



MEMORANDUM

From: Hsiaoling Wang, Ph.D.
CMC Reviewer
Laboratory of Analytical Chemistry and Blood Related Products (LACBRP)
Division of Biological Standards and Quality Control (DBSQC)
Office of Compliance and Biologics Quality (OCBQ)
Center for Biologics Evaluation and Research (CBER)
Food and Drug Administration (FDA)

To: Biologics License Application Submission Tracking Number 125611/0

Subject: Chemical Assays for Recombinant Coagulation Factor IX, pegylated (nonacog beta pegol)

Through: Lokesh Bhattacharyya, Ph.D., Lab Chief, LACBRP/DBSQC/OCBQ/CBER

William M. McCormick, Ph.D., Director, DBSQC/OCBQ/CBER

Applicant: Novo Nordisk Inc.

Submission Received by CBER: May 16, 2016

Summary:

A new BLA was submitted by Novo Nordisk for nonacog beta pegol, a glyco-pegylated recombinant human factor IX product, for use in adults and children with hemophilia B for control and prevention of bleeding episodes, perioperative management and routine prophylaxis.

This document constitutes the Primary Review Memo from DBSQC for the following analytical methods and their validations, which are proposed to be used for quality control of the (b) (4) drug product (DP).

1. Protein Content and (b) (4)
2. Identity, PEG Profile and Product Related Impurities by (b) (4)
3. Identity by Peptide Mapping by (b) (4)
4. Purity of rFIX (b) (4)
5. Product related impurities by (b) (4)
6. Water Determination By (b) (4)
7. Water Determination by (b) (4)

This reviewer found these seven analytical procedures are adequately described and validated for their intended uses. Approval is recommended for these assays.

Background

Nonacog beta pegol is a sterile lyophilized powder manufactured in three different strengths of factor IX: 500, 1000 or 2000 International Unit (IU) per vial. It is reconstituted in 10 mM histidine solution prior to intravenous injection.

Documents Reviewed

Original submission STN 125611/0 dated May 16, 2016

- Cover letter
- 3.2.S.4.1 Specification for drug substance
- 3.2.S.4.2 Analytical development for drug substance
- 3.2.S.4.4 Batch analyses (drug substance)
- 3.2.S.5 Reference standards or materials: Establishment of Primary Reference Material
- 3.2.S.5 Reference standards or materials: Establishment of Secondary Reference Material
- 3.2.P.5.1 Specification for drug substance
- 3.2.P.5.2 Analytical development for drug product
- 3.2.P.5.4 Batch analyses (drug product)
- Analytical Procedure (b) (4): Protein Content and (b) (4)
- Validation of Analytical Procedure (b) (4): Protein Content and (b) (4)
- Analytical Procedure (b) (4): Identity, PEG Profile and Product Related Impurities by (b) (4)
- Validation of Analytical Procedure (b) (4): Identity, PEG Profile and Product Related Impurities by (b) (4)
- Analytical Procedure (b) (4): Identity by (b) (4)
- Validation of Analytical Procedure (b) (4): Identity by (b) (4)
- Analytical Procedure (b) (4) Purity of rFIX (b) (4)
- Validation of Analytical Procedure (b) (4) Purity of rFIX (b) (4)
- Analytical Procedure (b) (4): Product related impurities by (b) (4)
- Validation of Analytical Procedure (b) (4) Product related impurities by (b) (4)
- Analytical Procedure (b) (4): Water Determination by (b) (4)
- Validation of Analytical Procedure (b) (4) Water Determination by (b) (4)

Amendment 17, dated Nov. 14, 2016

- Response to FDA Information Request
- Updated Analytical Procedure (b) (4): Identity by (b) (4)

Amendment 19, dated Nov. 28, 2016

- Response to FDA Information Request
- Analytical Procedure (b) (4)

Amendment 34, dated Jan. 17, 2017

- Response to FDA Information request
- Updated Analytical Procedure (b) (4): Identity, PEG Profile and Product Related Impurities by (b) (4)
- Updated Analytical Procedure (b) (4)

Amendment 40, dated Mar. 3, 2017

- Response to FDA Information request
- Validation of Analytical Procedure (b) (4)

Amendment 47, dated Apr. 10, 2017

- Response to FDA Information request


Review Narrative

Method and Method Validation


1. Protein Content and (b) (4) (Analytical Procedure (b) (4))

Method


(b) (4)




(b) (4)

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Validation

The method is validated by evaluating specificity, accuracy, precision, linearity, range and robustness for the protein content and by evaluating specificity, accuracy, precision and limit of quantitation (LOQ) for (b) (4) .


