Guideline on the regulation of unfractionated heparin product for human use in Egypt
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1. Introduction

Heparin is one of the most widely used medicines in the world, it has been described as a lifesaving as it plays a vital role in many serious conditions as kidney dialysis, surgery, cardiac-invasive, heart attack, cardiac arrhythmia, acute coronary syndrome, pulmonary embolism, stroke, deep vein thrombosis, blood clot prevention, and many other related conditions.

Unfractionated Heparin products are preparations that contain sodium or calcium salt of sulfated glycosaminoglycan, which are present as a heterogenous mixture of molecules with varying molecular weight in mammalian tissues (porcine & bovine) intestinal mucosa or other suitable tissues of domestic mammals used for food by humans, (UFH) is a fast-acting blood thinner that works together with antithrombin to block clot formation as it binds to antithrombin and enhances its ability to inhibit two of the body's most potent clotting factors (factor X_a and factor II_a).

UFH is a highly sulphated and heterogeneous member of the glycosaminoglycan family of carbohydrates consisting of various disaccharide units. The most common disaccharide unit is composed of α- D-glucosamine and uronic acid (α- L-iduronic acid or β- D-glucuronic acid) joined by glycosidic linkages, it possesses the highest negative charge density of all known biological molecules.

2. Scope

This guideline addresses the marketing authorization registration requirement of unfractionated heparins drug substance and drug product. Regardless of the origin of tissues either bovine or porcine origin to ensure safety, quality or efficacy of UFH products to be used in humans, these requirements cover:

- Control of starting materials
- Control of manufacturing process
- Control of drug substance and Drug product

-The principles described in this document apply to UFH of both bovine & porcine origins providing the recommended specifications that ensure minimum requirement of safety, quality and efficacy merged from different updated international pharmacopeias.

3. Definitions

1. **Crude heparin**: unpurified, unfractionated heparin (UFH) material which contains some impurities including peptides/ protein, lipids, oligonucleotides, and other non-heparin polysaccharides (e.g., heparan sulfate, dermatan sulfate, chondroitin sulfate) that requires further purification and processing before clinical use.

2. **Raw materials**: Substances such as reagents, culture media, fetal calf serum, additives, and buffers involved in chromatography, etc. used in the manufacturing or extraction of the active substance, but from which this active substance is not directly derived
3. **primary chemical reference substance**: A designated primary chemical reference substance is one that is widely acknowledged to have the appropriate qualities within a specified context, and whose assigned content when used as an assay standard is accepted without requiring comparison with another chemical substance.
4. **Secondary chemical reference substance**: a substance whose characteristics are assigned and/or calibrated by comparison with a primary chemical reference substance. The extent of characterization and testing of a secondary chemical reference substance may be less than for a primary chemical reference substance.
5. **Starting materials**: Any substance of biological origin such as micro-organisms, organs and tissues of either plant or animal origin, cells or fluids (including blood or plasma) of human or animal origin, and biotechnological cell constructs (cell substrates, whether they are recombinant or not, including primary cells)
6. **Process intermediate**: is a substance produced during the active substance's processing steps that undergo further molecular change or purification before becoming the active substance
7. **Source:** the supplier from which the starting material or intermediate is supplied. One source could include multiple slaughterhouses which are under the same pharmaceutical quality control system of the medicinal product manufacturer
8. **Applicant:** The Company requesting a Marketing Authorization for a medicinal product.
9. **OSCS impurities**: Over Sulfated Chondroitin Sulfate is a contaminant in heparin associated with adverse clinical events
10. **Certification of Suitability (CEP):** is a certificate that certifies compliance of the active pharmaceutical ingredients (API) or pharmaceutical ingredients with that of the rules laid down in the monograph of the European Pharmacopoeia (EP

4. Procedures

4.1 Control of starting materials

- Pooled intestinal mucosae either bovine or porcine are defined as the starting material for heparin. According to good manufacturing practice (GMP), the following steps should be considered before starting the manufacture of heparin:

1) Animal control

-The supply chain should be controlled with having farms that can supply animals (cows or pigs) (preferably closed herds) to slaughterhouses.

-All stages of production and sourcing are subject to a quality management system.

- Aspects with potential impact on product quality and safety need to be presented in sufficient details e.g., species and country of origin.

-The animals from which heparin is derived must meet the health requirements of animals suitable for human consumption following pre and post mortem inspection.
-Traceability of the animals back to the farm of origin should be documented.
-Animals should be inspected by health officials and veterinary certificates should be provided. --- The applicant must demonstrate the compliance of the starting materials (intestinal mucosa either bovine or porcine) with the note for Guidance on minimizing the risk of Transmitting Animal Spongiform Encephalopathy Agents via Medicinal Products (TSE certificate).

