

2ND WORKSHOP ON THE CHARACTERISATION OF HEPARIN PRODUCTS



Symposium organised by the European Directorate for the Quality of Medicines & HealthCare (Council of Europe), United States Pharmacopeia and the National Institute for Biological Standards & Control

19-20 June 2008
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SUMMARY OF WORKSHOP SESSIONS

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SESSION 1: CONTAMINATION OF HEPARIN – CURRENT SITUATION AND PERSPECTIVES

Recently, certain lots of heparin for intravenous administration have been associated with serious allergic-type adverse effects involving hypotension, resulting in a high number of patient deaths. These phenomena have occurred in the United States, in Germany and other European countries, turning this problem into an international health crisis. This sudden onset of adverse effects suggested the potential contamination of heparin batches. Extensive analysis, including screening for different typical biological contaminants such as DNA, lipids or proteins was carried out worldwide. In March 2008, the FDA posted the Capillary Electrophoresis (CE) and Proton Nuclear Magnetic Resonance (¹H-NMR) methods for screening heparin batches for the presence of the contaminant on their website. In April 2008, the FDA announced that the contaminant was identified as Oversulfated Chondroitin Sulphate (OSCS), a non-natural heparin-like glycosaminoglycan (GAGs). In addition to OSCS, the screening tests demonstrated the presence of natural GAGs in heparin products such as dermatan sulphate, which are process-related impurities. Although it was demonstrated that process-related impurities was not the cause of adverse effects, it was recognised that this issue should also be considered.

In response to this crisis, the European Pharmacopoeia (Ph. Eur.), United States Pharmacopoeia (USP) and Japanese Pharmacopoeia (JP) have reviewed their respective heparin monographs. The 2nd Workshop on the characterisation of heparin products was the opportunity to debate on the current situation and reactions of the different regulatory organisations and industries, and consider a harmonised approach between pharmacopoeias and licensing authorities to best handle this health problem.

Presentation of the situation

General European OMCL Network

The General European OMCL network (GEON), coordinated by EDQM, was given as an example of co-operation. It had made possible to share experimental data obtained with CE, NMR and other techniques, between the GEON members and their respective national competent authorities. It was highlighted that such a network was a benefit in terms of assistance in the interpretation of the results, making techniques available and having a rapid overview of the European situation.

Test methods used within the OMCL network included, in addition to the FDA screening methods and tests prescribed in the current Ph. Eur. monographs, near-infrared spectroscopy and size-exclusion chromatography after nitrous acid digestion (splitting). A prekallikrein activation test and a “classical electrophoresis” test were also being investigated.

Results obtained are summarised in the table below:

	API samples (292)	Product samples (288)
Dermatan sulphate (DS)	Up to 5% (107 batches)	Up to 2% (56 batches)
Over-sulphated chondroitin sulphate (OSCS)	Up to 17.5% (50 batches)	Up to 8% (51 batches)
Other impurities	51 batches	7 batches

Retrospective testing of safe “historical” batches was being carried out to help determine usual levels of manufacture/product-related impurities.

USA-FDA

Dr Ali Al-Hakim summed up the situation in the USA. Proof of intentional contamination had yet to be made. 70% of the heparin on the US market came from abroad, mostly from China. There were as many as 600,000 slaughterhouses for pigs in China, which made attempts to control the heparin supply chain extremely difficult. Deaths related to allergic or hypotensive reactions with the contamination had been noted from as early as 2006. A comparison of the prices for chondroitin sulphate and heparin showed chondroitin sulphate to be two hundred times cheaper.

It was not known for sure whether OSCS was the only contaminant linked to the adverse reactions. Its origin (species, organ) or the conditions and chemicals, solvents used to modify it were still unknown.

Safety measures taken by the licensing authorities

General considerations

It was noted that priority was given to keep heparin products safe instead of stopping their production. To stem the situation, different measures were taken among the different countries at several stages of the post-marketing life of heparin products:

- at the import level: when the product entered the country, alerts were launched. Initiative to identify the Active Pharmaceutical Ingredient (API) suppliers of a given Marketing Authorisation Holder (MAH) was also undertaken;
- at the post-marketing analysis level: both heparin APIs and final heparin products were analysed for the contaminant. Heparin products tested comprised unfractionated as well as fractionated heparins;
- at the pharmacovigilance level: adverse events were monitored and reviewed.