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2. Identity, PEG Profile and Product Related Impurities (b) (4)


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Method


(b) (4)

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Validation

The method is validated by evaluating specificity, linearity, precision, accuracy, range, LOQ and robustness.

(b) (4)

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(b) (4)



(b) (4)



(b) (4)



(b) (4)



3. Identity by (b) (4)


Method

(b) (4)



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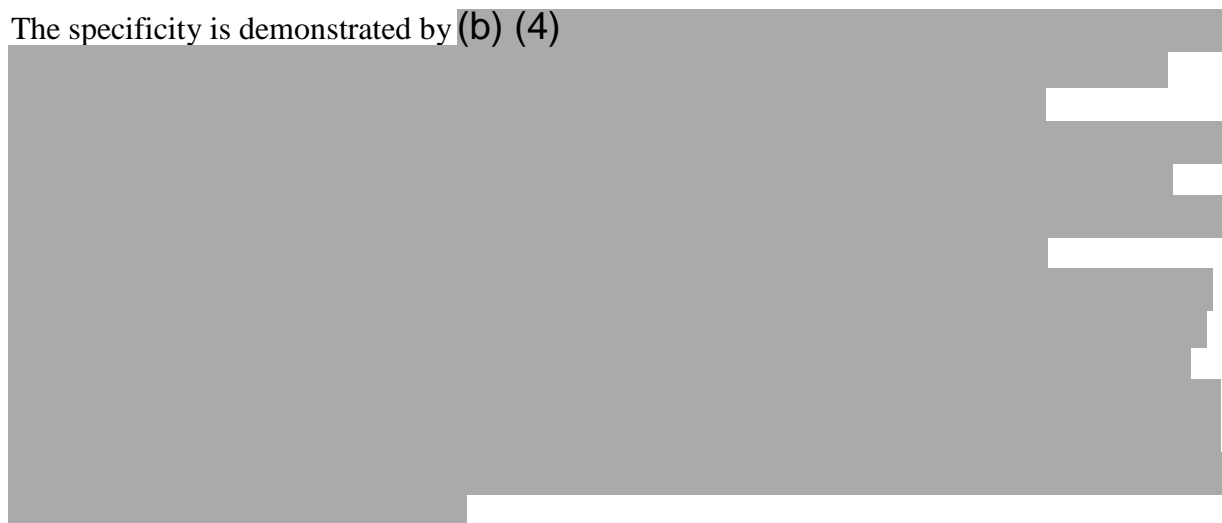
(b) (4)




Validation

This protein identification method is validated by evaluating specificity and robustness.


The specificity is demonstrated by (b) (4)



(b) (4)




4. Purity of rFIX (b) (4)




Method


(b) (4)



(b) (4)

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Validation

As a quantitative assay, the method is validated by evaluating specificity, precision, accuracy, linearity, range and robustness.


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(b) (4)

(b) (4)

5. Product related impurities (b) (4)

Method

(b) (4)

(b) (4)

Validation

As a quantitative method for impurities, it is validated by evaluating specificity, precision, accuracy, linearity, range, LOQ and robustness.

(b) (4)

(b) (4)

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(b) (4)

(b) (4)

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(b) (4)

6. Water Determination By (b) (4)

Method

(b) (4)

[REDACTED]

The proposed specification for the DP is (b) (4)

Validation

This validation report includes the (b) (4) model establishment and method validation with DP samples.

(b) (4)

[REDACTED]

The method is validated by evaluating specificity, precision, accuracy, linearity, range, LOQ and robustness.