2) Slaughterhouses

-Slaughterhouses should be dedicated to cows or pigs only. No other animals should be used in the same area so that the mucosa obtained from the animals cannot be mixed or contaminated with ruminants or tissues from animals of different species.
-These houses should be inspected by health officials (Vets) and veterinary certificates should be provided)

3) Mucosa extraction facilities

The extraction process should be performed by trained personnel using proper equipment, hygiene, clothes, etc. (personal qualifications and safety precautions and any certificate needed)

4) Storage and transportation of the mucosa

-The collected mucosa should be stored in appropriate containers with the addition of preservatives, usually sodium bisulfite, before shipping/transportation to the manufacturing facility. Which has a role in stabilizing the mucosa, limits microbial growth, and stops the intrinsic enzymatic digestion which liberates polysaccharide chains from proteoglycan complexes.
-Storing mucosa for an extended period may also result in the de-sulfation process due to natural enzymatic/ chemical reactions within the mucosa.

5) Mucosa arrival to the manufacturing site

Mucosa is usually transported by trucks to the manufacturing facility where it is pumped directly into reaction tanks/vessels where crude heparin is produced.

It is recommended for heparin manufacturer to test and confirm the species origin of crude heparin before use in manufacturing, the most common tests:

1) PCR for the identification of heparin animal species (porcine or bovine) and absence of any other species.
2) Biological activity to show anti-Xa activity, which is usually less than the purified API heparin due to the presence of impurities in the crude material (e.g., nucleotides, Proteins/peptides, dermatan sulfate, chondroitin sulfate).

3) NMR to confirm the basic features of heparin as a polysaccharide sulfated complex containing the main major repeating units of N-sulfo/N-acetylglucosamine linked 1 to 4 to uronic acid.
4) Test for OSCS in crude heparin in each lot of every shipment before use manufacturing process.

4.2 Control of manufacturing process

A combination of physicochemical-biological testing together with testing and control of the manufacturing process is needed for heparin characterization and determination of quality due to its complexity. Information about the manufacturing process, starting from the sourcing of the starting material (e.g., mucosa) should be given with sufficient information depending on the stage of the process, with a focus on critical quality attributes and critical process parameters and traceability of supply.

4.2.1 Drug substance

4.2.1.1 General Information

- **Nomenclature**
  Information on the nomenclature of the drug substance should be provided. For example
  -Recommended International Nonproprietary Name (INN)
  -Compendial name if relevant
  -Chemical name(s)
  - Company or laboratory code
  Other non-proprietary name(s), e.g., national name, United States Adopted Name (USAN), Japanese Accepted Name (JAN); British Approved Name (BAN
  Chemical Abstracts Service (CAS) registry number.

- **Structure**
  -Information on the structure should be provided including, structural formula (relative and absolute stereochemistry), molecular formula and the relative molecular mass.
- **General Properties**
  - A list of physicochemical properties and biological activity should be provided.

4.2.1.2 Manufacture

- **Manufacturer(s)**

-Information on the name, address, and responsibility of each manufacturer, including contractors, valid GMP certificate.

- **Description of Manufacturing Process and Process Controls**

-Narrative description of the manufacturing process including, quantities of raw materials, operating parameters (temperature, pressure, stirring rate, time and pH).

-In process control testing e.g. (sterility, bioburden, etc.) that when controlled result in consistent production of material(s) of appropriate quality.

-Flow diagram of the process including the purification steps, production batch size, reprocessing steps should be identified and justified,

-Batch numbering system.

- Relevant information for each stage (e.g., volumes, pH, critical processing time, holding times, temperatures, elution profiles, selection of fraction, storage of intermediate, if applicable).

- Information on filling process and shipping conditions for drug substance.

- **Control of Materials**

Materials used in the manufacture of the drug substance (e.g., raw materials, starting materials, solvents, reagents, catalysts) should be listed identifying where each material is used in the process. Information on the quality and control of these materials should be provided

- **Control of Critical Steps and Intermediates**

-Critical Steps: Tests and acceptance criteria (with justification including experimental data) performed at critical steps identified in 3.2.S.2.2 of the manufacturing process to ensure that the process is controlled should be provided.

Intermediates: Information on the quality and control of intermediates isolated during the process should be provided

- **Process Validation and/or Evaluation**

- Sufficient information should be provided on validation and evaluation studies to ensure process consistency and suitability for the intended use including, the validation plans, validation reports and conclusions from the study.

