparin sodium 40 mg/0.4 mL, solution for injection vs. Reference Medicinal Product Clexane, 40 mg/0.4 mL, solution for injection after subcutaneous administration in 20 healthy volunteers under fasting conditions. The subjects were confined in the Study Period 1 between October 11 and October 13, 2013 and for the Study Period 2 between October 19 and October 21, 2013.

Administration

Two single dose subcutaneous administrations in a cross-over design with a washout period of 8 days.

Study Participants

Inclusion Criteria

- Healthy males and females, age from 18 to 55 years, of Caucasian race. Non-smoker or past-smoker (at least 6 months before dosing).
- Body Mass Index (BMI) from 18.5 to 30 kg/m2, inclusive.
- Subject is available for the entire Study Period and has provided his/her written informed consent.
- Physical examination without significant deviations.
- Vital signs and ECG without significant deviations.

- All laboratory screening results within the normal range or being assessed as non-significant by the attending Clinical Investigator.
- Acceptance of use of contraceptive measures during the entire Study Period in subjects.
- Czech citizenship.

Exclusion Criteria

- Existing gastrointestinal diseases and/or pathological findings, which might influence the drug's safety, tolerability, absorption and/or pharmacokinetics.
- Acute or chronic diseases which may interfere with the aims of the study or with the bioavailability and/or pharmacokinetics of the IMP.
- History of severe allergy or allergic reactions to the study drugs or related drugs (e.g., enoxaparin sodium, heparin, other LMWHs).
- History or presence of serious clinical illness that can impact on the fate of drugs.
- Clinically significant illness within 4 weeks before the first dosing, including major surgery.
- Any significant clinical abnormality, including positive results of HBsAg and/or HCV and/or HIV test during screening procedure.
- Positive pregnancy testing, breast-feeding or lack of results of pregnancy test.
- Positive alcohol breath test.
- Positive urine screen for drugs of abuse.
- Serious mental disease or inability to cooperate with clinical team.
- Sitting blood pressure after a minimum of 5 minutes of rest is out of the range of 90-160/45- 100 mmHg or heart rate out of the range of 40-110 bpm during the screening procedure.
- Drug, alcohol (of 40 g g per day pure ethanol), solvents or caffeine abuse.
- Smoking.
- Use of organ-toxic drugs within 3 months before the first dosing i.e., any drug with a well defined potential for toxicity to a major organ or system (for example chloramphenicol, which may cause bone marrow suppression).
- Use of systemic drugs known to alter hepatic metabolism within 60 days prior to the first dosing.
- Any systemic prescription treatment within 28 days before the first dosing.
- Any systemic over-the-counter (OTC) drug treatment, vitamins or herbal treatment within 14 days before the first dosing.
- Donation or loss of: 1) at least 500 mL of blood within 60 days, or 2) 50 mL to 499 mL of blood within 30 days, or 3) plasma or platelets within 14 days before the first dosing.
- Participation in another clinical trial within 45 days prior to the first dosing.
- Significant bleeding disorders, thrombocytopenia, blood clothing disorders, increased risk of bleeding.

The inclusion and exclusion criteria are acceptable. The population recruited to the study is in line with the guidelines.

Treatments

Test Product

The test product used in the study: Name: Enoxaparin Sodium 40; Active ingredient: Enoxaparin sodium; Dosage form: Solution for injection; Strength: 40 mg (of salt)/0.4 mL; Batch Number: 4011CB 130203; Expiry Date: 20.02.2015; Manufacturer: Shenzhen Techdow Pharmaceutical Co., Ltd., China

Reference Product

The reference product used in the study: Name: Clexane; Active ingredient: Enoxaparin sodium; Dosage form: Solution for injection; Strength: 40 mg (of salt)/0.4 mL; MA Holder: Sanofi–Aventis France; Batch Number: 3CG92A; Expiry Date: 05.2016; Sourced market: Poland

Objectives

The objective of the study was to evaluate the pharmacokinetic/pharmacodynamic (PK/PD) parameters of the Test and Reference products both containing 40 mg of enoxaparin sodium in healthy volunteers under fasting conditions and to assess the bioequivalence of these products based on acceptance of confidence intervals of 80.00 % to 125.00 % for ln-transformed AUC(0-t) and Amax of enoxaparin anti-Xa and anti-IIa activity as primary parameters. Other PK/PD parameters were statistically evaluated as the secondary parameters.

Adverse events and clinically significant deviations from laboratory tests, physical examinations and vital signs were reported for the evaluation of safety.

Outcomes/endpoints

Primary parameters: AUC(0-t) and Amax (maximum activity observed) of anti-Xa and anti-IIa activity.

Equivalence criteria: The 95% confidence intervals for ln-transformed AUC(0-t) and Amax T/R ratio for the anti-Xa activity and anti-IIa activity have to lie within the predefined acceptance range of 80.00-125.00%.

Secondary PK/PD parameters: TFPI (Tissue Factor Pathway Inhibitor) activity; Rmin, Rmax and tmin, tmax of anti-Xa/anti-IIa activity ratio; AUC(0-∞), AUCres, tmax, t1/2, λz of anti-Xa, anti-IIa and TFPI activity;

Sampling: Blood collections for anti-Xa, anti-IIa and TFPI were performed prior to the administration of study medication and at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 8.0, 10.0, 12.0, 16.0, and 24.0 hours after drug administration (in total 18 blood samples of 6 ml each per period according to the study schedule).

Sample size

Based on the literature data the within-subject coefficient of variation (CV) of about 18% for enoxaparin anti-IIa activity (being more variable) was observed. The sample size was determined by a "test/reference" mean ratio between 95% and 105%, significance level of 5%, power of 80% to show bioequivalence. Based on these estimates and literature data the calculated sample size of 16 would have been sufficient to establish bioequivalence using the acceptance criteria of 80.00% to 125.00%, however, considering possible dropouts and withdrawals 20 subjects will be randomized and dosed.

Randomisation

The drugs, one injection of the test product or reference product, were administered subcutaneously by the Clinical Investigator to the study subjects according to the following randomized, two-treatment, two period, two-sequence, single dose, crossover design:

Table 3. Randomization and dosing schedule

Group	Number of Subjects	Treatments	
		Period 1	Period 2
RT	10	R	T
TR	10	T	R

Blinding (masking)

The study was open-labeled; however analysts did not have access to the randomization schedule to prevent bias during analyses.

Statistical Methodology

AUC(0-t) and Amax of anti-Xa and anti-IIa activities were used as primary parameters for bioequivalence assessment. Analyses of variance were performed on the pharmacokinetic parameters anti-IIa, anti-Xa and TFPI activity and anti-Xa/anti-IIa activity ratio:

- ln-transformed parameters of anti-Xa, anti-IIa and TFPI: AUC(0-t), AUC(0- inf,, Amax
- ln-transformed parameters of anti-Xa/anti-IIa activity ratio: Rmin, Rmax

The analysis of variance model included sequence, subject nested within sequence, period and treatment (i.e. drug formulation) as the sources of variance. The significance of the sequence effect was tested using the subject nested within sequence as the error term. A 5% level of significance was used. Each analysis of variance included calculation of least-squares means (LSM), estimates obtained for the adjusted differences between treatment means and the standard error associated with these differences. The above statistical analyses were done using the SAS® GLM procedure with fixed effects used for all terms. For the PK parameter of t1/2 ANOVA was performed on un-transformed data. In addition, a non-parametric Wilcoxon and median tests of treatment effect for tmax and tmin were performed.

The 90% confidence intervals were calculated for each parameter using LSM values. The confidence intervals were expressed as a percentage of the LSM for the Reference formulation. Percentage ratio of means (Test/Ref.) was also calculated for each parameter using LSM values. For ln-transformed parameters geometric means (i.e. antilog values of LSM on ln-transformed data) were used. Bioequivalence of the test product and the reference product was assessed on the basis of 90% confidence intervals for ln-transformed AUC(0-t) and Amax of enoxaparin activity parametres anti-Xa and anti-IIa as primary parameters. According to the Study Protocol standard bioequivalence acceptance range 80.00 % to 125.00 % was used.

Standard statistical methodology for the analyses of the primary endpoints was used. An ANOVA analysis, which included sequence, subject within sequence, period and formulation was used and all factors were specified as fixed term. This was considered appropriate as is the analysis of the primary endpoints on the log-transformed data, with subsequent back transformation.