The specificity is demonstrated by the water (b) (4)

[REDACTED]


(b) (4)

[REDACTED]


(b) (4)

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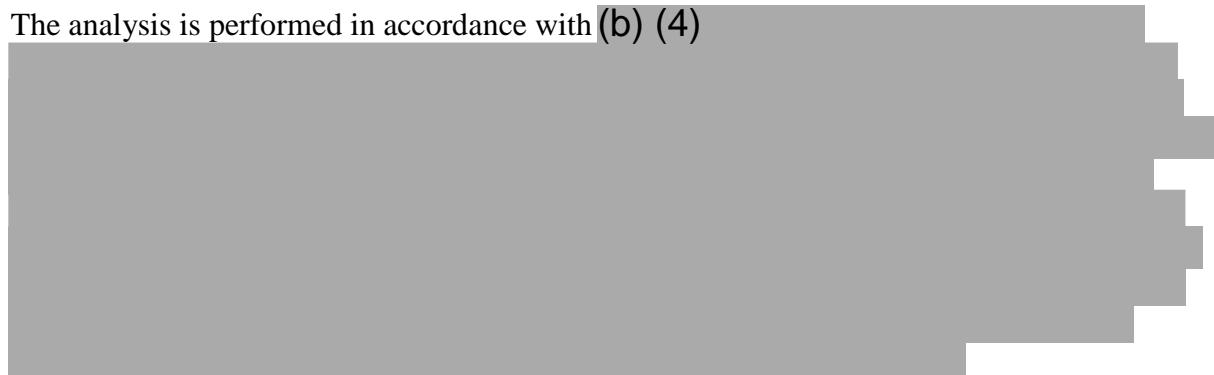
(b) (4)

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7. Water Determination by (b) (4)

Method

The analysis is performed in accordance with (b) (4)

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The proposed specification for water content of the DP sample is (b) (4).

Validation

(b) (4)

(b) (4)

(b) (4)

First Information Request (IR) and Review of Response

DBSQC IRs were sent to the sponsor as following on Oct. 24, 2016 after initial review. The responses were received on Nov. 14, 2016 and Nov. 28, 2016 in amendments 17 and 19 respectively.

1. Regarding analytical procedure (b) (4) "Protein Content and (b) (4) (novoDOCS ID 001357555):
 - a. A (b) (4) is used. Please provide data to demonstrate that (b) (4) of the protein is not affected by such high level of (b) (4) stress. In particular, we are concerned about the change in (b) (4) content of the sample. Please provide data that show that (b) (4) contents in (b) (4) DP are not altered by your assay method.
 - b. Please describe in detail how the peak area integration is done using (b) (4)

Review of the response

- a. (b) (4) representative lots of (b) (4) DP each and control sample were analyzed using (b) (4), DP and control samples are comparable at these (b) (4) levels of (b) (4). The results demonstrated that (b) (4) contents for (b) (4) DP are not affected by the (b) (4) containing (b) (4). The response is satisfactory.
- b. The request details were provided for the peak integration and the response is satisfactory.

2. Regarding "Validation of Analytical Procedure (b) (4) Protein Content and (b) (4) (novoDOCS ID 001713052)
 - a. We do not agree that accuracy can be inferred automatically from the results of the specificity, linearity and precision. You have not provided any data to demonstrate the accuracy of (b) (4) determination. Please provide details of your data analysis to show how you inferred accuracy of your method from the results of the specificity, linearity and precision. Alternatively, you may demonstrate the accuracy of the (b) (4) determination from (b) (4) studies or by comparing results obtained using an (b) (4) method.
 - b. In section 6.6 "Detection limit and quantitation limit (DL and QL)", you determined the QL to be (b) (4) by evaluating the precision of the (b) (4). We do not agree with your approach to determine QL/DL. Please provide data supporting QL by either using a drug product sample containing (b) (4) peak plus adequate precision and accuracy of the measurement, if such sample is available, or by plotting (b) (4) levels close to anticipated QL and using the equation (b) (4), where σ stands for the standard deviation of the peak area and S for the slope of the linear regression.
 - c. Please provide the actual test results and the statistical evaluation of your results to support your conclusion for the robustness study in section 6.7.