In parallel, communication to healthcare professionals and to the public via different means (internet, press release) was set up.

In case of contamination, despite the variability of the actions taken between countries, it was noted that a decision-making balance was always developed. The following strategies were observed:

- products were recalled. Quarantine was preferred to destruction to avoid shortage events.
- compensation with other MAH products was initiated ;
- acceptance criteria based on the threshold of OSCS percentage were set up to avoid shortage events ;
- to avoid a shortage of low-molecular-mass heparins (LMMH), information to healthcare practitioners not to use LMMH with the intravenous route was provided ;
- guidance to use alternative therapeutic protocols was provided to practitioners.

France

The LMMH market was 10 times higher than that for the unfractionated heparins. Enoxaparin accounted for 75% of the LMMH market. Requirements were less than 1% OSCS and less than 5% DS in batches (detected with the FDA NMR method), absence of unidentified impurities and non-porcine material was set as acceptance criterion.

Germany

50% heparins on the German market came from parallel importers. Measures to be taken in case of shortage were to tolerate up to 5% of OSCS, administration by the subcutaneous route only, apply a special warning sticker on (low-level) contaminated products and allow labelling in foreign languages for imported products. Traces of contaminants had still been found in batches received on 6 May. An NMR method for detection of OSCS in LMMH was under development.

Japan

Marketing authorisation holders were required to test their APIs with both FDA screening methods. Contaminants had been detected up to 2.2%, batches recalled. No spike in adverse events had been noted with these products.

Australia

All contaminated batches (whatever the contamination level) had been recalled. No regulatory action had been taken against products containing DS, however Dr. Kelly warned against homogeneity issues of the batches and hence problems of representative sampling: results (with the NMR and CE screening methods) had sometimes been shown to vary depending on whether the sample had been taken from the top or the bottom of the drum.

Heparin contaminant and impurities

It was agreed that a distinction had to be made between heparin natural impurities and non-natural contaminants like OSCS. OSCS is a non-natural glycosaminoglycan. It was commonly admitted that this substance could not be synthesised by biological enzymes and was therefore most probably the result of a counterfeiting action. Chondroitin Sulphate (CS), which is naturally present in cartilage, can acquire anticoagulant properties similar to heparin after chemical sulfation. It is supposed that the production of OSCS arose from a need to

compensate a shortage of starting material for heparin APIs production, caused by pig epidemics that occurred in China in 2007.

Apart from the contaminant, heparin products also contain process-related GAGs impurities such as dermatan sulphate. Up to now, no adverse effects were linked to these process-related impurities. However in the context of the heparin contamination crisis, scientists and regulators showed interest in debating the presence and impact of these substances in heparin products.

Possible solutions for the future – view points from industry and academia

General considerations

Methods of choice and quality requirements, which could be implemented for the quality control of heparin products, were largely discussed by industries and academia. Focus was put on the ability of the method to detect OSCS as well as process-related impurities.

CE and ¹H-NMR had been widely used by all of the stakeholders. From the data presented at this workshop it became obvious that ¹H-NMR, in comparison to CE was a powerful, robust and sensitive tool to screen heparin samples. It was noted that the CE method proposed by FDA would need some improvements as regards the resolution, separation of the peaks and suitability of the method for quantification.

In addition to these methods, other techniques were proposed such as Size Exclusion Chromatography (SEC), Anion exchange chromatography (AEC), ion-pairing chromatography (IPC) as potential candidate methods. These techniques could provide additional advantages over CE and ¹H-NMR (less expensive, quantification of highly sulphated GAGs).

Optical rotation was also mentioned but the usefulness of this technique was questioned.

Besides physicochemical methods, the use of biological assays, such as the anti-factor IIa and anti-factor Xa activity assays was also proposed as a possible tool to discriminate contaminated from non-contaminated batches.

Manufacturers and academia were encouraged to provide their methods to the Pharmacopoeias.

It was recognised that combining of methods should be considered. The main criterion for the choice of a method was its ability to detect and quantify all possible GAGs. However it was noted that the aim of the pharmacopoeial method was not to detect all possible contaminants but to detect any deviation in the quality of the product.