In the study report, the applicant discussed the study results based on 90% confidence intervals and this was not considered appropriate by the CHMP. Instead, a more stringent 95% confidence interval was required for the PD endpoints. Results pertaining to the 95% confidence intervals for the primary endpoints anti-Xa and anti-IIa (both AUC0-t and Amax) have however also been provided by the applicant (in an Appendix) and were shortly

presented in the Clinical Overview. Corresponding results for other endpoints were reported on request and altogether these post-hoc provisions of 95% confidence intervals allowed for standard assessment of the data.

The statistical analysis was conducted as planned in the study protocol, except that based on a U-shaped time-curve the analyzed endpoints for the anti-Xa/anti-IIa activity ratio were changed in a post-hoc amendment. This was done as anti-IIa values below LLOQ were observed, which resulted in infinite ratios and AUCs, which rendered the preplanned analysis not possible. Therefore this change was acceptable.

Results

Participant flow

Twenty (20) subjects and two (2) stand-by subjects participated in this study.

Twenty subjects were dosed according to the randomization schedule. The stand-by subjects were released from the clinic and no study medication was administered to them and no blood sample was taken. There were no drop-outs or withdrawal of subjects from the study (please see summary of subject disposition).

Twenty subjects were included in bioequivalence analysis according to the study protocol (please see table 4A and B).

Table 4A. Summary of subject disposition.

Item	Number of Subjects
Recruited	85
Informed consent signed	37
Eligible for the Study	23
Started Period 1	20
Drop-out during Period 1	0
Drop-out between both periods	0
Drop-out during Period 2	0
Completed	20

Table4 B. Summary of subject disposition.

	Sequence		Total
	TR	RT	
Subjects Randomized	10	10	20
Subjects Successfully Completed	10	10	20
Drop-out	0	0	0

T: Enoxaparin Sodium 40

Baseline data

Twenty (20) healthy subjects were included in the study (14 male and 6 female subjects, age 28.8 +-7.9)

Table 5. Baseline data

	All Subjects (N = 20)	Male subjects (N = 14)	Female subjects (N = 6)
Age (± SD)	28.8 (± 7.9)	29.9 (± 8.6)	26.2 (± 5.5)
BMI (± SD)	23.7 (± 3.0)	24.2 (± 2.6)	22.4 (± 3.6)

Numbers analysed

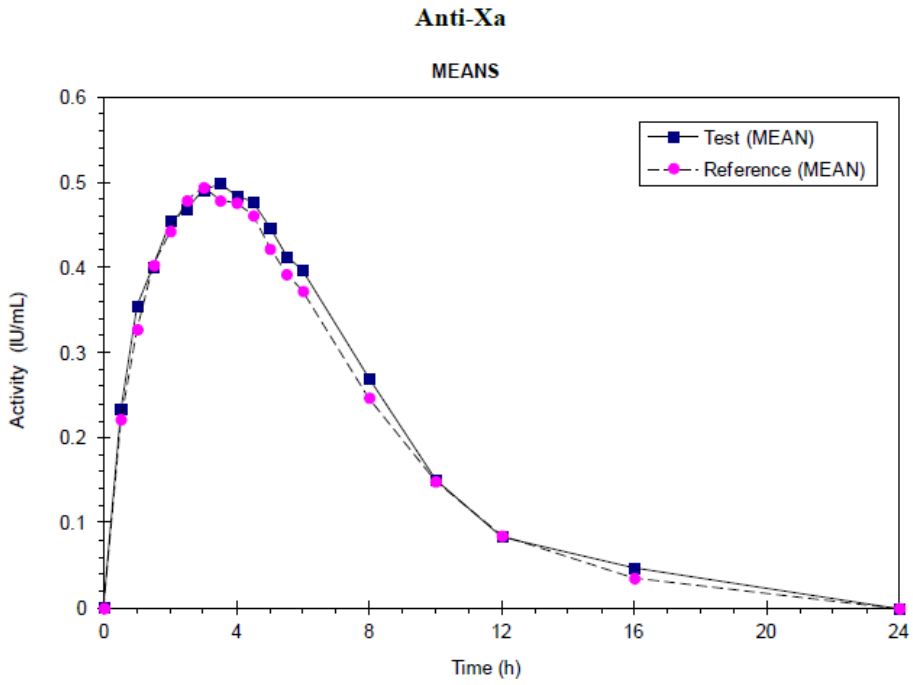
Twenty (20) subjects were included in bioequivalence analysis according to the study protocol.

Results of the primary endpoints

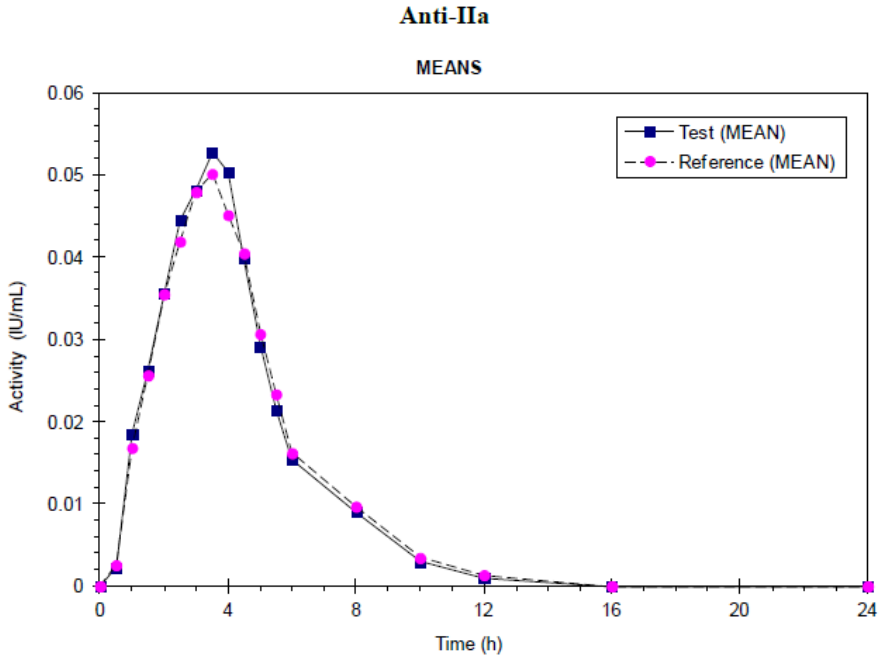
Table. Results of the primary endpoints anti-Xa activity and anti-IIa activity ($AUC_{(0-t)}$ and A_{max}):

Parameter	N	GEOMETRIC LEAST SQUARES MEANS		RATIO T/R (%)	95% CONFIDENCE LIMITS (%)	
		Test	Ref.		Lower	Upper
anti-Xa						
$AUC_{(0-t)}$ (UI·h/mL)	20	3.922	3.709	105.72	97.12	115.09
A_{max} (UI/mL)	20	0.524	0.500	104.79	99.45	110.43
anti-IIa						
$AUC_{(0-t)}$ (UI·h/mL)	20	0.2144	0.2070	103.58	90.34	118.76
A_{max} (UI/mL)	20	0.0563	0.0550	102.24	96.80	107.98

Pharmacokinetic Profile of Means:



Pharmacokinetic Profile of Means:



The 95% Confidence Interval of $AUC_{(0-t)}$ and A_{max} of the anti-Xa activity as well as the anti-IIa activity were well within the limits imposed for this variable (80.00—125.00%).

Based on these results, the test formulation of enoxaparin 40 mg/0.4 mL was considered bioequivalent to the reference formulation (Clexane 40 mg/0.4 mL) with respect to the primary endpoints.

