EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

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Assessment report for Protamine containing medicinal products

Review under Article 5(3) of Regulation (EC) No 726/2004

Procedure no: EMEA/H/A-5(3)/1341

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

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Table of contents

1. Background information on the procedure

Protamine sulphate is used to neutralise the anticoagulant action of heparin in the treatment of haemorrhage resulting from severe heparin or low-molecular weight heparin overdose. It is also used to neutralise the effect of heparin given before surgery and during extracorporeal circulation, particularly in cardiac surgery. Protamine containing medicinal products are marketed in this indication in many EU countries following national authorisation procedures.

In addition protamine sulphate has been identified as an excipient used in insulin containing products as well in a tick-born encephalitis vaccine. This use is for both national and centrally authorised medicinal products.

The active substance protamine sulphate is a purified mixture of simple proteins obtained from the sperm of a species of wild salmon fished off the coast of Japan (a specific area off the coast of Honshu).

Recently and due to the fishing restrictions in Japan following the earthquake and the tsunami in March 2011, sourcing of the raw material has been done in other fishing grounds (coast off Hokkaido). This new natural raw material has shown endogenous heterogeneity.

While the producer of the active substance is currently working to resolve the issue, the EMA was informed initially by member states, as well as from market authorisation holders that a potential supply shortage of the protamine sulphate containing medicinal products may occur as early as the end of 2012.

In order to better prepare in the case of potential future supply shortage in the European Market and in accordance with Article 5(3) of Regulation EC (No) 726/2004, the CHMP was asked by the Executive Director of EMA to give an opinion whether the variability currently observed in the protamine sulphate due to the different sources of the natural raw material has any consequence on the overall quality of the products where it is used and issue any recommendations that may be relevant to address the situation.

In addition the concerned MAHs informed the CHMP of their current and future position as regards product supply across the EU, the measures they are taking to maintain the supply and if any supply interruptions are anticipated. The CHMP was also requested to make further recommendations regarding the optimal medical use of the affected products, if necessary.

2. Scientific discussion

2.1. Introduction

Protamines are a group of small highly basic arginine-rich proteins associated with DNA in sperm in many species. Protamine sulphate consists of sulphates of these basic peptides extracted from the sperm or roe of fish. Protamines are mixtures of several similar peptides. The chum salmon protamine sulphate has four major species with different amino acid sequences of approximately 30 amino acids. The average molecular mass is estimated from 5,000 to 5,500.

Protamine sulphate has been used for over 60 years as a heparin antagonist and as an excipient in insulin formulations to prolong insulin action. The first insulin protamine preparations were in use by

the mid-1930s containing protamine sulphate produced from various sources including trout and salmon.

The European Pharmacopoeia (Ph. Eur.) monograph defines protamine sulphate as consisting of the sulphates of basic peptides extracted from the sperm or roe of fish, usually species of *Salmonidae* and *Clupeidae*. It binds with heparin in solution, inhibiting its anticoagulant activity; in the conditions of the assay this binding gives rise to a precipitate. Calculated with reference to the dried substance, 1 mg of protamine sulphate precipitates not less than 100 IU of heparin.

As already mentioned, the chum salmon protamine mixture is comprised of four main components. When subjected to reversed phase high performance liquid chromatography (RP-HPLC), the four main components are accompanied by a number of minor species. The amino acid sequences of the main components in the RP-HPLC elution order (peak A to peak D) are described in the literature (Hoffman *et al*, 1990).

Peak 1 (Peak A)	H-Pro AAAAA Ser Ser Ser A Pro Ile AAAAA Pro A Ala Ser AAAAA Gly Gly AAAA OH
Peak 2 (Peak B)	H-Pro AAAA Ser Ser AA Pro Val AAAAA Pro A Val Ser AAAAA Gly Gly AAAA OH
Peak 3 (Peak C)	H-Pro AAAA Ser Ser Ser A Pro Val AAAAA Pro A Val Ser AAAAA Gly Gly AAAA OH
Peak 4 (Peak D)	H-Pro AAAA Ala Ser AA Ile AAAAA Pro A Val Ser AAAAA Gly Gly AAAA OH
Where	A = Arginine, Ser = Serine, Pro = Proline, Ile = Isoleucine, Ala = Alanine, Gly = Glycine, Val = Valine

Table 1. *The four (A-D) main peptide sequences in chum salmon*

Based on a review of the literature, the composition of protamine changes as salmon move through different stages of spermatogenesis. The fishing season for wild spawning chum salmon covers several months with salmon at different stages of spermatogenesis being harvested with variations in their protamine composition (Ling *et al*, 1971; Lewis *et al*, 2003; Balhorn, 2007).

