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Heparins and substances of natural origin in the European Pharmacopoeia

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Presentation outline

• Substances of natural origin in the Ph. Eur.
• Recommendations for monographs of substances of natural origin
• Glycosaminoglycans (GAGs)
  • Heparin monographs
  • Sodium hyaluronate, chondroitin sulfate
• Urine-derived substances
• Protamine sulfate
Biological substances of natural origin in the Ph. Eur.

- An heterogeneous group
- **Glycosaminoglycans (GAGs):** heparins, sodium hyaluronate, chondroitin sulfate...
- **Other substances extracted from natural sources:** trypsin, protamine sulfate, pancreas powder...
- Human **urine-derived substances:** chorionic gonadotrophin, urofollitropin, urokinase
- **Substances derived from human plasma are excluded**

### Recommendations for the layout of monographs on substances of animal origin

#### Technical Guides

- **European Pharmacopoeia Style Guide:** European Pharmacopoeia Style Guide, August 2014
- **Structure Nomenclature:** Guide to the Graphic Representation and Nomenclature of Chemical Formulæ in the European Pharmacopoeia, 2nd Edition 2011
- **Elaboration of Monographs:**
  - Guide for the elaboration of monographs on synthetic peptides and recombinant DNA proteins (2010)
  - Guide for the elaboration of monographs on vaccines for veterinary use (2010)
  - Technical Guide for the elaboration of monographs on vaccines and other immunological human medicinal products
Specific monographs for substances of natural origin

- Not a single approach as heterogeneous class
  - For naturally-derived proteins, certain principles of the TG for the elaboration of monographs on [...] recombinant DNA proteins may apply
- Definition
  - States the source species and where applicable the organ or tissues from which the substance is derived
- Production
  - Viral and TSE safety: general chapters 5.1.7 Viral Safety, 5.2.8 Minimising the risk of transmitting animal spongiform encephalopathy agents via med products → not referred to in individual monographs!

Specific monographs for substances of natural origin

- Production (cont’d)
  - The health of animals used for production must fulfil the requirements for the health of animals suitable for human consumption. The quality of feeding stuff may be specified
  - The control of levels of contaminants (e.g. dioxins) through the manufacturing process may be required
- Labelling:
  - Origin only mentioned where necessary to demonstrate compliance with monograph requirements
  - Example: Chondroitin sulfate sodium (2064)
Specifying the origin: an example
Chondroitin sulfate sodium (2064)

- DEFINITION: It is obtained from cartilage of both terrestrial and marine origins
- **Specific optical rotation** (2.2.7):
  - −20 to −30 (terrestrial origin) or −12 to −19 (marine origin) (dried substance), determined on solution S1
- LABELLING: The label states the origin of the substance (marine or terrestrial)

Glycosaminoglycans

- Chondroitin 6-sulfate
- Keratan sulfate
- Heparin
- Dermatan sulfate
- Hyaluronate
Heparins

- **Clinical use**: used to prevent and treat blood clots
  - Widely used anticoagulants
- **Mechanism of action**: 
  - Catalyses the inhibition of thrombin and factor Xa by antithrombin
- **Unfractioned heparin**: 
  - Natural heparin, polydisperse (varying molecular masses)
  - Dose-response difficult to predict
- **Low molecular mass heparins** (e.g. Enoxaparin, Dalteparin): 
  - Have undergone fractionation or depolymerisation
  - Mw <8000 Da, NLT 60% of total mass has a relative molecular mass < 8000 Da
  - More predictable dose-response, longer duration of action

### Heparin monographs

- **Unfractionated heparin**: 
  - Heparin sodium (333)
  - Heparin calcium (332)
- **Assay of Heparin**: (2.7.5)
- **Low-molecular-mass heparins** (828)
  - **Common, general requirements**
  - **Specific requirements**
  - **Dalteparin sodium (1195)**
  - **Enoxaparin sodium (1097)**
  - **Nadroparin calcium (1134)**
  - **Parnaparin sodium (1252)**
  - **Tinzaparin sodium (1271)**
Heparin adulteration crisis (2007-2008)

- Serious allergic-type adverse effects resulting in a high number of deaths associated with lots for IV administration reported in the US, Germany and other European countries; Major recalls of heparin batches
- **Contaminant** identified as **Oversulfated Chondroitin Sulfate (OSCS)**, a non-natural heparin-like GAG
- Presence of natural GAGs such as Dermatan Sulfate and Chondroitin sulfate (process-related impurities), however not linked to adverse effects

Ph. Eur. response to the heparin crisis

- Revision of monographs on unfractioned heparin (Heparin sodium, Heparin calcium):
  - **Wave #1** (Suppl. 6.4): emergency measures: systemic screening for OSCS (FDA methods: CE and \(^1\text{H}-\text{NMR}\))
  - **Wave #2** (Suppl. 7.0): min potency, quality management system, \(^1\text{H}-\text{NMR}\) for IS, SAX-HPLC for ID & related substances, tightening of limits (nucleotidic impurities, nitrogen), Lowry test for total protein
  - **Wave #3** (Suppl. 8.3): restriction of scope (porcine origin), new assay method (chromogenic), requirement for ratio anti-Xa/anti-IIa, sodium range
Strengthened quality requirements

• **Scope**
  – Limited to material **from porcine origin** (reflects the market situation in Europe)

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**HEPARIN SODIUM**
Heparinum natricum

**DEFINITION**
Preparation containing the sodium salt of a sulfated glycosaminoglycan present in mammalian tissues. It is prepared from the intestinal mucosa of pigs. On complete hydrolysis, it liberates D-glucosamine, D-glucuronic acid, L-iduronic acid, acetic acid and sulfuric acid. It has the property of delaying the clotting of blood by catalysing the inhibition of thrombin and factor Xa by antithrombin.