Results of the secondary endpoints

As secondary endpoints TFPI (Tissue Factor Pathway Inhibitor) activity; Minimal and maximal response (Rmin, Rmax) and corresponding times (tmin, tmax) of anti-Xa/anti-IIa activity ratio; AUC(0-∞), AUCres, tmax, t1/2, λz of anti-Xa and anti-IIa have been evaluated with a 90% Confidence Limit and after request also with the more stringent 95% CI, as presented below:

Table 1 **Confidence Intervals**

Ln-transformed Distribution

Parameter	N	GEOMETRIC LEAST SQUARES MEANS		RATIO T/R (%)	95% CONFIDENCE LIMITS (%)		BE
		Test	Ref.		Lower	Upper	
anti-Xa							
$AUC_{(0-t)}$ (UI·h/mL)	20	3.922	3.709	105.72	97.12	115.09	YES
$AUC_{(0-\infty)}$ (UI·h/mL)	20	4.447	4.000	111.17	102.11	121.05	YES
A_{max} (UI/mL)	20	0.524	0.500	104.79	99.45	110.43	YES
anti-IIa							
$AUC_{(0-t)}$ (UI·h/mL)	20	0.2144	0.2070	103.58	90.34	118.76	YES
$AUC_{(0-\infty)}$ (UI·h/mL)	20	0.2434	0.2348	103.67	90.45	118.81	YES
A_{max} (UI/mL)	20	0.0563	0.0550	102.24	96.80	107.98	YES
TFPI							
$AUC_{(0-t)}$ (UI·h/mL)	20	3.17	3.73	85.10	56.46	128.29	NO
$AUC_{(0-\infty)}$ (UI·h/mL)	18	4.21	5.13	82.06	48.35	139.25	NO
A_{max} (UI/mL)	20	0.74	0.81	91.58	73.38	114.28	NO
Ratio (anti-Xa/anti-IIa)							
R_{min} (-)	20	8.035	7.944	101.14	86.22	118.65	YES
R_{max} (-)	20	33.672	32.771	102.75	85.66	123.24	YES

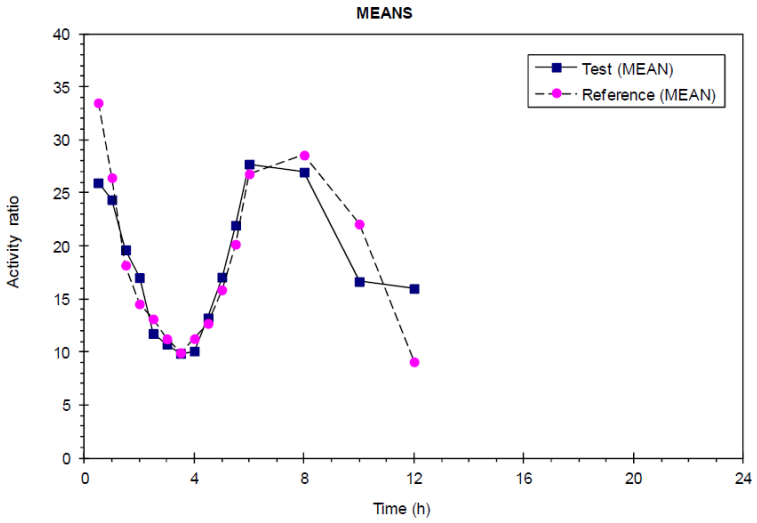
Non-transformed Distribution

Parameter	N	LEAST SQUARES MEANS		RATIO T/R (%)	95% CONFIDENCE LIMITS (%)		BE
		Test	Ref.		Lower	Upper	
anti-Xa							
$t_{1/2}$ (h)	20	3.94	3.27	120.70	89.36	152.04	NO
anti-IIa							
$t_{1/2}$ (h)	20	2.42	2.60	92.96	43.60	142.31	NO
TFPI							
$t_{1/2}$ (h)	18	7.11	9.41	75.53	1.26	149.79	NO

Anti-Xa/anti-IIa activity ratio

The applicant decided to change the analysis of the anti-Xa/anti-IIa activity ratio due to an U-shaped profile, which made it impossible to calculate the originally planned AUC(0-∞), AUCres, tmax, t1/2, λz.

Anti-Xa/anti-IIa – Means of 20 Subjects (linear and semi-logarithmic scale)



Minimal and maximal response (Rmin, Rmax) and corresponding times (tmin, tmax) were therefore presented instead, and ANOVA analysis of ln-transformed Rmin and Rmax were performed with a calculation of a 95% confidence interval through an post-hoc amendment after the last visit of the last patient (See table 1).

Summary Table of Results of Anti-Xa

Parameter	N	TEST		REFERENCE		RATIO T/R (%)
		MEAN	CV (%)	MEAN	CV (%)	
$AUC_{(0-t)}$ (UI·h/mL)	20	4.019	22.60	3.823	25.38	105.13
$\ln(AUC_{(0-t)})$ (UI·h/mL)	20	3.922	16.64	3.709	19.28	105.73
$AUC_{(0-\infty)}$ (UI·h/mL)	20	4.555	22.23	4.118	25.36	110.61
$\ln(AUC_{(0-\infty)})$ (UI·h/mL)	20	4.447	15.24	4.000	17.86	111.17
A_{max} (UI/mL)	20	0.531	16.47	0.509	19.37	104.23
$\ln(A_{max})$ (UI/mL)	20	0.524	-25.77	0.500	-28.66	104.79
t_{max} (h)	20	3.23	23.94	3.03	17.31	106.63
λ_z (h^{-1})	20	0.20858	42.72	0.22869	31.63	91.21
$t_{1/2}$ (h)	20	3.94	49.61	3.27	26.96	120.70

Notes:
- geometric means and ratio of geometric means are presented for ln-transformed parameters (i.e. exp(mean) instead of just mean of ln-transformed data) to enable easy comparisons
- these geometric means are equal to geometric least squares means because this study was balanced (10 RT and 10 TR sequences)

Summary Table of Results of Anti-IIa

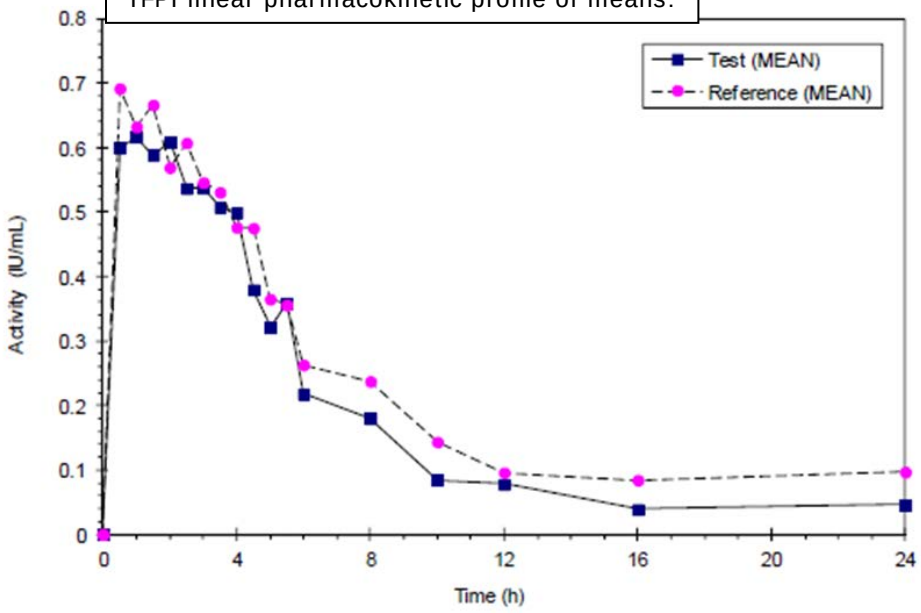
Parameter	N	TEST		REFERENCE		RATIO T/R (%)
		MEAN	CV (%)	MEAN	CV (%)	
$AUC_{(0-t)}$ (UI·h/mL)	20	0.2204	23.55	0.2210	38.96	99.73
$ln(AUC_{(0-t)})$ (UI·h/mL)	20	0.2144	-15.80	0.2070	-23.32	103.58
$AUC_{(0-\infty)}$ (UI·h/mL)	20	0.2497	22.53	0.2511	39.28	99.45
$ln(AUC_{(0-\infty)})$ (UI·h/mL)	20	0.2434	-16.59	0.2348	-25.72	103.67
A_{max} (UI/mL)	20	0.0570	16.25	0.0559	17.62	101.94
$ln(A_{max})$ (UI/mL)	20	0.0563	-5.70	0.0550	-6.34	102.24
t_{max} (h)	20	3.48	17.21	3.48	16.49	100.07
λ_z (h^{-1})	20	0.41983	80.18	0.43052	65.41	97.52
$t_{1/2}$ (h)	20	2.42	48.90	2.60	111.92	92.96

Notes:
- geometric means and ratio of geometric means are presented for ln-transformed parameters (i.e. exp(mean) instead of just mean of ln-transformed data) to enable easy comparisons
- these geometric means are equal to geometric least squares means because this study was balanced (10 RT and 10 TR sequences)

Summary Table of Results of TFPI

Parameter	N	TEST		REFERENCE		RATIO T/R (%)
		MEAN	CV (%)	MEAN	CV (%)	
$AUC_{(0-t)}$ (UI·h/mL)	20	4.10	64.89	5.12	80.70	80.05
$ln(AUC_{(0-t)})$ (UI·h/mL)	20	3.17	68.09	3.73	64.22	85.10
$AUC_{(0-\infty)}$ (UI·h/mL)	18	6.30	92.23	11.98	194.97	52.60
$ln(AUC_{(0-\infty)})$ (UI·h/mL)	18	4.21	67.11	5.65	63.47	74.64
A_{max} (UI/mL)	20	0.87	57.39	0.92	48.89	93.97
$ln(A_{max})$ (UI/mL)	20	0.74	-191.95	0.81	-256.98	91.58
t_{max} (h)	20	2.25	79.89	1.68	75.81	134.58
λ_z (h^{-1})	18	0.40310	107.81	0.30497	120.10	132.18
$t_{1/2}$ (h)	18	7.11	148.55	9.68	204.89	73.44

TFPI linear pharmacokinetic profile of means:



The selection of TFPI activity as well as the ratio of anti-Xa and anti-IIa activity was in line with the EMA *Guideline on Non-Clinical and Clinical Development of Similar Biological Medicinal Products containing Low-Molecular-Weight-Heparins (EMEA/CHMP/BMWP/118264/2007)*.