The product's natural source results in variability in the overall purity profile of the final product. This variability can be seen in the reversed phase HPLC (RP-HPLC) chromatogram overlay below, illustrating the results from three different suppliers of pharmaceutical grade protamine sulphate obtained from the family *Salmonidae*. Chromatograms 1, 2, and 3 are separate lots from one supplier and chromatograms 4 and 5 are lots from two other suppliers (Hvas *et al*, 2005).

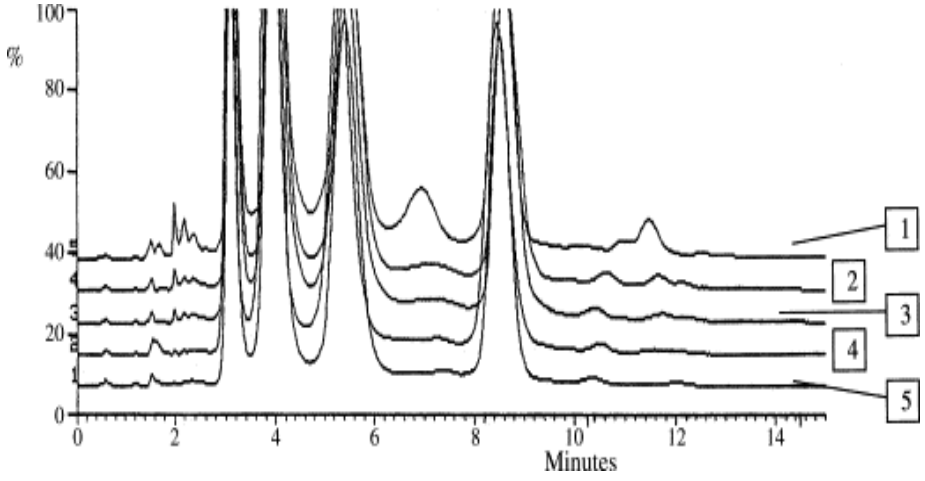

Figure 1. *Protamine sulphate from multiple suppliers*

This variability can also be seen in the RP-HPLC chromatograms (Figure 2, below) which shows the results of samples from three different lots of protamine sulphate produced by a supplier using chum salmon of *Oncorhynchus keta* as the source.

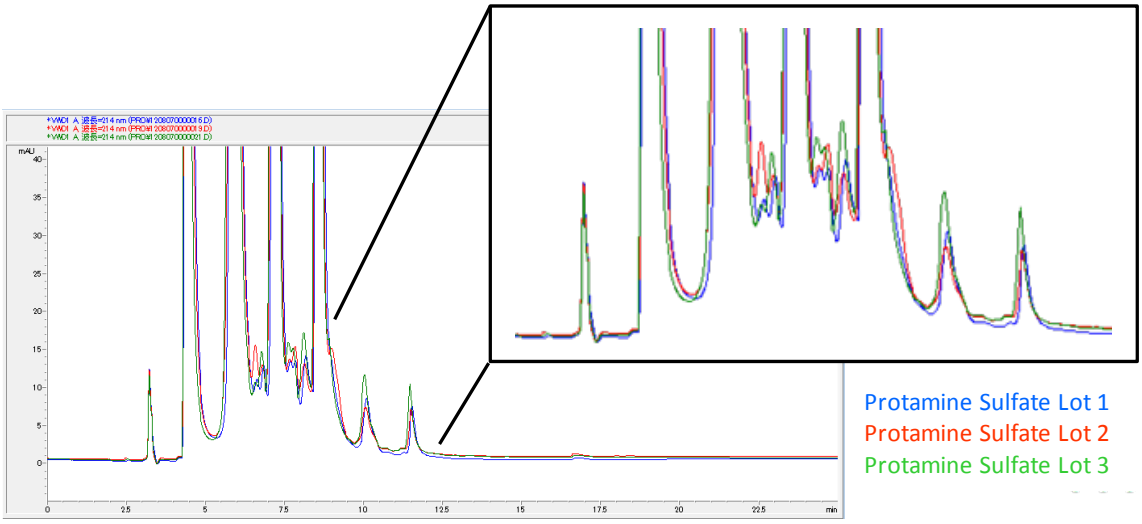

Figure 2. *Protamine Sulphate RP-HPLC results for three different lots*

Protamine principles have historically been obtained from the sperm (milt) of wild chum salmon fished off the Northeast coast of Honshu Island in Japan.