Dr Soon-Shiong from APP stated that a batch containing up to 30% of OSCS could pass the current compendial requirements. He advised to raise the lower potency limit to 175 USP U/mg and the minimum specific optical rotation to + 53°. Out of 94 commercial batches tested, only 4% would fail the higher potency requirement and 2% the higher optical rotation limit as proposed.

The *Baxter* representative reported that the CE method was being improved, notably to increase the resolution between OSCS and heparin, allowing for a better separation between glycosaminoglycans, reducing co-elution of the components and covering instruments other than those from Beckman's. As regards the NMR, the OSCS signal could vary around 2.15 ppm, depending on the purity of the test material. Several other possible methods were reviewed; cellulose acetate electrophoresis appeared to have the least drawbacks since it allowed quantification and was easily available and robust.

LEO had screened all their batches with the FDA methods. A GalN/GlcN method (in place for several years) was presented. They were currently investigating a potency method following complete depolymerisation. The usefulness of several methods in combination was highlighted.

Sandoz emphasised the importance of audits and close contact with local suppliers as well as tight collaboration with the authorities. Workshops close to a slaughterhouse were preferred since - at least - food regulations were more likely to be applied.

A SAX-HPLC method following nitrous acid and chondroitinase digestion was proposed by *Sanofi-Aventis* (LOQ 0.2%, LOD 0.05%). A very similar method was presented by *Gland Pharma* (India).

Dr Walenga from Loyola University gave an insight into the biological properties of OSCS. A superadditive anticoagulant effect was actually observed with a 50% heparin/OSCS mix so the observed potency was roughly the same as that of heparin alone. OSCS did not only bind to plasma proteins but reacted very strongly with HIT antibodies.

Rationale for limiting Dermatan Sulphate

Opinions on limiting the content in dermatan sulphate (DS) were divergent. On one hand, it was highlighted that DS contributed to the heparin activity and up to 16% had been found in "safe historical" batches. There was no reason to reduce DS from a pharmacological point of view. On the other hand, DS was an impurity. Ensuring low levels of DS reflected good process capability and, to other participants including a manufacturer, was not an issue. Besides, with such high levels, the product was no longer heparin but a mix of heparin and DS. However, caution should be exercised as trying to purify heparin too much may cause an unwanted modification of the molecular mass distribution. Two approaches could be considered:

- (1) to base the limit on current DS content of marketed heparins. This was the approach proposed, for example, by the representative of the OMCL network ;
- (2) to lower the limit to strengthen the quality of batches. However this approach might lead to the modification of the composition of the current marketed product and affect the molecular distribution of heparin products. Economical consequences would also arise.

Reconsidering Good Manufacturing Practices (GMP)

The heparin crisis has led to reconsider the GMP process. It was agreed that although OSCS did not emanate from a deviation in the manufacturing process, there was a need, as a long-term management, to run an inspection programme for controlling the manufacturing process, especially from the slaughterhouses to the production of heparin APIs.

The supply chain of heparin was a critical step in the production of heparin and therefore a potential way for contamination of batches. After processing of pig intestine mucosa in slaughterhouses, crude extracts were processed (extraction, purification, viral inactivation) in workshops. About 10 batches from different workshops were used to make one API batch.

Emphasis was put on the species of origin. An impurity originating from fish may not be detected by a method developed for the same impurity with a bovine origin. Traceability, as defined by GMP, was also evoked as an important element to ensure the quality of crude extracts. With regard to this issue, there was a request to consider the analysis of heparins for DNA and proteins impurities. Analysis of DNA by Q-PCR could be considered as a reliable technique to control the origin of the species and therefore detect heparins products prepared from different species origin.

Nevertheless, it was agreed that such proposals were GMP-related issues and were out of the scope of the Pharmacopoeia. Monographs should not be expected to cover any possible type of contaminants. A distinction had to be made between adulteration and unintentional contamination because of inadequate manufacturing practices.

Pharmacopoeias – plans for revision and perspectives of harmonisation

It was recognised that the different meetings with FDA and EDQM, besides the dissemination of information between countries had played an essential role in approaching the crisis in a relatively similar and global manner.