A discussion on the results of the secondary endpoints also using the more stringent 95% CI was provided by the applicant after request. The secondary endpoints AUC(0-∞) as well as the ratio of anti-Xa/anti-IIa activity demonstrated similarity.

t1/2 of anti Xa, anti IIa activity and all TFPI parameters could not fulfill the biosimilarity criteria. The large variability of the later time points may have affected the estimation of the elimination rate constant, thus the observed difference in t1/2 was not considered highly reliable. Apart from that the absolute difference in t1/2 was 40 minutes for anti-Xa and 11 minutes for anti-IIa activity which was not considered clinically meaningful. It was also noted that the anti Xa activity was below LLOQ at 24h in all subjects for Inhixa and Clexane, thus no accumulation of Inhixa is expected when administered repeatedly in clinical setting.

TFPI activity also failed to demonstrate similarity: the 95% CI for AUC0-t was 56.46 to 128.29, with a mean ratio of 85.10. for AUC(0-∞) 82.06 (48.35-139.25). For Amax the 95% CI was 73.38 to 114.28, with a mean ratio of 91.58. The upper as well as the lower boundary clearly crossed the acceptance margins of 80-125%, except for Amax, where only the lower boundary was crossed.

The applicant argued that TFPI serves only as an indirect parameter of enoxaparin activity (extrinsic pathway, released mainly by the endothelium); the variability of the TFPI parameters observed in the terminal part of the concentration time curve was very high; The sample size calculation was based on the primary parameters; Some level of endogenous activity was observed under physiological conditions; The varied source of TFPI, though not all susceptible to enoxaparin action, added a layer of potential variability in its measurement.

Endogenous TFPI activity exhibits a circadian rhythm (Pinotti et al 2005). The response of TFPI was not uniformly lower for Inhixa, also after Inhixa exposure nearly half of the subjects demonstrated a greater TFPI response. The variability for Clexane was higher than for Inhixa: variability of AUC0-t was 64.89% for Inhixa, while it was 80.70% for Clexane; the standard deviation was 2.66 and 4.13, respectively. For AUC0-∞, the

variability was 92.23% for Inhixa, while it was 194.97% for Clexane; the standard deviation was 5.81 and 23.36, respectively. Amax displayed the least amount of variability among the parameters for TFPI (CVintra of 34.29%), and it displayed the most favourable ratio (91.58%).

Safety Results

No subject experienced any adverse event. No serious adverse event (SAE) occurred. There were no clinically significant changes in the clinical laboratory measurements during the course of the study which could be reasonably associated with the formulations under investigation. All clinical laboratory values were reviewed by the Clinical Investigator and follow-up completed as requested. None of laboratory results deviations was considered clinically significant at the end of the study. For further details please, see Safety section of this AR.

Immunogenicity

No data presented.

Analytical Methods in Study No 411/13

Bioanalytical method and Validation for Factor IIa and Factor Xa

The analytical part of the study lasted from 25.10.2013 (sample receivement) till 02.12.2013; study samples were obtained stored at a nominal temperature of -70ºC.

720 samples from 20 subjects (18 time-points per subject, 2 periods) were analysed, the theoretical amount of samples is 720.

The analytes were coagulation factor IIa and factor Xa.

For both analytes the corresponding Ph. Eur. method was used and adapted regarding the volumes of the samples and reagents. Instead of reading the rate of change of absorbance, the reaction was stopped after 1.5 minutes for Factor IIa and after 2 minutes for factor Xa with sodium citrate and measured afterwards. Full method descriptions were provided.

As Factor IIa reference standard the *WHO International Standard for Low Molecular Weight Heparin* with an activity of 326 IU/ml was used and diluted adequately.

As Factor Xa reference standard the *WHO International Standard for Low Molecular Weight Heparin* with an activity of 1097 IU/ml was used and diluted adequately.

Incurred sample reanalysis for both methods

In order to provide sufficient confidence that the activity of samples being reported is accurate, 10% of study samples were re-analysed in separate runs at different days and the accuracy of factor-IIa activity and factor-Xa activity determination was assessed. Samples around A_{max} and in the elimination phase were selected for re-analysis.

Acceptance criteria

The difference between the two values obtained should be within 30% of the mean for at least 67% of the repeats.

Validation of the analytical methods for Factor II and Factor X

Adequate results obtained from both validations were presented in the dossier. The validations were in compliance with the *EMA "Guideline on bioanalytical method validation" (EMEA/CHMP/EWP/192217/2009)*

Bioanalytical method and Validation for TFPI

Analytical Methods

The analyte was Tissue factor pathway inhibitor.

The analytical method for determination of TFPI is performed as an indirect method via Factor VII and finally an FX-activation determined with a chromogenic reagent.

The method was adequately described in the dossier.

As reference standard TFPI reference plasma with 1 IU/mL is used, the linear range is between 0.02 and 0.2 IU/mL.

Incurred sample reanalysis for both methods

In order to provide sufficient confidence that the activity of samples being reported is accurate, 10% of study samples was re-analysed in separate runs at different days and the accuracy of TFPI activity determination was assessed. Samples around A_{max} and in the elimination phase were selected for re-analysis.

Acceptance criteria

The difference between the two values obtained should be within 30% of the mean for at least 67% of the repeats.

Validation of the analytical method for TFPI *was performed and* was acceptable for its intended purpose.

2.4.4. Discussion on clinical pharmacology

For low-molecular-weight heparin (LMWH) conventional pharmacokinetic (PK) studies cannot be performed. In line with the *EMA Guideline on non-clinical and clinical development of similar biological medicinal products containing low molecular-weight-heparins (EMEA/CHMP/BMWP/118264/2007 Rev. 1, 17 January 2013)* pharmacodynamic (PD) studies using surrogate markers such as anti FXa, anti-FIIa, Tissue Factor Pathway Inhibitor (TFPI) activity, as well as the ratio of anti-FXa and anti-FIIa activity are recommended.

According to this guideline PD studies by both the intravenous and subcutaneous routes are required, if the reference product can also be administered via the intravenous or intra-arterial route, which is here the case. The applicant did not discuss **the omission of an intravenous PD study** in the dossier but this approach was previously accepted during scientific advise (SA) provided by the CHMP. An intravenous study might not have additional value as subcutaneous PD data are regarded more sensitive. Therefore intravenous study was not considered necessary, provided that similarity in the primary endpoints will be shown in the subcutaneous administration as well as a satisfying comparative quality exercise. This approach is also supported by the *EMA Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues (EMEA/CHMP/BMWP/42832/2005 Rev. 1)* where it is written that: "*If the reference product can be administered both intravenously and subcutaneously, the evaluation of subcutaneous administration will usually be sufficient as it covers both absorption and elimination. Thus, it is possible to waive the evaluation of intravenous administration if comparability in both absorption and*

elimination has been demonstrated for the subcutaneous route". This approach was considered acceptable by the CHMP in particular given that the gaps in the knowledge of comparative immunogenicity between Inhixa and Clexane were addressed during the procedure.

Regarding the presented PD study, the **design and selection of the primary and secondary endpoints were appropriate** and in accordance to the *EMA Guideline on non-clinical and clinical development of similar biological medicinal products containing low molecular-weight-heparins (EMEA/CHMP/BMWP/118264/2007)*. The sampling time points were considered appropriate to reflect the absorption, Amax and the elimination period of enoxaparin, represented by surrogate PD parameters.

Biosimilarity was concluded for the primary endpoints AUC(0-t) and Amax as well as the secondary endpoints AUC(0-∞) and the ratio of anti-Xa/anti-IIa activity.