Review of the response

- a. The sponsor claimed that they have difficulty to perform (b) (4) study because of their inability to generate samples enriched in (b) (4). A discussion of how the accuracy can be theoretically inferred from linearity, specificity and precision was provided. Based on theoretical reasoning, the sponsor ruled out proportionality error, constant bias and non-linear bias for their assay results. This reviewer found that the response was not acceptable because the response lacked adequate experimental data for such a critical assay. A follow-up IR was sent to the sponsor to address the insufficient accuracy for this assay.
 - b. The (b) (4) values were calculated for the (b) (4) DP samples at appropriate (b) (4) range. The QL is conformed to be (b) (4). The response is acceptable.
 - c. The robustness data were provided in the response with statistic evaluation. The response is satisfactory.
3. Regarding “Validation of Analytical Procedure (b) (4) Identity, PEG Profile and Product Related Impurities by (b) (4) (novoDOCS ID 001745960):
- a. Please provide peak percentages of mono PEG rFIX, (b) (4) rFIX, rFIX (b) (4) and total impurities of the sample used for the linearity study in section 6.1.
 - b. As discussed above, accuracy of PEG rFIX, PEG rFIX related product and impurities cannot be automatically inferred from the outcome of linearity, specificity and precision for this critical assay. Please provide details of your data analysis to show how you inferred accuracy of your method from the results of the specificity, linearity and precision. Alternatively, you may demonstrate accuracy from your results of (b) (4) studies for each of rFIX, (b) (4) and total impurities for applicable ranges in reportable percentage up to their specification values for this product. Accuracy for these components may also be demonstrated by comparing the results of (b) (4) method(s).
 - c. In section 6.7 you determined rFIX QL to be (b) (4) by evaluating the precision of the (b) (4). We do not agree with such approach for the determination of QL. Please provide supporting data for QL for each of total impurities, rFIX PEG (b) (4), rFIX (b) (4) rFIX and rFIX separately for this assay. QL should be determined by using (b) (4) of these impurities with greater than (b) (4) of each designated peak and adequate precision and accuracy of the measurement, if such samples are available. Alternatively QL can be determined from the plot of peak area against peak percent of total impurities, rFIX PEG (b) (4), rFIX (b) (4) rFIX, each at least at three levels of peak area and using the equation, (b) (4), where σ stands for the standard deviation of the peak area and S for the slope of the linear regression.
 - d. Please provide the actual test results and the statistical evaluation to support your conclusion for the robustness study in section 6.8.

Review of the response

- a. The requested percentage data of the reference sample were provided in the response. The response is satisfactory.

- b. A response similar to Q2a (theoretical reasoning without experimental data) was provided. Follow-up IR was sent for the accuracy data for the validation of this assay.
 - c. The LOQs were re-estimated based on FDA suggested (b) (4) approach and summarized in the corresponding method validation. This reviewer agrees that total impurity is only obtained by calculation and does not need experimental data for its LOQ. The response is acceptable.
 - d. Robustness data were provided in the response. However, the analytical procedure in (b) (4) doesn't reflect the findings in the robustness study. A further IR regarding the column temperature as a critical separation parameter was sent to the sponsor.
4. Regarding analytical procedure (b) (4) "Identity by (b) (4) (novODOCS ID 001990709), please add acceptance criteria of (b) (4) performance check, such as (b) (4) as part of the SST in section 11.

Review of the response

In the updated SOP (b) (4) "Identity by (b) (4)", a new acceptance criterion of "Difference in RT for (b) (4)" is added to the section 11 based on 31 historical data sets of the control measurements to ensure adequate peak separation in the sample for this assay. Considering the qualitative nature and reference based identification for this assay, this change to the analytical procedure is sufficient without setting the acceptance criteria for tailing factor and theoretical plate number.

5. Regarding "Validation of Analytical Procedure (b) (4) Identity by (b) (4) (novODOCS ID 001990724), please provide details of test results and the statistical evaluation of the robustness study to support your conclusion in section 5.2.