-The heparin manufacturing process should be validated to show that relevant infectious and adventitious agents are cleared and inactivated (e.g., viruses, TSE agents) and also the process validated for lipid clearance.

- **Manufacturing Process Development**

- A description and discussion of the significant changes made to the manufacturing process should be also provided. For example: changes to the process or to critical equipment and the reason for the change and its impact on the drug substance quality should be explained

4.2.1.3 Characterization

- **Elucidation of structure and other characteristics**

-The structure and other characteristics of UFH can be elucidated using different suitable analytical methods to verify qualitative and quantitative analysis of amino sugars, di saccharide composition, free anions and combined sulfate analysis, molecular weight distribution.

-The structure verification can be performed by ion chromatography, NMR and IR or other suitable characterization methods.

-A valid biological assay to measure the biological activity should be provided by the manufacturer.

- **Impurities**

Information on impurities should be specified. Impurities can be classified into the following categories, organic impurities (process- and drug-related), inorganic impurities and residual solvents.

The manufacturer is recommended to follow reference ICH Guidelines: Q3A, Q3C, Q5C, Q6A, and Q6B and the below impurities specifications:

- **Limit of galactosamine in total hexosamine**: The percent galactosamine peak area of the total hexosamine of the Hydrolyzed sample solution must be NMT 1%.
- **Nucleotide impurities**: The absorbance measured at 260 nm is NMT 0.15%
- **Protein**: Maximum 0.5 %
- **Related dermatan sulfate and chondroitin sulfate substances**: Sum of dermatan sulfate and chondroitin sulphate is not more than the area of the corresponding peak in the chromatogram obtained with reference solution (2%).
- **Any other impurities (Over sulfated chondroitin sulfate):** No peak other than the peak due to dermatan sulphate +chondroitin sulphate are detected

4.2.1.4 Control of drug substance

- **Specification**
- **Analytical Procedures**

-Relevant & validated methods should be utilized to control quality attributes, including but not limited to the following:

1) **<u>Physicochemical Characters</u>**
   - Appearance
   - Solubility
   - loss on drying

2) **Identification**
   - Anti-factor Xa to anti-factor Ⅱa ratio (Complies with requirements under assay)
   - Molecular weight determinations
   - Chromatographic identity
   - H NMR spectroscopy

3) **Impurities:**
   - Nucleotide impurities
   - Protein impurities
   - Related dermatan sulfate and chondroitin sulfate substances
   - Absence of over sulfated chondroitin sulfate
   - Limit of galactosamine in total hexosamine
   - Residue on Ignition

4) **Microbial contamination**
   - Total aerobic bacteria count
   - Total mold and yeast count
   - Escherichia coli
   - Salmonella

5) **Bacterial endotoxin**
6) **Nitrogen**
7) **PH**
8) **Calcium**
9) **Sodium**
10) **Biological assay**

-Chromogenic assay of anti-factor II a in case of porcine origin

-Chromogenic assay of anti-factor IIa or plasma sheep clotting assay in case of bovine origin

-The manufacture should provide the justification & supportive data for the set specifications (Q6B)

- **Validation of Analytical Procedures**
- **Batch Analyses**
- **Justification of Specification(s)**

4.2.1.5 Reference Standards or Materials

-When a reference standard has been used for any of the assays, characterization data should be provided

-International standards should be used, when available, for establishing the in-house reference standard.

4.2.1.6 Container Closure System

A description of the container closure system(s) should be provided, including:

- The identity of materials of construction of each primary packaging component, and their specifications. The specifications should include description and identification (and critical dimensions with drawings, where appropriate). Non-compendial methods (with validation) should be included, where appropriate.
- For non-functional secondary packaging components (e.g., those that do not provide additional protection), only a brief description should be provided.
- For functional secondary packaging components, additional information should be provided. The suitability should be discussed with respect to, for example, choice of materials, protection from moisture and light, compatibility of the materials of construction with the drug substance, including sorption to container and leaching, and/or safety of materials of construction.

4.2.1.7 Stability

- **Stability Summary and Conclusions**

-The types of stability studies conducted, protocols used, and the results of the studies should be summarized.

-The summary should include conclusions with respect to storage conditions and shelf-life, in-use storage conditions and shelf-life should be provided.

- **Post-approval Stability Protocol and Stability**

-The post-approval stability protocol and stability commitment should be presented.

- **Stability Data**

Results of the stability studies should be presented in an appropriate format (e.g., tabular, graphical, narrative). Information on the analytical procedures used to generate the data and validation of these procedures.