In the light of the discussion that took place at the ad-hoc meeting organised by the European Pharmacopoeia Department in April 2008, the European Pharmacopoeia group of experts (group 6) responsible of heparin monographs agreed on a step-wise approach; (1) a short-term rapid reaction: introducing available screening methods (¹H-NMR and CE), with no detailed description in the monographs as a way of quickly dealing with the present situation, (2) a longer-term reaction: introducing a method to detect and quantify process-related impurities and setting-up limits for these impurities. The short-term approach had the advantage to be flexible. Furthermore the CE and ¹H-NMR methods had been developed as screening methods in an urgent situation and therefore would need to be fully validated to appreciate their suitability among other techniques for the purpose of detecting OSCS as well as process-related impurities. Due to difference in the market situation between European countries, acceptance criteria were at the discretion of the licensing authorities to avoid any shortage in heparin products.

The Japanese Pharmacopoeia (JP) was still working on a draft comprising two purity tests based on the FDA methods:

- identification of OSCS by $^1\text{H-NMR}$ (no signal between 2.13-2.17 ppm);
- detection and quantification of DS by CE (still under discussion).

Introduction of a comprehensive test or tests for glycan-related impurities was foreseen.

The United States Pharmacopoeia (USP) had just issued (18 June) official versions of the revised heparin sodium and heparin calcium monographs. They comprised both FDA NMR and CE methods for identity. Reference standards for use of these methods had been made available, too. Work on developing an anti-IIa potency assay was ongoing.

Both JP and USP had considered the use of specific Reference Material. While JP was currently working on establishing OSCS and DS standards, USP has already established Heparin and OSCS identification Reference Standards.

In this crisis context, the need for harmonisation of requirements and methods was strongly expressed and recommended. All participants were to consider co-operation in method elaboration and validation, as well as in establishment of specific reference materials. Sharing of data was also seen as an essential key in the completion of this work.

SESSION 2: GLOBAL HARMONISATION

The session began with an appreciation of the life and work of the late Dr Pietro Bianchini, presented by Dr Giuseppe Mascellani. Dr Bianchini's achievements over a long and productive career were outlined, including contributions to academic and medical science as well as commercial development of heparin products.

Heparin specifications before and after the adulteration tragedy: A US historical perspective

Dr Patrick Shaklee presented a historical perspective, concentrating on developments in the pharmacopoeial specifications for unfractionated heparin (UFH) over the previous ten years. The sourcing of material for UFH as a therapeutic agent in its own right as well as a source material for the manufacture of LMM heparin was also discussed. The benefits and costs of further tightening specifications for UFH were compared. The specification for anticoagulant activity should be increased, and assays specific for antithrombin-dependent activity used; if optical rotation is specified, the limit should be raised from the current Ph. Eur. level of 35° to at least 46°; a measure of GalN/GlcN ratio could be introduced, as well as a separation technique capable of distinguishing between heparin and other sulphated polysaccharides (for example by differential degradation of heparin using specific enzymes); limits on UV absorbance should be introduced (where they are not already present) to control protein and nucleic acid content.

Heparin contamination: FDA perspective. Characterisation and analytical consideration

Dr Ali Al-Hakim outlined the FDA's approach to characterisation and analysis of batches of unfractionated heparin, recalled because of their connection with the recent cluster of adverse events. In collaboration with a number of laboratories, after testing using numerous separation and characterisation methodologies, a sulphated heparin-like material was found to be present in several batches. Of the methods used, two (NMR spectroscopy and capillary electrophoresis) were posted on the FDA website for use in screening heparin batches for the contaminant. Detailed NMR investigations revealed that the contaminant was oversulphated chondroitin sulphate (OSCS). FDA will continue to work with other agencies and industry to ensure the supply of high quality heparin.

Possible “expander” contaminants of heparin preparations

Dr Barbara Mulloy pointed out that OSCS is only one of a number of highly sulphated polysaccharides with similar properties, which may not all be identifiable by tests closely optimised for OSCS alone.

NMR spectroscopic evaluation of heparin for pharmacopoeial purposes

Prof. Ulrike Holzgrabe and Dr. Bernd Diehl described proton NMR spectroscopy at 300 and 400 MHz of over 100 heparin batches collected by BfArM from international markets. OSCS could be detected with LOD 0.1%. Analysis of the 1D proton NMR spectra, using PCA and quantitative NMR strategies, can provide detailed descriptions of heparins and low molecular mass heparins with some level of contamination and dermatan sulfate content, as well as complex glycosaminoglycan mixtures.