Some of the secondary endpoints (AUC(0-t), AUC(0-∞) and Amax of TFPI (tissue factor pathway inhibitor) activity, T1/2 of anti-Xa, anti-IIa and TFPI activity), which were provided with a 95% CI after request, **could not fulfil the equivalence criteria**. The observed CV was very high. The applicant provided an extensive discussion on the investigations they performed to identify individual subjects, which may have contributed to the large variability of t1/2. Altogether it was argued that the estimation of the terminal parameters was prone to depend on small differences in the data points and this was agreed to. Since TFPI is physiologically available in plasma, a baseline **TFPI activity** was expected in pre-dose plasma samples, so an appropriate baseline correction for each subject and period has been performed by the applicant. The confidence intervals were very large as the CV was rather high. The upper as well as the lower boundary of TFPI AUC0-t and AUC0-∞ crossed the acceptance margins of 80-125%, except for Amax, where only the lower boundary was crossed and also a lower CV of 34% was observed. But also the point estimates (AUC0-t, AUC0-∞) were near the lower borders of the acceptance range. In case of AUC(0-t) the number of patients, in whom the TFPI activity after the administration of the reference product was higher than after the administration of the test product was 12 vs. 8. The mean AUC(0-t) covered less than 80% of AUC(0-∞) for both products, test and reference - baseline corrected. 8 subjects had values of the residual area >20% of the test vs. 7 subjects of the reference product. TFPI activity is highly variable for several reasons discussed above, and the population size was low, based on the expected CV of anti-IIa activity.

Nevertheless, in addition to their effects on factors Xa and IIa, heparins also achieve their **anticoagulant effects via other AT-mediated** (e.g. TAFI, reduced activation of thrombin activatable fibrinolytic inhibitor) **and non AT-mediated** (TFPI, acceleration of heparin cofactor II inhibition) **properties**. PD tests did not correspond to the whole polysaccharid mixture but only to parts of it (e.g. heparin molecules containing fewer than 18 monosaccharides do not catalyze inhibition of thrombin but still inactivate FXa). Similarly, polysaccharides bearing the anti-FXa and anti-FIIa activities, are only representative of about 20% of the overall polysaccharidic mixture in a given LMWH. Furthermore the **anti-FXa assay** is **an *in vitro* test** with plasma sample drawn from the subjects. On the contrary, **TFPI** indicates **the biological activity** in the vascular endothelium. It has already been shown that differences exist between LMWHs regarding TFPI release (Depasse et al., 2003). Therefore **this secondary parameter could add information** about the effect of tested product, especially when an efficacy study is omitted, as in this case. Unfortunately this parameter shows a high CV.

Further analyses and justifications were provided. As differences in TFPI release/ activity could result from structural differences of higher molecular weight saccharide chains, the applicant outlined that **the molecular mass distribution** of Inhixa was comparable to that of the reference product Clexane. The content of saccharide chains of molecular mass ≥ 2,000 Da in Inhixa was well within the range observed for different batches of Clexane, this also pertains to other fractions. In addition, the applicant analysed separately the

molecular weight parameters of Inhixa and Clexane fractions **of high-affinity (HA) and no-affinity (NA) to antithrombin** which showed no significant differences between the two products. Further oligosaccharides presence in Inhixa and Clexane were characterized. Comparative results showed essentially similarity between Inhixa and Clexane.

In addition, a comparative *in vitro* study showed comparable levels of TFPI release from human umbilical vein endothelial cells (HUVEC).

These results did not give base to conclude that there are any marked structural differences between the test and reference products which could affect their biological activity. Overall, investigations in factors which could influence TFPI activity/release showed no significant differences between Inhixa and Clexane.

The applicant decided to **change the analysis of the anti-Xa/anti-IIa activity ratio** due to an U-shaped profile, which made it impossible to calculate the originally planned AUC(0-∞), AUCres, tmax, t1/2, λz. Minimal and maximal response (Rmin, Rmax) and corresponding times (tmin, tmax) were therefore presented instead, and ANOVA analysis of ln-transformed Rmin and Rmax were performed through an post-hoc amendment after the last visit of the last patient. After request, also an analysis using a 95% CI was provided. The applicant explained that the rationale for non-calculation of the pre-planned AUC analysis for the ratio of anti-Xa/anti-IIa activity was that values for anti-IIa activity were below LLOQ. Therefore, the ratio and also the AUC for the ratio resulted in infinity. The applicant justified this approach with an increase of the scientific value of the study without impact on study safety and primary parameters evaluation. With this calculation, **the result supported the equivalence shown with the primary endpoints**. The provided explanation of the reason for this modification was accepted for the non-provision of the planned analysis.

2.4.5. Conclusions on clinical pharmacology

For LMWH conventional PK studies cannot be performed. The PD studies using surrogate markers such as anti FXa, anti-FIIa, tissue factor pathway inhibitor (TFPI) activity, as well as the ratio of anti-FXa and anti-FIIa activity are recommended in the *EMA Guideline on non-clinical and clinical development of similar biological medicinal products containing low molecular-weight-heparins (EMEA/CHMP/BMWP/118264/2007 Rev. 1, 17 January 2013).* The applicant submitted PK/PD study with the design and selection of the primary and secondary endpoints in accordance to this Guideline. The study demonstrated biosimilarity of tested product to Clexane, as the 95% CI of the primary parameters were well within the predefined boundaries, supported by the anti-Xa/anti-IIa activity ratio. Uncertainties regarding some secondary endpoints were sufficiently addressed by the applicant. The CHMP concluded that the clinical pharmacology data submitted as part of the strategy to demonstrate biosimilarity support the applicant's claim.

2.5. Clinical efficacy

No clinical efficacy study has been performed. The clinical similarity program was based on a comparative PK/PD study in healthy volunteers. No efficacy data has been provided, however, the dossier contained thorough discussion regarding publications on clinical efficacy of enoxaparin. This was considered of low relevance for a biosimilar application, since efficacy of the originator has already been demonstrated. Within the initially submitted application, the applicant did not discuss biosimilarity on an efficacy level in detail. However, additional justifications were provided during the procedure regarding the company strategy to demonstrate biosimilarity in particular the omission of the clinical study in this biosimilar application.

2.5.1. Discussion on clinical efficacy

The EMA Guideline on non-clinical and clinical development of similar biological medicinal products containing low-molecular-weight-heparins (EMEA/CHMP/BMWP/118264/2007 + Draft Rev. 1) foresees a clinical study comparing efficacy and safety of the biosimilar candidate and the reference product unless evidence for similar efficacy and safety of the biosimilar and the reference product could be convincingly deduced from the comparison of their physicochemical characteristics, biological activity/potency, using sensitive, orthogonal and state-of-the-art analytical methods, and from comparison of their PD profiles.

During the CHMP Scientific Advice (SA) procedures, the applicant claimed that **PK/PD parameters** such as anti-Xa, anti-IIa and TFPI activities **are more sensitive to detect potential differences** in efficacy than clinical equivalence. This was endorsed by the CHMP since these biomarkers are predictive indicators of the pharmacologic action of LMWH. Furthermore efficacy trials do not seem to have enough sensitivity or statistical power to detect differences in clinical endpoints, since they have never been able to detect differences between different LMWH with evident differences in PK/PD and anti-FXa activity.

Based on the submitted PK/PD study the test product was considered bioequivalent with the reference product with respect to primary PD parameters (anti- Xa and anti-IIa activity) as well as the Anti-Xa/anti-IIa activity ratio, but not secondary parameters TFPI and t1/2 for anti-Xa, anti-IIa activity and TFPI. TFPI parameter showed high variability which hampered a reliable conclusion on similarity regarding this endpoint. Comparative quality results including molecular mass distribution and the content of saccharide chains of molecular mass ≥ 2,000 Da, as well as comparative *in-vitro* TFPI release data sufficiently outweighed the slight uncertainty regarding TFPI activity. It was agreed that, PK or PD markers were more sensitive to detect potential differences in efficacy between two LMWHs than clinical equivalence. The CHMP concluded that bridging to clinical efficacy outcome was therefore acceptable.

2.5.2. Conclusions on clinical efficacy

There was no clinical efficacy study performed to support the biosimilarity claim. It was agreed that potential efficacy study would not be sensitive enough to reveal small differences between two similar enoxaparin-containing-products showing a similar PD profile. From this perspective, a stringent comparative quality documentation supported by a reduced (non-)clinical program was considered appropriate for showing equivalence of efficacy of LMWH. This was confirmed during the scientific advice (SA) procedure provided by the CHMP where it was outlined that "*if appropriately carried out, the comparability exercise at the quality level, including analysis of relevant quality attributes with sufficiently sensitive analytical tools, may allow a reduction of non-clinical and clinical data requirements*". The comparative quality program together with *in vitro* preclinical assays as well as the outcome of the primary endpoints of the clinical PD study provided comprehensive information for characterisation of the biosimilar candidate to conclude similarity regarding efficacy, however not, regarding safety (please, see the Clinical safety section, below).