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Strengthened quality requirements (cont’d)

• **Production**
  – **Source species identification; absence of material from possible contaminant species**
  – At a level of 0.1 per cent m/m (PCR-based methods widely used as a surrogate)

PRODUCTION
The animals from which heparin sodium is derived must fulfil the requirements for the health of animals suitable for human consumption. All stages of production and sourcing are subjected to a suitable quality management system. The identity of the source species and the absence of material from possible contaminant species such as cattle, sheep and goats, is verified by appropriate testing during production. The method used to confirm identity of the source species, and the point of application in the process, have been validated and shown to be capable of identifying the presence of material of other species at the level of 0.1 per cent (m/m). Species verification by methods based on polymerase chain reaction (PCR) amplification of species-specific DNA sequences has been widely shown to be an appropriate surrogate. If such a method has been chosen, it is also used to test for porcine DNA and to determine that it is present at a consistent level, in line with the manufacturing process used.
Strengthened quality requirements (cont’d)

- **Stricter limits** based on batch data
  - **Specific activity** (NLT 180 IU/mg)
  - **Nucleotidic and protein impurities** (A260: NMT 0.15; Lowry: NMT 0.5%)

Strengthened quality requirements (cont’d)

- **Assay** (2.7.5)
  - Need for a more specific assay that is not influenced by the presence of contaminating products with heparin-like activity (such as OSCS)
  - **Chromogenic assay** for determination of anti-factor IIa and anti-factor Xa activity
    - Designed to determine the anticoagulant activity that is specifically attributed to the action of heparin
2.7.5 Assay of heparin [UFH]  
(Suppl. 8.3)

- Clotting assay replaced by a chromogenic assay for anti-factor IIa activity (specificity)
- Assay for anti-factor Xa activity introduced to establish the ratio of anti-factor Xa to anti-factor IIa activity
- Based on the results of the international collaborative study to value assign the 6th international standard for unfractioned heparin

**Anti-IIa assay – Outline**

- New identification and purity tests
  - Anti-factor Xa/anti-factor IIa activity ratio
    - Narrow range characteristic of heparin, minimises the risk of adulteration
  - $^1$H-NMR
    - Selected for its ability not only to allow identification of heparin but also to alert users to possible contamination
Strengthened quality requirements (cont’d)

- New identification and purity tests
  - SAX-HPLC (dermatan sulphate + chondroitin sulphate: NMT 2.0%; no other peaks)
    - Allows the differentiation of natural contaminants linked to the production process such as DS and CS from chemically synthesised contaminants
  - Updated sodium range

Are requirements up-to-date for Low Molecular Mass Heparins?

- LMMHs are prepared from UFH. Quality requirements applying to them were thus strengthened through the revision of monographs on UFH
- Monograph on LMMHs (0828):
  "[LMMHs] are obtained by fractionation or depolymerisation of heparin of natural origin that complies with the monograph on Heparin sodium (0333) or Heparin calcium (0332), whichever is appropriate, [...] unless otherwise justified and authorised".
Sodium Hyaluronate (1472) & Chondroitin Sulfate (2064)

- No longer considered to meet the EU definition for biological AS (Dir 2001/83/EC)
- Consider a revision to mirror state-of-the-art testing (EMA request)
  - Sodium Hyaluronate: determination of molecular mass and polydispersity
  - Chondroitin Sulfate: determination of fine structure, distinguish between sources

→ Monograph update under discussion

Urine-derived substances
Urine-derived substances

- **hCG & FSH** (hormones), **Urokinase** (enzyme)
- **Ongoing update** (*Pharmeuropa 28.3*)
  - Revision to update and harmonise requirements on **viral safety** (EMA request)
  - The emphasis is now placed on the Production i.e. the clearance of viruses by the manufacturing process, rather than on tests for specific viruses/virus antigens. The manufacturing process should be demonstrated to inactivate and/or remove extraneous agents.

Urine-derived substances

- Revision in line with the new EMA guideline on the viral safety of urine-derived medicinal products (EMA/CHMP/BWP/126802/2012).
Other substances extracted from natural sources

Protamine Sulfate (0569)

- Sulfates of basic peptides extracted from the sperm or roe of fish – composed of four major peptides
- Antidote to heparin, critical excipient in insulin preparations (isophane insulin)
- EMA request to modernise the monograph
- Work on a general revision is underway:
  - HPLC method for identification (based on peptide composition), purity
  - Characterisation of heparin-binding properties
Thank you for your attention!