Advanced techniques suitable for commercial heparin and LMM heparin characterisation

Dr Giangiacomo Torri gave further examples of the application of NMR spectroscopic techniques to analysis of heparin preparations, for example distinguishing between sulphation patterns of porcine and bovine heparins. Both proton and carbon NMR can be used, and the powerful heteronuclear correlated spectroscopic experiments can combine both to give excellent spectral dispersion without the need to use a costly, very high field instrument. This technique can resolve signals from the OSCS contaminant in heparin. NMR spectroscopy can provide sensitive and quantifiable ways to detect contamination and the method can be adapted for contaminants other than OSCS.

Oversulphated chondroitin sulphate (OSCS) in heparin. NMR analyses

Dr Ian McEwen reported on the use of 1D and 2D proton NMR spectroscopy to identify the OSCS contaminant by comparison with published data for OSCS. The LOD was found to be at least as low as 0.5% using a 300 MHz spectrometer, with further improvements possible. The chemical shift of the methyl signal for OSCS appears to alter with its concentration in a LMM heparin sample. The reliability and rapidity of NMR analysis was pointed out.

Electrophoretic measurement to separate and quantify contaminants in heparin

Dr Thomas Freudemann presented a method for the determination of dermatan sulfate in heparin by one-dimensional cellulose acetate electrophoresis, which can also identify the OSCS contaminant. The method uses on-plate nitrous acid degradation of heparin and separation of nitrous acid resistant GAGs, with detection and quantification by densitometry of the stained bands. This method does not require expensive equipment and gives baseline separation between bands; the LOD for OSCS is 0.4%.

Method for determination of galactosamine as part of total hexosamine

Dr Rhonda Lecky described a method for chromatographic analysis of hexosamines resulting from acid hydrolysis of heparin samples. This method detects galactosamine, the hexosamine present in chondroitin and dermatan sulphate, with LOD 0.24% of total hexosamine. Experiments in which a contaminated heparin sample was completely depolymerised by heparinase were also described, showing that the resulting pure OSCS had negligible activity in a chromogenic anti-IIa assay, compared with nearly 60 IU/mg by the Ph. Eur. plasma based assay.

Replacement of the 5th International Standard for Unfractionated Heparin

Dr Elaine Gray announced the forthcoming collaborative study to replace the 5th International Standard for Unfractionated Heparin. The samples and protocol for the study will be dispatched to participants in November 2008 and it is envisaged that the new standard will be established by the WHO in October 2009. This study will also give comparative data on different anticoagulant assays for unfractionated heparin. Laboratories interested in taking part should contact Dr E Gray at NIBSC (egray@nibsc.ac.uk).

A general chromogenic substrate-based heparin assay

Dr Craig M. Jackson described an antithrombin dependent single stage chromogenic assay that can be adapted for measurement of anti-Xa and anti-IIa activity of heparin. The reaction mixture contains heparin, purified antithrombin, a suitable chromogenic substrate in appropriate concentrations. The reaction is started by the addition of the relevant proteinase, Xa or IIa. The reaction is permitted to proceed to completion after which the p-nitroaniline (pNA) produced from the hydrolysis of the chromogenic substrate is measured spectrophotometrically. The concentration of active (high affinity) heparin is related to the reciprocal of the pNA concentration. The basis for the use of this method for heparin, data from the measurement of both UFH and LMM heparins and validation data for the method were presented.

Potency assays: anti-factor IIa assay for UFH

Drs Kristian B. Johansen and Elaine Gray presented the rationale for the replacement of clot based anticoagulant assay with an antithrombin dependent anti-IIa assay for potency measurement of UFH. A proposed anti-IIa method based on the protocol detailed by the WHO Drafting Group (part of the WHO Consultation Group on Biological Standardization of Unfractionated Heparin) was qualified by the USP in 2004. In 2007 Pharmacopeial Forum published an "In-Process Revision" where the Anti-IIa procedure was proposed as a replacement for the sheep-plasma Assay of Heparin Sodium. This method is now in the process of being validated by the USP.

Harmonization of Pharmacopoeial methods

Drs Kristian B. Johansen and Elaine Gray tabulated the current EP and USP monographs for Heparin Sodium. The specifications of the different methods were compared and proposals for harmonization and improvements were presented and opened for discussion.