2.6. Clinical safety

Patient exposure

Twenty (20) healthy volunteers received a single dose of 40 mg enoxaparin s.c. (test and reference product in a cross-over design) in each of 2 study periods with a wash-out period of 8 days in between. All subjects screened were exposed to investigational product in period 01 and period 02 as foreseen. There were no drop outs or withdrawals.

Adverse events

No subject experienced any adverse event. No immunogenicity findings were provided.

Serious adverse event/deaths/other significant events

No serious adverse event (SAE) or death occurred.

Laboratory findings

In the PK/PD study there were a few laboratory results deviations however they were considered clinically insignificant by the Investigator. No laboratory finding was considered to be reasonably associated with the formulations under investigation. Activities of hepatic enzymes were in normal range in all subjects. The platelets count measured at the follow up visit were in normal range in all subjects. All clinical laboratory values were reviewed by the Investigator and follow-up completed as requested.

Safety in special populations

Only healthy volunteers were included in the PK/PD study.

Safety related to drug-drug interactions and other interactions

N/A

Discontinuation due to adverse events

None

2.6.1. Discussion on clinical safety

The safety evaluation was based on a crossover **PK/PD study in healthy subjects** and on **set of bioassays**, submitted with the responses during the procedure.

Overall, patient **exposure was low**. Only 20 subjects were randomized to receive test and reference product in an open label, single dose, two-treatment, two-period, two-sequence, crossover, comparative bioavailability and pharmacodynamic bioequivalence study of enoxaparin sodium 40 vs. reference medicinal product Clexane after subcutaneous administration in healthy volunteers under fasting conditions. There were no adverse events reported in the PK/PD study. In addition laboratory results deviations were considered clinically insignificant as assessed by the investigators. The short treatment duration, and the crossover design made it hard to comparatively assess clinical safety from such a limited dataset. In could be **acceptable to waive a comparative phase 3 study, in case sufficient reassurance concerning comparative safety and immunogenicity** is provided from quality, preclinical, clinical PK/PD and/or an additional clinical safety trial. However, initially, the applicant has provided **no discussion on how their development strategy might allow for extrapolation** of clinical safety data. The initial submission almost **exclusively focused on the safety and efficacy of enoxaparin sodium instead of the comparability between test and reference products**, which should be the actual focus of the dossier.

Within the PK/PD study no treatment related adverse events and consecutively, no discontinuations due to adverse events were noted. From the clinical data presented no differences between test and reference medicinal products could be derived in terms of safety. However, in the light of an abbreviated clinical

comparability exercise (one cross over PK/PD trial), the applicant was asked to present and discuss during the procedure **a strategy that could allow for waiving of clinical safety data**.

In particular**, the lack of an immunogenicity testing strategy** has not been initially addressed in the non-clinical and clinical parts of the dossier. This issue was considered a major concern since a rare but life-threatening immune-driven AE displayed by LMWHs is HITT (heparin induced thrombocytopenia and thrombosis). HITT is caused by formation and binding of antibodies to epitopes on PF4 (platelet factor 4), released from activated platelets, that develop upon formation of complexes with heparin (heparin/LMWH-PF4 complexes; Cines DB, 2007). The assessment of antibody formation was limited by the sample size of the PD trial (20 subjects) as well as the low frequency of immune-mediated events (HITT). In general the prevalence of antibody formation is 1-10%, the risk of HIT in patients treated with enoxaparin was estimated to be 0.2 percent (Martel N, 2005). The development of antibodies to LMWH-PF4-complexes appears to be also influenced by the clinical context of the treatment. Since **no data on the comparability of formation of PF4 antibodies** was available **on clinical level**, **comparative data on *in vitro* level** was considered to be of great interest.

In applicant's responses, a discussion and a new bioassay strategy on immunogenicity was provided. The applicants' discussion claiming biosimilarity for the candidate product was based on **qualitative comparability for a range of assays**, as well as **results of *in-vitro* anti-FXa and anti-FIIa assays and *in-vivo* anti-FXa, anti-FIIa assays** with samples derived from clinical studies, which generally brought no novel insight, concerning comparability of safety and immunogenicity. Regarding the concern for HIT and its published evidence for a link with platelet-activating IgG antibodies recognizing ultralarge complexes (ULC) composed of PF4 bound to heparin the applicant tried to characterize PF4-enoxaparin complexes formed by the reference medicinal product or the biosimilar candidate by SEC. The provided set of bioassays was not able to show its product-specific discriminatory capacity to detect clinically relevant differences. Thus, in view of the CHMP, the results – although not indicating differences – did not provide relevant supportive value. The gaps in the knowledge of comparability in terms of safety remained.

The applicant tried to address further the limitations of performed *in vitro* assays which were highlighted by the CHMP. The efforts undertaken by the applicant were well noticed by the CHMP, although constraints based on the exploratory nature of the testing approaches were experienced. In addition to efforts to underline robustness of *in vitro* based human peripheral blood mononuclear cell (PBMC) assays additional examinations based on TFPI release from HUVEC cells, circular dichroism, SPR (surface plasmon resonance) - based PF4 complex-binding studies and PCS-based particle size determination were submitted. Also these additional examinations were not able to indicate differences regarding antigenicity between test and reference medicinal products.

In light of a reduced clinical development program, out of which no relevant information on the comparability of safety between test and reference medicinal products could be derived, **the enhanced assay strategy** provided by the applicant during the procedure gives reassurance, in the opinion of the CHMP, that the most prominent safety concern associated with LMWHs, HP4 complex binding, is most likely similar between Clexane and Inhixa.

2.6.2. Conclusions on the clinical safety

The presented clinical safety data derived from a comparative PK/PD study were too scarce to conclude on a comparable safety profile of test and reference medicinal products. In particular **immunogenicity** has not been comparatively assessed and initially, the applicant did not present a strategy of *in vitro* and/or *in vivo* assays to allow for waiving of clinical safety data. In light of a reduced clinical development program, out of which no

relevant information on the comparability of safety between test and reference medicinal products could be derived, the enhanced assay strategy provided by the applicant during the procedure gave reassurance that the most prominent safety concern associated with LMWHs, HP4 complex binding is most likely similar between both tested products. In light of established biosimilarity on quality level, the remaining uncertainty that the safety profile of Inhixa and Clexane differs significantly was considered low enough to conclude on similarity.

2.7. Risk Management Plan

The CHMP received the following PRAC Advice on the submitted Risk Management Plan (RMP):

The PRAC considered that the RMP version 1.0 (dated 02 February 2015) could be acceptable if the applicant implements the changes to the RMP as described in the PRAC endorsed PRAC Rapporteur updated assessment report dated 09 July 2015.

The CHMP endorsed this advice.

The applicant implemented the changes in the RMP as requested by PRAC and CHMP.

The CHMP endorsed the RMP version 1.0 (dated 25 July 2016) with the following content:

Table – Summary of the Safety concerns

Important identified risks	Haemorrhages Heparin induced thrombocytopenia (HIT) Anaphylactoid and anaphylactic reactions Liver injury Hyperkalaemia
Important potential risks	Valve thrombosis in patients with prosthetic heart valves Osteoporosis Medication errors
Missing information	Use in paediatric patients Use in patients with hepatic impairment Use during pregnancy Use during lactation Use in obese patients (BMI >30 kg/m^2)

Pharmacovigilance plan

Details of outstanding additional pharmacovigilance activities

Not applicable

Risk Minimisation Measures

Table – Summary table of risk minimisation measures

Safety concern	Routine risk minimization measures	Additional risk minimization measures
Important Identified Risks		
Haemorrhages	SmPC sections 4.3, 4.4, 4.5, 4.8 and 4.9 PIL sections 2 and 4 *Other routine risk minimisation measures:* • Prescription only medicine	None proposed
Heparin induced thrombocytopenia (HIT)	SmPC sections 4.3, 4.4 and 4.8 PIL sections 2 and 4 *Other routine risk minimisation measures:* • Prescription only medicine • Targeted follow-up questionnaire	None proposed
Anaphylactoid and anaphylactic reactions	SmPC sections 4.3 and 4.8 PIL section 4 *Other routine risk minimisation measures:* • Prescription only medicine • Targeted follow-up questionnaire	None proposed
Liver injury	SmPC section 4.8 PIL section 4 *Other routine risk minimisation measures:* • Prescription only medicine	None proposed
Hyperkalaemia	SmPC sections 4.4 and 4.8 PIL section 4 *Other routine risk minimisation measures:* • Prescription only medicine	None proposed
Important Potential Risks		
Valve thrombosis in patients with prosthetic valves	SmPC sections 4.4and 4.6 PIL sections 2 and 4 *Other routine risk minimisation measures:* • Prescription only medicine	None proposed
Osteoporosis	SmPC section 4.8	None proposed