Review of the response

Robustness data were provided in the response and the response is satisfactory.

6. Regarding "Validation of Analytical Procedure (b) (4) Purity of (b) (4) (novODOCS ID 002326601):
- a. Please provide the correlation coefficient value for Figure 3 (page 9) in the linearity study.
 - b. Accuracy cannot be automatically inferred from the outcome of linearity and precision studies. Please provide details of your data analysis to show how you inferred accuracy of your method from the results of the specificity, linearity and precision. Alternatively, you may demonstrate accuracy of the rFIX purity from (b) (4) studies or by comparing results obtained using an (b) (4) method.
 - c. Please provide details of the test results and the statistical evaluation of the robustness study to support your conclusion in section 6.7.

Review of the response

- a. The requested correlation coefficient for linearity study was provided. The response is satisfactory.

- b. Accuracy data were not provided and a follow-up IR was sent to the sponsor for the validation of the assay.
 - c. Robustness data were provided in the response. The response is satisfactory.
7. Regarding analytical procedure (b) (4) “Product related impurities by (b) (4) (novoDOCS ID 001893633), please identify the rFIX (b) (4) in the chromatograms of (b) (4), DP and control shown in appendixes B-E and provide data in support of your identification.

Review of the response

The (b) (4), DP and control samples, which are analyzed by (b) (4)

. But only results from (b) (4) samples were provided. A follow-up IR was sent for the results from DP and control samples.

8. Regarding “Validation of Analytical Procedure (b) (4) Product related impurities by (b) (4) (novoDOCS ID001893669):
- a. You plotted total peak area of protein (b) (4) vs. protein load, which does not show linearity of individual impurities. This method is used for the determination of product-related impurities for rFIX (b) (4) and PEGylated rFIX (b) (4) separately. Therefore, please provide linearity plots of peak area of rFIX (b) (4) vs. protein load and peak area of PEG rFIX (b) (4) vs. protein load separately, including their slopes and their respective correlation coefficients to support linearity for both impurities.
 - b. You have not provided any data to demonstrate the accuracy of the assay. Please provide accuracy data from appropriately conducted studies. We suggest that you either perform (b) (4) study or use an (b) (4) method to support the accuracy of the method.
 - c. Please provide details of the test results and the statistical evaluation of the robustness study to support your conclusion in section 6.6.

Review of the response

- a. The requested plots were provided with the regression analysis results. The response is satisfactory.
 - b. The accuracy data was not provided and a follow-up IR was sent to the sponsor.
 - c. The robustness data were provided in the response. The response is satisfactory.
9. Please provide the analytical procedure/standard operating procedure of “Water Determination by (b) (4)

Review of the response

The requested analytical procedure “Analytical Procedure (b) (4)” was provided. The response is satisfactory.

10. We do not agree that Water Determination by (b) (4) is a (b) (4) method and verification (novoDOCS ID 002260532) of the method is sufficient. For a method to be (b) (4), there has to be a monograph of the article (b) (4) drug product) in (b) (4) to which the method is referenced. Please provide a complete validation of the (b) (4) method for your drug product. The range of water determination in weight should have adequate coverage of the water level in a typical DP sample. Your validation should include results for the determination of QL of the method because this is a quantitative test for residual moisture and is used as a reference/alternative method for lot release.

Review of the response

The sponsor still considered the method as a (b) (4) one and the verification data from water standards would be sufficient. The request validation report was not provided. The response is not acceptable, particularly without accuracy results from DP samples. A follow-up IR was sent to the sponsor to acquire validation report of (b) (4) method.

11. Regarding “Validation of Analytical Procedure (b) (4) Water Determination by (b) (4)” (novoDOCS ID 002013793):

Please provide the variation ranges of matrix components, especially the contents of mannitol, sucrose and polysorbate 80 in the samples used for calibration (Table 17, page 37). It is important for the established model to have a full coverage of the proposed specification ranges (polysorbate 80 (b) (4), sucrose (b) (4) and mannitol (b) (4) for these three OH containing components in the DP matrix to demonstrate the specificity of the method. (b) (4) moisture determination is only applicable to DP samples with matrix component ranges covered by the calibration model.