SESSION 3: LOW MOLECULAR MASS HEPARIN

This session focused on control of the quality of LMM heparin products and their starting material (unfractionated heparins). New additional characterisation methods were presented during the first part of the session. Compendial aspects of the monograph specifications in the three pharmacopoeia (USP, JP, Ph. Eur.), in particular with regard to the control of the quality of all marketed products (innovators and biosimilars) were discussed during the second part.

Unfractionated heparin as starting material for LMM heparins

PCR to control origin of species

P. Anger (Sanofi Aventis, France) presented quantitative PCR (QPCR) as a technique to control the species of origin of the starting material for LMM heparin.

QPCR is a very sensitive method that allows the detection of low levels of DNA from different species. The accessibility to the method is facilitated by the availability of all reagents, including primers for different species. A method validation is nevertheless needed, as a preliminary treatment with heparinase is required to remove the strong PCR inhibitor activity of heparin.

The inability of QPCR to detect DNA-free products such as hemi-synthetic products limits its use to product identity testing. Another limitation of the method is that it can only be applied on crude heparin and hence cannot be used for batch release. However, QPCR constitutes an interesting complementary method to NMR that is difficult to perform on crude heparin preparations. At Sanofi Aventis, the use of QPCR on batches of crude heparin has allowed the detection of batches contaminated by material from non-porcine origin.

During the discussion that followed the presentation, M.-H. Tissier (AFSSAPS, France) underlined that LMM heparin products are not restricted to those with porcine origin unless it is mentioned in the product specification. USP and JP confirmed that the same approach applies for their monographs and that the species of origin must be specified on the product label. Products irregularly containing heparin from different origins are thus to be considered as adulterated but not necessarily counterfeit products.

P. Jongen (Chair of Ph. Eur. Group of Experts on Biologicals) mentioned that GMP inspections need to be stricter to detect risks of contamination with products from other species. P. Anger (Sanofi Aventis) insisted that QPCR is not meant to substitute Ph. Eur. methods in the manufacturing section.

Characterisation of LMM heparins

Low molecular mass heparin characterisation: past and current views

Dr. Christian Viskov (Sanofi Aventis, France) presented an overview of the variety of LMM heparins resulting from different chemical or enzymatic cleavage production processes and,

taking the studies performed at Sanofi for enoxaparin, the current methods used to characterize them.

The differences between LMM heparins induced by depolymerisation processes are located:

1. at the structural level around the cleavage point,
2. at the endogenous disaccharide backbone level, with side-reactions generating non-natural products and inducing specific fingerprints of LMM heparins.
3. at the antithrombin binding site (ATIII binding site), where variation in pentasaccharide sequence alter interaction with antithrombin (AT) and flanking disaccharides units can modulate the affinity to AT. This results in the modification of the biological activity (anti-Xa activity) of LMM heparin.

Strategies to accurately analyse the LMM heparin mixtures are:

- HPLC analysis of the disaccharide building blocks after enzymatic digestion by a heparinase mixture. This method however gives an incomplete view due to lack of information about the sequence of the obtained fragments within the LMM heparin.
- analysis of the ATIII binding sequence after enzymatic digestion. This does however not give accurate data on ATIII binding site amount and diversity.
- analysis of the ATIII binding affinity by chromatography and evaluation of the anti-Xa and anti-IIa activities.

Laser light scattering characterization of LMMH: comparison of molecular weight distribution methods

Dr. Gyöngyi Gratzl (Ben Venue Laboratories, Boehringer Ingelheim, USA) presented the technique of Size-Exclusion chromatography coupled to Laser Light Scattering (SEC-MALLS) and its use for the characterization of LMM Heparins.

The method allows for the analysis of the distribution of molecular weights. Basic principles, calibration, normalization methods, system suitability requirements for SEC-MALLS were detailed. Results with two products (Dalteparin and Enoxaparin), using the *1st IRP Low Molecular Weight Heparin for molecular weight calibration*, were presented as an example of method validation.

The method gives similar results to a GPC method for the molecular weight determination of LMM heparins using the *1st IRP LMW Heparin for MW calibration* as a Broad Standard calibrant, but does not need relative heparin standards for daily calibration.

Compendial aspects: are current monograph specifications of LMM heparins sufficient to control the quality of both innovator and biosimilar products?