As a result of the March 2011 earthquake and subsequent tsunami the Japanese fishing industry essentially was not able to operate in their former fishing grounds. To maintain a protamine supply the fishing grounds for wild chum salmon were moved northward to Hokkaido Island. This change in the fishing grounds revealed endogenous heterogeneity in the product which was therefore not used in the manufacturing of medicinal products until an investigation would be complete.

European supply situation summary

The EMA requested information on the supply issue from all member states for use of protamine both as active substance and as excipient. From the collected information it was shown that several member states will have a supply shortage within the first quarter of 2013 with regards to protamine from the Honshu area and therefore there is need for assessment of the impact of using protamine from another source.

2.2. Discussion of the analytical results

Quality related data has been provided by the main EU manufacturer of the active substance while several MAHs have provided information on their supply. Regarding the quality related data various analyses have been performed by the manufacturer in order to identify and potentially characterise any differences in the raw material as it is discussed below.

2.2.1. Retrospective analysis

Firstly, historical protamine sulphate RP-HPLC data (2002 – 2012) were submitted for analysis. A review of the Total Area (%) of the four main peaks (A, B, C and D) showed variability between years and between lots within a year. The trend in the data between years showed shifts that would not be unexpected and could be attributed to environmental factors. The variability between lots within a given year is also not unexpected and could be attributed to the different stages of chum salmon spermatogenesis.

A chromatographic analysis for different lots of protamine sulphate was performed from different suppliers, different sources and year; the responses were expressed in Area Percent (%) of total.

This analysis of the chromatograms show that the variability of protamine sulphate derived from the Hokkaido fishing grounds is within the variability observed in historical results derived from Honshu fishing grounds with the exception of the sum of Area between Peaks C-D, for which results are slightly higher than the historical range (increase by ~0.5%).

Closer analysis of the individual relative retention time peaks in the C-D region reveals that the area percentages are not significantly different from historical results.

In addition a peak late-eluting region after main peak D was suspected which needed further investigation. This as it is explained below and it seems to be consistent with historical data.

2.2.2. Analysis of retained samples

Moreover, an additional study was designed by the manufacturer in order to conduct a head-to-head comparison of the purity profile of historical material (Honshu-sourced) vs. new material (Hokkaido-sourced). This study utilised retained samples of starting material, R-Pro (refined protamine sulphate starting material), and Protamine Sulphate from both sources which were analysed on a single chromatographic run.

i. Comparison of retained samples of protamine sulphate

Several samples of Protamine sulphate, including material from years 2003, 2007, and 2011 (Honshu-sourced material) and from 2012 (Hokkaido-sourced material) were co-analysed on this same chromatographic set-up.

The review of the full chromatograms show that the variability of the purity profiles seen in the regions among and in-between the four main peaks is much more significant than any small changes in the purity profile recently observed in the late-eluting region after main peak D.

This analysis confirms that the sum of area between the peaks C and D is found in higher levels in Hokkaido sourced protamine. However, as individual fractions of this C to D region have been shown to be present in similar level in historical batches, this point is not considered as a concern. However the manufacturers are encouraged to perform further qualitative and quantitative analyses of these fractions in the future.

ii. Comparison of retained samples of refined protamine sulphate starting material

Several other samples of R-Pro (refined protamine sulphate starting material), including from years 2007, 2010, and 2011 (Honshu-sourced material) and from 2012 (Hokkaido sourced-material) were analysed on a single chromatographic set-up using standard RP-HPLC method of analysis and quantitation.

Individual chromatograms from each of the batches of Honshu-sourced R-Pro were overlayed with the chromatogram obtained from Hokkaido-sourced R-Pro which contained a suspected new peak. The initially suspected new peak elutes at a later stage than the four major protamine peaks.

The comparison of chromatograms of historical Honshu-sourced material vs recent Hokkaido batches show that the suspected new late-eluting peak had been present in several of the Honshu R-Pro batches including 2007 and 2010 batches. A review of the historical chromatograms was completed and determined that the highest level of this minor component was actually observed in 2003 batches.

Based on the data provided, it can be concluded that the late eluting peak was present in variable level in historical batches that have been marketed for the last 10 years. Although the specie(s) present(s) in this peak has not been characterised, the presence of peak in the Hokkaido batches is not considered as concern as the level detected remains low. Furthermore, the highest level tested is not significantly different with those measured in batches produced in 2003. Nevertheless, the manufacturers are encouraged to further characterise the content of this peak in the future.