Review of the response

The sponsor stated that the (b) (4) calibration model for nonacog beta pegol DP samples was not verified for samples within the entire specification ranges for polysorbate 80, sucrose and mannitol. The sample formulation covers the contents of these three excipients within (b) (4) of their respective target values. Thus, the sponsor decided that (b) (4) method for moisture determination will be used for samples, in which the content of these three excipients are all within (b) (4) of their respective target values and (b) (4) will be used for water determination of samples outside the (b) (4) interval. The response is acceptable.

Second Information Request (IR) and Review of Response

The follow-up IRs were sent to the sponsor as following on Jan. 17, 2016. The responses were received on Jan. 31, 2017 and Mar. 3, 2017 in amendments 34 and 40 respectively.

1. In the response to the question 2a of our IR dated Oct. 24, 2016, you provided a discussion of three different types of bias to justify your inference of accuracy from linearity,

specificity and precision, and data to show that the constant bias is small for this assay. The figure associated with your response 2a is cut off in such a way that it is not possible for us to have an estimate of constant bias of this assay. In addition, you have not provided R2 value for line B [forced through (0,0)], which did not permit us to assess correlation between line B and actual data points. Furthermore, the three types of bias you indicated in your response assumes no interaction between the analyte and the stationary phase under the elution conditions. However, there is ample literature reference indicating interactions between the (b) (4)

Thus, you have not provided any information that would conclusively justify inferring accuracy of this assay based on linearity, precision and specificity of the method. Please provide the accuracy data as we requested on Oct. 24, 2016 to permit us to complete our review on time. Furthermore, to the best of our knowledge, the (b) (4) rich sample can be generated easily. Several methods have been reported in the literature. Samples, enriched in (b) (4), may be spiked for accuracy evaluation.

Review of the response

Accuracy data for both (b) (4) DP were provided from (b) (4) studies, which were summarized in the method validation above. The response is satisfactory.

2. Please provide appropriate data to show that (b) (4) in your product are not retained by the column under the proposed chromatography condition in the analytical procedure (b) (4) "Protein Content and (b) (4) (novoDOCS ID 001357555).

Review of the response

In response, (b) (4)

It showed no reduction in the total peak area for samples with (b) (4) up to (b) (4). Thus, it demonstrated that the (b) (4) in the product is not retained by the (b) (4). The response is satisfactory.

3. In the response to the question 2b of our IR dated Oct. 24, 2016, we do not agree with your calculation of the (b) (4) in your precision study. In addition, the formula of (b) (4) cannot be used for the LOQ calculation from multiple measurements of the same sample. Both σ and S values are determined from a linear plot of peak area versus (b) (4) peak percent with at least (b) (4) peak percents in the samples. (You may consult ICH Q2(R1) (p. 12) for more details on how to determine LOQ from (b) (4) Please provide LOQ for (b) (4) from either appropriately determined (b) (4) You may use the method for the determination of (b) (4) described in (b) (4)

Review of the response

The LOQ of (b) (4) was determined by (b) (4) using (b) (4) DP samples containing (b) (4) as requested. The results confirmed LOQ to be (b) (4). The response is acceptable.

4. In the response to the question 3b of our IR dated Oct. 24, 2016, you did not provide the accuracy data as requested. We do not agree with your justification supporting inference of accuracy from linearity, specificity and precision, the details of which we have discussed above under question #1. Please provide the accuracy data as we requested on Oct. 24, 2016.

Review of the response

Accuracy data for components of rFIX, (b) (4) were provided for both (b) (4) DP samples. They are summarized in the corresponding validation report. However, the accuracy data for mono-PEG rFIX was not provided. A further IR was made for the accuracy of mono-PEG rFIX.

5. Please provide appropriate data to show that the chromatogram shows all impurities present in (b) (4) DP samples and none of them are retained by the column for the analytical procedure (b) (4) "Identity, PEG Profile and Product Related Impurities by (b) (4) (novoDOCS ID001742468).