United States Pharmacopeia (USP) (Dr. A. Szajek)

The revised LMW heparin monograph will refer to the Heparin sodium monograph for the quality control of the heparin used as source material. The very limited definition of the specifications for the acceptance criteria and the absence of analytical characterisation of crude heparin call for a more strict control of suppliers, point of entry and manufacturers.

The presentation focused mainly on enoxaparin. Specific methods and reference standards are under development at USP for enoxaparin (for example, the 1, 6-Anhydro method). However, it is clear that a harmonisation of methods is needed for all LMM heparin products. A molecular weight determination test and anti-IIa and anti-Xa assays for LMWH will be proposed in the USP. As to the potency assignment, it was underlined that the Ph. Eur. applies the same potency standard to all products in line with WHO policy and International Unit (IU) definition.

Japanese Pharmacopoeia (JP) (Dr. N. Kawazaki)

Four LMM heparins are marketed in Japan. JP contains a specific monograph for parnaparin sodium and is developing a monograph for dalteparin.

The parnaparin monograph includes SE-HPLC/UV for the analysis of molecular mass distribution and the anti-Xa activity bioassay with the ratio to the anti-IIa activity for potency assignment.

Discussions are ongoing as to how to assess comparability of dalteparin products and how to discriminate the different LMM heparin products. Additional purity tests for OSCS and DS by NMR and/or CE will be added to the monograph for Heparin sodium, non-fractionated.

Other approaches, such as LC/ESI MS, monosaccharide composition analysis by HPAEC-PAD and glycan mapping are evaluated for the identification, comparability and purity testing.

European Pharmacopoeia (Ph. Eur.) (P. Jongen)

From the European regulatory perspective, LMM heparins are not chemicals but biological substances. Because of the complexity of the substance due to the starting material but also from the different production processes, compliance with pharmacopoeial monographs of LMM heparins is not sufficient to demonstrate biosimilarity. Additional chemical, biological and/or clinical testing may be needed. An EU regulatory guideline on similar biological medicinal products containing LMM heparin is currently under preparation at EMEA.

The Ph. Eur. includes a type monograph for LMM heparins and five specific monographs. These two types of monographs are linked by cross-references so that all LMM heparins need to fulfil the same general requirements unless stated differently in their specific monograph.

The Ph. Eur. is carefully considering any relevant new analytical tool that could be added to the monographs.

Although amendable as scientific and technical knowledge progresses, the current monographs may be sufficient to control the quality of both innovator and biosimilar products. They however do not ensure adequate characterization of substances as additional physico-chemical and biological tests need to be performed.

Need for harmonisation. Comparison of pharmacopoeial methods. Discussion.

The large global market of LMM heparins calls for a harmonised approach for the selection of analytical and biological assessment methods and for reference standards.

The need for the 1, 6-anhydro method for enoxaparin that verifies substance structure, a feature usually not included in monographs, was discussed. Sanofi and USP mentioned that it is a key test for enoxaparin because 1, 6 derivatives impact on its activity and efficacy. It was questioned whether the test would be used during development or for routine testing.

Differences between LMM heparin products other than anticoagulant activities exist, but are not supported by clinical data.

The wording in the monographs might need to be reconsidered during revisions to clearly indicate that the specifications set in the monograph on heparin (UFH) have to be met for the source material for LMM heparin.

The same heparin is used with similar processes by innovators and biosimilar producers. The lot-to-lot variability is however not known.

All Pharmacopoeias agreed that they are willing to include relevant new methods in their monographs. It was also noted that these methods should, as far as possible, avoid being available through a unique equipment manufacturer.

Until now, the anticoagulant activities of all LMM heparins have been expressed against the International Standards (IS) in IU. Although new products arising on the market challenge the use of the IU, it was emphasized that the use of a harmonised unitage is critical for the quality control and safety of all LMM heparin products as well as for their clinical use, the dosing of which is based on IUs, and their global market.

Open Discussion & Conclusions

Harmonisation of the different pharmacopoeial requirements is difficult, but is definitely in progress and is a prerequisite of the global market of such products. The timing of the decisions concerning heparin products is so far compatible between the different Pharmacopoeias and allows optimal harmonisation.

The three Pharmacopoeias are committed to proactively seek harmonisation. The present meeting, which involved 147 participants from 25 different countries, was an ideal opportunity for such a harmonisation.