	PIL section 4 *Other routine risk minimisation measures:* • Prescription only medicine	
Medication errors	SmPC sections 2 and 4.2 PIL sections 3 and 6 *Other routine risk minimisation measures:* • Prescription only medicine	None proposed
Missing Information		
Use in paediatric patients	SmPC section 4.2 *Other routine risk minimisation measures:* • Prescription only medicine	None proposed
Use in patients with hepatic impairment	SmPC section 4.2 *Other routine risk minimisation measures:* • Prescription only medicine	None proposed
Use during pregnancy	SmPC sections 4.4, 4.62 and 5.3 PIL section 2 *Other routine risk minimisation measures:* • Prescription only medicine	None proposed
Use during lactation	SmPC section 4.6 PIL section 2 *Other routine risk minimisation measures:* • Prescription only medicine	None proposed
Use in obese patients (BMI > 30kg/m^2)	SmPC section 4.4 PIL section 2 *Other routine risk minimisation measures:* • Prescription only medicine	None proposed

Conclusion

The CHMP and PRAC considered that the RMP version 1.0 (dated 25 July 2016) is acceptable.

The MAH was reminded that, within 30 calendar days of the receipt of the Opinion, an updated version of Annex I of the RMP template, reflecting the final RMP agreed at the time of the Opinion should be submitted to h-eurmp-evinterface@emea.europa.eu.

2.8. Pharmacovigilance

Pharmacovigilance system

The CHMP considered that the pharmacovigilance system summary submitted by the applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

2.9. New Active Substance

The CHMP, based on the available data, considers that enoxaparin sodium is not a new active substance, as it is a constituent of a medicinal product previously authorised within the European Union. Enoxaparin sodium is contained in the marketing authorisation Clexane which was authorised in the Union on 22 October 1990.

2.10. Product information

The MAH has the obligation to ensure that their product information is kept up-to-date as stated in Article 3(b) of Regulation (EC) No 726/2004.

2.10.1. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

2.10.2. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, Inhixa (enoxaparin sodium) is included in the additional monitoring list as it is a biological product that is not covered by the previous category and authorised after 1 January 2011.

Therefore the summary of product characteristics and the package leaflet includes a statement that this medicinal product is subject to additional monitoring and that this will allow quick identification of new safety information. The statement is preceded by an inverted equilateral black triangle.

3. Benefit-Risk Balance

The benefits and risks of a biosimilar medicinal product need to be evaluated from a different perspective as done for a new medicinal product, where the benefits of treating a certain disease are balanced against the risks which this new treatment may bring about. In the case of a biosimilar medicinal product the beneficial and adverse effects of the reference product have previously been demonstrated and are now sought to be extrapolated to the biosimilar product, but need not be replicated. Therefore, benefits and risks in the context of a biosimilar approach are discussed from the view of successful or failed confirmation of comparability between the test and the reference products.

Benefits

Beneficial effects

The pharmaceutical form of biosimilar enoxaparin sodium, Inhixa is identical to the pharmaceutical form of the reference medicinal product (RMP), Clexane (solution for injection in ready to use pre filled syringe). The establishment of the formulation has been based on the listed product information of the RMP.

Enoxaparin sodium has a well-established efficacy, safety and tolerability profile. The claimed indications and the dose recommendations are similar to the indications of the RMP.

From a **quality point of view**, it was considered that similarity between the biosimilar and the RMP was sufficiently showed. The performed comparability studies encompassed comparison of heparin source material and mode of depolymerisation, physico-chemical properties, structural comparisons (similarity of oligosaccharides sequence, similarity of oligosaccharide fragments, similarity of disaccharide building blocks and similarity of high/non-affinity components), in-vitro biological (clotting tests such as activated partial thromboplastin time [aPTT] and HEPTEST) and biochemical activity (inhibition of coagulation factors Xa [anti-FXa] and IIa [anti-FIIa]). The provided data were considered sufficient to demonstrate that both products are essentially biosimilar.

From **the preclinical perspective**, similarity between the biosimilar and the RMP was demonstrated with regards to *in vitro* bioassays on primary pharmacology. Comparative *in vitro* tests assessing the inhibition of coagulation factors Xa (anti-FXa) and IIa (anti-IIa), and clotting tests such as activated partial thromboplastin time (aPTT) and Heptest were applied and demonstrated biosimilarity between the test and reference medicinal products.

From **the clinical perspective** similarity was demonstrated in the single clinical study (comparative PK/PD Study No 411/13) for all primary endpoints (AUC0-t and Amax of anti Xa and anti IIa activity) as well as the anti-Xa/anti-IIa activity ratio. The PD markers were considered valid for pharmacokinetic and pharmacological action of enoxaparin sodium. The point estimates as well as the predefined 90% CIs (and the additional analysis with the 95% CI) were well within the 80-125% acceptance range.

Uncertainty in the knowledge about the beneficial effects

From **the quality point of view**, the chosen quality approach does sufficiently demonstrate that the test and the reference products are essentially biosimilar.

From **the preclinical perspective**, the absence of non-clinical *in vivo* studies was not of concern per se as relevant PD endpoints (anti-FXa, anti-FIIa and TFPI) were tested in the PK/PD clinical trial.

Some of the **secondary endpoints** in the comparative **PK/PD clinical trial** (AUC(0-t), AUC(0-∞) and Amax of TFPI (tissue factor pathway inhibitor) activity, T1/2 of anti-Xa, anti-IIa and TFPI activity), which were provided

on request with a 95% CI, could not fulfil the equivalence criteria. TFPI indicate the biological activity in the vascular endothelium and it has already been shown that differences exist between LMWHs regarding TFPI release. Therefore this secondary parameter could add additional information about the effect of the tested product. Unfortunately TFPI parameters showed a high variability and the study sample size was low, based on the expected coefficient of variation (CV) of anti-IIa activity. Therefore reliable assessment of the results for this parameter was not possible. Comparative quality results including molecular mass distribution and the content of saccharide chains of molecular mass $\geqslant$ 2,000 Da, as well as comparative *in vitro* TFPI release data sufficiently outweigh the slight uncertainty regarding TFPI activity. The clinical relevance of the not fulfilment of the equivalence criteria for T1/2 of anti-Xa, anti-IIa and TFPI activity was considered minor, given that the low absolute difference in T1/2 was 40 minutes for anti-Xa and 11 minutes for anti-IIa activities. Furthermore the large variability resulted in big differences when calculating the elimination rate constant, which hampered a solid comparative assessment.

Risks

Unfavourable effects

It was expected that unfavourable effects are similar to those of the reference product provided that the biosimilarity was established. Enoxaparin sodium is associated with the risk of bleeding which could be fatal. Other adverse reactions include thrombocytopenia (including immuno-allergic thrombocytopenia), allergic reactions (including anaphylactic /anaphylactoid reaction), hyperkaliemia, injection site reactions, hepatic enzymes increase, hepatitis, skin and subcutaneous disorders (urticaria, pruritus, erythema bullous dermatitis) and osteoporosis following long-term therapy.

Essential biosimilarity of Inhixa and the reference product Clexane was sufficiently demonstrated. The most prominent safety concern associated with LMWHs, heparin induced thrombocytopenia and thrombosis (HITT) is most likely similar between both products. HITT is caused by formation and binding of antibodies to epitopes on PF4 (platelet factor 4), released from activated platelets, that develop upon formation of complexes with heparin (heparin/LMWH-PF4 complexes). No additional risk regarding unfavourable effects as compared to the RMP were identified.

Uncertainty in the knowledge about the unfavourable effects

The comparative PK/PD trial, provided only limited safety data, since the short treatment duration, and the crossover design make it hard to comparatively assess clinical safety.