Review of the response

An experiment was designed to evaluate whether impurities are retained by column under proposed separation condition. (b) (4)

. The total area recoveries in (b) (4) method (b) (4) were between (b) (4) for tested other three samples. Thus, it demonstrated that samples containing (b) (4) show consistent total protein peak area as those containing (b) (4). This approach seems to be debatable whether (b) (4) methods are true (b) (4) because different detection wavelengths are used in (b) (4). It partially provides the evidence of the total protein peak equivalence in samples with different impurity levels.

On the other hand as described in method, (b) (4)

In addition, the SOP has a requirement for (b) (4) carryover from a blank following a control injection which assures limited impurity species retained on the column. During DBSQC confirmatory test of this assay, the carryover was found to be (b) (4) and the

contribution from the impurities is less than (b) (4). Above experimental results confirm that there is acceptable level of the impurities retained by the (b) (4) using the proposed chromatographic condition. Thus results from the assay adequately determine the impurities in the sample. The chromatographic method is acceptable.

6. In the response to the question 3c of our IR dated Oct. 24, 2016, we do not agree with the calculations for the (b) (4), as discussed above under Question 3. Please provide appropriate data on the determination of the LOQ values for rFIX, (b) (4) (all in peak percentage).

Review of the response

LOQ values based on appropriate (b) (4) calculation were provided and summarized in the corresponding method validation. However, LOQs are defined as (b) (4) rFIX and (b) (4)

. The LOQ of (b) (4)

because sample containing low %rFIX forms is not available. This approach for impurity LOQ determination is questionable and was not found in the literature. We suggested the sponsor to get LOQ estimation from accuracy experiments in the 3rd IR.

7. In the response to the question 3d of our IR dated Oct. 24, 2016, you submitted robustness study results, which show that (b) (4) has a significant influence on the results. The lower temperature (b) (4) shows poor separation of (b) (4) (unknown impurity). However, your analytical procedure (b) (4) continues to allow the (b) (4) of (b) (4) which is not supported by your robustness study. Please revise your analytical procedure (b) (4) as per your robustness evaluation results.

Review of the response

The SOP (b) (4) "Identity, PEG Profile and Product Related Impurities by (b) (4)" was updated with the (b) (4) to reflect the finding in the robustness study. The response is satisfactory.

8. In the response to the question 6b of our IR dated Oct. 24, 2016, you did not provide the accuracy data as requested. We do not agree with your justification supporting inference of accuracy from linearity, specificity and precision, the details of which we have discussed above under question #1. Please provide data as we requested on Oct. 24, 2016.

Review of the response

The accuracy data was provided by (b) (4) study using an in-process sample (b) (4). The response is satisfactory.

9. In the response to the question 7 of our IR dated Oct. 24, 2016, you stated that you collected (b) (4), DP and the control samples. However, you only provided the (b) (4) test results for (b) (4). Please provide the test results from DP and the control (b) (4) to support your conclusion.

Review of the response

The sponsor clarified that (b) (4) were only collected from (b) (4)

(b) (4) DP (b) (4) batches) samples showed equivalent amount of shoulder peaks in chromatograms. This is justified because, in general, assessment of impurities is performed using (b) (4) samples for product related impurities if the level of the impurity is similar to the level in the DP sample. The response is acceptable.

10. In the response to the question 8b of our IR dated Oct. 24, 2016, you did not provide accuracy data as requested. We do not agree with your justification supporting inference of accuracy from linearity, specificity and precision, the details of which we have discussed above under question #1. Please provide data as we requested on Oct. 24, 2016.

Review of the response

The accuracy data was provided by (b) (4) study of (b) (4) (b) (4) /DP samples. The results are included in the corresponding method validation. However, the sponsor stated that they were unable to obtain a sample (b) (4) during manufacture or by manipulations of (b) (4) DP material. Considering there is no specification set for DP of the impurity of PEG rFIX (b) (4) and the sponsor has showed due diligence to generate (b) (4) material of PEG rFIX (b) (4) the accuracy evaluation from rFIX (b) (4) only is acceptable.