2.2.3. Purity acceptance criteria

When the protamine sulphate purity specification of Not Less Than (NLT) 93% was proposed by the manufacturer in 2010, the considered protamine sulphate batches used were from October 2005 to July 2009, and at that time indicated a process operating above 93%. A 99/99% statistical tolerance interval was calculated resulting in a lower specification limit of NLT 92.5%. Given the interpretation of a tolerance interval, i.e. there is 99% confidence that at least 99% of future values will be above 92.5%, the protamine sulphate purity specification of NLT 93% was reasonable at the time based upon the considered data.

At present there are insufficient data to calculate a specification for purity based on Hokkaido data alone. It is recommended that when sufficient data are available, an analysis based entirely on Hokkaido data be performed.

Moreover, the historical data of the protamine sulphate purity specification showed NLT level as low as 91.4% in March 2002. An analysis of all historical data using 99/99% tolerance interval estimates a lower limit of 90.9%. As a result of the additional analysis a new limit for protamine sulphate purity of NLT 90.5% is proposed. Currently the batches sourced from the Hokkaido area display results ranging between 92.1-92.4% purity.

Taking into account the potential of shortage and the analysis of historical batches, the new limit of ≥90.5% is considered acceptable.

It is noted that the revision of Ph. Eur. monograph for protamine sulphate aiming at introducing the RP-HPLC method and a purity specification limit has been initiated. CHMP takes the opportunity to inform EDQM on the outcome of this Art. 5(3) procedure by the provision of the BWP report, in case it could be of interest in the current revision of the Ph. Eur. monograph.

2.3. Clinical aspects

Protamine sulphate is authorised for instant neutralisation of the anticoagulation induced by heparin. It is used in case of bleeding in patients who had received heparin, but also routinely and extensively in extracorporeal circulation (ECC) for heart/aorta surgery and more rarely in case of bleeding during percutaneous neurovascular interventions. It can also be used in other indications (e.g. vascular surgery).

A supply shortage of protamine sulphate in Europe could have a major impact, mainly for heart surgery. The common practice is to perform ECC with the use of heparin and it is of paramount importance to be able to neutralise the anticoagulation when the ECC is stopped at the end of the surgery. There is no easy alternative to the use of protamine sulphate in routine ECC procedure.

During the procedure several MAHs have confirmed that there are no reports of immune-allergic adverse reactions. A search concerning protamine sulphate in a European pharmacovigilance database since 2000 revealed 47 different adverse events concerning 26 patients. Due to this low number of cases no conclusions can be drawn regarding the effect of the historical variability on adverse events. It is nevertheless recommended that the marketing authorisation holders proactively collect pharmacovigilance information on potential hypersensitivity adverse reactions.

3. Overall conclusion and CHMP recommendation

Analysis of historical chromatograms and testing of retained samples of protamine showed that there are no significant differences in the new material (sourced from the Hokkaido fishing ground) for both the starting material and final protamine sulphate product compared to the ones produced from the old fishing source, and its level of impurity is not significantly higher than those observed in historical batches.

An increase in the sum of areas between major peaks C and D is observed in the Hokkaido derived batches. Although the sum of all this area is slightly higher than the levels that have been detected in historical batches, the analysis of individual fractions of this C-D region were shown to be present in similar level in historical batches.

A comprehensive analysis of batches produced between 2002 and 2011 demonstrates that several batches with purity level between 91 and 93% have been marketed without triggering any pharmacovigilance signal.

In conclusion, the quality profile of the protamine sulphate derived from the Hokkaido fishing ground is not considered to be significantly different from the one of batches produced between 2002 and 2011 derived from the Honshu fishing grounds.

Furthermore, this new material continues to comply with the current Ph. Eur. monograph definition of protamine sulphate.

As a consequence and in the absence of significant differences the CHMP consider that protamine protamine sulphate sourced from the Hokkaido fishing grounds can be used for the manufacturing of medicinal products both as active substance and as excipient.

Moreover the CHMP recommend that the marketing authorisation holders of protamine containing products proactively collect any pharmacovigilance information related to immune-allergic events.

In addition, acknowledging the need of continued protamine supply, the CHMP would like to emphasise to MAHs of medicinal products containing protamine of the importance of having alternative authorised suppliers of protamine to better manage any future issues of supply.

4. References

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