4.2.2 Drug product

4.2.2.1 Description and Composition of the Drug Product

A description of the drug product, description of the dosage form and its composition should be provided, their amount on a per unit basis (including overages, if any), the function of the components, and a reference to their quality standards (e.g., compendial monographs or manufacturer's specifications).

4.2.2.2 Pharmaceutical Development

-The development studies conducted to establish that the dosage form, the formulation and manufacturing process, container closure system, microbiological attributes, usage instructions.

-Identification and description of the formulation and process attributes (critical parameters) that can influence batch reproducibility, product performance and drug product quality.

-Supportive data and results from specific studies or published literature.

-Additional supportive data can be referenced to the relative non-clinical or clinical sections.

- **Components of the drug product**

-Discussion of the compatibility of the drug substance with excipients.

-Discussion of key physicochemical characteristics of the drug substance that can influence the performance of the drug product.

-The choice of excipients in the formulation their concentration, their characteristics that can influence the drug product performance should be discussed relative to their respective functions.

- **Formulation Development**

-Summary for the development of the drug product including the proposed route of administration and usage

-Differences between clinical formulations and the commercial formulation in formulation.

-Results from comparative in vitro studies or comparative in vivo studies should be discussed when appropriate.

- **Overages**

Justification of any overages in the formulation.

- **Physicochemical and biological properties**

Parameters relevant to the performance of the drug product, such as pH, ionic strength, biological activity or potency, and/or immunological activity

- **Manufacturing process development**

-The selection and optimization of the manufacturing process (critical aspects).

-Method of sterilization should be explained and justified.

-Differences between the manufacturing processes used to produce pivotal clinical batches and commercial batches that can influence the performance of the product.

- **Container closure system**

The suitability of the container closure system used for the storage, transportation and use of the drug product considering choice of materials, protection from moisture and light, compatibility of the materials with the dosage form (sorption and leaching).

- **Microbiological attributes**

Microbiological attributes of the dosage form (the rationale for not performing microbial limits testing for non-sterile products, selection and effectiveness of preservative systems in products containing antimicrobial preservatives, the integrity of the container closure system to prevent microbial contamination in case of sterile product)

- **Compatibility**

The compatibility of the drug product with reconstitution diluent(s) or dosage devices (e.g., precipitation of drug substance in solution, sorption on injection vessels, stability)

4.2.2.3 Manufacture

- **Manufacturer**

Information on the manufacturer includes the name, address and responsibilities of each manufacturer; each proposed production site or facility involved in the manufacturing and testing should be provided also GMP certificate should be provided.

- **Batch formula**

- A batch formula should be provided that includes a list of all components of the dosage form to be used in the manufacturing process, their amounts on a per batch basis, including overages, and a reference to their quality standards.

- **Description of manufacturing process and Process controls**

- A flow diagram including:
- Narrative description of the manufacturing process and process controls should be stated including in process testing and operating parameters performed at each step of manufacturing.

-The critical steps and points at which process controls, intermediate tests or final product controls are conducted

-Novel processes or technologies that directly affect product quality.

-Equipment should be identified by type and working capacity where relevant and proposals for the reprocessing of materials.

-Process parameters of all steps as: PH, temperature, time represented as numeric values, whereas numeric ranges for critical steps should be justified

- **Control of critical steps and intermediates**

-Information on the quality and control of critical steps and intermediates isolated during the process

- **Process validation and/or evaluation**

-Sufficient information should be provided on validation and evaluation studies to ensure process consistency and suitability for the intended use including, the validation plans, validation reports and conclusions from the study.

-Process validation studies for aseptic processing and sterilization should be included. Bracketing is acceptable if more than two fill volumes are included.

4.2.2.4 Control of Excipients

- **Specifications**
- **Analytical Procedures**
- **Analytical validation**
- **Justification for Specifications**
- **Excipient of human or animal origin, information including adventitious agents**

For excipients of human or animal origin, information should be provided regarding adventitious agents (e.g., sources, specifications; description of the testing performed; viral safety data).

- **Novel excipients**

In case of using excipient(s) used for the first time (novel excipients) in a drug product or by a new route of administration, full details of manufacture, characterization, and controls with cross references to supporting safety data should be provided.