11. Please provide appropriate data to show that the chromatogram shows all impurities present in (b) (4) DP samples and none of them are retained by the column for the analytical procedure (b) (4) "Product Related Impurities by (b) (4)" (novoDOCS ID001893633).

Review of the response

An approach similar to the response to Q5 above was provided. A sample (b) (4) was analyzed together with representative (b) (4) DP samples, using (b) (4). For (b) (4) analytical procedures the total protein peak area for each sample relative to the total protein peak area in the (b) (4) sample will be determined. Because (b) (4) method represents an (b) (4) method compared to (b) (4) method, an appropriate recovery of normalized total protein peak area by (b) (4) relative to (b) (4) is considered adequate for concluding that the components separated in the analytical procedure (b) (4) are not being partly retained on the (b) (4). The total area recoveries in (b) (4) method (b) (4) were between (b) (4) for tested other two samples. Thus, it demonstrated that samples containing high impurities show consistent total protein peak area as those containing low impurities.

On the other hand as described in method, all peaks (b) (4)

. In addition, the SOP has a requirement for (b) (4) carryover from a blank following a control injection which only allows limited impurity species retained on the column. Above experimental results and requirement assure there is acceptable level of the impurities retained by the column using the proposed chromatographic condition. Thus result from the assay adequately determines the impurities in the sample. The chromatographic method is acceptable.

12. The chromatograms you provided in the analytical procedure (b) (4) “Product Related Impurities by (b) (4)” show a significant shoulder peak for the main peak of nonacog beta pegol for (b) (4) DP samples. Please identify the shoulder peak with the supporting data. Furthermore, please explain the reason that the chromatograms obtained by the analytical method (b) (4) “Identity, PEG Profile and Product Related Impurities by (b) (4) (novDOCS ID001742468 Figures 4 and 5) doesn’t have the same peak for the nonacog beta pegol samples.

Review of the response

The (b) (4) method (b) (4) “Product Related Impurities by (b) (4)” was developed specifically for the detection of the product related impurities: (b) (4)

The main peak from the (b) (4) method described in the document (b) (4) shows the pattern consisting of a (b) (4)

assay, which can be utilized for the separation of different protein species selected. The response is satisfactory.

13. Please add details of your typical sample injection sequence including blank, control and sample injections and the procedure for sample mass determination to your “Analytical Procedure (b) (4) (novDOCS ID003214103) and submit for review.

Review of the response

The updated SOP “Analytical Procedure (b) (4) (version 2.0)” has added a sample injection sequence in the section 8.1. The response is satisfactory.

14. We do not agree that (b) (4) method is a (b) (4) method as has been discussed in the question 10 of our IR dated Oct. 24, 2016. Please provide a complete validation for the method with your drug product samples.

Review of the response

The validation report was submitted in the response. The response is satisfactory.

Third Information Request (IR) and Review of Response

The 3rd IRs were sent to the sponsor as following on Mar. 27, 2017. The responses were received on Apr. 10, 2017 in amendment 47.

1. In the response to the question 4 of our IR dated Jan. 17, 2017, you provided accuracy data for components of rFIX, (b) (4). This assay is used as PEG profile method for the determination of (b) (4) rFIX, in addition to the product related impurities mentioned above, please provide the accuracy data for mono-PEG rFIX.

Review of the response

The results of (b) (4) for the (b) (4) rFIX data was provided in the response for (b) (4) DP samples. They are included in the method validation. The response is satisfactory.

2. We do not agree with scaling down approach of LOQ determination for %rFIX (b) (4), %rFIX and (b) (4) rFIX in your response for our Q6 because (b) (4) does not change linearly with concentration or amount injected. Please provide appropriate results of evaluation of LOQ of the three above-mentioned impurities. We suggest you calculate the LOQ by plotting impurity peak area against %impurity from corresponding accuracy data for Q4 and using the formula of (b) (4), where σ stands for the standard deviation of the peak area and S for the slope of the linear regression.

Review of the response

The LOQs were calculated according to FDA reviewer's suggestion. The results are included in the method validation. The response is satisfactory.

Conclusion

The analytical procedures of (b) (4) are adequately described and validated after several IRs and additional experimental data.