Initially the **lack of an immunogenicity testing strategy** has not been addressed in the non-clinical and clinical parts of the dossier. The assessment of antibody formation was **limited by the sample size** of the PD trial (20 subjects) as well as the **low frequency of immune-mediated events** (HITT). The development of antibodies to LMWH-PF4-complexes appears to be also influenced by the clinical context of the treatment. Since no data on the comparability of formation of PF4 antibodies was available on clinical level, comparative data on *in vitro* level were considered. Also these additional examinations were not able to indicate differences regarding antigenicity between test and the RMP.

Benefit-risk balance

This is a biosimilar application. Positive benefit-risk balance was earlier demonstrated for the RMP Clexane. Given the nature of current application the confirmation of the positive benefit-risk balance focused on demonstrating similarity to the RMP throughout the development program and could not be outbalanced by other factors. A thorough discussion of the overall evaluation of biosimilarity is provided below.

Discussion on the benefit-risk assessment

From the quality point of view, the approach chosen sufficiently demonstrates that Inhixa and Clexane are essentially biosimilar. The performed comparability studies involved comparisons of broad physico-chemical properties, degree of sulfation and sodium content of the oligosaccharide chains, molecular weight determinations using HP-SEC with Triple Detector Arrays (HP-SEC/TDA), assessment of various monosaccharides that form an integral part of the disaccharide building blocks in enoxaparin chain including assessment of residues at both reducing and non-reducing ends, qualitative and quantitative comparisons of various saccharides following enzymatic digestions with heparinases. Equivalence in the heparin intermediate and its respective mode of depolymerisation was also carried out.

In addition, comparative assessment of Antithrombin III (AT-III) affinity chromatography derived High Affinity (HA) and No Affinity (NA) oligosaccharide fractions was also carried out. HA fractions isolated from both test and reference medicinal products have been compared in terms of decasaccharides. An in-depth comparison of tetrasaccharides, as well as NMR, heparinase mixture digestion, heparinase I digestion, heparinase II digestion, heparinase III digestion and chain mapping of isolated hexasaccharides, octasaccharides and decasaccharides from both test and reference medicinal products have been also carried out. Quantification of impurity profiles was as well part of the biosimilarity exercise.

A number of *in vitro* tests that assess inhibition of coagulation factors Xa (anti-FXa) and IIa (anti-FIIa), and clotting tests such as activated partial thromboplastin time (aPTT) and Heptest have also been compared between Inhixa and Clexane formulations. The major objection that has been raised during the procedure which questioned several aspects (e.g. differences in the link region and its safety implications) but the provided data were sufficient to demonstrate that both products are essentially biosimilar.

The pre-clinical and clinical requirements related to the development of biosimilar LMWHs are outlined in the *Guideline on biosimilar low molecular weight heparins (EMEA/CHMP/BMWP/118264/2007 Rev. 1).* This Guideline foresees the situation where a clinical program consisting only of a single PK/PD study could be acceptable. It states that: *"Only if similar efficacy of the biosimilar and the reference product can be convincingly deduced from the comparison of their physicochemical characteristics, biological activity/potency and PD fingerprint profiles, based on the use of highly sensitive and specific methods, then a dedicated efficacy trial may be waived. It is expected that this is an exceptional scenario since the required amount of reassurance from analytical data and bioassays would be considerable*."

This approach was also confirmed in the context of the scientific advice (SA) procedure provided by the CHMP. In terms of clinical efficacy, the applicant provided relevant assays on pre-clinical level (inhibition of coagulation factors Xa [anti-FXa] and IIa [anti-FIIa], and clotting tests such as activated partial thromboplastin time [aPTT] and HEPTEST). The applicant had furthermore demonstrated PK/PD comparability in the predefined co-primary endpoints (anti-Xa and anti-IIa activity) which were considered relevant in terms of pharmacological action of enoxaparin sodium (which is one of the prerequisites to waive an efficacy trial according to the *Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues (EMEA/CHMP/BMWP/42832/2005 Rev1)*. TFPI parameters showed high variability which hampered a reliable conclusion on similarity regarding these endpoints. Comparative quality results as well as comparative *in vitro* TFPI release data sufficiently outweigh the slight uncertainty regarding TFPI activity observed in the clinical PK/PD study.

It was hence accepted, that the applicant's approach with a single clinical trial was sufficient to conclude on similar efficacy. The initially submitted dossier lacked a certain bridging strategy that would allow for waiving of a confirmative phase 3 clinical study or in this case, a dedicated safety study. The applicant initially did not

provide sufficient discussion on how bridging data on quality, pre-clinical, PK/PD level on the one side and comparative safety and immunogenicity level on the other side, could be established. The comparative PK/PD trial, performed by the applicant provided only limited safety data, since the short treatment duration, and the crossover design made it hard to comparatively assess clinical safety. Consecutively, within this study no treatment related adverse events and no discontinuations due to adverse events were noted. Regarding clinical safety the *Guideline on biosimilar low molecular weight heparins (EMEA/CHMP/BMWP/118264/2007 Rev. 1)* states that "*Human safety data on the biosimilar will usually be needed pre-authorisation, even if similar efficacy can be concluded from the comparative data on physicochemical characteristics, biological activity/potency and PD fingerprint."* and "*Sufficient reassurance will be needed that the biosimilar LMWH is not associated with excessive immunogenicity compared to the reference product."*

Particularly the lack of any strategy to comparatively assess immunogenicity was considered a major concern, especially in the light of a rare but life-threatening immune-driven AE displayed by LMWHs i.e. HITT (heparin induced thrombocytopenia and thrombosis). This is a condition caused by formation and binding of antibodies to epitopes on PF4 (platelet factor 4), released from activated platelets, that develop upon formation of complexes with heparin (heparin/LMWH-PF4 complexes).

Since no data on the comparability of formation of HP4 (Heparin Platelet Factor 4) antibodies were available on the clinical level, comparative *in vitro* data on were considered of interest, since due to the low incidence of HITT, the conduct of a comparative clinical safety study was considered insensitive and unfeasible. However, the bioassays provided together with the initial responses were not judged to be sensitive to allow for the detection of potential differences between biosimilar candidate and originator. Further responses provided by the applicant addressed the limitations of performed *in vitro* assays which were highlighted by CHMP. Some constraints based on the exploratory nature of the testing approaches were experienced. Nevertheless, besides the efforts to underline robustness of the *in vitro* based human PBMC assays additional examinations based on TFPI release from HUVEC cells, circular dichroism, SPR based PF4 complex-binding studies and PCS - based particle size determination were submitted. Also these additional examinations were not able to indicate differences regarding antigenicity between test and reference compound. In light of a reduced clinical development program, the enhanced assay strategy provided reassurance that the remaining uncertainties regarding the safety profile are small enough to conclude that the safety profile of test and reference medicinal products are comparable.

Based on the totality of evidence, the established biosimilarity on quality level, together with the presented clinical data derived from a comparative PK/PD trial complemented by a set of *in vitro* assays the CHMP concluded on similar clinical safety profile between Inhixa and Clexane.

Conclusions

The overall benefit-risk balance of Inhixa is positive.

4. Recommendations

Outcome

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by consensus that the risk-benefit balance of Inhixa is favourable in the following indication:

Inhixa is indicated for adults for:

- Prophylaxis of venous thromboembolism, particularly in patients undergoing orthopaedic, general or oncological surgery.
- Prophylaxis of venous thromboembolism in patients bedridden due to acute illnesses including acute heart failure, acute respiratory failure, severe infections, as well as exacerbation of rheumatic diseases causing immobilisation of the patient (applies to strengths of 40 mg/0.4 mL).
- Treatment of deep vein thrombosis (DVT), complicated or uncomplicated by pulmonary embolism.
- Treatment of unstable angina and non-Q-wave myocardial infarction, in combination with acetylsalicylic acid (ASA).
- Treatment of acute ST-segment Elevation Myocardial Infarction (STEMI) including patients who will be treated conservatively or who will later undergo percutaneous coronary angioplasty (applies to strengths of 60 mg/0.6 mL, 80 mg/0.8 mL, and 100 mg/1 mL).
- Blood clot prevention in the extracorporeal circulation during haemodialysis.

The CHMP therefore recommends the granting of the marketing authorisation subject to the following conditions:

Other conditions or restrictions regarding supply and use

Medicinal product subject to medical prescription.

Conditions and requirements of the marketing authorisation

Periodic Safety Update Reports

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

The marketing authorisation holder shall submit the first periodic safety update report for this product within 6 months following authorisation.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

Risk Management Plan (RMP)

The MAH shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the marketing authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

Conditions or restrictions with regard to the safe and effective use of the medicinal product to be implemented by the Member States.

Not applicable.

These conditions fully reflect the advice received from the PRAC.