4.2.2.5 Control of Drug Product

- **Specifications**
- **Analytical Procedures**

The following analytical methods used for the testing of the Product should be described:

<u>1) Physicochemical characteristics:</u>

PH, Appearance, Color

2) Biological assay

- Chromogenic assay of anti-factor II a in case of porcine origin
- Chromogenic assay of anti-factor IIa or plasma sheep clotting assay in case of bovine Origin
- The manufacture should provide the justification & supportive data for the set specifications (Q6B)

3) Microbiological testing

Sterility

Bacterial endotoxin

- **Analytical validation**
- **Description of batches and result of batch analysis**
- **The Characterization of impurities**

Information on the characterization of impurities should be provided, if not previously provided in "3.2. S.3.2 Impurities"

- **Justification for Specifications**

4.2.2.6 Reference standard or materials

Information on the reference standards or reference materials used for testing of the drug product should be provided, if not previously provided in "3.2. S.5 Reference Standards or Materials"

4.2.2.7 Container Closure System

- A description of the container closure systems should be provided, including the identity of materials of construction of each primary packaging component and its specification. The specifications should include description, identification (and critical dimensions, with drawings where appropriate). Non-compendial methods (with validation) should be included where appropriate.
- For non-functional secondary packaging components (e.g., those that neither provide additional protection nor serve to deliver the product), only a brief description should be provided.
- For functional secondary packaging components, additional information should be provided. The suitability should be discussed with respect to, for example, choice of materials, protection from moisture and light, compatibility of the materials of construction with the drug substance, including sorption to container and leaching, and/or safety of materials of construction.

4.2.2.8 Stability

- **Stability Summary and Conclusion**
  The types of stability studies conducted, protocols used, and the results of the studies should be summarized.
  The summary should include conclusions with respect to storage conditions and shelf-life, in-use storage conditions and shelf-life should be provided.
- **Post-approval Stability Protocol and Stability Commitment**
  The post-approval stability protocol and stability commitment should be presented.
- **Stability Data**

  Results of the stability studies should be presented in an appropriate format (e.g., tabular, graphical, narrative). Information on the analytical procedures used to generate the data and validation of these procedures

5. Glossary:

API: Active Pharmaceutical product
CPP: Certificate of Pharmaceutical Product
CTD: Common Technical Document
DP: Drug Product
DS: Drug Substance
EDA: Egyptian Drug Authority
EMA: European Medicine Agency
FDA: Food & Drug Administration
FPP: Finished Pharmaceutical product
GMP: Good Manufacturing Practice
MA: Marketing Authorization
NRA: National Regulatory Authority
SOP: Standard Operating Procedures
WD: Working Day
WHO: World Health Organization
TSE: Transmissible Spongiform Encephalopathies
PCR: Polymerase Chain Reaction
NMR: Nuclear magnetic resonance
IR: Infra-Red
OSCS: Over sulfated chondroitin sulfate

6. References:

- Guideline on the use of starting materials and intermediates collected from different sources in the manufacturing of non-recombinant biological medicinal products (EMA/CHMP/BWP/429241/2013)
- Guideline on Active Substance Master File Procedure (EMA/ CHMP/QWP/227/02 Rev 3/Corr *)
- Annex I to Directive 2001/83/EC as amended Part I, 3.2 Basic principles and requirements, (8) Active Substance Master File (for Human medicinal products)
- Note for guidance on biotechnological/biological products subject to changes in their manufacturing process (CPMP/ICH/5721/03)
- Common technical document for the registration of pharmaceuticals for human use quality overall summary of module 2 and module 3: quality (CPMP/ICH/2887/99 - quality)
- Note for Guidance on minimizing the risk of Transmitting Animal Spongiform Encephalopathy Agents via Medicinal Products (EMA/410/01 rev.3) (2011/C 73/01)
- FDA Guidance for Industry Heparin for Drug and Medical Device Use: Monitoring Crude Heparin for Quality (2013)
- WHO Annex 3 General guidelines for the establishment, maintenance and distribution of chemical reference substances
- International Organization for Standardization. Certification of reference materials – general and statistical principles. 2006, ISO Guide 35.
- Reference ICH Guideline Q3A, Q3C, Q5C, Q6A, and Q6B
- British pharmacopoeia monograph for heparin sodium
- British pharmacopoeia monograph for heparin calcium (monograph 0332)
- British pharmacopoeia monograph for heparin injection
- USP 43monograph for heparin sodium
- USP 43 monograph for heparin calcium
- USP 43 monograph for heparin injection
- European pharmacopoeia monograph for heparin sodium EP10(01/2017:0333)
- European pharmacopoeia monograph for heparin calcium EP10
- Brazilian pharmacopoeia, Bovine heparin and porcine heparin monograph, 6th edition 2019

7. Annexes

None